EXHIBIT 183
Incidence and Prevalence of Juvenile Idiopathic Arthritis Among Children in a Managed Care Population, 1996–2009

Leslie R. Harrold, Craig Salman, Stanford Shoor, Jeffrey R. Curtis, Maryam M. Asgari, Joel M. Gelfand, Jashin J. Wu, and Lisa J. Herrinton

ABSTRACT. Objective. Few studies based in well-defined North American populations have examined the occurrence of juvenile idiopathic arthritis (JIA), and none has been based in an ethnically diverse population. We used computerized healthcare information from the Kaiser Permanente Northern California membership to validate JIA diagnoses and estimate the incidence and prevalence of the disease in this well-characterized population.

Methods. We identified children aged ≤15 years with ≥1 relevant International Classification of Diseases, 9th edition, diagnosis code of 696.0, 714, or 720 in computerized clinical encounter data during 1996–2009. In a random sample, we then reviewed the medical records to confirm the diagnosis and diagnosis date and to identify the best-performing case-finding algorithms. Finally, we used the case-finding algorithms to estimate the incidence rate and point prevalence of JIA.

Results. A diagnosis of JIA was confirmed in 69% of individuals with at least 1 relevant code. Forty-five percent were newly diagnosed during the study period. The age- and sex-standardized incidence rate of JIA per 100,000 person-years was 11.9 (95% CI 10.9–12.9). It was 16.4 (95% CI 14.6–18.1) in girls and 7.7 (95% CI 6.5–8.9) in boys. The peak incidence rate occurred in children aged 11–15 years. The prevalence of JIA per 100,000 persons was 44.7 (95% CI 39.1–50.2) on December 31, 2009.

Conclusion. The incidence rate of JIA observed in the Kaiser Permanente population, 1996–2009, was similar to that reported in Rochester, Minnesota, USA, but 2 to 3 times higher than Canadian estimates. (First Release April 15 2013; J Rheumatol 2013;40:1218-25; doi:10.3899/jrheum.120661)

Key Indexing Terms: JUVENILE IDIOPATHIC ARTHRITIS EPIDEMIOLOGY INCIDENCE PREVALENCE HEALTH MAINTENANCE ORGANIZATIONS COMPUTERIZED MEDICAL INFORMATION

Juvenile idiopathic arthritis (JIA) is one of the more common chronic diseases in childhood\(^1\). It is defined by the International League of Associations for Rheumatology (ILAR) as arthritis that begins before the 16th birthday and persists for at least 6 weeks, with other known conditions excluded\(^2\). In addition to arthritis, extraarticular manifestations such as uveitis can occur\(^3\). JIA often persists into adulthood and the resulting inflammation and joint damage

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can lead to substantial longterm morbidity and physical disability. The ILAR classification includes 7 subtypes of JIA, which are defined on the basis of symptoms and the number of involved joints. The 7 subtypes include systemic arthritis, oligoarthritis, polyarthritis [rheumatoid factor (RF)-negative and RF-positive], psoriatic arthritis (PsA), enthesitis-related arthritis, and undifferentiated arthritis. Prior to the introduction of the ILAR classification, the most common term used in North America to describe chronic arthritis in childhood was “juvenile rheumatoid arthritis” (JRA). However, this rubric included only 4 of the subtypes defined by ILAR: systemic arthritis, oligoarthritis, and polyarthritis (RF-negative and RF-positive).

Population-based registries are important for accurately identifying cases for epidemiologic studies. These registries are used to set research priorities, investigate potentially causal associations, describe prognosis, and identify patient subgroups who share etiologic or clinical characteristics that influence treatment. Examining the frequency of JIA in diverse populations of patients is important because chronic arthritis has been shown to vary according to race and location. In Europe, incidence rates per 100,000 range from 22.6 in Norway to 3.5 in the former East Berlin area of Germany. Prevalence rates in Sweden are 86 per 100,000 as compared to 31 per 100,000 in Costa Rica and 0.83 per 100,000 in Japan. Previous studies of JIA occurrence in the United States and Canada were conducted in somewhat homogeneous populations; their results may not be generalizable to contemporary cohorts. The Kaiser Permanente Northern California Autoimmune Disease Registry, containing information for patients seen from 1996 through 2009, includes patients with JIA using the ILAR definition and those diagnosed using older rubrics (including JRA), as well as those with ankylosing spondylitis (AS), PsA, and inflammatory bowel disease. In this report, we describe case-finding for JIA and provide estimates of the incidence, prevalence, and clinical characteristics of JIA in the Kaiser Permanente membership.

MATERIALS AND METHODS

We conducted our study with the approval of the Kaiser Foundation Research Institute Institutional Review Board.

Study population. Kaiser Permanente is a prepaid, comprehensive, integrated care organization that maintains computerized clinical data of all visits, procedures, pharmacy dispensings, and other medical goods and services provided to its 8.5 million members across the United States, including the 3.2 million members in Northern California. Health plan enrollees are 41.9% white, 13.5% Asian, 6.5% black, and 16.7% multiracial or other race. Hispanic ethnicity was reported by 16.1%. These databases, comprising a variety of computerized information systems as well as an electronic medical record, provide the opportunity to build disease registries for efficient study of chronic diseases that otherwise cannot be easily identified in a stable and well-characterized population. Kaiser Permanente did not have a pediatric rheumatologist on staff during much of the study period, but pediatricians and adult rheumatologists were free to refer patients to outside pediatric rheumatologists when needed. When outside referrals were made, they typically were to nearby academic centers and children’s hospitals, with claims captured by a Kaiser Permanente information system. Usually referrals were made at the time of diagnosis and during periods of disease exacerbations that were difficult for non-sub-specialists to manage.

The present study included persons with >12 months of enrollment in Kaiser Permanente Northern California between 1996 and 2009. Cases of preliminary JIA in patients age ≤15 years were identified using age on the date of the first diagnosis recorded during the period of observation. The upper age limit of 15 years was selected on the basis of the diagnostic criteria used by ILAR. As indicated in Figure 1, we identified children aged ≤15 years with at least 1 International Classification of Diseases, 9th edition (ICD-9) code of interest. Because there was no single code for JIA in the computerized outpatient or inpatient database, we used codes for chronic arthritis including 696.0 (PsA), 714 (which includes RA and JRA), and 720 (AS) to identify preliminary cases. We did not use codes for inflammatory bowel disease because few of those patients would be confirmed with JIA. A random sample of about 10% of all preliminary cases was selected, by assigning random numbers from 0 to 1.00 and selecting cases with numbers from 0 to 0.10 for validation, using detailed review of the medical record; the actual random sample was 8.4% of the total population.

Data collection. Data collection was accomplished during 2010. The period of observation began on the later of the patient’s first enrollment or January 1, 1996, and ended on the earlier of their disenrollment or December 31, 2009. Relevant computerized medical information was obtained for all preliminary cases and included clinical and membership data recorded during 1996–2009. These computerized data were recorded to provide clinical care and not to administer insurance claims. For care received between 2004 and 2006, electronic medical record information was available. Before the electronic medical record was established, the health plan maintained several computerized information systems that were accessed for our study. These information systems included outpatient encounters, hospital diagnoses, laboratory results, pharmacy information, and imaging examinations, among others. We also accessed outside claims that were generated when patients were referred out of the plan, for example, to children’s hospitals and academic medical centers.

Manual chart review was performed on the random sample of preliminary cases. The primary purpose of the chart review was to confirm the diagnosis recorded in the computerized data and to establish the initial diagnosis date. The initial diagnosis date was the date the patient was first diagnosed with JIA, whether or not the diagnosis was made within Kaiser Permanente or within the study period. A secondary purpose of the chart review was to obtain information on clinical characteristics of the disease. A single trained medical record abstractor reviewed each medical record after extensive training supervised by a rheumatologist. Every chart was discussed with the rheumatologist until the rheumatologist was satisfied with the quality of the medical record abstractor’s work. The abstractor accessed information from the electronic medical record, computerized information systems, and paper-based medical records. She reviewed outpatient clinic notes, hospital discharge summaries, laboratory results, radiology reports, and any other information in the medical record that was pertinent to the study. A board-certified rheumatologist was consulted when the abstractor was unsure how to interpret the medical record. Case definition. ILAR defines JIA as being present when disease symptoms persist for ≥6 weeks. Disease is classified as follows: (1) systemic onset; (2) oligoarticular (“persistent” if ≤4 joints over the course of disease and “extended” if >4 joints after 6 months); (3) polyarticular (>4 joints) and RF-negative; (4) polyarticular (>4 joints) and RF-positive; (5) PsA; (6) enthesitis-related arthritis; and (7) undifferentiated (not meeting the criteria of any of the other subgroups, or of multiple subgroups).

For the purpose of our study, we defined a preliminary case of JIA as one for whom a diagnosis of JIA was recorded by an adult or pediatric rheumatologist. A diagnosis recorded by a primary care provider required supporting information, such as documentation of a telephone conversation...
between the primary care provider and a rheumatologist. Clinic notes by a primary care provider indicating joint inflammation (including synovitis or dactylitis on physical examination and lasting at least 6 weeks) in children aged ≤ 15 years were considered to be confirmed as JIA. Terms such as RA and PsA were included as cases of JIA. In addition, notes such as “history of JIA,” “JIA in remission,” or “RA diagnosed at age 15” were accepted as confirmation of JIA for all providers.

**Disease manifestations.** Disease manifestations ascertained from chart review included JIA subtype; joint involvement (hand/wrist, shoulder, hip, knee, feet/ankle, sacroiliac, cervical spine/neck, thoracic spine, lumbar spine, spine not otherwise specified, and jaw); systemic symptoms (fever, hepatosplenomegaly, lymphadenopathy, rash, and arthritis); associated autoimmune diseases (inflammatory bowel disease, other); radiographic evidence of sacroiliitis, spondylitis, or juxtaarticular new bone formation; and presence of uveitis/iritis. We also obtained laboratory findings recorded in the computerized laboratory data.

**Validity of computerized data for identifying incident and prevalent JIA.** A case-finding algorithm was developed based on clinical experience and input from researchers with expertise using administrative data for this purpose. In addition, we developed a second algorithm to identify confirmed cases that were newly diagnosed, or incident. The variables examined for inclusion in the case-finding and incidence algorithms included (1) inpatient and outpatient visits with relevant codes; (2) visits specifically to a rheumatologist (adult or pediatric); (3) use of relevant drugs; (4) use of relevant radiology services; and (5) use of relevant laboratory services. We evaluated multiple possible case-finding algorithms, with “1 or more relevant diagnosis codes recorded in the inpatient or outpatient setting by a primary care physician, pediatrician, or rheumatologist (adult or pediatric)” as the basis for comparison with all other algorithms.

The sensitivity and positive predictive value (PPV) were determined for each of the algorithms under consideration. The sensitivity of the case-finding algorithms was defined as the number of confirmed cases captured by the algorithm divided by the number of confirmed cases with 1 or more relevant diagnosis codes, as described. The PPV was defined as the proportion of cases identified by the algorithm that were confirmed with JIA during the chart review. We did not compute the specificity and negative predicted value of the diagnostic codes because the focus of the study was on evaluating case-finding algorithms, and given the rarity of JIA, pursuing identification of patients without a diagnosis would have had an exceedingly low yield. The 95% CI for the rate was computed assuming a Poisson distribution. All analyses were conducted using SAS version 9.13 (SAS Institute Inc.).

**Estimation of the standardized incidence rate and point prevalence.** We applied the best case-finding algorithm to all children aged ≤ 15 years in the Kaiser Permanente membership. The best algorithm was defined as the one that provided the fewest falsely classified cases, including both false-positive and false-negative cases. The false-negative cases were those not captured by the algorithm. For example, an algorithm requiring 2 or more rheumatologist diagnoses would not capture patients with only a single rheumatologist diagnosis, even if the diagnosis was correct. This would represent a false-negative case when assessing the algorithm “≥ 2 rheumatologist diagnoses.”

The incidence and point prevalence estimates were corrected by first multiplying by the PPV and then dividing by the sensitivity. For example, an uncorrected incidence rate of 13.9 per 100,000 person-years times PPV of 0.80 divided by a sensitivity of 0.95 yields a corrected incidence rate of 11.7 per 100,000 person-years.

The incidence rate of JIA among health plan members ≤ 15 years of age was computed as the number of newly diagnosed cases divided by the
RESULTS

Validation of the case-finding algorithm. The number of children with \( \geq 1 \) ICD-9 diagnosis code of 696.0, 714, or 720 in the computerized data was 1153. They were enrolled in the study for an average of 8 years following their first relevant diagnosis code. Among these, 97 children were randomly sampled for chart review, of which 67 (69% with CI 59%–78%) were determined to have JIA documented in the medical record (Figure 1 and Table 1). Requiring a relevant computerized diagnosis by a rheumatologist reduced the sensitivity of case-finding from 67 true cases to 54 true cases (change in sensitivity from 100% to 81% with CI 69%–89%), but it also reduced the selection of false-positive cases from 30 to 6 (change in PPV, from 69% to 90% with CI 79%–96%). Of the 13 patients identified in the computerized data as having a primary care diagnosis, 7 had seen a pediatric rheumatologist at an academic center or children’s hospital, 4 had documentation of a diagnosis by a pediatric or adult rheumatologist that was not accurately captured in the computerized data, and 2 had longstanding disease diagnosed before their enrollment, with multiple mentions by their pediatricians and ophthalmologic evaluations for iritis. The best-performing case-finding algorithm to confirm a diagnosis of JIA required \( \geq 2 \) diagnoses of diagnosis codes 696.0, 714, or 720 by any provider type (e.g., primary care or pediatric or adult rheumatology). Of the 1153 children, 772 had \( \geq 2 \) diagnoses. This algorithm, which identified both incident and prevalent cases of JIA, had a sensitivity of 87% with CI 76%–93%, capturing 58 of 67 true cases (9 false-negatives); it had a PPV of 91% with CI 80%–96%, with 58 of the 64 cases in the sample captured being true cases (6 false-positive).

Validation of incident JIA. Of the 67 children confirmed with JIA, 44 (66% with CI 53%–77%) had incident disease diagnosed while they were a Kaiser Permanente member and during the study period, while 23 (34% with CI 23%–47%) were diagnosed before the patient enrolled in Kaiser Permanente or before the study period. The best algorithm required 12 months of enrollment before the first diagnosis, a diagnosis by a rheumatologist, and 2 or more diagnostic laboratory tests. This algorithm, which identified incident JIA only, had a sensitivity of 95% with CI 81%–99%, capturing 37 of 44 true cases (9 false-negatives); it had a PPV of 82% with CI 67%–91%, with 37 of the 45 cases in the sample captured being true cases (8 false-positive).

Table 1. Sensitivity and PPV of various algorithms for confirming JIA and the incidence date.

<table>
<thead>
<tr>
<th>Concept</th>
<th>Operational Definition of Preliminary Case-finding Algorithm</th>
<th>No. in Population</th>
<th>No. in Random Sample*</th>
<th>No. True Positives</th>
<th>Using Chart Review as the Gold Standard</th>
<th>95% CI (%)</th>
<th>95% CI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease confirmation</td>
<td>( \geq 1 ) diagnosis code from any provider</td>
<td>1153</td>
<td>97</td>
<td>67</td>
<td>30</td>
<td>100 by definition</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>( \geq 2 ) diagnosis codes from any provider**</td>
<td>772</td>
<td>64</td>
<td>58</td>
<td>6</td>
<td>87</td>
<td>76–93</td>
</tr>
<tr>
<td></td>
<td>( \geq 1 ) diagnosis code from rheumatology</td>
<td>696</td>
<td>60</td>
<td>54</td>
<td>6</td>
<td>81</td>
<td>69–89</td>
</tr>
<tr>
<td>New-onset disease</td>
<td>( \geq 1 ) diagnosis code from any provider</td>
<td>1153</td>
<td>97</td>
<td>44</td>
<td>53</td>
<td>100 by definition</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>( \geq 1 ) diagnosis code from rheumatology</td>
<td>696</td>
<td>60</td>
<td>42</td>
<td>18</td>
<td>95</td>
<td>84–99</td>
</tr>
<tr>
<td></td>
<td>( \geq 1 ) diagnosis code from rheumatology, ( \geq 2 ) laboratory tests performed (ANA, RF, HLA-B27)</td>
<td>581</td>
<td>52</td>
<td>39</td>
<td>13</td>
<td>93</td>
<td>79–98</td>
</tr>
<tr>
<td></td>
<td>( \geq 1 ) diagnosis code from rheumatology, ( \geq 2 ) laboratory tests performed (ANA, RF, HLA-B27), ( \geq 12 ) months of prediagnostic enrollment</td>
<td>488</td>
<td>45</td>
<td>37</td>
<td>8</td>
<td>95</td>
<td>81–99</td>
</tr>
</tbody>
</table>

* A random sample of about 10% of all preliminary cases was selected by assigning random numbers from 0–1.00 to the 1153 cases and selecting all cases with numbers from 0–0.10 for validation using medical record review. The number in the random sample was 97 of 1153, with subsets of the 97 meeting various operational definitions tested as preliminary case-finding algorithms. PPV: positive predictive value; JIA: juvenile idiopathic arthritis; ANA: anti-nuclear antibody; RF: rheumatoid factor.
Incidence and prevalence of JIA. We calculated the age- and sex-standardized incidence rate of JIA among children ≤ 15 years of age by applying the optimal incidence algorithm (determined through chart review of 97 children and requiring 12 months of enrollment before the first diagnosis, a diagnosis by a rheumatologist, and 2 or more diagnostic laboratory tests) to the computerized database that included 1153 persons with a relevant diagnosis code. We then corrected the number of estimated cases obtained using this algorithm by accounting for the sensitivity (95%) and PPV (82%). The corrected incidence rate per 100,000 person-years was 11.9 (CI 10.9–12.9). It was 16.4 (CI 14.6–18.1) in girls and 7.7 (CI 6.5–8.9) in boys (Table 2). Using the optimal case-finding algorithm to identify all cases (which included those with at least 1 diagnosis from any provider), the corrected prevalence of JIA among those aged ≤ 15 years among Kaiser Permanente Northern California enrollees, standardized to the 2000 U.S. Census, was 44.7 (CI 39.1–50.2) per 100,000 persons on December 31, 2009 (Table 2).

Characteristics of JIA cases. Demographic characteristics of the 67 children confirmed with JIA on chart review are shown in Table 3. Forty percent were white, 26% Hispanic, 6% Asian, and 3% African American, with 25% being of other races, multiracial, or of unknown race. Forty-eight percent had oligoarticular disease, 31% had polyarticular disease, 9% systemic, 3% AS, and 8% psoriatic. The proportion receiving an antinuclear antibody test was 97%, with 70% having a positive result; for RF, 82% were tested with 9% positive; and for HLA-B27, 24% were tested with 2% positive. Among the 5 patients who were RF-positive, 4 were among the 22 patients (18%) with polyarticular disease, while 1 was among the 45 patients (4%) without polyarticular disease. The knee was involved in 72% of cases, the hand in 43%, and the feet in 37%. No associated autoimmune disease was recorded for 82% of children. Of note, 3% had uveitis/iritis recorded during the course of their disease.

DISCUSSION

We estimated the incidence of JIA among pediatric enrollees of Kaiser Permanente Northern California during the period 1996–2009 at 11.9 cases (CI 10.9–12.9) per 100,000 person-years. This rate is consistent with population-based studies that have been conducted during the past 20 years (Table 4). Most were performed in Europe and North America, with the number of cases identified ranging from 4 to 488 (the present study).

In Europe, studies have reported annual incidence rates per 100,000 in Scandinavia of 14 to 22.6,19,20,21, similar to the rate of 11.7 (CI 8.7–14.8) per 100,000 person-years. This rate is consistent with population-based studies that have been conducted during the past 20 years (Table 4). Most were performed in Europe and North America, with the number of cases identified ranging from 4 to 488 (the present study).

In North America, the incidence rate reported for Canada was 4.1 to 5.3 per 100,00013,14, lower than the incidence rate estimated in our study using our case-finding algorithm, which was nearly identical to the rate of 11.7 (CI 8.7–14.8) per 100,000 person-years reported for Rochester, New York.

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Table 2. Standardized incidence (per 100,000 person-years), 1996–2009, and point prevalence (per 100,000), December 31, 2009, of juvenile idiopathic arthritis, by sex and age.

<table>
<thead>
<tr>
<th>Incidence</th>
<th>Boys Cases per 100,000 (95% CI)</th>
<th>Girls Cases per 100,000 (95% CI)</th>
<th>Boys and Girls Cases per 100,000 (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, yrs</td>
<td>N Person-yrs</td>
<td>N Person-yrs</td>
<td>N Person-yrs</td>
</tr>
<tr>
<td>0–5</td>
<td>51</td>
<td>781,699</td>
<td>5.6 (1.3–9.9)</td>
</tr>
<tr>
<td>6–10</td>
<td>51</td>
<td>522,150</td>
<td>8.5 (3.1–13.7)</td>
</tr>
<tr>
<td>11–15</td>
<td>58</td>
<td>543,446</td>
<td>9.2 (3.7–14.8)</td>
</tr>
<tr>
<td>Overall</td>
<td>160</td>
<td>1,847,295</td>
<td>7.7 (6.5–8.9)</td>
</tr>
</tbody>
</table>

Prevalence | N Population Cases per 100,000 (95% CI) | N Population Cases per 100,000 (95% CI) | N Population Cases per 100,000 (95% CI) |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, yrs</td>
<td>N Person-yrs</td>
<td>N Person-yrs</td>
<td>N Person-yrs</td>
</tr>
<tr>
<td>0–5</td>
<td>7</td>
<td>85,578</td>
<td>8.6 (2.2–14.9)</td>
</tr>
<tr>
<td>6–10</td>
<td>31</td>
<td>95,340</td>
<td>34.0 (22.1–46.0)</td>
</tr>
<tr>
<td>11–15</td>
<td>46</td>
<td>105,366</td>
<td>45.7 (32.4–58.9)</td>
</tr>
<tr>
<td>Overall</td>
<td>84</td>
<td>286,284</td>
<td>28.6 (22.4–34.6)</td>
</tr>
</tbody>
</table>

1 The age and sex distribution were standardized to that of the 2000 Census for the entire US population. The Kaiser Permanente population included 102,652 boys and 98,238 girls aged 0–5, 101,000 boys and 96,888 girls aged 6–10, and 108,747 boys and 104,399 girls aged 11–15. 2 The incidence rate was corrected for the sensitivity (0.95) and positive predictive value (0.82) of the validated incidence algorithm. 3 The point prevalence was corrected for the sensitivity (0.87) and positive predictive value (0.91) of the validated case-finding algorithm.
Differences in incidence rates reported across studies may be the result of many factors including small numbers of cases, differences in methods of ascertainment, true population differences based on exposure to precipitating factors, or genetic predisposition. In addition, changes over time in the classification of pediatric rheumatic diseases (the adoption of the ILAR classification of JIA), greater awareness of the condition by providers in certain regions, and varying access to pediatric rheumatologists to diagnose the condition may partly explain these results.

Despite the consistency of our study with other reports, our study has limitations. Computerized information has well-known limitations relating to accuracy and completeness; it was for this reason that we validated our data and corrected our estimates for the sensitivity and PPV of case-finding. It is possible that we underascertained mild disease, particularly among those who were enrolled in the health plan for a short time. However, the study was restricted to those Kaiser Permanente Northern California members with at least 12 months of enrollment (both to be identified as a case and to be included in the denominators of the incidence and prevalence calculations). In addition, it is possible that some patients with JIA never received a diagnosis by their providers and thus were not ascertained for this study. Because the computerized data did not contain information on symptoms recorded in a standardized manner, we cannot confirm the presence of synovitis, enthesitis, or associated autoimmune conditions.

The Kaiser Permanente Northern California population is quite representative of the general population of the state of California. The health plan’s data have been linked to the California Health Interview Survey of California residents age 20–79 years living in those postal codes served by Kaiser Permanente. When compared with persons who have medical insurance through other providers, the Kaiser Permanente membership has greater racial diversity (nonwhite, 43% vs 34%). When compared with persons who were not Kaiser Permanente members, including those who were uninsured or insured by others, Kaiser Permanente members have similar racial diversity (nonwhite, 43% vs 45%), although with fewer Latinos (16% vs 23%).

The point prevalence observed in the Kaiser Permanente Northern California population (44.7 per 100,000) estimates the disease burden among children ≤ 15 years of age only and does not include the burden among adults, although the disease is chronic in most. With respect to disease manifestations, we observed 48% of children with oligoarticular disease, 31% with polyarticular, and 9% systemic. The Rochester, Minnesota, cohort differed, with 72% of patients having oligoarticular, 17% polyarticular, and 11% systemic disease at onset, with progression of oligoarticular to polyarticular disease in 11% of the cases. The prospective design they used could account for the difference, with the present study including prevalent cases, for whom disease manifestation may have been recorded following the diagnosis. Our report of uveitis/iritis in 3% of children is a bit lower than the Scandinavian study that observed uveitis/iritis in 8.6% of children, which may be due to differences in race and ethnicity. Medical record review revealed a diagnosis of AS in only 3% of our study population. While this may reflect the true prevalence of the

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Proportion of Confirmed JIA Cases, n = 67 %</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, yrs</strong></td>
<td></td>
</tr>
<tr>
<td>0–5</td>
<td>33</td>
</tr>
<tr>
<td>6–10</td>
<td>21</td>
</tr>
<tr>
<td>11–15</td>
<td>46</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>64</td>
</tr>
<tr>
<td><strong>Race/ethnicity</strong></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>40</td>
</tr>
<tr>
<td>African American</td>
<td>3</td>
</tr>
<tr>
<td>Asian</td>
<td>6</td>
</tr>
<tr>
<td>Hispanic</td>
<td>26</td>
</tr>
<tr>
<td>Other/multiracial/unknown</td>
<td>25</td>
</tr>
<tr>
<td><strong>Type</strong></td>
<td></td>
</tr>
<tr>
<td>Oligoarticular</td>
<td>48</td>
</tr>
<tr>
<td>Polyarticular</td>
<td>31</td>
</tr>
<tr>
<td>Systemic</td>
<td>9</td>
</tr>
<tr>
<td>Psoriatic arthritis</td>
<td>8</td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>3</td>
</tr>
<tr>
<td>Unknown</td>
<td>1</td>
</tr>
<tr>
<td><strong>Laboratory findings (no. who received test)</strong></td>
<td></td>
</tr>
<tr>
<td>Antinuclear antibody (n = 65)</td>
<td>70% positive</td>
</tr>
<tr>
<td>Rheumatoid factor (n = 55)</td>
<td>9% positive</td>
</tr>
<tr>
<td>HLA-B27 (n = 16)</td>
<td>2% positive</td>
</tr>
<tr>
<td><strong>Joint involvement</strong></td>
<td></td>
</tr>
<tr>
<td>Knee</td>
<td>72</td>
</tr>
<tr>
<td>Hand</td>
<td>43</td>
</tr>
<tr>
<td>Feet</td>
<td>37</td>
</tr>
<tr>
<td>Hip</td>
<td>9</td>
</tr>
<tr>
<td>Shoulder</td>
<td>7</td>
</tr>
<tr>
<td>Cervical spine/neck</td>
<td>3</td>
</tr>
<tr>
<td>Spine</td>
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<tr>
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<td>7</td>
</tr>
<tr>
<td>Not recorded</td>
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<tr>
<td>Associated autoimmune disease</td>
<td>82</td>
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<tr>
<td>Negative/not mentioned</td>
<td>13</td>
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<tr>
<td><strong>Presence during course of the disease</strong></td>
<td></td>
</tr>
<tr>
<td>Uveitis/iritis</td>
<td>3</td>
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</table>

* Age at the time of identification of an incident or prevalent case, thus not correspond to the age of diagnosis for the entire population.
** These conditions include type 1 diabetes, asthma, autoimmune hepatitis, and ulcerative colitis. JIA: juvenile idiopathic arthritis.

Table 3. Demographic and disease manifestations of confirmed cases of JIA, Kaiser Permanente, Northern California, 1996–2009.
disease, it may also have been influenced by changes in the diagnosis and/or coding of the condition. More recently, physicians are considering spondyloarthropathies as a group rather than individual conditions and may include it as part of the JIA classification. Thus, physicians may report a diagnosis of JIA rather than AS in these patients.

The methods used for case-finding in any particular study will depend on the nature of the research question, the research setting, and the relative costs of overascertainment and underascertainment with respect to study validity and precision. A case-finding strategy that has poor sensitivity but a high PPV, such as through recruitment of pediatric rheumatology clinics, likely will yield more severe cases. Milder cases, and those of children whose families cannot afford the time or expense to travel to specialty clinics, are more likely to be managed by adult rheumatologists or primary care providers including pediatricians and family practitioners.

The incidence rate of JIA estimated for children aged \( \leq 15 \) years in the Kaiser Permanente population, 1996–2009, was similar to that reported in Rochester, Minnesota, but 2 to 3 times greater than Canadian estimates. Key strengths of our study include the size of the population, the diversity of the population, and the use of chart review to validate the diagnosis of JIA. Identification of an algorithm for use with computerized data enabled efficient identification of JIA cases. As a result, our study provides a foundation for further investigation including elucidation of the environmental and genetic influences on incident JIA, current treatment patterns, healthcare use, and longterm outcomes, as well as the safety of current treatments for JIA.

**REFERENCES**


EXHIBIT 184
Prevalence of and Annual Ambulatory Health Care Visits for Pediatric Arthritis and Other Rheumatologic Conditions in the United States in 2001–2004

JEFFREY J. SACKS,1 CHARLES G. HELMICK,1 YAO-HUA LUO,2 NORMAN T. ILOWITE,3 AND SUZANNE BOWYER4

Objective. To estimate the prevalence of and the annual number of ambulatory health care visits for pediatric arthritis and other rheumatologic conditions.

Methods. We used physician office visit, outpatient department visit, and emergency department visit data from the 2001–2004 National Ambulatory Medical Care Survey and 2001–2004 National Hospital Ambulatory Medical Care Survey to estimate annual visits for the International Classification of Diseases, Ninth Revision, Clinical Modification codes thought to represent significant pediatric arthritis and other rheumatologic conditions (SPARC). We converted visit estimates into prevalence estimates using data on the number of prior annual visits per patient. Synthetic estimates for states were produced using national rates.

Results. The average annualized estimate of the number of children with SPARC was 294,000 (95% confidence interval [95% CI] 188,000–400,000). The annualized number of ambulatory health care visits for SPARC was 827,000 (95% CI 609,000–1,044,000).

Conclusion. Pediatric arthritis estimates have varied widely because it is an umbrella term for which there are many definitions and because it is a relatively uncommon condition from a population surveillance perspective. Our estimates suggest that arthritis-related health care visits impose a substantial burden on the pediatric health care system. One advantage of this surveillance paradigm is that it has established a starting point for tracking the national prevalence of arthritis and rheumatologic conditions in children on an ongoing basis using existing infrastructure rather than expensive new surveys. This surveillance system will help us monitor and predict the health care needs of patients with these conditions.

KEY WORDS. Pediatric rheumatology; Juvenile arthritis; Prevalence; Surveillance; Epidemiology.

INTRODUCTION

The prevalence of childhood arthritis in the US is not currently known. Data reported from 34 worldwide studies conducted between 1966 and 1998 showed a juvenile arthritis prevalence range of 7–401 per 100,000 children (1,2). Applying these ranges to the almost 73 million children younger than age 18 years in the US during 2002–2003 (3,4) yields estimates of 5,100–292,600 children with arthritis. Using only studies conducted in the mainland US (1,2), the prevalence rate range is 9.2–220 per 100,000. Applying these ranges yields estimates of 6,700–160,500 US children with arthritis during 2002–2003. These wide ranges result from the following: 1) differing study definitions and criteria for what childhood arthritis is, including “ever affected” or “currently affected”; 2) differing ways of ascertaining cases (e.g., hospital based versus community based); 3) differing definitions of what a child is (age cutoffs ranged from 12 to 18 years old); 4) small sample sizes or durations in studies leading to chance variation in rates; and 5) temporal trends and geographic differences.
The introduction of the Arthritis Prevention, Control and Cure Act of 2004 (S. 2338), although not passed, provided impetus for the Centers for Disease Control and Prevention (CDC) to conduct studies on the prevalence of arthritis and other rheumatic diseases and report data on juvenile arthritis. In response, CDC and the American College of Rheumatology co-hosted in December 2004 a 1-day summit of surveillance experts, pediatric rheumatologists, and key stakeholders to review available data, consider options for estimating prevalence, and draft a list of conditions for ongoing surveillance. In June 2006, after a year-and-a-half process of gathering and considering additional input, seeking comments on varying surveillance case definitions, testing possibilities, and consulting with key constituents and partners (e.g., the Arthritis Foundation; the American Academy of Pediatrics; and the National Institute of Arthritis and Musculoskeletal and Skin Diseases, National Institutes of Health), the CDC Arthritis Program finalized a paradigm for ongoing surveillance of pediatric arthritis. Mirroring that of adult surveillance, the method uses selected International Classification of Diseases (ICD) diagnostic codes from health care and other diagnostically based data systems. The present study defined this new approach and used it to estimate the national (and synthetic state) prevalence and number of ambulatory health care encounters for pediatric arthritis and other rheumatologic conditions.

**MATERIALS AND METHODS**

**Case definition.** The International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) code set for surveillance of pediatric arthritis was developed over the period December 2004 to June 2006. Various approaches were considered (e.g., using the codes from an existing pediatric arthritis registry) (5) and comments were sought from the American College of Rheumatology Section on Pediatric Rheumatology, the American Academy of Pediatrics Section on Rheumatology, and individual consultants in rheumatology and surveillance. Our intention was to focus on capturing conditions that met the following criteria: 1) expected duration of ≥3 months; 2) likely to significantly impact a child’s life; and 3) likely to involve or already involving joint, cartilage, or muscle. As options were considered, it was noted that for well over 10 years, a set of ICD-9-CM codes developed by the National Arthritis Data Workgroup (NADW) had been used to analyze and report arthritis burden for adults (6). Ultimately, we decided that maintaining parallelism in adult and pediatric arthritis surveillance had far more benefits and fewer downsides than other available options. Therefore, the final codes chosen were: 1) the subset of adult NADW ICD-9-CM codes thought to capture conditions, such as juvenile rheumatoid arthritis (JRA), that are most relevant and likely to represent significant pediatric arthritis or other rheumatologic conditions in children younger than 18 years old and 2) additional ICD-9-CM codes for significant diseases (not symptoms or signs) frequently and consistently requested for surveillance by the American College of Rheumatology Section on Pediatric Rheumatology, the American Academy of Pediatrics Section on Rheumatology, and pediatric rheumatologist and surveillance consultants to the CDC (Table 1). These selected codes were termed significant pediatric arthritis and other rheumatologic conditions (SPARC) codes.

**Data source and approach.** We used physician office visit, hospital outpatient department visit, and emergency department (ED) visit data from the 2001–2004 National Ambulatory Medical Care Survey (NAMCS) and National Hospital Ambulatory Medical Care Survey (NHAMCS) (7) to estimate annual visits for the SPARC ICD-9-CM codes (Table 1). These surveys sample visits to physician offices and hospital clinics (not individuals), include up to 3 diagnostic codes assigned by health care professionals, and allow for disease-specific estimations. Because the physician office and outpatient clinic (but not the ED) visit data also include information on the number of past visits

<table>
<thead>
<tr>
<th>ICD-9-CM codes</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>099.3</td>
<td>Reactive arthritis</td>
</tr>
<tr>
<td>136.1</td>
<td>Behçet’s syndrome</td>
</tr>
<tr>
<td>274</td>
<td>Gout</td>
</tr>
<tr>
<td>277.3</td>
<td>Amyloidosis (includes familial Mediterranean fever)*</td>
</tr>
<tr>
<td>287.0</td>
<td>Allergic purpura</td>
</tr>
<tr>
<td>390</td>
<td>Rheumatic fever without mention of heart involvement</td>
</tr>
<tr>
<td>391</td>
<td>Rheumatic fever with heart involvement</td>
</tr>
<tr>
<td>437.4</td>
<td>Cerebral arteritis</td>
</tr>
<tr>
<td>443.0</td>
<td>Raynaud’s syndrome</td>
</tr>
<tr>
<td>446</td>
<td>Polyarteritis nodosa and allied conditions</td>
</tr>
<tr>
<td>447.6</td>
<td>Arteritis, unspecified</td>
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<tr>
<td>695.2</td>
<td>Erythema nodosum*</td>
</tr>
<tr>
<td>696.0</td>
<td>Psoriatic arthropathy</td>
</tr>
<tr>
<td>701.0</td>
<td>Circumscribed scleroderma*</td>
</tr>
<tr>
<td>710</td>
<td>Diffuse diseases of connective tissue</td>
</tr>
<tr>
<td>711</td>
<td>Arthropathy associated with infections</td>
</tr>
<tr>
<td>712</td>
<td>Crystal arthropathies</td>
</tr>
<tr>
<td>713</td>
<td>Arthropathy associated with other disorders classified elsewhere</td>
</tr>
<tr>
<td>714</td>
<td>Rheumatoid arthritis and other inflammatory polyarthropathies</td>
</tr>
<tr>
<td>715</td>
<td>Osteoarthritis and allied disorders</td>
</tr>
<tr>
<td>716</td>
<td>Other and unspecified arthropathies</td>
</tr>
<tr>
<td>719.2</td>
<td>Villonodular synovitis</td>
</tr>
<tr>
<td>719.3</td>
<td>Palindromic rheumatism</td>
</tr>
<tr>
<td>720</td>
<td>Ankylosing spondylitis and other inflammatory spondylopathies</td>
</tr>
<tr>
<td>727.0</td>
<td>Synovitis and tenosynovitis</td>
</tr>
<tr>
<td>729.0</td>
<td>Rheumatism, unspecified and fibrositis</td>
</tr>
<tr>
<td>729.1</td>
<td>Myalgia and myositis, unspecified</td>
</tr>
</tbody>
</table>

* The only conditions that are not a subset of the National Arthritis Data Workgroup adult definition of arthritis and rheumatologic conditions and were the ones frequently requested by consultants.
within the last 12 months for the patient represented by the record (categorized as 0, 1–2, 3–5 or ≥6 and excluding the current visit), we used those survey data to estimate the prevalence of children with SPARC in the manner suggested by Burt and Hing (8). Conceptually, the method assumes that the weighted number of visits for 1 record divided by the number of annual visits for the patient reflected in the record (visits/patient) equals the number of patients with that condition (i.e., visits / [visits/patient] = patients). Thus, if a sampled visit was for a new patient with JRA who had a current visit and the weighted number of visits estimated for that record was 1,000, then 1,000/1 = an estimated 1,000 patients with JRA, where 1 is the total number of annual visits for that patient (1 current visit + 0 prior visits). Similarly, for a sampled visit of a patient with JRA who had been seen 3–5 times previously during the past 12 months, if the weighted number of visits estimated for that record was 1,000, then 1,000/5 = 200 estimated patients with JRA (5 is the total number of annual visits for that patient: 1 current visit + 4 prior visits [the midpoint of 3–5]). Using the midpoint for the number of prior visits, the total number of visits was calculated as follows: 0 prior visits = 1 total visit; 1–2 prior visits = 2.5 total visits; 3–5 prior visits = 5 total visits; and ≥6 prior visits = 8 total visits.

We used the SAS software, version 9.1 (SAS Institute, Cary, NC) for data extraction and analysis. We estimated the weighted number of visits and patients for each year for each diagnostic code and averaged the estimates to produce an annualized 4-year estimate. We made these estimates when a SPARC code was a listed diagnosis anywhere on the record. Programming steps were taken to avoid double counting of any records. In the case where more than one SPARC code was present on a record, the first instance was used to assign the condition. Standard errors and 95% confidence intervals (95% CIs) for both the average annual visit and prevalence estimates were calculated based on the complex survey design variables (CSTRATM and CPSUM) using SUDAAN software (Research Triangle Institute, Research Triangle Park, NC). Because the 2001 data set did not contain these variables, they were created from other variables present in the data set (Schappert SM: personal communication).

National estimates were rounded to thousands. Because NAMCS and NHAMCS estimates based on <30 records or with relative standard errors >30% are considered unreliable (9), we clarified how estimates for specific conditions relate to these criteria.

Synthetic state-based prevalence estimates. State health departments have requested prevalence data on juvenile arthritis from the CDC because they lack such data in their own states. To address this data gap, we converted the above national ICD-based estimate into a rate by dividing the national estimate by the average number of US children younger than age 18 years during 2002–2003 (3,4). We multiplied that rate by the average in the year 2002 and year 2003 state-specific population estimates (3,4) of the number of children younger than age 18 years to produce state-specific estimates. We then applied the upper and lower 95% CI estimates for the national prevalence estimates to produce the range in rates to use for the state estimates. All state estimates were rounded to the nearest hundred.

**RESULTS**

The estimated number of children with SPARC was 236,000 in 2001; 364,000 in 2002; 292,000 in 2003; and

| Table 2. ICD-9-CM code-specific proportion of prevalence and ambulatory care visits for SPARC* |
|---------------------------------|----------------|----------------|
| ICD-9-CM code and condition† | Percent of prevalence | Percent of visits‡ |
| 727.0 Synovitis and tenosynovitis | 31.3 | 23.2 |
| 729.1 Myalgia and myositis, unspecified | 22.9 | 26.9 |
| 715 Osteoarthritis and allied disorders | 10.9 | 6.7 |
| 710 Diffuse diseases of connective tissue | 7.2 | 6.4 |
| 714 Rheumatoid arthritis and other inflammatory polyarthropathies | 5.4 | 7.4 |
| 287.0 Allergic purpura | 5.3 | 6.9 |
| 711 Arthropathy associated with infections | 5.2 | 6.9 |
| 716 Other and unspecified arthropathies | 3.0 | 3.0 |
| 446 Polyarteritis nodosa and allied conditions | 2.4 | 3.4 |
| Remaining SPARC codes§ | 6.4 | 9.2 |

* ICD-9-CM = International Classification of Diseases, Ninth Revision, Clinical Modification; SPARC = significant pediatric arthritis and other rheumatologic conditions.
† Estimates for 727.0, 729.1, 710, and 714 are based on samples of 42–104 records, but relative standard errors exceed 30%. All the remaining code estimates are based on <30 records.
‡ Visit data include 83,000 emergency department visits that were not used to estimate prevalence.
§ Data not shown individually for codes with estimated proportions <3% for prevalence or visits. Such codes include 099.3 reactive arthritis; 136.1 Behçet’s syndrome; 274 gout; 277.3 amyloidosis; 390–391 rheumatic fever; 437.4 cerebral arteritis; 443.0 Raynaud’s syndrome; 447.6 arteritis, unspecified; 695.2 erythema nodosum; 696.0 psoriatic arthropathy; 701.0 circumscribed scleroderma; 712 crystal arthropathies; 713 arthropathy associated with other disorders classified elsewhere; 719.2 villonodular synovitis; 719.3 polyarteritis nodosa; 720 ankylosing spondylitis and other inflammatory spondylopathies; and 729.0 rheumatism, unspecified and fibrositis.
284,000 in 2004, for an average annualized estimate of 294,000 children with SPARC (95% CI 188,000–400,000). The corresponding prevalence rate was 403 per 100,000 (95% CI 257–548 per 100,000). The top 5 most common conditions were synovitis and tenosynovitis, myalgia and myositis (includes codes for fibromyalgia), osteoarthritis and allied disorders, diffuse diseases of connective tissue, and rheumatoid arthritis and other inflammatory polyarthropathies (includes all codes for JRA and related conditions) (Table 2).

The number of ambulatory care visits for SPARC increased from 665,000 in 2001 to 813,000 in 2002 to 828,000 in 2003 to 1,000,000 in 2004, for an average annualized estimate of 827,000 visits (95% CI 609,000–1,044,000), including an average annualized estimate of 83,000 ED visits. State estimates based on the ICD-9-CM case definition ranged from 500 children with SPARC in Wyoming to 38,000 children in California (Table 3).

DISCUSSION

The national annualized prevalence estimate for 2001–2004 was 294,000 children with SPARC in the US. Childhood arthritis-related ambulatory health care visits were estimated to be 827,000, imposing a substantial burden on pediatric health care systems.

These ICD-based estimates have a number of limitations. First and foremost, much disagreement exists among experts about clinical case definitions of childhood arthritis. Indeed, our prolonged efforts at developing a surveillance case definition revealed much variation about what consultants thought should be counted as pediatric arthritis. In response, we also provide estimates for conditions that some constituents unsuccessfully nominated for SPARC (see Appendix A). Thus, a constituent who thought that what they consider to be childhood arthritis, may also have not been counted. In addition, because there are pockets of specialists, e.g., pediatric rheumatologists, whose practices may not have been included in the sample, there may be undercounting of cases. In contrast, children with SPARC who saw more than one physician may have been overcounted if the survey samples included both of these practitioners.

Fourth, our ICD-based estimate is based on diagnoses listed in any of 3 diagnostic fields of the sampled visit, as opposed to only the primary diagnosis for the visit. We chose to use any listed diagnosis as the basis for estimation, because although a SPARC code may not have been the primary reason for a visit, by virtue of it being listed one can assume it impacted on the visit in some way. For example, if a child with JRA went to a physician for an acute infection, the infection might be coded as the primary reason for the visit and JRA as a secondary reason. We did not want to miss counting such children. This decision may have caused some overestimation. If we were to use only the first-listed (i.e., primary) diagnosis, the annualized estimate would be 23% less (i.e., 226,000 children with SPARC [185,000 in 2001; 227,000 in 2002; 262,000 in 2003; 230,000 in 2004]). The annualized number of pediatric outpatient visits for SPARC using only the first-listed (primary) diagnosis would be 24% less (i.e., 627,000 [65,000 of which were ED visits] with 474,000 in 2001; 647,000 in 2002; 668,000 in 2003; and 719,000 in 2004). In contrast, using any diagnosis meant that in the case where a child had ≥2 SPARC codes, only the first encountered was used to assign the condition. Thus, a child with tenosynovitis and JRA listed in that order might be assigned to tenosynovitis, with JRA underestimated.

Fifth, we were unable to convert ED visits into prevalence estimates, which no doubt led to an undercount. Using the most conservative assumption that if the data on prior visits had been present and that each ED visit had the upper range (n = 8) of prior visits (for conversion of visits to children), then an additional 10,000 cases were not counted because we were unable to convert the ED visit data into prevalence. In contrast, if each such ED visit represented the only annual visit for that child, then an additional 83,000 children with SPARC were not counted in the estimate.

Sixth, the conversion of visits to prevalence assumes that the prior annual visit data are accurate. To the extent this parameter is mistaken, the estimates are imprecise. Another limitation is that misclassification and incorrect ICD coding can lead to estimate errors. We note that osteoarthritis and allied disorders was one of the more frequently coded diagnoses (usually not as the first-listed diagnosis); however, osteoarthritis is most unusual in childhood and suggests misclassification or misdiagnosis. These problems of misclassification and misdiagnosis continue to be an issue, especially considering that the vast majority of these children were not diagnosed by a physician with specialty training in pediatric rheumatology. Previous studies have shown that adult rheumatologists see many children with arthritis and joint symptoms in general because of the lack of adequate numbers of pediatric rheumatologists. The obvious miscoding of the diagnoses found in this survey points out once again the need for more pediatric rheumatologists who can make the cor-
Table 3. Synthetic state-specific prevalence estimates of pediatric arthritis, 2001–2004*

<table>
<thead>
<tr>
<th>Children &lt;18 years old, no.†</th>
<th>ICD-9-CM–based estimates, no. (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>72,969,000</td>
</tr>
<tr>
<td>Alabama</td>
<td>1,107,500</td>
</tr>
<tr>
<td>Alaska</td>
<td>190,900</td>
</tr>
<tr>
<td>Arizona</td>
<td>1,498,100</td>
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<tr>
<td>Arkansas</td>
<td>679,800</td>
</tr>
<tr>
<td>California</td>
<td>9,436,200</td>
</tr>
<tr>
<td>Colorado</td>
<td>1,151,900</td>
</tr>
<tr>
<td>Connecticut</td>
<td>854,100</td>
</tr>
<tr>
<td>Delaware</td>
<td>194,300</td>
</tr>
<tr>
<td>Dist. of Columbia</td>
<td>110,300</td>
</tr>
<tr>
<td>Florida</td>
<td>3,903,200</td>
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<tr>
<td>Georgia</td>
<td>2,282,600</td>
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<td>Hawaii</td>
<td>296,300</td>
</tr>
<tr>
<td>Idaho</td>
<td>371,200</td>
</tr>
<tr>
<td>Illinois</td>
<td>3,254,600</td>
</tr>
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<td>Indiana</td>
<td>1,599,400</td>
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<tr>
<td>Iowa</td>
<td>695,700</td>
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<td>Kansas</td>
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<td>Kentucky</td>
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<tr>
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<td>1,181,600</td>
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<tr>
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<tr>
<td>Maryland</td>
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<tr>
<td>Minnesota</td>
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<td>Mississippi</td>
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<td>Missouri</td>
<td>1,402,400</td>
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<td>Montana</td>
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<td>Nebraska</td>
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<td>Nevada</td>
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<td>New Hampshire</td>
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<td>New Jersey</td>
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<td>146,800</td>
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<td>Tennessee</td>
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<td>Texas</td>
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<td>Utah</td>
<td>728,000</td>
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<td>Vermont</td>
<td>138,600</td>
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<td>Virginia</td>
<td>1,789,100</td>
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<td>Washington</td>
<td>1,505,000</td>
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<td>West Virginia</td>
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<tr>
<td>Wisconsin</td>
<td>1,335,500</td>
</tr>
<tr>
<td>Wyoming</td>
<td>121,700</td>
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</table>

* ICD-9-CM = International Classification of Diseases, Ninth Revision, Clinical Modification; 95% CI = 95% confidence interval.
† Estimates based on dividing national ICD-9-CM–based any diagnosis significant pediatric arthritis and other rheumatologic conditions prevalence by the child population younger than 18 years of age and applying that rate to state-specific populations (3,4). All estimates rounded to the nearest 100.

rect diagnosis of joint pain and swelling in the pediatric population. This fact again speaks to the other issue addressed by the Arthritis Prevention, Control and Cure Act of 2004: the critical undersupply and maldistribution of pediatric rheumatologists.

Beyond how SPARC is defined, at least 3 clinical clas-
sification schemes for childhood arthritis exist: JRA, juvenile chronic arthritis, and juvenile idiopathic arthritis. All 3 schemes do not include many of the conditions considered as arthritis and other rheumatic conditions in adults, and while a case counted in 1 classification system may not be a case in another system, all schemes define childhood arthritis as occurring in children younger than 16 years of age. SPARC, however, includes 16- and 17-year-olds in the estimates because we elected to maintain national arthritis surveillance across the complete age spectrum (adult surveillance starts at age 18 years). Regardless of the classification schemes, the purpose of SPARC is for ongoing surveillance and trend analysis with a broad population focus and not clinical diagnosis of individual cases of illness.

To gauge the reasonableness of the SPARC estimates, we looked at 2 other data sources. First, we used self-reported data from the 2001–2004 National Health Interview Survey (NHIS). In 2001, adults were asked, “Have you ever been told by a doctor or other health professional that you have arthritis?” In 2002–2004, adults were asked, “Have you ever been told by a doctor or other health professional that you have arthritis, rheumatoid arthritis, gout, lupus or fibromyalgia?” Assuming the questions were, for practical purposes, identical, we summed the weighted annual 18-year-old prevalences for each year and averaged them. From a total of 3,428 sampled 18-year-olds, the average annualized estimate of self-reported doctor-diagnosed arthritis for 18-year-olds was 119,600 (95% CI 79,400–159,900). Second, the 2001–2004 NHIS also contained a sampling of children in households. Adult respondents were asked, “Has a doctor or health professional ever told you that (sampled child’s name) had any of these conditions?” The respondent was given a list of 10 conditions, one of which was arthritis. From child samples ranging from 12,249 to 13,579 over the 4 years, the average annualized estimate of arthritis prevalence among children younger than 18 years old was 80,100 (95% CI 51,500–108,600) (11).

That both of these self-report–based estimates are lower than the ICD-based estimates is not surprising. The SPARC definition includes many other conditions that the respondent may not think of as the conditions mentioned in the questions. For example, a respondent with tenosynovitis or polyarteritis nodosa might have answered “no” to the self-report question. For the 18-year-old–based estimate, poor recall may underestimate prevalence; the estimate also reflects a cohort experience and, therefore, is a lagging indicator of the current prevalence. For the proxy report on the child having arthritis, studies have shown that parental reporting of child arthritis is imprecise (12). Nevertheless, both forms of self-report provide a potential tool to monitor national trends over time. Although the adult self-report question is also used in the state-based Behavioral Risk Factor Surveillance System (BRFSS), state-specific 18-year-old BRFSS sample sizes averaged only 42 and, therefore, are not likely to be useful for state surveillance.

One aspect of the Arthritis Prevention, Control and Cure Act of 2004 was to address the need for pediatric rheumatologists. Although the estimates here give a data-based estimate of the burden of rheumatologic diseases in childhood, they cannot be used to estimate the need for pediatric rheumatologists. These practitioners diagnose and treat a broad range of conditions in addition to those considered to be classic rheumatic diseases. Whereas internal medicine-trained rheumatologists often confine their practices to the diagnoses listed in the NADW list, pediatric rheumatologists often serve as diagnosticians in their respective departments, seeing patients with a much wider spectrum of diagnoses. Indeed, a number of pediatric rheumatologists asked for the inclusion of conditions listed in the Appendix A, because they believed that these diagnoses comprised a large number of the patients seen in their practices, even if they were not defined as SPARC. However defined, pediatric arthritis-related health care visits clearly impose a substantial burden on pediatric health care systems, with more than three-quarters of a million visits annually. Adding in the visits for just a few of the conditions that pediatric practitioners see in their practice (see Appendix A) suggests that annual ambulatory care visits for conditions needing evaluation easily top 1 million.

Pediatric arthritis estimates vary widely, and therefore much difficulty is encountered in describing its epidemiology. The reasons for this are 3-fold: pediatric arthritis is an umbrella term covering a number of types of arthritis and related rheumatic conditions, there are a number of different clinical case definitions for pediatric arthritis, and pediatric arthritis is a relatively uncommon condition from a population surveillance perspective. These difficulties have inhibited progress in the field. The strength/merit of this surveillance paradigm is that in this contentious field, it has established a starting point for tracking the prevalence of arthritis and rheumatologic conditions in children on an ongoing basis using existing infrastructure rather than expensive new surveys. Its advantage over prior estimates is that these estimates are data based and national in scope. This surveillance system will help us monitor and predict the health care needs of patients with these conditions.

ACKNOWLEDGMENTS

We thank Gary Langmaid for computer programming, and Brian Feldman, MD, MSc, FRCPC (Hospital for Sick Children, Toronto); Rosemarie Hirsch, MD, MPH (National Center for Health Statistics); Reva Lawrence, MPH (National Institute of Arthritis and Musculoskeletal Diseases); and Patience White, MD, MA, FAAP (Arthritis Foundation) for work at the December 2004 meeting on considering surveillance options and the case definition.

AUTHOR CONTRIBUTIONS

Dr. Sacks had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study design. Sacks, Helmick, Ilowite, Bowyer.

Acquisition of data. Sacks, Luo.

Analysis and interpretation of data. Sacks, Helmick, Luo, Ilowite, Bowyer.


Statistical analysis. Sacks, Luo.
REFERENCES


Appendix A: Potential addition to estimated prevalence and outpatient visits for SPARC from selected pediatric conditions

<table>
<thead>
<tr>
<th>ICD-9-CM code and condition†</th>
<th>Percent addition to prevalence</th>
<th>Percent addition to visits‡</th>
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<tr>
<td>719.4 Pain in joint</td>
<td>97.7</td>
<td>94.8</td>
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<tr>
<td>726 Peripheral enthesopathies and allied syndromes</td>
<td>53.1</td>
<td>37.6</td>
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<tr>
<td>729.5 Pain in limb§</td>
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<td>727.4 Ganglion and cyst of synovium, tendon, and bursa</td>
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<tr>
<td>719.0 Effusion of joint</td>
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<td>13.0</td>
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<tr>
<td>728.8 Other disorders of muscle, ligament, and fascia</td>
<td>19.1</td>
<td>18.9</td>
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<tr>
<td>729.8 Other musculoskeletal symptoms referable to limbs§</td>
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<tr>
<td>719.9 Unspecified disorder of joint</td>
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<td>088.81 Lyme disease§</td>
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<td>717.7 Chondromalacia of patella§</td>
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<td>750.82 Marfan’s syndrome§</td>
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<td>719.7 Difficulty in walking</td>
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<td>279.4 Autoimmune disease, not elsewhere classified§</td>
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<td>756.83 Ehlers Danlos syndrome§</td>
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<tr>
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<tr>
<td>337.2 Reflex sympathetic dystrophy§</td>
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<td>4.0</td>
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<tr>
<td>728.9 Unspecified disorder of muscle, ligament, and fascia</td>
<td>2.8</td>
<td>5.4</td>
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* Because some of the input during our case definition development process requested inclusion of some conditions other than the final ones chosen for SPARC, we used a similar approach (any listed diagnosis) to calculate outpatient visits and estimated prevalence for the conditions above and below. Programming steps were taken to avoid double counting of any records. Any cases of SPARC were removed from the data set before this analysis was conducted. In the case where more than I of the codes of interest below was present on a record, the first instance found when proceeding from primary diagnosis to last listed diagnosis was used to assign the condition. We then calculated what percentage the estimate for SPARC would increase if the specific condition had been included in SPARC. The following ICD-9-CM codes all contributed <3% to either prevalence or visit estimates: 3.238, 36.825, 56.718, 55.6, 95.7, 98.5, 1355, 272.86, 277.2, 279.8, 344.6, 353.0, 354.0, 355.5, 357.1, 364.18, 443.89, 719.1, 719.5, 721, 727.1, 727.2, 727.3, 727.5, 727.9, 728.0, 728.1, 728.2, 728.3, 728.4, 728.5, 728.6, 728.7, 728.9, 729.38, and 733.48. SPARC = significant pediatric arthritis and other rheumatologic conditions; ICD-9-CM = International Classification of Diseases, Ninth Revision, Clinical Modification. Example: to add ICD-9-CM 726 (peripheral enthesopathies and allied syndromes) to the national estimate, multiply 294,000 (the national prevalence estimate) times 0.531 (the percent addition to prevalence found in the column) to yield 156,000 (the estimate of cases of ICD-9-CM 726). Add 156,000 to 294,000 to arrive at a sum of 450,000 cases of SPARC including peripheral enthesopathies and allied syndromes.

† Estimate for 719.4 based on >30 records and has relative standard error less than 30%; estimates for 726, 729.5, 727.4, 719.0, 728.8, 729.8, and 719.9 are based on >30 records but have relative standard errors greater than 30%. All other estimates are based on less than 30 records.

‡ Visit data include the following emergency department visits that were not used to estimate prevalence: 719.4 = 139,000; 729.5 = 108,000; 728.8 = 40,000; 719.0 = 23,000; 728.8 = 15,000. The first 2 estimates were based on >30 records; the latter 2 were not and should be considered unreliable.

The remaining codes with emergency department visits all had estimates <15,000.

§ Conditions that are not a subset of National Arthritis Data Workgroup adult definition.
EXHIBIT 185
What is childhood arthritis?

Arthritis in children is called childhood arthritis or juvenile arthritis. The most common type of childhood arthritis is juvenile idiopathic arthritis (JIA), also known as juvenile rheumatoid arthritis.

Childhood arthritis can cause permanent physical damage to joints. This damage can make it hard for the child to do everyday things like walking or dressing and can result in disability.

Is there a cure for childhood arthritis?

Although there is no cure, some children with arthritis achieve permanent remission, which means the disease is no longer active. Any physical damage to the joint will remain.

What are the signs and symptoms of childhood arthritis?

Symptoms may come and go over time. There may be times when symptoms get worse, known as flares, and times when symptoms get better, known as remission. Signs and symptoms include:

- Joint pain.
- Swelling.
- Fever.
- Stiffness.
- Rash.
- Fatigue (tiredness).
- Loss of appetite.
Inflammation of the eye.

Difficulty with daily living activities such as walking, dressing, and playing.

What causes childhood arthritis?

The exact cause of childhood arthritis is unknown. In childhood arthritis the immune system may not work right which causes the inflammation in the joints and other body systems.

How is childhood arthritis diagnosed?

Childhood arthritis is diagnosed through a physical examination and review of symptoms, X-rays, and lab tests. A doctor should make this diagnosis, particularly a rheumatologist who specializes in arthritis and other related conditions in children. These doctors are called pediatric rheumatologists.

Who gets childhood arthritis?

Childhood arthritis can affect children of all ages, races and ethnic backgrounds.

Learn more about childhood arthritis

- Juvenile Arthritis from National Institute of Arthritis and Musculoskeletal and Skin Diseases
- Juvenile Arthritis: Fast Facts for Patients and Caregivers from the American College of Rheumatology
- Childhood Arthritis and Rheumatology Research Alliance (CARRA)
- Kids Get Arthritis Too from the Arthritis Foundation

Learn more about arthritis

- Arthritis Types
- Physical Activity for Arthritis
- Frequently Asked Questions (FAQs)
- Arthritis-Related Statistics
EXHIBIT 186
Systemic Lupus Erythematosus (SLE)

(Disseminated Lupus Erythematosus or Lupus)

By Alana M. Nevares, MD, The University of Vermont Medical Center

Last full review/revision Apr 2020 | Content last modified Apr 2020

Systemic lupus erythematosus is a chronic autoimmune inflammatory connective tissue disorder that can involve joints, kidneys, skin, mucous membranes, and blood vessel walls.

- Problems in the joints, nervous system, blood, skin, kidneys, gastrointestinal tract, lungs, and other tissues and organs can develop.
- Blood tests and sometimes other tests are done to make the diagnosis.
- All people with lupus need hydroxychloroquine and people with lupus that is continuing to cause damage (active lupus) also need corticosteroids and other drugs that suppress the immune system.

About 70 to 90% of people who have lupus are women of child-bearing age, but children (mostly girls), older men and women, and even newborns can also be affected. Lupus occurs in all parts of the world but may be more common among blacks and Asians than whites.

The cause of lupus is usually not known. Occasionally, the use of certain drugs (such as hydralazine and procainamide, which are used to treat heart conditions, and isoniazid, which is used to treat tuberculosis) can cause lupus. Drug-induced lupus usually disappears after the drug is stopped.

The number and variety of antibodies that can appear in lupus are greater than those in any other disorder. These antibodies may sometimes determine which symptoms develop. However, the levels of these antibodies may not always be proportional to the person's symptoms.

Discoid lupus erythematosus (DLE), sometimes called chronic cutaneous lupus erythematosus, is a form of lupus that affects only the skin. In this condition, raised, round, red rashes occur, sometimes progressing to some loss of the skin with scarring and hair loss in affected areas. The rash clusters on light-exposed areas of the skin, such as the face, scalp, and ears. Sometimes a rash or sores also affect the mucous membranes, especially in the mouth. In 10% of people, manifestations of systemic lupus—for example, those affecting the joints, kidneys, and brain—may occur.
Subacute cutaneous lupus erythematosus (SCLE) is a form of lupus that affects mostly the skin, causing various rashes that are widespread, come and go, and may worsen with exposure to sunlight. Red and ring-shaped or psoriasis-like patches may form on the arms, face, and trunk. SCLE differs from DLE because SCLE rarely causes scarring. People commonly have fatigue and joint pains but usually do not have the serious damage to internal organs that can occur in SLE.

Symptoms

Symptoms of lupus vary greatly from person to person. Symptoms may begin suddenly with fever, resembling a sudden infection. Or symptoms may develop gradually over months or years with episodes (called flare-ups) of fever, feeling unwell, or any of the symptoms discussed below alternating with periods when symptoms are absent or minimal. Most people with lupus have mild symptoms affecting mostly the skin and joints. Migraine-type headaches, epilepsy, or severe mental disorders (psychoses) may be the first abnormalities that are noticed. Eventually, however, symptoms may affect any organ system.

Joint problems

Joint symptoms, ranging from intermittent joint pains (arthralgias) to sudden inflammation of multiple joints (acute polyarthritis), occur in about 90% of people and may exist for years before other symptoms appear. In long-standing disease, marked joint looseness and deformity may occur (Jaccoud arthropathy) but is rare. However, joint inflammation is generally intermittent and usually does not damage the joints.

Skin and mucous membrane problems

Rashes include a butterfly-shaped redness across the nose and cheeks (called a malar rash or butterfly rash), raised bumps or patches of thin skin, and flat or raised red areas on exposed areas of the face and neck, upper chest, and elbows. Blisters and skin ulcers (sores) are rare, but ulcers do commonly occur on mucous membranes, particularly on the roof of the mouth, on the inside of the cheeks, on the gums, and inside the nose.
Generalized or patchy loss of hair (alopecia) is common during flare-ups. Mottled red areas on the sides of the palms and up the fingers, redness and swelling around the nails, and flat, reddish purple blotches between the knuckles on the inner surfaces of the fingers also may occur. Purplish spots (petechiae) may occur because of bleeding in the skin as a result of low platelet levels in the blood. Long-lasting rashes resulting from exposure to sunlight (photosensitivity) occur in some people with lupus, particularly fair-skinned people.
People with lupus who have Raynaud syndrome have very pale or bluish fingers and toes when they are exposed to cold.

**Lung problems**

It is common for people with lupus to feel pain when breathing deeply. The pain is due to recurring inflammation of the sac around the lungs (pleurisy), with or without fluid (effusion—see symptoms of pleural effusion) inside this sac. Inflammation of the lungs (lupus pneumonitis), resulting in breathlessness, is rare, although minor abnormalities in lung function are common. Life-threatening bleeding into the lungs may rarely occur. Blockage of arteries in the lung caused by blood clots (thrombosis) can also occur.

**Heart problems**

People with lupus may have chest pain due to inflammation of the sac around the heart (pericarditis). More serious but rare effects on the heart are inflammation of the walls of the coronary arteries (coronary artery vasculitis), which can lead to angina, and inflammation of the heart muscle (myocarditis), which can lead to heart failure. The valves of the heart can rarely be involved and may need to be surgically repaired. People are at increased risk of coronary artery disease.

An infant whose mother has lupus and has a certain type of antibody (anti-Rho/SSA antibody) can be born with heart block.

**Lymph node and spleen problems**

Widespread enlargement of the lymph nodes is common, particularly among children, young adults, and blacks of all ages. Enlargement of the spleen (splenomegaly) occurs in about 10% of people.

**Nervous system problems**

Involvement of the brain (neuropsychiatric lupus) can cause headaches, mild impairment of thinking, personality changes, stroke, seizures, severe mental disorders (psychoses), or a condition in which a number of physical changes may occur in the brain, resulting in disorders such as dementia. The nerves in the body or spinal cord may also be damaged.
Kidney problems

Kidney involvement may be minor and without symptoms or may be progressive and fatal. People may develop kidney failure that requires dialysis. The kidneys can be affected at any time and may be the only organ affected by lupus. The most common results of kidney impairment are high blood pressure and protein in the urine that leads to swelling (edema) in the legs.

Blood problems

The numbers of red blood cells, white blood cells, and platelets may decrease. Platelets assist in blood clotting, so if these numbers decrease greatly, bleeding may occur. Also, and for other reasons, the blood may clot too easily, which accounts for many of the problems that can affect other organs (such as strokes and blood clots to the lungs or repeated miscarriages).

Gastrointestinal tract problems

People may have nausea, diarrhea, and vague abdominal discomfort. The occurrence of these symptoms may be the forewarning of a flare-up. Impairment of blood supply to various parts of the gastrointestinal tract may result in more severe abdominal pain, damage to the liver or pancreas (pancreatitis), or a blockage or hole (perforation) of the gastrointestinal tract.

Pregnancy problems

Pregnant women have a higher-than-normal risk of miscarriage and stillbirth. Flare-ups are common during pregnancy or immediately after delivery. Doctors do not advise women to conceive if their lupus has not been controlled during the prior 6 months.

Diagnosis

- A doctor's examination
- Laboratory tests

Doctors suspect lupus mainly on the basis of the person's symptoms during a thorough physical examination, particularly in a young woman. To help confirm the diagnosis, doctors do several laboratory tests. Although there is no single laboratory test that confirms the diagnosis of lupus, doctors do these tests to rule out other connective tissue disorders. Doctors then base the diagnosis of lupus on all of the information they gather, including symptoms, physical examination results, and all test results. Doctors use this information to help them determine whether people meet specific, established criteria that are used to confirm lupus. Nonetheless, because of the wide range of symptoms, distinguishing lupus from similar diseases and making the diagnosis can be difficult.

Laboratory tests

Although blood test results can help doctors diagnose lupus, they alone cannot confirm a definite diagnosis of lupus because sometimes the abnormalities they detect are present in healthy people or in people who have other disorders. A blood test can detect antinuclear antibodies (ANA), which are present in almost all people who have lupus. However, these antibodies also occur in other diseases. Therefore, if antinuclear antibodies are detected, a test for antibodies to double-stranded DNA as well as a test for other autoimmune antibodies (autoantibodies) are done. A high level of these antibodies to DNA strongly supports the diagnosis of lupus, but not all people who have lupus have these antibodies.
Other blood tests, such as measuring the level of complement proteins (proteins with various immune functions, such as killing bacteria), are also done and can help predict the activity and course of the disease in some people. Women with lupus who have repeated miscarriages or have had problems with blood clots should be tested for antiphospholipid antibodies. This is an important test when planning contraceptive methods or pregnancy. This blood test, which detects antibodies to phospholipids, can also help identify people at risk of repeated blood clots. Women with positive antibodies to phospholipids should not take estrogen-containing oral contraceptives and should choose other methods of contraception.

Blood tests can also indicate anemia, a low white blood cell count, or a low platelet count. People who have anemia undergo a direct Coombs test. This test is used to detect increased amounts of certain antibodies that are attached to the surface of red blood cells and can destroy red blood cells, causing anemia.

Additional laboratory tests are done to detect the presence of protein or red blood cells in the urine (urinalysis) or an elevation of creatinine in the blood. These findings indicate kidney inflammation of the filtering structure in the kidneys (glomeruli), a condition referred to as glomerulonephritis. Sometimes a kidney biopsy (removal of tissue for examination and testing) is done to help the doctor plan treatment. People who have lupus should be tested frequently for kidney damage even if they have no symptoms (see Kidney Function Tests).

Prognosis

Lupus tends to be chronic and relapsing, often with symptom-free periods (remissions) that can last for years. Flare-ups can be triggered by sun exposure, infection, surgery, or pregnancy. Flare-ups occur less often after menopause. Many people are being diagnosed earlier and with milder lupus than in the past, and better treatment is available. As a result, in most developed countries, more than 95% of people live for at least 10 years after the diagnosis is made. However, because the course of lupus is unpredictable, the prognosis varies widely. Usually, if the initial inflammation is controlled, the long-term prognosis is good. Early detection and treatment of kidney damage reduce the incidence of severe kidney disease. However, people who have lupus have an increased risk of heart disease.

Treatment
Hydroxychloroquine (an antimalarial drug) for all affected people.

Nonsteroidal anti-inflammatory drugs for mild joint symptoms and corticosteroid creams for mild skin symptoms.

Corticosteroids, immunosuppressive drugs, and antimalarial drugs for severe disease.

Treatment of lupus depends on which organs are affected and how active the inflammation is. The severity of organ damage in lupus is not necessarily the same as the activity of inflammation. For example, organs may be permanently damaged and scarred from lupus that caused inflammation in the past. Such damage may be referred to as “severe,” even if the lupus is not active (that is, it is not causing any inflammation or any further damage at this time). The goal of treatment is to decrease the activity of lupus—that is, to decrease inflammation, which in turn should prevent new or further damage.

The antimalarial drug hydroxychloroquine is given by mouth to all people who have lupus regardless of whether their disease is mild or severe because it decreases flares-ups and decreases their risk of death. However, hydroxychloroquine is not given to people who have G6PD deficiency (G6PD is an enzyme that protects red blood cells from certain toxic chemicals) because the drug can rapidly destroy red blood cells. People who take hydroxychloroquine should have periodic eye examinations because this drug slightly increases the risk of damage to the back of the eye when it is taken for many years.

Mild lupus

If lupus is not very active, causing mild joint or skin symptoms, treatment may not need to be intensive. Nonsteroidal anti-inflammatory drugs (NSAIDs) often can relieve joint pain but should usually not be taken for long periods of time uninterrupted. Antimalarial drugs, such as hydroxychloroquine, chloroquine, or quinacrine, help relieve skin and joint symptoms and reduce the frequency of flares-ups.

People who have rashes or sores should stay out of direct sunlight and use strong sunscreens (with a sun protection factor of at least 30) when outside. Rashes may also be treated with corticosteroid creams or ointments. If skin symptoms are not relieved by corticosteroid creams or ointments and hydroxychloroquine, people are given a combination of hydroxychloroquine and quinacrine or a combination of hydroxychloroquine and methotrexate, mycophenolate mofetil, or azathioprine.

Severe lupus

People who have severe, active lupus affecting the kidneys or brain, or causing lung bleeding are treated immediately, usually with the corticosteroid methylprednisolone given by vein (see Corticosteroids: Uses and Side Effects). Then people are given the corticosteroid prednisone taken by mouth. The dose and duration of treatment depend on which organs are affected. The immunosuppressive drug cyclophosphamide is also given to suppress the body's autoimmune attack. Mycophenolate mofetil is a commonly used alternative for severe lupus affecting the kidneys because it is as effective as and less toxic than cyclophosphamide.

People who have end-stage kidney disease can undergo a kidney transplantation as an alternative to dialysis. People who have certain blood problems are given moderate or high doses of corticosteroids by mouth along with an immunosuppressive drug such as azathioprine or mycophenolate mofetil. They may be given immune globulin (a substance that contains large quantities of many antibodies) by vein. People who are not helped by those treatments may be given rituximab.

People who have nervous system problems may be given cyclophosphamide or rituximab by vein. People who develop blood clots are given heparin, warfarin, or other anticoagulants (drugs that are sometimes called blood thinners).

People with severe lupus often notice their symptoms have lessened after 4 to 12 weeks of treatment.

Maintenance drug therapy

Once the initial inflammation is controlled, a doctor determines the lowest dose of corticosteroids and other drugs that control inflammation (such as antimalarial drugs and immunosuppressants) that is needed to most effectively suppress inflammation over the long term. Usually, the dose of prednisone is gradually decreased when symptoms are controlled and laboratory test results show improvement. Relapses or flares-ups can occur during this process. For most
people who have lupus, the dose of prednisone can eventually be decreased or eventually discontinued. Because the long-term use of high doses of corticosteroids leads to many side effects, people who need to take high doses of corticosteroids for a long time are given an alternative drug such as azathioprine, methotrexate, or mycophenolate mofetil. People who take corticosteroids should be tested periodically and, if necessary, treated for osteoporosis, which can occur with chronic corticosteroid use. People who take high doses of corticosteroids for long periods may be given calcium and vitamin D supplements and bisphosphonates to help prevent osteoporosis even if their bone density is normal.

Other medical conditions and pregnancy

All people should be monitored closely by a doctor for heart disease. Common risk factors for coronary artery disease (for example, high blood pressure, diabetes, and high cholesterol levels) should be controlled as well as possible. Surgical procedures and pregnancy may be more complicated for people who have lupus, and they require close medical supervision. If pregnant, women should remain on hydroxychloroquine throughout their pregnancy and may be given low-dose aspirin as well. Pregnant women who are at risk of blood clots may be given heparin. Miscarriages and flare-ups during pregnancy are common. Women should avoid becoming pregnant during a flare-up. Because mycophenolate mofetil and other drugs cause birth defects, women should wait to become pregnant until their disease has been well controlled for 6 months or longer (see lupus during pregnancy). Women who are in remission and who are thinking about becoming pregnant but who need to keep taking maintenance drugs are usually switched from mycophenolate mofetil to azathioprine at least 6 months before conceiving.

More Information

Lupus Foundation of America

Drugs Mentioned In This Article

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EXHIBIT 187
Lupus

Systemic Lupus Erythematosus (SLE)

What is SLE?

Systemic lupus erythematosus (SLE), is the most common type of lupus. SLE is an autoimmune disease in which the immune system attacks its own tissues, causing widespread inflammation and tissue damage in the affected organs. It can affect the joints, skin, brain, lungs, kidneys, and blood vessels. There is no cure for lupus, but medical interventions and lifestyle changes can help control it.

How serious is SLE?

The seriousness of SLE can range from mild to life-threatening. The disease should be treated by a doctor or a team of doctors who specialize in care of SLE patients. People with lupus that get proper medical care, preventive care, and education can significantly improve function and quality of life.

What causes SLE?

The causes of SLE are unknown, but are believed to be linked to environmental, genetic, and hormonal factors.

What are the signs and symptoms?

People with SLE may experience a variety of symptoms that include fatigue, skin rashes, fevers, and pain or swelling in the joints. Among some adults, having a period of SLE symptoms—called flares—may happen every so often, sometimes even years apart, and go away at other times—called remission. However, other adults may experience SLE flares more frequently throughout their life.

Other symptoms can include sun sensitivity, oral ulcers, arthritis, lung problems, heart problems, kidney problems, seizures, psychosis, and blood cell and immunological abnormalities.
What are the complications of SLE?

SLE can have both short- and long-term effects on a person's life. Early diagnosis and effective treatments can help reduce the damaging effects of SLE and improve the chance to have better function and quality of life. Poor access to care, late diagnosis, less effective treatments, and poor adherence to therapeutic regimens may increase the damaging effects of SLE, causing more complications and an increased risk of death.¹

SLE can limit a person's physical, mental, and social functioning. These limitations experienced by people with SLE can impact their quality of life, especially if they experience fatigue. Fatigue is the most common symptom negatively affecting the quality of life of people with SLE.²³

Many studies use employment as a measure to determine the quality of life of people with SLE, as employment is central to a person's life.³ Some studies have shown that the longer a person has had SLE, the less likely they are to be a part of the workforce. On average, only 46% of people with SLE of working age report being employed.³

Adherence to treatment regimens is often a problem, especially among young women of childbearing age (15 to 44 years). Because SLE treatment may require the use of strong immunosuppressive medications that can have serious side effects, female patients must stop taking the medication before and during pregnancy to protect unborn children from harm.

Can a woman with SLE have a healthy pregnancy?

Women with lupus can safely get pregnant and most will have normal pregnancies and healthy babies. However all women with lupus who get pregnant are considered to have a “high risk pregnancy.”

How is SLE diagnosed?

SLE is diagnosed by a health care provider using symptom assessments, physical examination, X-rays, and lab tests. SLE may be difficult to diagnose because its early signs and symptoms are not specific and can look like signs and symptoms of other diseases.¹ SLE may also be misdiagnosed if only a blood test is used for diagnosis. Because diagnosis can be challenging, it is important to see a doctor specializing in rheumatology for a final diagnosis. Rheumatologists sometimes use specific criteria [PDF -510KB] to classify SLE for research purposes.
Who is at risk for SLE?

SLE can affect people of all ages, including children. However, women of childbearing ages—15 to 44 years—are at greatest risk of developing SLE. Women of all ages are affected far more than men (estimates range from 4 to 12 women for every 1 man).

Learn more about lupus in women.

Minority racial and ethnic groups—blacks/African Americans, Hispanics/Latinos, Asians, and American Indians/Alaska Natives—are affected more than whites/Caucasians.

Does SLE run in families?

Most people with SLE do not have family members with the disease; however, some people with SLE do have a family history of the disease. Men and women with an immediate family member with SLE have only a slightly higher risk for the disease.

How is SLE treated?

Treating SLE often requires a team approach because of the number of organs that can be affected.

SLE treatment consists primarily of immunosuppressive drugs that inhibit activity of the immune system. Hydroxychloroquine and corticosteroids (e.g., prednisone) are often used to treat SLE. The FDA approved belimumab in 2011, the first new drug for SLE in more than 50 years.

SLE also may occur with other autoimmune conditions that require additional treatments, like Sjogren’s syndrome, antiphospholipid syndrome, thyroiditis, hemolytic anemia, and idiopathic thrombocytopenia purpura.

Learn more about lupus treatment.

How many people have SLE?

Incidence and prevalence are terms commonly used to describe how many people have a disease or condition.

CDC uses the latest available data for important research questions. Recent national estimates of prevalence and incidence are not available for SLE. SLE is relatively uncommon, is difficult to diagnose, and is not a reportable disease, so it is expensive to capture all diagnosed cases reliably for epidemiologic studies. There are no recent studies to determine
CDC funded several population-based patient registries to better estimate how many people have doctor-diagnosed SLE in certain racial/ethnic groups. The registries provide the most recent available prevalence and incidence estimates for SLE for whites, blacks, and American Indians/Alaska Natives was published in 2014, and those for Hispanics and Asians were published in 2017. The CDC-funded lupus registries used similar intensive methods for case finding (hospitals, specialists’ practices, health department data) and for seeing if possible cases met standard classification criteria (i.e., medical record review). See the Lupus Studies page for more information.

Prevalence
Prevalence is a measurement of all individuals affected by a disease at a particular time, usually a year.

Older national prevalence estimates vary widely due to differences in case definitions, small study populations, and study methods. A conservative estimate suggests a prevalence of 161,000 with definite SLE and 322,000 with definite or probable SLE.4

Results from the CDC Lupus registries estimated that annual prevalence from 2002–2004 was much higher for blacks than whites in Michigan (Washtenaw and Wayne Counties) (111.6 vs 47.5 per 100,000 people)5 and in Georgia (DeKalb and Fulton Counties) (128.0 vs 39.9 per 100,000 people).6 Annual prevalence from 2007–2009 for American Indians/Alaska Natives was 178 per 100,000 people.7 Registries in California (San Francisco County) and New York City (Manhattan) provided 2007-2009 prevalence estimates for Hispanics (90.5 and 82.2 per 100,000 people, respectively) and Asians (94.7 and 56.2 per 100,000 people, respectively).8,9

Annual prevalence estimates were much higher among women than men in Michigan (9.3 vs 1.5 per 100,000 people),5 in Georgia (145.8 vs 17.5 per 100,000 people),6 and in the American Indian/Alaska Native population (271 vs 54 per 100,000 people).7 From 2007–2009, in San Francisco County and Manhattan, estimates were higher among women than men for Hispanics (CA: 149.7 vs 22.9; NYC: 138.3 vs 19.4 per 100,000 people) and Asians (CA: 177.9 vs 20.1; NYC: 91.2 vs 14.2 per 100,000 people).8,9

Incidence
Incidence is a measurement of the number of new cases of individuals who contract a disease during a particular period of time, often a year.

Recent national incidence estimates are not available for SLE. National incidence data are difficult to obtain because it is relatively expensive to capture all diagnosed cases reliably (learn more about SLE prevalence and incidence above) and the year of onset is hard to determine (slowly developing, non-specific symptoms and signs), so resource-intense studies must be done in small areas.1

SLE incidence estimates are available from the five CDC-funded lupus registries. Annual incidence for different racial/ethnic groups from 2002–2004 was much higher for blacks than whites in Michigan (7.9 vs 3.7 per 100,000 people)5 and in Georgia (9.4 vs 3.2 per 100,000 people).6 Annual incidence from 2007–2009 for American Indians/Alaska Natives was 7.4 per 100,000 people).7 From 2007–2009, incidence for Hispanics in San Francisco County and Manhattan was 4.1 and 4.0 per 100,000 people, respectively, and for Asians, incidence was 4.2 and 3.8 per 100,000 people, respectively.8,9
Annual incidence estimates were much higher for women than men in Michigan (9.3 vs 1.5 per 100,000 people), Georgia (10.6 vs 1.9 per 100,000 people) and the American Indian/Alaska Native population (unadjusted 8.4 vs 2.7 per 100,000 people). Hispanic women had higher incidence estimates than men in San Francisco County (7.2 vs 0.6 per 100,000 people) and Manhattan (6.5 vs 1.3 per 100,000 people), as well as Asian women in San Francisco County (8.9 vs 0.3 per 100,000 people) and Manhattan (6.6 vs 0.5s per 100,000 people).

Can a person die from SLE?

Causes of premature death associated with SLE are mainly active disease, organ failure (e.g., kidneys), infection, or cardiovascular disease from accelerated atherosclerosis. In a large international SLE cohort with average follow-up of over 8 years during a 1958–2001 observation interval, observed deaths were much higher than expected for all causes, and in particular for circulatory disease, infections, renal disease, and some cancers. Those who were female, younger, and had SLE of short duration were at higher risk of SLE-associated mortality.

Using death certificates for US residents, SLE was identified as the underlying cause of death for an average of 1,176 deaths per year from 2010–2016. SLE was identified as a contributing cause of death (one of multiple causes of death, including underlying cause of death) for an average of 2,061 deaths per year during that 7-year-period.

What is CDC doing about SLE?

CDC has previously funded five lupus registries and the development of a public health agenda to guide public health efforts. Currently, CDC is funding work on several SLE-relevant activities, such as three follow-up studies and research for self-management. For more information, visit the CDC-funded activities page.

Other Types of Lupus

SLE is the most common and most serious type of lupus. Other types of lupus include the following:

**Cutaneous lupus** (skin lupus) is lupus that affects the skin in the form of a rash or lesions. This type of lupus can occur on any part of the body, but usually appears where the skin is exposed to sunlight.

**Drug-induced lupus** is similar to SLE, but occurs as the result of an overreaction to certain medications. Symptoms usually occur 3 to 6 months after starting a medication, and disappear once the medicine is stopped. Learn more about drug-induced lupus on the Medline Plus website.

**Neonatal lupus** occurs when an infant passively acquires auto-antibodies from a mother with SLE. The skin, liver, and blood problems resolve by 6 months, but the most serious problem—congenital heart block—requires a pacemaker and has a mortality rate of about 20%.
Additional Information

CDC Resources

- Lupus Basics
- CDC-Funded Lupus Activities
- CDC-Recommended Intervention Programs for Arthritis and other Rheumatologic Conditions

External Resources

- National Resource Center on Lupus
- Lupus Research Alliance
- American College of Rheumatology–Lupus [En–Español]
- The Lupus Initiative
- National Institute of Arthritis and Musculoskeletal and Skin Diseases [En–Español]

References


BRIEF REPORT


Eric Y. Yen and Ram R. Singh

Objective. Mortality statistics from the Centers for Disease Control and Prevention (CDC) are used for planning health care policy and allocating resources. The CDC uses these data to compile its annual ranking of leading causes of death based on a selected list of 113 causes. Systemic lupus erythematosus (SLE) is not included on this list. Since the ranking is a useful tool for assessing the relative burden of cause-specific mortality, this study was undertaken to rank SLE deaths among the CDC’s leading causes of death to see whether SLE is a significant cause of death among females.

Methods. Death counts for the female population of the US were obtained from the CDC’s Wide-ranging Online Data for Epidemiologic Research database and then grouped by age and race/ethnicity. Data on the leading causes of death were obtained from the Web-based Injury Statistics Query and Reporting System database.

Results. During 2000–2015, there were 28,411 deaths of females with SLE recorded as an underlying or contributing cause of death. SLE ranked among the top 20 leading causes of death in females between 5 and 64 years of age. SLE ranked tenth among those ages 15–24 years, fourteenth among those ages 25–34 years and 35–44 years, and fifteenth among those ages 10–14 years. For African American and Hispanic females, SLE ranked fifth among those ages 15–24 years, sixth among those ages 25–34 years, and eighth or ninth among those ages 35–44 years, after the 3 common external injury causes of death were excluded from the analysis.

Conclusion. SLE is among the leading causes of death in young females, underscoring its impact as an important public health issue.

Systemic lupus erythematosus (SLE) is a chronic inflammatory disease that predominantly affects females and can involve virtually any organ. We recently analyzed secular trends and population characteristics associated with SLE mortality using the US nationwide mortality database comprising 62,843 deaths from SLE, of which 84% were in females (1). We found that although SLE mortality rates have decreased over the past 5 decades, they remain high relative to the mortality rate for all causes other than SLE (non-SLE). In fact, the ratio of the SLE mortality rate to the non-SLE mortality rate was 34.6% higher in 2013 than in 1968. Thus, SLE mortality remains high in the US population.

The National Vital Statistics System of the Centers for Disease Control and Prevention (CDC) maintains a mortality database, with data provided by various jurisdictions that are legally responsible for the registration of vital events and information extracted from death certificates. This database encompasses more than 99% of the deaths of US residents in all 50 states and the District of Columbia. Mortality statistics data from this database serve as important indicators of the health of the US population and are used to estimate the burden of specific diseases. Mortality statistics are also used for health care policy planning and resource allocation.

Using the National Vital Statistics System mortality database, the CDC compiles an annual leading causes of death ranking based on a selected list of 113 causes (2). SLE is not included on this list. The cause of death ranking is a useful tool for assessing the relative burden of cause-specific mortality. Hence, we ranked SLE deaths among the CDC’s leading causes of death to determine the relative burden of SLE deaths in females.
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<tr>
<th>Rank</th>
<th>Ages 5–9 years</th>
<th>Ages 10–14 years</th>
<th>Ages 15–24 years</th>
<th>Ages 25–34 years</th>
<th>Ages 35–44 years</th>
<th>Ages 45–54 years</th>
<th>Ages 55–64 years</th>
<th>Age ≥65 years</th>
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* There were 8 deaths from systemic lupus erythematosus (SLE) among those ages 0–4 years. Values in parentheses are the number of deaths.
† External injury causes of death.
SUBJECTS AND METHODS

We conducted a population-based study using nationwide mortality counts for all female residents of the US from 2000 to 2015. Data on SLE deaths were obtained from the CDC Wide-ranging OnLine Data for Epidemiologic Research (WONDER) Multiple Cause of Death database (3). Death certificates in the US provide the International Classification of Diseases (ICD) code for the underlying or contributing causes of death (see Supplementary Figure 1, available on the Arthritis & Rheumatology web site at http://onlinelibrary.wiley.com/doi/10.1002/art.40512/abstract). The underlying cause of death is defined as “the disease or injury that initiated the events resulting in death” (4). The contributing causes of death are defined as “other significant conditions contributing to death but not resulting in the underlying cause.” Deaths were attributed to SLE if an ICD-10 code for SLE (M32 [SLE], M32.1 [SLE with organ involvement], M32.8 [other forms of SLE], or M32.9 [SLE, unspecified]) was listed as an underlying or contributing cause of death on the death certificate.

Age, race, and ethnicity were ascertained using standard methods described in the CDC’s Vital Statistics technical appendix (5). Race was classified as white, black or African-American, Asian or Pacific Islander, or American Indian or Alaska Native. Ethnicity was classified as Hispanic or non-Hispanic.

Death counts were obtained, using WONDER, in the female population of the US by age groups and race/ethnicity. Data on the leading causes of death were obtained from the CDC WONDER Web-based Injury Statistics Query and Reporting System database (3).

RESULTS

During 2000–2015, there were a total of 28,411 deaths of females with SLE recorded as the underlying or a contributing cause of death. The largest number of SLE deaths was in the group ages 65 years and older (Table 1). There were 8 SLE deaths among those ages 0–4 years, 18 among those ages 5–9 years, and 78 among those ages 10–14 years.

The ranking of SLE deaths relative to the 20 official leading causes of death in females is displayed in Table 1. SLE is among the top 20 leading causes of death in females between 5 and 64 years of age. SLE ranked tenth among those ages 15–24 years, fourteenth among those ages 25–34 years and those ages 35–44 years, and fifteenth among those ages 10–14 years. In those ages 15–24 years, SLE was the number one cause of death among chronic inflammatory diseases, ranking higher than diabetes mellitus, HIV, chronic lower respiratory disease, nephritis, pneumonitis, and liver disease.

Since the SLE mortality rate is independently associated with female sex and nonwhite race (1), we assessed the relative burden of SLE mortality in minority females of reproductive age (Figure 1). To focus on the organic causes of death, the 3 common external injury causes of death, namely, unintentional injury, homicide, and suicide, were excluded from this analysis. For females of all races/ethnicities, SLE ranked seventh as the leading cause of death among those ages 15–24 years and eleventh among both those ages 25–34 years and those ages 35–44 years. Among black and Hispanic females, the rankings for SLE were higher: fifth among those ages 15–24 years, sixth among those ages 25–34 years, and eighth or ninth among those ages 35–44 years.

DISCUSSION

This study illustrates that SLE is among the leading causes of death in young females. The actual rankings of SLE are likely even higher, because SLE may not be recorded on the death certificate for as many as 40% of patients with SLE in the US (6). Furthermore, the rankings of some other leading causes of death may be higher than their actual rank; for example, death certificates tend to overestimate cardiovascular disease mortality (7). The underreporting of SLE on death certificates may occur because patients with SLE die prematurely of complications such as cardiovascular events, infections, renal failure, and respiratory diseases (8). These proximate causes of death may be perceived to be unrelated to SLE, when in fact the disease or the medications used for it predispose to them. At the time of death, many SLE patients may be under the care of physicians who may have a limited awareness of SLE as the underlying cause of death. For example, 86% of 2,314 SLE deaths in Sweden occurred in hospital units other than rheumatology (9). Thus, many SLE patients may have only the proximate causes of death, and not SLE, recorded on their death certificates. Understanding the burden of SLE deaths will help improve this knowledge gap in health care workers. An awareness campaign to educate primary care physicians and internists about the multiorgan complications of SLE and its varying presentations at the time of death may be helpful in future studies to assess the true burden of SLE mortality.

We recently described a multiple regression analysis of SLE mortality risk stratified by race/ethnicity (1). That analysis showed that SLE mortality risk was higher in females than in men in all races/ethnicities, but both the adjusted odds ratio (OR) and predicted annual mortality differences were largest in African Americans, followed by Hispanics. The adjusted ORs for females relative to males were 6.49 (95% confidence interval [95% CI] 6.02–7.00) in African Americans, 5.81 (95% CI
5.19–6.51) in Hispanics, and 4.62 (95% CI 4.37–4.88) in whites. Consistently, SLE ranked higher among the leading causes of death in nonwhite females. Our data likely underestimate the true disease burden in minorities, given the underascertainment and underrecording of SLE deaths in less well-educated ethnic minorities (10) and uninsured patients (6). The higher rankings of SLE deaths in minority females are unlikely to be artifacts from misclassification of cause of death, because greater underreporting of SLE as the cause of death in underprivileged groups (6,10) would lead to greater underestimation of SLE deaths in the groups in which we found the ranking to be higher, namely African Americans and Hispanics. The difficulty in ascertaining the accuracy of the physician’s coding on death certificates still remains an important limitation of this study. However, it is less likely that SLE would be recorded as a cause of death on the death certificates of those who did not have SLE.

Several studies have suggested that older age is associated with lack of recording of SLE on death certificates. In the LUPus in Mi norities, NAture versus nurture (LUMINA) and Carolina Lupus Study cohorts, age at death was significantly higher among those for whom SLE was omitted on the death certificate compared to those who had SLE included on the death certificate (mean ± SD 50.9 ± 15.6 years versus 39.1 ± 18.6 years; \( P = 0.005 \); \( n = 76 \) SLE deaths) (6). The age at death was also significantly higher for SLE decedents who did not have SLE.

Figure 1. Leading causes of deaths of females of reproductive age by race/ethnicity and age. The ranking of systemic lupus erythematosus (SLE) relative to the 10 official leading causes of death in females of reproductive age in the US from 2000 to 2015 is shown. Rankings are shown for females of all races, non-Hispanic black females, and Hispanic females in each age group. SLE deaths include cases where SLE was listed as the underlying or contributing cause of death using International Statistical Classification of Diseases and Related Health Problems, Tenth Revision (ICD-10) code M32. (All deaths since 1999 have been coded using ICD-10.) To focus on the organic causes of death, we excluded the external injury causes of death, namely, unintentional injury, homicide, and suicide, from this analysis. CLRD = chronic lower respiratory disease.
recorded on the death certificate compared to those who did in the Georgia Lupus Registry (mean ± SD 55.5 ± 16.4 years versus 44.4 ± 17.6 years; P < 0.0001; n = 321 SLE deaths) (11). In a Swedish population-based study that included 1,802 SLE deaths, decedents 60–79 years old at death were ~2.5 times as likely to have SLE missing from their death certificates than those younger than 40 years (OR 2.48 [95% CI 1.34–4.58]) (12). Those studies also found that SLE patients who died of cancer or a cardiovascular event were more likely to be in the unreCORded group (6,12). Thus, the lower placement of SLE in the ranking of leading causes of death in older age groups may be due to omission of SLE on the death certificates of SLE decedents whose proximate causes of death were cancer or cardiovascular events.

Our findings underscore that SLE is an important public health issue among young females, which should be addressed by targeted public health and research programs. Increasing awareness among pediatricians and primary care physicians about the importance of early diagnosis and better management of SLE may help to reduce the high burden of SLE mortality. In recognition of the high mortality of SLE, in 2016 the National Institutes of Health increased research funding for SLE to $97 million annually. This is in comparison to $1,084 million for diabetes mellitus and $3,780 million for HIV (13). In light of our data showing a higher burden of SLE mortality in younger females than previously perceived, further increases in research funding for SLE are warranted.

In conclusion, the inclusion of SLE in the CDC’s selected list of causes of death for the annual ranking would highlight the importance of this disease as a major cause of death in young females. The recognition of SLE as a leading cause of death may influence physicians’ coding on death certificates, CDC reporting of death burden, government policy, and government research funding, which may eventually help to reduce the disease burden of SLE.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Singh had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.


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Human papillomavirus vaccine and systemic lupus erythematosus

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Abstract To investigate the association between human papillomavirus (HPV) vaccination and autoimmune manifestations compatible with systemic lupus erythematosus (SLE) or SLE-like disease, the medical history of six women who presented with SLE or SLE-like disease following HPV immunization was collected. Data regarding type of vaccine, number of immunization, family and personal, clinical and serological features, as well as response to treatments were analyzed. In the reported cases, several common features were observed, such as personal or familial susceptibility to autoimmunity or adverse response to a prior dose of the vaccine, both of which may be associated with a higher risk of post-vaccination autoimmunity. Favorable response to immunosuppressant was observed in all patients. In the current study, a temporal association between immunization with HPV vaccine and the appearance of a spectrum of SLE-like conditions is reported. Additionally, among the patients described, several common features were observed that may enable better identification of subjects at risk. Further studies are required to assess the safety of immunization with the HPV vaccine in patients with autoimmune-rheumatic diseases or in subject at risk of autoimmunity as well as the potential beneficial effect of preventive immunosuppressants.

Keywords Anti-phospholipids antibodies · ASIA · Autoimmunity · Human papillomavirus · Systemic lupus erythematosus · Vaccine

Introduction

Human papillomavirus (HPV) infection is widespread worldwide, affecting women of any age, with the highest risk of infection being reported among young women aged 15–19 years [1]. Nearly 80 % of women are predicted to encounter the infection within the first 5 decades of their lives, mostly through heterosexual activity [2].

HPV infects squamous epithelial cells (e.g., uterine cervix), which can be eventually pushed to neoplastic transformation. Nowadays, more than 100 HPV serotypes are known, among them, 40 have been shown to infect the human genital or oropharyngeal tracts [1]. HPV serotypes 16 and 18 are considered to be the most hazardous, being related to the majority (~80 %) of cervical cancer. Notably, cervical cancer is the third most frequent gynecological malignancy and is the fourth leading cause for cancer death in women worldwide [3], of which, the great majority of deaths occurring in developing countries.

In the last decade, two vaccines (Gardasil™ and Cervarix™) were developed for preventing HPV infection and its associated morbidity. Both vaccines (CervarixTM and Gardasil™) are composed of HPV-like proteins, such as personal or familial susceptibility to autoimmunity or adverse response to a prior dose of the vaccine, both of which may be associated with a higher risk of post-vaccination autoimmunity. Favorable response to immunosuppressant was observed in all patients. In the current study, a temporal association between immunization with HPV vaccine and the appearance of a spectrum of SLE-like conditions is reported. Additionally, among the patients described, several common features were observed that may enable better identification of subjects at risk. Further studies are required to assess the safety of immunization with the HPV vaccine in patients with autoimmune-rheumatic diseases or in subject at risk of autoimmunity as well as the potential beneficial effect of preventive immunosuppressants.

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as the L1 viral proteins. The vaccines differ in a way that Cervarix™ is a bivalent vaccine (directed at HPV serotypes 16 and 18) while Gardasil™ is quadrivalent (directed at HPV serotypes 16, 18, 6, 11). Different adjuvants were added to these vaccines, the Gardasil™ utilizes the hydroxyphosphate sulfate adjuvant whereas the Cervarix™ exploits a double-adjuvant system, ASO4 composed of 3-O-desacyl-4′ monophosphoryl lipid A and aluminum hydroxide [4]. Cervarix™ and Gardasil™ are administered through three boost intramuscular injections given within a period of 6 months (at 0, 1, and 6 months, and 0, 2, and 6 months respectively). Both HPV vaccines have been studied in terms of efficacy and safety [5–7]. They were found to be effective, providing a long-lasting protection against HPV infection and premalignant lesions (90–100 % of cases prevented) [6, 7]. Moreover, they may elicit a cross-protective antibody response against other HPV serotypes which are antigenically related to the vaccine-included ones [8, 9]. Both HPV vaccines are well tolerated, and in the general population, only mild local-site reactions and general symptoms such as fatigue, headache, and myalgia were reported following immunization [10, 11]. Nevertheless, it should be noted that a few serious adverse effects were also reported, including venous thrombosis, hypersensitivity reactions, anaphylaxis, motor neuron disease, and even deaths mainly in patients immunized with Gardasil™ [10, 12] of which, only the rate of venous thrombosis was significantly higher than that expected in the general population. Furthermore, an association between Gardasil™ and autoimmunity was suggested following reports of diverse post-vaccination autoimmune conditions [10, 13]. While considering Cervarix™, a large study of more than 60,000 subjects immunized with different ASO4 adjuvanted vaccines was conducted by GlaxoSmithKline Biologicals. In this study, the control groups received vaccines that were ASO4 free, non-adjuvanted, or adjuvanted with aluminum. The overall relative risk for developing an autoimmune disease was found to be 0.98, hence no direct statistically significant difference could be attributed to the ASO4 adjuvant. However, in the entire database, which included data for HPV as well as Hepatitis B and Herpes vaccines, the highest relative risk for an individual autoimmune event was for systemic lupus erythematosus (SLE) (RR-2.39) [14].

Methods

In the current study, we describe six patients who developed SLE or SLE-like disease after administration of the quadrivalent HPV vaccine. Data including demographics, disease manifestations, number of immunizations, family and personal medical history, serological features, as well as response to treatments were collected and analyzed.

Patients’ descriptions

Five patients presented with naïve autoimmune disease and one patient with SLE flare.

Patient n° 1

A 32-year-old woman was admitted to the hospital 5 days following the third immunization with Gardasil™. On admission, she suffered from general weakness, severe myalgia, polyarthritis, anorexia, sensitive skin rash (urticaria-like), malar rash, aphthous stomatitis, pharyngodynia, cervical lymphadenopathy (more than 3.5 cm), and hair loss. In addition, in the 4 weeks prior to her hospitalization she lost 10 kg of body weight. Laboratory tests demonstrated elevated erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP), anemia (without evidence of hemolysis), leukopenia, and lymphopenia. Autoantibodies screening showed positive antinuclear antibodies (ANA) with very high titers of anti-Ro (SSA) and anti-La (SSB) antibodies and high positive anti-dsDNA antibodies. Antiphospholipid antibodies were undetectable. Complement (C3) levels were very low. Urine analysis showed no active sediment and no evidence of infections was documented (i.e., normal blood and urine culture, negative serology for hepatitis, Epstein–Barr virus, cytomegalovirus, parvovirus). CPK and TSH levels were normal, as well as chest radiography and endoscopy. Of note, her medical history was unremarkable prior to immunization. However, mild weakness, facial malar rash, and hair loss were observed following the first immunization (6 months prior to hospitalization). Local reaction to vaccination, fever, fatigue, mild rash, and arthralgia were documented following the second dose but were interpreted as a “common cold.” Her family history was remarkable for autoimmune thyroid diseases. The patient was diagnosed as having SLE and treatment with high-dose prednisone (PDN) and hydroxychloroquine (HCQ) was commenced with gradual clinical improvement. PDN therapy was tapered slowly up to 5 mg/day, HCQ was continued at 400 mg/day along with supplementation of calcium and vitamin D. Eight months afterwards, the patient was in remission, with normalization of inflammatory laboratory parameters (CRP, ESR), as well as blood counts and complement levels.

Patient n° 2

A 29-year-old woman was admitted to the hospital 3 weeks following the second dose of Gardasil™ due to severe weakness, diarrhea, and elevated markers of inflammation. On admission, her physical examination revealed malar rash, photosensitivity, arthritis, and alopecia. In the next couple of months, she lost 30 % of her body weight and
remained hospitalized. Laboratory tests demonstrated elevated ESR, high titers of ANA, and anti-dsDNA antibodies, low levels of complement, and proteinuria of 1 g/day. The patient was diagnosed with SLE and severe protein-losing enteropathy.

Her medical history included immune thrombocytopenia diagnosed several years before immunization. At that time, she had normal bone marrow biopsies and no detectable serum autoantibodies, including ANA. She was treated with PDN and intravenous immunoglobulins. Two years prior to the administration of HPV vaccine, she underwent splenectomy with normalization of her platelet counts, and abortion of additional therapies. Of note, the patient was immunized with pneumococcal vaccine, as required, before splenectomy with no adverse events. In addition, the patient was diagnosed with early cervical intraepithelial neoplasia related to HPV months before immunization with Gardasil™. Her family medical history was unremarkable for autoimmune disorders.

Following immunization and diagnosis of SLE, she was treated with high-dose corticosteroids, azathioprine (AZA) and HCQ with gradual remission of her symptoms which were followed by slow down-tapering of PDN and AZA doses. Two years following the diagnosis of SLE, the patient achieved clinical and serological remission excluding ANA seropositivity and traces of protein in her urine. Her present therapy comprises of HCQ and calcium/vitamin D supplementation.

Patient n° 3

A 16-year-old high school girl was admitted to the Infectious Diseases Department because of high-grade fever (39.5 °C), generalized asthenia, diffuse polyarthralgia, and multiple erythematous annular cutaneous lesions on the face, trunk, and lower limbs which occurred 8 days after the first dose of Gardasil™ (Fig 1a–b). While she received the vaccine, she developed low-grade fever, which was interpreted as viral flu syndrome. Laboratory examinations revealed normochromic normocytic anemia with elevated CRP and ESR, an extensive bacterial and viral screening was negative, and her urine sediment was normal. Autoantibodies profile revealed seropositivity for ANA and lupus anticoagulant (LAC). Her medical and family histories were remarkable for Raynaud’s phenomenon while her maternal aunt was diagnosed with systemic sclerosis.

A diagnosis of “lupus-like” syndrome was determined and the patient was treated with intravenous high-dose methylprednisolone followed by oral PDN. Following initiation of treatment, her blood temperature normalized and her skin lesions significantly improved (Fig 1c–d), with almost complete resolution in a month, while receiving 50 mg of PDN (Fig 1e–f). The latter was tapered down within 6 months, and at 1 year following immunization, the patient was in good health.

Patient n°4

A 16-year-old high school girl was admitted to the hospital with a preliminary diagnosis of FUO (fever of unknown origin), which appeared for the first time 3 weeks after the second dose of Gardasil™. Fever was prolonged, mainly present in the morning, and rose up to 39 °C. In addition, pharyngodynia, erythematous skin lesions of elbows and knees, generalized asthenia, anorexia, polyarthralgia, and headaches were present. Pharyngeal culture and urine cultures excluded active infections. Laboratory workup revealed normochromic normocytic anemia, slight increase of serum amyloid A (SAA) levels of 9 mg/l (normal less than 6 mg/l). Proteinuria was absent. Autoantibodies profile demonstrated persistent positivity of anti-cardiolipin IgM and LAC. Magnetic resonance imaging of the brain excluded the presence of brain abnormalities consistent with antiphospholipid syndrome.

Her medical history was remarkable for recurrent tonsillitis during childhood and a streptococcus group B infection 1 year before admission, treated with penicillin. In addition, the patient suffered from Raynaud’s phenomenon grade II, defined by nailfold capillaroscopy. Her family history was also remarkable for Raynaud disease of patient's mother.

The patient was diagnosed with fever in a patient with antiphospholipid antibodies, possibly related to Gardasil™.
vaccination, compatible with the autoimmune/auto-inflammatory syndrome induced by adjuvants (ASIA). She was treated with naproxen 500 mg/day for 2 months and omega-3 polyunsaturated fatty acids 2,000 mg/day for 4 months; the doses were very gradually tapered down. She was discharged with instructions to avoid sun exposure and to avoid further vaccination. At follow-up visit, the patient was in remission and in good health.

Patient n° 5

A 19-year-old SLE patient was diagnosed with SLE flare 10 days following the second dose of Gardasil™, while in retrospect minor symptoms were already acknowledged following the first immunization. The patient was diagnosed with SLE 4 years prior due to the appearance of malar rash, typical SLE skin rash, arthritis, positive serology for ANA and anti-dsDNA antibodies, as well as very low C4 complement levels. She was treated with corticosteroid and HCQ and achieved a full clinical remission with normalization of complement and anti-dsDNA antibodies levels. Her maintenance therapy included low-dose HCQ and vitamin D. Following the first dose of HPV immunization, she experienced mild arthralgia, dyspnea (with no abnormalities on her chest x-ray), cervical lymphadenopathy, and skin rash. Treatment with PDN 40 mg/day was commenced with good response and the dose was slowly tapered down. Although otherwise advised, the patient decided to receive the second boost of the vaccine. This time SLE-symptoms were more pronounced with very notable malar rash, severe skin rash, cervical lymphadenopathy of more than 3 cm, alopecia, leucopenia, elevated ESR, and decreased complement levels. Corticosteroids dose was increased, and following discussions with the patient, therapy with belimumab (anti-BLyS) was commenced, which induced an improvement.

Patient n° 6

A 13-year-old African-American female approached her general physician 3 weeks following immunization with the second dose of Gardasil™ due to swelling of her index finger and a rash. During the following couple of months, she developed erythematous rash on her face, fever, periorbital edema, weight loss, malaise, fatigue, cervical, axillary and inguinal lymphadenopathy, as well as mild anemia. At this stage, she was referred to a rheumatologist who noted that she had a petechial rash, alopecia, leucopenia of 2,100 cells/mm³, and mild thrombocytopenia.

Further evaluation documented seropositivity for ANA, anti-RNP, anti-Smith and anti-RO/SSA antibodies as well as low C3 and C4, elevated ESR. The CRP level was normal. The patient was diagnosed with SLE. Hydroxychloroquine and prednisone treatment was started. Despite therapy, disease progression was documented with the appearance of CNS (i.e., seizures) and kidney involvement. Renal biopsy was compatible with mesangial proliferative glomerulonephritis, class II lupus nephritis. Thus, therapy was enhanced with high-dose steroids, cyclophosphamide, as well as antiepileptic medications. Under this therapy, there was gradual remission of the SLE. Notably, her personal medical history was remarkable only for common infections and a rash due to pityriasis rosea treated and resolved several months before immunization. Her family history revealed several members of the family with autoimmune diseases including SLE.

Discussion

The above reported cases reveal a temporal association between immunization with Gardasil™ and the appearance of a spectrum of SLE-like conditions. Lately, other cases of SLE onset or relapse following HPV vaccinations were reported [15]. Moreover, several features were common among patients (Table 1), and may shed some light on the link between HPV immunization and SLE.

In this study, all patients had a personal or family history of autoimmune-rheumatic conditions suggesting genetic or epigenetic contributing components. Genetic factors are key players in the mosaic of autoimmunity and complex genetic and epigenetic predisposition was defined in patients with SLE [16–19]. It has been noted that some vaccines may trigger autoimmunity in a predisposed recipient, since they widely stimulate the immune system [20, 21]. Specifically, SLE onset was reported after diverse vaccinations [22–24], while narcolepsy, another autoimmune disorder, was recently reported as having a strong link with genetic markers as well as the adjuvanted H1N1 vaccine [25]. In this context, it is important to mention that there are clinical data regarding using non-adjuvanted influenza A/H1N1 vaccine in a large cohort of autoimmune-rheumatic diseases, where the response to the vaccination was reduced, although adequate [26]. Intriguingly, adjuvants contained in many vaccines may per se taintalize both the innate and the adaptive immune response resulting in aberrant autoreactivity [27, 28]. Thus, one may suggest that a common denominator to post-vaccination autoimmunity is genetic or epigenetic vulnerability, and that personal or familial medical history of autoimmunity should be considered a risk factor for such adverse events. On the other hand, although vaccines related perturbations of the immune state may rarely unveil autoimmunity [21], patients affected with autoimmune-rheumatic diseases are at risk of infectious diseases owing both to impairment in their immune system and/or to the immunosuppressive therapy they often
As pointed above, in addition to our series, another case series of three patients from the Philippines with SLE flares was reported recently [15]. Moreover, other autoimmune and neurological immune-mediated conditions have been related to HPV vaccination [10, 36, 37, 38]. With regard to HPV vaccines, and particularly Gardasil™, no large studies have ever outlined a significant incidence of autoimmune disorders in immunized populations [39, 40]. However, limitations to these large studies, especially in assessing rare events, have been underlined before. Extremely large cohort studies are required for investigation of rare events, a longer follow-up duration may be needed for assessment of post-immunization effects and the lack of data regarding immune modulatory therapy taken at the time of immunization may be of importance. Performing such studies may be difficult, if not, impossible [40, 41]. Recently, official recommendations for vaccinations of patients affected with autoimmune-rheumatic disorders were drawn by a task force of the European League against Rheumatism [20, 42, 43]. By which, vaccination is recommended to these patients depending on the prevalence of the infective disease, the safety of the individual vaccine, and the ongoing activity of their autoimmune-rheumatic condition while hazardousness of some vaccines/adjuvants is still being explored [20, 21, 27, 28]. In our study, as well as in the study from the Philippines mentioned above, HPV vaccine, which is indicated mainly to young women, was recommended to relatively older patients at the age of 32, 45, and 58 years. Furthermore, in our cohort, one of the patients was immunized following a documented HPV-related cervical carcinoma in situ.

In summary, based on the current data, a causal link between HPV vaccination and onset or relapse of SLE is plausible. Therefore, although for most patients, the benefits of immunization outweigh its risks, clinicians must be aware of the odds for an autoimmune disease onset or exacerbation following HPV vaccination. A meticulous pre-vaccination risk-benefits assessment, close follow-up during and after each boost of vaccination, as well as assessment of concomitant therapy with immune-modulating agents such as HCQ, undergo [29]. Particularly, women affected with SLE display a higher prevalence of HPV infection as compared to the general population [30, 31]. As such, they should be carefully followed for HPV-related diseases (e.g., performing PAP smears regularly), as well as assessed for vaccination directed at HPV and others infectious agents. In summary, it seems that a careful individualized risk assessment is required regarding both the patient medical history of autoimmune and infectious diseases as well as history of adverse reactions to past vaccination is needed [20].

Another point for consideration was reported in four of the patients described. These patients received boost immunization (second or third vaccination) although mild adverse events were observed following a previous dose of Gardasil™ (Table 1). Notably, in most healthy subjects, mild adverse events following immunization are transient and can be disregarded. Alas, in a high-risk population, these mild events may be of significance, and although further studies are required, it seems that assessment following each boost of vaccination may be beneficial [23].

In this study, all patients responded favorably to therapy with corticosteroids, antimalarial drugs, and immunosuppressants, further supporting the notion that immune-mediated mechanisms underline these post-vaccination events. In addition, at the time of immunization, only one patient was treated with low-dose HCQ. The latter was found to be beneficial both for active therapy as well as for prevention of SLE exacerbations [32]. Thus, we could speculate that immune-modulatory therapy taken appropriately at the time of immunization may have a protective effect for patients at high risk for post-immunization adverse responses [33].

Notably, patients with autoimmune-rheumatic diseases are likely to achieve a less striking seroconversion (fourfold antibody increase after injection) as compared to healthy controls, especially while receiving immunosuppressants such as mycophenolate or azathioprine [34, 35]. Nonetheless, in most studies anti-infectious immunity following vaccination was achieved regardless of immunosuppressant use [20].
seems reasonable for patients with an autoimmune disease. Last but not least, a growing need to define risk factors (i.e., genetic susceptibility markers) and methods of intervention to decrease post-vaccination autoimmunity in healthy and diseased populations is yet unmet. Encouraging researchers to report similar cases and establishing active vaccines surveillance registries [44] may improve our knowledge and decision making regarding vaccinations in the future.

Disclosure  YS appears in court defending subjects afflicted by immunization. All other authors: disclosure: none.

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EXHIBIT 190
Self-Organized Criticality Theory of Autoimmunity

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Abstract

**Background:** The cause of autoimmunity, which is unknown, is investigated from a different angle, i.e., the defect in immune ‘system’, to explain the cause of autoimmunity.

**Methodology/Principal Findings:** Repeated immunization with antigen causes systemic autoimmunity in mice otherwise not prone to spontaneous autoimmune diseases. Overstimulation of CD4⁺ T cells led to the development of autoantibody-inducing CD4⁺ T (aiCD4⁺ T) which had undergone T cell receptor (TCR) revision and was capable of inducing autoantibodies. The aiCD4⁺ T cell was induced by de novo TCR revision but not by cross-reaction, and subsequently overstimulated CD8⁺ T cells, driving them to become antigen-specific cytotoxic T lymphocytes (CTL). These CTLs could be further matured by antigen cross-presentation, after which they caused autoimmune tissue injury akin to systemic lupus erythematosus (SLE).

**Conclusions/Significance:** Systemic autoimmunity appears to be the inevitable consequence of over-stimulating the host’s immune ‘system’ by repeated immunization with antigen, to the levels that surpass system’s self-organized criticality.


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Introduction

Since ‘clonal selection theory of immunity’ of F. Macfarlane Burnet and subsequent molecular biological discoveries on V(D)J recombination and the diversity and individuality of immune response, how autoimmunity arises remains unclear. Apart from the term ‘autoimmunity’ which is now ready-made, in the present study, we tried to see the pathogenesis of autoimmunity from different angle and test the integrity of immune ‘system’. The method we have chosen was to stimulate the system maximally by antigen to the levels far beyond its steady-state just like testing the capability of automobile. In a perfectly reproducible experiments in which the mice not prone to autoimmune diseases were immunized repeatedly with antigen, we have unexpectedly and surprisingly discovered that overstimulation of immune system beyond its self-organized criticality inevitably leads to systemic autoimmunity. Subsequent detailed molecular analyses revealed in the first that autoantibodies are induced not by cross reaction to antigen but by de novo T cell receptor (TCR) revision. Second, final maturation of effector cytotoxic T lymphocyte (CTL) via antigen cross-presentation is _sine qua non_ for generating autoimmune tissue injury. Most importantly, we now show that autoimmunity arises not from ‘autoimmunity’, but as a natural consequence of normal immune response when stimulated maximally beyond system’s self-organized criticality.

Results

Induction of Autoantibodies

Consistent with the common observation that T cells become anergic after strong stimulation with antigen [1], we observed that 2× immunization with staphylococcus enterotoxin B (SEB) caused SEB-reactive V88⁺CD4⁺ T cells from BALB/c mice to become anergized. However, these cells recovered from anergy to divide and produce IL-2 after further immunization 8× with SEB (Figure 1A). This was accompanied by the induction of autoantibodies, including IgG-, and IgM-rheumatoid factor (RF), anti-Sm antibody, and in particular, RF reactive against galactose-deficient IgG, typically found in human autoimmunity [2] (Figure 1A). Autoantibodies can also be induced by other conventional antigens, including ovalbumin (OVA) or keyhole limpet hemocyanin (KLH) (Figure S2) as long as immunizing antigen is correctly presented to T cells (Figure S1B). CD4⁺ T cells of repeatedly-immunized mice become fully matured, expressing CD45RB⁺, CD27⁺ and CD122⁺ (data not shown), and these primed CD4⁺ T cells can confer RF generation in naive recipients following adoptive transfer (Figure 1B). The induction of autoantibodies is independent of CD8⁺ T cells or MHC class I-restricted antigen presentation for the following reasons. First, both RF and anti-dsDNA antibody can be consistently induced upon repeated immunization of β₂-microglobulin (β₂m)-deficient BALB/c mice with OVA. β₂m-deficient mice are deficient in CD8⁺ T cells, which are reduced to <0.8% of splenic T cells [3] (Figure S3). Second, the ability to induce autoantibodies was transferable from OVA-immunized BALB/c mice to β₂m-deficient mice solely via CD4⁺ T cells (Figure 1C). Thus, CD4⁺ T cells from repeatedly-immunized mice acquire the ability to induce autoantibodies. We refer these as autoantibody-inducing CD4⁺ T (aiCD4⁺ T) cells in this communication.
Figure 1. Induction of autoantibodies and proteinuria. BALB/c mice were repeatedly injected i.p. with 25 μg SEB, 500 μg OVA or PBS every 5 d. (A) Serum IgG- and IgM-RFs, anti-galactose-deficient IgG and anti-Sm antibodies were measured using ELISA. The arbitrary unit (AU) of 1.0 is equivalent to the titer obtained from sera of prototypic autoimmune MRL/lpr mice. Data from each mouse are connected by dotted lines. (B) Adoptive transfer of splenic B, T, CD4+ and CD8+ T cells of SEB-, OVA- or PBS-immunized BALB/c mice into naïve BALB/c mice. The recipient mice were given single i.p. injection of 25 μg SEB or 500 μg OVA 24 h after cell transfer, and autoantibodies were measured 2 weeks later. (C) Adoptive transfer of cells from OVA-immunized BALB/c mice into β2m-deficient mice.

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Mechanism of Autoantibody Induction

To further clarify the characteristics of CD4⁺ T cells, we examined their TCR repertoire by spectratyping of their complementarity determining region 3 (CDR3) [4]. Combinatorial assessment of Vβ and Jβ showed that the CDR3 length profiles of CD4⁺ splenocytes in mice immunized either 8× with PBS or 2× with SEB fit a normal Gaussian curve, typical of a diverse and unbiased TCR repertoire (Figure 2A). However, splenocytes, but not thymocytes, from mice immunized 8× with SEB showed skewed length profiles, suggesting that TCR revision was in progress at periphery of the spleen. Genes encoding components of the V(D)J recombinase complex were specifically re-expressed in mice immunized 8× with SEB, including the recombination-activating genes 1 and 2 (RAG1/2), terminal deoxynucleotidyl transferase (TdT) and surrogate TCRα chain (pTα) [5] (Figure 2B). The RAG1 gene is expressed in vivo after immunization 8× with

Figure 2. TCR revision upon repeated immunization with antigen. (A) TCR CDR3 length profiles of mice immunized 8× with PBS, 2× or 8× with SEB. TCR repertoire of splenic CD4⁺ T cell was skewed only after immunization 8× with SEB. (B) Expression of V(D)J recombinase complex and related molecules in the spleen of PBS- or SEB-injected BALB/c mice. (C) GFP⁺ cells in the Vβ8⁺ CD4⁺ T population of rag1/gfp knock-in mice. IgG-RF as induced in rag1/gfp knock-in mice after immunization 8× with SEB (lower left). The GFP⁺ T cell fraction was also increased among Vβ8⁺ CD4⁺ T cells (mean ± SD, 4–5 mice/group). (D) TCRα chain revision in the spleen of mice immunized 8× with SEB was determined by LM-PCR detection of dsDNA breaks at the RSS flanking the TCRAV2, with PCR-amplified TCRα constant region (TCRAC) as a DNA quality control.

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SEB in rag1/gfp knock-in mice [6] (Figure 2C). In these mice, serum RF was increased in conjunction with an increase of GFP-expressing Vβ8.5 CD4+ T cells in the spleen. To directly prove that V(D)J recombination took place at the periphery in spleen, we used ligation-mediated PCR (LM-PCR) to detect blunt-end DNA fragments harboring a rearranged coding V region flanked by recombination signal sequences (RSS) [7,8]. We identified rearranged intermediates corresponding to the TCRβ variable region 2 (TCRV2) in the splenocytes of mice immunized 8× with SEB (Figure 2D). These findings indicate that repeated immunization with conventional antigen can induce the generation of α/CD4+ T cells which have undergone TCR revision and are capable of stimulating B cells [9]. This observation is in line with previous findings showing that such somatic mutations occur often in lymphocytes, a process which is considered to be a major stochastic element in the pathogenesis of autoimmunity [10,11]. Thus, overstimulation of CD4+ T cells by repeated immunization with antigen and induction of full maturation inevitably leads to the generation of α/CD4+ T cells which have undergone TCR revision and are capable of inducing autoantibodies. Importantly, the present study shows that such α/CD4+ T cells are induced by de novo TCR revision but not by cross-reaction to antigen.

Induction of Autoimmune Tissue Injury

Repeated immunization with OVA can also lead to autoimmune tissue injury and the production of autoantibodies reactive against IgG, Sm and dsDNA (Figure 3 and Figure S2A). Serum immune complex (IC), proteinuria, and the deposition of IC and OVA in the kidney were noted in mice immunized 12× with OVA (Figure 3A). Typical diffuse proliferative glomerular lesions were seen in the kidney, and these glomeruli were infiltrated with CD8+ T cells. These observations resemble the clinical features observed in lupus patients, who typically exhibit an increase in CD8+ T cells in the peripheral blood and infiltration of CD8+ T cells in kidney [12,13]. Immunization of mice 12× with OVA led to re-expression of the V(D)J recombinate complex and enlargement of the spleen (Figure S4A), and an increase in anti-dsDNA antibody, which is uniquely linked to autoimmune tissue injury in lupus nephritis [14] (Figure S2A). Pathological findings included diffuse membranous (wire-loop) and/or proliferative glomerulonephritis in the kidney (Figure 3A), infiltration of plasma cells around hepatic bile ducts (Figure S4B), enlarged lymphoid follicles with marked germinal center in spleen (Figure S4B), occasional lymphocyte infiltration into the salivary glands (data not shown), lymphoid cell infiltration into the thyroid, and perivascular infiltration of neutrophils and macrophages into the skin dermis of the auricle (Figure S4B). The lupus band test, diagnostic of SLE, was positive in the skin at the epidermal-dermal junction (Figure 3B).

Mechanism of Autoimmune Tissue Injury

It has been shown previously that IFNγ is increased in association with autoimmune tissue injury [15–17]. Consistent with this, we found that the number of IFNγ+CD8+ T cells, but not regulatory T or T helper 17 cells, was increased following immunization 12× with OVA (Figure 4A and data not shown). We also observed an expansion of IFNγ-producing effector/memory CD8+ T cells, which are necessary for adaptive immunity [18] (Figure 4A). These IFNγ-producing CD8+ T cells were observed to have infiltrated into OVA-deposited glomeruli of OVA-immunized mice (Figure 3A). CD8+ T cells are required for tissue injury based on the following observations. First, the transfer of CD8+ T cells can induce renal lesions in mice (Figure 4B), as well as the generation of new IFNγ+CD8+ T cells in the spleens of recipient mice following cell transfer (Figure S5). Second, autoimmune tissue injury is not induced by the transfer of CD8+ T cells from OVA-immunized wild-type mice into β2m-deficient mice (Figure 1C). And finally, CD8+ T cell transfer must be accompanied by at least a 1× booster immunization with OVA to induce autoimmune tissue injury in the recipient mice (Figure S6). The findings indicate that full-matured, IFNγ-producing effector CD8+ T cells are required for the induction of autoimmune tissue injury, provided that the relevant antigen is correctly presented on the target organs. These are well-established characteristics of CTL and not novel. We show, however, that (i) CTL is induced through an immune, but not ‘autoimmune’, process, and that (ii) autoimmune tissue injury inevitably occurs when CD8+ T cells are overstimulated to become matured effector CTLs. The latter means that regardless of how CTL is induced, the consequence of CTL over-induction is immune tissue injury.

Antigen Cross-Presentation

We next show that antigen cross-presentation is required for the induction of CTL and tissue injury. To test this, we co-cultured OVA-pulsed dendritic cells (DC) from mice immunized 12× with OVA together with T cells from OVA-TCR transgenic DO11.10 mice exclusively expressing OVA-reactive TCR [19]. We show that OVA-reactive DO11.10 CD8+ T cells are activated upon coculture with OVA-pulsed DCs (Figure 4C and Figure S7). Further, autoimmune tissue injury and the increase in IFNγ+CD8+ T cells, but not of autoantibody generation, were both abrogated by adding chloroquine (CQ), an inhibitor of antigen cross-presentation (Figure 4C). This indicates that antigen cross-presentation is required for the expansion of IFNγ-producing CD8+ T cells and autoimmune tissue injury.

α/CD4+ T Cell Helps CD8+ T Cell to Induce Tissue Injury

Since CTL appear to play a rather passive role in autoimmunity, we next studied whether or not α/CD4+ T cell help is required for the induction of autoimmune tissue injury. Since anti-CD4 treatment almost abrogates generation of IFNγ-producing CD8+ T cell and autoimmune tissue injury in OVA-immunized BALB/c mice (Figure S8), to test whether this CD4+ T cell-mediated help is mediated by αT cells or antigen-specific T cells, we have transferred CD4+ T cells from mice immunized 12× with KLH into CD4+ T-depleted BALB/c mice immunized 8× with OVA (Figure 4D). Because full-matured IFNγ+ CTLs do not develop with less than 8× immunization with OVA (Figure S9), this experiment can test the ability of α/CD4+ T cells that have undergone TCR revision to promote the maturation of OVA-specific CTL. The result showed that both autoimmune tissue injury and OVA-specific IFNγ+CD8+ T cells arose in these mice after transfer, indicating that α/CD4+ T cells with de novo TCR revision are required for the maturation of CD8+ T cell and autoimmune tissue injury (Figure 4D).

Discussion

The present findings are consistent with the current consensus that CD4+ T cells normally die via activation-induced cell death (AICD) after repeated exposure to a single antigen, while naive CD4+ T cells having a ‘cross-reactive’ TCR with lower affinity can be activated through repeated exposure to the same antigen and survive due to weak TCR signaling, ultimately acquiring autoreactivity [20]. We show here, however, that α/CD4+ T cells are induced not by cross-reaction, but by de novo TCR revision. The α/CD4+ T cells thus generated induce not only autoantibodies but also full-maturation of CD8+ T cells leading to autoimmune...
Figure 3. Induction of autoimmune tissue injury. BALB/c mice were injected i.p. with 500 μg OVA every 5 d. (A) Serum IC measured 2 d after final immunization, expressed as AU. Proteinuria assessed 9 d after final immunization: grades 1, 2 and 3 represent 30–100 mg/dl, 100–300 mg/dl and 300–1000 mg/dl of urinary protein, respectively (upper left). Representative histopathology of kidneys from mice immunized 12× with PBS or OVA (lower left) (H&E staining, bar = 20 μm; original magnification ×400): glomerular expansion with cellular infiltration including eosinophils seen under the same magnification. Immunohistochemistry for deposited IC, IgG, C3 and OVA (upper right) (bar = 50 μm; original magnification ×200), and cells infiltrated into glomeruli (bar = 20 μm; original magnification ×300), stained in serial tissue sections using anti-CD8α (53–6.7) and anti-IFNγ (R4-6A2) monoclonal antibodies, in the specimens of mice immunized 12× with OVA (lower right). (B) Lupus band test stained with anti-IgG and anti-C3 antibodies (bar = 20 μm; original magnification ×400).

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Figure 4. Expansion of CD8⁺ T cells and antigen cross-presentation. (A) Spleen cells stimulated with 50 ng/ml phorbol myristate acetate (PMA) and 500 ng/ml ionomycin for 4 h in the presence of brefeldin A (10 μg/ml) and stained for intracellular IFNγ (upper). Subsets of CD8⁺ T cells categorized into naïve (CD44lowCD62Lhigh), effector (CD44highCD62Llow), and memory (CD44highCD62Lhigh) fractions (middle). Flow cytometry of IFNγ⁺ cells within naïve or effector/memory CD8⁺ T cell populations. Spleen cells were separated into naïve (CD44low) and effector/memory (CD44high) cells using CD44 MACS beads, and IFNγ⁺ cells within the CD8⁺ T population was evaluated (lower). (B) Adoptive transfer of splenocytes of OVA-immunized BALB/c mice into naïve recipients. The recipients were injected with 500 μg OVA 24 h after cell transfer, and proteinuria examined 2 weeks later. (C) Cross-presentation of OVA to CD8⁺ T cells. Splenic CD11c⁺ DC from OVA-immunized or control mice were incubated in the presence (OVA(+)) or absence (OVA(−)) of 1 mg/ml OVA with or without chloroquine (CQ) (20 μg/ml) for 3 h, followed by a co-culture with KJ1-26⁺CD8⁺ T cells of DO11.10 transgenic mice to examine surface expression of CD69 (upper). Inhibition of cross-presentation in vivo by administration of 250 μg CQ per mouse 3 h prior to immunization with OVA or PBS. IFNγ⁺CD68⁺ T cells (middle), autoantibodies and proteinuria (lower) after 12× immunization. (D) Requirement of autoantibody-inducing CD4⁺ T cells for CD8⁺ T cell-mediated autoimmune tissue injury. BALB/c mice were immunized 12× with KLH, and CD4⁺ T cells were isolated using MACS beads. Cells were transferred into the anti-CD4 antibody-treated recipient mice immunized 8× with OVA. Percent matured CTL, i.e., IFNγ⁺CD8⁺ T cells, and proteinuria were measured 2 weeks after booster immunization 1× with KLH.

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tissue injury akin to human SLE. Thus, induction of αCD4+ T cells is a critical step, and subsequent induction of effector CTL is a critical next step in the development of autoimmunity [21,22]. The question of how autoimmunity is triggered can therefore be deduced to the quantitative response of host against immunizing antigen, i.e., the ability of host to present and/or cross-present antigen. It then follows that the ability of certain antigens such as measles virus to cause autoimmunity may be due to their ability, in conjunction with its ability to present antigen, to overstimulate CD4+ and/or CD8+ T cells of certain hosts beyond integrity of their immune system. Living organisms are constantly exposed to a broad range of environmental antigens, as exemplified by the recent re-emergence of measles virus infection among a subpopulation of Japanese young adults who were not vaccinated against the virus. We therefore conclude that systemic autoimmunity necessarily takes place when host’s immune ‘system’ is overstimulated by external disturbance, i.e., repeated exposure to antigen, to the levels that surpass system’s self-organized criticality, and propose here ‘self-organized criticality theory’ explaining the cause of autoimmunity.

Materials and Methods

Ethics Statement

This study was approved by the Institutional Animal Care and Use Committee and carried out according to the Kobe University Animal Experimental Regulations.

Reagents

APC (allophycocyanin)-conjugated antibody against CD4 (RM4-5), and PE-conjugated antibodies against CD62L (MEL-14), CD69 (H1.2F3) and were purchased from BioLegend (San Diego, CA); FITC-conjugated antibodies against CD44 (IM7.8.1) and DO 11.10 clonotypic TCR (KJ1-26) and PE-conjugated rat IgG1 isotype control from CALTAG Laboratories (Burlingame, CA); PE-Cy5 (phycocerythrin-cyanin 5)-conjugated antibody against CD8α (53-6.7), PE-conjugated antibodies against Vβ8 TCR (F23.1) and IFNγ (XMGl.2) from BD Pharmingen (San Diego, CA).

Animal Studies

Animal studies with BALB/c female mice (Japan SLC, Inc., Hamamatsu, Japan) and DO11.10 TCR transgenic mice [19] (Jackson Laboratory, Bar Harbor, ME), β2m-deficient mice [3] and rag1/gfp knock-in mice [6] of BALB/c background were performed with the approval of the Institutional Review Board. Mice (8 weeks-old) were immunized with 25 μg SEB or 500 μg OVA 24 h after cell transfer, and sera, urine and organ of recipients were studied 2 weeks afterwards.

BALB/c mice were injected i.p. with 200 μg anti-CD4 antibody (GK1.5; BioLegend) to deplete CD4+ T cell 24 h after immunization 8× with OVA. Four days later, CD4+ T cells from mice immunized 12× with KLH were transferred to the CD4+ T-depleted mice. The recipient mice received a single i.p. injection of 100 μg KLH 24 h after the cell transfer.

Assay for Mediators

Sera were assayed for anti-Sm antibody using Sm antigen (ImmunoVision, Springdale, AR), RF (Shibayagi Co., Gumma, Japan), RF for galactose-deficient IgG (Eisai Co., Ltd., Tokyo, Japan) and anti-dsDNA antibody using dsDNA (Worthington Biochemical Co., Lakewood, NJ) after digestion by S1 nuclease (Promega, Madison, WI). Serum IgC was detected using goat anti-C3 antibody (Bethyl Lab.).

CDR3 Length Spectratyping

cDNAs from thymocytes and CD4+ splenocytes were subjected to PCR amplification using Cβ- and VB8-specific primers. Amplified products were subjected to run-off reactions using three fluorophore-labeled Jβ primers, Jβ1.1, Jβ1.3 and Jβ2.4, and analyzed by GeneScan software (Perkin-Elmer Applied Biosystems, Emeryville, CA) [4].

RT-PCR

Total RNA was reversely transcribed to cDNA and amplified by PCR [23]. The products were fractionated by electrophoresis and transferred to nylon membranes (Roche Diagnostics, Mannheim, Germany). The membranes were hybridized to fluorescein end-labeled probes and visualized by alkaline phosphatase (ALP)-labeled anti-fluorescein antibody and Gene Images CDP-Star chemiluminescence reaction [Amersham Pharmacia Biotech, Piscataway, NJ]. The primers and probes were: 5’-CACAGGTGACACACAGTTCACAGTAC3’ (forward), 5’-AAAGTACATGTCCGGCTCT-3’ (reverse) and 5’-AACAGTTGCTGCGCTCT-3’ (probe) for RAG1 [24]; 5’-CAGACCTCAACAGGAAGTGACAG-3’ (forward), 5’-CCGCTACGTCAGTACAG-3’ (reverse) and 5’-CATCTCTAGTAAAG-3’ (probe) for TdT; 5’-CAGAGCTGCCATCT-3’ (forward), 5’-CATCTCTGCTCT-3’ (reverse) and 5’-GGTGCTGCTCCAGTGAGAT-3’ (probe) for pTα.

LM-PCR

DNA (1 μg) was ligated to 20 μM BW linker using T4 ligase (Takara Bio Inc., Shiga, Japan) [25]. Primary PCR was performed using 200 ng ligated DNA, BW-1HR primer (5’-CGGCTGATCCTGCGTGCGCTGC-3’) [24], primer specific for 3′ flanking sequence of TCRJF2 (5’-AGATGATACTAGAGACAAATGTGAG-3’) and 2 U of AmpliTaq Gold DNA polymerase (Applied
Antigen Cross-Presentation

OVA-reactive CD8+ T cells were isolated from spleens of DO 11.10 mice using MACS beads (Miltenyi Biotec), CD11c+ DCs (4×10^5/well) were isolated using MACS beads (Miltenyi Biotec) and incubated with 1 mg/ml OVA for 3 h, then co-cultured with DO11.10 CD8+ T (KJ1-26; C57BL/6) cells (2×10^5/well) for 24 h, and the expression of CD69 on DO11.10 CD8+ T cells was examined. IL-2 and IFNγ in culture supernatants were measured by ELISA (Biosource, Camarillo, CA).

To inhibit cross-presentation, mice were immunized in vivo with 250 µg of chloroquine (Sigma) 3 h prior to immunization with 500 µg OVA or PBS every 5 d. Presence of autoantibodies was analyzed 2 d after each immunization, and proteinuria and IFNγ/CD8+ T cells were examined 9 d after the final immunization.

Statistical Analysis

Statistical analyses were performed using Student’s t test, and the data are expressed as the mean ± SD.

Supporting Information

Figure S1 Induction of autoantibodies depends on correct presentation of antigen to T cells. (A) BALB/c mice were repeatedly injected i.p. with 25 µg of SEB or PBS every 5 d. Sorted Vβ8+CD4+ splenocytes obtained 9 d after the final immunization were stimulated in vitro with plate-bound 2 µg/ml anti-CD3 (145-2C11; Cederlane, Ontario, Canada) and 5 µg/ml anti-CD28 (37.51; BD PharMingen) antibodies for 24 h, and the expression of CD69 on DO11.10 CD8+ T cells was examined. IL-2 and IFNγ in culture supernatants were measured by ELISA (Biosource, Camarillo, CA).

(A) BALB/c mice were repeatedly injected i.p. with 25 µg of SEB or PBS every 5 d. Sorted Vβ8+CD4+ splenocytes obtained 9 d after the final immunization were stimulated in vitro with plate-bound 2 µg/ml anti-CD3 (145-2C11; Cederlane, Ontario, Canada) and 5 µg/ml anti-CD28 (37.51; BD PharMingen) antibodies for 24 h, and the expression of CD69 on DO11.10 CD8+ T cells was examined. IL-2 and IFNγ in culture supernatants were measured by ELISA (Biosource, Camarillo, CA).

(Figure S2) Generation of autoantibodies after repeated immunization with antigen. (A) The 8 week-old BALB/c mice were injected i.p. with 500 µg OVA every 5 d, and serum RF and anti-Sm, and anti-dsDNA antibodies (upper), and serum IgG and anti-OVA antibodies (lower) were quantified by ELISA 2 d after respective immunization. An arbitrary unit (AU) of 1.0 is the equivalent titer in sera of MRL/lpr mice. Serum IgG was quantified by ELISA (Bethyl Laboratories), and anti-OVA antibody was quantified using mouse anti-OVA monoclonal antibody (OVA-14; Sigma) as reference. (B) BALB/c mice were immunized i.p. with 100 µg KLH every 5 d. Serum RF and anti-Sm antibodies were measured by ELISA 2 d after respective immunization, AU 1.0 = equivalent detected in sera of MRL/lpr mice.

Figure S3 Induction of autoantibodies in CD8+ T cell-deficient mice. β2m-deficient mice were immunized with 500 µg OVA i.p. injection every 5 d, and IgG-RF, anti-dsDNA antibody, and proteinuria were measured.

Figure S4 Expression of V(D)J recombinease complex and histopathology of OVA-immunized BALB/c mice. (A) Expression of V(D)J recombination complex after immunization 12× with OVA as detected using RT-PCR (upper left), GFP+ cells in the CD4+ T cell of rog1/gfp knockout mice after immunization 12× with OVA (lower left), Appearance and weights of spleens and a representative low-magnification view of the spleens from PBS- and OVA-immunized mice (right, mean ± SD, 9 mice/group). Enlarged lymphoid follicles with marked germinal centers were seen in mice immunized with OVA (H&E staining, bar = 200 µm; original magnification ×20). (B) Representative renal and extra-renal histopathology in the mice immunized 12× with OVA. A wire-loop-like massive membranous glomerulonephritis in the kidney (upper left) (PAS staining, bar = 20 µm; original magnification ×400), plasma cell infiltrates around bile ducts (upper middle) (bar = 20 µm; original magnification ×100), expansion of lymphoid follicle in the white pulp of spleen (upper right) (bar = 200 µm; original magnification ×40), focal infiltrates of mononuclear cells to thyroid (lower left) (bar = 50 µm; original magnification ×100), and diffuse infiltration of inflammatory cells into auricular subcutaneous tissue (upper right) (bar = 50 µm; original magnification ×200).

Figure S5 The de novo generation of IFNγ-producing CD8+ T cells in recipient mice after cell transfer. Percentage of IFNγ+ cells within the CD8+ T cell population of the recipient mice was examined 2 weeks after cell transfer (mean ± SD, 5 mice/group).

Figure S6 Transfer of the ability to induce anti-ds DNA antibody or tissue injury by transfer of CD4+ or CD8+ T cells, respectively. Adoptive transfer of cells from OVA-immunized mice into naive BALB/c mice, with or without 1× booster injection of OVA (500 µg, 24 h post-transfer). Autoantibodies and proteinuria measured 2 weeks later.

Figure S7 Antigen-specific activation of T cells and the expression of MHC class I on DC. (A) Spleen cells were cultured with or without 1 mg/ml of OVA for 24 h, and the expression of CD69 on CD4+ T or CD8+ T cells was examined by flow cytometry. (B) DC from PBS- or OVA-immunized mice (PBS DC or OVA DC) were incubated in the presence or absence of chloroquine (CQ) (20 µg/ml) for 2 h and OVA (1 mg/ml) for 3h. OVA- and/or CQ-pulsed DCs were stained with biotin-conjugated anti-H-2k antibody (SFI-1;1; BD PharMingen) and PE-conjugated streptavidin (BioLegend).

Figure S8 Requirement of CD4+ T cell help for inducing autoimmune tissue injury. The mice were depleted of CD4+ T cells by treatment with 200 µg anti-CD4 antibody (Ab) (GK1.5, BioLegend) 24 h prior to 6×, 9× and 12× immunization with OVA. Control mice were injected with 200 µg rat IgG (CALTAG Lab.). (A) A representative flow cytometry plot showing that CD4+ T cells were depleted to 5.56±2.30% in the spleen and
3.42 ± 1.02% in peripheral blood mononuclear cells (PBMC) 9 d after 3rd treatment with anti-CD4 Ab. (B) Mice were immunized with OVA with or without adding anti-CD4 antibodies, and the number of IFNγ+ cells within the CD8+ T population (upper and lower left) (mean ± SD, 3 mice/group) and proteinuria (lower right) were evaluated. Figure S9 Study on the requirement of autoantibody-inducing CD4+ T cells for autoimmune tissue injury. Neither OVA-specific matured IFNγ+CD8+ T cells or autoimmune tissue injury were observed until BALT/c mice were immunized at least 10× with OVA. The percent splenic IFNγ+CD8+ T cells (left, mean ± SD, 4 or 5 mice/group) and proteinuria (right) were examined after immunization 6×, 8×, 10× and 12× with OVA. Acknowledgments We dedicate this work to the Late Professor Emeritus Atsushi Okabayashi, a mentor to SS, who introduced us to this area of study. We thank Prof. Masaaki Miyazawa, Department of Immunology, Kinki University School of Medicine, and Prof. Nobuo Saaguchi, Department of Immunology, Kumamoto University Graduate School of Medicine, for useful advice, authorization of results and providing mice. W. J. also thank Dr. Sachiyo Tsuji-Kawahara, Kinki University, for kindly providing β2m-deficient mice and Dr. Hideya Igarashi, Kumamoto University Graduate School of Medicine, for kindly providing mlg1/gfp knock-in mice. We thank Mai Takimoto and Toshie Nakashima, graduate students of our department, for studies of mlg1/gfp knock-in mice, CD3R3 spectratyping and LM-PCR, Dr. Akira Hashiramoto, Division of Rheumatology, Kobe University, for helpful discussions, and Dr. Marc Lampher for reviewing the manuscript. Author Contributions Conceived and designed the experiments: SS. Performed the experiments: KT YM. Analyzed the data: KT YM SS. Wrote the paper: KT SS.

References
Sjögren’s Syndrome

COVID-19 is an emerging, rapidly evolving situation.

Get the latest public health information from CDC: https://www.coronavirus.gov
Get the latest research information from NIH: https://www.nih.gov/coronavirus

Points To Remember About Sjögren’s Syndrome

- Sjögren’s syndrome is a disease that affects the glands that make moisture. It most often causes dryness in the mouth and eyes.

- The main symptoms of Sjögren's syndrome are dry eyes and dry mouth.

- Sjögren’s syndrome is an autoimmune disease. In Sjögren's syndrome, your immune system attacks the glands that make tears and saliva (spit).

- Doctors diagnose Sjögren's using a medical history, physical exam, certain eye and mouth tests, and blood tests.

- Treatment differs for each person and depends on what parts of your body are affected and focuses on getting rid of symptoms.

- Living with Sjögren's syndrome can be easier by taking good care of your eyes and mouth, by protecting your voice, and understanding what medicines cause dryness.

What is Sjögren’s syndrome?

https://www.niams.nih.gov/health-topics/sjogrens-syndrome
Sjögren’s syndrome is a disease that affects the glands that make moisture. It most often causes dryness in the mouth and eyes. It can also lead to dryness in other places that need moisture, such as the nose, throat, and skin.

Sjögren’s syndrome is also a rheumatic disease, which affects:

- Joints.
- Tendons.
- Ligaments.
- Bones.
- Muscles.

The signs and symptoms of rheumatic diseases can include:

- Redness or heat.
- Swelling.
- Pain.
- Loss of function.

**Primary Versus Secondary Sjögren’s Syndrome**

Doctors have two categories for Sjögren’s syndrome:

- **Primary form**: Occurs if you do not have other rheumatic diseases.
- **Secondary form**: Occurs if you already have another rheumatic disease, such as [rheumatoid arthritis](https://www.niams.nih.gov/health-topics/rheumatoid-arthritis) or [systemic lupus erythematosus](https://www.niams.nih.gov/health-topics/systemic-lupus-erythematosus), [scleroderma](https://www.niams.nih.gov/health-topics/scleroderma), or polymyositis.

**Who gets Sjögren’s syndrome?**
Most people with Sjögren's syndrome are women. It can occur at any age and in any race. But it is rare in children and most often shows up after age 40.

What are the symptoms of Sjögren’s syndrome?

The main symptoms are:

- Dry eyes. Your eyes may burn or itch or feel like they have sand in them. Sometimes Sjögren's syndrome can cause blurry vision or sensitivity to bright light, especially fluorescent lighting.

- Dry mouth. Your mouth may feel chalky or like it is full of cotton.

- You may have trouble:
  - Swallowing.
  - Speaking.
  - Tasting.

- Because you lack the protective effects of saliva, you may develop more dental decay (cavities) and mouth infections.

Sjögren’s syndrome also can affect other parts of the body, including:

- Skin.
- Joints.
- Lungs.
- Kidneys.
- Blood vessels.
- Digestive organs.
Symptoms can include:

- Nerves.

People with dry mouth can easily get mouth infections. Tell your doctor if you have any of these symptoms in your mouth:

- White patches.
- Red patches.
- Burning areas.

What causes Sjögren’s syndrome?

Sjögren's syndrome is an autoimmune disease. The immune system is supposed to fight disease by killing off harmful viruses and bacteria. But with autoimmune diseases, your immune system attacks parts of your own body by mistake.

In Sjögren’s syndrome, your immune system attacks the glands that make tears and saliva (spit). The damage keeps these glands from working right and causes dry eyes and dry mouth.
Doctors don’t know the exact cause of Sjögren's syndrome. They think it may be caused by a combination of two things:

- Genes.
- Exposure to something like a virus or bacteria.

Is there a test for Sjögren’s syndrome?

Doctors diagnose Sjögren's using:

- Medical history.
- Physical exam.
- Certain eye and mouth tests.
- Blood tests.

Doctors may also order:

- A urine test.
- A chest x-ray.

How is Sjögren’s syndrome treated?

Treatment differs for each person and depends on what parts of your body are affected. Treatment will focus on getting rid of symptoms and may include:

- Medicines for joint or muscle pain (such as aspirin and ibuprofen).
- Medicines that help you make more saliva.
- Medicines that suppress inflammation (such as corticosteroids).
Medicines that suppress the immune system.

Treatment for dry eyes may include:

- Eye drops that come in different thicknesses. You may have to try a few to find the right one.
- Eye ointments. These are thicker than eye drops. They protect the eyes and keep them wet for several hours. They can blur your vision, so you may want to use them while you sleep.
- Medicines to reduce inflammation in the eye.
- A chemical that wets the surface of the eye and keeps the natural tears from drying out so fast. It comes in a small pellet that you put in your lower eyelid. When you add eye drops, the pellet melts. This forms a film over your own tears and traps the moisture.
- Surgery to shut the tear ducts that drain tears from the eye.

Treatment for dry mouth may include:

- Chewing gum or sucking on hard candy helps your glands make more saliva. Use sugar-free gum and candy.
- Sipping water or a sugar-free drink often to keep your mouth wet.
- Using oil or petroleum-based lip balm or lipstick to help dry, cracked lips feel better.
- Using a saliva substitute prescribed by a doctor to make the mouth feel wet.
- Using medicine to help your mouth make more saliva.

Who treats Sjögren’s syndrome?

Because the symptoms of Sjögren's syndrome develop gradually and are similar to those of many other diseases, getting a diagnosis can take time. A person could see a number of doctors, any of whom could diagnose the disease and be involved in its treatment. These might
include:

- A rheumatologist, a doctor who specializes in diseases of the joints, muscles, and bones.
- A primary care physician.
- An internist.
- An ophthalmologist, a doctor who specializes in the care of the eyes.
- An otolaryngologist, a doctor who specializes in caring for ears, nose, and throat.

Usually a rheumatologist will coordinate treatment among a number of specialists.

Living with Sjögren’s syndrome

Living with Sjögren’s syndrome can be easier by following some tips for:

- Eye care.
- Mouth care.
- Protecting your voice.
- Understanding medicines that cause dryness.

General Tips for Eye Care

- Don’t use eye drops that irritate your eyes. If one brand or prescription bothers you, try another. Eye drops that do not contain preservatives are usually essential for long-term use.
- Practice blinking. You tend to blink less when reading or using the computer. Remember to blink 5 to 6 times a minute.
- Protect your eyes from drafts, breezes, and wind.
- Put humidifiers in the rooms where you spend the most time, including the bedroom, or...
Importance of Mouth Care

Natural saliva contains substances that help get rid of bacteria that can cause cavities and mouth infections. Good oral hygiene or mouth care is extremely important when you have dry mouth. Here’s what you can do to prevent cavities and infections:

- Visit a dentist regularly, at least twice a year, to have your teeth examined and cleaned.
- Rinse your mouth with water several times a day. Don’t use mouthwash that contains alcohol, because alcohol is drying.
- Use toothpaste that contains fluoride to gently brush your teeth, gums, and tongue after each meal and before bedtime. Non-foaming toothpaste is less drying.
- Floss your teeth every day.
- Avoid sugar between meals. That means choosing sugar-free gum, candy, and soda. If you do eat or drink sugary foods, brush your teeth immediately afterward.
- See a dentist right away if you notice anything unusual or have continuous burning or other oral symptoms.
- Ask your dentist whether you need to take fluoride supplements, use a fluoride gel at night, or have a varnish put on your teeth to protect the enamel.

Protect Your Voice
You can develop hoarseness if their vocal cords become inflamed or become irritated from throat dryness or coughing. To prevent further strain on your vocal cords, try not to clear your throat before speaking. Clearing your throat is hard on the vocal cords. To avoid irritating your vocal cords:

- Sip water.
- Chew sugar-free gum.
- Suck on sugar-free candy.
- Make an “h” sound, hum, or laugh to gently bring the vocal cords together.

**Medicines and Dryness**

Some medicines can cause eye and mouth dryness. If you are taking one of the drugs listed below, talk to your doctor about adjusting the dose or finding a different medicine. Don't stop taking any medicine without asking your doctor. These can include medicines that you take for:

- Allergies and colds (antihistamines and decongestants).
- Getting rid of extra fluids in your body (diuretics).
- Diarrhea.
- High blood pressure.

Some type of medicines that can cause dryness include:

- Antipsychotic medicines.
- Tranquilizers.
- Antidepressants.

**Other medical problems related to Sjögren’s syndrome**
A small number of people with Sjögren's syndrome may develop lymphoma. A form of cancer, lymphoma can affect the salivary glands, lymph nodes, the gastrointestinal tract, or the lungs. If you have enlargement of a salivary gland, you should contact your doctor. Other symptoms may include the following:

- Unexplained fever.
- Night sweats.
- Constant fatigue.
- Unexplained weight loss.
- Itchy skin.
- Reddened patches on the skin.

Many of these can be symptoms of other problems, including Sjögren's syndrome itself. Nevertheless, it is important to see your doctor if you have any of these symptoms.

For more info

**U.S. Food and Drug Administration**
Toll free: 888-INFO-FDA (888-463-6332)
Website: [https://www.fda.gov](https://www.fda.gov)

Drugs@FDA at [https://www.accessdata.fda.gov/scripts/cder/daf](https://www.accessdata.fda.gov/scripts/cder/daf) Drugs@FDA is a searchable catalog of FDA-approved drug products.

**Centers for Disease Control and Prevention, National Center for Health Statistics**
Website: [https://www.cdc.gov/nchs](https://www.cdc.gov/nchs)

**National Eye Institute**
Website: [https://www.nei.nih.gov](https://www.nei.nih.gov)

**National Institute of Dental and Craniofacial Research (NIDCR)**
Website: [https://www.nidcr.nih.gov](https://www.nidcr.nih.gov)
NIDCR Sjögren’s Syndrome Clinic
Website: https://www.nidcr.nih.gov/Research/NIDCRLaboratories/MolecularPhysiology/SjogrensSyndrome/

National Institute of Neurological Disorders and Stroke
Website: https://www.ninds.nih.gov

American Academy of Dermatology
Website: https://www.aad.org

American Academy of Ophthalmology
Website: http://www.aaoe.org

American Association for Dental Research and International Association for Dental Research
Website: http://www.iadr.org

American College of Rheumatology
Website: https://www.rheumatology.org

American Dental Association
Website: https://www.ada.org

American Autoimmune Related Diseases Association, Inc.
Website: https://www.aarda.org

Arthritis Foundation
Website: https://www.arthritis.org

Lupus Foundation of America
Website: https://www.lupus.org

The Myositis Association
Website: https://www.myositis.org
If you need more information about available resources in your language or other languages, please visit our webpages below or contact the NIAMS Information Clearinghouse at NIAMSInfo@mail.nih.gov.

- Asian Language Health Information
- Spanish Language Health Information
Sjögren's Syndrome

Fast Facts

- Sjögren's syndrome is an autoimmune condition that can occur at any age, but is most common in older women. Many patients develop Sjögren’s syndrome as a complication of another autoimmune disease, such as rheumatoid arthritis or lupus.

- Symptoms vary in type and intensity, but many people with Sjögren's are able to live normal lives.

- Most of the treatment for Sjögren's syndrome is aimed at relieving symptoms of dry eyes and mouth and preventing and treating long-term complications such as infection and dental disease. Treatments often do not completely eliminate the symptoms of dryness.

- Most patients with Sjögren's syndrome remain healthy, but some rare complications have been described, including an increased risk for cancer of the lymph glands (lymphoma). Thus, regular medical care and follow up is important for all patients.

Between 400,000 and 3.1 million adults have Sjögren’s syndrome. This condition can affect people of any age, but symptoms usually appear between the ages of 45 and 55. It affects ten times as many women as men. About half of patients also have rheumatoid arthritis or other connective tissue diseases, such as lupus.

In the early 1900s, Swedish physician Henrik Sjögren (SHOW-gren) first described a group of women whose chronic arthritis was accompanied by dry eyes and dry mouth. Today, rheumatologists know more about the syndrome that is named for Sjögren and—most significantly for patients—can offer advice about how to live with it.

What is Sjögren's syndrome?
Sjögren’s syndrome is an inflammatory disease that can affect many different parts of the body, but most often affects the tear and saliva glands. Patients with this condition may notice irritation, a gritty feeling, or painful burning in the eyes. Dry mouth (or difficulty eating dry foods) and swelling of the glands around the face and neck are also common. Some patients experience dryness in the nasal passages, throat, vagina and skin. Swallowing difficulty and symptoms of acid reflux are also common.

“Primary” Sjögren’s syndrome occurs in people with no other rheumatic disease. “Secondary” Sjögren’s occurs in people who have another rheumatologic disease, most often systemic lupus erythematosus and rheumatoid arthritis. It can occasionally be confused with a newly described syndrome call IGG4 disease.

Most of the complications of Sjögren’s syndrome occur because of decreased tears and saliva. Patients with dry eyes are at increased risk for infections around the eye and may have damage to the cornea. Dry mouth may cause an increase in dental decay, gingivitis (gum inflammation), and oral yeast infections (thrush) that may cause pain and burning. Some patients have episodes of painful swelling in the saliva glands around the face.

Complications in other parts of the body can occur. Pain and stiffness in the joints with mild swelling may occur in some patients, even in those without rheumatoid arthritis or lupus. Rashes on the arms and legs related to inflammation in small blood vessels (vasculitis) and inflammation in the lungs, liver, and kidney may occur rarely and be difficult to diagnose. Numbness, tingling, and weakness also have been described in some patients. The parotid gland is at the edge of the jaw and can become swollen and inflamed in some people with Sjögren’s Syndrome.

**What causes Sjögren’s syndrome?**

The cause of Sjögren’s syndrome is not known, but it is an autoimmune disorder. People with this disease have abnormal proteins in their blood. This suggests that the immune system, which normally functions to protect the body against cancers and infections, is reacting against its own tissue. The decrease in tears and saliva seen in Sjögren’s syndrome occurs when the glands that produce these fluids are damaged by inflammation. Research suggests that genetic factors and possibly viral infections may predispose people to developing this condition.

**How is Sjögren’s syndrome diagnosed?**

Diagnosis depends on a combination of symptoms, physical examination, blood tests, and sometimes special studies. Dry eyes and mouth may be early signs of the condition but require further investigation, because these symptoms can be caused by many other conditions or medications. Special tests may assess any decrease in tear or saliva production. An eye examination is helpful in detecting any eye changes seen in Sjögren’s. Blood tests can determine the presence of antibodies (immune system proteins that help destroy...
foreign invaders) typical of the disease. Typical antibodies include anti-nuclear antibodies (ANA), anti-SSA and SSB antibodies or rheumatoid factor, but these are not always present. Biopsies of saliva glands around the face or under the surface of the inner lip also may be used to make a diagnosis.

How is Sjögren's syndrome treated?

Treatment is designed to lessen the most bothersome symptoms. Dry eyes usually respond to artificial tears applied regularly during the day or to gels applied at night. Other measures, such as plugging or blocking tear ducts, can be used in more severe cases. Eye drops that reduce inflammation in the glands around the eyes, such as cyclosporine (Restasis), may be used to increase tear production. Drinking water, chewing gum, or using saliva substitutes may relieve dry mouth. Some patients benefit from using prescription medications that stimulate saliva flow, such as pilocarpine (Salagen) or cevimuline (Evoxac). If patients develop yeast infections, anti-fungal therapies may be used. Humidifiers and nasal saline irrigation may improve nasal dryness. Medications that reduce gastric acid (such as proton-pump inhibitors and H2 blockers) may lessen symptoms of acid reflux. Treatments may help relieve some of the dryness, but usually some dryness persists.

All patients should receive regular dental care to prevent cavities and tooth loss that may occur as a complication of Sjögren's. Patients with dry eyes should see an ophthalmologist (eye doctor) regularly for signs of damage to the cornea. Patients with excessive redness and pain in the eyes should be evaluated for infections.

Hydroxychloroquine (Plaquenil), an antimalarial drug used in lupus and rheumatoid arthritis, may be helpful in some patients with Sjögren's syndrome by reducing joint pain and rash experienced by some patients. Patients with rare but serious systemic symptoms, such as fever, rashes, abdominal pain, or lung or kidney problems, may require treatment with corticosteroids such as prednisone (Deltasone and others) and/or immunosuppressive agents like methotrexate (Rheumatrex), azathioprine (Imuran), mycophenolate (Cellcept) or cyclophosphamide (Cytoxan). In addition, researchers are evaluating rituximab (Rituxan) and other biological therapies to treat cases of Sjögren's that affect the entire body.

Broader health impact of Sjögren's syndrome

A vast majority of patients with Sjögren’s syndrome remain very healthy, without any serious complications. Patients should know that they face an increased risk for infections in and around the eyes and an increased risk for dental problems due to the long-term decrease in tears and saliva.

Rarely, patients may have complications related to inflammation in other body systems, including:

- Lung problems that may mimic pneumonia
- Abnormal liver and kidney function tests
Skin rashes related to inflammation of small blood vessels
Neurologic problems causing weakness and numbness

In a small number of people, Sjögren's syndrome may be associated with lymphoma, a cancer of the lymph glands.

Living with Sjögren's syndrome

Sjögren's syndrome cannot be cured, but in many cases proper treatment helps to alleviate symptoms. Rheumatologists are specialists in musculoskeletal disorders and, therefore, are more likely to make a proper diagnosis. They also can advise patients about the best treatment options available.

People with Sjögren's syndrome usually are able to live normal lives with very few adjustments. When a diagnosis is made, many patients must focus a great deal of attention dealing with dry eyes and dry mouth, but these symptoms tend to subside with time. Any pain or redness in the eyes should be evaluated promptly, as this may signal an infection. To reduce risk for cavities and other dental problems, patients must pay close attention to proper oral hygiene and regular dental care.

Patients should see their physician regularly for general health screening. They also should pay close attention to any abnormal swelling in the glands around the face or neck, under the arms, or in the groin areas, as this may be a sign of lymphoma.

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EXHIBIT 193
Alum, an Aluminum Based Adjuvant, Induces Sjögren’s Syndrome-like Disorder in Mice

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Abstract

Objective—Adjuvant induced innate immune responses have been suspected to play a role in the initiation of certain autoimmune disorders. This study investigates the role of alum, an aluminum based adjuvant in the induction of Sjögren’s syndrome-like disorder in mice.

Methods—Inbred, female New Zealand Mixed (NZM) 2758 strain of mice were injected with alum. Control mice were treated similarly with PBS. Mice were monitored for salivary gland dysfunction by measuring pilocarpine induced salivation. Presence of lymphocytic infiltrates within the submandibular glands was studied by histopathology. Autoantibodies to Ro and La proteins were analyzed by ELISA and the presence of anti-nuclear antibodies (ANA) was analyzed by indirect immunofluorescence.

Results—By eight weeks after treatment, the saliva production in alum treated mice was significantly decreased in comparison to the PBS treated mice. This functional loss persisted till the termination of experiments at 20wks. The incidence and severity of sialoadenitis was significantly higher in the alum treated mice. Although there were no differences in the levels of anti-Ro/La autoantibodies in sera of alum and PBS treated groups, the alum group showed higher ANA reactivity.

Conclusion—in the NZM2758 mice, alum induces a Sjögren’s syndrome-like disorder that is characterized by chronic salivary gland dysfunction and the presence of lymphocytic infiltrates within the salivary glands. Thus, the potential of aluminum based adjuvants for induction of autoimmunity should be closely monitored in individuals genetically susceptible to developing autoimmune disorders.

Keywords

Adjuvants; Sialoadenitis; Sjogren's syndrome

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INTRODUCTION

Sjögren’s syndrome (SS) is a chronic autoimmune disorder characterized by lymphocytic infiltration in the exocrine glands, loss of salivary and/or lacrimal gland function and the presence of circulating autoantibodies [1]. While role of adaptive immunity has been extensively investigated in SS, it is now clear that activation of innate immunity is also important for SS pathogenesis [2]. Using mouse models, we have previously reported the role of innate immunity, specifically the type I interferon pathway in SS [3–5]. Treatment of NZB/W F1 mice with incomplete Freund’s adjuvant (IFA) led to an accelerated SS-like disease with increased infiltration of type I IFN producing plasmacytoid dendritic cells in the salivary glands [3]. A similar exacerbation of SS was seen in mice treated repeatedly with poly(I:C), a potent inducer of type I interferon [4]. Furthermore, genetic ablation of type I interferon signaling in the B6.Aec1.Aec2 mice, a spontaneous mouse model of SS, resulted in a complete protection from the disease [5]. All these studies support the essential role for innate immune activation and the type I IFN pathway in the disease process.

Another pathway for activation of innate immunity involves the Nucleotide-binding oligomerization domain (NOD) like receptors (NLRs) [6]. Aluminum based adjuvants, such as alum, can stimulate innate immunity through NOD-like receptor pyrin containing domain 3 (Nlrp3) which associates with Caspase-1 to form an inflammasome complex [7]. The Nlrp3 inflammasome regulates cleavage of pro-inflammatory cytokines like IL-1β, IL-18 and IL-33 into their active forms, thereby activating multiple inflammatory processes. However, whether inflammasome activation can initiate SS is not known. Thus, in this study, we investigated the effects of alum, on development of SS-like disease in female NZM2758 mice. The NZM2758 mouse is an inbred strain generated by in-breeding of the NZB and NZW mice [8]. These mice do not spontaneously develop SS-like disease. However, they are genetically susceptible and develop reduced salivary gland function and sialoadenitis following injection with IFA [9].

MATERIALS AND METHODS

Mice and experimental design

All procedures were in accordance to NIH guidelines for humane use of laboratory animals and were approved by the Institutional Animal Care and Use Committee. NZM2758 mice were generated in a breeder colony and housed under specific pathogen free conditions. Eight to ten week old female mice were injected subcutaneously with a 50% alum (Pierce Chemical Company, IL, USA) suspension mixed with phosphate buffered saline (0.1ml/mouse). Additional intraperitoneal injections of alum (0.05ml/mouse) were given 4wks and 8wks later. Age matched control groups were injected similarly with PBS alone. Pilocarpine induced saliva production was measured at different time points as described previously [4]. Mice were euthanized at 20wks of age and salivary glands collected in 10% buffered formalin for histopathologic analysis.

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Histopathologic analysis of salivary glands

Submandibular salivary glands (SMG) were embedded in paraffin blocks and sections stained with hematoxylin and eosin using standard procedures. Three micron sections were cut from each block and two sections approximately 50 microns apart were evaluated for severity of sialoadenitis as previously described [3].

Analysis of serum autoantibodies

Sera from terminal bleeds were used for all autoantibody analyses. Serum antibodies to Ro60 and La antigens were measured in an ELISA described previously [3], except plates were coated with purified recombinant Ro60 and La expressed as fusion proteins with maltose binding protein. Maltose binding protein (MBP) alone was used as a control. Anti-nuclear antibodies were measured by indirect immuno-fluorescence staining on HeLa cells grown on coverslips using standard protocols [5]. Intensity of staining was scored by an observer blinded to experimental details.

Statistical Methods

Mann-Whitney non-parametric t test, paired t test, and One-way ANOVA with Bonferroni post-test for multiple comparisons were used to calculate p values as indicated. A confidence level of 95% was used for all tests and analyses were carried out using Graph Pad Prism 6.0 software.

RESULTS

The NZM inbred strains of mice carry genes that render them susceptible to different autoimmune diseases such as lupus (NZM2328, NZM2410) and Sjögren’s syndrome (NZM2758) [8, 9]. For the present study, NZM2758 female mice were injected either with alum or PBS. Pilocarpine induced saliva production was measured at 8wks after the first injection. Figure 1A shows that in comparison with PBS treated control mice, just two injections with alum induced a significant drop (p<0.0001) in the mean saliva volume. To further investigate the kinetics and persistence of functional loss, additional cohorts of mice were injected with alum and PBS at 0wks, 4wks and 8wks. At 8wks after treatment, the mean saliva volume in alum treated group was significantly (p=0.0103) reduced by 27%, compared to mean saliva volume on day 0 (Figure 1B). This drop in function persisted at 13wks (p=0.003) and at 20wks (p=0.0005), when the experiment was terminated. In contrast, the PBS treated mice did not show any statistically significant changes in saliva production over time. Thus, alum induces a persistent drop in saliva production in the NZM2758 mice.

SMG were harvested from mice after 20wks of treatment and evaluated for development of inflammatory infiltrates. Representative photomicrographs are shown in Figure 2. Only one out of four mice injected with PBS showed the presence of a small inflammatory focus in the SMG. In contrast, all five mice injected with alum developed multiple inflammatory foci of different sizes. The larger infiltrates were predominantly located in the peri-vascular or peri-ductal regions (arrows), although smaller foci were also seen surrounding secretory acini (arrowheads). The severity of sialoadenitis was scored by an observer blinded to
experimental details. As shown in Figure 2, mice treated with alum developed significantly higher (p=0.012) inflammation of the SMG.

Presence of circulating autoantibodies to Ro and La proteins is a hallmark of SS [1]. Therefore, terminal bleeds were studied for reactivity to Ro60 and La expressed as fusion proteins with MBP. As shown in Figure 3, alum failed to exacerbate the anti-Ro/La autoantibody response.

Anti-nuclear antibodies were studied by indirect immunofluorescence using HeLa cells as substrates (Figure 4). The ANA reactivity in alum group was higher than that in the PBS group. NZM2758 are autoimmune prone mice and develop a low level of circulating autoantibodies with age. Thus, detection of some ANA reactivity in the PBS treated group is not surprising. However, collectively these data demonstrate that alum exacerbates autoimmune responses in NZM2758 mice.

DISCUSSION

In the present study, we show that alum treatment of NZM2758 mice, leads to reduced salivary gland function, increased SMG inflammation and ANA production. This study is the first report demonstrating induction of SS-like disorder in a genetically susceptible mouse strain by an aluminum based adjuvant, alum.

Alum is responsible for the activation of inflammasome pathway leading to the production of pro-inflammatory cytokines like IL-1β and IL-18. Although increased levels of IL-1β [10] and IL-18 [11] has been reported in SS patients, the role of inflammasome components in pathogenesis of SS is only recently being investigated. Baldini et al show that the expression of P2X7 receptor (P2X7R), is increased in salivary glands of SS patients [12]. The P2X7R is involved in the activation of NLRP3 inflammasome. A concomitant increase in the gene expression of inflammasome components (NLRP3, ASC and caspase-1) in these patients is suggestive of their involvement in disease process. Interestingly, another recent study also suggests that P2X7R polymorphisms might be involved in SS pathogenesis [13]. These reports and the data from our investigation suggests that inflammasome activation plays an important role in the induction and progression of SS.

A surprising observation in the present study was that alum did not induce an exacerbation of anti-Ro/La autoantibody response. Moreover, the reactivity to recombinant Ro and La antigens was not very robust, in comparison to our previous study of IFA treatment of NZB/W F1 mice [3]. These data suggest a dissociation between circulating autoantibodies to Ro and La from other SS characteristics in alum treated NZM2758 mice. However, the ANA data suggests the possibility that exacerbation of autoimmune responses in this mouse following alum injections may target other antigens. The precise mechanisms responsible for increased sialoadenitis and salivary gland dysfunction in this model are not known. They might involve all or some of the pro-inflammatory mediators induced by activation of inflammasome as well as localized apoptosis in the salivary glands and chemokine production leading to inflammatory cell infiltration. Clearly, the alum-NZM2758 model will
be valuable for evaluating the role of different components of the inflammasome pathway in SS pathogenesis.

Alum is commonly used in animals and humans. It has a well-established record of safety and efficacy as an adjuvant. However, autoimmune/autoinflammatory syndrome induced by adjuvant (ASIA) has been recently described in humans [14]. Shoenfeld and colleagues suggest that alum or other stimulators of innate immunity may trigger different clinical entities including macrophagic myofascitits, the gulf war syndrome, siliconosis, and post-vaccination phenomenon. The recent reports of adjuvant mediated acceleration of autoimmunity in spontaneous murine models of SLE [9, 15] and this study with alum, support the hypothesis that activation of innate and adaptive immunity by adjuvants can initiate and exacerbate autoimmunity in genetically susceptible individuals.

However, there are major differences between administration of alum in mice and in humans [15]. Firstly, the dose in mice is significantly higher than in humans. Secondly, the route of administration in mice is subcutaneous and intraperitoneal, while in humans, it is intramuscular. These factors may influence the immune responses and add to the complexity of translating the observations from rodents directly to humans. Nevertheless, the studies in autoimmune prone mice clearly demonstrate the importance of investigating possible side effects of adjuvants, especially in individuals who repeatedly receive vaccinations in a short period of time. The study also underscores the need for developing newer and safer adjuvants for human use.

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Figure 1. Alum injection leads to loss of salivary gland function in NZM2758 mice

(A): The mean saliva level in alum treated NZM2758 mice (●, n=15) is significantly lower (p<0.0001; Mann-Whitney test) than that in PBS (○, n=11) treated group. Each data point represents one mouse. (B): In an additional cohort, mice were injected with PBS (n=4) or Alum (n=5) at 0, 4, and 8wk time points and saliva was measured at indicated time points. Compared to day 0, in alum injected mice, a significant drop in saliva production was seen at 8, 13 and 20 wks. Statistical analyses was done by paired t test (*p<0.05, **p<0.01, ***p<0.001). Analyses by One–way ANOVA with Bonferroni post-test for multiple comparisons showed that the saliva production in alum treated mice was significantly lower than age matched PBS treated group. Data are mean ± SEM saliva volumes for each group.
Figure 2. Alum injected mice show increased sialoadenitis
Representative photomicrographs of SMG gland sections stained with H & E are shown. Arrows indicate a large, and arrowheads indicate small inflammatory foci in alum injected mouse. Scale bar is 50µm. The severity of sialoadenitis was scored on a scale of 0 (no inflammation) to 5 (severe inflammation and gland destruction) and data are represented as mean ± SEM severity score for each group. Alum injected mice have a significantly (p=0.012) higher disease score compared to PBS injected mice Mann-Whitney test was used for statistical analysis.
Figure 3. Exacerbation of SS-like disease with alum injections is not associated with increase in circulating autoantibodies to mouse La and Ro60 proteins

ELISA plates were coated with recombinant mLa-MBP, mRo60-MBP or MBP alone. Sera from terminal bleeds were tested at a 1:100 dilution and bound IgG antibodies were detected. Data are shown as mean ± SEM of absorbance at 450nM.
Figure 4. Comparison of ANA reactivity between alum and PBS treated mice show higher ANA in the alum group. HeLa cells coated coverslips were used as substrate for indirect immunofluorescence. All sera were used at 1:50 dilution and bound IgG antibodies were revealed by goat anti-FITC conjugate. The numbers indicate individual mice. Scale bar is 20µm.
EXHIBIT 194
**1. Introduction**

Recently, a new syndrome, namely the “Autoimmune/inflammatory syndrome induced by adjuvants” (ASIA) [1–3] has been defined. The conditions included in ASIA syndrome share different common signs and symptoms and seem to be induced by the presence of adjuvants. These so-called adjuvants are substances able to boost the immune response and act as a trigger in the development of autoimmune diseases. Post-vaccination autoimmune phenomena represent a major issue of ASIA. Indeed, despite vaccines represent a mainstay in the improvement of human health, several of these have been implicated as a potential trigger for autoimmune diseases. Sjögren’s Syndrome (SjS) is a systemic chronic autoimmune inflammatory disease characterized by the presence of an inflammatory involvement of exocrine glands accompanied by systemic manifestations. Own to the straight association between infectious agents exposure (mainly viruses) and sicca syndrome development, the possible link between vaccine and SjS is not surprising. Indeed, a few cases of SjS following vaccine delivery have been reported. At the same extent, the induction of SjS following silicone exposure has been described too. Thus, the aim of this review was to focus on SjS and its possible development following vaccine or silicone exposure in order to define another possible facet of the ASIA syndrome.
The most commonly reported signs or symptoms of ASIA syndrome are fatigue, mialgia, myositis, arthralgia, neurological manifestations, fever, dry mouth and cognitive alterations. Fatigue represents one of the most disturbing and disabling manifestations of ASIA being associated in several occasions with sleep disturbances or non restful sleep [17].

The evidence of shared manifestations among all the different conditions included in ASIA syndrome suggests the existence of a common denominator which has been subsequently identified in exposure to adjuvants [4]. These belong to the family of the environmental agents that, as we know, together with a complex interaction with genetic, immune defects and hormonal factors, are able to drive ADs development [18]. The syndromes included in ASIA are immune-mediated conditions that usually appear after a challenge [44]. Nowadays, the most commonly used criteria are the “American-European Consensus Group Criteria” [45], developed in 2002 after a revision of the “Preliminary European criteria” dated 1993 [46,47].

Concerning SjS pathogenesis, the role of environmental agents, specifically infectious agents, in the presence of a predisposing genetic background, has been hypothesized [44,46,48–50]. Interestingly, particular infections may mimic SjS such as tuberculosis, leprosy, syringoches, hepatitis A, B or C, parvovirus B19, Dengue fever, malaria, subacute bacterial endocarditis, and HIV [10]. Certain viruses seem to express a tropism for salivary and lachrymal glandular tissue. These viruses include the Herpesvirdae family, especially the cytomegalovirus (CMV), Epstein–Barr virus (EBV), and human herpes virus (HVH)-6,7,8 [51]. A role for retroviral infections in particular human T lymphotropic virus type I (HTLV-I), has been suggested as well [52]. Viruses affecting exocrine tissues seem to be able to determine an activation of the innate immune system via Toll-like receptor (TLR) pathways. Indeed, TLRs recognize microbial molecular patterns stimulating the production of chemokines/cytokines including type I interferon (IFN–I) whose expression seems to be up-regulated in labial salivary glands, plasma, and peripheral blood cells of patients with SjS [10,53]. According to such findings, it is not surprising the possible development of SjS following the administration of a vaccine. Fig. 1 depicts a possible pathogenic scenario of SjS. On the other hand, when the immunogenicity and safety of a number of vaccines have been addressed in SjS as well as in patients with other connective tissue diseases, no particular concerns have been clearly demonstrated. This, for instance, was the case of the 2009 influenza A/H1N1 which seems to trigger no autoimmune condition [54]. The modern influenza vaccine seems also to be effective, as well as to have a sufficient immunogenicity and a good safety profile in patients with ADs, including SjS [55]. Nonetheless, Pasoto et al. recently evaluated the role of the A/California/7/2009/H1N1-like virus vaccination in pSjS patients [56] and detected a significant increase in the mean levels of anti-SSA/Ro and anti-SSB/La up to 1-year after vaccination with no changes in the other tested autoantibodies. This study was the first indicating that influenza A/H1N1 pdm09 vaccine may induce long-term changes in autoantibody profile restricted to SjS spectrum without a deleterious effect on the disease course. However, in another study on patients with systemic lupus erythematosus, no changes in the generation of anti-SSA/Ro and anti-SSB/La were observed after influenza vaccination [57]. Thus, since there are still points of controversy, an expert committee of the European League against Rheumatism (EULAR) has addressed the role of vaccines in patients with rheumatic diseases including SjS [58]. Such recommendations state that in SjS, as well as in the other ADs, despite several vaccines should be strongly
considered such as influenzae, the 23-valent polysaccharidase pneumococcal, herpes zoster and tetanus toxoid vaccinations, other should be avoided or at least considered only in selected patients. These investigated vaccines comprise the Bacillus Calmette–Guérin (BCG) vaccine, which is not recommended at all in patients with ADs, while the hepatitis A and/or B and the HPV vaccination should be administered only to selected patients [59]. We also note the critical role of sex and hormones on disease pathogenesis [60–63].

4. The cross-road between Sjögren’s syndrome and ASIA

The onset of SjS following vaccine delivery has been already described several years ago [64]. In this case, a young woman developed arthralgia, fatigue, dry mouth and dry eyes (confirmed by a positive Shirmer’s test) one month after Hepatitis B vaccination. The development of positive Rheumatoid Factor (RF), Anti-nuclear antibodies (ANA) and anti-SSA/Ro antibodies was documented and a lip biopsy confirmed the presence of inflammatory cells infiltrates. Later on, other cases of SjS following vaccine administration have been reported. In 2003, Narvaez et al. described the case of a 59 years-old woman who developed signs and symptoms of pSjS following BCG immunotherapy [65]. In this case, the patient was treated with BCG because of a localized superficial transitional cell carcinoma of the bladder. Several weeks after the first course of treatment she developed a keratoconjunctivitis sicca (confirmed by a positive Shirmer’s test), oral dryness (confirmed by a positive scintigraphy) and painful salivary gland enlargement. Serological exams tested negative for the presence of auto-antibodies, thus a lip biopsy was performed showing the typical histological signs of SjS. Furthermore, the case of a patient with pSjS following H1N1 vaccination has been recently described [66]. This was the case of a 30 years-old Caucasian woman who received one dose of H1N1 vaccination and seven days later developed arthralgia mainly involving shoulders, knees, ankles, wrists and fingers. Three months later, dry mouth and dry eyes (with positive Shirmer’s test) appeared. RF, ANA and anti-SSA/Ro antibodies tested positive and a gland biopsy was compatible with SjS. Interestingly, in this case the genetic background (HLA DRB1*03, *15, DQB1 *02, *06) was in agreement with pSjS development. Not only the association between pSjS and vaccines, but also between silicone exposure and pSjS development has been described in the literature. In 2003, Astudillo et al. reported the case of a 72 year-old retired male dental technician who developed pSjS after silicone exposure. In this patient, oral and ocular dryness (confirmed by positive Shirmer’s test), arthralgia and Raynaud’s phenomenon appeared. The presence of positive ANA and anti-SSA/Ro antibodies and positive gland biopsy confirmed the diagnosis of pSjS [11].

The onset of pSjS following silicone exposure has been also reported in other six cases—in coal-miners and in workers from a factory producing scouring powder. Indeed, in a study of 50 workers exposed to silicone, Sanchez-Roman et al. [67] found 64% of workers affected by autoimmune processes (3 cases with pSjS and 3 cases with secondary SjS). Later on, Puiseux et al. [68] described 3 cases of pSjS in silicotic coal miners, while in 1997, Orriols et al. [69] reported one case of sicca syndrome and silicoproteinosis in a dental technician. Furthermore, one interesting case was reported three years ago by an Italian group. In this patient, a 27 year-old woman who developed lupus nephritis after the administration of hepatitis B vaccine, the HLA antigen expression on lymphocytes and genomic haplotype were studied. The serological haplotyping, which was HLA A2425, B18 (Bw6)−, C−/−, Bw30, DR7−/−, DR11(5)/52, while the genomic haplotype was A*2403/2504, B*1825/1825, C*1207/1207, DRB1*1102/1132, DRB3*0202/0202, DQA1*0505/0505, DQB1*0301/0301, suggested that these HLA alleles were typical both of systemic lupus erythematosus as well as of SjS. Moreover, the patient had a persistent positivity of anti-SSA/Ro which was highly suspicious for the presence of an overlap syndrome. This interesting case strengthens the inking that post-vaccination autoimmune phenomena, i.e. ASIA
syndrome, rather occur in genetically predisposed individuals [70].
To corroborate these evidences, the association between hepatitis B
vaccine and autoimmunity has been recently included as part of the
spectrum of ASIA manifestations [71,72].

5. Sjögren’s syndrome and ASIA: shared pathogenic aspects

Even though SjS pathogenesis has not been fully unveiled,
several aspects have been delineated and animal models have provided
some important insights. Innate immunity has been
called to play a role since the type I IFN pathway is highly expressed
as documented by an increased circulating type I IFN activity and an
IFN ‘signature’ in peripheral blood mononuclear cells and minor
salivary gland biopsies from the patients. Once activated, salivary
gland epithelial cells express TLKs and MHC class I and II molecules,
and they can present autoantigens and produce proinflammatory
cytokines initiating the inflammatory process. The production of B-
cell activating factor (BAFF) by epithelial cells leading, together
with autoantigen presentation on salivary gland epithelial cells,
leads finally to the stimulation of the adaptive immune system [73]
and potential lymphoma [74]. Although T cells were originally
considered to play the initiating role in the autoimmune process
[75], recent evidences suggest that B lymphocytes have a central
role in the development of the disease [34]. The occurrence of an
autoimmune sialadenitis can be triggered by viral infections and is
characterized by the presence of several auto-antibodies including
anti-SSA/Ro and anti-SSB/La. These are present in up to 75% and
40% of patients, respectively. Moreover, ANA and RF are of frequent
observation. The disease is characterized by a marked hyper-
gammaglobulinemia and by frequent observation of monoclonal
igmopathy which seems to be a key marker of disease prognosis
and outcomes [74]. It is a cousin of primary biliary cirrhosis [76].

Mechanisms of apoptosis seem to play a role in glan
dular epithelial cell dysfunction [77] as well as metalloprotei
es which seem to give their contribute in the degradation of extracellular
matrix and in the destruction of the glandular architecture [78].
The existence of a strong autoantibody response and the presence of
germinal center—like structures in the salivary glands of SjS pa
patients imply that the aberrant immune response may be directed
against one or multiple autoantigens. The focal infiltration is
constituted initially of T cells, but then B Cell become predominant
and tend to aggregate in germinal center—like structure. Macro
phages and plasma cells can be recognized as well. It has been
hypothesized that primary events (e.g. infections) may occur in the
glands themselves, followed by an autoimmune attack. Whether B
cell activation is a primary cause or a secondary effect in SjS is not
known [79]. As mentioned above, viruses seem to give a great
contribute to the disease development. In particular, SjS onset
could be linked to EBV aggression and salivary glands can represent
a sites for latent EBV infection [80].

Evidence of a local response to autoantigens is derived from
studies demonstrating local antibody synthesis in salivary glands.
Specifically, the local production of anti—52-kD Ro and an aberrant
expression of SJ/SB in acinar epithelial cells have been demonstrated
[81,82]. Concerning such infiltrates, a Th1-driven cytokinic
response seems to be predominant, albeit Th2 and Th17 responses
have been suggested to participate in the pathogenic process [63].
Recent reports underlined the possible pathogenic role played by B
cells describing also a sort of B cells re—distribution in course of SjS
[83]. Indeed, an abnormal differentiation of B cells has been
described together with a decline in circulating memory B cells and a
subsequent increase in plasma cells and long-lived plasma cells.
Moreover, human salivary gland B-lymphocytes may participate
to glandular damage and seem to be able to induce apoptosis in
epithelial cells [84]. In line with such findings, a role for BAFF in
pSjS has been suggested. BAFF is produced by macrophages, DCs,
epithelial cells and activated T lymphocytes and seems to be a key
molecule in B-cell homeostasis [85]. BAFF is a member of the TNF
family, which is essential for the development and survival of B
lymphocytes; BAFF is elevated in SjS serum in association with
antibodies against Ro/SSA and La/SSB antigens [86]. BAFF may
determine an aberrant B cells maturation leading to self-reactive B
cells and an increased local production of autoantibodies [87].
New insights into interconnected cytokines in primary SjS showed that
the T-cell subsets in SJ is highly related with Th17 cells as well as
with B cell subpopulation [88].

The minor salivary glands epithelium is also involved in the
initiation and perpetuation of the local autoimmune response. Such
epithelial cells seem to be able to mediate the recruitment, homing,
activation, proliferation and differentiation of immune cells [84].
Epithelial cells are further activated by pro-inflammatory cytokines
(IL-18, IFN—γ, and TNFs) which are produced by adjacent T cells.
Dendritic cells (DC) are one of the main source of interferon (IFN)—α
which seems to be a powerful stimulator of the production of BAFF
by the epithelial cells, T cells and DC (Fig. 1). A typical clinical
feature of SjS is the possible evolution into lymphoma, mainly non
-Hodgkin lymphoma; nodal marginal zonal lymphomas and diffuse
large B cell lymphomas (DLBCL) have been also reported, and
mucosa-associated lymphoid tissue lymphomas represent one of
the major causes [89].

At the same extent, it could be of interest that some cases of
breast lymphomas, including non-Hodgkin lymphomas, have been
reported in women who have received silicone breast implants. In
these cases, the most common is the anaplastic large cell lym
phoma. However, B cell lymphoma has been far rarely described
but only in patients with compromised silicone implants [90].
Moreover, the DLBCL has been described also in subjects with other
implants than breast prostheses [91]. These two evidences may
suggest that chronic inflammation or viral infections (as per the
case of DLBCL) may be the shared trigger between ASIA and SjS
[92].

6. Final remarks

It is evident that many other dowels need to be added to
elucidate the pathogenesis of SjS. Even so, it is possible that, beside
infectious diseases, at least some cases may be ascribed to the
presence of an adjuvant as the smoking bullet, resembling the
features of ASIA syndrome. It could be hypothesized that the
adjuvant may elicit an abnormal immune response directed
towards the epithelium of the salivary and lacrimal glands thus
provoking tissue damage. Till now, most of the evidence is anec
todal. Furthermore, the association between vaccinations as well as
exposure to silicone is time-related rather than causal. In this view,
the efforts of researchers should aim at the clarification of the
shared mechanisms possibly underlining the two conditions, ASIA
and SjS. Of particular interest are the common clinical features
overlapping between these conditions. Indeed, as shown in Table 1,
many of the clinical and laboratory aspects that have been
described occurring during ASIA syndrome are also frequently
observed in patients with SjS. The proposed diagnostic criteria for
ASIA include the presence of “typical” clinical manifestations that
often include the presence of dry mouth and/or dry eyes, myalgia
and/or muscle weakness, and arthralgia and/or arthritis. Fatigue is
another key feature that is usually present in both conditions.
Chronic fatigue syndrome (CSF) is a pathological condition charac
terized by invalidating fatigue and a wide range of aspecific
symptoms. One of the etiological hypothesis proposed that EBV or
other infectious agents may be responsible for disease onset [93].
An immune activation with production of cytokines is observed in
the prevalence of signs and symptoms was de-
ated with de-
found that the anti-SSA/Ro and anti-SSB/La antibodies are associ-
Ro and anti-SSB/La, has been recently addressed[98]. The authors
proposed [94]. CSF has been associated with cases of L-triptophane-
infectious agent, a role for certain drugs and for silicone has been
this condition. Besides a specific autoimmune response against an
fectant, a role for certain drugs and for silicone has been
proposed [94], CSF has been associated with cases of L-triptophane-
duced eosinophilia and with silicone-induced human adjuvant
disease, that may be considered an
induced eosinophilia and with silicone-induced human adjuvant
in the development of ASIA and
moderately in the development of ASIA and

c autoantibodies, encompassing anti-SSA/
Ro and anti-SSB/La, has been recently addressed [98]. The authors
found that the anti-SSA/Ro and anti-SSB/La antibodies are associ-
c with definite gene variants and haplotypes including HLA-
DQB1*0201, DQB1*0202, DQB1*0301, DRB1*03, DPB1*0101, DR3,
B8, DQ2, DQ3, BANK1, LYN, TAP2 IL-10, TNFa, TREX1, DNase I, PKX
and STAT4 for anti-SSA/Ro and HLA-DQB1*0301, DQB1*0201,
DPB1*0101, DR3 DR2, DQ2, IL-10, TNFa, IRF7/KIAA1542, PKX,
DNMT1, FAS for anti-SSB/La. Some of these genes have been further
linked with a predisposition to autoimmunity. Thus, it could be of
interest to assess any association with adjuvant-triggered autoim-
immunity also in patients with SJ. Of interest is the fact that SJ usually
occurs in adults and can present with a late onset (after 65
years of age). Many patients have associated clinical issues of low
vitamin D levels, neuropathies and an interesting elevation of sera
IgG4 [99–101]. It is evident that in such cases the assessment of any,
eventual, time-relationship with vaccines could be difficult or
impossible. On the other hand, it is well known that post-vaccine
clinical manifestations can occur after several years. This is the
reason why even those anecdotal cases in which specific autoan-
tibodies, namely anti-SSA/Ro and anti-SSB/La, appear after vacci-
nations in the absence of a well-defined AD, are of great
importance. Indeed, the fact that specific autoantibodies arise even
years before diseases diagnosis has been extensively addressed in
a number of previous studies [102].

An intriguing premise is the observation that several vaccines may contain Saccharomyces cerevisiae residual traces as the result of
vaccine production. Saccharomyces cerevisiae is a possible trigger
for autoimmunity as it was demonstrated in animal models and by
the presence of anti-Saccharomyces cerevisiae in several autoim-
mune diseases [103,104]. Moreover, in a recent paper, homologies
between the structure of the Saccharomyces cerevisiae wall
(mannan) and the antigens SSA/Ro and SSB/La have been identified
with a percentage of possible relationship ranging between 46%
and 69% [105]. Thus, the administration of vaccines containing
Saccharomyces cerevisiae should be carefully evaluated in patients
with SJ since there is the possibility that such vaccines may elicit
the production of autoantibodies in these patients. Moreover, the
question whether vaccines may cause an autoimmune disease in
otherwise healthy subjects is still unanswered. The conclusions
advise that vaccines benefits largely overwhelm the risks [106]. Nonetheless, the possible association between vaccination and
autoimmune diseases, including SJ, is a matter of which physicians
should be aware of.

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implantation-induced scleroderma: description of four patients and a critical

Table 1
Prevalence of typical signs and symptoms in the Autoimmune/inflammatory syn-
drome induced by adjuvants (ASH) and in Sjögren’s Syndrome (SJ)4

<table>
<thead>
<tr>
<th>Signs and symptoms</th>
<th>ASH (post-vaccination phenomena) b</th>
<th>SJ a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connective tissue diseases specific</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arthralgia/Arthritis</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Myalgia/myopathy/muscle weakness</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Raynaud’s phenomenon</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Leukopenia</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Xerophthalmia</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Xerostomia</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Serositis</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Neurological/cognitive impairment</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Aspecific Fever</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Chronic fatigue/sleep disturbances</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Asthenia</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Gastro-intestinal</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>ANA</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Anti-SSA</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Anti-SSB</td>
<td>±</td>
<td></td>
</tr>
<tr>
<td>Rheumatoid factor</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Exposure to an external stimuli</td>
<td>&gt;99.9% d (Infection, vaccine, silicone, adjuvant)</td>
<td></td>
</tr>
<tr>
<td>Linkage with HLA</td>
<td>+ (HLA-DRw52 and HLA-DR3)</td>
<td></td>
</tr>
</tbody>
</table>

The prevalence of signs and symptoms was defined as (±) case reports or <1%, (++) if reported in <30% of subjects, (+) in 30–60% and (+++) if present in more than
60% of subjects.

a The highest prevalence observed in the literature of each sign and symptom is reported.
b Modified from Ref. [68].
c Modified from Ref. [107].
d Unknown.

1

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EXHIBIT 195
Autoimmune/Inflammatory Syndrome Induced by Adjuvants and Sjögren's Syndrome

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PMID: 27228631

Free article

Abstract

Sjögren's syndrome (SS), a chronic systemic autoimmune inflammatory condition involving the exocrine glands, has been suggested to be part of the spectrum of the Autoimmune/Inflammatory Syndrome Induced by Adjuvants (ASIA). ASIA incorporates an umbrella of clinical conditions including siliconosis, macrophage myofasciitis syndrome, and post-vaccination phenomena that occur after the exposure to a substance, namely the adjuvant. Interestingly, SS and ASIA share several common features. Firstly, a shared pathogenic mechanism involving a disruption of the immune system balance, with B cell proliferation, cytokine production and tissue infiltration, has been proposed. Patients with ASIA often present clinical features resembling those of SS; dry mouth and dry eyes have also been included in the proposed classification criteria for ASIA. Finally, several case reports have suggested that both vaccines and silicone may trigger the development of SS. Unveiling these common pathways will contribute considerably to our understanding and management of both conditions.
Increasing incidence of celiac disease in a North American population

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Abstract

OBJECTIVES—The prevalence of celiac disease (CD) varies greatly, potentially because of incomplete ascertainment of cases and small study samples with limited statistical power. Previous reports indicate that the incidence of CD is increasing. We examined the prevalence of CD in a well-defined US county.

METHODS—Population-based study in Olmsted County, Minnesota, US. Using the infrastructure of the Rochester Epidemiology Project, medical, histopathology, and CD serology records were used to identify all new cases of CD in Olmsted County since 2000. Age- and sex-specific and adjusted (to the US white 2000 population) incidence rates for CD were estimated. Clinical presentation at diagnosis was also assessed.

RESULTS—Between 2000 and 2010, 249 individuals (157 female or 63%, median age 37.9 years) were diagnosed with CD in Olmsted County. The overall age- and sex-adjusted incidence of CD in the study period was 17.4 (95% confidence interval [CI] = 15.2–19.6) per 100,000 person-years, increasing from 11.1 (95% CI=6.8–15.5) in 2000–2001 to 17.3 (95% CI=13.3–21.3)
in 2008–2010. The temporal trend in incidence rates was modeled as a two-slope pattern, with the incidence leveling off after 2004. Based on the two classic CD symptoms of diarrhea and weight loss, the relative frequency of classical CD among incident cases decreased over time between 2000 and 2010 (p=0.044).

CONCLUSION—The incidence of CD has continued to increase in the past decade in a North American population.

Keywords
autoimmunity; celiac; coeliac; epidemiology; prevalence

INTRODUCTION
Celiac disease (CD) is a chronic immune-mediated disease characterized by small intestinal inflammation.(1) Triggered by gluten exposure in genetically sensitive individuals,(2) this disease is associated with excess mortality as well as a number of complications, including type 1 diabetes(3) and lymphoproliferative disease.(4, 5) These and other high-risk groups, such as anemia, osteoporosis, and irritable bowel syndrome (IBS), have been identified(6) and patients in these risk groups are increasingly tested for CD. Even so, the prevalence of diagnosed CD is low. In a recent European multicenter study the prevalence of diagnosed CD was 0.18%; however, if Finland is excluded, where awareness is especially high, only 0.07% of the population had been diagnosed with CD.(7) Most previous American data indicate a true overall CD prevalence based on screening of about or slightly less than 1%. (8–12)

Prevalence studies based exclusively on serologic CD screening often report higher figures. (6) However, real differences may also exist between regions(7) and races, as higher levels of CD have also been shown outside Europe and the US among populations of predominantly European extraction (e.g., New Zealand and Australia).(13, 14)

Some prevalence data imply a true increase in CD or celiac autoimmunity over time,(15–17) but data are scarce on the actual incidence of CD.(18, 19) Such data are important in that temporal trends in CD incidence could provide insight into the environmental factors that trigger CD, as well as serve in planning for the health care needs of CD patients. For instance, the “Swedish epidemic,” with a sudden increase in CD incidence,(20) helped identify the interplay between gluten introduction and breastfeeding as important for the risk of CD in early age.(21) In this regard, we previously documented a strong increase in CD incidence in one US county in the 1990s(18) but it is unknown whether this increase was transient. The primary aim of this study was to estimate the incidence of CD in Olmsted County, Minnesota between 2000 and 2010.

METHODS
Setting
Olmsted County is situated in the upper Midwest, where whites make up over 85% of the population.(22) This community is ideally suited for epidemiologic studies because the Rochester Epidemiologic Project (REP) collects essentially all data on medical care for Olmsted County residents, focusing on the two major providers of health care in the community: the Mayo Clinic and its component hospitals (Saint Mary’s and Rochester Methodist) along with the Olmsted Medical Center with its affiliated community hospital and outpatient clinics. This medical records linkage system, which has been described elsewhere,(23) was used in our previous paper on CD trends in the area.(18) Using the
infrastructure of the REP, comprehensive (inpatient and outpatient) community medical records, together with CD serology and histopathology data, were reviewed to estimate the incidence of diagnosed CD in this well-defined geographic region. Because of this unique medical records linkage system, complete ascertainment of diagnosed CD cases is possible.

Several data sources were used to identify patients with CD: the REP medical index, as was used in previous studies(9, 18, 24), active recruitment of patients known to be undergoing assessment and potential diagnosis of the disease at the time of their clinical visit, and the electronic medical record (the Mayo Clinic Life Sciences System, begun in 1994), which also contains information on disease symptoms, histopathology records, and serology data. We also reviewed the patient charts of all individuals with a diagnosis of either CD (ICD-9: 579.0) or DH (ICD-9: 694.0).

All of the above searches included a check for Olmsted County residency based on zip codes. Because the county boundary passes through some small towns, the patient’s street address was reviewed in some instances to determine if he or she lived within Olmsted County.

Data collection

Following approval by the Institutional Review Boards of Mayo Clinic and the Olmsted Medical Center, one co-author (CvD) extracted data on CD from the clinical records and then classified patients according to age, sex, classical symptoms (presence of diarrhea or weight loss)(25), and year of diagnosis. In accordance with Minnesota law, patients who declined to provide authorization for the use of their medical records for research purposes were not included in this study (or any other medical study).

Statistical analyses

Incidence rates of clinically-diagnosed CD were estimated using the number of new cases in each age-, sex-, and calendar year-specific stratum as the numerator, with corresponding denominators derived from annual census figures for Olmsted County, assuming the entire population was at risk(22). Age- and sex-adjusted incidence rates were computed based on direct standardization against the 2000 US white population. We estimated 95% confidence intervals (CIs) assuming the Poisson distribution for numbers of cases. Multiple variable Poisson regression was used to examine the association of age, sex, and calendar year with incidence rates. Measured per individual year, both age and calendar year were fit as continuous variables in the modeling process, with smoothing loess plots applied to assess their functional form. For brevity, we tabulated incidence rates in Table 2 according to age categories (0–3, 4–18, 19–44, 45–64, and ≥65 years) and periods of 3-year intervals (except for the first two years, 2000–01, which overlap with our previously published incidence study).(18) A logistic regression model (using a generalized logit link function) was employed to assess the association of calendar year on the proportion of incidence cases presenting with classical symptoms (diarrhea with or without weight loss(25)) over the study period, adjusted for age and sex. The different models used were selected based on simple plots of the data in order to reflect the observed patterns in the data.

All analyses were performed using SAS/STAT software, version 9.2 (SAS Institute, Cary, NC). A two-sided alpha level of 0.05 was used to determine statistical significance.
RESULTS

Study subjects

From 2000 to 2010, 249 individuals were diagnosed with CD in Olmsted County. Some 22 of these had been identified through our family screening study in 2001–05. Of the 249, 157 (63%) were females. The median age at diagnosis was 37.9 (range, 1.2–84.9 years). Concerning presenting symptoms, 79 (32%) patients had diarrhea and 25 (10%) had weight loss, although only 14 (6%) had both diarrhea and weight loss at CD diagnosis and were therefore classified as having “classical” CD.

Of the 248 individuals with more extensive data available on symptoms, 99 (40%) had abdominal pain/cramping, 69 (28%) had anemia, 41 (17%) suffered from malaise, and 36 reported nausea/vomiting (15%). A positive family history for CD was seen in 72 individuals (29%). Of individuals tested for either tissue transglutaminase (TTG) or endomysial antibodies (EMA), 89% (166/187) were positive for at least one of these antibodies. Supplementary Table 1 shows the prevalence of CD in Olmsted county on Jan 1, 2010.

Temporal trends in CD incidence

The overall age- and sex-adjusted incidence of clinically diagnosed CD over the study period was 17.4 (95% CI = 15.2–19.6) per 100,000 person-years (p-y), increasing from 11.1 (95% CI = 6.8–15.5) per 100,000 p-y in 2000–2001 to 17.3 (95% CI = 13.3–21.3) per 100,000 p-y in 2008–2010 (Table 1 and Figure 1). Various options for the functional form of the relationship with calendar year were considered based on Figure 2. In particular, a loess smooth of incidence rate was plotted against calendar year to explore the linearity of their relationship. Because the incidence of CD appeared to increase in the first several years and then flatten after that (Figure 2, thin solid line), a model that assumed a linear increase from years 2000 to 2004 and a leveling off thereafter (Figure 2, thick dashed line) was eventually adopted. Based on a comparison of log-likelihood statistics, the model with this two-slope time trend provided a significantly better fit of the data than a model with a linear (one-slope) time effect (p=0.021).

In the two-slope model a higher incidence of CD was associated with female sex (p<0.001) and increasing age (p=0.034). Over the entire period, the age-adjusted incidence of CD in females and males was 21.3 (95% CI = 18.0–24.7) and 13.6 (95% CI = 10.8–16.5) per 100,000 p-y, respectively (p<0.001, adjusted for age and calendar year effects) (Table 1). With the possible exception of the last period (2008–2010), in which incidence rates were relatively high in individuals aged 4–18 years (Table 1 and Figure 3), the incidence of CD tended to be highest in the older age groups (45–64 years and 65–85 years). Controlling for sex and calendar year effects, the CD incidence rate increased about 6.5% (95% CI = 0.5%–12.8%) per 10-year increment in age (p=0.034).

CD incidence according to patient characteristics

Neither the percentage nor the incidence of CD characterized by diarrhea without weight loss increased significantly over the study period (p=0.557 and 0.136, respectively, adjusted for age and sex) (Table 2). In contrast, and as illustrated in Figure 4, the proportion of cases presenting with both diarrhea and weight loss decreased significantly over time (15% in 2000–2001 down to 3% in 2008–2010, p =0.044), although this did not result in a significant drop in the incidence of classical CD over the study period (p=0.168).
DISCUSSION

This study is potentially the most complete assessment of the recent incidence of CD in a North American population. Between 2000 and 2010, some 249 individuals were diagnosed with CD in Olmsted County. The corresponding overall incidence was 17.4 per 100,000 p-y, which, to our knowledge, is one of the highest figures reported anywhere in a carefully enumerated population that includes both adults and children. Adding to this our recent National Health and Nutrition Examination Survey (NHANES) data showing an overall prevalence of CD of 0.71% in the US, it is evident that CD is swiftly becoming a major health problem in this country. Although earlier efforts have been made to identify CD through screening of risk groups and certain age strata, as well as through screening in a primary care setting or in connection with upper endoscopy, few studies have used an integrative approach to determine the incidence of CD.

We found that the previously described increase in CD incidence (0.9 per 100,000 p-y in the 1960s, rising to 3.3 per 100,000 p-y in the 1990s) has not abated. As documented in the present study, the incidence of CD has continued to rise since then but seems to have leveled off at about 20 per 100,000 p-y after 2004. Although the high (and increasing) incidence may partly be explained by high physician awareness and large-scale efforts to screen at-risk groups (increasing detection rate), we believe that other factors play a substantial role since the rising incidence in the 1990s, when serological screening tools (e.g., endomysial and tissue transglutaminase antibodies) became readily available, was not followed by a drop in incidence after 2000, as would be expected if the Olmsted County population had merely been swept of unrecognized prevalent CD cases. Still we cannot rule out that external factors influenced case-finding. For instance it is possible that the publication of the first major US study of celiac seroprevalence in 2003 led to heightened awareness of CD and contributed to the rise in CD incidence seen in 2004–2006.

Several studies have shown changing incidence in CD. Considering the predominance of a Caucasian population in Olmsted County now, as in the 1950s, the rise of CD cannot be explained by a change in the underlying genetic makeup of the community. Instead, an environmental factor(s) is likely. Because we and others have found an association between infectious disease (especially gastroenteritis) and CD, a changing pattern in infections may have contributed to the rise in CD in Olmsted County. Another explanation concerns amount, timing, and frequency of gluten consumption. For instance, Ivarsson et al reported that high amounts of gluten increased the risk of CD. Unfortunately, we have no data on gluten consumption in individuals from Olmsted County, although gluten enriched foods (e.g., pizza, bagels, and high protein and high fiber bread) are increasingly ingested in the US.

In contrast to Swedish data showing very high incidence rates in children <2 years of age between 1985 and 1995 (about 200 per 100,000 p-y), we found a low incidence of CD in young US children. Interestingly, however, high incidence figures in the most recent period (2008–2010) were noted in the age groups born in the years of the Swedish epidemic (late 1980s and early 1990s). Therefore, we cannot rule out that the same environmental factors have been present both in Sweden and in the US.

The majority of newly diagnosed CD patients were females. This sex pattern is consistent with previous data. The female predominance is noteworthy given that population-based screening in our area has previously found a more neutral female-male ratio. Some data suggest that sex affects the clinical presentation of CD with women more often diagnosed with CD, whereas men remain undiagnosed. During their fertile years, women are also more likely to encounter health care and incidentally be diagnosed with CD.
Previous data from our center(18) and elsewhere(38) have otherwise established that classical CD (diarrhea and weight loss) has become less prominent. The current study was able to confirm that the proportion of newly diagnosed patients with both diarrhea and weight loss decreased from 2000 to 2010 but the absolute incidence of diarrhea-associated CD did not decrease. This observation suggests that the lower proportion of diarrhea in CD patients is probably due to testing of new risk groups,(39) such as patients with other immune-mediated diseases(40, 41) or osteoporosis,(42) first-degree relatives,(26) or a more extensive use of CD serology to identify early onset patients before classical symptoms can develop. Yet, the persistence of a steady rate of more classic disease may suggest that the true incidence of CD may be increasing.

The main strength of this paper is its population-based design. The well-established collaboration between the major health care providers in the area should virtually guarantee that we have identified all cases with diagnosed CD. Moreover, we had access not only to clinical registers but also to serology and histopathology data to ascertain patients. Finally, the large number of diagnosed cases means that we could better estimate the incidence rates (i.e. calculate narrow CIs). This study has some limitations that need to be considered when interpreting the results. The predominant population of Olmsted County consists of non-Hispanic whites and thus the number of Hispanics, Blacks and Asians are limited. We cannot therefore extrapolate our findings to these latter populations. CD has a marked predominance among non-Hispanic whites and may be less common among other minority groups.(11) We were also unable to determine the relative incidence of both diagnosed and undiagnosed CD since this was not a screening study. Finally, Olmsted county is dominated by the Mayo Clinic, which is also the main provider of health care for the county inhabitants. There is a strong interest in CD at the Mayo clinic, and it cannot be ruled out that this has influenced the diagnostic rate of CD in the Olmsted county.

In conclusion, the increase in incidence of CD between 1950 and 2001 has continued in the past decade but probably has leveled since 2004. The high CD incidence (17 per 100,000 p-y in the past decade) points towards a change in environmental exposures, potentially responsible for triggering CD not only in children but also, and particularly, in adults.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

**Acknowledgments**

Grant Support (Funding):

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The work at Mayo Clinic was supported by a grant from the National Institutes of Health – DK057892 and made possible by the Rochester Epidemiology Project (R01 AG034676 from the National Institute on Aging). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

**Abbreviations**

<table>
<thead>
<tr>
<th>CD</th>
<th>Celiac disease</th>
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<tr>
<td>CI</td>
<td>Confidence interval</td>
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</table>
HR  Hazard ratio
VA  Villous atrophy

REFERENCES


STUDY HIGHLIGHTS

WHAT IS THE CURRENT KNOWLEDGE

- The prevalence of celiac disease varies greatly
- Previous reports indicate that the incidence of celiac disease is increasing
- There is a paucity of research on CD incidence in the past decade

WHAT IS NEW HERE

- The incidence of celiac disease has continued to increase in the past decade in a North American population, but seems to have levelled off after 2004
- The absolute frequency of classical celiac disease did not change
- However, the relative frequency of classical celiac disease among incident cases has decreased
Figure 1.
Rates of CD Incidence in Olmsted County, MN from 2000 to 2010 (Loess Smooth).
Figure 2.
Modeling the Temporal Trend in CD Incidence between 2000 and 2010
Modeled incidence of CD, assuming a linear increase from 2000 to 2004 and constant thereafter, is shown in comparison with a loess smooth of the observed incidence (with shaded 95% confidence limits).
Figure 3.
Age Trends in CD Incidence by Calendar Year (Loess Smooth).

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Figure 4.
Temporal Trend in the Proportion of Classical Symptoms in Incidence CD Cases (Regression Lines).
Table 1

Incidence of celiac disease in Olmsted County, MN according to age and sex (2000–2010).

<table>
<thead>
<tr>
<th>Age group</th>
<th>Period</th>
<th>Females</th>
<th></th>
<th></th>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th></th>
<th>All ages</th>
<th></th>
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<tr>
<td></td>
<td>(n=26)</td>
<td>(n=67)</td>
<td>(n=83)</td>
<td>(n=73)</td>
<td>(n=26)</td>
<td>(n=67)</td>
<td>(n=83)</td>
<td>(n=73)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>0–3</td>
<td>0 (0.0)</td>
<td>1 (9.1)</td>
<td>1 (8.6)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>2 (16.8)</td>
<td>0 (0.0)</td>
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<tr>
<td>4–18</td>
<td>2 (7.3)</td>
<td>7 (16.2)</td>
<td>9 (19.9)</td>
<td>15 (31.8)</td>
<td>3 (10.3)</td>
<td>7 (15.3)</td>
<td>4 (8.3)</td>
<td>9 (18.0)</td>
<td></td>
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</tr>
<tr>
<td>19–44</td>
<td>5 (10.1)</td>
<td>17 (21.9)</td>
<td>28 (34.5)</td>
<td>21 (24.8)</td>
<td>2 (4.2)</td>
<td>11 (14.6)</td>
<td>6 (7.6)</td>
<td>7 (8.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>45–64</td>
<td>8 (29.0)</td>
<td>9 (20.8)</td>
<td>12 (26.5)</td>
<td>4 (8.5)</td>
<td>2 (7.7)</td>
<td>7 (17.2)</td>
<td>9 (21.1)</td>
<td>8 (18.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65–85</td>
<td>2 (15.0)</td>
<td>7 (33.5)</td>
<td>5 (22.8)</td>
<td>4 (17.5)</td>
<td>2 (19.7)</td>
<td>1 (6.3)</td>
<td>7 (41.9)</td>
<td>5 (28.7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All ages</td>
<td>14.2 (7.4–21.0)</td>
<td>21.4 (14.8–28.0)</td>
<td>26.8 (19.7–33.9)</td>
<td>20.1 (14.1–26.1)</td>
<td>8.1 (2.6–13.5)</td>
<td>13.5 (8.3–18.8)</td>
<td>15.9 (9.9–22.0)</td>
<td>14.9 (9.3–20.5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Age-specific results summarized with count and incidence rates (per 100,000 person-years, unadjusted), while bolded across-all-age results are described with the age-adjusted (and sex-adjusted for “Total” section) incidence rates and 95% confidence intervals.

* Rate = incidence rate per 100,000 person-years adjusted to the US white population in 2000.
## Table 2
Temporal trends of celiac disease in patients with diarrhea or weight loss (classical symptoms)

<table>
<thead>
<tr>
<th>CD Symptoms</th>
<th>2000±01 (n=26)</th>
<th>2002±04 (n=67)</th>
<th>2005±07 (n=83)</th>
<th>2008±10 (n=73)</th>
<th>Temporal trend p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea WITHOUT Weight Loss</td>
<td>Proportion of Cases with Symptom</td>
<td>Total</td>
<td>4 (15%)</td>
<td>22 (33%)</td>
<td>16 (19%)</td>
</tr>
<tr>
<td></td>
<td>Incidence of Symptom-Related CD</td>
<td>Total</td>
<td>1.6 (0.0–3.8)</td>
<td>6.6 (3.0–10.1)</td>
<td>4.4 (2.2–6.5)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>0.8 (0.0–2.3)</td>
<td>4.6 (1.6–7.7)</td>
<td>4.9 (1.4–8.5)</td>
<td>4.8 (1.6–8.1)</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>2.2 (0.0–5.3)</td>
<td>2.0 (0.0–2.2)</td>
<td>0.9 (0.0–2.2)</td>
<td>0.5 (0.0–1.4)</td>
</tr>
<tr>
<td>Diarrhea WITH Weight Loss</td>
<td>Proportion of Cases with Symptom</td>
<td>Total</td>
<td>4 (15%)</td>
<td>4 (6%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td></td>
<td>Incidence of Symptom-Related CD</td>
<td>Total</td>
<td>1.9 (0.0–3.8)</td>
<td>1.0 (0.0–1.9)</td>
<td>1.2 (0.0–2.3)</td>
</tr>
<tr>
<td></td>
<td>Females</td>
<td>1.7 (0.0–4.2)</td>
<td>1.0 (0.0–2.4)</td>
<td>1.6 (0.0–3.3)</td>
<td>0.6 (0.0–1.7)</td>
</tr>
<tr>
<td></td>
<td>Males</td>
<td>2.2 (0.0–5.3)</td>
<td>0.9 (0.0–2.2)</td>
<td>0.8 (0.0–2.3)</td>
<td>0.5 (0.0–1.4)</td>
</tr>
</tbody>
</table>

†Frequency of classical symptoms in CD cases summarized with counts and percentages and tested for a temporal trend using a generalized log model after adjusting for age and gender.

‡Incidence described with age-adjusted and sex-adjusted for bolded “Total” respectively. Incidence rates and 95% confidence intervals (per 100,000 person-years adjusted to the US white population in 2000), with rates tested for a temporal trend via Poisson regression after adjusting for age and gender.

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Background & Aims: Celiac disease is considered rare in North America. However, an increasing incidence and widening clinical spectrum have been reported in many countries, and serologic screening suggests a higher prevalence of minimally symptomatic disease. This study reports temporal trends in the incidence of celiac disease in Olmsted County, Minnesota. Methods: All county residents diagnosed with celiac disease between 1950 and 2001 were identified through the Rochester Epidemiology Project. Incidence rates were calculated assuming a Poisson distribution, and changes in incidence by calendar year, age, and gender were assessed by using Poisson regression. Results: Altogether, 82 new cases of celiac disease were identified during the 50-year period. There was a marked female predominance (P < 0.005), and the incidence rates increased with age (P < 0.001) and calendar period (P < 0.001). The overall annual incidence of celiac disease was 2.1 per 100,000 (95% confidence interval [CI], 1.7–2.6) but increased from 0.9 per 100,000 (CI, 0.5–1.2) in 1950–1989 to 3.3 per 100,000 (95% CI, 2.2–4.4) in the 1990s. The incidence was 9.1 per 100,000 (95% CI, 5.2–13.0) in the final 2 years of the study. Serology prompted biopsy in a substantial proportion of recent diagnoses. Clinical features also changed over time, with less diarrhea and weight loss at presentation. Conclusions: Celiac disease has increased recently in this well-characterized population. Milder clinical features and use of serology suggest an increased detection rate, although a true increase in incidence may have also occurred. Celiac disease is not rare in North America.

Celiac disease is a chronic inflammatory disorder of the small intestine resulting from the ingestion of gluten and related substances found in cereal grains such as wheat, barley, and rye.1,2 The prevalence of diagnosed celiac disease varies widely from 1:5000 to 1:200, with the lowest of these estimates reported from the United States.3,4 Considered a truly rare disease in this country, celiac disease is rarely considered by clinicians,5 despite the fact that the condition predominantly affects whites, who comprise more than 190 million residents of the United States. The long delays in diagnosis, the lack of understanding of the disease process, and the low research activity regarding celiac disease are largely a result of the perceived rarity of the disorder.6 This apparent rarity has been used as a justification for not routinely including celiac disease in the investigation of patients presenting with symptoms compatible with irritable bowel syndrome.7 A crucial basis for this assumption has been the single study in Olmsted County, Minnesota, which provided the only true population-based incidence and prevalence rates in North America.4 Since that report, however, there has been much literature describing atypical forms of celiac disease, and serologic tools have made diagnostic testing more accessible.8–10 Many factors seem to affect the rate of diagnosis including severity of the disease, availability of diagnostic facilities, awareness of the diagnosis, and testing strategies such as active case finding or screening.6,11,12 Because these advances may have led to an increased detection of celiac disease, we updated the earlier study to evaluate time trends in the incidence of celiac disease in this North American community during a 51-year period. The main aim of this study was to evaluate time trends in the identification and incidence in the past 50 years.

Methods

Study Setting

Olmsted County, Minnesota, is a medically well-defined population in the upper Midwest. The population is
largely white of Northern European extraction. Until recently, the population was almost entirely composed of whites of European extraction. In the last decade, there has been a recent influx of first Southeast Asian and then African ethnic groups so that the nonwhite group is about 10%. Population-based research is feasible in Olmsted County because medical care is virtually self-contained within the community and there are relatively few providers. The major provider is the Mayo Clinic, which has maintained a common medical record with its 2 affiliated hospitals, St. Mary’s and Rochester Methodist, for more than 90 years. Recorded diagnoses and surgical procedures are indexed, including the diagnoses made for outpatients seen in clinic consultations, emergency department visits, or nursing home care, as well as the diagnoses recorded for hospital inpatients, at autopsy examination, or on death certificates. Medical records of the other providers who serve the local population, most notably the Olmsted Medical Group and its affiliated Olmsted Community Hospital (Olmsted Medical Center), are also indexed and retrievable. Thus, details of the medical care provided to local residents are available for study through this medical records linkage system (the Rochester Epidemiology Project) as described elsewhere. This population has been extensively studied for the purposes of reporting the epidemiology of both common and rare diseases.

**Case Identification**

After approval by the institutional review boards of both the Mayo Clinic and the Olmsted Medical Center, we used the Rochester Epidemiology Project to identify all Olmsted County residents with a potential diagnosis of celiac disease or dermatitis herpetiformis who were evaluated between January 1, 1950, and December 31, 2001. In addition, the membership rolls of the local celiac disease support group and the records of the Mayo Department of Pathology and Laboratory Medicine were scrutinized. The complete inpatient and outpatient medical records of each candidate case were reviewed, as were the biopsy slides of the small intestinal samples taken at the time of the original diagnosis. The following information was abstracted from the records: (1) clinical features at or preceding diagnosis such as diarrhea, weight loss, body mass index, steatorrhea, iron deficiency anemia, neuromuscular symptoms, bone disease, and dermatitis herpetiformis; (2) clinical or histologic improvement after gluten-free diet; (3) antiendomysial, antigliadin, and antigliadin antibody status, if performed; (4) comorbid conditions and family history; and (5) cause of death if deceased. To be defined as celiac disease, each case was required to have (1) jejunal or duodenal biopsies with partial or complete villous atrophy associated with crypt hyperplasia and a lymphoplasmacytic infiltration in the lamina propria, and (2) clinical or histologic improvement with a gluten-free diet. Dermatitis herpetiformis was defined by characteristic pruritic rash, pathology (including immunofluorescence after 1980), and a response to dapsone. Medical records were not reviewed for the single patient who declined to provide an authorization for the use of his or her records in research as required by state law.

**Statistical Analysis**

To calculate incidence and prevalence rates, the entire population of Olmsted County was considered to be at risk; the denominator age- and gender-specific person-years were derived from decennial census figures. To be considered an incidence case, the patient must have been residing in Olmsted County at the time of initial diagnosis. All Olmsted County residents with a history of celiac disease on January 1, 2001, were included among the prevalence cases, regardless of the place of diagnosis. Rates were directly age- or age- and gender-adjusted by using the population structure of US whites in 2000 as the standard population because this is what the Olmsted population resembled until very recently and all identified patients were white. Ninety-five percent confidence intervals (95% CI) for the rates were estimated, assuming that the incidence cases followed a Poisson distribution.


The associations of presenting symptoms at diagnosis with age at diagnosis, gender, and calendar year of diagnosis were assessed by using logistic regression models. In these analyses (with the SAS procedure LOGISTIC) the (binary) dependent variable was presence versus absence of each presenting symptom at diagnosis, and the patient-specific age, gender, and year of diagnosis were included as the independent (predictor) variables. Potential differences in the associations (e.g., males over time versus females over time) were examined by incorporating specific interaction terms as predictors.

The association of body mass index (BMI) with age at diagnosis, gender, and year of diagnosis was assessed by using a multiple linear regression model (SAS procedure REG) with BMI as the dependent variable. An extension of the Fisher exact test (exact-Wilcoxon test) was used to assess the association between time period (pre-1990 vs. post-1990) and changes in the biopsies (ordered categories).

**Results**

Eighty-two Olmsted County residents were diagnosed with biopsy-proven celiac disease between 1950 and 2001. There were substantially more females than males (58 vs. 24; P < 0.005). All patients were white of European extraction. There was a marked adult predominance (median age at diagnosis, 46
years; range, 1–84 years) (Figure 1). Less than 15% (12 of 82) of the patients were 18 years of age or younger at the time of diagnosis (Table 1). During the same period, a total of 14 additional cases were classified as having possible celiac disease, on the basis of either history of a beneficial response to the gluten-free diet without confirmation by intestinal biopsy or biopsies consistent with celiac disease without an alternate diagnosis but not treatment with a gluten-free diet. The frequency of this type of patient did not change over time, and their clinical features were not different from the definite cases. The remainder of the analysis is restricted to the biopsy-proven cases.

**Temporal Trends in Incidence**

The overall age- and gender-adjusted incidence of celiac disease was 2.1 per 100,000 person-years (95% CI, 1.7–2.6). Age-adjusted annual incidence rates were greater in women than in men (2.8 per 100,000; 95% CI, 2.1–3.6 vs. 1.4 per 100,000; 95% CI, 0.8–2.0; \( P < 0.005 \)) for a female to male ratio of 2:1. Rates increased with age in both genders as delineated in Table 1 and illustrated in Figure 1. As shown in Figure 2, there was a dramatic increase in incidence after 1989. Between 1950 and 1989, the annual age- and gender-adjusted incidence rate was 0.9 per 100,000 (95% CI, 0.5–1.2), whereas the rate rose to 3.3 per 100,000 (95% CI, 2.2–4.4) in the 1990s and, in the last 2 years of the study, reached a level of 9.1 per 100,000 (95% CI, 5.2–13.0). The increase was seen for men as well as women and was evident in all age groups, except infants of 3 years of age or younger (Figures 3 and 4, Table 1). Women older than 44 years of age continued to make up the majority of new cases, although this trend did not reach significance.

**Clinical Features**

All subjects were identified after presentation to their physicians with symptoms. In most of these pa-

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**Table 1. Incidence of Celiac Disease in Olmsted County, Minnesota, 1950–2001 by Age Group, Gender, and Time Period**

<table>
<thead>
<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>n</td>
<td>Rate *</td>
<td>n</td>
<td>Rate *</td>
<td>n</td>
<td>Rate *</td>
<td>n</td>
</tr>
<tr>
<td>0–3 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>6</td>
<td>1.8 (0.3, 3.3)</td>
<td>6</td>
<td>1.4 (0.2, 2.5)</td>
<td>5</td>
</tr>
<tr>
<td>4–18 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
<td>0.6 (0.0, 1.8)</td>
<td>2</td>
</tr>
<tr>
<td>19–44 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
<td>0.6 (0.0, 1.8)</td>
<td>1</td>
<td>1.5 (0.5, 3.7)</td>
<td>2</td>
</tr>
<tr>
<td>45–64 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
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<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
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<tr>
<td>≥65 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
</tr>
<tr>
<td>All ages</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>6</td>
<td>1.8 (0.3, 3.3)</td>
<td>6</td>
<td>1.4 (0.2, 2.5)</td>
<td>5</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Males</th>
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<tr>
<td>0–3 yr</td>
<td>1</td>
<td>3.4 (0.2, 7.9)</td>
<td>1</td>
<td>0.4 (0.0, 1.2)</td>
<td>3</td>
<td>1.1 (0.0, 2.4)</td>
<td>4</td>
<td>1.0 (0.0, 2.2)</td>
<td>9</td>
<td>1.8 (0.6, 3.1)</td>
<td>6</td>
<td>5.8 (1.0, 10.6)</td>
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<tr>
<td>4–18 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
<td>2.1 (1.0, 3.2)</td>
<td>2</td>
<td>1.5 (1.0, 2.0)</td>
<td>4</td>
<td>2.5 (1.8, 3.4)</td>
</tr>
<tr>
<td>19–44 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
<td>0.6 (0.0, 1.8)</td>
<td>1</td>
<td>1.5 (1.0, 2.0)</td>
<td>4</td>
<td>3.7 (2.6, 5.0)</td>
<td>3</td>
<td>1.7 (0.8, 3.6)</td>
</tr>
<tr>
<td>45–64 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
<td>0.6 (0.0, 1.8)</td>
<td>1</td>
<td>1.5 (0.5, 3.7)</td>
<td>2</td>
<td>1.5 (1.0, 2.0)</td>
<td>4</td>
<td>2.5 (1.8, 3.4)</td>
<td>9</td>
<td>4.0 (2.5, 6.0)</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>0</td>
<td>0.0 (0.0, 0.0)</td>
<td>1</td>
<td>2.1 (1.0, 3.2)</td>
<td>2</td>
<td>1.5 (1.0, 2.0)</td>
<td>4</td>
<td>2.5 (1.8, 3.4)</td>
</tr>
<tr>
<td>All ages</td>
<td>1</td>
<td>0.2 (0.0, 0.5)</td>
<td>1</td>
<td>0.4 (0.0, 1.2)</td>
<td>3</td>
<td>1.1 (0.0, 2.4)</td>
<td>4</td>
<td>1.0 (0.0, 2.2)</td>
<td>9</td>
<td>1.8 (0.6, 3.1)</td>
<td>6</td>
<td>5.8 (1.0, 10.6)</td>
</tr>
</tbody>
</table>

*Incidence per 100,000 person-years.

*Incidence per 100,000 person-yr directly age-adjusted to the 2000 US white population.

*Incidence per 100,000 person-yr directly age- and gender-adjusted to the 2000 US white population.
patients (~90%) the diagnosis was considered or diagnostic investigations were initiated by primary care practitioners in community practice. Only a small minority (10%) had sought direct self-referral to a gastroenterologist. Diarrhea was present at diagnosis in just over half (54%) of the patients (Table 2). Steatorrhea stools were reported in 26%, abdominal pain in 34%, and bloating in 30%. Anemia had been diagnosed previously in 29 cases (35%). The median BMI was 21 (range, 13–40), and 21 (27%) patients were overweight at the time of diagnosis. Gender did not influence most of the presenting symptoms, except that stools consistent with steatorrhea were reported more commonly in men than in women (42% vs 19%; odds ratio [OR], 3.53; 95% CI, 1.09–12.03; \( P = 0.037 \)) (Table 3). Four of the 82 patients had type 1 diabetes mellitus (IDDM). In 3 of the 4, the diagnosis of IDDM preceded that of celiac disease by an average of 21 years. In a single patient, celiac disease preceded the diabetes diagnosis by 2 months. Two patients had type 2 diabetes mellitus, whereas 5 patients had hypothyroidism, and 2 others had hyperthyroidism. Four patients had osteoporosis, and of these one also had osteomalacia. Twelve patients had a clinical diagnosis of depression and one of schizophrenia. Two had oral aphthous ulcers, and one each had a diagnosis of chronic active hepatitis, Crohn’s disease, chronic myopathy, and pancreatic insufficiency.

**Temporal Trends in Clinical Features**

Several of the clinical features changed over time (Table 2). Diarrhea and steatorrhea affected most of the cases in the earlier decades but only a minority in the most recent time period (OR for diarrhea, 0.5; 95% CI, 0.3–0.76; \( P < 0.005 \)) (Table 3). The BMI at diagnosis increased over time (\( P < 0.01 \)), and a history of weight loss affected less than one third of the recently diagnosed patients (OR, 0.6; 95% CI, 0.39–0.88; \( P = 0.01 \)) (Table 3). Although diarrhea and weight loss comprised a lower proportion of new cases in the recent year, the number of new cases with these symptoms increased in absolute terms (Table 2). Almost one third of the patients diagnosed since 1990 were overweight at the time of diagnosis. Anemia remained common and was often the sole presentation of celiac disease.

**Pathologic Findings**

Seventy-six of the original biopsy specimens were available for systematic re-review. The others had either been lost or had degraded to the point at which they were
Most samples were obtained by suction biopsy methods up to 1990. Subsequent to that time, all samples were obtained by endoscopic biopsies in the postbulbar duodenum. Altogether, 67% of the biopsies showed complete villous atrophy, and 33% revealed partial villous atrophy; 97% showed crypt hyperplasia. The intraepithelial lymphocyte (IEL) to enterocyte (E) ratio was increased in these samples (mean 8.11006 ± 3.2 IELS/100 E). The orientation of the biopsies was judged to be excellent (several areas with at least 5 sets of parallel crypts with intact lamina propria and surface epithelium) in 64% of cases. However, there was a decrease in the quality of orientation of the samples with the advent of endoscopic biopsies (P < 0.001, exact-Wilcoxon test).

Serology was only used in the most recent 10 years. Consequently, only 14 of the 82 patients had serologic tests performed in conjunction with their diagnosis. Of these, 13 of the 14 had positive endomysial antibodies, and 3 of 3 had positive gliadin immunoglobulin A antibodies. In these 13 patients, the positive serologic test prompted the subsequent duodenal biopsies. In 13 additional patients, serology was performed after the biopsies were done as a confirmation of disease. Endomysial antibodies were positive in 12 of 15 of these other patients.

### Dermatitis Herpetiformis

Thirty-four Olmsted County residents had dermatitis herpetiformis initially diagnosed during the same time period and of these, 28 had dermatitis herpetiformis without a confirmed diagnosis of celiac disease. Most patients with dermatitis herpetiformis did not undergo small intestinal biopsy. The overall age- and gender-adjusted incidence of dermatitis herpetiformis was 0.9 per 100,000 person-years (95% CI, 0.6–1.3). The incidence rate remained stable over time as contrasted with celiac disease (Figure 2). There were 20 women and 14 men. The predominance of female to male cases was less marked than that of celiac disease.

### Table 2. Temporal Changes in Symptoms Among Olmsted County, Minnesota, Residents Diagnosed With Celiac Disease in 1950–2001, by Time Period

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhea</td>
<td>8 (100)</td>
<td>12 (67)</td>
<td>17 (49)</td>
<td>7 (33)</td>
<td>44 (54)</td>
</tr>
<tr>
<td>Steatorrhea</td>
<td>6 (75)</td>
<td>6 (33)</td>
<td>7 (20)</td>
<td>2 (10)</td>
<td>21 (26)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>5 (63)</td>
<td>10 (56)</td>
<td>18 (51)</td>
<td>3 (14)</td>
<td>36 (44)</td>
</tr>
<tr>
<td>Bloating</td>
<td>4 (50)</td>
<td>2 (11)</td>
<td>11 (31)</td>
<td>7 (33)</td>
<td>24 (30)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>2 (25)</td>
<td>3 (17)</td>
<td>7 (20)</td>
<td>4 (19)</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>4 (50)</td>
<td>8 (44)</td>
<td>8 (23)</td>
<td>7 (33)</td>
<td>28 (34)</td>
</tr>
<tr>
<td>Flatulence</td>
<td>2 (25)</td>
<td>3 (17)</td>
<td>8 (23)</td>
<td>3 (14)</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>18</td>
<td>35</td>
<td>21</td>
<td>82</td>
</tr>
<tr>
<td>BMI median</td>
<td>19 (17,21)</td>
<td>20 (16,23)</td>
<td>21 (18,25)</td>
<td>22 (20,30)</td>
<td>21 (18,25)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>BMI quartile</th>
<th>(n)</th>
<th>(n)</th>
<th>(n)</th>
<th>(n)</th>
<th>(n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>q1, twenty-fifth percentile</td>
<td>7</td>
<td>16</td>
<td>35</td>
<td>21</td>
<td>79</td>
</tr>
<tr>
<td>q3, seventy-fifth percentile</td>
<td>(19)</td>
<td>(20,23)</td>
<td>(18,25)</td>
<td>(20,30)</td>
<td>(18,25)</td>
</tr>
</tbody>
</table>

### Table 3. Association of Symptoms and BMI at Diagnosis With Age, Gender, and Calendar Period Among Olmsted County, Minnesota, Residents Diagnosed With Celiac Disease, 1950–2001

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Age at diagnosis</th>
<th>Gender</th>
<th>Calendar period</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1.2 (0.97–1.53)</td>
<td>0.1085</td>
<td>0.64 (0.22–1.79)</td>
</tr>
<tr>
<td>Bloating</td>
<td>1.03 (0.83–1.3)</td>
<td>0.7872</td>
<td>0.98 (0.33–2.74)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>1.11 (0.89–1.39)</td>
<td>0.3737</td>
<td>0.68 (0.24–1.91)</td>
</tr>
<tr>
<td>Steatorrhea</td>
<td>0.94 (0.72–1.23)</td>
<td>0.6548</td>
<td>3.53 (1.09–1.03)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>0.88 (0.68–1.14)</td>
<td>0.3343</td>
<td>1.66 (0.5–5.24)</td>
</tr>
<tr>
<td>Weight loss</td>
<td>1.06 (0.85–1.32)</td>
<td>0.6196</td>
<td>1.32 (0.48–3.61)</td>
</tr>
<tr>
<td>BMI</td>
<td>0.06 (0.07–1.13)</td>
<td>0.0304</td>
<td>1.7 (0.83–4.23)</td>
</tr>
</tbody>
</table>

NOTE. OR and 95% CIs were estimated from logistic regression model parameters to predict presence of symptoms at diagnosis (or linear regression model for BMI at diagnosis). Parameter estimate (PE) indicates changes in BMI with age at diagnosis and successive calendar period. *Per 10 years.
Prevalence of Celiac Disease Among Olmsted County, Minnesota, Residents by Time Period

<table>
<thead>
<tr>
<th></th>
<th>January 1, 1991</th>
<th>January 1, 2001</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Ratea</td>
</tr>
<tr>
<td><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 yr</td>
<td>3</td>
<td>83.1</td>
</tr>
<tr>
<td>4–18 yr</td>
<td>0</td>
<td>0.0</td>
</tr>
<tr>
<td>19–44 yr</td>
<td>3</td>
<td>13.0</td>
</tr>
<tr>
<td>45–64 yr</td>
<td>5</td>
<td>53.1</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>1</td>
<td>15.2</td>
</tr>
<tr>
<td>All agesb</td>
<td>12</td>
<td>22.1 (9.6,34.7)</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 yr</td>
<td>1</td>
<td>27.3</td>
</tr>
<tr>
<td>4–18 yr</td>
<td>1</td>
<td>8.3</td>
</tr>
<tr>
<td>19–44 yr</td>
<td>2</td>
<td>9.3</td>
</tr>
<tr>
<td>45–64 yr</td>
<td>2</td>
<td>21.7</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>1</td>
<td>25.0</td>
</tr>
<tr>
<td>All agesb</td>
<td>7</td>
<td>14.4 (3.5,25.4)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3 yr</td>
<td>4</td>
<td>55.0</td>
</tr>
<tr>
<td>4–18 yr</td>
<td>1</td>
<td>4.3</td>
</tr>
<tr>
<td>19–44 yr</td>
<td>5</td>
<td>11.2</td>
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<tr>
<td>45–64 yr</td>
<td>7</td>
<td>37.6</td>
</tr>
<tr>
<td>≥65 yr</td>
<td>2</td>
<td>18.9</td>
</tr>
<tr>
<td>All agesc</td>
<td>19</td>
<td>18.3 (10.0,26.5)</td>
</tr>
</tbody>
</table>

aPrevalence per 100,000 person-yr.
bPrevalence per 100,000 person-yr directly age-adjusted to the 2000 US white population.
cPrevalence per 100,000 person-yr directly age- and gender-adjusted to the 2000 US white population.

Table 4. Prevalence of Celiac Disease Among Olmsted County, Minnesota, Residents by Time Period

Disease (age-adjusted annual incidence of 1.0 per 100,000; 95% CI, 0.6–1.4 vs. 0.9 per 100,000; 95% CI, 0.4–1.4). The mean age at the diagnosis of dermatitis herpetiformis was 50 years. Very few of these patients had any recorded gastrointestinal symptoms.

Prevalence of Celiac Disease and Dermatitis Herpetiformis

A total of 52 patients with celiac disease were living in Olmsted County on January 1, 2001, for an age- and gender-adjusted prevalence of 44.1 per 100,000 person-years (95% CI, 32.0–56.2). This represents a 2-fold increase in the prevalence of celiac disease during a single decade from that reported previously (Table 4). There were an additional 15 patients with dermatitis herpetiformis (without celiac disease) living in the county at the same time, for a prevalence of 13.2 per 100,000 (95% CI, 6.4–19.9). The total prevalence for both diseases combined on January 1, 2001, was 57.2 per 100,000 (95% CI, 43.4–71.1).

Discussion

The incidence of celiac disease has been increasing in most European countries where it has been measured.12,21–23 Consistent with these results, we have identified a dramatic increase, predominantly in the last decade, in this study from the upper Midwest of the United States. Our recent incidence rates are much greater than those reported previously.4 However, the recent incidence rates are equivalent to those for Crohn’s disease and ulcerative colitis in the same population24,25 and are similar to those reported for IDDM in this community.26 This suggests that celiac disease is not as rare as has been believed.10 Indeed, the recent incidence figures from Olmsted County may be similar to those in Europe27 and higher than a recent study from the South Glamorgan area of Great Britain and in Denmark.11,28

Little information is available about the incidence of celiac disease across the entire age spectrum.11,12 This study is unusual in tracking secular changes in both adult and childhood cases of celiac disease. Many European studies have focused on the incidence in children without accounting for the adult occurrence of the disease.29–31 Indeed, adults predominated in our population. The rate of childhood diagnosis in Olmsted County has been consistently low for the last 50 years in contrast to the dramatic changes in childhood diagnosis in Ireland and Sweden.21,32 It has previously been suggested that unidentified environmental factors might affect the incidence of the disease in specific birth cohorts.33 For example, these geographic differences may be related to infant feeding practices or relatively high gluten content in the infant diet.34,35 In Denmark, where infant feeding practices differed dramatically from Sweden, a much lower incidence of celiac disease was seen and the age of diagnosis was 40 years, more closely approximating the United States data.28,36 The female predominance of celiac disease, as seen in our study, has been previously described.28,37 There are a number of possible explanations. First, the challenges of pregnancy and menstruation may accentuate anemia and exacerbate any effects of celiac disease. In addition, women may be more likely to have medical contact and hence have an opportunity to have anemia detected and investigated; alternatively, symptoms may be investigated because of symptom reporting.37 Iron deficiency was a common presenting feature in this cohort, reflecting the frequent occurrence of unsuspected celiac disease in anemic patients.38 The other reason is that other immune-mediated diseases have a female predominance generally.39

Perhaps a central issue is whether the apparent increase in incidence represents a true increase in new cases or better detection of cases. We did observe some change over time in the clinical spectrum of newly diagnosed cases. For example, BMI was higher in the more recently
diagnosed cases, suggesting that there may not have been the same degree of malnutrition, whereas diarrhea and steatorrhea made up a lower proportion of new cases. However, the significant increase in absolute numbers of new patients with diarrhea and weight loss at diagnosis suggests the possibility that a true increase in incidence may also be occurring in adults. The reason for this is unknown. Similarly, the increased numbers of patients with anemia at diagnosis and a similar degree of villous change on biopsy would suggest that we are not identifying less severe disease. Another factor that may influence the detection of celiac disease is the availability of serologic tests. Serologic tests have been useful in detecting cases of celiac disease in the primary care setting.

The advent of particularly specific and sensitive serologic tests such as endomyosial antibodies and tissue transglutaminase antibodies has provided a powerful tool for selection of patients for intestinal biopsy. Serology contributed to the diagnosis of 50% of the new patients in the last 2 years of the study, suggesting a substantial impact on the rate of recent new case detection. It was not a factor in the diagnosis of any of the patients detected in the preceding years, although it had been available for at least 10 years.

Dermatitis herpetiformis represents a small but very visible aspect of enteric gluten sensitivity. The incidence of dermatitis herpetiformis in this study is similar to that reported from Utah some years ago, although the female predominance in our study differs from the usual male predominance seen in Utah. Accurate incidence figures for dermatitis herpetiformis are unavailable from European centers; however, the prevalence of dermatitis herpetiformis in our community approximates that reported from Scotland. Although we have seen a dramatic increase in celiac disease, we did not see a change in the incidence of dermatitis herpetiformis. In most studies, dermatitis herpetiformis has made up 5%–10% of celiac disease and dermatitis herpetiformis combined, compared to Olmsted County where 23% of prevalent cases have dermatitis herpetiformis alone. It is remarkable that there were 35 incidence cases of dermatitis herpetiformis of which only 6 had documented celiac disease based on small bowel biopsy. The others had not undergone small intestinal biopsy. However, the high ratio of dermatitis herpetiformis to celiac disease suggests that either there is a predilection for dermatitis herpetiformis as a sole manifestation of gluten sensitivity in this population or that the celiac disease remains hidden or underdiagnosed. The incidence of dermatitis herpetiformis did not change over time in Olmsted County, and the current ratio of dermatitis herpetiformis to celiac disease seemed to approximate the ratio reported from other European centers, supporting the latter hypothesis.

This study had a number of strengths, including the ability to identify all cases, both adults and children, diagnosed during a 50-year period. It was possible to apply the same rigid criteria for diagnosis of celiac disease to all of the cases over the entire time span. Moreover, because this is a population-based study, any potential for referral bias has been removed. Of course, it is likely that these numbers may still underestimate the true incidence or prevalence of celiac disease in the community because results of population-based serologic screening surveys in both the United States and European populations have shown a much higher prevalence of minimally symptomatic or asymptomatic disease. Indeed, a recent serologic screening survey in Denmark, which has low incidence rates, suggested that there might be a higher prevalence of undiagnosed celiac disease than previously suspected; however, these cases were not biopsied. However, although serologic screening studies provide important prevalence data on both clinically apparent and covert disease in a population, they do not provide temporal trend information on disease as this longitudinal study does. It is not widely accepted that widespread screening should be undertaken in the general population; however, active case finding by studying select at-risk groups may show a greater frequency of disease than detected clinically. Such a study is underway in Olmsted County. Many of the true population-based screening studies suffer from either a lack of biopsy confirmation of the cases identified or a small population sample. These shortcoming are not applicable in our study because the sample size, the population of Olmsted County, is more than 100,000 and all cases have been verified by standard diagnostic criteria rigidly applied over time.

In summary, celiac disease is not as rare as previously thought, especially in adults and women. More work is needed to account for the lower incidence of childhood celiac disease compared to some European countries, like Sweden, and to explain the different secular trends seen in various regions of the world. There may be both an increase in true incidence of disease in adults and an increased awareness of the milder expressions of disease among primary care practitioners. That has lead to dramatically increased rate of diagnosis.

References


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EXHIBIT 198
The economics of coeliac disease: a population-based study

K. H. Long*, A. Rubio-Tapia†, A. E. Wagie*, L. J. Melton III‡, B. D. Lahr§, C. T. Van Dyke† & J. A. Murray†

SUMMARY

Background
Despite increasing prevalence, the economic implications of coeliac disease are just emerging.

Aims
To assess the impact of coeliac disease diagnosis on healthcare costs and the incremental costs associated with coeliac disease.

Methods
Administrative data for a population-based cohort of coeliac disease cases and matched controls from Olmsted County, Minnesota were used to compare (i) direct medical costs 1 year pre- and post-coeliac disease diagnosis for 133 index cases and (ii) 4-year cumulative direct medical costs incurred by 153 index cases vs. 153 controls. Analyses exclude diagnostic-related and out-patient pharmaceutical costs.

Results
Average total costs were reduced by $1764 in the year following diagnosis (pre-diagnosis cost of $5023 vs. $3259; 95% CI of difference: $688 to $2993). Over a 4-year period, coeliac disease cases experienced higher outpatient costs (mean difference of $1457; \( P = 0.016 \)) and higher total costs than controls (mean difference of $3964; \( P = 0.053 \)). Excess average total costs were concentrated among males with coeliac disease ($14 191 vs. $4019 for male controls; 95% CI of difference: $2334 to $20 309).

Conclusions
Coeliac disease-associated costs indicate a significant economic burden of disease, particularly for diseased males. Diagnosis and treatment of coeliac disease reduce medical costs of care suggesting an economic advantage to earlier detection and treatment.

Aliment Pharmacol Ther 2010; 32: 261–269
INTRODUCTION

Coeliac disease (CD) is defined as a permanent intolerance to ingested gluten that damages the small intestine and resolves with the removal of gluten from the diet.\(^1\)\(^2\) Classically, CD causes diarrhoea and malabsorption with resulting malnutrition and deficiency of micronutrients (iron, folate, and the fat-soluble vitamins). However, the disorder frequently presents with only the vaguest gastrointestinal symptoms and a variety of extraintestinal manifestations (so-called ‘atypical’ CD). CD may remain entirely silent for many years despite much damage to the intestine.\(^1\) Although the wide spectrum of clinical manifestations of CD is better recognized in the United States now than decades ago, patients with CD may still have a long duration of symptoms and often undergo extensive and expensive medical investigations prior to an accurate diagnosis.\(^3\)

Once thought to be a rare disease, CD is now recognized as a common chronic disorder affecting ~1% of the general population in Western countries.\(^1\)\(^2\)\(^4\)\(^5\) Thus, the condition is of increasing public health concern in the United States, given the increasing prevalence and a belief that early diagnosis and treatment may prevent complications and reduce the economic burden of the disease.\(^6\) Much of the economic literature in this area has, in fact, focused on estimating costs associated with CD screening or diagnosis and patient dietary costs.\(^7\)\(^8\)\(^9\)\(^10\)\(^11\)\(^12\)\(^13\)\(^14\) However, research on the effect that detecting and treating CD has on health care resource use and the overall economic burden of illness is scarce and just emerging.\(^15\) Taking advantage of unique resources linking medical record and administrative data for a geographically defined population, we assessed the impact of CD diagnosis on costs of care and also provide longitudinal estimates of the incremental costs associated with CD prior to diagnosis.

METHODS

Study setting

A unique set of circumstances exist in Olmsted County, Minnesota, that facilitate population-based epidemiological and health services research. The county is relatively geographically isolated with few medical providers in the area. Under the auspices of the Rochester Epidemiology Project (REP), medical records for care received at Mayo Clinic and Olmsted Medical Center since 1966 have been successfully linked to provide medical record data from essentially all sources of medical care utilized by members of this local population.\(^16\) More recently, an electronic data sharing agreement was signed by these providers to also provide research access to patient level administrative data, which are archived within the Olmsted County Health Care Expenditure and Utilization Database (OCHEUD). OCHEUD currently provides billing information on all hospital and ambulatory care delivered to Olmsted County residents from 1987 to 2008.

Because of well-known discrepancies between billed charges and true resource use, utilization in OCHEUD is valued using standard methods by grouping services into the Medicare Part A and B classifications; Part A billed charges (hospital-billed services and procedures) are adjusted using hospital cost-to-charge ratios at the departmental level and wage indexes. Medicare Part B items (primarily physician-billed services) are valued using national average Medicare reimbursement rates. Costs are adjusted for inflation to express costs in constant dollars. The combination of OCHEUD and REP resources makes population-based health economic research feasible for numerous medical conditions.\(^17\)\(^18\)

Identification of CD cases and controls

Cases of CD were initially identified by searching the REP diagnostic index for Olmsted County residents who were assigned a diagnosis of CD (ICD-9 code 579) or dermatitis herpetiformis (ICD-9 codes: 694, 694.2) within calendar years 1989–2006. The complete in-patient and out-patient medical record for each candidate case was then thoroughly reviewed by a physician investigator to confirm that individuals met conventional diagnostic criteria for CD.\(^5\)\(^19\) CD cases included those with a positive biopsy (jejunal or duodenal biopsies with partial or total villous atrophy associated with crypt hyperplasia and an increased number of intraepithelial lymphocytes) and either clinical or histological improvement after the introduction of a gluten-free diet or a positive endomysial or tissue transglutaminase antibody IgA. Note that serological testing was not widely performed in Olmsted County until 1995.\(^20\) The diagnostic approach for CD at Mayo Clinic follows conventional recommendations and does not routinely include additional testing such as HLA genotyping.\(^2\)\(^20\) The date of diagnosis and year of symptom onset were recorded and residency at the time of diagnosis was confirmed through record review.

Each incident CD case was matched to a general population control residing in Olmsted County, identified by the REP, who was free of CD as indicated by diagnostic coding. Controls were matched for gender, age (birth year ± 1 year), a service date within 1 year of the CD
index case diagnosis, and a Mayo registration date within 2 years of the index case initial visit (this matches for the deviation of prior medical record documentation). The complete medical records of potential controls were reviewed by a physician investigator to exclude the presence of CD.

Residency status verification
Complete capture of health care utilization and associated cost data probably hinged on residency status. Thus, we further verified residency in Olmsted County for both CD cases and controls throughout the observation duration for economic analysis. Initially, this verification was conducted electronically by searching REP databases for health care encounters with county zip codes. If at least one provider visit as a county resident was found during a year, then residency was assumed throughout that year. Cases of uncertain residency underwent further review by a REP residency specialist who reviewed the detailed provider-linked medical records (including correspondence), serial telephone books, maps and plat books, city directories and public record databases to validate residency throughout the period of interest.

Ethical issues
The study was approved by Mayo Clinic and Olmsted Medical Center Institutional Review Boards. All patients who did not grant authorization to use their medical records for research were excluded from analyses as per Minnesota law.21

Economic outcomes and statistical analyses
Impact of CD diagnosis on direct medical costs. Health care utilization and associated costs were tracked in OCHEUd for the identified CD cases to estimate the impact of diagnosis on total direct medical costs of care. The impact of diagnosis on costs was determined by comparing the costs incurred by cases in the year prior to and the year following their diagnosis. Thus, only cases with verified local residency during this observation period were included in this analysis. Analyses were conducted including and excluding diagnosis-related costs as well as stratified by cost categories of interest (in-patient, outpatient, total costs of care). Services and costs we identified as related to the diagnosis of CD included serological testing (endomysial, gliadin, anti-reticulin antibody, and tissue transglutaminase antibodies), HLA typing, upper GI endoscopy with intestinal biopsy, surgical pathology and consultation, and bone densitometry. These services were considered related to the CD diagnosis if they occurred within 90 days of the recorded diagnosis date. Similarly, we considered any Gastroenterology office visit within 15 days of diagnosis to be a diagnosis-related cost of care.

Incremental costs associated with CD. We estimated the direct medical costs attributable to CD by comparing the cumulative costs incurred by CD cases with those incurred by the matched controls over equivalent periods of observation in the OCHEUd database. In an attempt to estimate the economic burden of undiagnosed and untreated CD, we focused our analysis on 4 years of observation prior to the year before CD diagnosis for cases and the 4 years prior to that index year for control subjects. For a patient diagnosed with CD in 2003, for instance, we estimated and compared his/her estimated cumulative costs incurred between 1999 and 2002 with the costs incurred by the matched control over the same time frame. We conducted analyses stratified by cost categories of interest [in-patient episodes, total out-patient care and subcategories (surgery, radiology, laboratory services, office visits), total costs] and by gender and age at index (i.e. ≤40 years and >40 years). As pre-existing disease may confound assessments of incremental costs, we compared CD cases and matched controls using a validated systematic method of classifying comorbidity when diagnostic codes from administrative databases are utilized (Deyo adaption of Charlson comorbidity index) based on diagnoses assigned in the 4-year observation period of interest.22

In a post hoc analysis, CD cases were categorized by severity and clinical presentation to assess the impact of these variables on incremental costs associated with CD. Severe CD was defined by the presence of both diarrhoea and weight loss. ‘Classical’ CD include patients with a diarrhoea-predominant syndrome and ‘atypical’ CD was used for patients with nonspecific gastrointestinal symptoms (including bloating or abdominal pain) and/or extraintestinal manifestations (e.g. iron-deficiency anaemia, abnormal liver function tests, osteoporosis, etc.).2 Patients with refractory coeliac disease were identified by conventional criteria.23, 24

Statistical analysis
Continuous data are summarized as mean ± standard deviation. Discrete data are presented as frequency (percentage). Characteristics of CD cases and non-CD controls as of index were compared using Student’s t-test for continuous data and Pearson’s chi-squared test for categorical data, as appropriate. In all economic analyses, observed
costs were compared using paired Student’s *t*-tests and nonparametric bootstrapped confidence intervals. All statistical tests were two-sided, and *P* values less than 0.05 were considered significant. SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) was used in the analyses.

**RESULTS**

**Patients**

We initially identified 168 CD patients who were Olmsted County residents at the time of CD diagnosis. Six patients declined to provide research authorization and were excluded. Nine additional patients were not local residents during the entire 4 years prior to the CD diagnosis year. Thus, the final analytical cohort for analysis of incremental cost associated with CD consisted of 153 cases and 153 controls (153 matched pairs). Twenty-nine additional patients may not have been local residents during the year prior to and the year following diagnosis and thus were excluded from the cohort used to assess the impact of CD diagnosis on direct medical costs (final cohort of 133 CD cases).

The mean age for the 133 CD patients included to assess the impact of CD diagnosis on direct medical cost was 41 years, with the majority (66%) of female gender. Sixty (45%) patients had one or more Charlson comorbidities in the years since first electronically available diagnosis at Mayo clinic to CD diagnosis date (16.6 ± 8.7 years). Table 1 provides a summary of clinical characteristics of CD patients and between-group comparisons for the 153 CD patients and non-CD matched controls. Gender and age distributions did not differ between cases and controls (by virtue of the study design) nor did the estimated summary measures of comorbidity during the 4-year observation period of interest.

Sufficient information for assessment of severity and clinical presentation was available in 151 (99%) patients. Severe CD was present in 22 (15%) patients and CD was nonsevere in 129 (85%) patients. Classical CD was observed in 58 (38%) patients and atypical CD in 93 (62%) patients. Two patients met criteria for refractory coeliac disease. The diagnosis of the refractory state was made after 4 years and 5 years of CD diagnosis.

<table>
<thead>
<tr>
<th>Variable</th>
<th>CD cases (n = 153)</th>
<th>Controls (n = 153)</th>
<th><em>P</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>40.2 ± 20.5</td>
<td>40.2 ± 20.5</td>
<td></td>
</tr>
<tr>
<td>Male gender, n (%)</td>
<td>51 (33)</td>
<td>51 (33)</td>
<td></td>
</tr>
<tr>
<td>Charlson comorbidity count</td>
<td>0.46 ± 0.92</td>
<td>0.33 ± 0.68</td>
<td>0.16</td>
</tr>
<tr>
<td>Value &gt; 1, n (%)</td>
<td>45 (29)</td>
<td>38 (25)</td>
<td>0.37</td>
</tr>
<tr>
<td>Charlson comorbidity index†</td>
<td>0.60 ± 1.41</td>
<td>0.73 ± 0.91</td>
<td>0.18</td>
</tr>
<tr>
<td>Value &gt; 1, n (%)</td>
<td>15 (10)</td>
<td>17 (11)</td>
<td>0.71</td>
</tr>
<tr>
<td>Clinical characteristics of CD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Villous atrophy in diagnostic biopsy</td>
<td>153/153 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive tTGA or EMA at diagnosis</td>
<td>100/114 (88%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Classical presentation</td>
<td>58/151 (38%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atypical presentation</td>
<td>93/151 (62%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe CD</td>
<td>22/151 (15%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment with a gluten-free diet</td>
<td>142/147 (98%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical response to gluten-free diet</td>
<td>109/109 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HLA-DQ2 or DQ8</td>
<td>69/69 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Index was defined as date of CD diagnosis for cases and the same year for matched controls.
† Charlson comorbidity index was calculated using the Deyo adaption based on diagnoses in the 4 years of observation prior to the year before diagnosis for cases and the 4 years prior to that same year for controls.

CD, coeliac disease; tTGA, tissue transglutaminase antibodies; EMA, endomysial antibodies; HLA, human leucocyte antigens.
respectively because of recurrence of a severe malabsorptive syndrome despite strict adherence to GFD.

Impact of coeliac disease diagnosis on direct medical costs
Significant cost differences in in-patient, out-patient and total direct medical costs were observed when comparing costs in the year prior to and the year following CD diagnosis (Table 2). Total annual direct medical costs were reduced by $2118, on average, following CD diagnosis ($5457 vs. $3339; bootstrapped 95% CI of difference: $922–3417). This difference was driven by both reduced in-patient costs and out-patient (professional service) costs in the year following diagnosis (mean reductions: $1338 and $742, respectively). Observed reductions in in-patient and total costs of care following diagnosis persisted even with the exclusion of services deemed to be related to the diagnostic process itself (Table 2). Mean total direct medical costs were reduced by $1764, on average, in the year following diagnosis with exclusion of CD-diagnostic costs of care ($5023 vs. $3259; bootstrapped 95% CI of difference: $688–2993).

Incremental cost associated with coeliac disease
Table 3 compares mean costs incurred by CD patients and non-CD matched control subjects over the 4-year observation period (before CD diagnosis for cases and prior to corresponding index year for controls). Over the 4 years, CD cases experienced significantly higher outpatient costs of care and borderline significantly higher total costs of nearly $4000, on average, compared with control subjects (mean total costs: $11 037 vs. $7073; bootstrapped 95% CI of difference: $65–8020). The higher out-patient costs observed among CD patients relative to non-CD controls (mean difference: $1457; \( P = 0.02 \)) was driven by increased radiological and laboratory service use, as well as increased costs related to office visits (office visit mean cost difference: $466; \( P = 0.002 \)).

Total cumulative 4-year costs were observed to be higher both for the 76 CD patients aged ≤ 40 compared with their controls (mean cost difference for case minus control of $1856, \( P = 0.40 \)) as well as for the 77 CD patients and matched controls over 40 years of age at index (mean cost difference for case minus control of $6044, \( P = 0.08 \)). These differences, however, did not reach statistical significance. Cumulative costs were similar for the 102 female CD patients and matched controls ($9460 vs. $8600; \( P = 0.66 \)). In contrast, cumulative 4-year costs for the 51 male CD patients were significantly higher than those incurred by their non-CD controls ($14 191 vs. $4018; \( P = 0.03 \)).

The distribution of 4-year costs for CD cases stratified by severity and clinical presentation and for matched controls are presented in Figure 1. Post hoc analyses comparing 4-year costs in these CD subgroups with

### Table 2 | Annual direct medical costs in the year prior to and following the diagnosis of coeliac disease*

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Before diagnosis (( n = 133 ))</th>
<th>After diagnosis (( n = 133 ))</th>
<th>Mean difference (95% CI)( ^\dagger )</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Diagnosis costs included</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total costs</td>
<td>$5457 ($4659, $7395)</td>
<td>$3339 ($2551, $4895)</td>
<td>$2118 ($922, $3417)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>In-patient costs( ^\ddagger )</td>
<td>$2851 ($2079, $4138)</td>
<td>$1512 ($955, $2543)</td>
<td>$1339 ($400, $2260)</td>
<td>0.003</td>
</tr>
<tr>
<td>Out-patient costs( ^$ )</td>
<td>$2598 ($2412, $3266)</td>
<td>$1856 ($1551, $2415)</td>
<td>$742 ($389, $1274)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>**Diagnosis costs excluded( ^*$ )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total costs</td>
<td>$5023 ($4037, $6296)</td>
<td>$3259 ($2377, $4538)</td>
<td>$1764 ($688, $2993)</td>
<td>0.003</td>
</tr>
<tr>
<td>In-patient costs( ^\ddagger )</td>
<td>$2851 ($2079, $4138)</td>
<td>$1512 ($955, $2543)</td>
<td>$1339 ($400, $2260)</td>
<td>0.003</td>
</tr>
<tr>
<td>Out-patient costs( ^$ )</td>
<td>$2154 ($1816, $2533)</td>
<td>$1773 ($1428, $2200)</td>
<td>$381 ($36, $796)</td>
<td>0.054</td>
</tr>
</tbody>
</table>

* Mean costs per patient in 2007 constant dollars.
\( ^\dagger \) Bootstrap 95% CI using the percentile method.
\( ^\ddagger \) Hospital (Medicare Part A) costs.
\( ^\$ \) Professional (Medicare Part B) costs.
\( ^\*$ Diagnostic services of interest included serologic testing, endoscopy with biopsy and pathology, bone density radiography, and gastroenterology office visits.
matched controls find patients with classical CD presentation to have higher out-patient costs as compared with controls \((P = 0.05)\), but individuals with atypical CD presentation incurred similar costs of care. Severe CD patient cumulative costs were similar to those of the matched controls; however, patients with nonsevere CD had significantly higher out-patient costs \((\$5181 \text{ vs. } \$3475; \ P = 0.01)\).

**DISCUSSION**

This population-based study estimates the impact of CD diagnosis on direct medical costs of care as well as the incremental costs associated with CD prior to diagnosis. Even with the exclusion of costs related to the diagnostic process, we find that direct medical out-patient and total costs were significantly reduced for patients in the year following CD diagnosis. Furthermore, CD patients

| Table 3 | Direct medical costs incurred by Olmsted County, Minnesota, residents with coeliac disease diagnosed in 1989–2006 and controls without coeliac disease* |
|---|---|---|---|
| Endpoint | CD cases \((n = 153)\) | Controls \((n = 153)\) | Mean difference \((95\% \ CI)\)† | \(P\)-value |
| Total costs | \$11 037 \((\$7866, \$14 793)\) | \$7073 \((\$5211, \$9483)\) | \$3964 \((\$65, \$8020)\) | 0.053 |
| In-patient costs‡ | \$5976 \((\$3625, \$8762)\) | \$3497 \((\$2206, \$5311)\) | \$2478 \((\$–402, \$5532)\) | 0.106 |
| Out-patient costs§ | \$4937 \((\$3921, \$6082)\) | \$3480 \((\$2816, \$4253)\) | \$1457 \((\$293, \$2689)\) | 0.016 |
| Surgery | \$795 \((\$615, \$1114)\) | \$685 \((\$503, \$991)\) | \$110 \((\$–208, \$453)\) | 0.469 |
| Radiology | \$910 \((\$671, \$1351)\) | \$546 \((\$406, \$770)\) | \$364 \((\$101, \$702)\) | 0.009 |
| Laboratory | \$546 \((\$476, \$723)\) | \$377 \((\$304, \$481)\) | \$169 \((\$73, \$351)\) | 0.006 |
| Office visits | \$1472 \((\$1215, \$1969)\) | \$1006 \((\$847, \$1253)\) | \$466 \((\$157, \$945)\) | 0.002 |
| Other medical | \$981 \((\$709, \$1465)\) | \$724 \((\$464, \$1090)\) | \$256 \((\$–178, \$781)\) | 0.234 |

* Mean 4-year cumulative costs per patient in 2007 constant dollars.
† Bootstrap 95% CI using the percentile method.
‡ Hospital (Medicare Part A) costs.
§ Professional (Medicare Part B) costs.

CD, coeliac disease.

![Figure 1](image-url)
experienced significantly higher direct medical costs during the 4-year observation period (nearly $4000, on average) compared with unaffected peers, suggesting a substantial economic burden of disease.

These results are consistent with those recently published by Green et al. (2008), who used out-patient administrative claims data to assess the economic implications of CD diagnosis. A particular strength of our study was our ability to link detailed medical records data (including laboratory data) to line item utilization and cost data regardless of payer type. In our population-based design, the diagnosis of CD was confirmed by re-review of the medical record and was not only based on diagnostic codes. Thus, this study design decreased bias induced by referral, missing data and inaccurate coding. Our results suggest a 29% reduction in outpatient costs in the year following diagnosis as well as an overall 39% reduction in total medical cost of care. Our decision to limit costs that relate to the diagnosis of CD to 90 days for tests and 15 days for office visits is consistent with our experience in a major tertiary referral centre with expertise in CD, but may be too restrictive in other settings or populations.

To our knowledge, ours is the first study to assess incremental costs attributable to CD despite the fact that cost-of-illness studies are increasingly needed for modelling the cost-effectiveness of alternative treatments and for informing clinical and public health policy. In our sample, undiagnosed and untreated CD patients incurred nearly $4000 in excess 4-year medical costs compared with non-CD matched peers. This significant economic burden was even more pronounced among males with CD, who incurred over $10 000 more, on average, in medical costs during the study period than their matched non-diseased controls. The reasons for this finding are not totally clear, but clinically detected CD is twice as frequent among women as men and it is possible that male symptoms could more often be attributed to causes other than CD (such as irritable bowel syndrome), increasing both the number of clinic visits to make the correct diagnosis and the cost of care. Gender-related differences in both clinical presentation of CD and comorbidity may also affect estimates of cost of care, including previous evidence of more severe disease in males.

Our post hoc analyses of incremental costs between CD cases and controls stratified by severity and clinical presentation suggest that the excess total costs associated with CD we observed are largely driven by significant excess out-patient costs in patients with classical CD (characterized by a diarrhoea-predominant syndrome) and in patients with nonsevere CD (defined by the absence of a combination of diarrhoea and weight loss). Reasons to explain these differences remain unknown, but we can speculate that it may be related to a more extensive differential diagnosis in non-severe CD, expedited diagnosis of severe cases, cost inherent to the approach of undiagnosed chronic diarrhoea, and serology-driven diagnosis of atypical cases. Further studies to address these hypotheses are not only necessary but may also be clinically relevant.

Although not statistically significant, we also found it curious that in-patient costs were observed to be over $2400 higher (P = 0.11), on average, for CD patients in our sample compared with controls as anecdotally, CD does not seem to be a condition with an acute need for in-patient care. To investigate this further, we performed additional subgroup analyses stratifying by whether in-patient events were considered CD-related (or not) based on a blinded clinical review of primary discharge diagnoses. Examples of episodes considered CD-related include a diagnosis of anaemia, unspecified constipation, generalized abdominal pain and intussusception. Interestingly, in-patient costs for CD-related episodes were significantly higher, on average, for CD cases than for controls ($392 vs. $33; P = 0.05). Costs for non-CD in-patient episodes were also observed to be $1565 higher, on average, for CD cases compared with controls, but this difference was not statistically significant. Thus, at least some of the higher in-patient cost in CD cases are explained by higher CD-related in-patient episodes.

Our study also has potential limitations to note. The present study was conducted in a single geographical setting, Olmsted County, where the population is predominately of white ethnicity and much of the care obtained by study participants occurred at a high volume referral centre. Patients and results may differ in other practice settings and locales. We also acknowledge our relatively small sample sizes, particularly in stratified analyses, hindering cost comparisons, given the high variability and skewed nature of this outcome. Furthermore, while we did restrict our sample to individuals with verified county residency to limit missing data concerns, the possibility still remains that some individuals may have obtained healthcare outside our local setting. Finally, a significant limitation of this study design is that our analysis was focused on costs from the health care provider perspective, and it does not include pharmaceutical costs, decreased work productivity, or the very significant
out-of-pocket cost associated with gluten-free food that can be 2–4 times as expensive as similar wheat-based food.\textsuperscript{13, 14} It remains possible that our observed reduction in medical cost after CD diagnosis and treatment may have shifted the economic burden to patients as the excess cost associated with the gluten-free diet is not reimbursed by payers in the U.S unlike in other countries such as Finland where a monthly compensation (‘dietary grant’) has been paid by a Social Insurance Institution since 2002.\textsuperscript{32} Finally, quality of life implications associated with a gluten-free diet or CD that may be of significant concern from a patient perspective were not analysed.\textsuperscript{34–36}

In conclusion, using a population-based study design, our estimates of CD-associated costs indicate a significant economic burden of disease, particularly for men with CD. Diagnosis and treatment of CD significantly reduces direct medical costs of care, suggesting an economic advantage to earlier detection and treatment.

**ACKNOWLEDGEMENTS**

**Declaration of personal interests:** Dr. Murray is a consultant for Ironwood Inc, Flamentera, Actogenix, Ferring Pharmaceuticals Inc and Nexpep. Dr. Long, Dr. Rubio-Tapia, A. Wagie, Dr. Melton, B. Lahr, and C. Van Dyke declare no competing interests. Kirsten Hall Long: design, collection and interpretation of data, statistical analysis, and manuscript preparation; Alberto Rubio-Tapia: conception, design, funding, collection and interpretation of data, and manuscript preparation; Brian D. Lahr: collection and interpretation of data, statistical analysis; Carol T. Van Dyke: data collection; Joseph A. Murray: conception, design, funding, collection and interpretation of data, and manuscript preparation. Guarantor of the article: Kirsten Hall Long, Ph.D. All authors have reviewed and approved the final draft submitted.

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**REFERENCES**

Human papillomavirus vaccination of adult women and risk of autoimmune and neurological diseases

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Background. Since 2006, human papillomavirus (HPV) vaccines have been introduced in many countries worldwide. Whilst safety studies have been reassuring, focus has been on the primary target group, the young adolescent girls. However, it is also important to evaluate safety in adult women where background disease rates and safety issues could differ significantly.

Objective. We took advantage of the unique Danish and Swedish nationwide healthcare registers to conduct a cohort study comparing incidence rate ratios (RRs) of 45 preselected serious chronic diseases in quadrivalent HPV (qHPV)-vaccinated and qHPV-unvaccinated adult women 18–44 years of age.

Methods. We used Poisson regression to estimate RRs according to qHPV vaccination status with two-sided 95% confidence intervals (95% CIs).

Results. The study cohort comprised 3 126 790 women (1 195 865 [38%] Danish and 1 930 925 [62%] Swedish) followed for 16 386 459 person-years. Vaccine uptake of at least one dose of qHPV vaccine was 8% in the cohort: 18% amongst Danish women and 2% amongst Swedish. We identified seven adverse events with statistically significant increased risks following vaccination—Hashimoto’s thyroiditis, coeliac disease, localized lupus erythematosus, pemphigus vulgaris, Addison’s disease, Raynaud’s disease and other encephalitis, myelitis or encephalomyelitis. After taking multiple testing into account and conducting self-controlled case series analyses, coeliac disease (RR 1.56 [95% confidence interval 1.29–1.89]) was the only remaining association.

Conclusion. Unmasking of conditions at vaccination visits is a plausible explanation for the increased risk associated with qHPV in this study because coeliac disease is underdiagnosed in Scandinavian populations. In conclusion, our study of serious adverse event rates in qHPV-vaccinated and qHPV-unvaccinated adult women 18–44 years of age did not raise any safety issues of concern.

Keywords: Cohort study, Epidemiology, Human papillomavirus, Vaccine Safety.

Since 2006, human papillomavirus (HPV) vaccines have been introduced in many countries worldwide including all of North America and most of Western Europe [1]. The peak incidence of HPV infection occurs soon after sexual debut, and to increase vaccine effectiveness, national immunization programmes target the youngest adolescent girls of 9–12 years of age. Consequently, the majority of postlicensure evidence of the safety of HPV vaccines comes from young adolescents [2]. However, adult women are also getting HPV vaccinated through catch-up programmes or by choice at their own expenses. Indeed, the Advisory Committee on Immunization Practices recommends HPV vaccination of females through the age of 26 years, and recent estimates indicate that amongst HPV-vaccinated women aged 19–26 years approximately 20% received their first vaccine dose at the age of 19 or older [3, 4]. In adult women, evidence of vaccine safety is limited to primarily prelicensure phase II/III randomized clinical trials including women 15–26 years of age [5]. These studies have been reassuring, but only statistically powered to evaluate common adverse events such as local
reactions and general symptoms such as fever and nausea. In addition, randomized trials are being conducted in women older than 25 years of age with favourable vaccine efficacy estimates against infection and cervical abnormalities up to 45 years of age, suggesting that the age indication might be expanded in future [6, 7].

When a large population is vaccinated, cases of serious and rare chronic disease are expected to occur in temporal relationship to vaccination purely by chance [8]. Despite this fact, sensationalist reporting of such cases in traditional and social media channels is frequently the source of public concern and anti-vaccine sentiment [9]. To properly address vaccine safety concerns and contribute relevant safety data to help inform clinical practice and assessment by drug authorities, carefully designed and well-conducted postlicensure studies are vital. Furthermore, many chronic diseases, such as autoimmune or neurological diseases, that are often allegedly linked to HPV vaccination occur at markedly different rates in young girls and adult women. Thus, it is also important to evaluate HPV vaccine safety in all target groups and not just the primary group of young girls.

We took advantage of the unique Danish and Swedish nationwide healthcare registers to conduct a postlicensure cohort study comparing rates of serious chronic disease in quadrivalent HPV (qHPV)-vaccinated and qHPV-unvaccinated adult women 18–44 years of age.

**Materials and methods**

We designed a register-based cohort study including all Danish and Swedish women 18–44 years of age in the period from 1 October 2006 to 30 June 2013 for Danish women and to 31 December 2012 for Swedish women (we did not have access to Swedish data beyond this date). We accomplished this using register data from the Danish Civil Registration System and Statistics Sweden, respectively [10]. These registers contain basic demographic information such as date of birth, date of potential loss to follow-up by, for example, emigration or death and a unique personal identifier for each resident. Both Denmark and Sweden keep nationwide registers of demographic and health-related information on all residents. In both countries, a unique personal identifier is assigned to all residents and is used to access information in national registers [11]. We used this identifier to obtain information on HPV vaccination status, serious chronic disease and possible confounders for all women in the cohort.

**HPV vaccination**

The qHPV vaccine (Gardasil; Sanofi Pasteur MSD SNC, Lyon, France) was licensed in Europe in September 2006. In Denmark, the qHPV vaccine has been included in the national vaccination programme since 2009 for 12-year-old girls, with catch-up vaccination of girls 13–15 years of age from October 2008 and of young women 20–27 years of age from August 2012. In Sweden, the qHPV vaccine was available from May 2007 as a subsidized vaccine for 13- to 17-year-old girls outside the national programme. In January 2012, the vaccine was included in the national vaccination programme for 10- to 12-year-old girls together with catch-up vaccination of 13- to 18-year-old girls. Since licensure, the qHPV vaccine has also been available in both countries for adult women at their own expense.

In both Denmark and Sweden, we obtained information on qHPV vaccinations primarily from the national prescription registers (Anatomic Therapeutic Chemical [ATC] code J07BM01) [12, 13]. Both registers contain individual-level information on all prescriptions filled at Danish and Swedish pharmacies, respectively. We supplemented this with information on catch-up vaccinations of young women given within the national vaccination programmes. We obtained this information from national vaccination registers. In Denmark, we used the Childhood Vaccination Database, which contains information on all vaccinations administered in the national childhood vaccination programme [14]. In Sweden, we used the Swedish HPV vaccination register, which was launched in parallel with the start of opportunistic HPV vaccination [15]. For vaccinations identified through prescription data, we defined the date of vaccination as the date of filling the prescription plus 2 days, which was the average time to administration amongst a subset of vaccinations with registrations in both prescription and national registers. Because the use of the bivalent HPV vaccine is rare in both Denmark and Sweden (given to <1% of all vaccines), it was not evaluated in the present study. The 9-valent HPV vaccine was not in use during the study period.
Serious adverse events

Before conducting the study, we predefined a number of serious chronic disease outcomes using International Classification of Diseases 10th revision (ICD-10) codes—see Table S1 for all study outcomes with corresponding ICD-10 codes. We included a wide range of autoimmune diseases because these are often claimed to be adverse effects of vaccinations. We also included a selection of neurological diseases, mainly based on recent reports of increased risks of neurological diseases such as Guillain–Barré syndrome and narcolepsy after pandemic influenza vaccination. In addition, many of the predefined outcomes have been linked with vaccination in traditional and social media channels in reports of disease occurring in the immediate time-period following vaccination. Finally, the selected study outcomes are similar to what has previously been included in studies of HPV vaccine safety [16]. In total, we evaluated 45 outcomes.

In both Denmark and Sweden, we used hospital patient registers to obtain the predefined diagnoses (coded using ICD-10 with dates of hospital contacts) for all women in the cohort [17, 18]. Both registers contain nationwide individual-level information on all hospital contacts in the study period including inpatient and outpatient visits. We had no information from primary health care. However, in both countries, primary health care acts as a gatekeeper to further care within specialized departments in the hospital setting.

Statistical methods

Study follow-up started on the date of becoming 18 years of age or 1 October 2006, whichever came last. Women who received their first vaccination prior to this date were excluded from the study cohort. We chose this new user design to remove women with a history of HPV vaccination without any adverse events as they might have a lower risk of developing adverse events after subsequent HPV vaccinations. Follow-up ended on the date of loss to follow-up (emigration, death or disappearance), vaccination with the bivalent HPV vaccine, first hospitalization with the outcome under study, 45 years of age or end of follow-up (1 July 2013 for Danish women or 1 January 2013 for Swedish), whichever came first. For each disease, this yielded outcome counts together with the corresponding number of person-years of follow-up, according to age, calendar year, qHPV vaccination status and country of residence. We then used Poisson regression (log-linear regression of the counts with the logarithm of person-time as offset) to estimate incidence rate ratios (RRs) according to qHPV vaccination status together with diagnostic investigation. This is such to allow for the insidious onset of disease and we considered hospitalization register data in the preceding 10 years. RRs were adjusted for age (categories 18–24, 25–29, 30–34, 35–39 and 40–44 years of age), calendar year (2-year intervals) and country of residence (Denmark or Sweden).

We subdivided all follow-up times as qHPV vaccinated into two risk periods: an acute period comprising the first 179 days after vaccination and a long-term period comprising all vaccinated follow-up times in the study after the first 180 days. These periods were defined according to the latest vaccine administration. Thus, after each dose, the vaccinated women re-entered the acute risk period. The duration of the acute period was defined as such to allow for the insidious onset of disease without diagnostic investigation. This is similar to risk periods after HPV vaccination in comparable studies. We estimated adjusted RRs for the acute period, the period after the first 180 days and the combined period following qHPV vaccination.

Sensitivity analyses

For each significantly increased RR (lower bound of 95% CI > 1.0), we (i) reanalysed the association using the self-controlled case series (SCCS) method to take potential confounding by all unmeasured time-invariant confounders into account [19], (ii) compared RRs between Denmark and Sweden for consistency of association and (iii) recalculated the confidence interval using the Bonferroni correction to take multiple testing into account. For any associations that remained after these three
criteria were applied, we additionally assessed clustering of events in time by plotting cases according to time since vaccination.

For the SCCS analysis, we used the cases and follow-up from the cohort analysis, except that when becoming a case, follow-up was not terminated but continued until any of the other conditions for terminating follow-up in the cohort analysis were met. We then used a conditional Poisson model to derive incidence ratios (IRs) by comparing the rate of events during the period under investigation with the rate of events in the unvaccinated period for each case. The IRs derived from the SCCS analysis were adjusted for age and calendar year periods similar to the main analysis.

Given the large number of study outcomes and two risk periods under investigation, we conducted a significant number of separate analyses (n = 345). However, as different risk period analyses for a given study outcome cannot be assumed statistically independent, we conservatively used the number of study outcomes (n = 45) for Bonferroni-corrected confidence intervals corresponding to 1 – (0.05/45)% confidence intervals.

Results

The study cohort comprised 3,126,790 women (1,195,865 [38%] Danish and 1,930,925 [62%] Swedish) aged 18–44 years with a mean age of entry into the study of 29.8 years (Table 1).

Table 1 Descriptive characteristics of Danish and Swedish women 18–44 years of age in the period from 1 October 2006 to 30 June 2013 for Danish women or 31 December 2012 for Swedish women

<table>
<thead>
<tr>
<th>Overall (n = 3,126,790)</th>
<th>Denmark (n = 1,195,865)</th>
<th>Sweden (n = 1,930,925)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person-years of follow-up</td>
<td>16,386,459</td>
<td>6,536,547</td>
</tr>
<tr>
<td>Age at entry, mean (SD)</td>
<td>29.8 (8.7)</td>
<td>30.2 (8.6)</td>
</tr>
<tr>
<td>Year of study entry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>2,708,683 (87)</td>
<td>1,057,223 (88)</td>
</tr>
<tr>
<td>2007</td>
<td>101,127 (3)</td>
<td>38,369 (3)</td>
</tr>
<tr>
<td>2008</td>
<td>94,342 (3)</td>
<td>35,538 (3)</td>
</tr>
<tr>
<td>2009</td>
<td>77,239 (2)</td>
<td>29,345 (2)</td>
</tr>
<tr>
<td>2010</td>
<td>66,038 (2)</td>
<td>24,845 (2)</td>
</tr>
<tr>
<td>2011</td>
<td>43,151 (1)</td>
<td>49,11 (&lt;0.5)</td>
</tr>
<tr>
<td>2012</td>
<td>34,509 (1)</td>
<td>39,33 (&lt;0.5)</td>
</tr>
<tr>
<td>2013</td>
<td>17,01 (&lt;0.5)</td>
<td>17,01 (&lt;0.5)</td>
</tr>
<tr>
<td>Age at vaccination, mean (SD)</td>
<td>24.6 (4.9)</td>
<td>25.1 (4.7)</td>
</tr>
<tr>
<td>Vaccinated with quadrivalent HPV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First dose</td>
<td>242,720 (8)</td>
<td>211,188 (18)</td>
</tr>
<tr>
<td>Second dose</td>
<td>201,965 (6)</td>
<td>176,196 (15)</td>
</tr>
<tr>
<td>Third dose</td>
<td>135,885 (4)</td>
<td>116,806 (10)</td>
</tr>
<tr>
<td>Year of vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2006</td>
<td>342 (&lt;0.5)</td>
<td>119 (&lt;0.5)</td>
</tr>
<tr>
<td>2007</td>
<td>8,385 (3)</td>
<td>3,947 (2)</td>
</tr>
<tr>
<td>2008</td>
<td>13,927 (6)</td>
<td>9,879 (5)</td>
</tr>
<tr>
<td>2009</td>
<td>12,915 (5)</td>
<td>9,787 (5)</td>
</tr>
<tr>
<td>2010</td>
<td>7,755 (3)</td>
<td>5,968 (3)</td>
</tr>
<tr>
<td>2011</td>
<td>12,184 (5)</td>
<td>8,936 (4)</td>
</tr>
<tr>
<td>2012</td>
<td>132,756 (55)</td>
<td>118,096 (56)</td>
</tr>
<tr>
<td>2013</td>
<td>54,456 (22)</td>
<td>54,456 (26)</td>
</tr>
</tbody>
</table>

*Values are numbers (percentages) unless otherwise stated.*
Vaccine uptake of at least one dose of qHPV vaccine was 8% in the cohort: 18% amongst Danish women and 2% amongst Swedish. Amongst Danish women, 10% received all three doses, and amongst Swedish women, 1% received all three doses during the study period. The mean age at vaccination was 25.1 years in Denmark and 21.2 years in Sweden. For both Denmark and Sweden, the majority of all vaccinations were administered in 2012 (56% and 46%, respectively).

We followed women in the cohort for 16 386 459 person-years according to qHPV vaccination status: 319 298 years amongst vaccinated and 16 067 162 years amongst unvaccinated. During the study period, 150 629 women were lost to follow-up (emigration $N = 142 127$, death $N = 7281$ or unexplained disappearance from the source registers $N = 1221$) and 591 were vaccinated with the bivalent HPV vaccine and consequently censored. This yielded crude incidence rates for 45 autoimmune and neurological study outcomes amongst qHPV-vaccinated and qHPV-unvaccinated women (Fig. 1).

We identified seven adverse events with statistically significant increased RRs associated with qHPV vaccination compared with four adverse events with statistically significant reduced RRs (Table 2). RRs for Hashimoto’s thyroiditis were increased for both the period any time after vaccination (RR 1.35, 95% confidence interval 1.10–1.67) and the more than 180 days after vaccination period (1.42, 1.08–1.88). RRs for coeliac disease were increased for both the period any time after vaccination (RR 1.56, 1.29–1.89), the first 179 days (1.54, 1.16–2.03) and the more than 180 days after vaccination period (1.58, 1.22–2.05). The RR for localized lupus erythematosus was increased for the period any time after vaccination (1.70, 1.01–2.86). For pemphigus vulgaris, the RR for the first 179 days was increased (8.75, 1.04–73.99), whereas the RR for Addison’s disease was increased in the more than 180 days after vaccination period (2.25, 1.10–4.59) and the RR for Raynaud’s disease was increased for the period any time after vaccination (1.46, 1.02–2.09). Finally, the RR for other encephalitis, myelitis or encephalomyelitis was increased in the more than 180 days after vaccination period (4.27, 1.00–18.35). The associations with pemphigus vulgaris, Addison’s disease and other encephalitis, myelitis or encephalomyelitis were based on few vaccinated cases ($n = 1$, 2 and 8, respectively, Table 2).

We conducted a number of sensitivity analyses (Table 3). Taking multiple testing into account and comparing the original cohort estimates with estimates derived from the SCCS method, only coeliac disease was still associated with qHPV vaccination. The RR was 1.56 (1.29–1.89) and 1.65 (1.20–2.27) for the period any time after vaccination for the cohort method and the SCCS method, respectively. This association was present throughout the vaccination period and appeared to be stronger in Denmark (1.74, 1.35–2.25) than in Sweden (1.21, 0.87–1.68). The crude incidence rates for coeliac disease in Denmark were 13.9 and 31.1 per 100 000 person-years for the unvaccinated and vaccinated periods, respectively. The corresponding Swedish rates were 34.2 and 50.9 per 100 000 person-years. In Fig. 2, we present coeliac cases occurring after vaccination ordered according to timing. Visual inspection reveals clustering in the first year after the first dose.

Discussion

In this large cohort study comprising more than 3 million Danish and Swedish adult women, we evaluated possible associations between qHPV vaccination and 45 preselected autoimmune and neurological outcomes. Comparing incidence rates in qHPV-vaccinated and qHPV-unvaccinated women, we identified seven adverse events with statistically significant increased risks following vaccination—Hashimoto’s thyroiditis, coeliac disease, localized lupus erythematosus, pemphigus vulgaris, Addison’s disease, Raynaud’s disease and other encephalitis, myelitis or encephalomyelitis. These increased risks were offset by four adverse events with significantly reduced risks. After sensitivity analyses, the association between qHPV vaccination and coeliac disease was the most robust; the remaining six associations with increased risks were not statistically significant when taking multiple testing into account or when using a case-only analytical approach. The observed association of a 56% increased risk of coeliac disease after qHPV vaccination was strong, and the increase was strikingly similar in both risk periods after vaccination. We did not see any evidence of consistency of effect because the increase was only observed in Denmark. However, a plot of the timing of coeliac diagnoses in relation to HPV vaccination dates reveals clustering extending beyond the first 179 days of the acute risk period. In our study, approximately half of all coeliac cases occurring after vaccination occurred
within 1 year of the first dose. To our knowledge, no previous study has linked qHPV vaccination and coeliac disease. Coeliac disease is an autoimmune condition triggered by dietary gluten in susceptible individuals with an estimated prevalence of 1% worldwide [20]. Scandinavia is
Table 2 Rate ratios of adverse events according to quadrivalent human papillomavirus vaccination status in a cohort of Danish and Swedish women 18–44 years of age in the period from 1 October 2006 to 30 June 2013 for Danish women or 31 December 2012 for Swedish women

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Unvaccinated Events</th>
<th>Any time after vaccination Events</th>
<th>0–179 days since vaccination Adjusted RR (95% CI)</th>
<th>≥180 days since vaccination Adjusted RR (95% CI)</th>
<th>Adjusted RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Autoimmune</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thyroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grave’s disease</td>
<td>7923</td>
<td>93</td>
<td>0.79 (0.64–0.97)</td>
<td>53</td>
<td>0.93 (0.71–1.22)</td>
</tr>
<tr>
<td>Hashimoto’s thyroiditis</td>
<td>4329</td>
<td>97</td>
<td>1.35 (1.10–1.67)</td>
<td>45</td>
<td>1.28 (0.94–1.72)</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>28,023</td>
<td>348</td>
<td>0.88 (0.79–0.98)</td>
<td>120</td>
<td>0.65 (0.54–0.78)</td>
</tr>
<tr>
<td>Other hyperthyroidisms</td>
<td>8,117</td>
<td>150</td>
<td>1.04 (0.88–1.22)</td>
<td>71</td>
<td>0.98 (0.77–1.25)</td>
</tr>
<tr>
<td><strong>Gastrointestinal</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Celiac disease</td>
<td>4,204</td>
<td>113</td>
<td>1.56 (1.29–1.89)</td>
<td>53</td>
<td>1.54 (1.16–2.03)</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>5,048</td>
<td>134</td>
<td>1.07 (0.90–1.28)</td>
<td>77</td>
<td>1.22 (0.97–1.54)</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>3,898</td>
<td>62</td>
<td>0.86 (0.66–1.11)</td>
<td>31</td>
<td>0.87 (0.61–1.24)</td>
</tr>
<tr>
<td>Primary biliary cirrhosis</td>
<td>182</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ulcerative colitis</td>
<td>7,727</td>
<td>187</td>
<td>1.09 (0.94–1.26)</td>
<td>100</td>
<td>1.16 (0.95–1.42)</td>
</tr>
<tr>
<td><strong>Musculoskeletal or systemic</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ankylosing spondylitis</td>
<td>1,919</td>
<td>41</td>
<td>1.20 (0.87–1.65)</td>
<td>17</td>
<td>1.00 (0.62–1.63)</td>
</tr>
<tr>
<td>Behcet’s syndrome</td>
<td>190</td>
<td>2</td>
<td>0.62 (0.15–2.56)</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Henoch–Schönlein’s purpura</td>
<td>341</td>
<td>9</td>
<td>0.90 (0.46–1.79)</td>
<td>7</td>
<td>1.40 (0.64–3.04)</td>
</tr>
<tr>
<td>Myositis</td>
<td>918</td>
<td>9</td>
<td>0.76 (0.39–1.47)</td>
<td>4</td>
<td>0.71 (0.26–1.92)</td>
</tr>
<tr>
<td>Polymyositis/dermatomyositis</td>
<td>173</td>
<td>2</td>
<td>0.68 (0.17–2.83)</td>
<td>2</td>
<td>–</td>
</tr>
<tr>
<td>Reiter’s syndrome</td>
<td>29</td>
<td>1</td>
<td>1.96 (0.24–15.92)</td>
<td>0</td>
<td>–</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>6,251</td>
<td>84</td>
<td>0.98 (0.79–1.22)</td>
<td>38</td>
<td>0.92 (0.66–1.27)</td>
</tr>
<tr>
<td>Sjögren’s syndrome</td>
<td>1,342</td>
<td>13</td>
<td>1.25 (0.72–2.19)</td>
<td>7</td>
<td>1.48 (0.70–3.15)</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>1,447</td>
<td>18</td>
<td>0.80 (0.50–1.28)</td>
<td>8</td>
<td>0.73 (0.36–1.47)</td>
</tr>
<tr>
<td>Systemic sclerosis (scleroderma)</td>
<td>297</td>
<td>7</td>
<td>1.83 (0.84–3.99)</td>
<td>4</td>
<td>2.13 (0.77–5.91)</td>
</tr>
<tr>
<td>Vasculitis—unspecified</td>
<td>1,301</td>
<td>23</td>
<td>1.03 (0.68–1.58)</td>
<td>11</td>
<td>1.01 (0.55–1.85)</td>
</tr>
<tr>
<td>Wegener’s granulomatosis</td>
<td>171</td>
<td>4</td>
<td>1.44 (0.52–4.04)</td>
<td>1</td>
<td>0.72 (0.10–5.27)</td>
</tr>
<tr>
<td><strong>Haematological</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Autoimmune haemolytic anaemia</td>
<td>159</td>
<td>3</td>
<td>1.36 (0.42–4.42)</td>
<td>2</td>
<td>1.89 (0.45–7.92)</td>
</tr>
<tr>
<td>Idiopathic thrombocytopenic purpura</td>
<td>797</td>
<td>23</td>
<td>1.38 (0.90–2.12)</td>
<td>14</td>
<td>1.70 (0.98–2.94)</td>
</tr>
<tr>
<td>Pernicious anaemia</td>
<td>310</td>
<td>8</td>
<td>1.57 (0.76–3.27)</td>
<td>5</td>
<td>2.04 (0.82–5.10)</td>
</tr>
</tbody>
</table>
Table 2 (Continued)

<table>
<thead>
<tr>
<th>Adverse events</th>
<th>Unvaccinated</th>
<th>Any time after vaccination</th>
<th>0–179 days since vaccination</th>
<th>≥180 days since vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Events</td>
<td>Adjusted RR (95% CI)</td>
<td>Events</td>
<td>Adjusted RR (95% CI)</td>
</tr>
<tr>
<td>Dermatological</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythema nodosum</td>
<td>1047</td>
<td>29 (1.26 (0.86–1.85))</td>
<td>17 (1.50 (0.92–2.46))</td>
<td>12 (1.04 (0.58–1.84))</td>
</tr>
<tr>
<td>Localized lupus erythematosus</td>
<td>619</td>
<td>16 (1.70 (1.01–2.86))</td>
<td>8 (1.71 (0.84–3.51))</td>
<td>8 (1.69 (0.83–3.44))</td>
</tr>
<tr>
<td>Localized scleroderma</td>
<td>418</td>
<td>9 (1.44 (0.73–2.85))</td>
<td>6 (2.07 (0.90–4.75))</td>
<td>3 (0.90 (0.29–2.84))</td>
</tr>
<tr>
<td>Pemphigoid</td>
<td>75</td>
<td>2 (2.48 (0.57–10.71))</td>
<td>1 (2.80 (0.37–21.47))</td>
<td>1 (2.22 (0.30–16.55))</td>
</tr>
<tr>
<td>Pemphigus foliaceus</td>
<td>12</td>
<td>0 –</td>
<td>0 –</td>
<td>0 –</td>
</tr>
<tr>
<td>Pemphigus vulgaris</td>
<td>29</td>
<td>1 (3.42 (0.42–27.81))</td>
<td>1 (8.75 (1.04–73.99))</td>
<td>0 –</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>11 138</td>
<td>116 (0.96 (0.80–1.16))</td>
<td>57 (1.06 (0.82–1.38))</td>
<td>59 (0.88 (0.68–1.14))</td>
</tr>
<tr>
<td>Vitiligo</td>
<td>1382</td>
<td>14</td>
<td>1.00 (0.59–1.71)</td>
<td>10</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute rheumatic fever</td>
<td>72</td>
<td>1 (0.50 (0.07–3.75))</td>
<td>0 –</td>
<td>1 –</td>
</tr>
<tr>
<td>Addison’s disease</td>
<td>427</td>
<td>12</td>
<td>1.71 (0.94–3.12)</td>
<td>4</td>
</tr>
<tr>
<td>Raynaud’s disease</td>
<td>1367</td>
<td>33</td>
<td>1.46 (1.02–2.09)</td>
<td>17</td>
</tr>
<tr>
<td>Sarcoidosis</td>
<td>1784</td>
<td>36</td>
<td>1.31 (0.93–1.85)</td>
<td>16</td>
</tr>
<tr>
<td>Type 1 diabetes</td>
<td>4318</td>
<td>63</td>
<td>0.93 (0.72–1.19)</td>
<td>28</td>
</tr>
<tr>
<td>Neurological</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Bell’s palsy</td>
<td>4515</td>
<td>53</td>
<td>0.72 (0.55–0.95)</td>
<td>21</td>
</tr>
<tr>
<td>Epilepsy</td>
<td>7878</td>
<td>151</td>
<td>0.91 (0.77–1.07)</td>
<td>84</td>
</tr>
<tr>
<td>Guillain–Barré syndrome</td>
<td>194</td>
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<td>0 –</td>
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<tr>
<td>Myasthenia gravis</td>
<td>227</td>
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<td>1.07 (0.39–2.96)</td>
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<tr>
<td>Narcolepsy</td>
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<td>21</td>
<td>1.42 (0.89–2.24)</td>
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<tr>
<td>Other encephalitis, myelitis, encephalomyelitis</td>
<td>62</td>
<td>3</td>
<td>3.40 (0.99–11.75)</td>
<td>1</td>
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<tr>
<td>Paralysis</td>
<td>2574</td>
<td>18</td>
<td>0.52 (0.32–0.83)</td>
<td>7</td>
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</tbody>
</table>

Rate ratios are adjusted for age, calendar period and country of residence. Significant associations are in bold.
considered a high-prevalence area, and it has been suggested that the condition is underdiagnosed in Denmark when using hospital register data for case ascertainment. In a small screening study, Danish colleagues concluded that coeliac disease was markedly underdiagnosed in the general adult population and estimated a prevalence of 0.48% that was several times higher than the registry-based prevalence in Denmark [21].

Unmasking of pre-existing conditions at vaccination visits has been described for adolescents and young adults in the context of qHPV vaccination; the vaccination visit triggers a work-up of symptoms that later result in a diagnosis [22]. Unmasking of an underreported disease such as coeliac disease in qHPV-vaccinated Danish women is a possible explanation for the increased RR and is also consistent with the clustering after the first dose that we observed.

We have previously evaluated the risk of serious chronic disease in qHPV-vaccinated compared to qHPV-unvaccinated girls aged 10–17 in a similar setting [16]. In line with the present study, we found no support for associations between qHPV vaccination and autoimmune and neurological adverse events. To our knowledge, no previous study has evaluated qHPV vaccine safety in women older than 26 years of age [23, 24]. In our study, the mean age at vaccination was 25.1 years, and

### Table 3  Sensitivity analyses. Adjusted rate ratios with 95% confidence intervals for different analytical scenarios

<table>
<thead>
<tr>
<th>Association</th>
<th>Original estimate</th>
<th>With Bonferroni correction</th>
<th>Only Danish data</th>
<th>Only Swedish data</th>
<th>Self-controlled data case series method</th>
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</thead>
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<tr>
<td>Hashimoto’s thyroiditis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any time after vaccination</td>
<td>1.35 (1.10–1.67)</td>
<td>1.35 (0.96–1.91)</td>
<td>1.18 (0.93–1.51)</td>
<td>1.81 (1.20–2.74)</td>
<td>1.30 (0.94–1.80)</td>
</tr>
<tr>
<td>≥180 days since vaccination</td>
<td>1.42 (1.08–1.88)</td>
<td>1.42 (0.90–2.26)</td>
<td>1.27 (0.91–1.77)</td>
<td>1.70 (1.02–2.84)</td>
<td>1.30 (0.80–2.11)</td>
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<tr>
<td>Coeliac disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Any time after vaccination</td>
<td>1.56 (1.29–1.89)</td>
<td>1.56 (1.13–2.15)</td>
<td>1.74 (1.35–2.25)</td>
<td>1.21 (0.87–1.68)</td>
<td>1.65 (1.20–2.27)</td>
</tr>
<tr>
<td>0–179 days since vaccination</td>
<td>1.54 (1.16–2.03)</td>
<td>1.54 (0.97–2.44)</td>
<td>1.66 (1.20–2.31)</td>
<td>1.05 (0.58–1.89)</td>
<td>1.64 (1.17–2.30)</td>
</tr>
<tr>
<td>≥180 days since vaccination</td>
<td>1.58 (1.22–2.05)</td>
<td>1.58 (1.03–2.43)</td>
<td>1.85 (1.30–2.61)</td>
<td>1.30 (0.87–1.93)</td>
<td>1.68 (1.06–2.67)</td>
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<td>Localized lupus erythematosus</td>
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<tr>
<td>Any time after vaccination</td>
<td>1.70 (1.01–2.86)</td>
<td>1.70 (0.72–4.03)</td>
<td>1.93 (1.10–3.39)</td>
<td>0.55 (0.08–3.91)</td>
<td>1.95 (0.93–4.08)</td>
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<td>Pemphigus vulgaris</td>
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<td>0–179 days since vaccination</td>
<td>8.75 (1.04–73.99)</td>
<td>8.75 (0.25–305.06)</td>
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<td>Not estimable</td>
<td>Not estimable</td>
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<td>Addison’s disease</td>
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<td></td>
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<tr>
<td>≥180 days since vaccination</td>
<td>2.25 (1.10–4.59)</td>
<td>2.25 (0.68–7.38)</td>
<td>2.93 (1.41–6.10)</td>
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<td>Raynaud’s disease</td>
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<tr>
<td>Any time after vaccination</td>
<td>1.46 (1.02–2.09)</td>
<td>1.46 (0.81–2.65)</td>
<td>1.16 (0.73–1.84)</td>
<td>2.04 (1.14–3.64)</td>
<td>1.17 (0.67–2.05)</td>
</tr>
<tr>
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<td></td>
<td></td>
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</tr>
<tr>
<td>≥180 days since vaccination</td>
<td>4.27 (1.00–18.35)</td>
<td>4.27 (0.38–48.28)</td>
<td>2.81 (0.34–23.46)</td>
<td>Not estimable</td>
<td>2.79 (0.07–116.95)</td>
</tr>
</tbody>
</table>
our study is the first to provide extensive safety information in women 26–44 years of age. Baseline disease risks can vary greatly in girls compared to adult women, and it is not unreasonable to expect that different safety issues will arise in different age groups. It should also be noted that HPV vaccination of older women is not primarily an issue in the introductory phase of HPV vaccination programmes, but a continuing issue with less than optimal coverage or delayed vaccination because of vaccine safety scares. Consequently, studies such as ours are an important supplement to HPV vaccine safety studies in the younger programme girls.

Our study has a number of strengths. We were able to combine nationwide individual-level data from two Scandinavian countries with similar health care. This not only provided us with an impressive cohort size of more than 3 million women, but also reduced the potential for forms of selection and ascertainment bias seen with, for example, case–control studies. Adult women actively seeking HPV vaccination could be a selected group with respect to comorbidity, socio-economic and lifestyle factors compared to the general population of women. We were able to address confounding by conducting sensitivity analyses with the SCCS method, which takes into account confounding and bias by factors that do not vary during the study period. Although the cohort results do not suggest a clear pattern of bias with seven increased risks and four reduced of 45 possible, we cannot exclude that unmeasured confounding is present in some form. However, we are not aware of any obvious sources of bias.

Our study relies on identifying serious chronic disease through hospital registers. In Scandinavia, serious chronic disease will be diagnosed in the hospital setting and captured in our study if it is diagnosed. We expect the majority of outcomes in our study to be captured in hospital data, but some less severe conditions might only be diagnosed in primary care or remain undiagnosed entirely. All subsidized vaccinations and vaccinations obtained through prescriptions will be captured by the respective vaccination and prescription registers. Exposure ascertainment in this study can thus be considered close to complete. For many of the included outcomes, there will be a delay between onset and diagnosis. We included two risk periods of interest in our study: a 180-day period for outcomes with little delay between onset and diagnosis, and a period following 180 days for outcomes with a more insidious onset. Although our study cohort was large, many of the included outcomes are rare and null findings should be interpreted in the context of statistical power. It will be important to continue to monitor HPV vaccine safety in future when statistical power is increased and more definite conclusions can be reached.
In conclusion, our study of serious adverse event rates in qHPV-vaccinated and qHPV-unvaccinated adult women 18–44 years of age did not raise any safety issues of concern.

Author contributions

Hviid had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Hviid conceived of and designed the study. Scheller, Svanström, Arnheim-Dahlström and Hviid obtained the data. Svanström conducted the statistical analyses. All authors contributed to the interpretation of results. Hviid drafted the manuscript. Scheller, Pasternak, Svanström, Arnheim-Dahlström and Grönlund critically revised the manuscript. Hviid and Arnheim-Dahlström obtained the funding for the study.

Conflict of interest statement

Scheller, Hviid, Svanström, Pasternak have no conflicts of interest to report. Arnheim-Dahlström has obtained funding from MDS Sanofi Pasteur and GlaxoSmithKline for unrelated studies.

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Role of sponsor

The funding agencies had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

References


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**Supporting Information**

Additional Supporting Information may be found in the online version of this article:

**Table S1.** Study outcomes with ICD-10 codes.
Inflammatory bowel disease (IBD) is a broad term that describes conditions characterized by chronic inflammation of the gastrointestinal tract. The two most common inflammatory bowel diseases are ulcerative colitis and Crohn's disease. Inflammation affects the entire digestive tract in Crohn's disease and only the large intestine (also called the colon) in ulcerative colitis. Both illnesses involved an abnormal response to the body's immune system.
DATA & STATISTICS

RESOURCES

Highlights

Hospitalizations for IBD Among Older Adults

MMWR: Prevalence of Inflammatory Bowel Disease Among Adults Aged ≥ 18 Years — United States, 2015

Featured Topics
The CDC Inflammatory Bowel Disease (IBD) Program creates featured topics in order to bring attention to important IBD issues and information.
Inflammatory bowel disease (IBD)

Data and Statistics

Inflammatory Bowel Disease Prevalence (IBD) in the United States

In 2015, an estimated 1.3% of US adults (3 million) reported being diagnosed with IBD (either Crohn's disease or ulcerative colitis). This was a large increase from 1999 (0.9% or 2 million adults).

Some people were more likely to report having IBD, including those:

- Aged 45 years or older.
- Hispanic or non-Hispanic white.
- With less than a high school level of education.
- Not currently employed.
- Born in the United States (compared with adults born outside of the United States).
- Living in poverty.
- Living in suburban areas.

This estimate does not include children younger than 18 years, who may also have IBD. Most people with IBD are diagnosed in their 20s and 30s.

On the basis of the National Inpatient Sample data, there was no significant change in the hospitalization rate when Crohn's disease was the primary diagnosis from 2003 to 2013. The hospitalization rate, however, increased significantly during this period from 44.2 to 59.7 per 100,000 population when it was listed as any secondary diagnosis. The mean hospitalization costs were $11,345 for Crohn's disease and $13,412 for ulcerative colitis. From 2003 to 2008, total hospitalization costs increased annually by 3% for Crohn's disease and 4% for ulcerative colitis but remained unchanged for both diseases from 2008 to 2014.

Compared with adults without IBD, those with IBD are more likely to have certain chronic health conditions that include:

- Cardiovascular disease (CVD).
- Respiratory disease.
- Cancer.
- Arthritis.
- Kidney disease.
- Liver disease.
In addition, clinicians should be aware of potential health-risk behaviors that are more prevalent among adults with IBD than those without, such as:

- Not getting enough sleep.
- Not meeting aerobic nor muscle-strengthening physical activity guidelines.

Since IBD is associated with various chronic and infectious conditions, preventive care is an essential aspect of lifelong disease management. The American College of Gastroenterology published clinical guidelines for disease prevention among people with IBD. The first U.S. population-based study using 2015 and 2016 National Health Interview Survey results reported that adults with IBD were more likely than adults without IBD to receive preventive care services, which included:

- Receiving medical advice about smoking cessation and healthy diet.
- Having colon cancer screening in the past 12 months.
- Having ever had an HIV test.
- Having ever had a pneumococcal vaccine.
- Having received a flu vaccine in the past 12 months.
- Having received a tetanus vaccine in the past 10 years.

References


Content source: National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention
EXHIBIT 202
Inflammatory Bowel Disease in Children and Adolescents

Michael J. Rosen, MD, MSCI; Ashish Dhawan, MBBS, MSPH; Shehzad A. Saeed, MD

Epidemiologic Features
The inflammatory bowel diseases (IBDs), including ulcerative colitis and Crohn disease, are chronic inflammatory disorders of the gastrointestinal tract that most often diagnosed in adolescence and young adulthood, with a rising incidence in pediatric populations. These disorders are common enough in children that most pediatricians and other pediatric clinicians will encounter children with IBD in their general practice. Inflammatory bowel disease is caused by a dysregulated mucosal immune response to the intestinal microflora in genetically predisposed hosts. Although children can present with the classic symptoms of weight loss, abdominal pain, and bloody diarrhea, many present with nonclassic symptoms of isolated poor growth, anemia, or other extraintestinal manifestations. Once IBD is diagnosed, the goals of therapy consist of eliminating symptoms, normalizing quality of life, restoring growth, and preventing complications while minimizing the adverse effects of medications. Unique considerations when treating children and adolescents with IBD include attention to the effects of the disease on growth and development, bone health, and psychosocial functioning. The purpose of this review is to provide a contemporary overview of the epidemiologic features, pathogenesis, diagnosis, and management of IBD in children and adolescents.

Pathogenesis
In patients with IBD, host genetic, environmental, and microbial influences converge and result in a dysregulated mucosal immune response against the commensal intestinal microbiota. Recent technological advances have led to an explosion of discovery of the genetic and microbial influences on IBD. Genome-wide association studies have identified common variants in more than 150 genes that confer risk for IBD. Risk variants can be grouped into biological pathways that shed light on IBD pathogenesis, including innate and adaptive immunity and epithelial function. No difference exists in the common risk genes between pediatric- and adult-onset IBD; however, early-onset IBD may be associated with a higher burden of common risk variants and rarer variants with high penetrance.

Three important observations underscore the importance of the environment on the development of IBD. First, the concordance rate for CD in monozygotic twins is only 50% and even less for UC. Second, the rising incidence of IBD during the past 60 years is too fast to be explained by changes in our genetic makeup. Third, IBD is less common in developing countries, but, as countries become more developed, the incidence of IBD also rises. Furthermore, children of those who immigrate from developing countries to Western countries exhibit an incidence of IBD similar to that of Western populations. Therefore, early-life environmental factors associated with a Western lifestyle may predispose to IBD. In fact, cesarean delivery, lack of exposure to breast milk, dietary fat intake, and early exposure to antibiotics have all been implicated as risk factors for IBD.

Most humans live in harmony each day with the 10 trillion bacteria and fungi that constitute our intestinal microbiome, a relationship that is quite remarkable when one considers that only a single layer of intestinal epithelial cells separates these organisms from circulating mucosal immune cells. Thus, investigators have an intense interest in understanding how the gut microbiome contributes to IBD. Animal studies have demonstrated the fundamental role of the microbiome in the development of IBD because intestinal inflammation does not develop in most rodent models of IBD raised in germ-free conditions. Children and adults with IBD are known to exhibit a dysbiosis with an overall restriction in the diversity of intestinal bacterial species and overrepresentation and underrepresentation of specific taxa.

Disease Classification
Inflammatory bowel disease is classified into UC and CD. Ulcerative colitis is characterized by diffuse, continuous inflammation of the colon extending from the rectum proximally. Patients with UC and diffuse pancolitis can exhibit mild inflammation of the ileum termed backwash ileitis. In addition, 40% to 70% of patients with UC exhibit mild inflammation in the upper gastrointestinal tract.
Crohn disease can involve any area in the gastrointestinal tract from the mouth to the anus but most commonly involves the terminal ileum and colon and can present with an inflammatory, penetrating, stricturing, or combination phenotype. Endoscopic features that distinguish CD from UC include discontinuous inflammation and discrete aphthous or linear ulceration (Figure). In addition, 20% of children with CD will have perianal involvement, including skin tags, fissures, fistulas, and/or abscesses. 

Histologic features common to CD and UC include evidence of active inflammation (ie, neutrophils) and chronicity (ie, crypt loss or branching, mucin depletion, and/or lamina propria lymphocytosis). Although the inflammation and injury in UC is limited to the mucosa, CD can be a transmural process. Noncaseating granulomas are observed in as many as 60% of pediatric patients with CD and, in the right clinicopathologic setting, can distinguish CD from UC.

Crohn disease involving the colon only is more common in children than adults, which makes it difficult to distinguish CD and UC in some patients. The term IBD unspecified (previously called indeterminate colitis) is reserved for patients who cannot be classified definitively as having UC or CD.

### Diagnosis

#### Clinical Presentation

The presentation of IBD in children and adolescents can be variable. The reported incidence of presenting signs and symptoms is detailed in the Table. Pediatricians and other primary care clinicians should become familiar with the atypical presentations of IBD because 22% of children present with growth failure, anemia, perianal disease, or other extraintestinal manifestations as the only predominant initial feature. Extraintestinal manifestations associated with IBD are detailed in Box 1. A detailed family history should be obtained because 20% of children with IBD have an affected relative.

#### Physical Examination

A careful physical examination may provide clues to underlying pathologic features. Assessment of growth curves is critical; although some patients may present with acute weight loss, others will present with a more insidious chronic flattening of their weight and height curves (Figure). Conversely, the possibility of IBD in obese patients should not be dismissed because 25% of children with IBD are obese. Abdominal examination may reveal focal tenderness or fullness related to the distribution of their disease. Rebound tenderness and guarding may indicate perforation or abscess that should be evaluated promptly with imaging. The perianal region should be examined for tags, fissures, fistulas, or abscesses. Digital rectal examination may provide information regarding anal strictures, fluctuance from an abscess, or occult blood. Other potential findings of the physical examination may include oral aphthous ulcers, clubbing, signs of delayed puberty, and skin lesions, such as erythema nodosum and pyoderma gangrenosum.

#### Laboratory Examinations

Common abnormal laboratory findings in children with IBD at diagnosis include anemia, thrombocytosis, hypoalbuminemia, and elevated levels of inflammatory markers. The recommended initial laboratory evaluation in a patient with suspected IBD is detailed in Box 2. A normal laboratory evaluation result does not exclude a diagnosis of IBD because approximately 10% to 20% of children with IBD will have normal laboratory results. stool should be examined for occult blood, bacterial pathogens (including Escherichia coli, Salmonella spp, Campylobacter jejuni, and Yersinia enterocolitica). Fecal calprotectin, a neutrophil-derived protein with elevated concentrations in the setting of intestinal inflammation, is emerging as a useful biomarker, with 98% sensitivity and 68% specificity in children with suspected IBD.

#### Endoscopy

Prompt referral to a pediatric gastroenterologist for endoscopic evaluation is appropriate when a primary care clinician suspects IBD in a child based on clinical and laboratory findings. Esophagogastroduodenoscopy and ileocolonoscopy with biopsy remain the criterion standard for the diagnosis and classification of IBD in children. Gross and histopathologic findings are crucial for determining disease severity and extent and for distinguishing UC from CD. Video capsule endoscopy may be indicated to evaluate the proximal small intestine when a high suspicion for CD exists and the diagnosis cannot be confirmed by results of conventional endoscopy and imaging.

#### Imaging

Small-bowel imaging is essential for mapping disease location, assessing severity, and identifying complications, such as fistulas, abscesses, and intestinal strictures. Imaging should be performed after endoscopic diagnosis because imaging is not as sensitive for colonic and mild small-bowel disease, and specialized imaging protocols are used to evaluate IBD. Cross-sectional enterography, including computed tomographic enterography and magnetic resonance enterography, has replaced fluoroscopic small-bowel follow-through as the modality of choice. Both modes of enterography permit assessment of the lumen, the mucosa, the bowel wall,
and intra-abdominal complications. Pelvic magnetic resonance imaging and rectal endoscopic ultrasonography in centers with expertise are the preferred modalities for evaluating perianal abscesses and fistulas.32

Figure. Representative Endoscopic Images of Normal and Inflamed Gastrointestinal Mucosa From Pediatric Patients With and Without Inflammatory Bowel Disease

A. Normal terminal ileal mucosa
B. Normal colonic mucosa
C. Severely inflamed terminal ileum
D. Deep linear ulceration
E. Scattered aphthous ulcers
F. Inflamed colonic mucosa

A, A vascular pattern, villous epithelium, and normal lymphoid nodularity are visible. B, A thin transparent glistening mucosa and delicate vascular network are visible. C, Terminal ileum in a child with Crohn disease (CD) shows mucosal thickening and erythema, complete loss of vascular pattern, pseudopolyps surrounded by deep ulceration, and luminal narrowing. D, Linear ulcer directly adjacent to normal colon mucosa in a young child with CD. E, Tissue in the stomach antrum of a 10-year-old child with CD. F, Adolescent with ulcerative colitis has diffuse erythema, loss of vascular pattern, and granular-appearing superficial ulceration.

Treatment
Overall Treatment Goals and Strategy
The goals of treatment of IBD in children have changed dramatically in the past 15 years. When treatment options were limited, the primary goal was the reduction of symptoms. Now, with biologics targeting tumor necrosis factor (TNF) that can heal the mucosa and augment growth, we have the opportunity to modify the natural history of the disease. Therefore, the current goals of treatment are to (1) eliminate symptoms and restore quality of life, (2) restore normal growth, and (3) eliminate complications.

Therapies for IBD may be broadly classified according to their ability to induce remission of active disease and maintain remission in patients with quiescent disease. Some therapies are effective only for remission induction or maintenance, whereas others are appropriate for both indications.
Corticosteroids

Corticosteroids are effective for the induction of clinical remission in CD and UC in children; however, approximately half of the patients will become dependent on corticosteroids or require surgery.33 Fewer than one-third of patients with CD in clinical remission with corticosteroid treatment will achieve mucosal healing.34 Corticosteroids are not appropriate as maintenance therapy owing to the panoply of well-established adverse effects with long-term use.

Budesonide is a high potency corticosteroid that undergoes extensive first-pass metabolism in the liver, limiting the systemic bioavailability and adverse effects. Controlled-release budesonide formulations are effective for induction of remission in UC and CD; however, they are not effective as maintenance therapy.35,36 Although budesonide formulations are appealing owing to their reduced adverse effect profile, they are not as effective as conventional corticosteroids and are reserved for mild to moderately active disease.

Enteral Nutrition Therapy

Treatment with exclusive enteral nutrition (EEN), defined as the provision of essentially 100% of caloric needs by liquid formula, is as effective as corticosteroid therapy for inducing clinical remission in children with CD.37 Duration of EEN therapy typically is 8 to 12 weeks. Advantages of this approach compared with corticosteroids include support of growth by EEN, avoidance of corticosteroid-associated adverse effects, and more effective healing of the mucosa.37 The primary disadvantage of EEN is the strict liquid formula diet, which requires many patients to place a nasogastric tube each evening (or keep it in all day) for nocturnal continuous feeding. Exclusive enteral nutrition is widely used as first-line induction therapy in Europe and is gaining increasing traction in the United States. Inflammation and symptoms will return with discontinuation of EEN therapy; therefore, EEN is often used in combination with maintenance medical therapy. Some success has been reported using various partial enteral nutrition regimens as maintenance therapy, such as overnight feedings with a normal daytime diet or nasogastric feeding for 1 of every 4 months.38

Aminosalicylates

Aminosalicylates exert a topical anti-inflammatory effect on the intestinal mucosa. They can be administered orally in formulations that release the active moiety 5-aminosalicylic acid (5-ASA) in the ileum and colon or topically via enema or suppository. Sulfasalazine has been used for more than 40 years to treat IBD, but many patients cannot tolerate sulfa-related adverse effects (ie, nausea, headache, fever, and rash). Therefore, newer sulfa-free 5-ASA drugs (mesalamine, balsalazide disodium, and osalazine sodium) have been developed that deliver high concentrations of 5-ASA to the intestinal mucosa with fewer adverse effects. The 5-ASA drugs are effective for the induction and maintenance of remission in adults with mildly to moderately active UC, but few clinical trials have been conducted in children.39 Balsalazide, the only 5-ASA agent with an indication from the US Food and Drug Administration in children, induces a clinical response at 8 weeks in 45% of children with mildly to moderately active UC and remission in 12%.40 A large observational study showed that 30% of children with UC will maintain remission with administration of 5-ASA drugs alone.41 Although 5-ASA drugs are still commonly prescribed for CD, systematic reviews do not support their efficacy.42 Rare adverse effects of 5-ASA include paradoxical exacerbation of colitis, interstitial nephritis, pericarditis, and pneumonitis.

### Table. Clinical Presentation of IBD in Children and Adolescents

<table>
<thead>
<tr>
<th>Presenting Symptom</th>
<th>Classification of IBD, % of Patientsa</th>
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</thead>
<tbody>
<tr>
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<td>Crohn Disease</td>
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<td>General</td>
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<td>Weight loss</td>
<td>55-80</td>
</tr>
<tr>
<td>Fever</td>
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<tr>
<td>Anorexia</td>
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<tr>
<td>Growth retardation</td>
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<tr>
<td>Lethargy</td>
<td>13-27</td>
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<tr>
<td>Diarrhea</td>
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</tr>
<tr>
<td>Rectal bleeding</td>
<td>22-49</td>
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<td>Constipation</td>
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<tr>
<td>Perianal disease</td>
<td>6-15</td>
</tr>
<tr>
<td>Mouth ulcers</td>
<td>5-28</td>
</tr>
</tbody>
</table>

Abbreviations: IBD, inflammatory bowel disease; NA, not applicable.

* Ranges are derived from data reported by Kugathasan et al,20 Griffiths,24 and Sawczenko and Sandhu.22

### Box 1. Extraintestinal Manifestations of Inflammatory Bowel Disease in Children and Adolescents

<table>
<thead>
<tr>
<th>Dermatologic</th>
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<tbody>
<tr>
<td>Erythema nodosum</td>
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<td>Pyoderma gangrenosum</td>
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<table>
<thead>
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<table>
<thead>
<tr>
<th>Renal</th>
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<tbody>
<tr>
<td>Nephrolithiasis</td>
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<th>Pancreatic</th>
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<tr>
<td>Pancreatitis</td>
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<th>Hematologic</th>
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<tr>
<td>Anemia</td>
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<td>Venous thromboembolism</td>
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Immunomodulators

Thiopurine drugs, including azathioprine sodium and its active metabolite mercaptopurine (6-MP), have been used for the treatment of IBD for more than 30 years. Given their delayed onset of effect of several weeks, thiopurines are mainly effective as maintenance therapies. A landmark multicenter, randomized clinical trial demonstrated that early (within the first 8 weeks of a diagnosis) introduction of 6-MP reduces corticosteroid exposure and improves the maintenance of clinical remission in children with CD.43 In a similar fashion, observational studies support the use of thiopurines in children with UC refractory to 5-ASA drugs.44 Adverse effects associated with thiopurines include myelosuppression, elevated transaminase levels, and pancreatitis. A small increased risk for lymphoma associated with thiopurines has been noted, with an absolute risk of 4.5 per 10,000 patient-years in children receiving thiopurines compared with 0.6 per 10,000 patient-years in the general pediatric population.45

Methotrexate sodium is another immunomodulator being used with increased frequency given concerns regarding the modest risk for lymphoma with thiopurines. Large retrospective cohort studies support the use of methotrexate as effective in maintaining clinical remission in about one-third of children with CD.46 Methotrexate may also be used for maintenance of remission in pediatric UC. Adverse effects of methotrexate include nausea, hepatotoxicity, and myelosuppression. Patients should take a daily folic acid supplement when receiving methotrexate.

Anti-TNF Therapy

The introduction of therapeutic monoclonal antibodies directed against TNF, a major proinflammatory pathogenic cytokine in CD and UC, has revolutionized the treatment of IBD. These anti-TNF biologics are administered by infusion (infliximab) or subcutaneous injection (adalimumab, certolizumab pegol, and golimumab). Infliximab, the first anti-TNF drug introduced in 1998, has been studied in well-designed trials in children and has been indicated by the US Food and Drug Administration for the treatment of moderately to severely active CD and UC in children. In children with CD, 88% respond to infliximab with 56% in remission at 1 year.47 For UC, 73% respond with 39% in remission at 1 year.48 Adalimumab also has demonstrated efficacy for the treatment of moderately to severely active CD in children and is approved by the US Food and Drug Administration for this indication.49 Anti-TNF drugs are typically used in children with IBD refractory to corticosteroids or in those who are corticosteroid dependent despite immunomodulator therapy. They are sometimes used in conjunction with immunomodulators, and, in adults, the combination of infliximab and azathioprine is more effective than either agent alone.50

Anti-TNF agents are superior to thiopurines for inducing complete mucosal healing of the intestine and are the only class of drugs with demonstrated ability to heal perianal fistulas completely in CD.51 Furthermore, infliximab has been shown to improve linear growth in children with associated growth failure.52 For these reasons, anti-TNF agents may be prescribed as first-line therapy for CD in children with severe deep mucosal ulcerations, perianal fistulas, and/or significant growth failure.

Adverse effects of anti-TNF biologics include infusion or injection site reactions and a psoriasis-like rash. Anti-TNF drugs increase the risk for infection, in particular fungal, viral, and mycobacterial infections. All patients must be screened for latent tuberculosis infection before initiation of anti-TNF therapy.

Any risk for lymphoma associated with anti-TNF therapy alone has been difficult to discern because, in adults, anti-TNF agents are commonly prescribed in conjunction with thiopurines or in patients with previous exposure to thiopurines. A recent systematic review in children with IBD treated with anti-TNF biologics identified 2 cases of lymphoma during 9516 patient-years of follow-up. This risk of 2.1 per 10,000 patient-years in patients treated with anti-TNF drugs was statistically similar to the 0.6 per 10,000 patient-years in the general pediatric population.55

Since 1996, reports of a rare and particularly lethal form of hepatosplenic T-cell lymphoma in patients treated with anti-TNF drugs and thiopurines have accumulated. From 1996 through 2010, 36 cases of hepatosplenic T-cell lymphoma were reported in patients with IBD. All had received combination therapy with an anti-TNF drug and thiopurine or treatment with thiopurine alone, and none received anti-TNF drugs alone. Most of the patients had received thiopurines for at least 2 years and were males younger than 35 years.52 Therefore, the risks of combination therapy with an anti-TNF drug and thiopurine, particularly in young men, must be weighed carefully against the anticipated benefits.

Surgery

Surgery is an important therapeutic option in the comprehensive management of UC and CD in children. Total colectomy with ileal pouch anal anastomosis is indicated in children with UC refractory to medical therapy. In this procedure, the diseased colon is removed, and a pouch reservoir is constructed from the distal ileum and anastomosed to a short cuff of remaining rectum to preserve continuity and avoid a permanent ileostomy. Children have excellent long-term outcomes after this procedure, with a quality of life similar to that of the general population.53

Owing to the transmural nature of the inflammation in CD, complications such as fistulas, intra-abdominal abscesses, and bowel strictures can arise that require surgery. Surgery may also be indicated in CD when disease is refractory to medical therapy. Among children with CD, 14% require intra-abdominal surgery within 5 years of diagnosis.54
Long-term Sequelae

Micronutrient Deficiencies

Disease factors that include chronic blood loss, intestinal malabsorption, decreased intake, and chronic inflammation place patients with IBD at risk for deficiencies of various micronutrients, such as iron, folate, vitamin B₁₂, and vitamin D. Vitamin D deficiency (25-hydroxyvitamin D level, <15 ng/mL [to convert to nanomoles per liter, multiply by 2.496]) occurs in 35% of children with IBD, and 60% exhibit suboptimal levels (25-hydroxyvitamin D level, <30 ng/mL). Although the role of vitamin D for supporting intestinal calcium absorption and bone health is well established, mounting evidence suggests that vitamin D also maintains intestinal immune homeostasis and epithelial integrity. Children with low vitamin D levels are more likely to have disease recurrence, and maintaining serum vitamin D levels greater than 30 ng/mL increases the likelihood of maintaining clinical remission.

Growth and Bone Health

Growth failure occurs in approximately 40% and 10% of children with CD and UC, respectively. The cause of growth failure in pediatric IBD is multifactorial and includes decreased intake, increased metabolic demand, malabsorption, cytokine-induced growth hormone resistance, and corticosteroids. Nineteen percent of children with CD achieve an adult height 8 cm shorter than expected. Thus, close monitoring of linear growth is imperative, and treatment should be directed at restoring normal growth.

Intricately linked to growth deficits are significant abnormalities in bone metabolism in children with IBD. Although many disease factors, including malnutrition, delayed puberty, decreased physical activity, malabsorption, and corticosteroid use, have a negative effect on bone metabolism, chronic inflammation itself may exert the most profound effects. Because most adult bone mass is achieved by 16 and 18 years of age in boys and girls, respectively, without appropriate monitoring and treatment, children with IBD may not achieve optimal adult bone mass, which places them at risk for fracture as they age. Vertebral compression fractures have been reported in children with IBD and low levels of bone mineral density. Dual-energy x-ray absorptiometry of the total body is recommended in children with IBD and growth impairment, delayed puberty, prolonged corticosteroid use, or severe inflammation. A dietary intake of 1000 to 1600 mg of elemental calcium and 800 to 1000 IU of vitamin D is recommended for children and adolescents with IBD.

Colon Cancer

Owing to chronic inflammation, patients with UC and CD involving the colon have an increased risk for colon cancer. The cumulative incidence of colon cancer in patients with UC from population-based studies is 13 per 1000 patients. The risk increases with time from diagnosis, and higher-risk groups include those younger at diagnosis, with pancolitis, or with associated primary sclerosing cholangitis. Therefore, beginning 7 to 10 years after the diagnosis, children with UC and Crohn colitis should undergo colonoscopy with surveillance biopsy every 1 to 2 years.

Psychosocial Function

Children with IBD have higher rates of depressive and anxiety disorders compared with children with other chronic conditions. Depressive and anxiety symptoms correlate with disease activity, and factors such as the effect of proinflammatory cytokines on the brain, sleep disturbance, and corticosteroids may contribute. Cognitive behavioral therapy is effective, and, at times, pharmacotherapy may be helpful as an adjunct to therapy. Clinicians should be alert for symptoms of depression and anxiety and refer patients for treatment when indicated.

Symptoms such as abdominal pain, fatigue, and diarrhea also affect the patients’ quality of life and social functioning. Pediatricians and other primary care clinicians should support families in establishing a formal plan at school (eg, a 504 plan at US public schools for students with disabilities) to ensure appropriate accommodations are made for IBD symptoms (eg, unfettered access to restrooms and extended time to complete assignments after periods of absence).

The Future of Pediatric IBD

The care of children with IBD is being advanced by new drug development and large collaborative research efforts. Vedolizumab, a monoclonal antibody against α4β7 integrin that inhibits lymphocyte migration to the intestine, is the most recently approved treatment for CD and UC in adults and is starting to be used in older children with disease refractory to anti-TNF agents. The established collaborative research networks have led to increased remission rates through quality improvement, the discovery of new pediatric IBD risk genes, and insights into the microbiome and molecular pathogenesis of pediatric IBD. Thus, we hope that the translation of recent discoveries will lead to a day when most children with IBD achieve a sustained remission, enjoy a healthy childhood, and are free to pursue their goals unhindered by disease.


43. Markowitz J, Gancher K, Kohn N, Lesser M, Daum F. A multicenter trial of 6-mercaptopurine...


EXHIBIT 203
What is IBD?

Inflammatory bowel disease (IBD) is a term for two conditions (Crohn’s disease and ulcerative colitis) that are characterized by chronic inflammation of the gastrointestinal (GI) tract. Prolonged inflammation results in damage to the GI tract. Some of the differences between Crohn’s disease and ulcerative colitis:

<table>
<thead>
<tr>
<th>Crohn’s Disease</th>
<th>Ulcerative Colitis</th>
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<tbody>
<tr>
<td>Can affect any part of the GI tract (from the mouth to the anus)—Most often it affects the portion of the small intestine before the large intestine/colon.</td>
<td>Occurs in the large intestine (colon) and the rectum</td>
</tr>
</tbody>
</table>
What is inflammatory bowel disease (IBD)?

Inflammatory Bowel Disease - Division of Population Health

Page 2 of 3

https://www.cdc.gov/ibd/what-is-IBD.htm

What are the symptoms of IBD?

Some common symptoms are:

- Persistent diarrhea
- Abdominal pain
- Rectal bleeding/bloody stools
- Weight loss
- Fatigue

What causes IBD?

The exact cause of IBD is unknown, but IBD is the result of a defective immune system. A properly functioning immune system attacks foreign organisms, such as viruses and bacteria, to protect the body. In IBD, the immune system responds incorrectly to environmental triggers, which causes inflammation of the gastrointestinal tract. There also appears to be a genetic component—someone with a family history of IBD is more likely to develop this inappropriate immune response.

How is IBD diagnosed?

IBD is diagnosed using a combination of endoscopy (for Crohn’s disease) or colonoscopy (for ulcerative colitis) and imaging studies, such as contrast radiography, magnetic resonance imaging (MRI), or computed tomography (CT). Physicians may also check stool samples to make sure symptoms are not being caused by an infection or run blood tests.
to help confirm the diagnosis.

How is IBD treated?

Several types of medications may be used to treat IBD: aminosalicylates, corticosteroids (such as prednisone), immunomodulators, and the newest class approved for IBD—the "biologics". Several vaccinations for patients with IBD are recommended to prevent infections. Severe IBD may require surgery to remove damaged portions of the gastrointestinal tract, but advances in treatment with medications mean that surgery is less common than it was a few decades ago. Since Crohn's disease and ulcerative colitis affect different parts of the GI tract, the surgical procedures are different for the two conditions.

IBD is not...

IBD is not Irritable Bowel Syndrome (IBS)...

IBD should not be confused with irritable bowel syndrome or IBS. Although people with IBS may experience some similar symptoms to IBD, IBD and IBS are very different. Irritable bowel syndrome is not caused by inflammation and the tissues of the bowel are not damaged the way they are in IBD. Treatment is also different. To learn more about the difference between inflammatory bowel disease and irritable bowel syndrome, go to http://www.crohnscolitisfoundation.org/ibd-and-irritable-bowel.pdf

IBD is not celiac disease...

Celiac disease is another condition with similar symptoms to IBD. It is also characterized by inflammation of the intestines. However, the cause of celiac disease is known and is very specific. It is an inflammatory response to gluten (a group of proteins found in wheat and similar grains). The symptoms of celiac disease will go away after starting a gluten-free diet, although it usually will be months before the full effects of the new diet will be reached.

References


Content source: National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention
Influence of environmental factors on the onset and course of inflammatory bowel disease

Amit Kumar Dutta, Ashok Chacko

Abstract

Numerous environmental factors have been linked with inflammatory bowel disease. These include smoking, diet, hygiene, drugs, geographical and psychosocial factors. These factors may either increase the risk of or protect against developing this condition and can also affect the course of illness in a positive or negative manner. A number of studies have examined the influence of environmental factors on inflammatory bowel diseases as a whole as well as on ulcerative colitis and Crohn’s disease separately. As there are differences in the pathogenesis of ulcerative colitis and Crohn’s disease, the effect of environmental factors on their onset and course is not always similar. Some factors have shown a consistent association, while reports on others have been conflicting. In this article we discuss the current evidence on the roles of these factors on inflammatory bowel disease, both as causative/protective agents and as modifiers of disease course.

Key words: Environmental factors; Crohn’s disease; Ulcerative colitis; Etiology; Outcome

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Core tip: Environmental factors have an important influence on the onset and course of inflammatory bowel disease. Multiple factors have been implicated with some showing a consistent effect, while the roles of others have been variable. The current evidence on their role in inflammatory bowel disease is discussed. A better understanding of these factors may help plan future preventive strategies.

Dutta AK, Chacko A. Influence of environmental factors on the onset and course of inflammatory bowel disease. World J
INTRODUCTION

The latter half of the twentieth century witnessed a steep increase in the prevalence of inflammatory bowel disease (IBD) in developed nations of North America and Europe. During the last three decades, populations previously considered to have low risk such as in Asia and eastern Europe are witnessing a substantial increase in this disease\[1\]. This may be explained by changes in environmental factors in these regions. Environmental factors that have been proposed to play a role in the emergence of IBD are smoking, diet, drugs, major life stressors, hygiene and lifestyle[1,2]. In this paper we review the role of environmental risk factors on the onset and course of ulcerative colitis (UC) and Crohn’s disease (CD), the two main types of IBD.

ENVIRONMENTAL FACTORS IN THE PATHOGENESIS OF IBD

IBD is a complex disorder where interplay between host genetics, gut microbiota and environmental factors are regarded as drivers of chronic inflammation in the gut[3]. Genetic factors play an important role with more than 150 IBD susceptibility gene loci identified to date. However, approximately two-thirds of patients with IBD have no identifiable genetic defect, which suggests that gut microbiota and environmental factors play an important role[4]. Studies on individuals migrating from countries with low prevalence of IBD to regions with high prevalence have shown an increased risk of IBD among migrants further supporting the role for environmental factors[5-8]. These factors include smoking, diet, drugs, psychosocial factors, climate, pollution and hygiene[9]. The composition of gut microbiota, currently considered a key factor in the pathogenesis of IBD, is affected by environmental factors such as breast feeding, antibiotics, smoking, obesity and diet[10,11]. For example, breast fed and formula fed infants showed a difference in the quantity of Bifidobacteria in the gut[12]. Smoking cessation changes the gut flora by increasing the proportion of Firmicutes, reducing Proteobacteria and increasing microbial diversity making the flora different from gut microbiota in IBD where there is an abundance of Proteobacteria and Actinobacteria, reduced Firmicutes along with reduced microbial diversity[10,13]. These data suggest that an alteration in microbial composition of the intestine by environmental factors is one mechanism by which environmental factors increase susceptibility to IBD.

Environmental factors may also directly act on the intestinal mucosa and alter immune function and gene expression. This can be due to a change in intestinal permeability or an alteration in host gene expression by epigenetic modification or other mechanisms[14-16]. The end result is an abnormal host immune function and chronic inflammation in the gut.

A interesting example of how environmental factors affect gut immune function is provided by studies on transcription factor aryl hydrocarbon receptor (AhR)[17]. This transcription factor which is altered by dietary and environmental factors affects innate immunity in gut and immune cells (T cells and natural killer cells). Intestinal T cells and natural killer cells isolated from Crohn’s disease patients have shown low levels of AhR expression and these receptors respond to AhR ligands by upregulating interleukin-22 and downregulating inflammatory cytokines[17]. Therefore, it is plausible that environmental factors that downregulate AhR alter immune function and predispose to CD[17]. Smoking has also been shown to affect gene expression and immune function in the gut[18]. The complex interaction between host genes and environmental factors works both ways[19]. While the above examples demonstrate the effect of the environment on host gene expression, host genes can also influence the composition of microbiota which forms the local gut environment. NOD2 gene mutation predisposes to the development of IBD. A possible mechanism may be an alteration in gut microbiota in NOD2 gene mutation as shown in animal studies[20]. Mutation in autophagy-related 16-like 1 gene (ATG16L1) has also been found to increase the risk of IBD and it is possible that defective autophagy may alter the gut microbiome[19]. Further insights into the gene-environment interaction will lead to a better understanding of the pathogenesis of IBD.

The “hygiene hypothesis” has been commonly cited as the reason for the difference in IBD prevalence in different regions[21]. Better hygiene in developed regions leads to reduced microbial exposure in childhood which may affect development of the gut immune system and immune tolerance. Helminth infestation in animal models has been shown to upregulate Th2 cytokines and attenuate the Th1 pathway in the intestinal mucosa leading to suppression of inflammation and enhancement of the mucosal barrier[22,23]. Reduced exposure to helminths in developed societies has been suggested to be a risk factor for CD. Other factors such as stress, linked to exacerbation of IBD, may affect immune function by altering gut permeability and non-steroidal anti-inflammatory drugs (NSAIDs) by non-selective inhibition of cyclo-oxygenase[24,25].

Dietary factors may affect gut immune function directly in addition to their effect on microbiota[26]. There has been a recent increase in interest in the role of vitamin D in CD[27]. Our group and others have shown that vitamin D levels are reduced in patients with CD and levels correlate negatively with disease activity[28]. Data obtained mainly from animal studies have shown that vitamin D has immune regulating properties[29]. This includes maintenance of CD8+ T
cells in the quiescent stage, shifting the cytokine profile to anti-inflammatory type and inhibition of epithelial cell apoptosis mediated by the vitamin D receptor\(^\text{29}\). Chen et al\(^\text{30}\) have shown that TNF-\(\alpha\) downregulates the vitamin D receptor, which in turn may promote inflammation. A high fibre diet protects against IBD by promoting the formation of short chain fatty acids like butyrate, which are a source of energy for colonocytes and by regulating T cell function\(^\text{31}\). The Nurses Health Study showed that soluble dietary fiber (fruits and vegetables) was associated with a reduced risk of CD\(^\text{31}\). The protective effect of fruits and vegetables may be through their antioxidant properties and clearing of reactive oxygen species\(^\text{32}\). Red meat has been associated with increased risk of IBD. Linoleic acid (long-chain omega-6 fatty acid) found in red meat and food oils is metabolized to arachidonic acid metabolites which are involved in the production of inflammatory mediators such as leukotrienes and prostaglandins\(^\text{33}\). Higher consumption of fish oils made up of omega-3 fatty acids (higher omega-3 to omega-6 fatty acid ratio) has been shown to be protective in children with CD\(^\text{32}\). There is some evidence that phytochemicals, such as curcumin found in turmeric, have antioxidant and free radical scavenging properties which may limit inflammation and help maintain remission in IBD\(^\text{34,35}\). These data suggest that environmental factors play a role in the pathogenesis of IBD by altering gut microbiota and affecting gut immunity by numerous mechanisms.

**INTERPRETING THE AVAILABLE EVIDENCE**

There are a large number of publications exploring the link between environmental factors and IBD. With the exception of smoking and appendectomy, the roles of other risk factors have been inconsistent and it is important to understand the type of study design when interpreting the results of these studies. Many of these are case-control studies which are relatively easy to perform and require few resources. However, an important limitation is the recall bias which affects the accuracy of ascertaining risk factors. Several prospective cohort studies have been carried out and data obtained from them are more robust. An important cohort study of note in this regard is the Nurses Health Study (NHS) I and II from the United States\(^\text{31}\). NHS I was initiated in 1976 and included 121700 subjects and NHS II was initiated in 1989 and included 116000 subjects. Periodic assessment of factors such as smoking, oral contraceptive pills (OCPs), alcohol and diet were carried out prospectively and occurrence of disease was noted. While this was mainly initiated for the outcomes of cardiovascular illness and cancer, a number of studies have been published on the role of these factors and IBD. The main limitation is that the subjects were women and most were white and generalisability across race, gender and various socioeconomic strata was difficult. Some of the studies have made use of a population-based registry to minimise referral bias and reflect population characteristics\(^\text{36}\). Finally, a number of meta-analyses have been published on various risk factors and IBD and these represent a higher quality of evidence. One must be cautious in interpreting the results of a meta-analysis as inclusion of low quality studies and heterogeneity among studies may affect the outcome.

**ENVIRONMENTAL FACTORS AND ONSET OF IBD**

A large number of environmental factors have been proposed to have a causative or protective effect on the onset of both CD and UC. Available evidence from many of these studies is summarised in Tables 1, 2 and 3. These tables have been grouped according to the study design to keep the quality of evidence in perspective. Table 1 includes meta-analysis, Table 2 cohort studies and Table 3 summarises data from case-control studies.

**Smoking**

There is adequate evidence linking smoking with IBD. It has opposing effects on CD and UC. The meta-analysis by Mahid et al\(^\text{37}\) showed that current smoking increases the risk of CD, but has a protective effect on the onset of UC. Interestingly, former smokers had an increased risk of developing UC. Data from the NHS cohort showed that both current and former smoking was associated with increased risk of CD\(^\text{38}\). Unlike the result from meta-analysis, the study showed current smoking was not protective against UC, but former smoking was a risk factor. Population-based case-control studies from New Zealand, Hungary and Sweden have also shown increased risk of CD and decreased risk of UC with smoking\(^\text{39-41}\). The strong data linking smoking to IBD suggests that prenatal and childhood exposure to passive smoking may predispose to CD. However, a meta-analysis which included 13 studies did not show any significant impact of prenatal and childhood exposure to smoking on the occurrence of CD or protection against UC\(^\text{42}\). Based on these data, there is a strong case to recommend smoking cessation to reduce the risk of CD.

**Diet, vitamin D and breast feeding**

Western diet which is high in refined sugar and low in fibre has been proposed as a risk factor for IBD\(^\text{26}\). Increasing consumption of western diet is considered a reason for the rising incidence of IBD in Asia. The NHS data on 170776 subjects showed that intake of a median of 24.3 g of fibre per day reduced the risk of CD by about 40%\(^\text{41}\). Further analysis showed that this benefit was highest for soluble fibre in fruits, while
insoluble fibre from legumes, whole grains and cereals did not affect the risk. Interestingly the amount and type of fibre had no significant impact on the risk of UC[31]. A case-control study from Canada exploring dietary pattern and risk of CD among subjects up to 20 years of age found a diet containing vegetables, fish, olive oil, fruit, grain and nuts was negatively associated with CD[43]. Another case-control study from Denmark showed an increased risk of both CD and UC in patients on a diet containing low fibre and high sugar[44]. A study from our center showed that regular fish consumption reduces the risk of CD[45]. Tjonneland et al[46] performed a nested case-control study, which included participants in the EPIC (European Prospective Investigation into Cancer and Nutrition) study, to assess the link between dietary linoleic acid (source of arachidonic acid whose metabolites encourage inflammation) and UC. Dietary linoleic acid was found to be associated with an increased risk of developing UC and the effect was greater with higher intake[46]. There has been increasing reports of vitamin D deficiency among patients with IBD, especially CD[27]. While this may be a consequence of the disease, vitamin D may also play a role in modulating gut immune function and have an effect on the onset of IBD[47]. A prospective study of 72719 subjects in

Table 1  Environmental factors and onset of inflammatory bowel disease - meta-analyses

<table>
<thead>
<tr>
<th>Author et al[39], 2012</th>
<th>Study setting</th>
<th>Effect on CD</th>
<th>Effect on UC</th>
<th>Effect on IBD overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soon et al[39], 2012</td>
<td>Urban living and CD and UC</td>
<td>IR (incident rate ratio, 1.42; 95%CI: 1.26-1.6)</td>
<td>IR (incident rate ratio, 1.17; 95%CI: 1.03-1.32)</td>
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<tr>
<td>Luther et al[39], 2010</td>
<td>H. pylori infection and risk of IBD (23 studies)</td>
<td></td>
<td></td>
<td>DR (RR = 0.64; 95%CI: 0.54-0.75)</td>
</tr>
<tr>
<td>Barclay et al[39], 2009</td>
<td>Breast feeding and early onset IBD (7 studies)</td>
<td>NA</td>
<td>NA</td>
<td>DR (OR = 0.69; 95%CI: 0.51-0.94)</td>
</tr>
<tr>
<td>Jones et al[39], 2008</td>
<td>Prenatal or childhood passive smoking and risk of IBD (13 studies)</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Cornish et al[39], 2008</td>
<td>OCP and risk of IBD (14 studies)</td>
<td>IR (RR = 1.46; 95%CI: 1.26-1.70)</td>
<td>IR (RR = 1.28; 95%CI: 1.06-1.54)</td>
<td></td>
</tr>
<tr>
<td>Mahid et al[39], 2007</td>
<td>Smoking and risk of IBD (13 studies related to UC and 9 related to CD)</td>
<td>IR with current smoking (OR = 1.76; 95%CI: 1.40-2.22)</td>
<td>DR with current smoking (OR = 0.58; 95%CI: 0.45-0.75)</td>
<td>IR with former smoking (OR = 1.79; 95%CI: 1.37-2.34)</td>
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</table>

<table>
<thead>
<tr>
<th>Author Study setting</th>
<th>Study subjects</th>
<th>Effect on CD</th>
<th>Effect on UC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timm et al[39], 2014, Europe</td>
<td>Population-based cohort</td>
<td>DR with being born and living on livestock farm for first 5 yr of life</td>
<td></td>
</tr>
<tr>
<td>Khalili et al[39], 2013, United States</td>
<td>146681 subjects from NHS I and II</td>
<td>NA - Breastfeeding, low or high birth weight, preterm birth</td>
<td>NA - Breastfeeding, low or high birth weight, preterm birth</td>
</tr>
<tr>
<td>Ananthakrishnan et al[39], 2013, United States</td>
<td>337376 person-years of follow-up</td>
<td>DR - Long term intake of higher dietary fibre especially from fruit</td>
<td>NA with dietary fibre</td>
</tr>
<tr>
<td>Ananthakrishnan et al[39], 2013, United States</td>
<td>170776 subjects from NHS I and II</td>
<td>IR with recent and baseline depressive symptoms</td>
<td>NA with recent and baseline depressive symptoms</td>
</tr>
<tr>
<td>Levi et al[39], 2013, Israel</td>
<td>Population-based cohort of 953684 Jewish adolescents</td>
<td>IR with high socioeconomic status, western origin, male sex</td>
<td>DR with four or more children in childhood</td>
</tr>
<tr>
<td>Higuchi et al[39], 2012, United States</td>
<td>229111 subjects from NHS I and II</td>
<td>IR - Current smoker, former smoker</td>
<td>NA - Current smoker</td>
</tr>
<tr>
<td>Ananthakrishnan et al[39], 2012, United States</td>
<td>76795 subjects from NHS I</td>
<td>IR - frequent use of NSAID</td>
<td>IR - frequent use of NSAID</td>
</tr>
<tr>
<td>Ananthakrishnan et al[39], 2012, United States</td>
<td>1295317 person-years of follow-up</td>
<td>NA - Aspirin</td>
<td>NA - Aspirin</td>
</tr>
<tr>
<td>Ananthakrishnan et al[39], 2012, United States</td>
<td>72719 subjects from NHS</td>
<td>DR - Higher predicted level of plasma Vitamin D</td>
<td>NA - Vitamin D level in plasma</td>
</tr>
</tbody>
</table>

1European Community Respiratory Health Survey. IR: Increased risk; DR: Decreased risk; NA: No association; CD: Crohn’s disease; UC: Ulcerative colitis; RR: Relative risk; IBD: Inflammatory bowel disease; OCP: Oral contraceptive pill.
<table>
<thead>
<tr>
<th>Author</th>
<th>Study setting</th>
<th>Effect on CD</th>
<th>Effect on UC</th>
</tr>
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<tbody>
<tr>
<td>Ng et al[51], 2014,</td>
<td>CD - 186</td>
<td>DR with breast feeding for &gt; 12 mo, antibiotic use, having dogs, daily tea intake, daily physical activity</td>
<td>DR with breast feeding for &gt; 12 mo, antibiotic use, daily tea and coffee intake, presence of hot water tap, flushing toilet in childhood with smoking</td>
</tr>
<tr>
<td>Asia Pacific</td>
<td>UC - 256 Controls - 940</td>
<td></td>
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<td></td>
<td></td>
<td>Outcome - environmental risk factors and IBD</td>
<td>IR with smoking</td>
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<tr>
<td>Sood et al[64], 2014,</td>
<td>UC - 516 Controls - 188</td>
<td></td>
<td></td>
</tr>
<tr>
<td>India</td>
<td></td>
<td>Outcome - environmental risk factors and UC</td>
<td>IR with owning a pet and stressful events</td>
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<td></td>
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<td>DR with better toilet facilities and having private bed</td>
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<tr>
<td>Chu et al[62], 2013,</td>
<td>CD - 88</td>
<td>DR - Helminth infection, shared housing, raw beef consumption</td>
<td>DR - Helminth infection, mixed race, smoking, shared housing, raw beef consumption</td>
</tr>
<tr>
<td>South Africa</td>
<td>UC - 63</td>
<td>IR - Urban dwelling, parental tertiary education</td>
<td>IR - parental tertiary education</td>
</tr>
<tr>
<td></td>
<td>Control - 219</td>
<td></td>
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</tr>
<tr>
<td>Jakobsen et al[114],</td>
<td>CD - 59</td>
<td>IR with bedroom sharing, prior hospitalisation with gastrointestinal infection, family history</td>
<td>IR with prior hospitalisation with gastrointestinal infection, family history</td>
</tr>
<tr>
<td>2013, Denmark</td>
<td>UC - 56</td>
<td>DR with wholemeal bread consumption</td>
<td>DR with daily vegetable consumption</td>
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<td></td>
<td>Controls - 477</td>
<td></td>
<td></td>
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<tr>
<td>Hlavaty et al[61], 2013,</td>
<td>CD - 190</td>
<td>IR with short duration of breast feeding, infrequent childhood sports activity, smoking, infrequent contact with animals in childhood</td>
<td>IR with short duration of breast feeding, infrequent childhood sports activity, smaller family size in childhood</td>
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<tr>
<td>Slovakia</td>
<td>UC - 148</td>
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<td></td>
<td>Controls - 355</td>
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<td>Outcome - environmental risk factors and pediatric IBD</td>
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<tr>
<td>Pugazhendhi et al[45],</td>
<td>CD - 200</td>
<td>IR with safe drinking water</td>
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<tr>
<td>2012, India</td>
<td>Controls - 200</td>
<td>DR with regular fish consumption and presence of cattle in house</td>
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<td></td>
<td></td>
<td>Outcome - environmental risk factors and CD</td>
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<tr>
<td>Castiglione et al[115],</td>
<td>CD - 468</td>
<td>NA with any factors except IR with smoking and appendectomy</td>
<td>NA with any factors except DR with smoking and appendectomy</td>
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<tr>
<td>2012, Italy</td>
<td>UC - 527</td>
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<td></td>
<td>Controls - 562</td>
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<tr>
<td>Hansen et al[44], 2011,</td>
<td>CD - 123</td>
<td>DR with breast feeding, tonsillectomy,</td>
<td>DR with breast feeding, tonsillectomy, appendectomy, smoking</td>
</tr>
<tr>
<td>Denmark</td>
<td>UC - 144</td>
<td></td>
<td>IR with pertussis and polio vaccine, measles infection, low fibre and high sugar</td>
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<td></td>
<td>Controls - 267</td>
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<tr>
<td>López-Serrano et al[31]</td>
<td>124 CD and 235 controls</td>
<td>IR with pertussis and polio vaccine, measles infection, smoking, low fibre and high sugar</td>
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</tr>
<tr>
<td>2010, Spain</td>
<td>146 UC and 278 controls</td>
<td>IR - Living in urban area, high educational level, social status</td>
<td>IR - Living in urban area, high educational level, social status</td>
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<td></td>
<td></td>
<td>DR - Childhood respiratory infection and gastroenteritis</td>
<td>DR - Childhood respiratory infection and gastroenteritis, appendectomy, current smoking</td>
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<tr>
<td>Geary et al[39], 2010,</td>
<td>Population-based case-control study</td>
<td>IR with smoking, high social class at birth, Caucasian ethnicity</td>
<td>IR with high social class at birth, Caucasian ethnicity, migrant</td>
</tr>
<tr>
<td>New Zealand</td>
<td>CD - 636, UC - 653 Controls - 600</td>
<td>DR with breastfeeding and childhood vegetable garden</td>
<td>DR with smoking, breast feeding and childhood vegetable garden</td>
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<tr>
<td>Joseph et al[28], 2009,</td>
<td>CD - 34</td>
<td>IR - lower levels of Vitamin D</td>
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<tr>
<td>India</td>
<td>Controls - 34</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amre et al[32], 2007,</td>
<td>CD - 130</td>
<td>DR - higher consumption of vegetables, fruit, fibre, fish, long chain omega three fatty acid</td>
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<tr>
<td>Canada</td>
<td>Controls - 202</td>
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<td></td>
<td></td>
<td>Outcome - diet and pediatric CD</td>
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the NHS cohort showed a protective role for a higher predicted vitamin D level against the development of CD\textsuperscript{40}. A case-control study by our group in India, which included 34 patients with CD and 34 controls found significantly lower levels of serum 25(OH) vitamin D in patients compared with controls (16.3 ± 10.8 ng/mL vs 22.8 ± 11.9 ng/mL, \( P < 0.05 \))\textsuperscript{28}. Disease severity was negatively correlated with vitamin D levels. Lower duration of sunlight exposure with consequent vitamin D deficiency in northern latitudes might be a factor contributing to the north-south gradient of IBD, but this needs to be confirmed. In contrast to the above positive studies, a case-control study from the United States failed to show a significant difference in the vitamin D levels between IBD subjects and controls\textsuperscript{40}.

The data on breast feeding and onset of IBD are conflicting. The meta-analysis by Barclay et al\textsuperscript{52} showed that breast feeding reduced the overall risk of early onset IBD, but had no impact on the onset of CD or UC separately. The recently published case-control study from the Asia Pacific region which included subjects from different Asian countries and Australia showed that breast feeding for more than 1 year reduced the risk of both CD and UC\textsuperscript{51}. The case-control studies from Slovakia in 2013 and Denmark in 2011 also suggested that breast feeding may be protective\textsuperscript{164,52}. In contrast, data from 146681 subjects in the NHS cohort did not show any association between breast feeding and onset of CD or UC\textsuperscript{53}. Interestingly, a study from France showed that breast feeding may increase the risk of CD.

The evidence for benefits of high fibre and low fat diet, longer period of breast feeding and correcting vitamin D deficiency in preventing IBD is not conclusive. However, as some studies show that they may be beneficial and as they also have other health benefits, it may be reasonable to encourage these interventions.

**Drugs**

Among the drugs available, OCPs, NSAIDs and antibiotics have often been linked to the onset and course of IBD. The meta-analysis by Cornish et al\textsuperscript{36}, which included 14 studies with a total of 75815 subjects showed an increased risk of CD with the use of OCPs and risk increased with longer duration of use. There was also an increased risk of developing UC, but the effect was less than in CD. A large prospective cohort study involving 232452 women (NHS 1 and 2) also showed that oral contraceptive use was associated with CD. The association between OCPs and UC was restricted to women with a history of smoking\textsuperscript{55}. Although current evidence suggests that there is a moderate association between exposure to OCPs and the development of CD, no conclusions can be made regarding the use of OCPs and the risk of developing IBD.

A study which explored the risk of IBD with NSAIDs and aspirin intake among 76795 subjects from the NHS cohort found an increased risk of developing CD and UC among those who used NSAIDs for at least 15 d every month. No association between aspirin use and IBD was found\textsuperscript{56}. Antibiotics, by affecting gut microbiota, may modulate gut immune response and might be a risk factor for IBD. A nested case-control study from Canada (2234 patients and 22346 controls) which assessed the risk of IBD with antibiotic use (2-5 years pre-diagnosis) found a positive association between antibiotic use and the risk of both CD and UC\textsuperscript{57}. In a case-control study involving 587 patients with CD, antibiotic use 2-5 years pre-diagnosis was found more often in patients than controls\textsuperscript{58}. Virta et al\textsuperscript{58}, from Finland used the National Register to explore the link between antibiotics and risk of UC and CD. They found an increased risk of pediatric CD, but no added risk for pediatric UC with the use of antibiotics. The study also showed a stronger association of CD in boys and with the use of cephalosporins\textsuperscript{59}. Interestingly, a study from Asia Pacific showed a decreased risk of CD and UC with antibiotic usage\textsuperscript{51}. Data on the association between IBD and specific antibiotics are limited to the pediatric literature. Penicillins, cephalosporins, and tetracyclines have been linked with the development of CD, but the exact mechanism is not well understood\textsuperscript{59,60}. Although studies support a link between antibiotic exposure and the onset of CD, causality has not been firmly established. However, prudent use of antibiotics is good clinical practice. Interpreting the association of drugs with IBD is challenging due to the wide variety of antibiotics, NSAIDs and OCPs available as well as the difficulty in determining the magnitude, type of exposure and duration of use of the drugs.

**Hygiene**

A number of studies have explored surrogate factors associated with the “hygiene hypothesis” and the risk of IBD. These include urban living, family size, toilet facilities, helminth infestation, drinking water facilities, etc\textsuperscript{21}. Most were case-control studies, and the results
from some of these studies are summarised in Table 3. While the results are quite variable, some studies showed urban living, high social status, high social class and safe drinking water to be associated with an increased risk of IBD. A meta-analysis which included 40 studies also showed a positive association between urban living and both CD and UC. Pugazhenthi et al. from our center showed a positive association between safe drinking water and CD, but not with urban living. Various studies have found that childhood respiratory and gastrointestinal infection, childhood helminth infestation, pet exposure and shared housing reduce the risk. In contrast, the Asia Pacific study which showed reduced association of UC with the presence of a hot water tap and flushing toilet in childhood and the recent study from Northern India which showed reduced risk of UC with better toilet facilities and a private bed refute the hygiene hypothesis. Evidence for the hygiene hypothesis is conflicting. The reasons may be the inclusion of a wide variety of factors under this category, lack of large prospective cohort studies, presence of confounders or a true lack of association.

Other factors
Several other factors such as appendectomy, infections, air pollution, seasonal variation, physical activity, vaccination, and psychological factors have been implicated in the etiology of IBD. A large Swedish study showed reduced risk of UC in patients whose appendix was removed for inflammatory pathology before the age of 20 years. Other studies have also shown that appendectomy was associated with decreased risk of UC. Unlike in UC, some studies including a meta-analysis showed that appendectomy increases the risk of CD up to 5 years after surgery and thereafter the risk falls to that seen in the general population. As clinical symptoms of CD may be similar to acute appendicitis, some of the association seen during the initial time period after appendectomy may be related to an erroneous diagnosis. Helicobacter pylori (H. pylori) infection was shown to have a protective association with IBD in a meta-analysis of 23 studies. It is unclear whether this is a reflection of overcrowding and low socioeconomic status associated with H. pylori infection or an effect of this bacterium on gut immunity. On the other hand, a population-based cohort study from Denmark showed that past infection with Salmonella and Campylobacter was associated with an increased risk of both UC and CD. In the past, Mycobacterium avium subspecies paratuberculosis was considered an etiological agent for CD, but recent data does not support this. Large cohort studies have also shown a reduced incidence of IBD in subjects with more siblings and those who lived on a farm with livestock in childhood. In a study from the United Kingdom, air pollution did not affect the overall onset of IBD; however, subset analysis showed that there was an increased risk of early onset CD with exposure to nitrogen dioxide and early onset UC after exposure to sulphur dioxide. The north-south gradient of IBD observed in some regions may be related to differences in climate. In a Norwegian cohort, Aamodt et al. studied the influence of temperature, altitude and precipitation to assess the impact of latitude on the incidence of UC. Temperature had a negative association with UC, while the other factors had no significant effect. Others have shown an association between IBD and both childhood vaccines and physical activity. Thompson and colleagues were the first to suggest that measles vaccination was associated with a 3-fold increased risk of CD and UC compared to unvaccinated controls. Subsequent studies have not confirmed these findings. Available data provide no firm evidence to suggest that routine vaccinations have an effect on the development of CD. Psychological factors have also been linked with the onset and course of IBD. Smoking seems to have a definite, detrimental effect on the course of CD. Several studies have shown an increased risk of flares, more active disease, increased hospitalisation rates, increased risk of surgery and post-operative recurrence in patients with CD who are smokers compared with non-smokers. Smoking also affects disease behaviour and is associated with a higher risk of penetrating disease and extra-intestinal manifestations. There is a strong case for smoking cessation in CD as shown in an interventional study from France where patients who quit smoking had a reduced rate of disease exacerbations compared to smokers. Based on these findings, smoking cessation should be strongly encouraged in CD. A recent meta-analysis of 20 studies on UC showed lower colectomy rates in active smokers. Another population-based cohort study which included 771 patients with UC from seven European countries and Israel found lower relapse rates in current smokers. The reason for the differential effect of smoking on CD and UC is unclear.

ENVIRONMENTAL FACTORS AND THE COURSE OF IBD
The usual reasons for disease exacerbation in IBD are natural history of the disease, non-compliance with drugs and gastrointestinal infections; environmental factors may also influence the course of disease. Table 4 summarises data from some of the studies evaluating environmental factors and the course of IBD.

Smoking seems to have a definite, detrimental effect on the course of CD. Several studies have shown an increased risk of flares, more active disease, increased hospitalisation rates, increased risk of surgery and post-operative recurrence in patients with CD who are smokers compared with non-smokers. Smoking also affects disease behaviour and is associated with a higher risk of penetrating disease and extra-intestinal manifestations. There is a strong case for smoking cessation in CD as shown in an interventional study from France where patients who quit smoking had a reduced rate of disease exacerbations compared to smokers. Based on these findings, smoking cessation should be strongly encouraged in CD. A recent meta-analysis of 20 studies on UC showed lower colectomy rates in active smokers. Another population-based cohort study which included 771 patients with UC from seven European countries and Israel found lower relapse rates in current smokers. The reason for the differential effect of smoking on CD and UC is unclear.

Psychological factors have been proposed to have
a greater role in influencing the course of IBD as compared to their etiological role. A large population-based cohort study from Canada showed an increased risk of flare in IBD patients with high perceived stress at one year follow-up\(^\text{(93)}\). A couple of other cohort studies, one of which included patients with CD and the other with UC, also showed that stress was associated with increased disease exacerbation\(^\text{(94,95)}\). A prospective observational study from Belgium showed that major depressive disorder was a risk factor for failure to achieve remission with infliximab and for earlier relapse in patients with active CD\(^\text{(96)}\). In addition to flares, psychiatric comorbidity may also affect the risk of surgery. A multi-institutional cohort study showed that psychiatric comorbidity increased the risk of surgery in patients with CD, but no association was observed with UC\(^\text{(97)}\). In contrast, a systematic review which included 12 studies showed a lack of convincing evidence that therapy of depression and anxiety alters the disease course of IBD\(^\text{(98)}\). Although the evidence for psychological factors influencing the course of IBD is not robust, it may be prudent to treat these patients to improve their quality of life.

Drugs may also influence the course of IBD. Data on NSAIDs as a trigger for disease relapse in IBD is conflicting. Case reports and small series suggest that nonselective NSAIDS trigger disease relapse\(^\text{(99)}\). In an uncontrolled study, Takeuchi et al\(^\text{(25)}\), found an increased risk of flares in IBD patients taking non-selective NSAIDs, but not with selective COX-1 or COX-2 inhibitors. A randomised controlled trial of celecoxib and placebo in UC did not show a significant

<table>
<thead>
<tr>
<th>Author</th>
<th>Study setting</th>
<th>Effect on CD</th>
<th>Effect on UC</th>
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<tbody>
<tr>
<td>Ott et al(^\text{[99]}), 2014, Germany</td>
<td>Cohort study</td>
<td>IR of EIM</td>
<td>NA</td>
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<td></td>
<td></td>
<td>CD - 161</td>
<td></td>
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<td></td>
<td></td>
<td>UC - 96</td>
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<td></td>
<td></td>
<td>Outcome: Smoking and EIM</td>
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<tr>
<td>Feagins et al(^\text{[100]}), 2014, United States</td>
<td>Case-control study</td>
<td>NA with NSAID, antibiotics, stress, smoking, infection and travel in past 3 mo</td>
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<td></td>
<td></td>
<td>Active IBD - 166</td>
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<td></td>
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<td>IBD in remission - 68</td>
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<td></td>
<td></td>
<td>Outcome: triggers for flare of IBD</td>
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<tr>
<td>Ananthakrishnan et al(^\text{[101]}), 2013, United States</td>
<td>Multi-institutional cohort study, CD - 5403, UC - 5429</td>
<td>IR of surgery with psychiatric comorbidity</td>
<td>NA of surgery with psychiatric comorbidity</td>
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<td></td>
<td></td>
<td>Outcome: psychiatric comorbidity and surgery and hospitalisation in CD and UC</td>
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<tr>
<td>Bernstein et al(^\text{[102]}), 2010, Canada</td>
<td>Population-based cohort</td>
<td>IR of flare - High perceived stress</td>
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<td></td>
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<td>IBD - 704</td>
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<td>Outcome: risk factors for flare</td>
<td></td>
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<tr>
<td>Packer et al(^\text{[103]}), 2010</td>
<td>Systematic review, 7 studies</td>
<td>Physical activity significantly increased quality of life and decreased disease activity</td>
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<td></td>
<td></td>
<td>Outcome: Physical activity and course of IBD</td>
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<tr>
<td>Bitton et al(^\text{[104]}), 2008, Canada</td>
<td>Cohort study</td>
<td>IR with stress/avoidance coping, higher</td>
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<td></td>
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<td>101 patients with CD in remission</td>
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<td></td>
<td></td>
<td>Outcome: biopsychosocial factors and relapse</td>
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<td>Follow-up - 1 yr</td>
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<tr>
<td>Takeuchi et al(^\text{[105]}), 2006, United Kingdom</td>
<td>Case series</td>
<td>IR of flare with non-selective NSAID</td>
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<tr>
<td></td>
<td></td>
<td>IBD - 209</td>
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<tr>
<td>Sandborn et al(^\text{[106]}), 2006, United States</td>
<td>Outcome: risk of flare with NSAID</td>
<td>No significant difference between celecoxib (3%) and placebo group (4%)</td>
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<td>RCT - Celecoxib vs placebo for 2 wk</td>
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<td>UC - 222</td>
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<td></td>
<td></td>
<td>Outcome: exacerbation during 2 wk</td>
<td></td>
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<tr>
<td>Persoons et al(^\text{[107]}), 2005, Belgium</td>
<td>Cohort study</td>
<td>Major depressive disorder associated with reduced response to infliximab</td>
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<td>CD - 100</td>
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<tr>
<td></td>
<td></td>
<td>Outcome: major depressive disorder and response to infliximab</td>
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<tr>
<td>Cosnes et al(^\text{[108]}), 1999, France</td>
<td>Cohort study</td>
<td>NA between OCP use and disease flare</td>
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<td></td>
<td>CD - 331</td>
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<td>Outcome: OCP and flare of CD</td>
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<td>Follow-up - 12 to 18 mo</td>
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<tr>
<td>Cosnes et al(^\text{[109]}), 1999, France</td>
<td>Cohort study</td>
<td>IR of flare - Current smokers</td>
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<td>CD - 622</td>
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<td>Outcome: risk factors for flare of CD</td>
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<td>Follow-up - 12 to 18 mo</td>
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<tr>
<td>Boyko et al(^\text{[110]}), 1998, United States</td>
<td>Outcome: Smoking and course of UC</td>
<td>DR of hospitalisation</td>
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<td>UC - 209, compared smokers with non-smokers</td>
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<td></td>
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<td>Outcome: Smoking and course of UC</td>
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IR: Increased risk; DR: Decreased risk; NA: No association; CD: Crohn’s disease; EIM: Extraintestinal manifestation; IBD: Inflammatory bowel disease; NSAID: Nonsteroidal anti-inflammatory drug; RCT: Randomised controlled trial; CRP: C reactive protein; OCP: Oral contraceptive pill.
difference in relapse rates between the two groups\textsuperscript{100}. The Canadian population-based cohort study and a recent study from the United States also showed no impact of NSAIDs on disease flare\textsuperscript{93,101}. Although the evidence is weak, the American College of Gastroenterology practice guidelines currently recognize NSAID use, including the use of COX-2 inhibitors, as a potential exacerbating factor for relapse of CD\textsuperscript{102}. The role of antibiotics and OCPs in modulating disease activity in IBD is unclear\textsuperscript{93,101,103}. A systematic review of 10 RCTs involving 1160 patients showed that antibiotics were more effective than placebo in inducing remission in active CD\textsuperscript{104}. Shortcomings of the study were moderate heterogeneity between studies and multiple antibiotics used either alone or in combination. As multiple antibiotics were used in different studies, the data are difficult to interpret, and additional studies are required to address the role of antibiotics in influencing the course of IBD. A systematic review that included 10 studies suggested that there is no risk of disease exacerbation in women with IBD who use oral contraceptives\textsuperscript{105}.

Dietary factors have been suggested as triggers for disease flares. Data on this subject are limited and confusing as patient surveys show heterogeneity regarding trigger foods\textsuperscript{106,107}. Fish oil which has omega-3 fatty acid with anti-inflammatory properties may be beneficial in maintaining remission in IBD. They may be of some utility in managing UC, but have not proven to be a substitute for conventional drugs\textsuperscript{108}. A prospective study of 191 patients with UC, who were followed-up for one year, to determine the effects of dietary factors on relapse, showed that higher meat and alcohol consumption was associated with an increased risk of relapse\textsuperscript{109}. The sulphur content in the food was proposed to be the likely trigger.

Other factors such as air pollution, exposure to ultraviolet light and physical activity have also been linked to the course of IBD. A systematic review of seven studies found physical activity to be associated with increased quality of life and decreased disease activity among patients with IBD\textsuperscript{110}. Cucino et al\textsuperscript{111}, found the manual work and farming were associated with decreased mortality in IBD. Low exposure to ultraviolet light has been associated with an increased risk of hospitalisation and surgery among IBD patients\textsuperscript{112}. Exposure to pollutants in air was also shown to increase hospitalisation rates\textsuperscript{113}. Although the environmental factors have not been as extensively evaluated with respect to their role on the course of IBD compared to their etiological role, there is modest evidence that some of these factors may influence the course of illness.

CONCLUSION

Data suggest that environmental factors play a significant role in the etiology of IBD and probably on the disease course. While the evidence for some factors is strong, many factors require further supportive data. Interventional studies assessing the effects of modifying these risk factors on natural history and patient outcomes are an important unmet need.

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EXHIBIT 205
Anti-Saccharomyces cerevisiae Autoantibodies in Autoimmune Diseases: from Bread Baking to Autoimmunity

Maurizio Rinaldi · Roberto Perricone · Miri Blank · Carlo Perricone · Yehuda Shoenfeld

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Abstract Saccharomyces cerevisiae is best known as the baker’s and brewer’s yeast, but its residual traces are also frequent excipients in some vaccines. Although anti-S. cerevisiae autoantibodies (ASCAs) are considered specific for Crohn’s disease, a growing number of studies have detected high levels of ASCAs in patients affected with autoimmune diseases as compared with healthy controls, including antiphospholipid syndrome, systemic lupus erythematosus, type 1 diabetes mellitus, and rheumatoid arthritis. Commensal microorganisms such as Saccharomyces are required for nutrition, proper development of Peyer’s aggregated lymphoid tissue, and tissue healing. However, even the commensal nonclassically pathogenic microbiota can trigger autoimmunity when fine regulation of immune tolerance does not work properly. For our purposes, the protein database of the National Center for Biotechnology Information (NCBI) was consulted, comparing Saccharomyces mannan to several molecules with a pathogenetic role in autoimmune diseases. Thanks to the NCBI bioinformation technology tool, several overlaps in molecular structures (50–100 %) were identified when yeast mannan, and the most common autoantigens were compared. The autoantigen U2 snRNP B″ was found to conserve a superfamily protein domain that shares 83 % of the S. cerevisiae mannan sequence. Furthermore, ASCAs may be present years before the diagnosis of some associated autoimmune diseases as they were retrospectively found in the preserved blood samples of soldiers who became affected by Crohn’s disease years later. Our results strongly suggest that ASCAs’ role in clinical practice should be better addressed in order to evaluate their predictive or prognostic relevance.

Keywords Anti-Saccharomyces cerevisiae autoantibodies · Autoimmune diseases · Molecular mimicry · Autoantigenicity · Vaccines

Introduction

Saccharomyces cerevisiae, also known as the baker’s or brewer’s yeast, has long been utilized to ferment the sugars of rice, wheat, barley, and corn to produce alcoholic beverages, and in the baking industry to raise dough. Thousands of years ago, yeasts accidentally "contaminated" flour or drinks, and the results were pleasant for the people who tasted them. As a consequence, we are now commonly exposed to yeast [1]. Nonetheless, anti-S. cerevisiae antibodies (ASCAs), directed against the phosphopeptidomannan part of the cell wall of the yeast, have been identified as an important and specific serological marker for Crohn’s disease (CD) [2]. Furthermore, ASCAs were detected retrospectively as being present years before CD clinical onset in 31.3 % of preserved blood samples of Israeli Defence Force soldiers compared to none of the controls [3]. The pathogenic significance of ASCAs is not yet fully understood, but molecular mimicry of self-antigens remains a possibility. Although ASCAs are considered specific for CD, a growing number of studies have individually identified high levels of ASCAs in other autoimmune diseases such as antiphospholipid syndrome (APS) [2], systemic
lupus erythematosus (SLE) [4], diabetes mellitus type 1 [5], and rheumatoid arthritis (RA) [6].

In recent decades, the pathogenesis of autoimmune diseases has increasingly come to be understood as a multifactorial process which develops through the interaction of the pebbles of genetic, immunological, environmental, and hormonal factors which combine to compose the complex mosaic of autoimmunity [7–10]. Evidence of the association between ASCAs and autoimmune disorders has increased over the past two decades, and to the best of our knowledge, this is the first review aimed at discussing these findings and providing the molecular background for further studies.

Environment/Infections and Autoimmunity

The immune system is exposed to different antigens from the very first stages of its development and maturation. Positive selection allows weakly self-binding lymphocytes to survive, while negative selection leads to the accomplishment of the death program for cells interacting too tightly with immunogenic molecules, with the aim of maintaining self-tolerance. Only a very small minority of lymphocytes will survive and interact with several microbial antigens that could potentially cause pathology. The burden of infections dealt with from childhood makes every individual’s microbial pattern unique [11]. Microbial agents can induce autoimmunity through four main mechanisms [12]: (1) molecular mimicry is perhaps the most likely mechanism and occurs when shared epitopes (carbohydrate, protein/peptides, or DNA) in the pathogen and host’s molecular structures cross-react in the presence of an active immune response. Cross-reactivity is confirmed by the association between the pathogen and the autoimmune disease, the elicitation of the specific immune response, active immunization or autoantigen-induced disease in animal models, and passive immunization if the disease occurs either after autorre cognition or autoantigen-induced disease in animal models, and autoimmune disorders has increased over the past two decades, and to the best of our knowledge, this is the first review aimed at discussing these findings and providing the molecular background for further studies.

Exhibit 205
overexpression has also been described in response to the eukaryotic yeast cells of *Candida albicans* [25]. Furthermore, miRNAs, especially miR155, are also involved in maintaining the proper T cell-dependent humoral response, certainly by modulating the release of cytokines and possibly through other mechanisms as well [26]. In a recent study by Iborra et al. increased miRNA expression was found in IBD, and the expression of miR192 and miR21 was also related to ulcerative colitis disease activity compared with healthy controls [23]. A prominent role, among several infective agents associated with autoimmune diseases, has been recognized for viruses and bacteria. Persistent Epstein–Barr virus (EBV) infection is linked with RA, where the EBV DNA load can increase by up to tenfold, and with SLE, where impaired T cell function was reported in addition to latently infected peripheral B cells [27]. Moreover, anti-EBV viral capsid and anti-EBV nuclear antigen 1 antibodies were also detected in almost all patients with multiple sclerosis (MS), and titers become two- to threefold higher than those of controls during the third decade of life, increasing in line with the MS risk [28]. Concerning bacterial infections, in a prospective study, half of 246 patients with inflammatory arthritis were found positive for RF, and their anti-*Proteus mirabilis* IgM and IgA titers were significantly higher than those in other groups of patients. Currently, there are no studies reviewing the impact of environmental exposure to fungal antigens on autoimmunity. However, Shinohara et al. induced coronary arteritis (a hallmark of Kawasaki arteritis) in mice by intraperitoneal injection of *C. albicans* water-soluble fraction, and the disease has been related to the activation of the complement lectin pathway [29], possibly through mannan-binding lectin (MBL), a pattern recognition molecule [30]. MBL can bind mannan which is a fungal cell wall antigenic element notably exposed on the yeast cell surface of *S. cerevisiae*. The detection of ASCAs typically associated with CD, perhaps more than just an epiphenomenon, may also be implicated in the pathogenesis of IBD [31]. Interestingly, *C. albicans* is able to induce the production of ASCAs (but *S. cerevisiae* does not elicit the reverse), as tested in rabbit experimental models and as shown by immunohistochemical stains from biopsies of patients with diagnosed systemic candidiasis [32].

**Immunological Aspects and Assays**

Yeasts are known as biological machines capable of producing antigenic components for vaccines needed to elicit protective immune responses. A question arises: is *S. cerevisiae* immunogenic itself? The use of *Saccharomyces*-based therapeutic vaccines is supported by the evidence of their ability to stimulate tumor or viral-specific CD4 and CD8 T cell responses. Heat-killed *S. cerevisiae* seems to be an attractive carrier because it can express different antigens and is cost-effective as it provides danger signals that lead dendritic cells (DCs) to maturation without the need of additional adjuvants. The phagocytosis of the yeast by DCs is triggered by the immunogenic cell wall molecules, such as β-1,3-D-glucan and mannan, which induce critical signals usually associated with microbial infection. This event represents the first step, and it is followed by antigen degradation and the presentation of their fragments on the APC surface by MHC I and MHC II molecules, interacting with the TCR and inducing co-stimulatory signals to lead the adaptive T cell (CD8+ or CD4+)-mediated response [33, 34]. *S. cerevisiae* is an effective inducer of innate and adaptive cellular immunity, and recombinant yeast cells (GI-5005) can lead to a potent antigen-specific proliferative and cytotoxic T cell response associated with Th1-type cytokine secretion [35].

Commensals such as *Saccharomyces* are required for nutrition, proper development of Peyer’s aggregated lymphoid tissue, and tissue healing. However, it is possible that in the proinflammatory pathological microenvironment that characterizes many immune-mediated diseases, notably IBD, the alteration of the finely regulated interaction between APCs, the nonclassically pathogenetic microbiota, and Th17 cells in the gut could trigger autoimmunity [20]. Moreover, MyD88, as an adaptor molecule shared by most TLRs, has been proven to play a key role in antifungal defense by several in vivo studies, underlining the relevance of TLRs in the host interaction with fungal microbial antigens [36].

The subsequent development of the humoral immune response leads to the production by B lymphocytes and plasma cells of antibodies against the yeast, so it is not yet clear whether it might simply represent an epiphenomenon or could have a direct pathogenic role through a co-stimulatory CD80/86-CD28-mediated effect. Indeed, it is plausible that in a proinflammatory state, a mechanism of molecular mimicry involving the eukaryotic microorganism and self-antigens takes over. Furthermore, infection by *C. albicans* can induce the production of ASCAs in humans, while *Saccharomyces* does not lead to the production of antibodies to *C. albicans* [29].

On the other hand, concerns have been raised regarding the current safety of vaccines due to the presence of adjuvants. Since heat-killed *Saccharomyces* can act like common adjuvants such as squalene, aluminum, and silicone (Table 1) when injected together with preventive vaccines, there is a risk of inducing an autoimmune disorder by administering *Saccharomyces*-based therapeutic or even preventive vaccines instead of traditional ones [37–39]. It is very difficult to find the ideal compromise between immune system stimulation and modulation. Indeed, procedures used to manufacture vaccines for hepatitis B and/or hepatitis A result in products that contain no more than 1–5% residual yeast proteins (Recombivax HB, Engerix B; Merck & Co.) [40]. Although the risk/benefit analysis still
Table 1 | Vaccines containing S. cerevisiae as an adjuvant

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Extract</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTaP-HepB-IPV (Pediatrix)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Hip/Hep B (Comvax)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Hep B (Engerix-B)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Hep B (Recombivax)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>HepA/HepB (Twinrix)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Meningococcal (Menveo)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Pneumococcal (Prevnar)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Pneumococcal (Prevnar13)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Typhoid (oral Ty21a)</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>HPV (Gardasil)</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>

indicates ensuring that vaccinations are used, we now face the challenge of developing risk-free vaccines.

Indeed, an additional risk is that of developing the recently identified syndrome, namely, autoimmune/inflammatory syndrome induced by adjuvants [37] that comprises several medical conditions characterized by hyperactive immune responses accompanied by a similar array of signs and symptoms. Since adjuvants are the common ground that could possibly induce autoimmune or autoinflammatory diseases in humans, the use of immunogenic matter as an adjuvant, such as S. cerevisiae, should be carefully evaluated.

ASCAs were found in several autoimmune diseases by means of ELISA tests used for the quantitative measurement of IgG- and IgA-class autoantibodies against mannan of S. cerevisiae in human sera or plasma. The assessment of ASCAs by ELISA resulted in 50–79 % sensitivity and 74–93 % specificity in Australian CD patients, depending on the commercial kits used [41].

ASCAs are directed against the cell wall mannan (phosphopeptidomannan) of the yeast S. cerevisiae. IgG as well as IgA ASCAs are held to be highly specific for CD. However, the determination of ASCAs is also reliable in other autoimmune disorders besides CD [3]. In a normal range study with serum samples from healthy blood donors, ASCA tests for either IgG or IgA subclasses were considered positive at a titer of >10 U/ml.

Nevertheless, positive results should be interpreted in the light of the patient’s clinical status. It is recommended that each laboratory establishes its own normal and pathological ranges for serum ASCAs. The lower detection limit for ASCA ELISA was determined at 1 U/ml. The solid phase is coated with mannan from S. cerevisiae (ORG 545 ASCA IgG/IgA, ORGENTEC Diagnostika GmbH, Germany). Therefore, the ASCA test kits only recognize autoantibodies specific for this phosphopeptide [3].

Database Searching Method and Results

For our purposes, we consulted the protein database of the National Center for Biotechnology Information (NCBI), comparing mannan of Saccharomyces to several molecules with a pathogenetic role in autoimmune diseases. We focused on specific and significant results (highest identity/positivity), displaying them in tables by pathological conditions. The related graphics show the location of the positive match, along sequence bars composed of ranges with different color shades, according to the resulting score.

We also evaluated the expectation or expect value (E value); this represents the number of different alignments with scores equivalent to or better than what is expected to occur by chance in a database search. The lower the E value, the more significant is the score and the alignment [54]. The E values for our results range between 0.17 and 9.90 with a mean of 3.11. This reliability index supports and reinforces the evidence of our findings.

Table 2 | Link between rheumatoid arthritis-associated antigens and ASCAs

<table>
<thead>
<tr>
<th>Autoimmune Disease</th>
<th>ASCA</th>
<th>Antigens (Homo sapiens)</th>
<th>Comparison to mannan; accession no. EDV13046.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rheumatoid arthritis</td>
<td>Neg</td>
<td>RF</td>
<td>5/10, 50 %</td>
</tr>
<tr>
<td></td>
<td>Pos</td>
<td>Anti-citrullinated collagen type 2</td>
<td>3/6, 50 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>gp130-RAPS</td>
<td>4/5, 80 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EIF4G1</td>
<td>6/8, 75 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Bip/GRP78</td>
<td>5/7, 71 %</td>
</tr>
</tbody>
</table>

The main autoantigens in RA with the highest sensitivity and specificity have been considered with the relative percentage of sequence identities and/or positive substitutions. Accession number EDV13046.1 is a code which corresponds to mannan molecular structure in the NCBI database, which was considered the referring element for each comparison.

RF rheumatoid factor, RAPS rheumatoid arthritis antigenic peptide-bearing soluble form, EIF4G1 eukaryotic translation initiation factor 4 gamma 1, Bip/GRP78 glucose-regulated protein 78

Autoimmune Diseases Associated with S. cerevisiae

The association between infectious and autoimmune diseases has been widely described, and S. cerevisiae may represent another dowel in this parquetry puzzle. In one study, ASCA IgA, IgG, and IgM levels were measured with ELISA in 30 patients affected with RA and in 152 healthy adult controls. ASCA IgA prevalence was significantly higher in RA patients (40 %) than in healthy subjects (5.3 %). In RA patients, ASCA IgA levels strongly correlated with C-reactive protein (CRP) (r = 0.695; p < 0.01) and erythrocyte sedimentation rate (r = 0.708; p < 0.01) [6]. As shown in Table 2, we observed significant similarities be-
Sequence bars are displayed as a group of bars in various shades of gray, each representing a result sequence that shows the position(s) where the result sequence is similar to the input sequence. Sequence bars show their score range by color shade variation, according to the alignment scores color key. The most similar hits are upmost (≥200). Fig. 1a Conserved domains for chain B anti-citrullinated peptide collagen type 2. Clusters of different arrowheads which belong to specific hits included in a superfamily protein domain are shown such as the heterodimer interface (polypeptide binding site) on conserved domain IgC_L that compose the conserved feature mapped on the query sequence. b Distribution of two hits for chain B anti-citrullinated peptide collagen type 2 compared to mannan. The alignments’ result came from the process of matching amino acid residues from both biosequences. In this sequence matching, consistent homology was detected showing the existing degree of similarity between the considered molecular structures.

Fig. 2a Conserved domains for U2 snRNP B*. Clusters of different arrowheads which belong to specific hits included in a superfamily protein domain are shown, namely, the RNA recognition motif (RRM) also known as RNA-binding domain (RBD) or ribonucleoprotein (RNP) domain, one of the most plenteous protein domains in eukaryotes. Compositionally biased region not used in this domain database search is highlighted on the query sequence. b Distribution of the hit for U2 snRNP B* compared to mannan. In this sequence match, a very high homology was detected showing the existing degree of similarity between the conserved molecular structures. Fig. 3a Conserved domains for ICAM 1 which play a central role in intercellular adhesion have been detected; the Ig2 ICAM-1-like-specific hit, included in the immunoglobulin superfamily protein domain, is displayed. Compositionally biased regions not used in this domain database search are highlighted on the query sequence. b Distribution of the hit for ICAM 1 compared to mannan. Fig. 4a Conserved domains for α-enolase 1, a glycolytic enzyme, have been detected compared to mannan. Clusters of different arrowheads which belong to specific hits included in the triosephosphate isomerase or TIM phosphate binding superfamily protein domain are shown such as dimer interface on conserved domain enolase; 37 of 37 of the residues that compose this conserved feature have been mapped to the query sequence. b Distribution of five hits for α-enolase 1. In this sequence match, a very high homology was detected showing the existing degree of similarity between the conserved molecular structures. Fig. 5a Conserved domains for anti-cardiolipin/beta-2-glycoprotein-I immunoglobulin light chain variable region have been detected compared to mannan. Compositionally biased region not used in this domain database search is highlighted on the query sequence. b Clusters of different arrowheads which belong to specific hits included are shown. In this sequence match, a very high homology was detected showing the existing degree of similarity between the considered molecular structures such as the antigen binding site on conserved domain IgV_L lambda where 6/6 residues that compose this conserved feature have been mapped to the query sequence between the sequence of autoantigens (Fig. 1a, b) and mannan expressed by the cell wall of S. cerevisiae.

In another study, serum samples from healthy volunteers (n=152) and patients with SLE (n=40) were compared for ASCA IgA, IgG, and IgM levels using ELISA. The prevalence of ASCA IgG, but not IgM and IgA, was significantly raised in active SLE patients (57.5 %) compared to healthy controls (8.5 %) (Fig. 2c). ASCA IgG levels in SLE patients during remission were relatively lower, indicating a possible correlation with disease activity [4]. Several SLE autoantigens have been found to share sequences with yeast mannan, and U2 snRNP B* (Fig. 2a, b) shows the best match (Table 3).

### Table 3: Link between systemic lupus erythematosus-associated antigens and ASCAs

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>ASCA Antigens (H. sapiens)</th>
<th>Comparison to mannan; accession no. EDV13046.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>IgA</td>
<td>Identities</td>
</tr>
<tr>
<td>Systemic lupus erythematosus</td>
<td>Pos Neg SSA (Ro), 5/10, 50 % 6/10, 60 %</td>
<td>5/10, 50 % 6/10, 60 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/13, 46 % 9/13, 69 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6/11, 55 % 7/11, 64 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/15, 33 % 8/15, 53 %</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5/6, 83 % 5/6, 83 %</td>
</tr>
</tbody>
</table>

The percentages of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related.

### ASCAs, Autoimmunity, and Atherosclerosis

The role of local and general inflammation in atherosclerosis is a major area of interest. In a case–control study, the ASCA IgG and IgA levels of patients with acute myocardial infarction (AMI) were compared with those of controls to investigate the possible role of ASCAs in atherosclerosis. AMI was diagnosed by electrocardiography and serial enzymes. Elevated ASCA IgA and IgG levels were found, suggesting that ASCA positivity in AMI could represent a useful marker for atherosclerotic plaque instability. It might also provide a link between inflammatory processes and increased cardiovascular risk. Interestingly, one of the most significant sequence matches with mannan is ICAM-1 (Fig. 3a, b). ICAM-1 and P-selectin (Table 4) are crucial molecules for transendothelial migration of leucocytes, playing an important role in the process of atherogenesis, especially in patients with chronic systemic inflammatory autoimmune diseases [42]. The role of macrophages expressing the LOX1 receptor for oxidized LDL is well-known in the pathogenesis of atherosclerosis, but more recently, a distinction has been proposed for the cells involved in the atherogenic process, especially when it is accelerated by systemic inflammatory.

### Table 4: Link between acute myocardial infarction-associated antigens and ASCAs

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>ASCA Antigens</th>
<th>Comparison to mannan; accession no. EDV13046.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiovascular diseases, acute myocardial infarction</td>
<td>Pos Pos P-selectin Interstitial adhesion molecule-1 Myosin</td>
<td>8/15, 53 % 11/15, 80 % 4/4, 100 % 4/4, 100 % 5/8, 63 % 7/8, 88 %</td>
</tr>
</tbody>
</table>

The percentage of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related.
disease: type 1 (M1) macrophage cells with a proinflammatory effect (expressing high levels of CD80–86, IL23, and MHC II) and type 2 CD163+ (M2) cells displaying a Th2-like phenotype (widely represented in atherosclerotic lesions of the vascular intimal layer). The M2a macrophage subtype is activated by signaling molecules such as IL-4 and IL-13 with anti-inflammatory and pro-B cell growth and activation properties. They are also characterized by the expression of mannose receptors and the production of TGF-β, dectin1, MBL, and IL-10 (among other secretory products), thus promoting immunoregulation, tissue remodeling, and fibrosis induction. Moreover, the dominance of the M1 over the M2 phenotype has been associated with progression of atherosclerotic disease [43].

In a randomized trial, *Saccharomyces boulardii* oral administration in patients with heart failure (NYHA I–II) ameliorated gastrointestinal symptoms (constipation, postprandial fullness, flatulence, and dyspepsia), lowering CRP, leukocyte count, and echocardiographic left atrial diameter compared to placebo. Increased ASCA levels were also reported in several studies on Behçet’s disease in which a prevalence of 44 % was shown in patients with intestinal involvement, compared with 3 % in patients without gastrointestinal symptoms [44]. Alpha-enolase 1 (Fig. 4a, b) is known as an autoantigen in Behçet’s disease with ocular involvement (uveitis). As reported in Table 5, the similarity of sequences suggests the presence of shared epitopes in different kinds of rheumatic conditions with immunologic ocular involvement. Cross-reactive epitopes on β2-glycoprotein-I (β2GPI) and *S. cerevisiae* (Fig. 5a, b; Table 6) were also found by our group in patients with APS. Anti-β2GPI antibodies were affinity purified from ASCA-positive APS patients and were proven to bind mannan in a dose-dependent manner [2]. Encompassing different autoimmune disorders associated with ASCA positivity [5, 45–53], we discovered other autoantigens that might cross-react with antibodies against mannan of *S. cerevisiae* (Table 7); the percentages of sequence identities (ID) and/or positive substitutions (PS) were pointed out for transglutaminase (ID and PS 60 %) in celiac disease, GAD65 (ID 35 %, PS 57 %) and zinc transporter 8 (ID 43 %, PS 57 %) for diabetes mellitus type 1, proteinase3 (ID 57 %, PS 86 %) and myeloperoxidase (ID 71 %, PS 86 %) for vasculitis, soluble liver/pancreas antigen (ID 40 %, PS 80 %) for autoimmune hepatitis, calprotectin or protein S100-A8 for Crohn’s disease (ID 60 % PS 100 %), and thyroglobulin (ID 35 %, PS 52 %) and thyroid peroxidase (ID and PS 71 %) for autoimmune thyroid disease.

Performing our database research of shared epitopes, we found very interesting results with regard to systemic sclerosis (SSc) (Table 8); high percentages of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related.

### Table 5 Link between Behçet’s disease, immunologic ocular disease–associated antigens, and ASCAs

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>ASCA Antigens (H. sapiens)</th>
<th>Comparison to mannan; accession no. EDV13046.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG IgA</td>
<td>Identities Positives</td>
<td></td>
</tr>
<tr>
<td>Behçet’s disease</td>
<td>Pos Pos</td>
<td>α-Enolase 1 (6/8, 75 %)</td>
</tr>
<tr>
<td>Immunologic ocular disease</td>
<td>Pos Pos</td>
<td>NKG5 (4/7, 57 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Granulysin (4/8, 50 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rhodopsin (5/10, 50 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycosaminoglycan (5/10, 50 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xylose kinase (6/8, 75 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>α-Enolase 1 (6/8, 75 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Retinal S-Ag (6/12, 50 %)</td>
</tr>
</tbody>
</table>

The percentage of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related.

### Table 6 Link between antiphospholipid syndrome–associated antigens and ASCAs

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>ASCA Antigens (H. sapiens)</th>
<th>Comparison to mannan; accession no. EDV13046.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG IgA</td>
<td>Identities Positives</td>
<td></td>
</tr>
<tr>
<td>Antiphospholipid syndrome</td>
<td>Pos Pos</td>
<td>β2-Glycoprotein-I (7/18, 39 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>β2-Glycoprotein-I precursor (11/18, 56 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Annexin A5 (5/8, 63 %)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti-CLβ-2GPI (7/11, 64 %)</td>
</tr>
</tbody>
</table>

The percentage of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related.

### Table 7 Link between AID-associated antigens and ASCAs

<table>
<thead>
<tr>
<th>Autoimmune disease</th>
<th>ASCA Antigens (H. sapiens)</th>
<th>Comparison to mannan; accession no. EDV13046.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG IgA</td>
<td>Identities Positives</td>
<td></td>
</tr>
<tr>
<td>Celiac disease</td>
<td>Neg Pos</td>
<td>Transglutaminase (6/10, 60 %)</td>
</tr>
<tr>
<td>Diabetes mellitus type 1</td>
<td>Pos Pos</td>
<td>GAD65 (8/23, 35 %)</td>
</tr>
<tr>
<td>Vasculitis</td>
<td>Pos Pos</td>
<td>Soluble liver/pancreas antigen (4/10, 40 %)</td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td>Pos Pos</td>
<td>Calprotectin (3/5, 60 %)</td>
</tr>
<tr>
<td>Autoimmune thyroid disease</td>
<td>Neg Pos</td>
<td>Thyroglobulin (8/23, 35 %)</td>
</tr>
</tbody>
</table>

The percentage of sequence identities and/or positive substitutions can express the extent to which the protein sequences are related.
The importance of improving and better defining the clinical use and reliability of ASCA tests in clinical practice for autoimmune diseases should certainly be underlined. ASCA positivity should be addressed in patients with different clinical courses who may deserve closer management of comorbidities, especially concerning increased cardiovascular risk.

### References


Vaccination and Risk for Developing Inflammatory Bowel Disease: A Meta-Analysis of Case-Control and Cohort Studies

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This article has an accompanying continuing medical education activity on page e130. Learning Objective—Upon completion of this activity, successful learners will be able to discuss the implication of vaccination and environmental factors in the development of inflammatory bowel disease.

BACKGROUND & AIMS: Environmental factors may play a key role in the pathogenesis of inflammatory bowel disease (IBD). Whether vaccination is associated causally with IBD is controversial. We performed a meta-analysis of case-control and cohort studies on the association between vaccination and the risk for IBD.

METHODS: Studies and abstracts investigating the relationship between vaccination and subsequent risk for developing IBD were reviewed. Childhood or adult immunizations with any vaccine type, at any dose, and with any vaccine schedule were used as inclusion criteria.

RESULTS: Eleven studies were included in the systematic review and meta-analysis: 8 case-control studies and 3 cohort studies. Studied vaccines were bacille Calmette-Guérin), vaccines against diphtheria, tetanus, smallpox, poliomyelitis, pertussis, H1N1, measles, rubella, mumps, and the combined meases, mumps, and rubella vaccine. Only a few details about vaccine type or route of administration were found in studies. Overall, there was no association between childhood immunization and risk for developing IBD: bacille Calmette-Guérin, relative risk (RR) of 1.04 (95% confidence interval [CI], 0.78–1.38), diphtheria, RR of 1.24 (95% CI, 0.80–1.94), tetanus, RR of 1.27 (95% CI, 0.77–2.08), smallpox, RR of 1.08 (95% CI, 0.70–1.67), poliomyelitis, RR of 1.79 (95% CI, 0.88–3.66), an measles containing vaccines, RR of 1.33 (95% CI, 0.31–5.80) in cohort studies, and RR of 0.85 (95% CI, 0.60–1.20) in case-control studies. Subgroup analysis for Crohn’s disease (CD) and ulcerative colitis (UC) found an association between the polioomyelitis vaccine and risk for developing CD (RR, 2.28; 95% CI, 1.12–4.63) or UC (RR, 3.48; 95% CI, 1.2–9.71). The RR of developing IBD after H1N1 vaccination was 1.13 (95% CI, 0.97–1.32).

CONCLUSIONS: Results of this meta-analysis show no evidence supporting an association between childhood immunization or H1N1 vaccination in adults and risk of developing IBD. The association between the poliomyelitis vaccine and the risk for CD or UC should be analyzed with caution because of study heterogeneity.

Keywords: Crohn’s Disease; Ulcerative Colitis; Poliomyelitis; BCG; Vaccine; Childhood.

The etiology of inflammatory bowel diseases (IBDs) including Crohn’s disease (CD) and ulcerative colitis (UC) remains unknown. The pathogenesis of IBD is thought to involve an altered immune response against gut microflora in genetically predisposed individuals, leading to mucosal inflammation and ulcerations.1,2 Currently, more than 100 susceptibility genes for CD and UC have been identified by a

Abbreviations used in this paper: BCG, bacille Calmette-Guérin; CD, Crohn’s disease; CI, confidence interval; IBD, inflammatory bowel disease; IPV, injected inactivated vaccine; MMR, measles, mumps, rubella; OR, odds ratio; OPV, oral live vaccine; RR, relative risk; UC, ulcerative colitis.
genome-wide association scan study.3 Although genetic influence may play an important role in the development of IBD, several observations plead for equal implication of environmental exposure.1–6 The lack of complete penetrance of UC and CD among monozygotic twins and the limited familial occurrence of IBD (5%–10%) indicates that environmental factors play a role in the development of these disorders.7,8 Many environmental factors have been proposed as etiologic factors of CD or UC.8–10

There is accumulating evidence that events early in life may have long-term effects on health and disease.9,14 In the same way, it has been suggested that attenuated live measles virus vaccine might lead to IBD.12 The first report on the risk of developing IBD after a measles vaccination in leads to international controversy and suspicion about vaccination safety. Since then, several population-based, case–control and cohort studies have investigated the potential link between childhood immunization and IBD with conflicting results.11–21 Different types of vaccines were studied such as poliomyelitis, measles, rubella, mumps, smallpox, pertussis, tetanus, diphtheria, and bacille Calmette–Guérin (BCG).

An attractive theory is that vaccination, which leads to a decrease in the prevalence of early childhood infections, may favor immunologic diseases.22 A direct effect of viral or bacterial components included in vaccines on the immune system also may be implicated.23 Finally, adjuvant contained in many vaccines, such as aluminum, could be at risk of overstimulating the immune system, leading to dysregulated inflammatory response.10,24 Overall, whether vaccination is associated causally with IBD remains controversial.

The aim of this study therefore was to perform a systematic review and meta-analysis of case–control and cohort studies on the association between vaccination and the risk of developing IBD.

Methods

Search Strategy and Study Selection

A systematic review and meta-analysis was conducted in accordance with guidelines for systematic reviews and meta-analyses published previously.25 A computerized search of the medical English and non-English literature was conducted using MEDLINE (1970 to June 2014), EMBASE, and the Cochrane central register of controlled trials. Studies and abstracts investigating the relationship between vaccination and subsequent risk for the development of IBD were reviewed. Only randomized controlled trials, controlled clinical trials, cohort studies, and case–control studies investigating the risk for IBD after vaccination were eligible for inclusion. Childhood or adult immunizations with any vaccine type, at any dose, and with any vaccine schedule were used as inclusion criteria. Potentially eligible studies were identified via a literature search using the terms ulcerative colitis, Crohn’s disease, inflammatory bowel disease, colitis, or ileitis. These were combined using the set operator AND with studies identified with the following terms: vaccine, vaccination, immunization, smallpox, poliomyelitis, tetanus, diphtheria, pertussis, mumps, rubella, measles, meases, mumps, rubella (MMR) vaccine, BCG, or influenza vaccine. Abstracts of the studies identified by the initial search were evaluated by the lead investigator for appropriateness to the study question, and all potentially relevant articles were obtained and evaluated in detail. We searched the bibliographies of all relevant articles obtained and any published reviews for additional studies. Abstract books of conference proceedings from major congresses in gastroenterology, Digestive Diseases Week, United European Gastroenterology Week, and the European Crohn’s and Colitis Organisation between 2002 and 2013 were hand-searched to identify potentially eligible studies published only in abstract form. Articles were assessed independently by 2 investigators using predesigned eligibility forms, according to the predefined eligibility criteria. Any disagreements between investigators were resolved by discussion.

Outcome Assessment

The outcome measures were defined a priori. The primary outcome assessed was the occurrence of IBD, CD, or UC in patients receiving vaccination compared with patients without any vaccination. The meta-analysis evaluated different types of vaccines against tetanus, poliomyelitis, diphtheria, pertussis, smallpox, measles, mumps, rubella, and tuberculosis (BCG). Most of the studies analyzed the risk of developing IBD independently for each vaccine in the same patient population without reporting a global risk after vaccination. In these studies, each patient may have received 1 or more vaccine types. Because it was not possible to pool the risk of all vaccines for each study, we performed the meta-analysis for each vaccine type separately.

Data Extraction

All data were extracted by the lead investigator to a Microsoft Excel spreadsheet (XP professional edition; Microsoft Corp, Redmond, WA) as an odds ratio (OR) of developing IBD for each vaccine type in each study. If not available, the OR was calculated using the number of cases and controls exposed or not. In addition, the following clinical data were extracted for each study, when available: number of centers, country of origin, geographic region, type of study, inclusion period, inclusion criteria, methods for collecting past vaccination data, matching characteristics for case–control studies,
disease type (UC or CD), total number of controls and IBD cases, and studied vaccines.

Statistical Analysis

Pooled results were expressed as the OR of IBD with vaccination compared with no vaccination, with 95% confidence intervals (CIs). Analyses were performed if at least 3 studies evaluating the same vaccination could be combined. If only 1 or 2 studies investigated the same vaccination, a description of studies was performed without a meta-analysis. Each meta-analysis was performed using only case–control studies, except for the measles vaccination, in which case–control and cohort studies were included in an analysis stratified by study design. For each meta-analysis, the method of Der Simonian and Laird was used. According to this method, studies were considered as a random sample from a population of studies. Statistical heterogeneity was tested for each analysis. Because of heterogeneity among studies, a random-effect model was used to analyze data. All analyses were performed using R software and metafor package (R Development Core Team, 2011; R Foundation for Statistical Computing, Vienna, Austria).77

Results

Literature Search

The search strategy identified 428 citations, and 412 articles were excluded after reviewing the title and abstract (Supplementary Figure 1). Sixteen studies were retrieved for evaluation and 5 studies were excluded. Three studies were epidemiologic studies on IBD incidence during a vaccination campaign without estimation of the risk, 1 study was a case report, and 1 study did not have a control group. Finally, 11 studies were included in this systematic review and meta-analysis, and the characteristics of these studies are presented in Table 1. There were 8 case–control studies, of which 6 were population-based and 2 were hospital-based, and 3 were population-based cohort studies, including 3 cohorts reporting data on measles and on H1N1 influenza. Two studies investigated the occurrence of IBD in the pediatric population only. These studies reported on 11 vaccine types, including measles, mumps, and rubella, and the MMR vaccine, and H1N1 influenza. All of these studies except 1 (H1N1 vaccine) investigated the effect of childhood vaccination. Because most of the studies analyzed the risk of developing IBD independently for each vaccine in the same patient population without reporting a global risk after vaccination, we performed the meta-analysis for each vaccine type separately (Table 2).

Bacille Calmette–Guérin

Three case–control studies (n = 963 patients) investigated the risk for IBD after BCG vaccination during childhood. The relative risk (RR) of developing IBD after BCG vaccination was 1.04 (95% CI, 0.78–1.38). These figures were 1.17 (95% CI, 0.58–2.38; n = 3 studies; 563 patients) and 0.98 (95% CI, 0.73–1.30; n = 2 studies; 400 patients) for CD and UC, respectively (Figure 1). There was heterogeneity only among studies on CD (I2 = 62%; P = .07).

Diphtheria- and Tetanus-Containing Vaccines

Three case–control studies (n = 524 patients) investigated the risk of developing IBD after diphtheria or tetanus vaccination during childhood with 1 of these studies investigating only pediatric IBD. The RRs of developing IBD after vaccination with diphtheria-containing vaccines and tetanus-containing vaccines were 1.24 (95% CI, 0.80–1.94) and 1.27 (95% CI, 0.77–2.08), respectively (Figure 1). No heterogeneity was observed between the 3 studies.

Poliomyelitis

Three case–control studies (n = 666 patients) investigated the risk of developing IBD after a poliomyelitis vaccination during childhood. A study was published only as an abstract. The pooled RR of developing IBD after poliomyelitis vaccination was 1.79 (95% CI, 0.88–3.66) (Figure 2A). The specific RRs of developing CD (n = 345) and UC (n = 174) were 2.28 (95% CI, 1.12–4.63) and 3.48 (95% CI, 1.24–9.71), respectively (Figure 2B). The exclusion of the study available only as an abstract and without details on risk for CD or UC did not change our results with a pooled RR of 2.61 (95% CI, 1.46–4.68; P = .0012; and I2 = 0%; P = .7817, respectively). There was significant heterogeneity between the studies (I2 = 67%; P = .049) (Figure 3).

Pertussis

Two case–control studies (n = 407 patients) investigated the risk of developing IBD after a pertussis vaccination during childhood. The first study, which included 140 IBD patients born after 1968 in the United Kingdom and receiving a pertussis vaccination until 6 years of age, did not find a significant association between vaccination and the risk of developing IBD (OR, 1.00; 95% CI, 0.62–1.62). The second study from Denmark investigated childhood pertussis vaccinations in 267 patients diagnosed with IBD during 2003 to 2004 and matched controls. In this study, vaccination against pertussis increased the risk of developing IBD, especially UC (combined OR, 2.08; 95% CI, 1.07–4.03). Because only 2 studies were available, we did not perform a meta-analysis.
<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Country</th>
<th>Type</th>
<th>Population studied</th>
<th>Outcome</th>
<th>Methods for vaccine recall</th>
<th>Matching</th>
<th>Disease</th>
<th>Type of vaccine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glat et al</td>
<td>1987</td>
<td>International (United States, United Kingdom, Sweden, Holland, Denmark, Israel, France, Italy)</td>
<td>Hospital-based</td>
<td>IBD patients started &lt;20 y, &lt;25 y at the time of the study, at least 6 months duration</td>
<td>Diagnosis of IBD</td>
<td>Self-completion questionnaire</td>
<td>1:2, sex, age</td>
<td>CD</td>
<td>Smallpox, polio, BCG, diphtheria, measles, pertussis, tetanus</td>
</tr>
<tr>
<td>Feeney et al</td>
<td>1997</td>
<td>UK monocentric</td>
<td>Hospital-based</td>
<td>Born in or after 1968</td>
<td>Diagnosis of IBD</td>
<td>Data extraction from database</td>
<td>1:2, sex and year of birth</td>
<td>IBD</td>
<td>Pertussis, diphtheria, tetanus, measles</td>
</tr>
<tr>
<td>Davis et al</td>
<td>2001</td>
<td>US multicenter</td>
<td>Population-based</td>
<td>IBD and cases from age 6 mo to index date (diagnosis) or reference date for controls</td>
<td>Diagnosis of IBD</td>
<td>Data extraction from database</td>
<td>1:5, sex, center, year of birth</td>
<td>IBD</td>
<td>Measles, MMR vaccine</td>
</tr>
<tr>
<td>Baron et al</td>
<td>2005</td>
<td>France multicenter</td>
<td>Population-based</td>
<td>IBD occurring before age 17 y</td>
<td>Personal interview by trained investigators of mother and child (vaccine certificate needed)</td>
<td>None</td>
<td>1:1, age, sex, living area</td>
<td>IBD</td>
<td>MMR vaccine, BCG, poliomyelitis, smallpox</td>
</tr>
<tr>
<td>Bernstein et al</td>
<td>2006</td>
<td>Canada monocentric</td>
<td>Population-based</td>
<td>IBD &lt; 50 y</td>
<td>Questionnaire and venipuncture</td>
<td>None</td>
<td>IBD</td>
<td>Measles, mumps, rubella</td>
<td></td>
</tr>
<tr>
<td>Hansen et al</td>
<td>2011</td>
<td>Denmark monocentric</td>
<td>Population-based</td>
<td>Diagnosis of IBD during inclusion period</td>
<td>Questionnaire-based study</td>
<td>Age, sex, ethnicity, geographic location</td>
<td>IBD</td>
<td>Pertussis, diphtheria, tetanus, poliomyelitis, BCG, measles, rubella</td>
<td></td>
</tr>
<tr>
<td>Shaw et al</td>
<td>2012</td>
<td>Canada multicenter</td>
<td>Population-based</td>
<td>IBD born after 1989 and diagnosis before 2008</td>
<td>Data extraction, vaccination complete vs incomplete or none by age 2 y</td>
<td>Age, sex, living area at time of diagnosis</td>
<td>IBD</td>
<td>Diphtheria, tetanus, poliomyelitis, MMR vaccine</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Characteristics of Case–Control and Cohort Studies Included in the Meta-Analysis
Smallpox

Three case-control studies (n = 1255 patients) investigated the risk of developing IBD after a smallpox vaccination.17,21 The pooled RR of developing IBD after a smallpox vaccination was 1.08 (95% CI, 0.70–1.67; P = .72), without heterogeneity between the studies (Figure 4).

Measles, Rubella, and Mumps Vaccines

Eight studies (n = 1366 patients) investigated the risk of developing IBD after measles or MMR vaccination.11,12,14–16,18–20 Four studies investigated vaccination against measles with only live or attenuated vaccines,12,16,18,19 2 studies investigated only MMR vaccine,11,20 and 1 study investigated both vaccination strategies (Table 2). One other study focused on measles, rubella, and mumps serology, considering that most of the patients were seropositive because of childhood immunization (Table 2).14 Because 2 studies were population-based cohort studies,12,19 a meta-analysis of measles-containing vaccines was stratified by study design.

The pooled RRs of developing IBD after measles-containing vaccines in cohort and case-control studies were 1.33 (95% CI, 0.31–5.80) and 0.85 (95% CI, 0.60–1.20), respectively (Figure 4). In the meta-regression, results of cohort and case-control studies were not statistically different. The pooled RR of developing IBD after measles-containing vaccines (MMR included) by pooling cohort and case-control studies was 0.97 (95% CI, 0.63–1.49; P = .89) (Figure 4). There was significant heterogeneity between the studies (I² = 74%; P < .001) (Figure 3); this heterogeneity was owing mostly to cohort studies with an I² of 91% (P < .001) and 45% (P = .1023) for cohort and case-control studies, respectively (Figure 4).

The RRs of developing IBD after a measles vaccination and MMR vaccination in case-control studies were 0.97 (95% CI, 0.73–1.30; P = .82) and 0.67 (95% CI, 0.36–1.24; P = .199), respectively. No heterogeneity was observed between the studies. The RR of developing IBD after vaccination with rubella-containing vaccines (MMR included) was 0.65 (95% CI, 0.33–1.25; P = .19). There was significant heterogeneity between the studies for rubella-containing vaccines (I² = 77%; P = .002). Sensitivity analysis excluding the study using serology and not vaccination14 did not change the results (data not shown).

H1N1 Vaccine

Only 1 study investigated the risk of developing IBD after vaccination against the H1N1 influenza virus (Pandemrix, GlaxoSmithKline, London, UK) during the 2009 vaccination campaign in Sweden.13 It was a prospective population-based cohort study comparing the frequency of incident autoimmune diseases in patients...
with or without vaccination. After adjusting the statistical model for age, sex, socioeconomic status, and health care consumption (number of hospital admissions and visits to specialist care 1 year before the pandemic period), the hazard ratio of developing IBD after the H1N1 vaccination was 1.13 (95% CI, 0.97–1.32).

## Discussion

Since the report by Thompson et al, the controversy about the risk of developing IBD after childhood immunization still is ongoing. This study reported that vaccination with the live measles vaccine was a risk for the development of consequent inflammatory disease, such as CD or UC. However, many publications after this report investigating vaccination with measles-containing vaccines did not show any association between immunization and IBD. Epidemiologic studies also investigated other vaccines such as BCG, diphtheria, tetanus, poliomyelitis, smallpox, pertussis, rubella, and mumps, reporting conflicting results. A positive association between BCG vaccination and CD were found in the study by Baron et al, with a high risk in patients with multiple immunization shots. This study also found a positive association between poliomyelitis and smallpox vaccination and risk for IBD. On the contrary, 1 hospital-based, case-control

### Table 2. ORs of Developing IBD According to Vaccine Type in Case–Control and Cohort Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Total cases, n</th>
<th>Vaccine type</th>
<th>Disease</th>
<th>Cases, n</th>
<th>Controls, n</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilat et al</td>
<td>499</td>
<td>Smallpox</td>
<td>CD</td>
<td>266</td>
<td>36</td>
<td>922</td>
<td>76</td>
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<tr>
<td>Thompson et al</td>
<td>54</td>
<td>Live measles</td>
<td>UC</td>
<td>11</td>
<td>14</td>
<td>3531</td>
<td>11393</td>
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<tr>
<td>Feeney et al</td>
<td>140</td>
<td>Pertussis</td>
<td>IBD</td>
<td>99</td>
<td>41</td>
<td>198</td>
<td>82</td>
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<td>Feeney et al</td>
<td>140</td>
<td>Diphtheria/tetanus</td>
<td>IBD</td>
<td>130</td>
<td>10</td>
<td>261</td>
<td>19</td>
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<tr>
<td>Feeney et al</td>
<td>140</td>
<td>Measles</td>
<td>IBD</td>
<td>79</td>
<td>61</td>
<td>160</td>
<td>120</td>
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<tr>
<td>Morris et al</td>
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<td>Measles</td>
<td>UC</td>
<td>20</td>
<td>10</td>
<td>7284</td>
<td>2428</td>
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<tr>
<td>Davis et al</td>
<td>142</td>
<td>MMR</td>
<td>IBD</td>
<td>35</td>
<td>17</td>
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<td>372</td>
<td>Measles-containing vaccines</td>
<td>CD</td>
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<td>304</td>
<td>6</td>
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<tr>
<td>Morris et al</td>
<td>372</td>
<td>Measles-containing vaccines</td>
<td>UC</td>
<td>131</td>
<td>6</td>
<td>304</td>
<td>6</td>
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<tr>
<td>Bernstein et al</td>
<td>282</td>
<td>MMR vaccine</td>
<td>CD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Bernstein et al</td>
<td>282</td>
<td>BCG</td>
<td>CD</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>Bernstein et al</td>
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<td>CD</td>
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<td>-</td>
<td>-</td>
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<tr>
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<td>CD</td>
<td>170</td>
<td>65</td>
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<td>67</td>
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<td>Hansen et al</td>
<td>372</td>
<td>Measles</td>
<td>UC</td>
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<td>7</td>
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<td>Hansen et al</td>
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<td>Measles</td>
<td>UC</td>
<td>128</td>
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<tr>
<td>Bardage et al</td>
<td>14,842</td>
<td>H1N1 influenza</td>
<td>UC</td>
<td>125</td>
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<td>100</td>
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<td>MMR vaccine</td>
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<td>14,842</td>
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<td>UC</td>
<td>186</td>
<td>70</td>
<td>132</td>
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*Results are shown for measles, mumps, and rubella serology.

*Controls are expressed in person-years.
Figure 1. Risk ratio of developing IBD after BCG, diphtheria, and tetanus vaccinations in case-control studies.

Figure 2. Risk ratio of developing IBD after poliomyelitis vaccination in case-control studies. (A) Meta-analysis using all studies reporting the risk for IBD. (B) Meta-analysis with studies reporting the specific risk for Crohn’s disease and ulcerative colitis.
study published in 1987 found a protective role of smallpox vaccination against CD.17

Including 11 studies, 8 case–control studies, of which 6 were population-based and 2 were hospital-based, and 3 population-based cohort studies, we performed a meta-analysis of the risk of developing IBD after childhood or adult vaccination. Of note, the majority of the studies included focused only on childhood vaccination and 1 study investigated the H1N1 vaccine in adults. Overall, our results did not find any significant increased risk of developing IBD after this vaccination. Regarding poliomyelitis vaccination, 2 of the 3 studies analyzed in the meta-analysis reported a significant risk of developing IBD after vaccination. The other study was published only as an abstract and did not provide details for CD and UC. When we performed a sensitivity analysis without the study published as an abstract, we found a positive association between poliomyelitis vaccination and the risk of developing CD or UC (2.28 [95% CI, 1.12–4.63] and 3.48 [95% CI, 1.24–9.71], respectively). Regarding pertussis vaccination, only 2 studies reported a risk of developing IBD, with conflicting results.16,18

The results of this meta-analysis are globally reassuring regarding the risk of developing IBD after childhood vaccination. Vaccines that are developed to protect against an infectious disease or its consequences are, for the majority, not a risk for the subsequent development of intestinal inflammatory disease. The strength of our study was the large number of patients included in the meta-analysis, with 2399 IBD patients and 33,747 controls in 10 studies investigating 10 vaccine types (BCG, diphtheria, tetanus, poliomyelitis, smallpox, pertussis, measles, rubella, mumps, and the MMR vaccine), although not all vaccines were investigated in each IBD patient. The study investigating the H1N1 vaccine included 14,842 patients with IBD. Limitations of our work mainly were owing to the fact that studies investigating the risk of developing IBD after vaccination were extremely heterogeneous regarding their study design, sample size, geographic location, and vaccination recall methods. Indeed, we found significant heterogeneity between the studies for poliomyelitis- and measles-containing vaccines. Vaccination protocols varied between countries and evolved through the years, with different types of vaccines and schedules leading to difficulties in risk evaluation. Moreover, some vaccines used were live attenuated vaccines such as measles, oral poliomyelitis, or whole-cell pertussis vaccines, and may have a different effect on immune system activation and dysregulation compared with other inactivated acellular vaccines. In addition, the method of recalling the vaccination status in each patient represented a limitation in these studies. Indeed, half of the studies used a database to collect the vaccination status of patient, but the other half used only a self-completed questionnaire or an interview, which may have lead to recall bias. Specific bias present only in certain studies could have led to heterogeneity as well as publication bias because statistically significant results come mostly from smaller studies,11,12,18 as illustrated by the Funnel plot profile for poliomyelitis. However, the small number of studies for most vaccines did not allow us to explore further publication bias and heterogeneity. Of note, the study by Gilat et al,17 which collected data about several vaccinations, only reported detailed positive results for smallpox vaccination, indicating that no association was found for the other vaccines without providing any detail. A major limitation of the study was that all the

Figure 3. Funnel plots for poliomyelitis and measles vaccines.
case–control studies included in the meta-analysis evaluated more than 1 vaccine at a time and that it was not possible to determine specifically the risk related to only 1 vaccine. Individuals with multiple vaccinations may be at higher risk for IBD, as suggested in the study by Baron et al.\textsuperscript{11}

In this meta-analysis, although the overall analysis of all studies investigating the poliomyelitis vaccination did not find any increased risk of developing IBD, sensitivity analysis indicated that patients receiving the poliomyelitis vaccine may be at risk for developing CD or UC. Indeed, 2 studies showed an increased risk of developing IBD after childhood vaccination with the poliomyelitis vaccine\textsuperscript{11,18} and only 1 study published as an abstract did not find a significantly increased risk.\textsuperscript{20} The poliomyelitis vaccine is used widely in developed countries and mostly combined with diphtheria- or tetanus-containing vaccines.\textsuperscript{28,29} These 3 national studies investigating the poliomyelitis vaccination did not provide any detail on the route of administration of the vaccine, which may be a critical factor to explain differences in results.\textsuperscript{11,18,20} Indeed, 2 types of poliomyelitis vaccine, an oral live vaccine (OPV) and an injected inactivated vaccine (IPV) have been used during the past decades worldwide to eradicate poliomyelitis.\textsuperscript{28} We may hypothesize that live attenuated OPV may have a greater impact on activating the mucosal immune system than the IPV, and have different effects on the risk of IBD. Although publications did not contain any information about the route of administration, extrapolation could be made by looking at public vaccination history in involved countries.\textsuperscript{28,30,31} In the French study by Baron et al\textsuperscript{11} patients were recruited beginning in 1988 and should have received the IPV because France had withdrawn the utilization of the OPV since 1983. In Denmark, combined vaccination with OPV and IPV has been practiced since 1966 and has been replaced only recently with IPV vaccination alone.\textsuperscript{30} Patients in the Danish study by Hansen et al\textsuperscript{18} all would have been vaccinated with the IPV. In Manitoba, Canada, after having adopted a mixed schedule of IPV and OPV since 1962, switched to an exclusive OPV schedule during the 1970s. Then, between 1994 and 1997 Manitoba transitioned to the exclusive use of the IPV.\textsuperscript{28} Therefore, we may hypothesize that the majority of patients in the Canadian study from Shaw et al\textsuperscript{20} (pediatric IBD cases in Manitoba born after 1989 and diagnosed before 2008) may have received only the OPV. This difference in vaccine type if true may explain the contradictory results of these studies. Heterogeneity in the meta-analysis results also could be explained by differences in study design (2 studies investigated only pediatric IBD patients\textsuperscript{11,20}) or in the samples (the study

![Figure 4. Risk ratio of developing IBD after smallpox and measles vaccinations in case–control and cohort studies.](image)

![Table 1. Smallpox vaccination and risk for IBD.](table)
by Shaw et al.18 had half as many IBD cases as the 2 other studies11,16. Moreover, methods for vaccination recall were different between these studies, with positive studies using questionnaires and the negative study using a population-based database of immunizations administered in Manitoba. In addition, the IPV contains many adjuvants that may be involved in stimulation of the immune system.24 Indeed, vaccine adjuvants such as thimerosal and aluminum, the 2 major salt-based adjuvants that have been used or still are used in vaccines, may participate in the development of inflammatory disorders.10,32 Thimerosal, an ethyl mercury-containing compound that has been used as a preservative inmultidose vials of vaccines to prevent bacterial and fungal contamination of those vials, now has been removed completely from vaccines whereas aluminum salts used to boost the immune response still are present in most of the vaccines used in children. Both thimerosal and aluminum have been suspected to be involved in various inflammatory or neurologic disorders.10,32 Thus, the lack of data about the exact composition of vaccines used in these studies has lead to difficulty in interpreting results.

We also found conflicting results on pertussis vaccination and the risk of developing IBD in 2 studies.16,18 There was no information about the type of pertussis vaccine, but during the study period in the United Kingdom and Denmark a whole-cell pertussis vaccine was used exclusively.33,34 Other differences in the design of these studies may explain the conflicting results because the UK study was hospital-based whereas the Danish study was population-based. Moreover, in the study by Hansen et al.,18 poliomyelitis virus found to be associated with an increased risk of IBD may act as a confounding factor. Interestingly, the UK study by Feeney et al.16 did not report on poliomyelitis vaccination and during the study period only the OPV was used in the United Kingdom.

In conclusion, results of this meta-analysis do not support a role of childhood immunization or H1N1 vaccination in the development of IBD. The association between the poliomyelitis vaccine and risk for CD or UC should be analyzed cautiously because of study heterogeneity and will require further investigation.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of Clinical Gastroenterology and Hepatology at www.cghjournal.org, and at http://dx.doi.org/10.1016/j.cgh.2015.04.179.

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20. Shaw SY, Blanchard JF, Bernstein CN. Early childhood immunizations are not associated with pediatric IBD: a population-based analysis. Gastroenterology 2012;142(Suppl 1);S-88.

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Conflicts of interest
The authors disclose no conflicts.
Supplementary Figure 1. Flow chart.

Studies identified in literature search (n=428)

Studies retrieved for evaluation (n=16)

Excluded after reviewing titles and abstracts (n=412)

11 studies investigating the risk of developing IBD after vaccination
- 8 case-control studies (6 population-based and 2 hospital-based)
- 3 population-based cohort studies

Five studies did not meet all inclusion criteria and were excluded:
- 3 epidemiological studies
- 1 case report
- 1 uncontrolled study

10 studies included in the meta-analysis with 10 vaccine types (Bacille Calmette-Guérin, diphtheria, tetanus, poliomyelitis, smallpox, pertussis, measles containing vaccines, MMR vaccines, rubella, mumps)

One study analysed H1N1 vaccination and was included in the systematic review
Immune Thrombocytopenia

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Synonyms of Immune Thrombocytopenia

- autoimmune thrombocytopenic purpura
- ITP
- idiopathic immune thrombocytopenia
- primary immune thrombocytopenia

General Discussion

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by abnormally low levels of blood cells called platelets, a situation which is referred to as thrombocytopenia. Platelets are specialized blood cells that help maintain the integrity of the walls of our blood vessels and help prevent and stop bleeding by accelerating clotting. A normal platelet count ranges from approximately 150,000 to 400,000 per microliter of blood depending on the laboratory. If someone has a platelet count lower than 100,000 per microliter of blood with no other reason for low platelets, that person might have ITP. There is currently no definitive laboratory test to diagnose ITP. Rather, ITP is considered a diagnosis of exclusion (see below) meaning that other causes have been eliminated or are unlikely. What tests to do to exclude other causes is not well-established and can differ among patients and hematologists.
As the platelet count falls, the risk of developing bleeding symptoms increases. ITP can be discovered incidentally based on a blood count ordered for other reasons, such as a routine yearly checkup. However, most patients with ITP present to their doctor with abnormal bleeding into the skin resulting in bruising, also known as purpura or tiny red dots on the skin called petechiae. Bleeding from mucous membranes also may occur and may subsequently result in low levels of circulating red blood cells (anemia; this usually means bleeding in the mouth and nose but could also include blood in the urine. Internal bleeding at presentation is very uncommon. ITP is generally called newly diagnosed when it has been present for less than 3 months, persistent when present for 3-12 months and chronic when present for longer. The term “acute” is not used currently. The clinical onset may be rapid with overnight onset of skin bleeding or gradual over months.

Eighty percent (80%) of children who present with ITP have a self-limited form that resolves with or without treatment (i.e. spontaneously) within 12 months and often sooner. In contrast, the proportion of adults with ITP who have a chronic condition is much higher, exceeding 50% in most series. ITP that develops in adolescents most often follows the clinical course seen in adults.

Mechanistically, the fundamental abnormality in ITP is that the patient’s immune system recognized their own platelets as “foreign” leading their B-lymphocytes to produce self-reactive anti-platelet antibodies that attach to platelet surfaces. A type of white blood cells in the spleen and in other organs, called macrophages (scavenger cells), recognize antibody-coated particles, in this case antibody-coated platelets, leading to their ingestion into the macrophages and subsequent destruction. The bone marrow attempts to compensate but is often unable, especially in severe cases, to keep up with the destruction. Platelet production may also be impaired when anti-platelet antibodies bind to the cells in the bone marrow that produce platelets, called megakaryocytes.

While it may seem like ITP is a simple disease, there are many nuances to diagnosis, mechanism of disease, and management, in addition to the variability of outcomes between and among children and adults. This includes variation in the severity of bleeding at any given platelet count as well as how individual patients respond to various forms of treatment.

In addition to serious physical bleeding-related manifestations of the disease, ITP is associated with debilitating fatigue (reported in up to 39% of adults with ITP), as well as impaired quality of life across domains of emotional, functional, and reproductive health, and work and social life. These symptoms that accompany the disease can interfere with daily activities and lead to anxiety, fear, depression, embarrassment of unexplained bruising and nosebleeding (epistaxis), isolation, inadequacy, and frustration with a patient’s inability to control their body and their health. This list does not even encompass the side effects of treatments which, while possibly improving part of the above problems, can be devastating in different ways. Together, these multi-faceted effects of ITP often take a significant toll on patients’ quality of life.

Management depends on severity of symptoms, platelet count, age, lifestyle, response to therapy and its side effects, the presence of other medical issues that affect the risk of bleeding, quality of life as discussed above, and, of course, personal preferences of both the patient and the doctor.

**Signs & Symptoms**

As mentioned, there is variation among individuals in their tendency to bruise and bleed when they have a low platelet count, i.e. some patients tolerate quite low platelet counts for long periods of time with minimal or no bruising and bleeding, while others have substantial bleeding at the same counts. A child or adult with immune thrombocytopenia may display no symptoms (be asymptomatic) or the symptoms may appear when the platelet count is very low. Such symptoms may include:
- Skin that bruises very easily and even spontaneously
- A rash consisting of small red dots (petechiae) that represent small hemorrhages caused by broken blood vessels or leaks in a capillary wall
- Bleeding from the gums
- Frequent and long-lasting nose bleeds that are hard to stop
- Blood blisters on the inside of cheeks
- Excessive and prolonged menstrual bleeding
- Less likely, signs of internal bleeding, with blood in urine, vomit, or bowel movements
- In rare patients, bleeding in the brain called intracranial hemorrhage that is very much like a stroke
- Debilitating fatigue, depression, low mental and physical energy

In severe cases, frequent bleeding episodes may result in low levels of circulating red blood cells (anemia), which may cause fatigue and impair response to exertion. In rare cases, serious bleeding into the brain (intracranial hemorrhage) may occur. Fatigue (even in the absence of anemia), anxiety, and depression are common experiences for some people with ITP resulting in difficulties at work or school and also in social difficulties. These symptoms may be caused by the disease itself, by anxiety, or as side effects of medical treatment.

Causes

Antibodies are normally produced by the body’s immune system only in response to foreign substances, known as antigens, for example on certain viruses and bacteria or on red blood cells or tissue from unrelated people. Immune thrombocytopenia belongs to a group of disorders in which the body’s natural immune defenses inappropriately act against its own cells or tissues (autoimmune disorders). In ITP, such an abnormal immune reaction leads to destruction of the individual’s own platelets. For reasons that remain as yet unknown, lymphocytes in the bone marrow, spleen, and elsewhere are stimulated to produce antibodies that attach to platelet surface antigens, and the platelets are recognized as foreign by the immune system. In most individuals, the platelets are the only target of the misdirected immune response. However, in some, ITP develops in the context of another disorder that predisposes to making autoantibodies. This is called secondary ITP and is discussed below. In most individuals, however, no such connection to another disease is evident and the cause of anti-platelet antibody production remains unknown.

The autoantibodies in ITP bind to otherwise normal platelets in the blood that then circulate through the spleen, the liver, and elsewhere. The antibody-platelet complexes are recognized by tissue macrophages, which ingest and destroy antibody-coated platelets as they would normally when they encounter any antibody-coated foreign particle. The bone marrow attempts to compensate by producing more platelets but the rate of platelet destruction may exceed the marrow capacity and thrombocytopenia develops. Platelet production by megakaryocytes in the bone marrow may also be impaired when the same autoantibodies that bind to the platelets attach to megakaryocytes, the platelet precursors in the bone marrow. Therefore, the mechanisms underlying ITP and the resulting very low platelet counts can be characterized as including increased platelet destruction, reduced or inadequate platelet production, or both. It is not currently possible to define the relative importance of these two possibilities in a specific patient.

In children, ITP often appears soon after an acute viral infection. This suggests that antibodies produced to fight foreign viral substances (antigens) may “cross-react” with similar appearing antigens on platelets, which in turn lead to platelet destruction. This has been shown in the case of chicken pox, for example. However, as mentioned, identification of the exact mechanism of ITP in a given patient is not possible and predicting in a child (or adult) who will get better and who will not, who will bleed and who will not, is not possible in a precise way.

It is quite rare for more than one family member or members of more than one generation to have ITP. When there is such a family history of thrombocytopenia, a genetic disorder involving platelets is much more likely.
As mentioned, some people have secondary ITP, meaning that their ITP is part of another condition. Secondary ITP can be caused by inherited immune disorders (such as autoimmune lymphoproliferative syndrome, ALPS, some of whom also have antibody-mediated red cell destruction), systemic autoimmunity such as systemic lupus (in which the immune system attacks other cells as well as platelets), persistent infections (such as HIV, hepatitis B or C, and the ulcer-causing stomach bacterium, *Helicobacter pylori*), and lymphoproliferative disorders such as chronic lymphocytic leukemia that impair the immune system. A few cases resembling ITP may result from the use of certain drugs, after a viral or bacterial infection or in approximately one in 40,000 children after vaccination for measles-mumps-rubella (MMR). The effect of drugs may be either to suppress the bone marrow from making platelets or to induce the formation of antibodies that attack platelets but involve the drug as a target. A good source of information on this subject is: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2682438/

### Affected Populations

The incidence (how many people are diagnosed each year) of ITP among adults in the USA is estimated to be 3.3 per 100,000 adults/year. The prevalence (how many adults have ITP at any time) is 9.5 cases per 100,000. The annual prevalence is estimated at 5.3 per 100,000 among children, because children with ITP usually recover, the number of children who have ITP at any one time is almost equal to those diagnosed annually. Worldwide, it is estimated that there are well over 200,000 people affected by ITP.

The incidence of ITP increases with age and is more common over the age of 60. Among adults (age 30-60) diagnosed with chronic ITP, there are 2.6 cases among women for every case involving a male. In older adults, about the same number of men and women are diagnosed with ITP. Among children diagnosed with acute ITP, the male to female ratio is also almost equal, with 52% male to 48% female. About 40% of all patients diagnosed with one or another form of ITP are children younger than 10 years of age. Among children, the incidence is greatest between 2 and 4 years of age. However, ITP can occur at any age from 3 months of age to over 100 years of age.

### Related Disorders

Thrombocytopenia due to the following disorders can resemble those of ITP. Comparisons may be useful for the doctor to develop a differential diagnosis (consider the full spectrum of what could be underlying the ITP):

Thrombocytopenia may be caused by other blood (hematological) disorders that affect the ability of the bone marrow to produce platelets.

As mentioned, immune thrombocytopenia can be secondary to a systemic autoimmune disorder such as systemic lupus erythematosus. Adverse drug reactions (drug-induced thrombocytopenia) are also potential causes of thrombocytopenia. Quinidine, quinine and heparin are examples of drugs associated with the development of immune thrombocytopenia.

Thrombotic thrombocytopenia purpura (TTP) is a rare, but quite serious blood disease. Major symptoms may include disturbances (interruptions) in blood supply due to clotting that can involve any of a number of organs including the brain, severe thrombocytopenia, accelerated destruction of red blood cells leading to their characteristic fragmented appearance on the blood film (microangiopathic hemolytic anemia). The cause of thrombotic thrombocytopenia purpura in most patients is an autoantibody that blocks the function of an protein known as ADAMTS13 that helps to control blood clotting. (For more information on this disorder, choose “thrombotic thrombocytopenia purpura” as your search term in the Rare Disease Database.)

Henoch-Schönlein purpura is a rare inflammatory disease of the small blood vessels (capillaries) that is mostly self-limited. It is the most common form of childhood blood vessel inflammation (vasculitis) and results in inflammatory changes in small
blood vessels. The symptoms of Henoch-Schonlein purpura usually begin suddenly and may include headache, fever, loss of appetite, cramping abdominal pain, and joint pain. The resemblance to ITP is that red or purple spots typically appear on the skin that may resemble bruises; however thrombocytopenia is not usually part of the disease. Inflammatory changes associated with Henoch-Schonlein purpura can also develop in the joints, kidneys, digestive system, and, in rare cases, the brain and spinal cord (central nervous system). The exact cause of Schonlein-Henoch purpura is not fully understood, although research suggests that it may be an autoimmune disease, e.g. a severe allergic reaction to offending substances (e.g., foods or drugs). (For more information on this disorder, choose “Henoch-Schonlein purpura” as your search term in the Rare Disease Database.)

Heparin-induced thrombocytopenia is the most common drug-induced, antibody-mediated thrombocytopenic disorder. Heparin is often used to prevent blood clotting (anticogulant). Heparin-induced thrombocytopenia is a complicated entity in which the antibodies bind to platelets and other cell types leading to the formation of blood clots (thrombosis). This often affects the limbs, but can also cause stroke or the formation of blood clots in the lungs (pulmonary emboli) resulting in chest pain, coughing, difficult or labored breathing (dyspnea), and coughing up blood (hemoptysis). Skin ulcerations may develop due to impaired blood flow. If severe enough, fingers and toes may be lost.

Hypersplenism, an overactive spleen usually from liver disease, may cause an isolated thrombocytopenia and thereby mimic ITP. Thrombocytopenia is usually not as severe. Either a history of known liver disease, such as hepatitis C, or feeling a very enlarged spleen on physical examination or findings on ultrasound leads to the diagnosis.

The antiphospholipid syndrome (APS) is a disorder associated with autoantibodies to various phospholipids that make up the membranes of cells, such as cardiolipin, and to proteins that bind to such phospholipids, such as beta-2-glycoprotein 1. Patients with APS may present with thrombosis in any organ or gestational complications, including recurrent miscarriages or fetal losses. Immune thrombocytopenia may be superimposed on the thrombotic complications. Some patients with ITP also have anti-phospholipid antibodies but do not necessarily develop thrombotic or gestational complications as a result.

Anti-thyroid autoantibodies may also be detected in patients with ITP; as many as 10% of women of child-bearing age may have both ITP and antibodies to the thyroid. Assessment of thyroid function may therefore be indicated in many patients. Other kinds of autoantibodies are less common.

**Diagnosis**

The diagnosis of ITP is made by excluding other causes of thrombocytopenia, including certain medications or disorders that affect the bone marrow and reduce platelet production, such as acute leukemia and aplastic anemia. On occasion, a low platelet count may be detected incidentally by blood tests such as a complete blood count (CBC) ordered for other purposes and the individual is without apparent symptoms (asymptomatic). Inspection of the blood smear under the microscope will verify the platelets are truly reduced in number and not simply clumped (stuck together so they are too big to be counted by automated machines as platelets), and that the platelets are not uniformly very small or exceeding large (giant platelets approximating the size of red blood cells). The red blood cells and white blood cells are normal in number and appear normal to the eye, which helps to exclude consideration of leukemia and/or aplastic anemia, among other causes of thrombocytopenia. The presence of unusual appearing cells in the blood or additional abnormalities in the blood counts, might indicate the need for a biopsy of the bone marrow to exclude other causes of impaired platelet production and/or consideration of secondary ITP.

In a patient who is otherwise in his/her usual state of good health who has not taken a new medication, has thrombocytopenia and no other abnormality found in a complete blood count or upon inspection of the blood smear, and has no family history of thrombocytopenia, the diagnosis of ITP is favored. There is no definitive
test (such as measurement of platelet autoantibodies) to make the diagnosis or to exclude the diagnosis of ITP. A robust response to ITP-specific treatments such as IVIG (intravenous immunoglobulin) or glucocorticoids (described below) provides strong evidence in favor of the diagnosis.

**Standard Therapies**

**Treatment Overview**

While there is no well-established cure for ITP, fortunately almost all patients find their platelet count improves following treatment. What proves difficult for many ITP patients is finding the treatment that works for them without unwanted side effects. In some individuals, the disease can go into remission for an extended period of time, perhaps for the remainder of a person's life. ITP can also recur at any time. There is currently no way to predict the course of the disease. Changes in diet or lifestyle may improve (or worsen) the sense of well-being.

**Criteria for Treatment**

In many children and some adults, therapy may not be necessary at the time they first see the doctor and the disorder may resolve spontaneously. The decision to initiate treatment depends on the severity of bleeding, the severity of the thrombocytopenia, the age of the patient (increased risk of bleeding in adults and especially in the elderly), coincidental disorders that might predispose to bleeding (tendency to fall, concurrent anti-platelet or anticoagulants), life-style (such as young and athletic) and risks and side effects of each intervention. These same factors can also contribute to deciding which treatment to use.

The goal of therapy is to prevent bleeding, to stabilize and hopefully to improve the platelet count, and help restore the patient's ability to have a normal lifestyle. When treatment is deemed necessary, there are many options that have proven successful. Treatments differ in likelihood of benefit and risks and some are considered more toxic are therefore, generally deferred unless it is proven they are needed. Treatments also differ in their intended effect: short term platelet increase versus long-term maintenance of a stable platelet count. It is important to understand both the success rate and potential side effects before beginning any form of treatment. Hematologists may even recommend several treatments at once to increase their success rate and minimize their side effects.

**First Line/Emergency Therapy**

Treatment with corticosteroid drugs (e.g., prednisone, dexamethasone, methylprednisolone) is usually the mainstay of initial therapy. These drugs function by suppressing the clearance of antibody-coated platelets and perhaps by increasing platelet production. They may also decrease the risk of bleeding by improving blood vessel lining cell function. Very high doses (especially of dexamethasone) may impair the production of anti-platele antibodies with the hope that the platelet count will remain elevated after the patient stops taking prednisone. However, additional studies are needed to affirm the long-term benefit of such a "high-dose" approach. In general, the duration and dose of corticosteroids should be minimized because of their immediate and long-term side effects. Therefore, corticosteroids are used to control the disease until a transition can be made to other forms of treatment in patients who do not achieve a spontaneous remission.

If platelet counts do not improve after corticosteroid treatment or if when individuals present with severe bleeding, treatment may include adding intravenous immunoglobulins (IVIG), usually by infusions given as needed based on the count and bleeding every 2-4 weeks, but this rarely leads to a cure. Platelet transfusions are reserved for emergent situations because they are likely to be destroyed relatively quickly by the autoantibodies.

The orphan drug anti-D (WinRho SDF, Rhophylac), a specific form of gamma globulin, was approved by the Food and Drug Administration (FDA) to treat ITP in individuals who are red blood cell RhD antigen positive, do not already have antibodies on their red cells, and have not undergone splenectomy. The drug can be
used repeatedly, including in children who have the acute or chronic form of ITP. However, concerns have been raised because of a small number of individuals who have had severe side effects from brisk red cell destruction and its consequences soon after infusion.

**Second Line Therapy**

The criteria for determining whether second line therapy is needed are the same as those involving initiation of treatment plus patients with suboptimal responses to first line approaches. As mentioned, corticosteroids should be used for the shortest duration possible to achieve these objectives and to provide a bridge to less toxic alternatives. Many adults and some children will require such long-term management because their platelet count will fall once the dose of corticosteroids is tapered.

One option in the second-line setting involves the use of thrombopoietin receptor agonists (TPO-RAs). TPO-RAs function by stimulating the body’s production of platelets by megakaryocytes in the bone marrow, which release proplatelets that mature into platelets. By increasing the rate at which platelets are produced in the body, TPO-RAs may overcome the heightened rate of platelet destruction caused by antiplatelet antibodies and their ability to impair megakaryocyte function. Three TPO-RAs approved by the FDA for use in ITP are eltrombopag (Promacta), romiplostim (Nplate) and avatrombopag (Doptelet), while others are in development or approved for other related indications.

In 2008, the FDA approved both romiplostim (Nplate) manufactured by Amgen Inc. and eltrombopag (Promacta) manufactured by GlaxoSmithKline (GSK) to treat both children and adults with ITP who have had an insufficient response to corticosteroids, immunoglobulins, or splenectomy. Romiplostim is typically given by weekly subcutaneous injection, most often in a doctor’s office. Novartis Pharmaceuticals Corporation acquired eltrombopag from GSK. There are a few important dietary restrictions that are needed to maximize the benefit of this oral agent, which is administered once daily. In 2015, eltrombopag was approved for the treatment of children 1 year and older with ITP who have had an insufficient response to corticosteroids, immunoglobulins or splenectomy; romiplostim was similarly approved in late 2018. Response rate, depending upon the definition of response, to both agents ranges from 40- 80% and, once obtained consistently, was durable with ongoing treatment. The drugs are generally well tolerated and long-term safety studies have mitigated initial concerns about thrombosis and bone marrow scarring. Some patients (an unknown percent) will experience sufficient improvement in their ITP over time to discontinue treatment. In 2019, the FDA approved avatrombopag (Doptelet) manufactured by Dova Pharmaceuticals to treat ITP in adults with chronic ITP who have had insufficient response to a previous treatment. This is the only oral TPO-RA medication approved to treat ITP that can be taken with food. Avatrombopag is generally considered safe and well tolerated.

Another option is anti-CD20 antibody, rituximab (Rituxan), which reduces IgG antibody production; there are now several biosimilars. About half the patients respond initially, but only 20-30% are cured in long-term outcome studies. Women of child-bearing age of duration of ITP < 1-2 years have an over 50% cure rate; all others have a very low rate of cure. Rituximab is generally well tolerated but infusion reactions can occur. Administration may be repeated when a durable response has been seen, but concern over repetitive administration of this immunosuppressant is warranted. One to 3-4-day courses of high dose dexamethasone have been used in an effort to increase responses. A third option is splenectomy, (typically laparoscopic) because the spleen plays a major role in destroying antibody-covered platelets and in making antiplatelet antibodies. Splenectomy improves platelet counts in approximately 70 percent of patients initially and can induce a long-term remission in 60 percent. The high long-term success rate must be weighed against the small but real increased risk of thrombosis and serious infection, which necessitates appropriate vaccinations and urgent evaluation for serious febrile illnesses. Most guidelines recommend deferral of splenectomy for a year from diagnosis in order to determine if a patient will go into remission. However, splenectomy remains an option in patients who fail other
forms of treatment or in resource challenged areas where more expensive alternatives are not available. Lastly, in 2018, Tavalisse ( fostamatinib disodium hexahydrate) was approved by the FDA for the treatment of thrombocytopenia in adults with ITP who have had insufficient response to a previous treatment. Tavalisse is manufactured by Rigel Pharmaceuticals, Inc. Approximately 20% of patients who are refractory to other forms of management responded based on pre-specified criteria but almost 40% did so using less stringent but clinically meaningful endpoints. It has a number of side effects (hypertension, diarrhea, headache, and abnormal liver tests) but one advantage is that it has the least risk of thrombosis of any licensed treatment of ITP.

**Third Line Therapy**

A small percentage of patients fail to respond or tolerate first or second line treatments. For those, options include dapsone, Imuran (azathioprine), Cytoxan (cyclophosphamide), Sandimmune (cyclosporine), Danocrine (janazol), Cellcept (mycophenolate mofetil), Vincristine (vinca alkaloids), or combinations. Several other novel forms of treatment are in clinical trials.

If the patient has antibodies or evidence of Helicobacter pylori infection, treatment with antibiotics and proton pump inhibitors may ameliorate the condition. Antibiotic associated remission of ITP is much more common in Asia and in some parts of Europe than in patients who have lived their entire life in North America.

Some patients report success with complementary therapies such as vitamins, supplements, diet changes, herbs, and energy work, such as Reiki. However, there are no controlled trials in ITP patients demonstrating utility or safety of any of these agents.

ITP treatments vary with the disease severity, age of the patient, experience of the hematologist, patient preference and other factors.

**Investigational Therapies**

Information on current clinical trials is posted on the Internet at www.clinicaltrials.gov (http://www.clinicaltrials.gov). All studies receiving U.S. government funding, and some supported by private industry, are posted on this government web site.

The PDSA website also lists the most current ITP clinical trials at https://pdsa.org/clinical-trials.html (https://pdsa.org/clinical-trials.html)

For information about clinical trials being conducted at the NIH Clinical Center in Bethesda, MD, contact the NIH Patient Recruitment Office:

Toll-free: (800) 411-1222  
TTY: (866) 411-1010  
Email: prpl@cc.nih.gov (mailto:prpl@cc.nih.gov)

Some current clinical trials also are posted on the following page on the NORD website:  

For information about clinical trials sponsored by private sources, contact:  
www.centerwatch.com (http://www.centerwatch.com)

For information about clinical trials conducted in Europe, contact:  
https://www.clinicaltrialsregister.eu/ (https://www.clinicaltrialsregister.eu/)

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JOURNAL ARTICLES
(http://www.ncbi.nlm.nih.gov/pubmed/20131303)


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EXHIBIT 208
What you need to know about alopecia areata

Alopecia areata is a common autoimmune skin disease, causing hair loss on the scalp, face and sometimes on other areas of the body. In fact, it affects as many as 6.8 million people in the U.S. with a lifetime risk of 2.1%

People of all ages, both sexes and all ethnic groups can develop alopecia areata. It often first appears during childhood and can be different for everyone who has it.

Will I have hairloss for life?

With alopecia areata, your hair follicles remain alive and hair can regrow at any time

Get the facts on hairloss associated with alopecia areata and your options for treatment

Learn more about what causes alopecia areata and why your family history doesn't always matter

Understand how alopecia areata affects children of different ages and how you can help them to talk about their condition with friends, teachers and classmates

Sign up to learn more about alopecia areata

And you'll also get the NAAF info pack with helpful tips and advice
Did you Know?

Alopecia areata is known as a “polygenic disease.” This means that, unlike a single-gene disease, both parents must contribute a number of specific genes in order for a child to develop it. Because of this, most parents will not pass alopecia areata along to their children. With identical twins — who share all of the same genes — there’s only a 55% chance that if one has alopecia areata, the other will, too. This is why scientists believe that it takes more than just genetics to cause the disease and that other environmental factors also contribute to people developing alopecia areata.

Are there different types of alopecia areata?

With all forms of alopecia areata, your body’s own immune system attacks your healthy hair follicles, causing them to become much smaller and drastically slow down production to the point that hair growth may stop.

Depending on which type and severity of the disease you have, you might experience hair loss in different areas and your hair loss and regrowth may be unpredictable and cyclical (happen over and over) for many years. Though for some people, hair may also regrow in a few months.

Three of the more well-known types of alopecia areata

- Alopecia areata patchy — The most common form, with one or more coin-sized hairless patches on the scalp or other areas of the body
- Alopecia totalis — Total loss of the hair on the scalp
- Alopecia universalis — Complete loss of hair on the scalp, face and body

Currently, there is no cure for alopecia areata. But the good news is that even when your disease is “active,” your hair follicles remain alive. This means that your hair can grow back again — even after a long period of time and even if you have more than 50% hair loss.

Learn more about all of the different types of alopecia areata.

What causes alopecia areata?

There are lots of factors that contribute to developing this complex condition. Alopecia areata is an autoimmune disease, which means your immune system mistakes the normal cells in your body as foreign invaders and attacks these cells.

Scientists aren’t exactly sure what “triggers” the immune system to attack healthy hair follicles when people have alopecia areata, or even if these triggers first happen inside the body (from a virus or bacteria), outside the body (from something in your surroundings) or if it’s a combination of both.
Will my child inherit alopecia areata from me?

It’s understandable that adults who have alopecia areata would be concerned about the risks of passing the disease to future children. However, because alopecia areata is so complex, it’s almost impossible to predict whether or not your child will develop the condition.

Scientists believe that multiple factors (both genetic and in the environment) are needed in order to trigger the disease, not just simply family heredity. In fact, most parents will not pass alopecia areata along to their children.

Did you know as many as 6.8 million people in the U.S. — 147 million people worldwide — are affected by alopecia areata with a lifetime risk of 2.1%?

What kinds of symptoms will I have with alopecia areata?

All types of alopecia areata result in some form of hair loss. There is no way to predict the pattern of hair loss and regrowth you will experience or how severe or long lasting it will be. It’s important to remember that alopecia areata is different for everyone who has it.

Still, there are some common symptoms of alopecia areata that are good to know and recognize. However, the only way to be sure you have alopecia areata is to make an appointment and get a diagnosis from a doctor.

**Symptoms of alopecia areata may include**

- Small, round (or oval) patches of hair loss on the scalp, beard area of the face or other areas of the body with hair
- Hair loss and regrowth at the same time in different areas of the body
- Significant hair loss in a very short period of time
- Hair loss that’s mostly on one side of the scalp, instead of both sides
- “Exclamation point” hairs that are narrow at the base/next to the scalp
- “Stippling” or “Pitting” (rows of tiny dents) on the fingernails
What are my options for treatment and care of affected areas?

Unlike many skin diseases, alopecia areata does not cause rashes, redness, hives or severe itching. Even so, some people with alopecia areata find it helpful to protect exposed skin — the head, ears and face — from damaging sun exposure or other harsh elements. A scalp without hair is more sensitive to cold as well.

Depending on which type of alopecia areata you or your child has, your age, and the extent of hair loss, there are a variety of treatment options available for disrupting or distracting the immune attack and/or stimulating the hair follicle — especially for those who have milder forms of the disease (less than 50% hair loss).

For those who have more than 50% hair loss on their scalp or other areas of the body, there are oral and injectable medications available. However, these medications do not work for everyone. That’s why it’s important to talk with your doctor to discuss the risks and benefits of using any medications.

Learn more about treatment options and products and accessories for people with alopecia areata.
EXHIBIT 209
Hair Loss After Routine Immunizations

Robert P. Wise, MD, MPH; Kitonga P. Kiminyo, MD; Marcel E. Salive, MD, MPH

Context.—Alopecia is a recognized adverse effect of numerous medications, but vaccines are not normally considered a cause for unexpected loss of hair.

Objective.—To describe case reports of hair loss after routine vaccines and to assess the hypothesis that vaccinations might induce hair loss.

Design.—Case series with telephone follow-up.

Methods.—Review of spontaneous reports to the Food and Drug Administration, the Centers for Disease Control and Prevention, and the Vaccine Adverse Event Reporting System.

Main Outcome Measure.—Loss of hair following immunization.

Results.—A total of 60 evaluable reports submitted since 1984 and coded for "alopecia" after immunizations included 16 with positive rechallenge (hair loss after vaccination on more than 1 occasion), 4 of which were definite and 12 possible or probable. Of the 60 cases, 46 had received hepatitis B vaccines. Both of the currently available recombinant products, as well as the former plasma-derived product, were represented. Females predominated in all age groups. The majority of patients recovered, but clinical features, such as intervals from vaccination until onset and the extent and reversibility of hair loss, varied widely. Nine patients reported previous medication allergy.

Conclusion.—There may be an association, probably very rare, between vaccinations and hair loss. More than 1 pathophysiologic mechanism may be responsible. Since apparently nonrandom distributions by vaccine, age, and sex could reflect biased case ascertainment, further research will be needed in defined populations with consistent case detection.

VACCINE coverage depends on public confidence in the safety of routine immunizations. Manufacturers and regulatory agencies attempt to identify risks of new pharmaceutical products before they are licensed, but constraints, such as the relatively small number of subjects in clinical trials, limit discovery of adverse effects. Therefore, postlicensure safety surveillance is essential.

In early 1994, a concerned mother telephoned the Food and Drug Administration (FDA), describing her 5-year-old daughter’s experience of nearly total loss of scalp hair after second and third doses of hepatitis B vaccine (HBV). Following this index report of recurrent hair loss after routine childhood vaccinations (patient 1, below), we reviewed national vaccine safety surveillance systems for additional cases.

Methods

We found cases of hair loss after vaccinations dating back to 1969 in national surveillance systems for vaccine adverse events: the current Vaccine Adverse Event Reporting System (VAERS) and prior data from the FDA, the Centers for Disease Control and Prevention (CDC), and product manufacturers. Five excluded reports lacked sufficient detail. In 60% of cases, we interviewed the patient, a parent, or a physician, usually by telephone, for further details.

Results

The investigation yielded 60 reports with varying degrees of hair loss. The index patient and 3 others described clear positive rechallenge for hair loss (recurrence following readministration: of a suspect vaccine).

Patient 1.—A 12-month-old white female infant in California began to lose scalp hair 10 days after her second immunization with HBV. During the next 3 months, she progressed to complete baldness, but regrowth was complete by the age of 18 months. Approximately 1 week after her third dose of HBV, given with oral polio vaccine (OPV) at the age of 18 months, her parents noticed recurrent hair loss, which again progressed to near total loss of hair and which regrew beginning at the age of 2 years. Extensive medical evaluation failed to identify a cause. Her physicians considered, but discounted, the possibility that recent vaccination might have contributed, since she had experienced no adverse event after her first dose of HBV at the age of 10 months, and there was no suggestion in the medical literature or product package labeling that hair loss might follow vaccination.

Patient 2.—A 17-day-old white female neonate received her first dose of HBV. Slight hair loss was observed 36 days later. She received first doses of diphtheria-tetanus-pertussis (DTP) vaccine, Haemophilus influenzae type B conjugate vaccine (HIB), and OPV at the age of 2 months without apparent incident. Her second doses of DTP, HBV, HIB, and OPV at the age of 4 months were followed after 1 to 2 weeks by development of complete alopecia. Third doses of HBV and HIB were given at 6 months of age with complete regrowth of hair by the age of 7 months, when her third dose of HBV, given alone, was followed by recurrence of complete alopecia within 1 week, as well as a dry, red, scaly, eczema-like rash on her left arm. She lost eyebrows and lashes at the age of 13 months, 5 weeks after her first tuberculosis tine test. Partial sparse hair regrowth was noted (but not eyebrows or lashes) at 16 months of age, but this hair was also shed 2 months later when she received DTP, HIB, and OPV. Her eyebrows later grew back, but not most of her lashes.

Patient 3.—Within 1 day after her first dose of HBV, a 30-year-old female nurse developed mild hair loss, arthralgias, fatigue, and weakness, which lasted 1 week. One month later, her second dose was followed 1 day later by recurrent onset of hair loss and, about 2 weeks later, by recurrent arthralgias, fatigue, and weakness. Alopecia progressed for a few months until she esti...
Hair loss and Positive Rechallenges in 60 Reports of Postvaccinal Hair Loss

<table>
<thead>
<tr>
<th>Vaccines Administered</th>
<th>Clear</th>
<th>Possible</th>
<th>No or Unknown</th>
<th>Total</th>
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<td>11</td>
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<tr>
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<tr>
<td>Total</td>
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<td>12</td>
<td>44</td>
<td>60</td>
</tr>
</tbody>
</table>

*HBV indicates hepatitis B vaccine. Positive rechallenge refers to hair loss more than once after vaccinations. Clear positive rechallenge reports specified that the first episode had resolved before onset of the second. Possible positive rechallenge cases described exacerbation or continuation of hair loss after a second immunization but lacked indication of prior recovery. No or unknown positive rechallenge indicates that further vaccine administration did not induce recurrent hair loss, that no further vaccination was given, or (most cases) information about subsequent immunizations was not available.

Reports to the Food and Drug Administration of hair loss after vaccination by year of onset.

The extent and duration of hair loss varied widely among 37 reports with sufficient data to classify. Sixteen patients reported severe alopecia (extensive hair loss over more than half of the head or body); 8 recovered most or all of their hair; and 4 had persistent baldness. Recovery status remains unknown in 4 cases. Eighteen patients reported mild to moderate hair loss (most hair still intact). Nine had full resolution, 1 did not, and 8 were unknown. Two of 3 ambiguous histories of hair loss included full recovery.

Three patients had histories of past hair loss without linkage to immunizations. Nine reported allergy to medications. Hepatitis B vaccine, identified in 46 cases, was the most frequently cited vaccine exposure (Table). It was given alone in 40 cases. Both recombinant products and the earlier plasma-derived vaccine were represented.

**Comment**

This case series, particularly the 4 cases of hair loss with clear positive rechallenge, provides evidence consistent with a causal relationship to vaccinations. Hair loss was the central feature in 45 cases, including all 4 of the clear positive rechallenges.

The heterogeneous vaccine exposures and subsequent clinical manifestations suggest that more than 1 vaccine and pathophysiologic mechanism could trigger or contribute to hair loss following vaccination. Hepatitis B vaccine alone preceded hair loss in 40 patients, but 14 reported no HBV exposure, including 1 with positive rechallenge after influenza virus vaccine. Cases varied markedly in hair loss severity, intervals to onset, and recovery. Several reported additional symptoms, notably arthralgia or arthritis in 9 patients.

Vaccines are not usually identified among antecedents to hair loss, although Petkov et al described a 22-year-old man with a history of neurodermatitis with initial onset of fever, lymphadenopathy, and alopecia areata 5 days after smallpox vaccination. We hypothesize that vaccine antigens may be capable of triggering hair loss, either via telogen effluvium or through a novel autoimmune-mediated mechanism.

Recent reviews of drug-induced hair loss implicate numerous other pharmaceutical agents via either of 2 pathophysiologic mechanisms. Anagen effluvium refers to a direct cytotoxic effect on the rapidly dividing hair follicle cells, common with chemotherapeutic agents for malignancies (which did not confound our cases). Onset of hair loss follows the drug insult after a short lag of days to weeks.

Telogen effluvium, a usually reversible response to pharmacologic or physiologic stress, could account for some of our cases. Large fractions of follicle cells enter the resting phase (telogen), followed some 1 to 3 months later by widespread shedding when they reenter the active hair synthesis phase (anagen) together. Recognized triggers include several medications, high fevers, hormonal changes, hemorrhage, and others. Although many of our cases had much

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**Exhibit 209**

**Hair Loss After Routine Immunizations—Wise et al**

JAMA, October 8, 1997—Vol 278, No. 14

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shorter intervals from vaccination to onset of hair loss than the weeks to months typical in telogen effluvium, most of our intervals were approximate. Further, intervals were likely to be reported with reference to the most recent vaccination. But telogen effluvium might have been triggered by a prior immunization. In patient 2, for example, the first episode of total hair loss began less than 2 weeks after second doses of 4 vaccine products, but this onset date also corresponds to a 4-month interval since the first doses of 3 vaccines. Similarly, the second episode followed HBV by less than a week, corresponding as well to a 3-month lag after third doses of DTP and HIBV. Thus, both episodes could be consistent with telogen effluvium rather than relatively acute responses to the more proximate doses of HBV.

In addition to the possible role of telogen effluvium, we propose that immunologic mechanisms should also be considered. Although other immunologic drug reactions are well known, autoimmunity is hypothesized as the underlying cause of alopeia areata. Immunologic mechanisms have not been implicated in hair loss as an adverse effect of medications. We speculate, however, that cell growth cycles might be pathologically modulated by vaccine-induced antibodies. This scenario suggests antigenic similarities between vaccines and hair follicles, at least in susceptible patients, that should be investigated.

Unexpected hair loss could occasionally follow vaccine exposures by chance alone, since vaccine exposures are ex-haemorrhagic, common, and unexplained hair loss (alopecia areata or other syndromes) is not rare. The reported rate of 20.2 cases of alopeia areata per 100,000 in Olmsted County, Minnesota, would translate to 50,000 US cases per year. However, the positive rechallenges and distinctive distributions by age, sex, and vaccines in our case series appear unlikely to have arisen by chance alone. Olmsted County age-adjusted rates did not differ by sex (20.3 for females and 19.9 for males), and most rates in 10-year age groups were between about 19 and 29 per 100,000 persons-year. In contrast, the majority of our cases (34 of 39 adults) were women whose hair loss followed immunizations with only one of several common vaccine types.

Hair loss is reported very rarely following immunizations. Although underreporting commonly characterizes passive surveillance, the FDA has learned of fewer than 5 cases per year during a decade in which Americans received roughly 1 billion vaccine doses. Even when hair loss occurs after vaccination, it is often mild or moderate and self-limited. Apart from cosmetic implications, the worst consequences may be anxiety and costs for medical evaluation, secondary effects that may be reduced through dissemination of this information.

We cannot dismiss possible biases in case ascertainment. Health care workers are a primary target group for HBV. Among 39 adult cases, half (19) were nurses, physicians, or other medical personnel; women accounted for 17 of the 19. Employees in this field may be more likely to suspect recent immunization when they develop hair loss, and they are familiar with the importance of reporting adverse events. Sex-specific bias might also have affected reporting. Men noticing hair loss might more often attribute it to male pattern baldness.

A patient with hair loss possibly linked to antecedent immunization must decide, with physician and family, whether to receive remaining routine vaccinations. At present, we believe that the risk of additional vaccinations for patients who have lost hair once after immunization should be judged individually, weighing an uncertain risk that hair loss could recur against clear benefits of protection from target diseases.

After reviewing 60 case reports, we believe that immunizations warrant consideration among potential causes of hair loss. Further investigation of possible associations between vaccines and loss of hair has begun in the Vaccine Safety Datalink, where several health maintenance organizations facilitate investigations of immunization safety by pooling vaccination, hospital discharge, and other health service data.

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References
Alopecia Areata After Vaccination: Recurrence with Rechallenge

Abstract: Alopecia areata (AA) is the most common form of hair loss in children. We report the case of a child who had two episodes of AA after two different vaccines with complete hair regrowth between the episodes. This case supports the concept that vaccination might be a trigger for the development of AA in genetically predisposed children.

Alopecia areata (AA) is a T-lymphocyte-mediated autoimmune disease and the most common form of alopecia seen in children. Although its pathogenesis is uncertain, a few reports describe AA after routine vaccinations (1,2). Herein we report the case of a child who had two episodes of AA after two different vaccines.

CASE REPORT

A 3-year-old boy with a history of glucose-6-phosphate dehydrogenase deficiency and atopic dermatitis experienced two episodes of AA after vaccinations. The vaccinations immediately preceded the onset of hair loss in both episodes. The first episode occurred at age 27 months, approximately 1 week after the third dose of Japanese encephalitis vaccine, as an isolated alopecic patch on the scalp that progressed to near-complete baldness over the subsequent month, followed by complete regrowth in 6 months. The second episode developed within 3 days after the third dose of influenza vaccine at age 36 months as a recurrence of progressive hair loss. The patient presented to our clinic at 39 months old. Physical examination showed complete loss of scalp hair and bilateral eyebrows (Fig. 1). Multiple regularly distributed yellow dots were found using dermoscopy (Fig. 2). His parents denied any change in medication or illness preceding these two episodes. Alopecia totalis was diagnosed. Topical fluocinonide 0.05% solution twice daily for 3 months produced no improvement. Topical immunotherapy with diphenylcyclopropenone was subsequently applied weekly for 2 months, with only sparse regrowth noted.

DISCUSSION

Wise et al (1), who collected 60 cases of alopecia after immunization using the Vaccine Adverse Event Reporting System (VAERS), first reported hair loss after vaccination in 1997. Two case–control studies using the VAERS database demonstrated that patients with zoster vaccination (odds ratio [OR] = 2.7) or quadrivalent human papillomavirus vaccination (OR = 8.3) had a greater risk of developing alopecia than unexposed individuals (3,4), although the investigators did not have detailed enough clinical information in either study to ascertain whether each case of “alopecia” was alopecia areata. Sundberg et al (5) tested this hypothesis in the C3H/HeJ spontaneous adult-onset AA mouse model. They found that older mice had significantly faster onset of AA after receiving the hepatitis B vaccine than controls (p = .03), suggesting that the vaccine may initiate disease in mice predisposed to AA.

Although vaccines play a major role in improvement of human health, multiple vaccines have been implicated as potential triggers for autoimmune diseases, probably in genetically predisposed individuals. Recently a new syndrome, termed autoimmune/inflammatory syndrome induced by adjuvants (AIDSIA) has been described. This syndrome, which includes AA as one of the primary features, is characterized by the development of a disease in response to an adjuvant present in a vaccine (5).

Figure 1. Clinical examination demonstrated diffuse hair loss over the whole scalp and both eyebrows.
(ASIA), has been introduced. The term implies that adjuvant is capable of boosting immune response and acting as a trigger in the development of autoimmune disease (6). Adjuvants and vaccine antigens may evoke T-cell-mediated immune reactions, which may trigger AA in genetically predisposed individuals.

The occurrence of AA 1 week after vaccination followed by regrowth followed by recurrence 3 days after a different vaccine strongly suggests a link between vaccination and the onset of alopecia in this child, who was probably genetically predisposed. Given that vaccination is nearly universal in developed countries and that occurrence of any type of hair loss after vaccination is rare, generalizable conclusions cannot be drawn from a single case.

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EXHIBIT 211
Autoimmune Bullous Dermatoses: A Review

Patrícia Patrício,a Carlos Ferreira,a Manuel Marques Gomes,b and Paulo Filipeb

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Bullous dermatoses are a variety of autoimmune skin diseases that are characterized by the presence of bullae or blisters. Most of these diseases are associated with substantial morbidity, and a few may result in death. Although most general approaches to the treatment and diagnosis of these entities are similar, the diagnosis of the specific disease is important, because the most appropriate dosage and timing of some commonly used medications vary considerably. The review covers the management of main autoimmune bullous dermatoses, including bullous pemphigoid and pemphigus vulgaris, linear IgA dermatosis, dermatitis herpetiformis, and bullous systemic lupus erythematosus.

Key words: autoimmune bullous dermatoses; bullous dermatoses; bullous pemphigoid and pemphigus vulgaris; linear IgA dermatosis; dermatitis herpetiformis and bullous systemic lupus erythematosus

Bullous Pemphigoid

Bullous pemphigoid (BP) is a chronic, autoimmune, subepidermal, blistering skin disease that rarely involves mucous membranes. It occurs mainly in the elderly and rarely in children. Onset is typically between 60 and 80 years of age. There is equal incidence in men and women, and there are no known racial or ethnic predilections.

The lesions of BP may initially start as an urticarial eruption, which over a course of weeks to months, develops into bullae. The lesions are usually pruritic, and there may be tenderness at the site of eroded lesions. Once formed, blisters are large and tense, with a round or oval shape. Discrete lesions arise on normal or erythematous skin and are scattered throughout the body, including the axillae, medial thighs, groin, abdomen, flexor forearms, and lower legs. The lesions may be localized or generalized. BP involves the mucosa in 10–25% of patients.

Histological examination of a skin biopsy from a bulla reveals a subepidermal blister with superficial dermal inflammation consisting of lymphocytes, histiocytes, and eosinophils. On electron microscopy, blister formation is found to occur within the lamina lucida of the basement membrane, causing a loss of anchoring filaments and hemidesmosomes. BP is characterized by the presence of immunoglobulin G (IgG) autoantibodies specific for the hemidesmosomal BP antigens BP230 (BPAg1) and BP180 (BPAg2). The binding of the antibodies at the basement membrane activates complement and inflammatory mediators. Serum levels of autoantibodies against BPAg2 are reportedly correlated with disease activity in some studies. The role of autoantibodies specific for BP antigens in the initiation and the perpetuation of disease is unknown. Although BPAg2 has been identified as the major antigen involved with BP disease development, autoantibodies against alpha 6 integrin...
and laminin-5, two other skin basement membrane components, were identified in human patients affected by BP. Eotaxin, an eosinophil-selective chemokine, is strongly expressed in the basal layer of the epidermis of lesional BP skin and parallels the accumulation of eosinophils in the skin basement membrane zone area. It may play a role in the recruitment of eosinophils to the skin basement membrane area. Moreover, IL-5, an interleukin with eosinophil chemoattractant and activation properties, has been found in the skin of patients with BP.

Interleukin 16, a chemotactic factor responsible for recruiting CD4+ T cells to the skin and for inducing functional interleukin 2 receptors for cellular activation and proliferation, was found to be expressed by epidermal cells and infiltrating CD4+ T cells in lesional skin. In addition, serum levels of different mediators inducting monokine induced by interferon gamma (MIG, a Th1-type chemokine), CCL17, and CCL22 (Th2-type chemokines) were also increased in BP patients compared with healthy subjects.

Matrix metalloproteinases MMP-2, MMP-9, and MMP-13 were significantly increased in lesional skin, with T cells comprising the majority of MMP cellular sources, suggesting a major role of MMP in the blistering of BP. The BAFF (B cell activating factor belonging to the tumor necrosis factor family) cytokine regulates B cell proliferation and survival and was found to be increased in sera of BP patients. To establish a diagnosis of BP, the following tests should be performed: histopathologic analysis from the edge of a blister and direct immunofluorescence (DIF) studies on normal-appearing perilesional skin. If the DIF result is positive, indirect immunofluorescence (IDIF) is performed using the patient's serum. The preferred substrate for IDIF is salt-split normal human skin substrate. DIF tests usually demonstrate IgG and complement C3 deposition in a linear band at the dermal-epidermal junction.

Rarely, skin biopsy samples placed in transport media (Michel buffer) may yield false-negative results. This observation makes the use of fresh tissue the preferred substrate for DIF studies. IDIF studies document the presence of IgG circulating autoantibodies in the patient's serum that target the skin basement membrane component.

Direct and indirect immuno-electron microscopy (immunoEM) ultrastructurally localize in vivo–bound IgG autoantibodies (direct immunoEM) or the binding site of circulating IgG autoantibodies (indirect immunoEM) at the lamina lucida of the basement membrane. The sensitivity of immunoblotting varies. In 75% of patients, a reaction occurs with the BP230 antigen, while, in 50% of patients, this is observed with the BP180 antigen. Immunoprecipitation, like immunoblotting, demonstrates reactivity with BP230 and BP180.

In several reports, ELISA has been demonstrated to be highly sensitive and specific. These assays are used only as investigational tools. For the diagnosis of bullous pemphigoid three major criteria must be met or one DIF criterion (major criterion 3) and 1 minor criterion. Major criteria are: (1) (clinical) polymorphic eruption with tense blisters and erosions in skin (rarely mucosa), (2) (histopathological) subepidermal blistering with eosinophils, (3) (DIF) deposition of Ig G and C3 along the basement membrane. Minor criteria are: (1) deposition of Ig G and C3 along the basement membrane (by immunofluorescence), (2) BP antigens 1 or 2 (by ELISA), (3) bands at 180 or 230 kDa (by immuno-blotting).

Treatment consists of systemic prednisone, alone or in combination with a steroid-sparing agent such as azathioprine, mycophenolate mofetil, or a tetracycline. These drugs are usually started simultaneously, followed by a gradual tapering of the prednisone and continuation of the steroid-sparing agent until clinical remission is achieved. Mild cases may require only topical potent corticosteroids. Methotrexate may be used in patients with severe disease who are unable to tolerate prednisone. In most patients who are treated, BP remits within 1.5–5 years.
**Pemphigus Vulgaris**

Pemphigus vulgaris (PV) is a mucocutaneous autoimmune disease that affects the skin, oral cavity, and other mucosal surfaces. Genetic predisposition is suspected because of the identification of certain major histocompatibility complex (MHC) class II molecules, such as DR4 (DRB1*0402) and DRw6 (DQB1*0503).

Blisters in PV are associated with the binding of IgG autoantibodies to keratinocyte cell-surface molecules. These intercellular or PV antibodies bind to keratinocyte desmosomes and to desmosome-free areas of the keratinocyte cell membrane. PV antibody binds to keratinocyte cell-surface molecules desmoglein 1 and desmoglein 3. Patients with active disease have circulating and tissue-bound autoantibodies of both the immunoglobulin G1 (IgG1) and immunoglobulin G4 (IgG4) subclasses. Disease activity correlates with antibody titer in some patients. Mucous membranes typically are affected in PV. Intact bullae are rare in the mouth, more commonly the lesions are ill-defined, irregular, gingival, oral or palatine erosions. Other areas may be affected, including the conjunctiva, esophagus, labia, vagina, cervix, penis, urethra, and anus. Mucosal lesions may precede cutaneous lesions by months. Lesions in skin folds can form vegetating granulations. The vegetating type of response can be more resistant to therapy and can remain in one place for long periods of time. In patients with active blistering, firm, sliding pressure with a finger separates normal-appearing epidermis, producing an erosion known as Nikolsky sign, which is not specific for PV and is found in other active blistering diseases. The Asboe–Hansen sign occurs when a lateral pressure on the edge of a blister spreads the blister into clinically unaffected skin. PV eventually has classic association with other autoimmune diseases, particularly myasthenia gravis and thymoma. There are some cases associated with drugs and paraneoplastic forms. Diagnosis of the disease is made on the basis of the following data: presence of current blister formation, erosions and crust; presence of Nikolsky sign; histological detection of intra-epidermal blistering; detection of acantholytic keratinocytes by the Tzanck test; detection of pemphigus antibodies; and DIF and/or IDIF positive.

Conventional therapy consists of high-dose corticosteroids, immunosuppressive agents, and intravenous immune globulin. In refractory PV, the combination of rituximab and immune globulin is effective.

**Linear Ig A Dermatosis**

Linear IgA dermatosis (LAD) is an autoimmune vesicobullous subepidermal dermatosis. Infections, drugs, or malignant processes may provoke it, but it sometimes has an idiopathic origin. The disease affects people of all ages, but two peaks can be observed: the chronic bullous disease of childhood appearing before the age of five and the adult linear IgA disease appearing after the age of 60 years. The lesions of linear IgA dermatosis consist of pruritic, annular papules, vesicles, and bullae that are found in groups. There is a predilection for the extensor surfaces, with symmetrical distribution. Lesions are seen on the elbows, knees, and buttocks. Because of itching, excoriations will lead to the formation of many crusted papules. Chronic bullous disease of childhood presents with abrupt onset of tense bullae on an inflamed, erythematous base and is accompanied by pruritus and a burning sensation. The lesions are most frequently found on or near the genitalia, but may also be found on other areas, including the face, especially the perioral region. Characteristic “collarettes” of vesicles or blisters often form as new lesions arise in the periphery of old lesions. In both forms of linear IgA dermatosis, mucous membrane involvement may occur and ranges in severity from mild oral ulcers to severe oral or conjunctival disease. On histopathology, in linear IgA dermatosis and chronic bullous disease of childhood, the bullae are subepidermal, with collections of
neutrophils along the basement membrane and occasionally in the dermal papillary tips.

The diagnosis can be confirmed by DIF, displaying linear IgA deposits along the epidermal basement membrane. A complexity and heterogeneity of the target antigens in different patients with LAD have been established. Incriminated antigens are proteins with molecular weight of 285 kDa, as well as 97-kDa and 120-kDa antigens, which appear to be fragments of extracellular domain of bullous pemphigoid antigen BP 180 (type XVII collagen). BP 230 antigen located in the lamina lucida of the basal membrane, collagen VII, which is a component of anchoring fibrils as well as some still unidentified antigens, may also be involved. A strong association between the disease and autoimmune haplotypes HLA-B8, CW7, and DR3 has been reported.

The disease is characterized by circulating and tissue-bound IgA antibodies against heterogeneous antigens located in the cutaneous basement membrane zone. Application of split skin technique has demonstrated that the majority of antibodies bind to the epidermal side of the lamina lucida, whereas the rest adheres to the dermal side of the artificial blister, and a few are of a combined pattern. In cases triggered by drugs, such as vancomycin, phenitoin, somatostatin, amiodarone, lithium, and captopril, remission may follow the withdrawal of the incriminated drug.

In idiopathic cases, the treatment should be started with a dose of 25–50 mg dapsone daily, which has to be increased stepwise to 100–150 mg daily. As dapsone may cause a hemolytic anemia, decreased hemoglobin values, or even methemoglobinemia, the enzyme glucose-6-phosphate dehydrogenase has to be assayed before beginning the treatment. As an alternative drug, sulphapyridine 250 mg to 3 g daily can be administered. Skin lesions in linear IgA dermatosis and chronic bullous disease of childhood respond rapidly when treated with dapsone or sulphapyridine. Some patients may require low-dose prednisone initially to suppress blister formation.

### Dermatitis Herpetiformis

Dermatitis herpetiformis (DH) is an autoimmune blistering disorder associated in most patients to a gluten-sensitive enteropathy (GSE). It is characterized by grouped excoriations; erythematous, urticarial plaques; and papules with vesicles. It is exquisitely pruritic, and the vesicles are often excoriated to erosions by the time of physical examination. Onset tends to be between 20 and 40 years of age but may occur at any age, including childhood, and there is a 2:1 preponderance for men. The lesions of dermatitis herpetiformis usually begin as a vesicle, but may also be erythematous papules, urticaria-like wheals, excoriations, crusts, or rarely, large bullae. The lesions may be grouped, giving a “herpetiform” appearance. Once the lesions have resolved, there may be transient hyper- or hypopigmentation. The lesions are usually intensely pruritic, accompanied by burning and stinging. Many patients experience localized burning, stinging, and pruritus approximately 8 to 12 h before the onset of lesions, and many are able to predict an eruption. There is symmetric distribution along the extensor surfaces, including the elbows, knees, buttocks, shoulders, and sacral areas. Less frequently, the lesions are found on the scalp, face, hairline, and the posterior neck. Involvement of the palms and soles is rare, and mucous membrane lesions are uncommon. More than 90% of patients have an associated GSE upon endoscopic examination even if asymptomatic.

Histopathologically there are neutrophilic micro-abscesses in dermal papillae, dermal infiltration of neutrophils and eosinophils, and the formation of subepidermal vesicles. Blisters form within the lamina lucida, the weakest portion of the dermo-epidermal vesicles, due to neutrophil lysosomal enzymes. Dermal blood vessels may be surrounded by a lymphohistiocytic infiltrate, as well. Granular IgA deposits alone or in association with C3 in dermal papillae of perilesional skin observed by DIF is the standard criterion of diagnosis. Because
deposits are found throughout normal-appearing skin, the standard practice is to obtain biopsy specimens from normal-appearing perilesional skin for DIF staining. In areas corresponding to IgA deposits, there may also be complement deposition. IgA and IgG antireticulin and anti-endomysial antibodies have been detected in DH patients’ sera. An increased incidence of antinuclear and antithyroid microsomal antibodies is also found in these patients. An underlying genetic predisposition to DH has been demonstrated. Both DH and celiac disease (CD) show an increased expression of HLA-A1, HLA-B8, HLA-DR3, and HLA-DQ2 haplotypes. Evidence is mounting that epidermal transglutaminase 3 (TGase3), a cytosolic enzyme involved in cell envelope formation during keratinocyte differentiation, is the autoantigen of DH. Theoretically, DH is caused by dermal deposition of circulating immune complexes containing both IgA and TGase3. This is supported by the finding that precipitates of skin-bound IgA from DH lesions contain TGase3. In addition, it has been demonstrated that serum from DH patients contains high-affinity anti-TGase IgA autoantibodies.

The leading theory for DH is that a genetic predisposition for gluten sensitivity, coupled with a diet high in gluten, leads to the formation of IgA antibodies to gluten-TGase complexes. These antibodies cross-react with TGase3, and IgA/TGase3 complexes deposit within the papillary dermis to cause the lesions of DH. These IgA deposits can disappear after long-term avoidance of dietary gluten. Cutaneous IgA deposits in DH have been shown to function in vitro as a ligand for neutrophil migration and attachment. Although IgA deposition is pivotal for disease, an increased serum IgA is not necessary for pathogenesis. When the disease is active, circulating neutrophils have a higher level of CD11b and an increased ability to bind IgA. Collagenase and stromelysin 1 may be induced in basal keratinocytes either by cytokines released from neutrophils or by contact with keratin from damaged basement membrane matrix. Stromelysin 1 may contribute to blister formation. Mild local trauma may also induce the release of cytokines and attract the partially primed or activated neutrophils, which is consistent with the typical location of DH lesions on frequently traumatized areas, such as the knees and elbows. Hormonal factors may also play a role in DH; recent reports describe DH induced by treatment with leuprolide acetate, a gonadotropin-releasing hormone analog. Androgens have a suppressive effect on immune activity, including decreased autoimmunity, and androgen-deficient states may be a potential trigger for DH exacerbation. Apoptosis may contribute to the pathogenesis of epidermal changes in DH, and recent research demonstrates a markedly increased apoptotic rate within the epidermal compartment in DH.

Most patients with DH have histologic evidence of enteropathy, even in the absence of symptoms of malabsorption. IgA circulating immune complexes are present in 25–35% of patients with DH, although no association with disease severity has been noted. IgA antibodies to gliadin (a portion of wheat protein), reticulum, and smooth muscle endomysium have also been noted in patients with DH and in those with isolated GSE. The presence of IgA anti-endomysial antibodies correlates with the extent of the gut disease.

Patients will experience prompt relief of lesions within 1 to 2 days of initializing treatment with dapsone or sulfapyridine. It is important to remember to always check G6PD and baseline complete blood count levels before starting dapsone. Other methods of treatment include dietary modification. One form is the gluten-free diet, which has been found to improve both intestinal and skin lesions. The onset is slow, taking from 5 months to 1 year before the effect is noted; however, close adherence to the diet will allow patients to stop or significantly decrease the medications. Alleviation of skin lesions can occur within a few weeks of starting the diet, even if the patient ingests large amounts of gluten, but this diet is difficult to tolerate. Control of the skin disease can be achieved with
medications, dietary avoidance of gluten, or both. Avoidance of dietary gluten for 10 years or more has resulted in loss of cutaneous IgA deposits, which then return upon reinstitution of gluten in the diet. Dapsone does not improve GI mucosal pathology. Other, less effective treatments for DH include colchicine, cyclosporine, azathioprine, and prednisone. UV light may provide some symptomatic relief. Cyclosporine should be used with caution in patients with DH because of a potential increase in the risk of developing intestinal lymphomas. DH responds well to medications and diet, and has a good prognosis. Association of DH with other GI conditions include gastric atrophy, gastric hypochlorhydria, and pernicious anemia. Associated autoimmune diseases include dermatomyositis, type 1 diabetes mellitus, myasthenia gravis, rheumatoid arthritis, Sjögren syndrome, systemic lupus erythematosus, and thyroid abnormalities. Thyroid abnormalities include hypothyroidism, hyperthyroidism, thyroid nodules, and thyroid cancer. Neoplastic conditions include GI lymphomas and non-Hodgkin’s lymphoma.

**Bullous Systemic Lupus Erythematosus**

Bullous systemic lupus erythematosus (BSLE) is an autoantibody-mediated subepidermal blistering disease that occurs in patients with systemic lupus erythematosus (SLE). These blisters result from toxic necrolysis of the skin, mediated by deposition of immunoreactants at the basement membrane, and underlying dermal vasculitis. Blisters may arise on erythematosus or normal skin and are nonscarring. Lesions occur on sun-exposed or flexural skin. Skin biopsy shows subepidermal vesicles containing neutrophils with micro-abscesses, nuclear dust, and fibrin. Blistering often parallels flares of SLE involving other organ systems, in particular renal disease. Camisa and Sharma proposed criteria for this distinct subset of vesiculobullous skin lesions occurring in patients with SLE: (1) a diagnosis of SLE based on American Rheumatism Association criteria; (2) vesicles and bullae arising upon but not limited to sun-exposed skin; (3) histopathology compatible with DH; (4) negative IDIF for circulating basement membrane zone antibodies; (5) DIF positive for IgG and/or IgM and often IgA at the basement membrane zone. Yell and colleagues suggested this classification be revised because of the heterogeneity of clinical and immunohistological presentation. They defined BSLE as an acquired subepidermal blistering disease in a patient with SLE, in which immune reactants are present at the basement membrane zone on direct or IDIF. DIF microscopy demonstrates immunoglobulin G (with or without immunoglobulin A [IgA] and immunoglobulin M) deposits at the basement membrane zone (BMZ). Evidence of antibodies to type VII collagen via DIF or IDIF on salt-split skin, immunoblotting, immunoprecipitation, ELISA, or immuno-electron microscopy can be demonstrated. All five criteria are needed for a diagnosis of type 1 BSLE, whereas only the first four criteria are needed to diagnose type 2 (undetermined location of antigen or dermal antigen other than type VII collagen) and type 3 (epidermal antigen) BSLE. Type VII collagen, a component of anchoring fibrils, is also targeted in epidermolysis bullosa acquisita (EBA). However, unlike EBA, BSLE tends to respond dramatically to treatment with dapsone, and these disease can usually be differentiated from lupus by characteristic changes (including immune deposits) and serum antibodies (which react with different parts of the dermis). Not all blistering eruptions that occur in patients with lupus erythematosus (LE) represent BSLE as defined above. Such patients may present with a severe form of acute or subacute cutaneous LE (SCLE) that resembles erythema multiforme (Rowell syndrome) or toxic epidermal necrolysis (TEN). Because EBA and BSLE share the same target antigen, distinguishing between the two may be difficult.

In patients with BSLE, antibodies directed at the BMZ likely mediate the blistering phenotype by directly interfering with adhesive connections at the dermo-epidermal junction and
through induction of complement-dependent inflammation that leads to tissue injury and dermo-epidermal separation. Proteolytic damage caused by recruited neutrophils contributes to the latter process.

In type 1 BSLE (which accounts for most cases), antibodies against type VII collagen may weaken or block anchoring fibril-mediated connections between the lamina densa of the basement membrane and the papillary dermis. In both EBA and BSLE, antigenic epitopes reside within the NC1 and NC2 domains of type VII collagen, which are localized to the lamina densa and the underlying dermis, respectively. Antibodies recognizing bullous pemphigoid antigen 1, laminin-5, and laminin-6 have also been described in patients with BSLE.

The term acute syndrome of apoptotic pan-epidermolysis (ASAP) has been proposed for the TEN-like cutaneous injury pattern that can occur in settings of LE, acute graft-versus-host disease, pseudoporphyria, and the classic drug-hypersensitivity syndrome. Fas–Fas ligand interactions have been implicated in the massive keratinocyte apoptosis that characterizes ASAP. TEN-like cutaneous LE must be differentiated from drug-induced TEN occurring in a patient with LE. Patients with TEN-like acute cutaneous LE often have significant systemic disease activity (such as lupus nephritis or cerebritis).

In LE-specific vesiculobullous skin disease, the lesions are distinct from BSLE, representing severe variants of acute, subacute, or (rarely) discoid cutaneous LE. The eruptions can develop rapidly or evolve over several weeks. In TEN-like acute cutaneous LE, photodistributed diffuse or patchy erythema evolves (usually rapidly) into flaccid bullae (positive Nikolsky sign, unlike BSLE) and widespread sheet-like, full-thickness epidermal detachment. Certain individuals may have a genetic predisposition to develop autoimmune to BMZ antigens and to SLE. For example, EBA, BSLE, and SLE are all associated with an increased prevalence of the HLA class II DR2 haplotype. The antigen-presenting protein encoded by the DR2-associated DRB1*1501 allele (found in both EBA and BSLE patients) has been postulated to be involved in presenting type VII collagen epitopes to T lymphocytes.

Histopathologically, a BSLE-like picture is seen in DH and DH-like drug eruption. The presence of mucin among the collagen bundles in the dermis, the depth of the infiltrate, and the thickened BMZ in BSLE differentiates it from DH and DH-like drug eruption.

BSLE occurs in the setting of SLE; thus, ANA test results generally are positive. Anti-dsDNA, anti-Smith, anti-Ro/SS-A, anti-La/SS-B, and anticardiolipin antibodies may also be detected. Other laboratory abnormalities related to SLE can include low levels of complement (C3, C4, CH50), anemia, leukopenia, thrombocytopenia, proteinuria or cellular casts upon urinalysis, and an elevated erythrocyte sedimentation rate. Anticardiolipin antibodies and lupus anticoagulant have also been reported in individuals with Rowell syndrome. Leukocytoclastic vasculitis (LCCV) and septic vasculitis have a histopathologic picture similar to BSLE, but in LCCV the infiltrate invades the walls of the blood vessels, while in BSLE it is perivascular. Clinically, generalized vesicles and bullae can occur in LCCV, but they usually become purpuric. In septic vasculitis thrombi are seen inside the blood vessels. BSLE generally responds well to medical therapy, and treatment with dapsone is particularly effective. Although type 1 BSLE and EBA are both characterized by antibodies targeting type VII collagen, EBA differs considerably in its marked resistance to therapy.

Dapsone is the initial treatment of choice for BSLE. The response is usually dramatic, with cessation of new blister formation within 1–2 days and rapid healing of existing lesions. Low doses (25–50 mg/day) are often effective, although a higher dose is sometimes required. Rapid recurrences may occur upon withdrawal of dapsone, with prompt remission after reinstitution of therapy. However, discontinuance of dapsone therapy is usually possible within a year. Prednisone may be effective in patients...
who are intolerant of dapsone, have a poor re-
response to dapsone, or require treatment of con-
current systemic manifestations of SLE. Com-
bination therapy with prednisone and dapsone
can also be beneficial. Methotrexate (MTX),
azathioprine, and mycophenolate mofetil
are additional therapeutic options. Extensive
eruptions of TEN-like LE require prompt
institution of therapy with intravenous im-
munoglobulin and/or systemic corticosteroids.
Less fulminant manifestations of erythema
multiforme–like LE can be treated with anti-
malarials, corticosteroids (topical or systemic),
and (in the presence of systemic disease) other
agents in the therapeutic armamentarium for
LE. 9

The basic understanding of inflammatory
dermatoses and autoimmune-mediated skin
disorders has greatly advanced and broadened
our understanding of the underlying immune
mechanisms that shape the complex network of
chronic inflammation and autoimmunity. New
treatments, including B-cell-directed therapy,
are the new therapeutic frontier for this kind of
diseases. With this resume, we summarize the
process of establishing and revising the diagno-
sis criteria and clinical and therapeutic aspects
of the main types of autoimmune bullous der-
matoses diseases.

Conflicts of Interest

The authors declare no conflicts of interest.

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Bullous pemphigoid in infants: characteristics, diagnosis and treatment

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Abstract

Background: Bullous pemphigoid (BP) in infants is a rare but increasingly reported autoimmune blistering skin disease. Autoantibody reactivity is usually poorly characterized. Current guidelines do not address specific aspects of the infantile form of BP. The objectives of this study are to define clinical and diagnostic characteristics of infantile BP and develop a treatment algorithm.

Methods: Detailed characterization of a current case series of five infants with BP from our departments. Comprehensive analysis of all reported cases (1–12 months) with respect to clinical and laboratory characteristics, treatment and outcome.

Results: In total 81 cases were identified (including our own). The mean age was 4.5 months. Moderately severe and severe disease was seen in 84% of cases. Involvement of hands and feet was present in all cases. Immunofluorescence microscopy was comparable with BP in adults. Where analyzed, the NC16A domain of bullous pemphigoid 180 kDa antigen/collagen XVII (BP180) was identified as the major target antigen. BP180 NC16A ELISA values in our cohort were significantly higher than in a control cohort of 28 newly diagnosed adult patients. 50% of patients were treated with systemic corticosteroids, 20% with a combination of systemic corticosteroids and dapsone or sulfapyridine and 10% with topical corticosteroids alone. 14% of patients needed a combination of multiple immunosuppressants. All but one patient reached remission. Relapses were rare.

Conclusions: Presentation of infantile BP is often severe with blistering of hands and feet present in all cases. Pathogenesis and diagnostic criteria are comparable to adult BP, yet BP180 NC16A ELISA levels seem to be significantly higher in infants. The overall disease outcome is favorable. Based on the results of this study we propose a treatment algorithm for infantile BP.

Keywords: Bullous skin disease, Skin blistering, Vaccination
Diagnostic results in infantile and adult BP are similar, but serological tests were not performed systematically in many of the reported cases [13]. The gold standard for diagnosis is direct immunofluorescence microscopy (DIF). However, little information is available on the interpretation of ELISA levels [14], inflammatory markers or blood cell counts in infants. Further knowledge, especially about the relevance of ELISA levels might help to assess disease severity and thus influence the choice of medication or duration of treatment.

Concerning the treatment of infantile BP, first line treatment usually consists of topical or systemic corticosteroids. However, there are no stringent therapeutic criteria and there has been very little discussion on the different options for second line treatment. Furthermore, in clinical consensus guidelines on treatment of BP, there is very little, if any, information on treatment in infants [15-18].

Here, we report the diagnostic results and disease course of five children with infantile BP in our care and a comprehensive analysis of all cases reported in the literature. Based on these data – and taking into account the published guidelines for adults as well as special circumstances of treating small infants – we propose a first treatment algorithm for infantile BP.

Methods
Infantile BP cohort and adult BP control cohort
Five infantile BP patients presented at or were referred to our departments. They were included in this study after we obtained parental informed consent for participation and took blood and skin samples for diagnostic and research purposes. As a control, BP180 NC16A ELISA levels of a cohort of 28 adult BP patients that were newly diagnosed in the same time period were determined after informed consent was provided. All investigations were conducted according to the declaration of Helsinki criteria.

Histopathology, immunofluorescence microscopy, immunoblotting and ELISA
Hematoxylin eosin staining of formalin fixed, paraffin embedded tissue sections was performed using standard methods. DIF and indirect immunofluorescence microscopy (IIF) were performed as previously described [19-21]. FITC labeled antibodies used for DIF were anti human IgG, IgA, IgM and C3c (Dako, Hamburg, Germany) at a dilution of 1:200, 1:50, 1:50 and 1:500 respectively. For IIF on split-skin, patient sera were diluted 1:10, secondary antibodies used were FITC labeled anti human IgG and IgA (Dako, Hamburg, Germany) at a dilution of 1:100 and 1:25 respectively. Immunoblotting of normal human keratinocyte extracts with patient sera at a 1:20 dilution and alkaline phosphatase anti human IgG (Sigma-Aldrich, Taufkirchen, Germany) secondary antibody was performed as previously described [20,21]. ELISA kits for the detection of BP180- and bullous pemphigoid 230 kDa antigen (BP230)-specific antibodies (MBL, Nagoya, Japan) were used according to the manufacturer’s protocol with the cut-off at 9 U/ml.

Statistical analysis
Boxplot descriptive statistics of BP180 NC16A ELISA values were performed using GraphPad Prism software (GraphPad Software, La Jolla, CA).

Literature search
We searched all retrievable English- and foreign-language medical literature using PubMed, PubMed Central, EMBASE, and Google Scholar databases as well as literature cited in the obtained reports. Relevant information was extracted and reviewed to avoid duplications of reports. We included only infants up to 12 months in our review and excluded cases of neonatal BP.

Results
Patient cohort/index case
The clinical and laboratory findings of the five patients in our cohort are presented in Table 1. Patient 1 (index case) showed characteristic infantile BP and was the most severely affected; his treatment proved to be the most challenging. He is therefore presented in more detail. The previously healthy three-month-old boy of Algerian descent presented with a one-week history of small blisters on hands and feet and urticarial plaques on the trunk. Impetigo had been ruled out at a nearby hospital but no diagnosis had been made. He had received one oral vaccination against Rotavirus one month prior. No other vaccinations had been given. Apart from mild eczema, there was no family history of skin disease. Over the course of one week the lesions increased in number and size. The patient was irritable and not feeding well.

On clinical examination, he had firm blisters and bullae predominantly on the hands and feet, as well as urticarial plaques with an elevated rim and a dusky center. These plaques were predominantly located on the trunk but also present on all other areas of the body (Figure 1A, B). The Nikolsky sign was negative; there were no mucosal lesions.

Blister fluid microscopy demonstrated mainly eosinophil granulocytes; cultures from blister fluid remained sterile. Blood testing, including a full blood count, showed normal values with the exception of a peripheral eosinophilia of 10%. Punch biopsies were performed for histologic and immunofluorescence analyses. Histology showed dermal edema and eosinophil inflammatory infiltrate (not shown). DIF revealed linear staining of IgG (Figure 1E) and complement component C3 (Figure 1F) along the basement membrane zone. IIF microscopy showed circulating IgG autoantibodies binding to the
### Table 1 Clinical and laboratory findings of the patient cohort

<table>
<thead>
<tr>
<th>Age (months)/Gender</th>
<th>Extent of Disease/Hands/Feet (HF)</th>
<th>OM DIF/IIF/Immunoblot</th>
<th>ELISA WBC (Eos in%)</th>
<th>Treatment</th>
<th>Time Until Remission/Relapse/Duration of Treatment</th>
<th>Special aspects</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 3/M Generalized</td>
<td>+</td>
<td>DIF: IgG, C3 (BM)</td>
<td>Anti BP180</td>
<td>a) Prednisolone</td>
<td>Initially rapid response with disease control</td>
<td>Family history of atopy</td>
</tr>
<tr>
<td></td>
<td>HF+</td>
<td>IIF: IgG (BR)</td>
<td>136 U/ml</td>
<td>2 mg/kg/d → 1 mg/kg/d</td>
<td>Relapse within 2 weeks after diagnosis on systemic prednisolone (2 mg/kg) and during respiratory tract infection</td>
<td>Rotavirus vaccine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IB: 180kD pos. (norm &lt; 9)</td>
<td>54 × 10⁹/l (52)</td>
<td>b) Dapsone 2 mg/kg/d</td>
<td>Slow response after relapse, need for multiple medications</td>
<td>4 weeks prior</td>
</tr>
<tr>
<td></td>
<td></td>
<td>At relapse:</td>
<td>Tc &gt;1000 × 10⁹/l</td>
<td>c) IVIG 1 g/kg × 3</td>
<td>Response to dapsone after 2.5 weeks</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti BP180</td>
<td>189 U/ml</td>
<td>d) MMF (2× 600 mg/m²/d)</td>
<td>Duration of treatment: 8 months</td>
<td></td>
</tr>
<tr>
<td>2) 3/M Localized with few disseminated lesions HF+</td>
<td>-</td>
<td>DIF: IgG, C3 (BM)</td>
<td>Anti BP180</td>
<td>a) Topical Prednicarbate</td>
<td>Good response to topical treatment within days</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IIF: IgG (BR)</td>
<td>90 U/ml</td>
<td>(mid-potency corticosteroid)</td>
<td>No relapse</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IB: 180kD pos. (norm &lt; 9)</td>
<td>Anti BP230 neg.</td>
<td>Duration of treatment: 4 weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3) 4/M Generalized</td>
<td></td>
<td>DIF: IgG, C3 (BM)</td>
<td>Anti BP180</td>
<td>a) Prednisolone</td>
<td>Complete remission within 1 week</td>
<td>Vaccination 4 weeks prior</td>
</tr>
<tr>
<td></td>
<td>HF+</td>
<td>IIF: IgG (BR)</td>
<td>156 U/ml</td>
<td>2 mg/kg/d → 1 mg/kg/d</td>
<td>Weaning of steroids within 3 months</td>
<td>(DPTP, HiB, HepB, Rotavirus)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IB: 180kD pos. (norm &lt; 9)</td>
<td>Anti BP230 neg.</td>
<td>b) Dapsone 1.5 mg/kg/d</td>
<td>No relapse</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti BP230 neg.</td>
<td>Duration of treatment: 6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4) 3/F Generalized</td>
<td>-</td>
<td>DIF: IgG, C3 (BM)</td>
<td>Anti BP180</td>
<td>a) Prednisolone</td>
<td>Slow response to prednisolone 1 mg/kg</td>
<td>Rotavirus vaccine</td>
</tr>
<tr>
<td></td>
<td>HF+</td>
<td>IIF: IgG (BR)</td>
<td>125U/ml</td>
<td>2 mg/kg/d → 1 mg/kg/d</td>
<td>Rapid response to oral betamethasone 0.3 mg/kg/d</td>
<td>4 weeks prior</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IB: 180kD pos. (norm &lt; 9)</td>
<td>Anti BP230 neg.</td>
<td>b) Systemic betamethasone</td>
<td>No relapse upon glucocorticoid tapering</td>
<td>Arterial hypertension</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anti BP230 neg.</td>
<td>0.3 mg/kg/d</td>
<td>c) Dapsone 1 mg/kg/d → 0.5 mg/kg/d</td>
<td>Complete remission under dapsone 0.5 mg/kg/d</td>
<td>Myocardial hypertrophy</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>d) Dapsone Treatment ongoing</td>
<td>→ Propranolol</td>
<td></td>
</tr>
</tbody>
</table>
Table 1 Clinical and laboratory findings of the patient cohort (Continued)

<p>| | | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5)</td>
<td>7/M</td>
<td>Generalized</td>
<td>-</td>
<td>DIF: IgG, C3 (BM)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HF+</td>
<td>IIF: IgG (BR)</td>
<td>154 U/ml</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>IB: 180kD pos. (norm &lt; 9)</td>
<td>Anti BP230 neg.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>a) Prednisolone 1 → 0.5 mg/kg/d</td>
<td>Rapid response to oral betamethasone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>b) Systemic betamethasone</td>
<td>Full remission after 2 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>c) Dapsone 0.5 mg/kg/d</td>
<td>Treatment ongoing</td>
</tr>
</tbody>
</table>

HF: Hands/Feet + present, − not present; OM: Involvement of oral mucosa; + present, − not present; DIF: Direct immunofluorescence microscopy; IIF: Indirect immunofluorescence microscopy; IB: Immunoblot; BM: basement membrane; BR: Blister roof; WBC: White blood cell count; Eos: eosinophil granulocytes; Tc: thrombocytes; DPTP: Diphteria, Pertussis, Tetanus, Poliovirus; HiB: Haemophilus influenzae type b; HepB: Hepatitis B.

Generalized disease = Moderately severe and severe disease.
epidermal side of the salt-split skin (Figure 1G). ELISA
testing with recombinant NC16A domain of BP180 was
strongly positive (136 U/ml, norm <9 U/ml). The find-
ings were diagnostic for BP.

Initially, we treated with potent topical corticosteroids
and oral antihistamines, which did not lead to significant
improvement. After confirmation of the diagnosis, a
treatment with prednisolone up to 2 mg/kg/day was ini-
tiated. After a brief period of clinical improvement and
disease control, the patient had a respiratory tract infec-
tion in the course of which he developed severe blister-
ing. At this time he was still on 2 mg/kg prednisolone
daily (Figure 1C, D). Peripheral blood count showed
leucocytosis with a maximum of 54 G/l (52% eosinophils)
and significant reactive thrombocytosis (>1000 G/l) with
signs of increased coagulation activity necessitating treat-
ment with acetylsalicylic acid. The ELISA value for BP180-
specific antibodies at this point was 189 U/ml. After
confirming normal glucose-6-phosphate dehydrogenase
levels, we added dapsone at a maximum dose of 2 mg/kg
daily, controlling for the development of methemoglobinemia.
As the blistering continued to progress, we added intravenous
immunoglobulins (IVIG) 1 g/kg three times. Yet the patient
developed more cutaneous and additionally intraoral blis-
ters causing refusal of oral intake. He also developed per-
sistent hoarseness, but laryngeal involvement of the BP
could be excluded.

After two weeks of worsening, we added oral myco-
phenolate mofetil (MMF) at a dose of 625 mg/m² twice
daily (MMF local dosing regimen, note that recom-
mended standard dose in children is 600 mg/m² twice
daily). Within days, the patient’s skin improved and the
number of new lesions decreased. We interpreted this
improvement as delayed response to dapsone rather
than response to MMF, which usually takes several weeks to set in. Over the following weeks, we slowly weaned the patient off systemic corticosteroids and then reduced the MMF dose in two steps over two months. After another two months of clinical remission, we also stopped treatment with dapsone. After 12 months the patient was off all medication. Anti-BP180 antibody values significantly decreased over the course of three months, parallel to clinical improvement. Also, the number of leukocytes (including eosinophils) and thrombocytes decreased and normalized. At the time of submission, the patient had been free of symptoms for two years. Due to parental fear of relapse, the patient had not received any further vaccinations.

Analysis of all reported infantile BP cases, including own patient cohort

Clinical characteristics

The literature review of all obtainable reports between the years 1977 and 2013 including our own cases revealed 53 reports [8,11-14,22-68] with a total of 81 cases of BP occurring in children within the first year of life but beyond the neonatal period (Additional file 1: Table S1). While very few cases were reported before the year 2000, there has been a significant increase since then (Additional file 2: Figure S1). The mean and median age was approximately four months with 64% of cases between three to five months. The gender ratio male to female was 39 to 38. In four cases gender was not stated. Moderately severe and severe (generalized) disease (>10% body surface area – BSA) was seen in 83.9% of cases (n = 68 of 81). All children showed at least some involvement of the hands and feet. Mucosal blistering was present in 14.8% of cases (n = 12 of 81); four of these patients had severe disease (Table 2).

98% (n = 79 of 81) of children affected had previously been healthy. One patient had a congenital T-cell lymphocytopenia and one child had been diagnosed with Hyper IgE-syndrome. The general condition at the time of presentation was good in the majority of cases; some patients were irritable, likely due to pruritus. However, one child with a very delayed initiation of appropriate treatment presented with significant morbidity, including severe weight loss, dehydration and failure to thrive, as well as developmental delay [41]. One of our own patients was also severely affected during a relapse where he refused oral intake and lost weight (see index case above). Both children improved quickly once sufficient treatment was established.

Twenty five children (30.8%) had been vaccinated within days or weeks prior to the onset of disease, the majority with the standard mix of passive vaccines recommended in this age group. Two of our five own cases had received a newly recommended oral vaccine against Rotavirus prior to disease onset. It is possible that the rotavirus vaccine may be causative in some instances [22-53].

Table 2 Clinical characteristics of all reported infantile BP cases, including own patient cohort

<table>
<thead>
<tr>
<th>No of cases</th>
<th>N = 81</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (median) age/age range</td>
<td>4.5 (4) months/1–12 months</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>39/38 (4 unknown)</td>
</tr>
<tr>
<td>Extent of skin involvement</td>
<td></td>
</tr>
<tr>
<td>• Localized/mild disease (+/- few disseminated plaques)</td>
<td>N = 10 (12.3%)</td>
</tr>
<tr>
<td>• Generalized/moderately severe and severe disease</td>
<td>N = 68 (83.9%)</td>
</tr>
<tr>
<td>• N/A</td>
<td>N = 3 (3.7%)</td>
</tr>
<tr>
<td>• Involvement of hands and feet</td>
<td>N = 81 (100%)</td>
</tr>
<tr>
<td>Involvement of oral mucosa (with generalized disease)</td>
<td>N = 12 (14.8%)</td>
</tr>
<tr>
<td>No of children vaccinated prior to onset</td>
<td>N = 25 (30.8%)</td>
</tr>
<tr>
<td>• DPTP +/- others,</td>
<td>N = 22</td>
</tr>
<tr>
<td>• Rotavirus,</td>
<td>N = 2</td>
</tr>
<tr>
<td>• DPTP plus Rotavirus</td>
<td>N = 1</td>
</tr>
<tr>
<td>No of patients with a relapse</td>
<td>N = 12 (14.8%)</td>
</tr>
<tr>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>• Cured</td>
<td>N = 76 (93.8%)</td>
</tr>
<tr>
<td>• In remission under treatment at time of report</td>
<td>N = 3 (3.7%)</td>
</tr>
<tr>
<td>• Still symptomatic at time of report</td>
<td>N = 1 (1.2%)</td>
</tr>
<tr>
<td>• Death</td>
<td>N = 1 (1.2%)</td>
</tr>
</tbody>
</table>

N/A: Not Available; DPTP: Diphteria, Pertussis, Tetanus, Poliovirus.
to the onset of disease. This has not been reported before. In two children a febrile infection was reported prior to the onset of disease [37] or prior to a relapse [27], this report.

Pathophysiology and diagnostic features
Histology, if reported, showed dermal edema, an inflammatory infiltrate dominated by eosinophils and subepidermal blistering. DIF showed IgG and/or C3 along the basement membrane in 72 cases (90%), in 12 cases (15%) there were additional IgA deposits, in four cases there were IgM- and in one case IgE-deposits. In immunoblot analyses reported in 20 patients, 15 sera recognized a 180 kDa protein, five sera recognized a 230 kDa protein, and one serum both.

ELISA values were reported in only 21 (25.9%) cases. All of these patients had antibodies against the NC16A-domain of BP180; two also had additional anti-BP 230 antibodies. Comparison of ELISA values of reported cases from different centers is not fully possible because of different commercial and non-commercial ELISA systems used. In our own cohort, BP180 NC16A ELISA values in infantile patients were significantly higher than in a control group of 28 adults newly diagnosed with BP in our center in the same time period (Figure 2). Extremely high values in our cohort and in reported patients seemed to be associated with more extensive disease and the need for systemic treatment.

A blood cell count was reported in 37 patients, the mean white blood cell count was 23.9 G/l (range <10-120G/l, median 19.4). The percentage of eosinophils had a mean of 23% (range 7-66%, median 19%).

Treatment modalities
The majority of patients were treated with systemic corticosteroids (50.6%) with or without additional erythromycin or other antibiotics. 19.8% of patients were treated with a combination of systemic corticosteroids and dapsone or sulfapyridine, and 9.9% were treated with topical corticosteroids alone. 13.7% of patients (n = 11) needed a combination of multiple agents (Table 3). All but one patient reached remission eventually. However, the patient with concomitant congenital T-cell lymphocytopenia died from unknown cause three months after having received two doses of rituximab for severe disease. Relapses were not common (14.8%, n = 12) (Table 3 and Additional file 3: Table S2).

Discussion
Infantile BP is considered very rare. Prospective studies are therefore difficult to perform. Incidence in Israel was estimated to be 2.36:100,000 per year [13]; however, in most countries no central registry exists and the disease might be under-recognized. We present a detailed characterization of a current cohort of five infants with BP from our departments. Furthermore we performed a comprehensive analysis of all cases reported in the literature (age 1–12 months) with respect to clinical and laboratory characteristics and treatment modalities. Taken together the results allow for the following conclusions.

Diagnostic features
Laboratory test results in infantile BP generally resemble those in adult BP. Linear IgG and/or C3 depositions at the basement membrane in DIF are the diagnostic hallmark. Autoantibody profiles, as detected by various methods, are comparable to those in adults with BP [69]: autoantibodies against the NC16A domain of BP180 are more frequent than anti-BP230 antibodies.

We propose the following minimal diagnostic criteria for infantile BP: typical clinical picture (urticarial plaques and blisters, acral distribution) and linear IgG and/or C3 deposition at the basement membrane in DIF. Further diagnostic pointers are the presence of serum autoantibodies against BP180 and/or BP230. and – even though less specific – subepidermal blistering with an eosinophil rich inflammatory infiltrate in conventional histology.

Even though ELISA results were only reported in a minority of cases, and different test systems used do not allow for direct comparison, the reported autoantibody levels in infants seem fairly high. Comparing ELISA values of our five infants with a control group of 28 adults newly diagnosed with BP in our center in the same time period, we found that the mean and median

![Figure 2 ELISA values in infantile and adult BP. Anti-BP180 ELISA values in our infantile BP cohort were significantly higher, compared to a control group of newly diagnosed adult BP patients (normal value <9 U/ml; boxplot analysis; whiskers: minimum and maximum values; bottom and top of boxes: first and third quartiles; band inside box: median; cross: mean).]( alta.org/article.png)
The levels of anti-BP180 NC16A antibody levels in infants were significantly higher. These ELISA values had been measured with the same test system (see Methods).

The clinical relevance of antibody testing in infantile BP has been contested [14]. Nevertheless – when tested – patients with a more recalcitrant disease course demonstrated high autoantibody levels. In our cohort, higher values at presentation correlated with the need for more aggressive and longer-term treatment, and values increased before relapses. Therefore, it appears reasonable to take into account the levels of BP180-specific autoantibodies in infantile BP when making treatment decisions.

### Patient characteristics/clinical features

At disease onset, the mean age of children was around four months. As opposed to previous reports [13], there was no significant female predominance.

No common trigger was identified. A large number of patients had either been vaccinated or suffered an infection prior to the onset or relapse of disease (Table 2, Additional file 1: Table S1 and Additional file 3: Table S2). The type of infection or vaccine varied. It can be speculated that a modulation of the immune system might play a role in triggering or unmasking an underlying subclinical BP. Nevertheless, especially due to the high number of infants receiving vaccination, this association might be purely coincidental and we believe that the term postvaccination infantile BP should be used with caution.

Cases of adult BP associated with malignancy exist, even though the causal relation remains unclear.

### Table 3 Treatment Modalities of Infantile BP Patients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No of cases (% of total N = 81)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Topical corticosteroids alone</td>
<td>N = 8 (9.9%)</td>
<td>Good response</td>
</tr>
<tr>
<td>Topical corticosteroids + IVIG</td>
<td>N = 1 (1.2%)</td>
<td>Several relapses for one year</td>
</tr>
<tr>
<td>Topical corticosteroids + erythromycin</td>
<td>N = 1 (1.2%)</td>
<td>Good response</td>
</tr>
<tr>
<td>Systemic +/- topical corticosteroids (+/- antibiotics)</td>
<td>N = 41 (50.6%)</td>
<td>Good response</td>
</tr>
<tr>
<td>Systemic corticosteroids + dapsone/ sulphapyridin (+/- antibodies)</td>
<td>N = 16 (19.8%)</td>
<td>Good response</td>
</tr>
<tr>
<td>Dapsone/ sulphapyridin alone</td>
<td>N = 2 (2.5%)</td>
<td>One relapse under treatment. Same treatment was attempted in one other patient without success, so steroids were added.</td>
</tr>
<tr>
<td>No treatment</td>
<td>N = 1</td>
<td></td>
</tr>
<tr>
<td>N/A</td>
<td>N = 1</td>
<td></td>
</tr>
<tr>
<td>Corticosteroids +/- dapsone plus other medications due to poor response</td>
<td>N = 11 (13.7%)</td>
<td></td>
</tr>
<tr>
<td>• Azathioprine</td>
<td>N = 1</td>
<td>No response</td>
</tr>
</tbody>
</table>
| • Cyclosporine                                  | N = 2                          | Good response in N = 1  
Partial response in N = 1 |
| • Mycophenolate mofetil                          | N = 7                          | Moderate response in N = 7 |
| • Erythromycin and nicotinamide                 | N = 8                          | Good response in N = 3  
Partial / uncertain response in N = 5 |
| • IVIG                                           | N = 8                          | Good response in N = 2  
Partial/ uncertain response in N = 6 |
| • Rituximab                                      | N = 3                          | Good response N = 2  
Partial response N = 1.  
One sudden death in one of those two patients after three months (child had congenital immune deficiency). |
| • Omalizumab                                     | N = 1                          | Good response |

**IVIG:** Intravenous immunoglobulins.

---

### Table 4 Important Differential Diagnoses of Infantile BP

**Autoimmune blistering skin diseases**

- Linear IgA dermatosis
- Epidermolysis bullosa acquisita
- Epidermolysis bullosa
- Porphyria
- Bullous impetigo
- Pompholyx
- Bullous mastocytosis
- Insect bites
- Insect bite like reaction of hematologic malignancy

**Hereditary**

**Infectious**

**Others**

---
contrast, no case of infantile BP in relation with a malignant neoplasm has been reported. Furthermore, unlike in adult BP [70,71], drugs do not seem to play a major role in triggering infantile BP.

Within the age group of four weeks to 12 months, the clinical picture was moderately severe to severe (generalized) in over 80% of cases. Acral blistering was present in all children, while mucosal involvement was uncommon. In
localized disease, hands and feet were usually affected. There was no case of isolated genital infantile BP. Taken together, involvement of the hands and feet can be considered as a clinical hallmark and diagnostic clue of infantile BP. This is in contrast to childhood and adult BP [1,4,69]. Important differential diagnoses of infantile BP are listed in Table 4.

Most infants were doing well at the time of presentation despite some irritability, likely due to pruritus. However, individual children with significant morbidity including difficulty breathing and feeding, and weight loss, have been reported.

Even though initial presentation is often severe, the prognosis of infantile BP is excellent, with all but one patient reaching complete remission. That child had only been followed up short-term at the time of publication [8] and subsequent remission is possible. One infant passed away shortly after having been discharged from hospital. This child had received several doses of rituximab and had an underlying immune deficiency, which might have played a role.

The number of relapses was low. It seems that relapses can be triggered by infections or that they occurred in patients where tapering of corticosteroids was started early. Also, relapses were more frequent in patients who did not receive systemic corticosteroids (Additional file 3: Table S2). Once the disease has been controlled for several months, the likelihood of a relapse is extremely small.

**Treatment algorithm**

In contrast to adult BP, no treatment guidelines for infantile BP exist [15–18,72], and there has been little discussion on possible criteria for choosing the right treatment. After a comprehensive analysis of reported treatments in all published cases of infantile BP — together with lessons learned from our own cohort — we propose a first treatment algorithm. This step-by-step diagnostic and treatment algorithm takes into account disease severity, response to initial treatment and specific practical aspects of steroid sparing agents. It is based on general experience with the different medications in infants and the treatment recommendations published for adult BP (Figure 3).

After the diagnosis is established, all patients should receive treatment with mid- to high-potency topical corticosteroids. Children with moderately severe or severe disease (generalized, >10% BSA) usually require additional treatment with systemic corticosteroids. If the treatment response is slow or high doses of corticosteroids are needed for disease control, additional steroid sparing agents should be considered. Dapsone seems to be the agent of choice as it is usually well tolerated, effective, and is frequently used for other autoimmune blistering diseases of infancy and childhood, such as linear IgA dermatosis. Other steroid sparing agents used are IVIGs and MMF. Little or no experience exists for erythromycin-, methotrexate-, cyclophosphamide or azathioprine treatment in infants with BP. Rituximab is to be reserved as rescue treatment for the most severe cases [49,67]. The full potential and dosing of omalizumab in infantile BP warrant further investigation [56,73].

After clinical remission for several months, treatment discontinuation can be considered. In our experience ELISA autoantibody values can take a long time to normalize and are therefore not always helpful for deciding when to end treatment.

**Conclusions**

Infantile BP is considered a rare disorder; however an increasing number of reports during the last years show that it might have been under-recognized. As the disorder is not well known to general pediatricians and dermatologists, most infants are not promptly diagnosed and undergo multiple examinations before establishment of the correct diagnosis.

Infantile BP presents with urticarial plaques and blisters. Involvement of hands and feet is present in all cases. The clinical picture of infantile BP is characteristic. It is therefore a realistic aim to make the diagnosis early, avoid unnecessary diagnostic measures, and treat appropriately to avoid severe morbidity.

Pathogenesis and diagnostic criteria are comparable to adult BP; yet ELISA levels seem to be higher in infants. The overall disease outcome is favorable. Based on the results of this study we have established a first step-by-step diagnostic and treatment algorithm, taking into account disease severity, response to initial treatment and specific aspects of steroid sparing agents.

**Additional files**

**Additional file 1:** Table S1. All cases of infantile BP in the literature and this study.

**Additional file 2:** Figure S1. The number of published infantile BP cases has significantly increased since 2000.

**Additional file 3:** Table S2. Relapses of infantile BP.

**Abbreviations**

BP: Bullous pemphigoid; BP180: Bullous pemphigoid 180 kDa antigen/collagen XVII; BP230: Bullous pemphigoid 230 kDa antigen; BSA: Body surface area; DIF: Direct immunofluorescence microscopy; IIF: Indirect immunofluorescence microscopy; IVIG: Intravenous immunoglobulins; MMF: Mycophenolate mofetil.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

ASB and JSK conceptualized and designed the study, collected and analyzed the entire data, drafted the manuscript and approved the final manuscript as submitted. CM, HO, BM, FS and DK contributed clinical data, helped with data analysis and approved the final manuscript as submitted. ES and CS contributed diagnostic data, helped with data analysis and approved the final manuscript as submitted.
Case
Document
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et al.2:20-cv-02470-WBS-JDP
Orphanet Journal of Rare Diseases (2014) 9:185

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version of the manuscript.
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8 Filed 12/29/20 Page 255 ofPage
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11

Exhibit 212


Case 2:20-cv-02470-WBS-JDP - Document 8 - Filed 12/29/20 - Page 256 of 341
Postvaccination Bullous Pemphigoid in Infancy: Report of Three New Cases and Literature Review

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Abstract: Bullous pemphigoid (BP) is an acquired autoimmune blistering skin disorder characterized by tissue-bound and circulating immunoglobulin G (IgG) autoantibodies directed against BP antigens 180 and 230 of the hemidesmosomes. We present three infants who developed BP shortly after vaccination and review similar cases in the literature.

Bullous pemphigoid (BP) is an autoimmune blistering skin disorder characterized by tissue-bound and circulating immunoglobulin G (IgG) autoantibodies directed against BP antigens 180 and 230 of the hemidesmosomes. We present three infants who developed BP shortly after vaccination and review similar cases in the literature.

CASE REPORTS
Three previously healthy children, ages 4 to 5 months, developed a blistering eruption appearing 1 to 10 days after receiving vaccination against hepatitis B, diphtheria, pertussis, tetanus, poliomyelitis, Haemophilus influenzae B, and meningococcus C. Our cases further reinforce the causal association between childhood BP and vaccination.
infiltrate, and direct immunofluorescence of perilesional skin showed linear IgG and C3 deposits along the basement membrane (Fig. 2). Treatment with oral prednisone at a dose of 1.5 mg/kg/day was instituted for 2 to 3 months and the lesions resolved. New attacks of lesions were not seen after subsequent vaccinations in any case.

**DISCUSSION**

Bullous pemphigoid in children under 1 year of age usually shows predominantly acral involvement and spares the mucosa and genital area. Unlike in adults, childhood BP has an excellent prognosis and resolves rapidly after initiation of treatment. Therapy is based on systemic steroids at doses ranging from 1 to 2 mg/kg/day of prednisone (1). There have been some 100 reported cases of childhood BP (2), of which 18 cases have been related to vaccine administration, of which only 9 cases occurred in infants younger than 6 months of age (2–9) (Table 1). The latency period ranged between a few hours and 3 weeks. All cases had palmoplantar involvement, and mucosal membranes were spared in all but one case. The tetanus, diphtheria, pertussis, and polio vaccine was administered in all cases (2–10), although in some patients, vaccinations against pneumococcus (4–9), *H. influenzae* B (3–7, 9), hepatitis B (3, 5, 7), and meningococcus (5, 9) were also administered and cannot be excluded as triggering factors. Systemic steroid treatment was successful in all but one case, in which high-potency topical steroids achieved resolution. The outcome was favorable in all patients, with resolution of lesions in 2 to 6 months. Three cases showed recurrence with subsequent vaccinations, although less severe than the initial outbreak (3, 5, 7), so the authors did not consider it necessary to avoid subsequent vaccinations. The etiology is uncertain. Some authors have suggested that certain vaccines may unmask subclinical BP by inducing a nonspecific immune reactivation in genetically predisposed patients (11), whereas others hypothesize that intrauterine transmitted maternal IgG antibodies might play a role (4). In some cases reported in the literature, vaccination was not considered as a triggering factor, but the lesions appeared shortly thereafter (12, 13). Although the high rate of vaccinations in the first year of life contrasts with the low number of reported cases of BP after vaccination, making it difficult to explain a causal relationship, recurrences and onset of lesions a few hours after vaccination seem to reinforce the hypothesis of a causal association.

<table>
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<tr>
<th>Characteristic</th>
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<th>Vaccination</th>
<th><strong>Latency</strong></th>
<th><strong>Location</strong></th>
<th><strong>Treatment</strong></th>
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**TABLE 1. Reported Cases of Postvaccination Infantile Bullous Pemphigoid**
Postvaccination childhood BP is an idiopathic infantile disorder that appears after administration of the tetanus, diphtheria, pertussis, and polio vaccine alone or in combination with other vaccines, shows prominent palmoplantar involvement, and responds well to systemic steroid therapy. Although it does not occur in all patients, the possibility of recurrence with subsequent vaccinations must be taken into account.

REFERENCES

Figure 1. Clinical manifestations of patients 1 (A, B), 2 (C, D), and 3 (E, F). Multiple vesicles and tense bullae on the palms and soles and erythematous and edematous wheals on the trunk.

Figure 2. (A) Histologic findings in patient 2 showing edema and a conspicuous eosinophilic inflammatory infiltrate within the papillar dermis. (B) Subepidermal blistering and eosinophilic infiltrates in patient 1. (C) Direct immunofluorescence of perilesional skin shows linear deposits of IgG along the basal membrane in patient 3.
EXHIBIT 214
Three case reports of post immunization and post viral Bullous Pemphigoid: looking for the right trigger

Luca Baroero¹, Paola Coppo¹, Laura Bertolino², Stefano Maccario³ and Francesco Savino¹*

Abstract

Background: Bullous pemphigoid (BP) is a blistering skin disorder infrequent in infancy and rarely reported in medical literature.

Case Presentation: Here we describe three cases of BP which were referred to our department in the last 15 years. Two of them developed an eruption of bullous lesions just a few days after vaccination for diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B and Haemophilus influenzae B. The third patient developed the same blistering lesions shortly after herpetic stomatitis. In all three cases, clinical diagnosis was confirmed by histological examination which showed subepidermal bullae with a dermal inflammatory infiltrate, and direct immunofluorescence of perilesional skin showed linear IgG and C3 deposits along the basement membrane zone. Immunoblot assay was positive for BP antigen 180. Treatment with oral prednisone was instituted and the lesions resolved in two out of three patients; the third one was treated with an immunosuppressive agent (tacrolimus) and corticosteroid and subsequently with intravenous immunoglobulin and plasmapheresis, due to an underlying complex autoimmune disease.

Conclusion: Although the mechanism of induction of BP is still unclear, the close relationship between trigger events (immunization or viral infection) and onset of the disease arises a possible association.

Keywords: Case report, Infant, Bullous Pemphigoid, Drug therapy, Vaccination

Background

Bullous pemphigoid (BP) is an autoimmune blistering skin disorder associated with presence of tissue-bound and circulating IgG autoantibodies directed against hemidesmosomal proteins, called BP antigen 180 and BP antigen 230 [1]. Bullous pemphigoid usually affects the elderly and is rare in childhood and infancy. BP is diagnosed on the basis of clinical, histologic and immunologic findings [2, 3]. Among possible trigger factors of BP, immunization and viral infections are mentioned in literature. Some cases of BP have been reported soon after vaccine administration, although the immunological mechanism underneath is still unclear [4–6].

The clinical presentation of BP amongst children differs from that seen in adults, notably in terms of acral involvement with predominance of palmoplantar lesions, sparing the mucosa and genital area, in children aged less than 1 year. Unlike in adults, childhood BP has usually a good prognosis and resolves quite rapidly after initiation of treatment [7].

Although a clear trigger is not well established for BP, especially in infancy, a combination of multiple factors can be postulated. We present here 3 cases of children younger than 2 years who were referred to our Hospital in the last 15 years after developing BP related in time with a previous episode of vaccination or viral infection.

Case Presentation

A previously healthy 3-month-old boy was referred to our Hospital with a 15-day history of a blistering eruption on his hands and feet. He received a first dose of combined vaccination against diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B and Haemophilus influenzae B 2 days before the onset of the bullous rash. He had been previously treated at home with topical
gentamicin and oral co-amoxiclavulante, without resolu-
tion of the skin eruption. There was no relevant family
history for autoimmune or blistering disorders and no risk
factors during pregnancy or delivery had been identified.
Infant was breast-fed and growing normally. Clinically he
presented with blistering lesions with a prevailing acral
distribution: large vesicles and tense bullae with surround-
ing erythema were seen on the palms and soles, whereas
widespread smaller blisters on erythematous skin could be
noticed on the trunk and abdomen (Figs. 1 and 2). Mucous
membranes were not involved and other systems' exami-
nation was unremarkable. Observations were within
normal limits and the patient was afebrile. Results of
laboratory investigations showed that the patient had
a mild eosinophilia (1.47 × 10^9/L, 12% of WBC count);
inflammatory markers and complement components
were normal. Bacteriology analysis of the fluid inside
blisters revealed no infections and blood virological
tests were negative. A first biopsy for histologic study
was taken from a recent vesicular lesion and showed
subepidermal blister with a mixed superficial perivascular
inflammatory infiltrate with abundant eosinophils. A sec-
ond biopsy for direct immunofluorescence (DIF) was taken
from uninvolved perilesional skin: the results of DIF
showed linear deposition of immunoglobulin G (IgG, faint deposits) and complement component 3 (C3, int-
tense deposits) along the basement membrane zone
leading to the diagnosis of bullous pemphigoid (Fig. 3).
The immunoblot assay was positive for BP antigen 180.
Oral steroids have been started with prednisone at
1.5 mg/kg/day for ten days. Once the development of
blisters was stopped and erythema had subsided, a careful
tapering of prednisone was started, following an alternate
day scheme. Considering the severity of the disease and
the young age of infants, we started with a higher dose
than suggested in guidelines [2]. For the whole duration of
steroid treatment, the patient was subjected to a strict
follow-up: therapy was well tolerated, with no adverse
effects, as hypertension, weight gain, hyperglycemia or
other blood test alterations. Prednisone was carefully
tapered off over a 2-month period with no evidence of
disease relapse and currently the patient is still in re-
mission. Resumption of the vaccination schedule did
not induce any recurrence of the disease.
A 17-month-old girl with a history of eczema and
autoimmune enteropathy developed a blistering eruption
on her hands and feet a few days after the second dose
of hexavalent vaccination. Considering the autoimmune
disorder affecting her gut, on the recommendation of
gastroenterologists, she was treated with an immunosup-
pressive agent (tacrolimus) and corticosteroid; during a
suspension of therapy for remission of gastrointestinal
symptoms, she received the first dose of hexavalent vac-
cination at the age of 15 months with appearance of a
single blister on the back of one hand 5 days later. At the age of 17 months 7 days after the second dose of vaccination, she developed a bullous rash on the limbs which subsequently spread to the whole body. Two punch biopsies were taken, one for the histologic examination and the other for DIF, and they led to the diagnosis of bullous pemphigoid. The girl was treated with oral prednisone 1 mg/kg/day; as the lesions did not improve the dose was increased to 2 mg/kg/day but still without benefit. Subsequently she received intravenous Ig and finally plasmapheresis (5 sessions) with full recovery within 6 months. The patient developed over the years an IPEX-like syndrome caused by deficiency of CD25 (IL2-RA), characterized by immunodeficiency and autoimmunity, which was genetically confirmed. She recently underwent a bone marrow transplant with success.

A 2-month-old girl with unremarkable family history for bullous diseases, developed an eruption of bullous lesions, on an erythematous base, confluent located in both hands and feet, with palm and sole involvement, together with multiple ovaloid erythematous plaques, some with vesicles, on the abdomen. Ten days before she had been diagnosed with acute gingivostomatitis subsequently confirmed by PCR detection of HSV-1 DNA as herpetic stomatitis. At the beginning, to avoid bullous impetigo, the infant was managed with intravenous co-amoxiclavulanate. IgM antibody titer against HSV-1 was positive and suggestive of recent infection. A skin biopsy subsequently confirmed BP, showing subepidermal blisters. A second biopsy for direct immunofluorescence DIF showed linear deposits of IgG and C3 at the epidermal BMZ, confirming the diagnosis of bullous pemphigoid. Immunoblot assay was positive for BP antigen 180. The infant was managed with oral prednisone 1 mg/kg/day with rapid improvement, and she became free of blisters after 3 weeks of treatment. Follow-up to 6 months was good.

All parents of the three reported cases provided written informed consent to the inclusion of data concerning their infants in the manuscript in compliance with the Helsinki Declaration.

Discussion
The reported cases are presentations of bullous pemphigoid, the most prevalent autoimmune blistering skin disease, presenting with tense blisters on erythematous skin, predominantly affecting elderly people and unusual in infancy. Bullous pemphigoid is usually a self-limiting disease with a clinical course that may last from months to years in adults. In childhood and infancy BP usually responds well to conventional treatments, with a good prognosis [8].

The etiopathogenesis of bullous pemphigoid is complex and during recent years much has been postulated regarding the trigger factors related to the development of this condition, as immunizations and viral infections [9–11]. None of our patients had a suggestive family history for blistering skin disease and specifically their mothers did not develop gestational pemphigoid during pregnancy.

Here we report the case of two infants who developed an eruption of bullous lesions just a few days after vaccination against diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B and Haemophilus influenzae B, while the third patient after a viral infection by HSV-1. The latency period ranged between 2 and 10 days. A too short interval from immunization to onset of skin lesions could be considered an argument against the existence of a true relationship: since IgG production begins 10–14 days post-immunization, a 2–3 day latency period would generally be considered too short a time-frame for auto-immune manifestations characterized by IgG deposition to develop. Some authors anyway have suggested that certain vaccines may unmask subclinical BP by inducing a nonspecific immune reactivation in genetically predisposed infants more sensitive to the stimulus [8]. Others hypothesized that intrauterine transmitted maternal IgG antibodies might play a role [4] but a vertical transference of antibodies seems unlikely since in all the cases of PB reported in the literature where tests on mother’s serum were performed, circulating anti-BMZ antibodies were not found [11]. Moreover, according to recent studies, the trauma caused by the vaccine injection may led to Th17 cell activation with increased of IL-17 which is able to release pro-inflammatory cytokines and proteolytic enzymes, which may result in blister formation [12]. Finally, CD25 deficiency may be related to BP, since the lack of CD25+ cells has been observed in bullous pemphigoid lesions [13].

In literature some tens of cases of childhood BP have been reported, of which about 20 have been related to vaccine administration, but only a few occurred in infants younger than 6 months of age [6, 10, 14]. Anyway, the association of BP and vaccination could be entirely coincidental, given that vaccination in infants is a usual and daily practice in developed countries while cases of BP reported in infants are really limited. The high rate of vaccinations in the first year of life in contrast with the low number of reported cases of BP after vaccination makes it difficult to explain a causal relationship, even if described cases of recurrence after a new dose of vaccination seem to reinforce the hypothesis of a causal association [8].

Although the histopathological and immunological features of infantile BP are indistinguishable from those of childhood BP and adult BP, age-related differences in regional distribution of lesions were demonstrated. A recent study found that lesions are more likely located
on the extremities during the first year of life [15]. For this reason, the clinical presentation of infantile BP seems to differ from that of childhood and adult BP, which are characterized by tense blisters predominantly appearing along folds in the skin on the lower abdomen, groin, upper thighs and arms. In our cases there was no correspondence between the location of the vaccine administration and the site of occurrence of the first lesions.

The diagnosis of BP in our three cases has been confirmed with DIF studies on perilesional skin, which showed linear deposits of IgG and/or C3 at the epidermal BMZ [16]. To perform the DIF, frozen sections fixed in acetone at a temperature of 4 °C were incubated with Ig fluorescein isothiocyanate in humid chamber (IgA, IgM, C3 dilute 1/10; IgG 1/20), then rinsed in PBS and covered with anti-fade mounting medium.

Laboratory investigations are nonspecific, while histopathologic analysis shows sub-epidermal blisters. Diagnostic findings for BP are listed in Table 1. In our cases indirect immunofluorescence and detection of circulating autoantibodies against PB antigens were not performed.

Regarding differential diagnosis, BP should be differentiated from other subepidermal diseases: most of all DIF is useful in distinguishing BP from epidermolysis bullosa acquisita, mucous membrane pemphigoid and linear IgA disease. Bullous lesions may also be caused by insect bites, burns, cellulitis and contact dermatitis. Viral and bacterial skin infections should be recognized and treated before starting immunosuppressive therapy [7, 16].

Treatment with oral prednisone was instituted and the lesions rapidly resolved in two out of three patients, with suppression of inflammation and blistering typically achieved in a period of a few weeks, after which the dose was gradually reduced; the third one was treated with an immunosuppressive agent (Tacrolimus) and corticosteroid and subsequently with intravenous immunoglobulin (IVIg) [18]. Reports have also described successful therapy of BP patients with rituximab in treatment-refractory forms [19].

Since up to 40% of patients with BP on systemic corticosteroids develop severe infectious complications resulting in hospitalization or death [20] we administered a broad-spectrum antibiotic therapy to our 3 patients.

**Conclusion**

In this article we reported two infants who developed an eruption of bullous lesions just a few days after vaccination against diphtheria, tetanus, pertussis, poliomyelitis, hepatitis B and Haemophilus influenzae B, while the third patient showed the same lesions after a viral infection by HSV-1. Although the mechanism of induction is unclear, the close relationship between trigger events and onset of the disease suggests that there may be an association. Treatment with oral prednisone was effective in achieving disease control in two out of three patients; the third one was treated with a combination of systemic corticosteroids and Tacrolimus and subsequently with intravenous immunoglobulin and plasmapheresis, due to a complex underlying autoimmune disease [7].

According to a Cochrane review by Kirtschig et al. oral corticosteroid drugs are the most common treatment regimens and starting doses of prednisolone of 0.75 mg/kg/day or less seem to be adequate to control disease and reduce the incidence and severity of adverse reactions [17].

Other treatments with reported benefit are potent topical steroids, azathioprine, mycophenolate mofetil, dapsone, methotrexate, cyclosporin, cyclophosphamide, plasma exchange, as well as erythromycin and tetracycline as monotherapy or with rituximab and corticosteroids. Reports have also described successful therapy of BP patients with rituximab in treatment-refractory forms [19].

Since up to 40% of patients with BP on systemic corticosteroids develop severe infectious complications resulting in hospitalization or death [20] we administered a broad-spectrum antibiotic therapy to our 3 patients.

**Table 1** Diagnostic findings for BP

<table>
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<th>Clinic</th>
<th>Blistering lesions on erythematous skin, with a prevailing acral distribution</th>
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<td>Histology</td>
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<td>Direct immunofluorescence microscopy</td>
<td>Linear deposits of IgG and C3 along the basement membrane</td>
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<td>Indirect immunofluorescence microscopy on salt-split skin</td>
<td>BP antibodies deposited primarily at the epidermal side of the induced blister</td>
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<td>ELISA</td>
<td>Presence of circulating antibodies against the 2 BP antigens (BP180 and BP230)</td>
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**Abbreviations**

BMZ: Basement membrane zone; BP: Bullous pemphigoid; C3: Complement component 3; DIF: Direct immunofluorescence; HSV-1: Herpes simplex virus type 1; IgG: Immunoglobulin G; IgM: Immunoglobulin M; PCR: Polymerase chain reaction

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**Availability of data and materials**

Raw data from the study are available upon request from the corresponding author.
**Authors’ contributions**

L.B. conceptualized and designed the study, drafted the initial manuscript and references, and revised and approved the final manuscript as submitted. P.C. carried out the initial analyses, drafted the initial manuscript, reviewed and revised the manuscript, and approved the final manuscript as submitted. L.B. performed the analyses with direct immunofluorescence, reviewed and revised the manuscript, and approved the final manuscript as submitted. S.M. critically reviewed the manuscript, edited the article and approved the final manuscript as submitted. F.S. designed and coordinated the data collection, wrote the initial manuscript, critically reviewed the manuscript, and approved the final manuscript as submitted.

**Competing interests**

The authors have no conflicts of interest relevant to this article to disclose. The authors have no financial relationships relevant to this article to disclose.

**Consent to publication**

Written informed consent for the publication of their details and clinical images was obtained from the parents of the patients.

**Ethics approval and consent to participate**

The study was approved by the local ethics committee (Comitato Interaziendale AA.SS.DO. O.I.R.M./S. Anna - Ordine Mauriziano di Torino prot. N 632/2015) before inclusion in the manuscript. AA.SS.OO. O.I.R.M./S. Anna - Ordine Mauriziano di Torino prot. N 632/2015) before

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**References**


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Otitis media: Inflammation of the middle ear characterized by the accumulation of infected fluid in the middle ear, bulging of the eardrum, pain in the ear and, if eardrum is perforated, drainage of purulent material (pus) into the ear canal.

Otitis media is the most frequent diagnosis in sick children in the U.S., especially affecting infants and preschoolers. Almost all children have one or more bouts of otitis media before age 6.

The eustachian tube is shorter in children than adults which allows easy entry of bacteria and viruses into the middle ear, resulting in acute otitis media. Bacteria such as *Streptococcus pneumoniae* (*strep*) and *Haemophilus influenzae* (*H. flu*) account for about 85% of cases of acute otitis media and viruses the remaining 15%. Babies under 6 weeks of age tend to have infections from different bacteria in the middle ear.

Bottle-feeding is a possible risk factor for otitis media. Breastfeeding temporarily passes the mother’s immunity to the baby which helps prevent acute otitis media. It is alleged by some investigators that the position of the breastfeeding child may be better than the bottle-feeding position for eustachian tube function. However, this has not been proven. If a child needs to be bottle-fed, holding the infant rather than allowing the child to lie down with the bottle is thought by some to be better. A child should not take the bottle to bed because falling asleep with milk in the mouth increases the incidence of tooth decay.

Upper respiratory infections are a prominent risk factor for acute otitis media so exposure to groups of children as in child-care centers results in more frequent colds and therefore more ear. Irritants such as tobacco smoke in the air also increase the chance of otitis
media. Children with **cleft palate** or **Down syndrome** are predisposed to **ear infections**. Children who have acute otitis media before 6 months of age have more frequent later **ear infections**.

Young children with otitis media may be irritable, fussy, or have problems feeding or sleeping. Older children may complain about **pain** and fullness in the ear. **Fever** may be present in a child of any age. These symptoms are often associated with signs of **upper respiratory infection** such as a runny or stuffy nose or a **cough**.

The buildup of pus within the middle ear causes pain and dampens the vibrations of the eardrum (so there is usually transient **hearing loss** during the infection). Severe **ear infections** may cause the eardrum to rupture. The pus then drains from the middle ear into the ear canal. The hole in the eardrum from the rupture usually heals with medical treatment.

The treatment for acute otitis media is antibiotics usually for 7-10 days. About 10% of children do not respond within the first 48 hours of treatment. Even after antibiotic treatment, 40% of children are left with some fluid in the middle ear which can cause **temporary hearing loss** lasting for up to 3-6 weeks. In most children, the fluid eventually disappears (resorbs) spontaneously (on its own). Children who have recurring bouts of otitis media may have a tympanostomy tube (ear tube) placed into the ear during surgery to permit fluid to drain from the middle ear. If a child has a bulging eardrum and is experiencing severe pain, a myringotomy (surgical incision of the eardrum) to release the pus may be done. The eardrum usually heals within a week.

Acute otitis media is not **contagious** (although the **cold** that preceded it may be). A child with otitis media can travel by airplane but, if the eustachian tube is not functioning well, changes in pressure (such as in a plane) can cause discomfort. A child with a draining ear should, however, not fly (or swim).
What is an ear infection?

An ear infection is an inflammation of the middle ear, usually caused by bacteria, that occurs when fluid builds up behind the eardrum. Anyone can get an ear infection, but children get them more often than adults. Three out of four children will have at least one ear infection by their third birthday. In fact, ear infections are the most common reason parents bring their child to a doctor. The scientific name for an ear infection is otitis media (OM).

What are the symptoms of an ear infection?

There are three main types of ear infections. Each has a different combination of symptoms.

- **Acute otitis media** (AOM) is the most common ear infection. Parts of the middle ear are infected and swollen and fluid is trapped behind the eardrum. This causes pain in the ear—commonly called an earache. Your child might also have a fever.
- **Otitis media with effusion** (OME) sometimes happens after an ear infection has run its course and fluid stays trapped behind the eardrum. A child with OME may have no symptoms, but a doctor will be able to see the fluid behind the eardrum with a special instrument.
- **Chronic otitis media with effusion** (COME) happens when fluid remains in the middle ear for a long time or returns over and over again, even though there is no infection. COME makes it harder for children to fight new infections and also can affect their hearing.

How can I tell if my child has an ear infection?

Most ear infections happen to children before they’ve learned how to talk. If your child isn’t old enough to say “My ear hurts,” here are a few things to look for:

- Tugging or pulling at the ear(s)
- Fussiness and crying
- Trouble sleeping
- Fever (especially in infants and younger children)
- Fluid draining from the ear
- Clumsiness or problems with balance
- Trouble hearing or responding to quiet sounds
What causes an ear infection?

An ear infection usually is caused by bacteria and often begins after a child has a sore throat, cold, or other upper respiratory infection. If the upper respiratory infection is bacterial, these same bacteria may spread to the middle ear; if the upper respiratory infection is caused by a virus, such as a cold, bacteria may be drawn to the microbe-friendly environment and move into the middle ear as a secondary infection. Because of the infection, fluid builds up behind the eardrum.

The ear has three major parts: the outer ear, the middle ear, and the inner ear (see figure, page 1). The outer ear, also called the pinna, includes everything we see on the outside—the curved flap of the ear leading down to the earlobe—but it also includes the ear canal, which begins at the opening to the ear and extends to the eardrum. The eardrum is a membrane that separates the outer ear from the middle ear.

The middle ear—which is where ear infections occur—is located between the eardrum and the inner ear. Within the middle ear are three tiny bones called the malleus, incus, and stapes that transmit sound vibrations from the eardrum to the inner ear. The bones of the middle ear are surrounded by air.

The inner ear contains the labyrinth, which help us keep our balance. The cochlea, a part of the labyrinth, is a snail-shaped organ that converts sound vibrations from the middle ear into electrical signals. The auditory nerve carries these signals from the cochlea to the brain.

Other nearby parts of the ear also can be involved in ear infections.

The eustachian tube is a small passageway that connects the upper part of the throat to the middle ear. Its job is to supply fresh air to the middle ear, drain fluid, and keep air pressure at a steady level between the nose and the ear.

Adenoids are small pads of tissue located behind the back of the nose, above the throat, and near the eustachian tubes. Adenoids are mostly made up of immune system cells. They fight off infection by trapping bacteria that enter through the mouth.

Why are children more likely than adults to get ear infections?

There are several reasons why children are more likely than adults to get ear infections.

Eustachian tubes are smaller and more level in children than they are in adults. This makes it difficult for fluid to drain out of the ear, even under normal conditions. If the eustachian tubes are swollen or blocked with mucus due to a cold or other respiratory illness, fluid may not be able to drain.

A child’s immune system isn’t as effective as an adult’s because it’s still developing. This makes it harder for children to fight infections.

As part of the immune system, the adenoids respond to bacteria passing through the nose and mouth. Sometimes bacteria get trapped in the adenoids, causing a chronic infection that can then pass on to the eustachian tubes and the middle ear.

How does a doctor diagnose a middle ear infection?

The first thing a doctor will do is ask you about your child’s health. Has your child had a head cold or sore throat recently? Is he having trouble sleeping? Is she pulling at her ears? If an ear infection seems likely, the simplest way for a doctor to tell is to use a lighted instrument, called an otoscope, to look at the eardrum. A red, bulging eardrum indicates an infection.
A doctor also may use a pneumatic otoscope, which blows a puff of air into the ear canal, to check for fluid behind the eardrum. A normal eardrum will move back and forth more easily than an eardrum with fluid behind it.

Tympanometry, which uses sound tones and air pressure, is a diagnostic test a doctor might use if the diagnosis still isn’t clear. A tympanometer is a small, soft plug that contains a tiny microphone and speaker as well as a device that varies air pressure in the ear. It measures how flexible the eardrum is at different pressures.

How is an acute middle ear infection treated?

Many doctors will prescribe an antibiotic, such as amoxicillin, to be taken over seven to 10 days. Your doctor also may recommend over-the-counter pain relievers such as acetaminophen or ibuprofen, or eardrops, to help with fever and pain. (Because aspirin is considered a major preventable risk factor for Reye’s syndrome, a child who has a fever or other flu-like symptoms should not be given aspirin unless instructed to by your doctor.)

If your doctor isn’t able to make a definite diagnosis of OM and your child doesn’t have severe ear pain or a fever, your doctor might ask you to wait a day or two to see if the earache goes away. The American Academy of Pediatrics issued guidelines in 2013 that encourage doctors to observe and closely follow these children with ear infections that can’t be definitively diagnosed, especially those between the ages of 6 months to 2 years. If there’s no improvement within 48 to 72 hours from when symptoms began, the guidelines recommend doctors start antibiotic therapy. Sometimes ear pain isn’t caused by infection, and some ear infections may get better without antibiotics. Using antibiotics cautiously and with good reason helps prevent the development of bacteria that become resistant to antibiotics.

If your doctor prescribes an antibiotic, it’s important to make sure your child takes it exactly as prescribed and for the full amount of time. Even though your child may seem better in a few days, the infection still hasn’t completely cleared from the ear. Stopping the medicine too soon could allow the infection to come back. It’s also important to return for your child’s follow-up visit, so that the doctor can check if the infection is gone.

How long will it take my child to get better?

Your child should start feeling better within a few days after visiting the doctor. If it’s been several days and your child still seems sick, call your doctor. Your child might need a different antibiotic. Once the infection clears, fluid may still remain in the middle ear but usually disappears within three to six weeks.

What happens if my child keeps getting ear infections?

To keep a middle ear infection from coming back, it helps to limit some of the factors that might put your child at risk, such as not being around people who smoke and not going to bed with a bottle. In spite of these precautions, some children may continue to have middle ear infections, sometimes as many as five or six a year. Your doctor may want to wait for several months to see if things get better on their own but, if the infections keep coming back and antibiotics aren’t helping, many doctors will recommend a surgical procedure that places a small ventilation tube in the eardrum to improve air flow and prevent fluid backup in the middle ear. The most commonly used tubes stay in place for six to nine months and require follow-up visits until they fall out.

If placement of the tubes still doesn’t prevent infections, a doctor may consider removing the adenoids to prevent infection from spreading to the eustachian tubes.
Can ear infections be prevented?

Currently, the best way to prevent ear infections is to reduce the risk factors associated with them. Here are some things you might want to do to lower your child’s risk for ear infections.

• Vaccinate your child against the flu. Make sure your child gets the influenza, or flu, vaccine every year.

• It is recommended that you vaccinate your child with the 13-valent pneumococcal conjugate vaccine (PCV13). The PCV13 protects against more types of infection-causing bacteria than the previous vaccine, the PCV7. If your child already has begun PCV7 vaccination, consult your physician about how to transition to PCV13. The Centers for Disease Control and Prevention (CDC) recommends that children under age 2 be vaccinated, starting at 2 months of age. Studies have shown that vaccinated children get far fewer ear infections than children who aren’t vaccinated. The vaccine is strongly recommended for children in daycare.

• Wash hands frequently. Washing hands prevents the spread of germs and can help keep your child from catching a cold or the flu.

• Avoid exposing your baby to cigarette smoke. Studies have shown that babies who are around smokers have more ear infections.

• Never put your baby down for a nap, or for the night, with a bottle.

• Don’t allow sick children to spend time together. As much as possible, limit your child’s exposure to other children when your child or your child’s playmates are sick.

What research is being done on middle ear infections?

Researchers sponsored by the National Institute on Deafness and Other Communication Disorders (NIDCD) are exploring many areas to improve the prevention, diagnosis, and treatment of middle ear infections. For example, finding better ways to predict which children are at higher risk of developing an ear infection could lead to successful prevention tactics.

Another area that needs exploration is why some children have more ear infections than others. For example, Native American and Hispanic children have more infections than do children in other ethnic groups. What kinds of preventive measures could be taken to lower the risks?

Doctors also are beginning to learn more about what happens in the ears of children who have recurring ear infections. They have identified colonies of antibiotic-resistant bacteria, called biofilms, that are present in the middle ears of most children with chronic ear infections. Understanding how to attack and kill these biofilms would be one way to successfully treat chronic ear infections and avoid surgery.

Understanding the impact that ear infections have on a child’s speech and language development is another important area of study. Creating more accurate methods to diagnose middle ear infections would help doctors prescribe more targeted treatments. Researchers also are evaluating drugs currently being used to treat ear infections, and developing new, more effective and easier ways to administer medicines.

NIDCD-supported investigators continue to explore vaccines against some of the most common bacteria and viruses that cause middle ear infections, such as nontypeable Haemophilus influenzae (NTHi) and Moraxella catarrhalis. One team is conducting studies on a method for delivering a possible vaccine without using a needle.
**Where can I find additional information?**

The NIDCD maintains a directory of organizations that provide information on the normal and disordered processes of hearing, balance, taste, smell, voice, speech, and language. Please visit the NIDCD website at [http://www.nidcd.nih.gov](http://www.nidcd.nih.gov) for the directory.

Use the following keywords to help you search for organizations that can answer questions and provide printed or electronic information on ear infections:

- Otitis media (ear infection)
- Speech-language development
- Early identification of hearing loss in children

For more information, additional addresses and phone numbers, or a printed list of organizations, contact:

**NIDCD Information Clearinghouse**

1 Communication Avenue  
Bethesda, MD 20892-3456  
Toll-free Voice: (800) 241-1044  
Toll-free TTY: (800) 241-1055  
Fax: (301) 770-8977  
E-mail: nidcdinfo@nidcd.nih.gov
NIDCD supports and conducts research and research training on the normal and disordered processes of hearing, balance, taste, smell, voice, speech, and language and provides health information, based upon scientific discovery, to the public.

NIDCD Fact Sheet: Ear Infections in Children
NIH Publication No. 13-4799
Updated March 2013

For more information, contact:
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NIH...Turning Discovery Into Health
EXHIBIT 217
What is the incidence and prevalence of acute otitis media (AOM) in the US?

Updated: Sep 25, 2019
Author: John D Donaldson, MD, FRCSC, FACS; Chief Editor: Arlen D Meyers, MD, MBA  more...

References

ANSWER

In the United States, 70% of all children experience one or more attacks of AOM before their second birthday. A study from Pittsburgh that prospectively followed urban and rural children for the first 2 years of life determined that the incidence of middle ear effusion episodes is approximately 48% at age 6 months, 79% at age 1 year, and 91% at age 2 years. [16]

The peak incidence of AOM is in children aged 3-18 months. Some infants may experience their first attack shortly after birth and are considered otitis-prone (ie, at risk for recurrent otitis media). A study by Megged et al found that 30% of pediatric patients who had neonatal AOM suffered from recurrent AOM later in childhood, compared with 10% of controls. [17]

In the Pittsburgh study, the incidence of AOM was highest among poor urban children. Differences in incidence between nations are influenced by racial, socioeconomic, and climatic factors.
What is the incidence and prevalence of acute otitis media (AOM) in the US?

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EXHIBIT 218
Kids' Ear Infections Cost Healthcare System Nearly $3 Billion Annually

January 10, 2014

Acute otitis media, or ear infection, is the most common ailment among kids of preschool age and younger in the U.S., primarily because these children have immature middle-ear drainage systems, higher exposure to respiratory illnesses and undeveloped immune systems.

And because it's also the most common reason for antibiotic use among all children, the costs associated with acute otitis media (AOM) are under more scrutiny than ever by healthcare and government administrators, especially given today's political and economic climate, strained healthcare resources and cost-containment efforts.

While estimates of the economic impact of AOM have been formulated in the past, a new study by UCLA and Harvard University researchers is the first to use a national population database that gives a direct, head-to-head comparison of expenditures for pediatric patients diagnosed with ear infections and similar patients without ear infections.
The findings show that AOM is associated with significant increases in direct costs incurred by consumers and the healthcare system. With its high prevalence across the U.S., pediatric AOM accounts for approximately $2.88 billion in added healthcare expenses annually and is a significant healthcare utilization concern.

The research is published in the current edition of the journal The Laryngoscope.

"Although the annual incidence of ear infection may be declining in the U.S., the number of kids affected remains high, and the public health implications of AOM are substantial," says study co-author Dr. Nina Shapiro, director of pediatric otolaryngology at Mattel Children's Hospital UCLA and a professor of head and neck surgery at the David Geffen School of Medicine at UCLA. "As our healthcare system continues to be vigorously discussed around the nation, efforts to control costs and allocate resources appropriately are of prime importance."

For the study, the researchers examined records of pediatric patients under the age of 18 culled from the 2009 Medical Expenditure Panel Survey, a national survey conducted by the Agency for Health Research and Quality which serves a benchmark data-set specifically designed for the assessment of healthcare costs.

Of the 81.5 million children the researchers sampled, 8.7 million had received care for ear infections. The rates of visits to the doctor's office, refills of prescription medications and healthcare costs associated with doctor visits were then compared between those with diagnosed ear infections and those without. The rates were adjusted for age, sex, region, race, ethnicity, insurance status and co-morbidities.

The researchers found that children with ear infections had an average of two additional outpatient visits, 0.2 emergency visits and 1.6
prescriptions filled, compared with those without ear infections.

Ear infections were associated with an incremental increase of $314 per child annually for outpatient healthcare and an average of $17 in additional costs for medications. This resulted in an overall $2.88 billion annual cost for ear infections.

"Although certain immunizations that target infection-causing bacteria may play a role in slightly reducing the overall rate of ear infections, millions of young kids will still have them," Shapiro says. "The take-home message is that the common ear infection is an extremely costly entity with significant financial burdens on the healthcare system."

Future studies on the healthcare cost associated with AOM may include analyzing the indirect costs, such as work and school days missed, gasoline costs and parking charges for outpatient visits, the researchers say.

Study co-authors included Dr. Sameer Ahmed of UCLA and Dr. Neil Bhattacharyya of Harvard Medical School.
Immunologic dysfunction contributes to the otitis prone condition

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Antigen presenting cells

SUMMARY

Acute Otitis Media (AOM) is a multifactorial disease occurring mostly in young children who are immunologically naïve to AOM pathogens. This review focuses on work from Rochester NY, USA over the past 12 years among young children who had AOM infections microbiologically-confirmed by tympanocentesis, so called “stringently-defined”. Among stringently-defined otitis prone children deficiencies in fundamental immune defense mechanisms have been identified that contribute to the propensity of young children to experience recurrent AOM. Dysfunction in innate immune responses that cause an immunopathological impact in the nasopharynx have been discovered including inadequate proinflammatory cytokine response and poor epithelial cell repair. Adaptive immunity defects in B cell function and immunologic memory resulting in low levels of antibody to otopathogen-specific antigens allows repeated infections. CD4+ and CD8+ T cell function and memory defects significantly contribute. The immune profile of an otitis prone child resembles that of a neonate through the first year of life. Immunologic defects in otitis prone children cause them to be unusually vulnerable to viral upper respiratory infections and respond inadequately to routine pediatric vaccines.

Acute otitis media (AOM) is extremely common, drives antibiotic use, emergence of antibiotic resistant bacteria and is costly. Temporary hearing loss is the most common complication; rarely there are intracranial complications.1 The World Health Organization estimates that 51,000 deaths/year are attributable to AOM in children younger than 5 years old and that chronic otitis media (occurring in 65–330 million people) is the major cause of hearing loss in developing countries.2,3 Each episode of AOM is typically followed by 4–12 weeks of otitis media with effusion (OME) during which time the child has diminished hearing and this often leads to temporary delayed speech and language development and can be associated with permanent hearing loss.4–6 In the US alone, the economic burden of otitis media exceeds $6 billion/year in medical treatment, surgical management, and loss of income for working parents.7

For several decades the underlying pathogenesis of AOM in children has been attributed to Eustachian tube dysfunction as most important.8 Children demonstrate a diminished propensity to develop AOM over time and this change has long been thought due to the Eustachian tube anatomy of children transitioning to more “adult-like” by age three to five years old to account for the frequency of AOM infections being outgrown. Direct evidence for this explanation of otitis proneness (OP) is lacking in very young children since Eustachian tube functional testing has not been reported in children below age three years old.9,10 Evidence supporting the role of Eustachian tube dysfunction derives mostly from studies in older children and adults11 although persistent dysfunction occurs after children have outgrown their propensity for recurrent AOM12 which argues against a causative link. OM has been described to be a heritable condition based on a twin study13 and single nucleotide polymorphisms involving immune mediating cytokines have been described in OP children.14–17 However, a clinical condition occurring as frequently as OP likely has other underlying causes.

Our work over the past decade brings forward an immunologic explanation for OP susceptibility and our work in Rochester, NY USA is the focus of this review. In our studies, clinically diagnosed children with AOM and confirmed by tympanocentesis were defined as stringently OP (sOP) if they experienced 3 separate AOM infections within a 6 month or 4 AOM infections within a 12 month time span. Children experiencing fewer AOM infections, confirmed by tympanocentesis, were defined as non-otitis prone (NOP). Other groups studying immunological characteristics of OP children have most often found deficiencies8–21 but those studies did not restrict the definition of OP to cases where microbiologic confirmation occurred.1 It is probable that a requirement for microbiologic proof of AOM refines the study population to allow clearer outcome differences during immunologic studies. Indeed, with a strict definition, we have identified multiple deficiencies in innate and adaptive immunity among young children who are sOP and introduced the term “prolonged neonatal-like immune profile

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(PNIP) because of striking similarities in immune responses seen in sOP children that resemble neonatal immunity.\textsuperscript{22,23} Specifically, we observed that peripheral blood mononuclear cells (PBMCs) from sOP children between the ages of 6 and 12 months display a general skewing away from Th1 and Th17 immunity and toward Th2 and Treg dominance and a lack of enhanced inflammatory cytokine production by antigen presenting cells (APCs).\textsuperscript{24,25} We found that the immune problems of sOP children were largely outgrown by age three years old coinciding with the epidemiologic observation of diminishing AOM in that age time frame. In previous studies, sOP children were not diagnosed by otolaryngologists. The clinical diagnoses were made by referring primary care physicians. Unfortunately, if 50\% of the children entering those prior studies did not have recurrent AOM and were not authentic OP children then those previous studies may have missed the immune deficiencies we identified because their study populations were significantly contaminated by non-ottitis prone (NOP) children. The innovation of our work has been to apply stringent diagnostic criteria and microbiologic verification prospectively in a longitudinal study design that eliminates children who are misdiagnosed.

The significance of our investigations extends beyond application to OP children. We have shown that sOP children are prone to more frequent clinically-diagnosed, (a subset confirmed by virus-specific detection) viral upper respiratory infections\textsuperscript{26} and 23\% of sOP children respond with sub-protective antibody levels after routine pediatric vaccinations.\textsuperscript{22,27} In this review we describe the accumulated results from 2006–2018, of studies involving 796 children, from whom we have prospectively collected about 20,000 samples that included blood, peripheral blood mononuclear cells (PBMCs), nasal swabs, nasal washes and/or oropharyngeal swabs during 3837 healthy planned visits at ages 6, 9, 12, 15, 18, 24, and 30–36 months of age and 1276 AOM visits, all before child age 36 months. 75 (∼10\%) children met the sOP definition. Children that did not meet the sOP criteria we classify as NOP. There are many factors that may contribute to children with recurrent AOM\textsuperscript{28} but our work suggests that primary among those factors are immunologic deficiencies described here (Table 1 summarizes the findings).

Mucosal nasopharyngeal compartment (Fig. 1)

**Innate responses**

**Neutrophils**

Neutrophil activation and bacterial ingestion represent part of the innate immune response. In a study of NP samples, we found that the neutrophil count increased roughly 600-fold during AOM in sOP and NOP children.\textsuperscript{29} We further conducted studies of neutrophil recruitment and inflammation in the NP in children during health, viral URI and AOM in the presence or absence of NP colonization by Streptococcus pneumoniae (Spn).\textsuperscript{29} We found no evidence of dysfunctional neutrophil recruitment or bactericidal ROS production by neutrophils.\textsuperscript{30} We compared difference of gene expression of inflammatory effectors from neutrophils of sOP and NOP children during AOM (Fig. 1). Real-Time PCR (RT-PCR) was used to assess gene expression of inflammatory effectors. We found that sOP children had decreased mRNA expression of cytokine IL-1β from neutrophils.\textsuperscript{29}

**Epithelial and cytokines**

We studied the role of epithelial and innate mucosal responses to NP colonization in our sOP child population during a viral URI that proceeded to cause AOM due to Spn.\textsuperscript{30} We found significantly lower epidermal growth factor, epidermal growth factor receptor and angiogenin concentrations in the NP of sOP compared with NOP children suggesting lower capacity for epithelial repair in sOP children. We also found significantly lower proinflammatory neutrophil chemotactic receptors such as MIP-1β, IL-8 and CXCL5 (ENA-78) in the NP of sOP children at onset of AOM. These findings of lower epithelial repair and impaired proinflammatory response provide other mechanisms as to why sOP children succumb to repeated AOM events.

In a separate study, we investigated whether compartmental responses differed by comparing the cytokine levels in NP, MEF and peripheral blood of sOP and NOP children during an AOM event caused by Spn.\textsuperscript{31} Nasal washes from sOP children contained significantly higher levels of proinflammatory cytokine IL-2 but lower levels of IL-7 and insulin-like growth factor-4 (IGFBP-4), both involved in proliferation and T-cell homeostasis, but similar expression levels of IFN-γ and IL-17a, both of which are normally protective responses to an infection from innate and adaptive cells. We compared the RNA expression of IFN-γ, IL-2 and IL-17a from the MEF. We found significantly heightened transcription of IL-2 in sOP children and no significant difference in IFN-γ or IL-17a transcript levels. However, plasma IFN-γ and IL-2 levels were significantly lower in sOP children while IL-17a was too low to measure in both cohorts. These results show that the middle ear cytokine response mirrored those of the nasal mucosa versus the peripheral blood, suggesting that proximal mucosal sites may better predict the quality of the middle ear response than peripheral blood. Our results also highlight the differences between local and systemic immune responses that could co-ordinate anti-bacterial immune responses in young children. In addition, this data further highlights that the sOP child has immune deficits that fail to increase recall responses after additional exposure to opothogen upon additional infections.

In another study NOP children had significantly higher levels of IL-6, IL-10, INF-γ, TNF-α, IL-1β, MCP-1, RANTES, IL-2 and IL-17 during viral URIs versus AOMs following the URIs, when compared to sOP children.\textsuperscript{26} In that same study, we analyzed the relationship between the NP cytokine/chemokine level and SOP rate using a logistic regression analysis, generalized additive model, we found that sOP children had significantly lower nasal proinflammatory levels of IL-6, IL-10, TNF-α and RANTES than NOP children during viral URIs.

Taken together, sOP children have more frequent viral URIs than NOP children, due to deficient antiviral nasopharyngeal proinflammatory cytokine and chemokine responses, and this facilitates the development of AOMs. Our findings of lower epidermal repair processes and lower inflammatory response in the nasal mucosa of NOP versus NOP children provide another possible mechanism which might contribute to the predisposition of the sOP child to repeated AOM events.

We studied mucosal antibodies levels in the NP to Spn. Specifically, we investigated mucosal IgG and IgA levels in the NP and MEF from children to three Spn antigens (PhdT, PcPA and Ply).\textsuperscript{32} We showed that higher naturally acquired mucosal antibody levels to these antigens was associated with reduced AOM caused by Spn. We then sought to correlate the mucosal antibody levels in sOP children to those same pneumococcal proteins with Spn NP colonization and the occurrence of AOM.\textsuperscript{33} We found that sOP children had significantly higher colonization frequency by Spn (p < 0.0001) and significantly lower IgG and IgA levels to all 3 Spn proteins compared with NOP children except IgG to Ply D1. Spn colonization in NOP children led to 2-fold to 5-fold increase in mucosal IgG and IgA levels to all 3 proteins, whereas Spn colonization in sOP children generally failed to elicit antibody responses. Taken together, these data on mucosal antibody supports our hypothesis that sOP children have an immunological defect in responding to natural immunization by NP colonization and AOM.
Table 1
Immunologic Measures in sOP vs NOP Children.

<table>
<thead>
<tr>
<th>MUCOSAL (NP &amp; MEF)</th>
<th>sOP:NOP</th>
<th>Visit Type(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Innate Responses</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immune modulators-NP</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIP-1β</td>
<td>↓</td>
<td>AOM</td>
<td>30</td>
</tr>
<tr>
<td>CXCL5</td>
<td>↓</td>
<td>AOM</td>
<td>30</td>
</tr>
<tr>
<td>IL-8</td>
<td>↓, NSD</td>
<td>AOM</td>
<td>26,30</td>
</tr>
<tr>
<td>IL-6</td>
<td>↓</td>
<td>AOM, URI@Healthy</td>
<td>24,26,30</td>
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<tr>
<td>EGF</td>
<td>↓</td>
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<td>EGF-R</td>
<td>↓</td>
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<tr>
<td>Angiogenin</td>
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<td>AOM</td>
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</tr>
<tr>
<td>ICAM-1</td>
<td>↓</td>
<td>AOM</td>
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</tr>
<tr>
<td>IL-7</td>
<td>↓</td>
<td>AOM</td>
<td>31</td>
</tr>
<tr>
<td>IFGBP-4</td>
<td>↓</td>
<td>AOM</td>
<td>31</td>
</tr>
<tr>
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</tr>
<tr>
<td>TNFα, IL-6, IL-10, RANTES</td>
<td>↓</td>
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<tr>
<td>MCP-1</td>
<td>NSD</td>
<td>AOM</td>
<td>26,30</td>
</tr>
<tr>
<td>IL-2</td>
<td>↑</td>
<td>AOM</td>
<td>26,31</td>
</tr>
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<td>NSD</td>
<td>URI@Healthy</td>
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<tr>
<td>IL-17a</td>
<td>NSD</td>
<td>AOM, URI@Healthy</td>
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<td>IFN-γ</td>
<td>NSD</td>
<td>AOM, URI@Healthy</td>
<td>26,31</td>
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<tr>
<td>IL-1β, IL-8, MIP-1α</td>
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<td>URI@Healthy</td>
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<tr>
<td>TLR2/4 (RNA)</td>
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<tr>
<td>TLR2 (RNA) (Epithelials &amp; Neutrophils)</td>
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<td>ICAM-1</td>
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<td>AOM</td>
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<tr>
<td>Immune modulators-MEF</td>
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<tr>
<td>IL-2 (RNA)</td>
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<tr>
<td>IL-8 (RNA)</td>
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<tr>
<td>SLPI (RNA)</td>
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<td>AOM</td>
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<tr>
<td>MIP-1α (RNA)</td>
<td>↑</td>
<td>AOM</td>
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</tr>
<tr>
<td>RANTES (RNA)</td>
<td>↓</td>
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<tr>
<td>IFNα1 (RNA)</td>
<td>↓</td>
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<tr>
<td>IFNβ (RNA)</td>
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<td>MAPK8 (RNA)</td>
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<tr>
<td>TICAM2 (RNA)</td>
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<td>AOM</td>
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<tr>
<td>ZBP1 (RNA)</td>
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<td>AOM</td>
<td>67</td>
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<tr>
<td><strong>Adaptive Responses</strong></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Antibodies</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG to Spn &amp; Mcat Ags</td>
<td>↓</td>
<td>Healthy</td>
<td>33,68</td>
</tr>
<tr>
<td>IgA to Spn &amp; Mcat Ags</td>
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<td>Healthy</td>
<td>33,68</td>
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<td><strong>Colonization</strong></td>
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<tr>
<td>Spn, NTHi, Mcat</td>
<td>↑</td>
<td>Healthy, URI@Healthy, AOM</td>
<td>26,33,68</td>
</tr>
<tr>
<td><strong>Viral Infection</strong></td>
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<tr>
<td>RSV</td>
<td>↑</td>
<td>AOM</td>
<td>48</td>
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<tr>
<td>URIs</td>
<td>↑</td>
<td>Healthy</td>
<td>26</td>
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<tr>
<td><strong>BLOOD</strong></td>
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<tr>
<td>Baseline Responses</td>
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<tr>
<td>PBMC (unstimulated)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>IL-2</td>
<td>↓</td>
<td>AOM</td>
<td>31</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>↓</td>
<td>AOM</td>
<td>31</td>
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<tr>
<td>BAFF, APRIL</td>
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<tr>
<td>APCs (unstimulated)</td>
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<td></td>
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<tr>
<td>Total Monocytes &amp; cDCs</td>
<td>↑</td>
<td>Healthy</td>
<td>25</td>
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<tr>
<td>MHC II</td>
<td>↓</td>
<td>Healthy &amp; AOM</td>
<td>69</td>
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<tr>
<td>PBMC (R848 stimulated)</td>
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<tr>
<td>IL-1β, IL-6, IL-8, IL-10, IL-12, IFN-α, TNF-α, IFN-γ, CCL2, CCL4, CCL5, CXCL10</td>
<td>NSD</td>
<td>Healthy</td>
<td>25</td>
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<tr>
<td>APCs (R848 stimulated)</td>
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</tr>
<tr>
<td>Intracellular IL-12, TNF-α, IFN-α</td>
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<td>Healthy</td>
<td>25</td>
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<tr>
<td><strong>Innate &amp; Adaptive Responses</strong></td>
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<tr>
<td>PBMC (HK-Spn stimulated)</td>
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<td>IL-2, IL-4, IL-6, IL-10, IL-13</td>
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<tr>
<td>IFN-γ</td>
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<td>Healthy</td>
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(continued on next page)
Table 1 (continued)

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<thead>
<tr>
<th>MUCOSAL (NP &amp; MEF) sOP:NOP</th>
<th>Visit Type(s)</th>
<th>Ref</th>
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<tr>
<td>IL-17A</td>
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<td>IL-21</td>
<td>↓ Healthy</td>
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<td>IL-23</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>IL-2 (RNA)</td>
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<td>24</td>
</tr>
<tr>
<td>IL-13 (RNA)</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>IL-17A (RNA)</td>
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<td>24</td>
</tr>
<tr>
<td>IL-23 (RNA)</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>TGF-β (RNA)</td>
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<td>24</td>
</tr>
<tr>
<td>IFN-γ (RNA)</td>
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</tr>
<tr>
<td>TBR21 (RNA)</td>
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<td>24</td>
</tr>
<tr>
<td>RORC (RNA)</td>
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</tr>
<tr>
<td>FOXP3 (RNA)</td>
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**Adaptive Responses**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Visit Type(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG to Spn, NTHi, Mcat Ags</td>
<td>↓ Healthy &amp; AOM</td>
<td>34,40,41</td>
</tr>
<tr>
<td>Antibodies to pediatric vaccine Ags (DTaP, Hib, Polio, Spn PSs)</td>
<td>↓ Healthy</td>
<td>22,41,42</td>
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<td>IgG to RSV</td>
<td>↓ Healthy</td>
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<tr>
<th>B cells</th>
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<tr>
<td>Total B cells</td>
<td>NSD Healthy</td>
<td>41</td>
</tr>
<tr>
<td>BAFFR, TACI</td>
<td>↓ Healthy</td>
<td>42</td>
</tr>
<tr>
<td>BCMA</td>
<td>NSD Healthy</td>
<td>42</td>
</tr>
<tr>
<td>BAFFR, TACI, B7-1, B7-2 (RNAs)</td>
<td>↓ Healthy</td>
<td>42</td>
</tr>
<tr>
<td>BCMA, CD40L (RNAs)</td>
<td>NSD Healthy</td>
<td>42</td>
</tr>
<tr>
<td>%Memory B cells</td>
<td>↓ Healthy</td>
<td>41,42</td>
</tr>
<tr>
<td>%Memory B cells response to DTaP</td>
<td>↓ Healthy</td>
<td>41,42</td>
</tr>
<tr>
<td>%Switched memory</td>
<td>↓ Healthy</td>
<td>41,42</td>
</tr>
<tr>
<td>%Plasma cells</td>
<td>↓ Healthy</td>
<td>42</td>
</tr>
<tr>
<td>Spn Ag-specific ASCs</td>
<td>↓ Healthy</td>
<td>42</td>
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<table>
<thead>
<tr>
<th>CD4⁺ T cells</th>
<th>Visit Type(s)</th>
<th>Ref</th>
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<tbody>
<tr>
<td>Total CD4⁺ T-cells</td>
<td>NSD Healthy</td>
<td>41</td>
</tr>
<tr>
<td>Naive CD4⁺ T-cells</td>
<td>NSD Healthy</td>
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</tr>
<tr>
<td>Memory CD4⁺ T-cells</td>
<td>NSD Healthy</td>
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<table>
<thead>
<tr>
<th>% CD4⁺ T-cells (HK-Spn stimulated)</th>
<th>Visit Type(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>pSTAT3</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>pSTAT3 (+Th17 cytokines)</td>
<td>NSD Healthy</td>
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<table>
<thead>
<tr>
<th>IFN-γ</th>
<th>Visit Type(s)</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>IL-17A</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↓ Healthy</td>
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<table>
<thead>
<tr>
<th>%Naive CD4⁺ T-cells (HK-Spn stimulated)</th>
<th>Visit Type(s)</th>
<th>Ref</th>
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</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↓ Healthy</td>
<td>24</td>
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</table>

<table>
<thead>
<tr>
<th>%Memory CD4⁺ T-cells (HK-Spn stimulated)</th>
<th>Visit Type(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>IL-2</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>IL-17A</td>
<td>↓ Healthy</td>
<td>24</td>
</tr>
<tr>
<td>TNF-α</td>
<td>↓ Healthy</td>
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<table>
<thead>
<tr>
<th>%Memory CD4⁺ T-cells (Spn or NTHi antigen stimulated)</th>
<th>Visit Type(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ</td>
<td>↓ Healthy</td>
<td>41</td>
</tr>
<tr>
<td>IL-2</td>
<td>↓ Healthy</td>
<td>41</td>
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<tr>
<td>IL-4</td>
<td>↓ Healthy</td>
<td>41</td>
</tr>
<tr>
<td>IL-17A</td>
<td>↓ Healthy</td>
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<table>
<thead>
<tr>
<th>%Memory CD4⁺ T-cells (SEB stimulated)</th>
<th>Visit Type(s)</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFN-γ, IL-2, IL-4, IL-17a</td>
<td>NSD Healthy</td>
<td>43</td>
</tr>
</tbody>
</table>

NSD: No statistical difference detected.
AOM: Child with acute otitis media (AOM).
Healthy: Child at a normal healthy visit checkup.
URI@Healthy: Child came in during a normal healthy visit checkup but was diagnosed with an upper respiratory infection (URI).
NP: Nasopharyngeal.
MEF: Middle ear fluid.
PBMC: Peripheral blood mononuclear cells.
Spn: S. pneumoniae; HK-Spn: heat killed Spn; NTHi: non-typeable H. influenzae; Mcat: Moraxella catarrhalis.
↑ ↓: relative increase or decrease response in sOP versus NOP children.
Fig. 1. Nasopharynx and middle ear responses in sOP versus NOP children. Relative levels that show a statistical difference between sOP versus NOP children are depicted with up or down arrows. Modulator measurements are of proteins except those in brackets are of mRNA.

Systemic blood compartment (Fig. 2)

Professional antigen presenting cells (APCs)

APCs bridge the innate immune system to the adaptive immune system by facilitating presentation of antigens to B cells and T cells. Therefore we sought to determine whether there might be defects in numbers, phenotype and/or function of APCs in the peripheral blood of sOP infants. APC phenotypic counts, MHC II expression and intracellular cytokine levels were determined in response to TLR 7/8 stimulation using R848. Innate immune mRNA expression was measured using RT-PCR and cytokines were measured using Luminex technology. We found significantly higher numbers of monocytes among conventional DCs but not plasmacytoid DCs in sOP children even when healthy compared to NOP age-matched infants. The presence of increased numbers of monocytes and cDCs in the blood we hypothesized was consistent with the existence of a heightened pro-inflammatory state in sOP children at recurrent AOMs. However, we found that sOP and NOP infants produce similar levels of innate associated cytokines upon PBMC stimulation with a TLR agonist (TLR7/8). Baseline cytokine/chemokine levels, as well as expression levels of TLRs and intracellular signaling molecules from PBMCs in response to TLR stimulation were similar among sOP and NOP children, suggesting that sOP APC function might not be a major contributor to sOP immune hyposresponsiveness (Fig. 2).

Adaptive responses

Antibody

The three major otopathogens causing recurrent OM in our study children have been Spn, nontypeable H. influenzae (NTHi) and Moraxella catarrhalis (Mcat). We have shown that nasopharyngeal (NP) colonization is not only a necessary first step toward infection, it also is a natural immunizing event. We hypothesized that sOP children would have reduced levels of antibodies to the principal opopathogens resulting in there being less adaptive immunity to control the growth and eventual spread of the bacterium from the NP to the middle ear (ME). To test that hypothesis, we determined serum IgG titers against Spn proteins PhtD, PhtE, LytB, PcpA, PldD1 rather than serotype-specific capsule polysaccharides. We found that sOP children had significantly lower IgG titers to PhtD, PhtE, LytB, PldD1 than NOP children at healthy visits with asymptomatic NP colonization and at onset of AOM, supporting our hypothesis.

In a similar study, we investigated antibody levels to NTHI protein antigens (P6, O, OMP26). Antibody levels to the three antigens measured longitudinally during NP colonization between age 6 and 24 months showed >2-fold increases over time in sOP children compared to >4-fold increases in NOP children. Similar to our findings for protein vaccine candidates of Spn and NTHI, sOP children displayed a later and a significantly lower peak of serum IgG antibody rise than NOP children for Mcat protein antigens (OppA,
OMP CD, Hag5-9) during NP colonization of Mcat. However, at time of AOM caused by Mcat, only serum IgG antibodies to OppA or Hag5-9 were significantly higher for NOP compared to SOP children.

Although these studies do not inform regarding protective levels of antibody, the fact that SOP children have significantly lower adaptive antibody levels to Spn, NTHi and Mcat antigens from infections by these pathogens support the finding as to why they also have significantly higher colonization frequency by Spn, NTHi and Mcat than NOP children.

To extend the findings regarding antibody hyporesponsiveness, we sought to determine whether SOP children would also have lower antibody responses to pediatric vaccine immunizations. We analyzed sera collected from SOP and age-matched NOP children age 6–24 months for IgG concentrations to the DTaP antigens (diphtheria toxoid (DT), tetanus toxoid (TT), pertussis toxoid (PT), filamentous hemagglutinin (FHA), and pertactin (PRN)), polio, hepatitis B, H. influenzae type b capsule polysaccharide-ribitol-phosphate (PRP) and Spn capsular polysaccharide conjugate vaccine. SOP children were significantly more likely to have non-protective responses against DT, TT, hepatitis B, polio 3, and Spn 23F, but not polio 1 and 2, PRP, or Spn 6B and 14. Lower putative protective responses to PT, FHA, PRN pertussis antigens were also observed. A high percentage of these SOP children had non-protective antibody titers to the pediatric vaccines tested and sub-protective levels persisted until 24 months of age in many SOP children despite routine vaccination boosters. Currently these vulnerable SOP children do not show evidence of higher rates of vaccine-preventable infections. However, we speculated that they are protected by herd immunity and in the United States and other countries where parent refusal of vaccines has increased or immunizations are limited, herd immunity may become threatened.

Memory B cells

Knowing that a high percentage of SOP children develop lower levels of antibody to otopathogenproteins during natural exposure via the mucosal route, we sought to determine the percentages of memory B cells to Spn antigens compared to NOP children. We found that SOP children had significantly lower frequencies of antigen-specific memory B cells against 3 Spn protein antigens (PhtD, PhtE, and PhtY). Additionally, these frequencies correlated positively with serum IgG levels to the same antigens. Since SOP children failed to develop protective antibody levels to standard pediatric vaccines when given parenterally, we examined memory B cell responses to the DTaP vaccine antigens (DT, TT, and PT) in healthy SOP and NOP children. We found the frequency of total memory CD19+ CD27+ CD24− B cells was significantly lower in SOP children. Further, SOP children had significantly fewer memory B cells specific for DT, TT, and PT, and antigen-specific B cell frequencies correlated with serum IgG titers as in the earlier study.

We further analyzed specific aspects of the B cells in SOP children. We found fewer switched memory B-cells as measured by CD19, CD27, IgG and IgM surface markers. The B-cells of SOP children also showed reduced levels of expression of co-stimulatory molecules and TNF family receptors: transmembrane activator and
calcium modulating cyclophilin-ligand interactor (TACI), together with B cell maturation antigen (BCMA) and B cell activating factor receptor (BAFFR).

Overall, our data indicate that sOP children have reduced memory B-cells to otopathogen antigens (and vaccines), reduced switched memory B-cells with IgM and IgG receptors and poor expression of TNF family receptors compared to NOP children which may lead to failure in generating Spn and NTHi specific or vaccine antigen specific antibody generation.

**CD4 T cells**

We hypothesized that alterations in CD4 T-cell subsets in sOP children might also contribute to immune hyporesponsiveness, as compared to NOP children. Using 5 Spn and 3 NTHi protein antigens, we enumerated Spn- and NTHi- specific functional CD4 T-helper memory cell subsets in the peripheral blood of cohorts of sOP and NOP children with AOM or NP colonized with either Spn or NTHi. We found significantly reduced percentages of functional CD45RAlow memory CD4 T cells producing cytokines (IFN-γ, IL-2, IL-4 and IL-17A) in sOP children following AOM and NP colonization with either Spn or NTHi. Thus we showed that sOP children also have a diminished ability to generate memory T cell responses after NP colonization and AOM. These data had particular importance with regard to IL-17A since data from mouse NP colonization models suggest that protection against Spn carriage is dependent on IL-17 expressing CD4 T cells by a mechanism involving IL-17A increasing Spn killing by neutrophils. However, the percentage of functional memory CD4 T-cells was similar for sOP and NOP when stimulated with SEB ruling out an intrinsic defect in CD4 T-cells of sOP children.

**Peripheral blood mononuclear cell (PBMC) cytokine production and T cell skewing**

Proinflammatory cytokines are known to be critical for protective immunity against Spn infection. Therefore, we compared extracellular cytokine levels from PBMCs of sOP and NOP children in response to Spn stimulation. sOP children produced significantly lower levels of IFN-γ, IL-17A, IL-21 and IL-23 than NOP children. RNA analysis of Spn stimulated PBMCs also showed significantly lower expression levels of IFN-γ, IL-2, IL-13, IL-17A, IL-23 and TGF-β. Furthermore, Th1 and Th17 fate-determining transcription factors T-box transcription factor 21 (TBX21) and RAR-related orphan receptor C (RORC) showed significantly lower expression levels in samples from sOP children compared with NOP children while Foxp3 (Treg cell) expression was significantly higher from sOP children.

In a subsequent study, we heat killed Spn stimulation for in vitro stimulation of T cells. We found significantly diminished memory CD4 (CD69+CD45RA−) T-cells producing IL-2, TNF-α and IL-17 in sOP children. Addition of exogenous Th17-prompting cytokines (IL-6, −1β, −23 and TGF-β) restored CD4+ Th17 cell function in cells from sOP children to levels measured in NOP children. We also observed that both CD4 naïve (CD69+CD45RA+) and memory populations were significantly less activated in sOP children. In additional unpublished experiments we stimulated T-cells with TCR stimulating reagents mimicking DC activation (anti-CD3/CD28 Dynabeads), and found sOP children (age 6–9 months) had significantly impaired responses as measured by CD69 expression. Taken together our results indicate that sOP children generate fewer memory T cells and their T cells have deficiencies in their T cell receptors or T cell signaling after antigen priming.

**Viral URI co-pathogenesis with otopathogens**

A risk factor known to be associated with AOM is a preceding or concurrent viral upper respiratory tract infection (URI). In our studies, at onset of AOM, 93% of the children had clinical signs of a viral URI. We examined the differential impact of respiratory syncytial virus (RSV) and parainfluenza virus (PIV) URIs on the frequency of AOM caused by Spn and NTHi in sOP and NOP children as a potential mechanism to explain increased susceptibility to AOM. RSV was substantially more likely to contribute to AOM in sOP than in NOP children, and additionally sOP children were significantly more likely to be infected by RSV. This corresponded with significantly lower serum antibody titers against RSV in sOP children. PIV infections did not differentially affect AOM events in sOP and NOP children. To investigate the interface of a diminished neutralizing antibody response to virus and the correlative heightened viral replication we detected with neutrophil phagocytic function during AOM, an ex vivo phagocytic assay was developed. RSV impaired the phagocytic activity of neutrophils isolated from infected children significantly more than PIV. These data suggested that a failure to neutralize RSV could disrupt the capacity of neutrophils to engulf the bacterial otopathogen, facilitating the development of AOM. Taken together, the data show that lower innate and adaptive immune responses to RSV in sOP children allowed for viral interference with innate antibacterial immune responses, thus contributing to increased frequency of AOMs.

**Correlation between sOP and neonatal immunity**

The immune system of sOP children clearly differs in many ways from that of NOP children. A terminology of “prolonged neonatal-like immune profile” was proposed to provide context to the overarching theme of the immune differences in sOP vs. NOP children. The neonatal immune system is required to handle the abrupt transition to multiple, simultaneous antigenic stimulations from microbial colonization, exposure to food antigens, etc., so the responses must be muted through mechanisms of immune suppression. Thus the neonatal immune response tends to be anti-inflammatory rather than pro-inflammatory, resulting in predominance of anti-inflammatory innate cytokines. In neonates responses to TLR signals are dampened. Newborn APCs are relatively lower in number with low basal levels of MHC-II surface expression and a decreased ability to produce cytokines. Neonatal CD4+ T cells show preferential differentiation of Th2 cells over Th1 cells and higher numbers of T reg cells. Effects in plasma cell differentiation occur due to limited T cell help. Neonates produce lower levels of antibody following antigenic stimulation. The ability of B cells to proliferate and differentiate into plasma cells and memory cells influences the levels of antibody and recall responses on antigen re-exposure, respectively. Neonatal B cells have decreased TNF family receptors. T NF super family members expressed by B cells include BAFF and APRIL and 3 receptors: BAFFR, BCMA and TACI. Preterm newborn neonatal B cells show reduced proliferation in response to BAFF and anti-IgM and express less TACI, BCMA and BAFFR than adult B cells. Taken together, these multiple features of neonatal immunity that have been identified in the sOP child provide the foundation for the proposed prolonged neonatal-like immune profile moniker.

Another conceptualization of the sOP child immune system might focus on immunosuppression and a chronic hyper-inflammatory state in the nasopharynx due to recurrent viral URI and otopathogens colonization. Early in life, the NP of children is colonized with multiple bacterial species, including potential otopathogens that occupy the nasal epithelial surface. NP colonization is the initial, necessary step in the pathogenesis of AOM. During viral URI, the major bacterial otopathogens can disseminate to the middle ear. Despite similar clinical care and demographic factors, we have shown that sOP children are more susceptible to bacterial AOM during viral URI than NOP children. This might be viewed as a failure of commensal-immune equilibrium. Both hu-
man and mouse studies have demonstrated that colonization is an immunizing event, generating both B and T cell memory to the colonizing bacteria. Accumulating data suggests adaptive immunity to NP colonizers requires boosting from successive encounters to reach a threshold of protection.\textsuperscript{57} This may indeed be the reason for reduced colonization rates in healthy adults compared to children. Th1 and Th17 memory cells that promote neutrophilia are protective against pathogens, with an additional role played by antibodies raised against capsular and protein antigens.\textsuperscript{58-62} When high levels of anti-capsular antibodies are generated via vaccination (as is the case with pneumococcal conjugate vaccine), the B cell response alone is sufficient to prevent future colonization with the immunizing serotypes.\textsuperscript{63} Cross-serotype protection is the ideal immune response and has mostly been associated with T cell memory.\textsuperscript{60,61} Overall, these data suggest both B and T cell responses are important for protection, and development of immune memory over successive childhood infections plays an important role in future protection from both colonization and subsequent AOM infections. In addition, the epithelial surface is the physical surface which bacteria utilize to colonize the NP, often forming biofilms to occupy a niche in this space. Epithelial cells provide the physical barrier, via tight and adherens junctions, that prevents pathogens colonization. In addition, epithelial-derived cytokines and chemokines are central initiators and modulators of the local innate immune response.\textsuperscript{64} Therefore, in addition to pathogen-specific memory T and B cells, epithelial barrier and immune function are likely a critical factor in determining the establishment and density of NP bacterial colonization, a necessary step in AOM pathogenesis. Taken together, observations from SOP children demonstrate an immunosuppressed state with its downstream effects on adaptive immune responses/immune memory and inadequate innate immune responses in the NP along with poor NP epithelial cell repair.

Conclusions

All infants are born immunologically naïve to respiratory viruses and potential bacterial otopathogens. All have Eustachian tube anatomy that favors reflux of nasopharyngeal secretions containing viruses and bacteria into the middle ear to cause AOM. Environmental risk to experience viral URI and NP colonization by potential bacterial otopathogens can be delayed by avoidance of exposure to others who harbor and can transmit the organisms. However, infants and young children cannot avoid their parents or siblings and interactions with the wider family group and others in society eventually occurs. AOM infection risk may be mitigated somewhat by avoidance of day care and pacifier use and providing breast feeding.\textsuperscript{65} Nevertheless, when children are exposed to respiratory viruses leading to occasional or recurrent viral URI, a subset subsequently experience AOM and about 10% of children experience recurrent AOM if stringently diagnosed. These children account for approximately half of all AOM cases observed, suggesting a high impact for improved understanding of the immune mechanisms contributing to cause susceptibility in this population of children. Infection history of a child early in life is known to have profound influences on later immune development.\textsuperscript{66} We recently found that SOP children experience NP colonization by Spn, NTHi, and Mcat at a substantially greater rate and at earlier ages than NOP children (unpublished). However, the immune responses against these pathogens is reduced at 6 months of age before onset of AOM, with lower bacterial otopathogens-specific antibody titers and weaker responses by T and B cells upon stimulation with bacterial antigens in vitro, Thus, the events that predispose children to experience recurrent AOM may occur earlier than we have studied them. Microbiome modulation of the immune response commencing shortly after birth is currently under investigation by our group.

Funding

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Declaration of Competing Interest

None.

Acknowledgements


References


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42. Basha S, Pichicher ME. Decreased TNF family receptor expression on B-cells is associated with reduced humoral responses to Streptococcus pneumoniae infections in young children. Cell Immunol 2017;320:11–19.


EXHIBIT 220
Vaccine

Brucella abortus v. a modified live virus vaccine of Brucella abortus strain 19, used for immunization of healthy calves against brucellosis.

bursal disease v. a modified live virus vaccine of chick embryo origin, used for immunization of flocks against infectious bursal disease.

Calmette v. BCG v. canine distemper v. a modified live virus vaccine consisting of an attenuated strain of canine distemper virus propagated in tissue culture, used for immunization of dogs against canine distemper.

coccidiosis v. live sporulated oocysts of chicken origin, used to introduce subclinical coccidial infection in chickens in order to establish immunity against clinical infections.

conjugate v. a vaccine composed of an immunogenic polysaccharide conjugated with a protein carrier.

diphtheria and tetanus toxoids v. diphtheria and tetanus toxoids.

diphtheria and tetanus toxoids and acellular pertussis v. DTP vaccine, a combination of diphtheria toxoid, tetanus toxoid, and acellular pertussis vaccine; adsorbed on an aluminum-adsorbing agent. It is administered intramuscularly to children younger than seven years of age, for simultaneous immunization against diphtheria, tetanus, and pertussis.

diphtheria, tetanus, and tetanus toxoids v. DTP vaccine, a combination of diphtheria toxoid, tetanus toxoid, and tetanus toxoid and diphtheria toxoid, and acellular pertussis vaccine.

diphtheria and tetanus toxoids and pertussis v. DTP vaccine, a combination of diphtheria toxoid, tetanus toxoid, and whooping cough vaccine; administered intramuscularly for simultaneous immunization against diphtheria, tetanus, and pertussis. It is no longer used in the United States. See diphtheria and tetanus toxoids and acellular pertussis v. and tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis vaccine.

distemper v. canine distemper v. a modified live virus vaccine of chick embryo of tissue culture origin, used for prevention of canine distemper in mink.

duck virus enteritis v. a modified live virus vaccine of chick embryo origin, used for prevention of duck virus enteritis.

duck virus hepatitis v. a modified live virus vaccine of chick embryo origin, used for prevention of duck virus hepatitis.

duck, fowl v. a modified live virus vaccine of chicken embryo origin, used for immunization of chickens against Eastern and Western equine encephalomyelitides.

duck, peste des petits poissons v. a modified live virus vaccine of chicken embryo origin, used for immunization of ducks against equine influenza due to influenza virus A equine strains 1 and 2.

duck, peste des petits poissons v. a modified live virus vaccine of chicken embryo origin, used for immunization of ducks against equine influenza due to influenza virus A equine strains 1 and 2.

duck, peste des petits poissons v. a modified live virus vaccine of chicken embryo origin, used for immunization of ducks against equine influenza due to influenza virus A equine strains 1 and 2.

duck, peste des petits poissons v. a modified live virus vaccine of chicken embryo origin, used for immunization of ducks against equine influenza due to influenza virus A equine strains 1 and 2.

encephalomyelitis v. equine encephalomyelitis.

equine encephalomyelitis v. a lethal killed virus vaccine of chicken tissue culture origin, used for immunization of horses against Eastern and Western equine encephalomyelitides.

equine influenza v. a lethal killed virus vaccine of chicken embryo origin, used for immunization of horses against equine influenza due to influenza virus A equine strains 1 and 2.

equine influenza v. a lethal killed virus vaccine of chicken embryo origin, used for immunization of horses against equine influenza due to influenza virus A equine strains 1 and 2.

equine influenza v. a lethal killed virus vaccine of chicken embryo origin, used for immunization of horses against equine influenza due to influenza virus A equine strains 1 and 2.

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equine influenza v. a lethal killed virus vaccine of chicken embryo origin, used for immunization of horses against equine influenza due to influenza virus A equine strains 1 and 2.

equine influenza v. a lethal killed virus vaccine of chicken embryo origin, used for immunization of horses against equine influenza due to influenza virus A equine strains 1 and 2.
EXHIBIT 221
Definition of unvaccinated in English:

unvaccinated

Translate unvaccinated into Spanish
Pronunciation /ənˈvækənˌtid/ /ənˈvækənˌted/

ADJECTIVE

(of a person) not inoculated with a vaccine to provide immunity against a disease.

‘pockets of unvaccinated children’

+ More example sentences

Pronunciation /ənˈvækənˌtid/ /ənˈvækənˌted/
State and local school vaccination requirements exist to ensure that students are protected from vaccine-preventable diseases (1). This report summarizes vaccination coverage and exemption estimates collected by state and local immunization programs* for children in kindergarten (kindergartners) in 49 states and the District of Columbia (DC) and kindergartners provisionally enrolled (attending school without complete vaccination or exemption while completing a catch-up vaccination schedule) or in a grace period (a set interval during which a student may be enrolled and attend school without proof of complete vaccination or exemption) for 28 states. Median vaccination coverage† was 95.1% for the state-required number of doses of diphtheria and tetanus toxoids, and acellular pertussis vaccine (DTaP); 94.3% for 2 doses of measles, mumps, and rubella vaccine (MMR); and 93.8% for 2 doses of varicella vaccine. The median percentage of kindergartners with an exemption from at least one vaccine§ was 2.2%, and the median percentage provisionally enrolled or attending school during a grace period was 1.8%. Vaccination coverage among kindergartners remained high; however, schools can improve coverage by following up with students who are provisionally enrolled, in a grace period, or lacking complete documentation of required vaccinations.

Federally funded immunization programs collaborate with departments of education, school nurses, and other school personnel to assess vaccination coverage and exemption status of children enrolled in public and private kindergartens. In accordance with state and local school entry requirements, parents and guardians submit children’s vaccination records or exemption forms to schools, or schools obtain records from state immunization information systems. During the 2017–18 school year, 49 states and DC reported coverage for all state-required vaccines and exemption data among public school kindergartners; 48 states and DC reported on private school kindergartners.** Median vaccination coverage for the state-required number of doses of DTaP, 2 doses of MMR, and 2 doses of varicella vaccine are reported. Coverage with hepatitis B and poliovirus vaccines, which are required in most states but not included in this report, are presented on SchoolVaxView (2). Twenty-eight states reported data on kindergartners who, at the time of assessment, attended school under a grace period or provisional enrollment. Immunization programs in U.S. territories also receive public funding for immunization and report vaccination coverage and exemptions to CDC; however, national medians and summary measures reported here include only the U.S. states and DC.

Vaccination coverage and exemption estimates were adjusted according to survey type and response rates.†† During the 2017–18 school year, vaccination coverage data were reported for approximately 3,988,127 kindergartners, exemption data for approximately 3,634,631, and grace period and provisional

* Federally funded immunization programs are located in the 50 states and DC, five cities, and eight U.S. territories and freely associated states (territories). Two cities reported data to CDC, which were included in their state data to calculate medians. Immunization programs in U.S. territories reported vaccination coverage and exemptions to CDC; however, these data were not included in median calculations.

† Median vaccination coverage was determined using estimates for 49 states and DC. Wyoming did not report data because of problems with the quality of data reported by schools. Data from cities were included with their state data. Data from territories were not included in median calculation.

§ Median exemption rate was determined using estimates for 49 states and DC. Wyoming did not report data because of problems with the quality of data reported by schools; Colorado, Illinois, Minnesota, and Missouri were included in the tables and figure but excluded from the median exemption rate because they did not collect information on the number of kindergartners with an exemption. Data from cities were included with their state data. Data from territories were not included in median calculation.

¶ Assessment date varied by state and area. Seven states assess on the first day of school; 18 states assess by December 31; 12 states assess by some other date, ranging from 30 days after admission to March 5; 12 states and DC assess on a rolling basis.

** Six states reported coverage and exemption data for at least some homeschooled kindergartners. California included data for 18 independent study schools and eight virtual schools in public school data and data for homeschooled with six or more students in private school data. North Dakota reported some homeschool data separately. Oregon reported some homeschool data separately; children enrolled in public online homeschools were included in the public school data. Pennsylvania included all homeschooled students in their public school data. Utah included some homeschooled students in public and private school data. Vermont included homeschooled students in their public and private school data if the students were enrolled in one or more classes at a school; homeschooled children who were exclusively homeschooled were not subject to vaccination requirements and were not included in these estimates.

†† Most immunization programs that used census or voluntary response provided CDC with data aggregated at the state or local (city or territory) level. Coverage and exemption data based on a census or voluntary response were adjusted for nonresponse using the inverse of the response rate, stratified by school type (public, private, and homeschool, where available). Programs that used complex sample surveys provided CDC with deidentified data aggregated at the school or county level for weighted analysis. Weights were calculated to account for sample design and adjusted for nonresponse for data collected through complex sample design wherever possible.

Vaccination Coverage for Selected Vaccines and Exemption Rates Among Children in Kindergarten — United States, 2017–18 School Year

Jenelle L. Mellerson, MPH1,2; Choppell B. Maxwell, DrPH1,2; Cynthia L. Knighton2; Jennifer L. Kriss, PhD2; Ranee Seither, MPH2; Carla L. Black, PhD2
enrollment data for approximately 2,825,691.\textsuperscript{55} Potentially achievable coverage for MMR was calculated for each state as the percentage of students vaccinated with 2 doses of MMR plus the percentage without 2 doses of MMR and no documented vaccination exemption. Nonexempt students included those provisionally enrolled, in a grace period, or otherwise without documentation of vaccination.

During the 2017–18 school year, vaccination assessments varied by immunization program because of differences in states’ required vaccines and doses, vaccines assessed, assessment methods, and data reported. Among the 49 states and DC reporting kindergarten vaccination data, 36 used a census; nine used a sample; three used a voluntary school response; and two used a mix of sampling methods.\textsuperscript{56} All states used the same methods to collect both vaccination coverage and exemption data except Alaska, Kansas, Virginia, and Wisconsin, where a sample was used for vaccination coverage data and a census for exemption data. Kindergartners were considered up to date and included in the coverage estimate for a given vaccine if they received all doses required for school entry,\textsuperscript{***} except in seven states††† that considered kindergartners up to date only if they received all doses of all vaccines required for school entry. Reporting of varicella vaccination status among kindergartners with a history of varicella disease varied within and among states; some were reported as vaccinated against varicella and others as medically exempt.

Among the 49 states and DC included in this analysis, median 2-dose MMR coverage was 94.3\% (range = 81.3\% [DC] to 99.4\% [Mississippi]), 23 states reported coverage ≥95\%, and three states and DC reported coverage <90\% (Table 1). Median DTaP coverage was 95.1\% (range = 79.7\% [DC] to 99.4\% [Mississippi]), 25 states reported coverage ≥95\%, and three states and DC reported coverage <90\%. Among the 41 states and DC that required and reported 2 doses of varicella vaccine, median coverage was 93.8\% (range = 80.5\% [DC] to ≥99.4\% [Mississippi]), 17 states reported coverage ≥95\%, and four states and DC reported coverage <90\%.

The median percentage of kindergartners with an exemption from one or more required vaccines (not limited to MMR, DTaP, and varicella vaccines) was 2.2\% (range = 0.1\% [Mississippi] to 7.6\% [Oregon]), compared with 2.0\% during the 2016–17 school year (Table 2). The median percentage of medical exemptions was 0.2\% (range = <0.1\% [Hawaii] to 0.8\% [Alaska]); the median percentage of nonmedical exemptions was 2.0\% (range = <0.1\% [California] to 7.5\% [Oregon]). Among the 29 states and DC with an increase in exemptions in 2017–18, vaccination coverage was ≥95\% in 15 states for MMR, 16 states for DTaP, and 11 states for 2 doses of varicella.

The median reported percentage of kindergartners attending school during a grace period or provisionally enrolled was 1.8\% (range = 0.2\% [Georgia and Hawaii] to 8.5\% [Arkansas]) (Table 2). In 11 of 28 states reporting for the 2017–18 school year, the percentage of children provisionally enrolled or within a grace period at the time of the assessment exceeded the percentage of children with exemptions from ≥1 vaccines. Among the 26 states and DC with MMR coverage <95\%, 20 could potentially achieve ≥95\% coverage if all nonexempt students who were provisionally enrolled, in a grace period, or otherwise without evidence of complete vaccination were vaccinated (Figure).

### Discussion

During the 2017–18 school year, median kindergarten vaccination coverage was close to 95\% for MMR, DTaP, and varicella vaccine. The number of states with coverage ≥95\% increased from 20 to 23 (MMR), 23 to 25 (DTaP), and 15 to 17 (2 varicella vaccine doses) since the 2016–17 school year (2,3). Coverage increases in selected states might result from modifications to state programs. For example, Pennsylvania reduced its provisional enrollment period from 240 days to 5 days with a medical certificate indicating the scheduling of missing vaccine doses. The Indiana State Department of Health initiated report cards for schools displaying kindergarten vaccination coverage rates and built a bidirectional interface that increased the amount of data in their immunization information system. Kentucky removed the provider signature requirement when printing a certificate of immunization status, allowing school nurses to use the immunization information system certificate to document vaccination history. In Virginia, the number of local health departments participating in back-to-school immunization clinics for children entering school

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\textsuperscript{55} The kindergarten population is an approximation provided by each immunization program. The totals reported here are the summations of the kindergarten population among programs reporting data for coverage, exemptions, and grace periods or provisional enrollment. Data from cities and territories were not included in these totals.

\textsuperscript{56} States using a census attempted to collect data from all kindergartners at all schools and succeeded in collecting data for ≥90\% of kindergartners. The type of sample employed by the nine states using a sample to collect coverage data varied and included a stratified two-stage cluster sample (eight states) and a stratified one-stage cluster sample (one state). A voluntary response of schools was defined as a census survey with a response rate <90\% of the known population of kindergartners. A mix of methods included two or more described sampling methods (a census for one school type and voluntary response for the other).

\textsuperscript{***} All 49 reporting states and DC required 2 doses of a measles-containing vaccine. Local DTaP requirements varied. Nebraska required 3 doses, four states (Illinois, Maryland, Virginia, and Wisconsin) required 4 doses, and all other states required 5 doses, unless the fourth dose was administered on or after the fourth birthday. The reported coverage estimates represent the percentage of kindergartners with the state-required number of DTaP doses, except for Kentucky, which required 5 doses of DTaP by age 5 years, but reported 4-dose coverage for kindergartners. Nine states required 1 dose of varicella vaccine; 41 states and DC required 2 doses.

\textsuperscript{†††} Alabama, Florida, Georgia, Iowa, Mississippi, New Hampshire, and New Jersey considered kindergartners up to date only if they had received all doses of all vaccines required for school entry.
# TABLE 1. Estimated vaccination coverage* for MMR, DTaP, and varicella vaccines among children enrolled in kindergarten, by vaccine and immunization program — United States and territories, 2017–18 school year

<table>
<thead>
<tr>
<th>Immunization program</th>
<th>Kindergarten population†</th>
<th>No. (%) surveyed</th>
<th>Type of survey conducted§</th>
<th>Local data available online¶</th>
<th>MMR**</th>
<th>2 doses (%)</th>
<th>4 or 5 doses (%)</th>
<th>1 dose (%)</th>
<th>2 doses (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alabama</strong></td>
<td>57,245</td>
<td>57,245 (100.0)</td>
<td>Census</td>
<td>Yes</td>
<td>94.3</td>
<td>95.1</td>
<td>96.2</td>
<td>93.8</td>
<td></td>
</tr>
<tr>
<td><strong>Alaska</strong></td>
<td>9,692</td>
<td>707 (7.3)</td>
<td>Stratified 2-stage cluster sample</td>
<td>No</td>
<td>91.6</td>
<td>91.1</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Arizona</strong></td>
<td>81,710</td>
<td>81,710 (100.0)</td>
<td>Census</td>
<td>Yes</td>
<td>93.4</td>
<td>93.5</td>
<td>96.2</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td><strong>Arkansas</strong></td>
<td>39,630</td>
<td>38,242 (96.5)</td>
<td>Census (public), voluntary response (private)</td>
<td>No</td>
<td>91.9</td>
<td>91.3</td>
<td>NA</td>
<td>91.6</td>
<td></td>
</tr>
</tbody>
</table>

See table footnotes on next page
TABLE 1. (Continued) Estimated vaccination coverage* for MMR, DTaP, and varicella vaccines among children enrolled in kindergarten, by vaccine and immunization program — United States and territories, 2017–18 school year

<table>
<thead>
<tr>
<th>Immunization program</th>
<th>Kindergarten population†</th>
<th>No. (%) surveyed</th>
<th>Type of survey conducted§</th>
<th>Local data available online¶</th>
<th>MMR**</th>
<th>DTaP††</th>
<th>Varicella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 doses (%)</td>
<td>4 or 5 doses (%)</td>
<td>1 dose (%)</td>
</tr>
<tr>
<td>Utah††</td>
<td>48,827</td>
<td>48,827 (100.0)</td>
<td>Census</td>
<td>Yes</td>
<td>93.4</td>
<td>93.2</td>
<td>NA</td>
</tr>
<tr>
<td>Vermont††</td>
<td>6,255</td>
<td>6,255 (100.0)</td>
<td>Census</td>
<td>Yes</td>
<td>94.1</td>
<td>94.0</td>
<td>NA</td>
</tr>
<tr>
<td>Virginia†††</td>
<td>100,581</td>
<td>4,224 (4.2)</td>
<td>Stratified 2-stage cluster sample</td>
<td>Yes</td>
<td>95.5</td>
<td>98.2</td>
<td>NA</td>
</tr>
<tr>
<td>Washington***</td>
<td>85,118</td>
<td>79,977 (94.0)</td>
<td>Census</td>
<td>Yes</td>
<td>90.6</td>
<td>90.7</td>
<td>NA</td>
</tr>
<tr>
<td>West Virginia****</td>
<td>19,519</td>
<td>15,120 (77.5)</td>
<td>Voluntary response</td>
<td>No</td>
<td>98.4</td>
<td>98.0</td>
<td>NA</td>
</tr>
<tr>
<td>Wisconsin**,††††§§§</td>
<td>66,178</td>
<td>1,223 (1.8)</td>
<td>Stratified 2-stage cluster sample</td>
<td>No</td>
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<td>96.5</td>
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</tr>
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<td>Wyoming</td>
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<td>Not conducted</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
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<tr>
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</tr>
<tr>
<td>American Samoa**,****</td>
<td>758</td>
<td>758 (100.0)</td>
<td>Census</td>
<td>No</td>
<td>90.9</td>
<td>81.8</td>
<td>NReq</td>
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<tr>
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<td>Census</td>
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<td>75.8</td>
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<tr>
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<td>Stratified 2-stage cluster sample</td>
<td>No</td>
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<td>92.0</td>
<td>NReq</td>
</tr>
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<td>No</td>
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<tr>
<td>Palau¶¶,¶¶¶</td>
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<td>313 (100.0)</td>
<td>Census</td>
<td>No</td>
<td>100.0</td>
<td>100.0</td>
<td>NReq</td>
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<tr>
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<td>NA</td>
<td>Not conducted</td>
<td>No</td>
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<tr>
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<td>NA</td>
<td>Not conducted</td>
<td>No</td>
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</tbody>
</table>

Abbreviations: DTaP/DT = diphtheria and tetanus toxoids (DT) and acellular pertussis vaccine; MMR = measles, mumps, and rubella vaccine; NA = not available; NReq = not required for school entry.

* Estimates are adjusted for nonresponse and weighted for sampling where appropriate. Estimates based on a completed vaccine series (i.e., not vaccine-specific) use the “≥” symbol. Coverage might include history of disease or laboratory evidence of immunity.

† The kindergarten population is an approximation provided by each program.
‡ Sample designs varied by state or area: census = program attempted to include all schools (public and private) and all children within schools in the assessment and had a student response rate of ≥90%; 1-stage or 2-stage cluster sample = schools were randomly selected, and all children in the selected schools were assessed (1-stage), or a random sample of children within the schools was selected (2-stage); voluntary response = a census with a student response rate of <90% (does not imply that participation was optional).
§ Some programs publish kindergarten vaccination data online that are more detailed than the state-level estimates in this table. Examples of more detailed data include county, parish, school district, and school-level estimates.
** Most states require 2 doses of MMR; Alaska, New Jersey, and Oregon require 2 doses of measles, 1 dose of mumps, and 1 dose of rubella vaccines. Georgia, New York, New York City, North Carolina, and Virginia require 2 doses of measles and mumps and 1 dose of rubella vaccines. Iowa requires 2 doses of measles and 2 doses of rubella vaccines.
†† Pertussis vaccination coverage might include some diphtheria, tetanus toxoids, and pertussis vaccine (DTP) vaccinations if administered in another country or by a vaccination provider who continued to use DTP after 2000. Most states require 5 doses of DTP for school entry, or 4 doses if the fourth dose was received on or after the fourth birthday; Illinois, Maryland, Virginia, and Wisconsin require 4 doses; Nebraska requires 3 doses. The reported coverage estimates represent the percentage of kindergartners with the state-required number of DTP doses, except for Kentucky, which requires ≥5 but reports ≥4 doses of DTP.
§§ Medians calculated from data from 49 states and the District of Columbia (i.e., does not include Wyoming, Houston, New York City, American Samoa, Federated States of Micronesia, Guam, Marshall Islands, Northern Mariana Islands, Palau, Puerto Rico, or U.S. Virgin Islands). Coverage data were reported for 3,988,127 kindergartners.
¶¶ The percentage surveyed likely was <100%, but is reported as 100% based on incomplete information about the actual current enrollment.
††† Did not include some types of schools, such as online schools or those located on military bases or in correctional facilities.
††‡ Kindergarten vaccination coverage data were collected from a sample, and exemption data were collected from a census of kindergartners.
§§§ Counted some or all vaccine doses received regardless of Advisory Committee on Immunization Practices recommended age and time interval; vaccination coverage rates reported might be higher than those for valid doses.
¶¶¶ For Palau, estimates represent coverage among children in first grade.
†††† Reported public school data only.
††††† Puerto Rico and U.S. Virgin Islands did not report data for the 2017–18 school year because of widespread logistical issues caused by Hurricane Maria.

increased, with most local health departments following up with parents about missing vaccinations before the clinics (J Mellerson, CDC, unpublished data, 2018).

Although the overall percentage of children with an exemption was low, this was the third consecutive school year that a slight increase was observed (2). Reasons for the increase cannot be determined from the data reported to CDC but could include the ease of the procedure for obtaining exemptions (4) or parental vaccine hesitancy (5). Reported exemptions do not distinguish between exemptions for one vaccine versus all vaccines. Previous studies indicate that most children with exemptions have received at least some vaccines (6–8). Recent data from the National Immunization Survey indicate the percentage of children reaching age 2 years without having received any vaccinations has increased gradually, from 0.9% for children born in 2011 to 1.3% for children born in 2015.
TABLE 2. Estimated number and percentage* of children enrolled in kindergarten with reported type of exemption from vaccination, and grace period/provisional enrollment, by immunization program†—United States and territories, 2017–18 school year

<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Nonmedical exemptions</td>
<td>Any exemption</td>
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See table footnotes on next page
TABLE 2. (Continued) Estimated number and percentage* of children enrolled in kindergarten with reported type of exemption from vaccination, and grace period/provisional enrollment, by immunization program1 — United States and territories, 2017–18 school year

<table>
<thead>
<tr>
<th>Immunization program</th>
<th>Medical exemptions, no. (%)</th>
<th>Religious no.</th>
<th>Philosophical no.</th>
<th>Total no. (%)</th>
<th>2017–18, no.</th>
<th>2017–18 %</th>
<th>2016–17 %</th>
<th>Percentage point difference (2016–17 to 2017–18)</th>
<th>Grace period or provisional enrollment§§§ no. (%)</th>
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</thead>
<tbody>
<tr>
<td><strong>Territories and associated states</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>American Samoa</td>
<td>0 (0.0)</td>
<td>0</td>
<td>—**</td>
<td>0 (0.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>None</td>
</tr>
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<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
<tr>
<td>Guam</td>
<td>0 (&lt;0.1)</td>
<td>10</td>
<td>—**</td>
<td>10 (0.4)</td>
<td>10</td>
<td>0.4</td>
<td>0.2</td>
<td>0.2</td>
<td>NR</td>
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<tr>
<td>Marshall Islands</td>
<td>0 (0.0)</td>
<td>—†††</td>
<td>—**</td>
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<td>0 (0.0)</td>
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<td>0</td>
<td>0</td>
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<td>None</td>
</tr>
<tr>
<td>Palau†††</td>
<td>0 (0.0)</td>
<td>— §§</td>
<td>— §§</td>
<td>0 (0.0)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.0</td>
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<tr>
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<td>NA</td>
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<tr>
<td>U.S. Virgin Islands§§§</td>
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<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: NA = not available (i.e., not collected); None = state does not allow grace period or provisional enrollment; NR = not reported to CDC.
* Estimates are adjusted for nonresponse and weighted for sampling where appropriate.
† Medical exemptions, nonmedical exemptions, and grace period or provisional enrollment status might not be mutually exclusive. Some children might have both medical and nonmedical exemptions, and some enrolled under a grace period or provisional enrollment might be exempt from one or more vaccinations.
‡ A grace period is a set number of days during which a student can be enrolled and attend school without proof of complete vaccination or exemption. Provisional enrollment allows a student without complete vaccination or exemption to attend school while completing a catch-up vaccination schedule. In states with one or both of these policies, the estimates represent the number of kindergartners within a grace period, provisionally enrolled, or some combination of these categories.
§ Medians calculated from data for 45 states and District of Columbia; states excluded were Colorado, Illinois, Minnesota, Missouri, and Wyoming. Houston, New York City, American Samoa, Federated States of Micronesia, Guam, Marshall Islands, Northern Mariana Islands, Palau, Puerto Rico, and U.S. Virgin Islands also were excluded. Exemption data were reported for 3,634,631 kindergartners. Grace period or provisional enrollment median was calculated from data from 28 states; data were reported for 2,825,691 kindergartners.
** Philosophical exemptions were not allowed.
†† Medical exemptions were not allowed.
§§ Religious and philosophical exemptions were not reported separately.
§§§ Program did not report the number of children with exemptions, but instead reported the number of exemptions for each vaccine, which could count some children more than once. Lower bounds of the percentage of children with any exemptions estimated using the individual vaccines with the highest number of exemptions are for Colorado, 0.2% with medical exemptions, 0.3% with religious exemptions, 4.2% with philosophical exemptions, and 4.7% with any exemptions; for Illinois, 0.2% with medical exemptions, 1.4% with religious exemptions, and 1.6% with any exemptions; for Minnesota, 0.2% with medical exemptions, 0.3% with religious exemptions, 4.2% with philosophical exemptions, and 4.7% with any exemptions; for Missouri, 0.2% with medical exemptions, and 3.5% with any exemptions; and for Puerto Rico, 0.2% with medical exemptions, 2.1% with religious exemptions, and 2.3% with any exemptions.
§§§§ For Palau, estimates represent exemptions among children in first grade.
§§§§§ Puerto Rico and U.S. Virgin Islands did not report data for the 2017–18 school year because of widespread logistical issues caused by Hurricane Maria.

(9) Two of the 10 states with <90% coverage for ≥1 dose of MMR among children aged 19–35 months in the 2014 National Immunization Survey (10) (the approximate cohort of children entering kindergarten in the 2017–18 school year) also had <90% coverage for ≥2 doses of MMR among kindergartners in 2017–18; in eight states, coverage with ≥2 doses of MMR was <95%, indicating that some children who were undervaccinated in early childhood do not catch up before kindergarten entry. This highlights the importance of school entry vaccination requirements to ensure catch-up vaccination of unvaccinated and undervaccinated children.

In 11 of the 28 states reporting 2017–18 grace period or provisional enrollment data, the percentage of kindergartners in these groups at the time of assessment exceeded the percentage with an exemption from one or more vaccines, representing a group of children who could be fully vaccinated with appropriate follow-up. CDC encourages programs to collect and use these data to identify populations of undervaccinated students. Almost all states could achieve ≥95% vaccination coverage if undervaccinated nonexempt children were vaccinated in accordance with local and state vaccination policies.

The findings in this report are subject to at least five limitations. First, comparability is limited because of variation in states’ requirements, data collection methods, and definitions of grace period and provisional enrollment. Second, representativeness might be negatively affected because of data collection methodologies that miss some schools or students or assess vaccination status at different times. Third, actual vaccination coverage, exemption rates, or both might be underestimated or overestimated because of inaccurate or absent documentation. Fourth, median coverage estimates include only 49 of 50 states and DC, median exemption estimates include only 45 states and DC, and the median grace period or provisional enrollment estimate includes only 28 states for the 2017–18 school year.
Finally, because most states do not report vaccine-specific exemptions, estimates of potentially achievable MMR coverage are approximations. However, if reported exemptions were for a vaccine or vaccines other than MMR, estimates of potentially achievable MMR coverage would be higher than those presented.

Kindergarten vaccination requirements help ensure that students are fully vaccinated with age-appropriate vaccines upon school entry. Although overall vaccination coverage is high, coverage could be improved in many states. CDC works with immunization programs to collect and report data on school vaccination coverage, exemption rates, and grace period and provisional enrollment each year. Immunization programs can use these data to understand and address undervaccination among kindergartners and to identify schools and communities where focused interventions could improve coverage with required vaccines.

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1Certified Technical Experts Inc., Montgomery, Alabama; 2National Center for Immunization and Respiratory Disease, Immunization Services Division, CDC.

All authors have completed and submitted the ICMJE form for disclosure of potential conflicts of interest. No potential conflicts of interest were disclosed.

References


Summary

What is already known about this topic?

Immunization programs conduct annual kindergarten vaccination assessments to monitor school-entry vaccination coverage for all state-required vaccines.

What is added by this report?

Median vaccination coverage was 94.3% for 2 doses of measles, mumps, and rubella vaccine; 95.1% for the state-required number of doses of diphtheria and tetanus toxoids and acellular pertussis vaccine; and 93.8% for 2 doses of varicella vaccine. Although the median exemption rate gradually increased for the third year in a row to 2.2%, most undervaccinated children did not have exemptions.

What are the implications for public health practice?

School assessment allows immunization programs to target interventions to schools with undervaccinated kindergartners to increase compliance with state and local vaccination requirements.

** Figure includes all states with reported MMR coverage for the 2017–18 school year of <95%, the Healthy People 2020 target for MMR vaccination coverage among kindergartners. http://www.healthypeople.gov.

Exhibit 222


ABSTRACT. Context. Each year 2.1 million children 19 to 35 months of age are undervaccinated. Among these are children who have received no vaccinations. Undervaccinated children are at increased risk of acquiring and transmitting vaccine-preventable diseases.

Objectives. To assess whether the characteristics of children with no vaccinations differ from those of undervaccinated children, to monitor trends in the numbers of unvaccinated children, and to identify states with high rates and counties with large numbers of unvaccinated children.

Design. A nationally representative probability sample of children 19 to 35 months of age was collected annually between 1995 and 2001. Vaccination histories were ascertained from children's medical providers. Undervaccinated children had received ≥1 dose of diphtheria-tetanus-pertussis, polio, measles, Haemophilus influenzae type b, hepatitis B, or varicella vaccine but were not fully vaccinated. Undervaccinated children were children who were reported as having no medical providers and having received no vaccinations or children whose medical providers reported administering no vaccinations.

Participants. A total of 151,720 children sampled between 1995 and 2001, 795 of whom were unvaccinated.

Results. Undervaccinated children tended to be black, to have a younger mother who was not married and did not have a college degree, to live in a household near the poverty level, and to live in a central city. Undervaccinated children tended to be white, to have a mother who was married and had a college degree, to live in a household with an annual income exceeding $75,000, and to have parents who expressed concerns regarding the safety of vaccines and indicated that medical doctors have little influence over vaccination decisions for their children. Undervaccinated children were more likely to be female than male. Annually, ~17,000 children were unvaccinated. The largest numbers of unvaccinated children lived in counties in California, Illinois, New York, Washington, Pennsylvania, Texas, Oklahoma, Colorado, Utah, and Michigan. States that allowed philosophical exemptions to laws mandating vaccinations for children had higher rates of unvaccinated children among racial/ethnic minorities living in those neighborhoods, reinforcing the assumption that children with no vaccinations have characteristics that are similar to those of undervaccinated children.

Conclusions. Undervaccinated children have characteristics that are distinctly different from those of undervaccinated children. Undervaccinated children are clustered geographically, increasing the risk of transmitting vaccine-preventable diseases to both unvaccinated and undervaccinated children. Pediatrics 2004;114:187–195; excerpt, undervaccinated, unvaccinated.

ABBREVIATIONS. CI, confidence interval; NIS, National Immunization Survey; PKAM, Parental Knowledge and Attitudes topical module; RDD, random-digit dialing; UTD, up-to-date; NUTD, not up-to-date; VPD, vaccine-preventable disease; MSA, metropolitan statistical area.

Children who have received no vaccinations have not been well studied in the United States. Many studies have assumed that children with no vaccinations are similar to undervaccinated children and thus have included children with no vaccinations among the undervaccinated, to investigate factors associated with being undervaccinated. Those studies have shown that being undervaccinated is significantly associated with belonging to a racial/ethnic minority, having a mother with low educational status, and belonging to a household that is living in poverty. A few studies conducted in inner-city locations have found high rates of unvaccinated children among racial/ethnic minorities living in those neighborhoods, reinforcing the assumption that children with no vaccinations have characteristics that are similar to those of undervaccinated children.

In 2003, reports from state and local health departments again illustrated the role that children with no vaccinations can play in vaccine-preventable disease (VPD) outbreaks. In Westchester County, New York, the county health commissioner said that an outbreak of pertussis started with children who were not vaccinated because their parents had decided against it. That outbreak subsequently spread into adjoining Putnam County. By the end of October 2003, 25 cases of pertussis were confirmed in Putnam County.

One purpose of this article is to determine the characteristics that distinguish unvaccinated children from undervaccinated children. This information is important in designing interventions that are tailored for differences between undervaccinated children. Specifically, we identify demographic and socioeconomic differences between these groups, as well as differences regarding safety concerns and people who are important in influencing parents' decision on whether to vaccinate their children. Also, we describe trends in the numbers of unvaccinated children between 1995 and 2001 and provide state esti-
mutes of the numbers of unvaccinated children per 100 000 children. Finally, because it is sensitive to target interventions in geographic areas where the largest numbers of unvaccinated children live, we present estimates of the numbers of children with no vaccinations in 50 counties in the United States with the largest communities of unvaccinated children.

METHODS

In our analyses, we use data from the National Immunization Survey (NIS), a survey of children 19 to 35 months of age. Each year, the NIS obtains an independent representative sample in 78 Immunization Action Plan areas. These areas include the 50 states, the District of Columbia, and 27 other large metropolitan statistical areas (MSAs). The NIS includes 2 phases of data collection. In the first phase, households with ≥1 age-eligible children are identified by using list-assisted, random-digit dialing (RDD). Cellular telephone numbers are excluded from the sampling frame. When a household with an age-eligible child is identified, the RDD interview is conducted to collect demographic information about each age-eligible child in the household, demographic and socioeconomic information about the age-eligible child’s mother, and sociodemographic information about the household. Also, information is obtained from the RDD respondent regarding the child’s vaccination history. At the end of the NIS RDD interview, consent is asked to contact the age-eligible children’s medical care providers. If consent is given, then the second data collection phase of the NIS is conducted. In the second phase, all of the providers named by the NIS RDD respondent are contacted by mail, to obtain the household’s age-eligible children’s provider-reported vaccination histories. Data from the provider-reported vaccination histories are used to evaluate sampled children’s vaccination status, ie, whether they had received all doses of recommended vaccines, and to estimate vaccination coverage rates. Zell et al and Smith et al provide a more detailed overview of the design and methods used in the NIS, and Frankel et al and Smith et al describe the statistical adjustment to the NIS sampling weights that account for households that do not have telephones.

Table 1 lists the 6 vaccines and the number of doses for each vaccine that were recommended for birth cohorts sampled by the 2001 NIS. For each of these vaccines, children were considered to be up-to-date (UTD) on a vaccine if their vaccination providers reported administering at least the recommended number for the vaccine; they were otherwise considered not up-to-date (NUTD). In our analyses, the vaccination status of a child was designated “fully vaccinated” if the vaccination providers reported administering at least the recommended number for all 6 vaccines, “undervaccinated” if the child was NUTD on ≥1 vaccine but had received ≥1 dose of any of the 6 recommended vaccines, or “unvaccinated” if the NIS RDD respondent reported that the child had received no vaccinations and the child had no vaccination providers or all providers identified by the household reported administering no vaccinations to the child.

To evaluate the associations of vaccination status with child, maternal, and household sociodemographic characteristics, we compared the distribution of each characteristic across vaccination status levels, using data from the 2001 NIS. To evaluate how the distributions of fully vaccinated children differed from those of undervaccinated children, we used logistic regression. To evaluate how the distributions for unvaccinated children differed from those for undervaccinated children, we subdivided undervaccinated children according to their degree of undervaccination, ie, whether they were UTD on ≥6 vaccines but NUTD on ≥1 vaccine, or NUTD on ≥1 vaccine but had received ≥1 dose of any of the 6 vaccines, or “unvaccinated” if the NIS RDD respondent reported that the child had received no vaccinations and the child had no vaccination providers or all providers identified by the household reported administering no vaccinations to the child.

RESULTS

Rates of Vaccination

In 2001, an estimated 62.8% of all children 19 to 35 months of age in the United States were fully vaccinated with ≥4 doses of diphtheria, tetanus, and acellular pertussis vaccine, ≥3 doses of polio vaccine, ≥1 dose of measles, mumps, and rubella vaccine, ≥3 doses of Haemophilus influenzae type b vaccine, ≥3 doses of hepatitis B vaccine, and ≥1 dose of varicella vaccine (Table 2). Among all children 19 to 35 months of age, an estimated 36.9% were undervaccinated. In the undervaccinated group, children were most frequently NUTD on varicella vaccine (23.5%), diphtheria, tetanus, and acellular pertussis vaccine (18.2%), hepatitis B vaccine (11.2%), and polio vaccine (11.0%). An estimated 0.3% of all children 19 to 35 months of age were unvaccinated.

Factors That Distinguish Fully Vaccinated Children From Undervaccinated Children (2001 NIS)

Table 3 gives the results of the logistic regression model analysis for evaluating factors associated with being undervaccinated, compared with fully vaccinated. These analyses indicated that, compared with fully vaccinated children, undervaccinated children were significantly more likely to be black than Hispanic or non-Hispanic white; younger; or foreign-born than born in the United States. Undervaccinated children were significantly more likely to have a mother who was young; widowed, divorced, or separated than married; had educational attainment that was high school or less than a college degree; and whose preferred language was English than

| TABLE 1. Recommended Vaccines for the Birth Cohorts Sampled by the 2001 NIS |
|-----------------------------|-----------------------|
| Vaccine                     | No. of Recommended Doses |
| Diphtheria-tetanus-acellular pertussis | 4 |
| Polio                       | 3 |
| Measles-mumps-rubella       | 1 |
| Haemophilus influenzae type b | 3 |
| Hepatitis b                 | 3 |
| Varicella                   | 1 |
Spanish. Finally, undervaccinated children were significantly more likely to live in a household with an annual income below the poverty level than in a household with an annual income greater than $75,000; in a household with ≥4 children than in a household in which he/she was the only child; in a household that had moved across state lines since the child’s birth than in a household that did not move across state lines; or to live in a non-MSA (nonurban/nonsuburban) than in the central city of a MSA.

Factors That Distinguish Unvaccinated Children From Fully Vaccinated Children (2001 NIS)

Table 4 presents the results of the 3 separate logistic regressions used to compare unvaccinated children with children at the 3 different levels of under-vaccination. These analyses indicated that, compared with undervaccinated children who were NUTD on ≥2 vaccines, unvaccinated children were significantly more likely to be non-Hispanic white than Hispanic, black, or non-Hispanic Asian. Compared with undervaccinated children who were NUTD on all 6 vaccines but had received ≥1 vaccine dose, unvaccinated children were significantly more likely to have a mother who had a college degree than ≤12 years of education. Compared with all undervaccinated children, unvaccinated children were significantly more likely to have a mother ≥30 years of age than 20 to 29 years of age. Finally, compared with undervaccinated children who were NUTD on all 6 vaccines but had received ≥1 vaccine dose, unvaccinated children were significantly more likely to live in a household with an annual income at or exceeding $75,000 than in a household with a lower annual income, and unvaccinated children were significantly more likely to live in a household with ≥4 children than in a household in which he/she was the only child.

Factors That Distinguish Unvaccinated Children From Fully Vaccinated Children (2001 NIS)

Table 4 presents the results of the logistic regression used to compare unvaccinated children with fully vaccinated children. These analyses indicated that, compared with fully vaccinated children, unvaccinated children were significantly more likely to be non-Hispanic white than Hispanic and more likely to live in a household with ≥4 children than in a household in which he/she was the only child. Unvaccinated children did not differ significantly from children who were fully vaccinated with respect to any other child, maternal, or household characteristics. Among unvaccinated children, the proportion of boys was 57.3% and significantly exceeded that of girls by 14.6% (P = .05).


Among parents of unvaccinated children, 47.5% expressed concerns regarding safety, compared with 5.1% of parents with undervaccinated children (relative risk: 17.0; 95% confidence interval [CI]: 5.2–55.7). Among parents of unvaccinated children, 70.9% said that a doctor was not influential in shaping their vaccination decisions for their children, compared with 22.9% among undervaccinated children (relative risk: 8.2; 95% CI: 2.6–25.8).


Figure 1 shows trends in the estimated numbers of unvaccinated 19- to 35-month-old children each year between 1995 and 2001. Between 1995 and 2000, the estimated numbers increased significantly, from 14,719 in 1995 to 24,073 in 2000 (P = .05).

Rates and Numbers of Unvaccinated Children According to State

Using data collected between 1995 and 2001, Fig 2 presents estimated rates of unvaccinated children per 100,000 children 19 to 35 months of age according to state, as well as the District of Columbia. Estimated rates ranged from a low of 60 per 100,000 (Rhode Island) to 1125 per 100,000 (Utah). Among the 10 states with the highest estimated rates per 100,000 children 19
to 35 months of age, 7 were western states (Utah, Montana, Oregon, Colorado, Washington, Alaska, and Idaho). Figure 3 shows that states that allowed philosophical exemptions to laws mandating vaccinations for children as they entered school had significantly higher estimated rates of unvaccinated children 19 to 35 months of age ($P < .05$).

Using data collected between 1995 and 2001, Fig 4 presents the estimated numbers of unvaccinated children 19 to 35 months of age according to county for the 50 counties in the United States with the greatest estimated numbers of unvaccinated children. Figure 4 shows that, among the 20 counties with the greatest estimated numbers of unvaccinated children, 7 counties were in California and 5 were in other western states. The counties with the largest numbers of un-

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**Table 3.** Percentiles of Children at Levels of Child, Maternal, and Household Characteristics According to NUTD Status: Results of the Logistic Regression Analysis (2001 NIS)

<table>
<thead>
<tr>
<th>Child characteristics</th>
<th>Unvaccinated Children</th>
<th>Undervaccinated Children</th>
<th>Fully Vaccinated Children</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Race/ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>6.8 (±5.9)</td>
<td>21.3 (±6.6)</td>
<td>20.9 (±2.5)</td>
</tr>
<tr>
<td>White, non-Hispanic*</td>
<td>82.0 (±10.3)</td>
<td>52.0 (±7.5)</td>
<td>54.0 (±3.1)</td>
</tr>
<tr>
<td>Black, non-Hispanic</td>
<td>9.3 (±8.8)</td>
<td>23.5 (±6.6)</td>
<td>19.4 (±2.6)</td>
</tr>
<tr>
<td>Asian, non-Hispanic</td>
<td>2.0 (±2.3)</td>
<td>2.5 (±1.7)</td>
<td>4.4 (±1.2)</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male*</td>
<td>57.3 (±13.1)</td>
<td>47.5 (±7.4)</td>
<td>52.9 (±3.0)</td>
</tr>
<tr>
<td>Female</td>
<td>42.7 (±13.1)</td>
<td>52.5 (±7.4)</td>
<td>47.1 (±3.0)</td>
</tr>
<tr>
<td>Age of child</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19–24 mo</td>
<td>36.7 (±13.0)</td>
<td>50.7 (±7.5)</td>
<td>45.6 (±3.0)</td>
</tr>
<tr>
<td>25–29 mo</td>
<td>28.2 (±12.1)</td>
<td>21.1 (±5.6)</td>
<td>26.4 (±2.5)</td>
</tr>
<tr>
<td>30–35 mo*</td>
<td>35.2 (±13.5)</td>
<td>28.1 (±6.5)</td>
<td>30.8 (±2.8)</td>
</tr>
<tr>
<td>Foreign born</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1.7 (±3.0)</td>
<td>0.5 (±0.7)</td>
<td>2.7 (±0.9)</td>
</tr>
<tr>
<td>No*</td>
<td>99.3 (±3.0)</td>
<td>99.5 (±0.7)</td>
<td>97.3 (±0.9)</td>
</tr>
<tr>
<td>Maternal characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Widowed/divorced/separated</td>
<td>6.4 (±9.1)</td>
<td>12.8 (±5.4)</td>
<td>10.1 (±1.9)</td>
</tr>
<tr>
<td>Never married</td>
<td>16.9 (±10.3)</td>
<td>27.0 (±6.7)</td>
<td>25.1 (±3.0)</td>
</tr>
<tr>
<td>Married*</td>
<td>76.8 (±12.6)</td>
<td>60.2 (±7.4)</td>
<td>64.8 (±3.1)</td>
</tr>
<tr>
<td>Educational attainment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12 y</td>
<td>16.0 (±10.9)</td>
<td>18.8 (±5.2)</td>
<td>20.6 (±2.8)</td>
</tr>
<tr>
<td>12 y</td>
<td>32.1 (±12.8)</td>
<td>48.8 (±7.5)</td>
<td>39.6 (±3.1)</td>
</tr>
<tr>
<td>&gt;12 y, non-college graduate</td>
<td>15.1 (±12.3)</td>
<td>13.7 (±5.5)</td>
<td>14.9 (±1.9)</td>
</tr>
<tr>
<td>College graduate*</td>
<td>36.8 (±12.2)</td>
<td>17.7 (±4.4)</td>
<td>24.8 (±2.3)</td>
</tr>
<tr>
<td>Preferred language</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>English</td>
<td>92.5 (±6.5)</td>
<td>89.6 (±4.1)</td>
<td>87.4 (±2.2)</td>
</tr>
<tr>
<td>Spanish*</td>
<td>5.0 (±5.5)</td>
<td>9.6 (±4.0)</td>
<td>10.1 (±1.9)</td>
</tr>
<tr>
<td>Other</td>
<td>2.6 (±3.6)</td>
<td>0.9 (±0.8)</td>
<td>2.5 (±1.2)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥19 y</td>
<td>5.7 (±8.6)</td>
<td>4.9 (±3.3)</td>
<td>4.8 (±1.8)</td>
</tr>
<tr>
<td>20–29 y</td>
<td>33.1 (±13.1)</td>
<td>59.8 (±7.1)</td>
<td>49.8 (±3.1)</td>
</tr>
<tr>
<td>≥30 y*</td>
<td>61.2 (±13.8)</td>
<td>35.3 (±6.8)</td>
<td>45.4 (±3.0)</td>
</tr>
<tr>
<td>Household characteristics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Annual family income</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Above, &gt;$75 000*</td>
<td>21.8 (±12.0)</td>
<td>5.8 (±2.4)</td>
<td>11.4 (±1.6)</td>
</tr>
<tr>
<td>Above, &lt;$75 000</td>
<td>39.5 (±12.9)</td>
<td>49.3 (±7.5)</td>
<td>47.9 (±3.0)</td>
</tr>
<tr>
<td>Below</td>
<td>19.0 (±11.0)</td>
<td>29.2 (±6.6)</td>
<td>25.8 (±2.9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>19.7 (±11.8)</td>
<td>15.7 (±7.0)</td>
<td>14.9 (±2.8)</td>
</tr>
<tr>
<td>No. of children ≤18 y in the household</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 child*</td>
<td>9.5 (±6.3)</td>
<td>22.2 (±6.2)</td>
<td>21.1 (±2.5)</td>
</tr>
<tr>
<td>2 or 3 children</td>
<td>43.3 (±13.4)</td>
<td>33.7 (±7.5)</td>
<td>60.8 (±3.0)</td>
</tr>
<tr>
<td>≥4 children</td>
<td>47.2 (±13.9)</td>
<td>24.1 (±6.7)</td>
<td>18.1 (±2.6)</td>
</tr>
<tr>
<td>Moved from different state since child’s birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moved from different state</td>
<td>15.4 (±10.0)</td>
<td>12.4 (±4.8)</td>
<td>12.8 (±1.9)</td>
</tr>
<tr>
<td>Did not move from different state*</td>
<td>84.6 (±10.0)</td>
<td>87.6 (±4.8)</td>
<td>87.2 (±1.9)</td>
</tr>
<tr>
<td>Living in a MSA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSA, central city</td>
<td>32.4 (±12.9)</td>
<td>44.5 (±7.2)</td>
<td>36.6 (±2.8)</td>
</tr>
<tr>
<td>MSA, non-central city*</td>
<td>41.2 (±13.3)</td>
<td>35.3 (±7.7)</td>
<td>45.4 (±3.1)</td>
</tr>
<tr>
<td>Non-MSA</td>
<td>26.4 (±12.6)</td>
<td>20.2 (±5.8)</td>
<td>18.0 (±2.1)</td>
</tr>
</tbody>
</table>

* Reference level for the logistic regression analysis.
The remaining counties among the 20 with the greatest numbers of unvaccinated children included the cities of Chicago, IL, Pittsburgh, PA, Dallas, TX, Houston, TX, Oklahoma City, OK, and Grand Rapids, MI. Also included among those counties were Westchester County, NY, and Lancaster County, PA. New York City was not among the 50 areas with the greatest estimated numbers of children with no vaccine doses.

**DISCUSSION**

Despite the efforts of state and federal agencies to increase vaccination coverage rates, 2.1 million children (36.9%) in the population of children 19 to 35 months of age were undervaccinated in 2001, and 17,000 children (0.3%) had not received any vacci-
CHILDREN WHO HAVE RECEIVED NO VACCINES

Fig 1. Estimates and 95% CIs of annual numbers of unvaccinated children in the US population.

States and the District of Columbia. In Colorado, the percentage of school-entry-aged children who were exempted from compulsory vaccinations increased from 1.37% in 1987 to 2.08% in 1998. In Washington state in 2000, among schools in which ≥5% of all children had been exempted, 38.8% had no records on file at the school of their having been administered any vaccinations. There is evidence that families with similar attitudes and beliefs regarding vaccinations cluster geographically. For example, 12.3% of all children attending public schools and 18.8% of children attending day care in Ashland, Oregon, in 2002 claimed exemptions from mandatory vaccination laws, compared with 2.4% for the entire state that year. Other research has shown that, once parents have established a decision not to vaccinate, they are unlikely to be persuaded to change their decision, regardless of the risks of VPDs. Our data show that parents who have children with no vaccinations are significantly more likely to report that doctors have little or no influence on their decision to vaccinate their children. Therefore, children who are not vaccinated by 19 to 35 months of age may remain unvaccinated up to and beyond school entry. As a result of parents claiming a medical, religious, or philosophical exemption to laws mandating compulsory vaccinations for their children, unvaccinated and undervaccinated children may accumulate with time and increase in numbers in the communities in which they live. Our study has shown that unvaccinated children are clustered in counties in MSAs in western states, although there are also large numbers of unvaccinated children in southern, eastern, and midwestern MSAs.

The consequences of being an “exemptor” were illustrated by a population-based, retrospective, cohort study of all reported measles and pertussis cases among children 3 to 18 years of age in Colorado in 1987–1998. Results from that study showed that exemptors were 22 times more likely to contract measles and 6 times more likely to contract pertussis than were vaccinated persons. Also, the majority of recent tetanus cases have occurred among unvaccinated children. In a measles outbreak among the Amish in 1987, the attack rate was 1.7% among vaccinated individuals and 73.8% among unvaccinated individuals. In the Netherlands, polio outbreaks among communities of religious people who frequently refuse vaccination have been reported, despite a national vaccination coverage rate of 97%. The risk of acquiring a VPD is also evident in the community in which exemptors live; in 1979, a polio outbreak paralyzed 14 Amish people in the United States and the outbreak spread to unvaccinated non-Amish neighbors. In states with loosely enforced state immunization laws, higher measles incidences have been observed. A mathematical model constructed using recent data from California indicated that the incidence of acquiring measles increased from 5.5% to 30.8% as the probability of contact between nonexemptors and exemptors increased from 20% to 60%. Because of the potential for unvaccinated exemptors to accumulate with time in the communities in which they live, there may be greater...
probabilities of contact between nonexemptors and exemptors and increases in the rates of VPDs in both groups. Our results show that the proportions of unvaccinated children are significantly greater in states that allow philosophical exemptions to laws that mandate vaccinations for children as they enter school.

Why do some parents avoid vaccinating their children? Our results indicate that parents of unvaccinated children are much more concerned about vaccine safety than are parents whose children receive ≥1 vaccine dose. In a survey of parent’s beliefs and practices regarding vaccinations and autism, siblings in families in which there was an autistic child were 3 times more likely to be unvaccinated, compared with siblings in families in which there was a child with attention-deficit/hyperactivity disorder. In response to concerns about the perceived risk of autism resulting from vaccinations, parents might have avoided having their sons vaccinated at a higher rate than their daughters, as a result of knowing that they have risk factors for autism and knowing that the rate of autism is 4 times greater for boys than for girls. Although this explanation is conjectural, it may explain why our results show that boys are significantly more likely to be unvaccinated than girls.

Safety concerns regarding alleged links between hepatitis B vaccine and multiple sclerosis or between diphtheria-tetanus-pertussis vaccine and sudden infant death syndrome may be among parent’s concerns that influence their decision not to vaccinate their children. Concerns regarding these issues continue to circulate, although current scientific evidence does not support an association between vaccines and these conditions. In addition, parents...
may choose not to vaccinate their children because of religious beliefs or because of misconceptions about immunizations.

The strengths of this study include a nationally representative sample obtained by the NIS, the large size of the sample, and the ongoing nature of the survey, which allows trends in vaccination coverage to be monitored. The limitations of this study include the possibility of errors in ascertaining whether children were unvaccinated. For example, parent’s reports that their children did not have a vaccination provider and had received no vaccinations may be unreliable. In that case, the estimates of the numbers of unvaccinated children that we report would overestimate the true values. Also, households that choose not to respond to the NIS may be more likely to have unvaccinated children. In that case, the estimates of the numbers of unvaccinated children that we report would underestimate the true values, particularly in geographic areas in which the NIS non-response rates are high because of negative attitudes toward vaccination surveys.

Finally, although we report on the 50 counties with the largest estimated numbers of children who received no vaccinations, it is possible that there are other counties in the United States where the proportions of children with no vaccinations are large. In our analyses, we noted several sparsely populated counties inhabited primarily by racial/ethnic minorities where the proportions of unvaccinated children were large. However, these estimated values were based on very small sample sizes, which precludes publication because of confidentiality concerns.

Our study suggests that the characteristics of children who are unvaccinated are different from those of children who are undervaccinated. Other research has shown that unvaccinated children are at greater risk of both acquiring and transmitting VPDs. Because of these differences, interventions need to be specifically designed and targeted toward parents who choose for their children not to receive any vaccinations.

Acknowledgments

We are grateful for many collegial discussions with Charles LeBaron, Frank DeStefano, Lance Rodewald, Pat Cummings, Beth Hibbs, Deborah Gust, Jeanne Santoli, and Emmanuel Maurice.

References

**Children Who Have Received No Vaccines: Who Are They and Where Do They Live?**

Philip J. Smith, Susan Y. Chu and Lawrence E. Barker

*Pediatrics* 2004;114;187

DOI: 10.1542/peds.114.1.187

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<tr>
<th>Updated Information &amp; Services</th>
<th>including high resolution figures, can be found at: <a href="http://pediatrics.aappublications.org/content/114/1/187">http://pediatrics.aappublications.org/content/114/1/187</a></th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>This article cites 28 articles, 2 of which you can access for free at: <a href="http://pediatrics.aappublications.org/content/114/1/187#BIBL">http://pediatrics.aappublications.org/content/114/1/187#BIBL</a></td>
</tr>
</tbody>
</table>
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Philip J. Smith, Susan Y. Chu and Lawrence E. Barker
_Pediatrics_ 2004;114;187
DOI: 10.1542/peds.114.1.187

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http://pediatrics.aappublications.org/content/114/1/187
Exhibit 224

**Exuvolemia** (ev-o-vo-le-me-a) [ev- + volume + -emia] normovolemia. evulamic adj.

**Exuvolia** (ev-o-vole-a) normal water content or volume of a given body compartment, e.g., extracellular evulida.

**Ev** electron volt.

**Exvacu-** (ex-vak-u-*) 1. emptying. 2. cathartic. (defs. 1 and 2), 3. a remedy that empties any organ, such as a cathartic, emetic, or diuretic.

**Exvacu-a-ton** (ex-vak-u-a-shon) 1. an emptying, 2. emptying of the bowels, as by a medicine; called also catharsis and purgation.

**Exvacu-a-tor** (ex-vak-u-a-tor) an instrument for removing fluid or small particles from a body cavity or container.

**Exag-i-na-tion** (ex-a-jin-a-shun) obstruction of a layer or part to form a pouch.

**Ex optic** e. see under optic.

**Ex-a-nec-cent** (ex-a-nec-cent) [L. exanecescere to vanish away] vanishing; passing away quickly; unstable; unfixed.

**Ex-ans syndrome** (ex-ans) [Robert Sherman Exans, American physician, 1912–1974] see under syndrome.

**Exap-o-ra-tion** (ex-ap-o-ra-shun) [L. e out + vaporare to steam] conversion of a liquid or solid into vapor.

**Exa-sion** (ex-a-shun) in psychiatry, suppression of an idea that would come near in a thought sequence and substitution of a closely related idea; a form of paralogy.

**Ex-ten-tration** (ex-ten-tration) [L. exentratzs] 1. protrusion of abdomin visceral. 2. removal of the abdominal viscera.

**Exdiaphragmatic** e. a congenital anomaly characterized by failure of muscular development of part or all of one (or occasionally both) hemidiaphragms, resulting in superior displacement of abdominal viscera and altered lung development.

**Exophthalmia** (ex-o-phalm-a) [Greek exo- outside + phalamos eye] protruding of eyeball.

**Ex-pa-sus** (ex-pa-sus) [Oskar Everbusch, German ophthalmologist, 1853–1912] see under operation.

**Ex-sion** (ex-sion) 1. a turning inside out. Called also eversion. 2. a turning outward, as of the sole of the foot or the eyelid.

**Extraction** (ex-trak-tsion) [L. exstrax] 1. a process of removing a tooth from its socket.

**Extraction** (ex-traction) [L. exstrax] 1. an act of extracting; the removal or extraction of a part or organ.

**Ex-sion** (ex-sion) 1. a cutting out; an excision.

**Ex-vascular** e. see under axillary.

**Ex-vio-late** (ex-vio-late) [L. exviosus] forcible excision; see excision.

**Ew-ar t sign** (ew-ar-t) [William Ewart, English physician, 1848–1929] see under sign.

**Ew-Ho** elbow-wrist-hand orthosis.

**Ewing sarcoma** (tumor) (tring) [James Ewing, American pathologist, 1866–1943] see under sarcoma.

**Ewing-ul-la** (ew-ing-ul-la) [William H. Ewing, American bacteriologist, 1911–1963] a genus of gram-negative, rod-shaped bacteria of the family Enterobacteriaceae, consisting of facultatively anaerobic, oxidase-negative, catalase-positive organisms that are motile by peritrichous flagella. The type species is *E. americana*.

**E. americana* a species that is a rare cause of human infection, including nosocomial bacteremia, peritonitis associated with peritoneal dialysis, and conjunctivitis.

**Ex** [L. ex out of, away from] a prefix meaning away from, without, or outside; it is sometimes used to denote completely, as in extravasation.

**Excit** [Gr. exkiva because it is sixth in the series of prefixes for multiples] a combining form used in naming units of measurement to indicate a quantity 1 quintillion (10^18) times the unit designated by the root with which it is combined. Symbol E.

**Exc-er-ba-tion** (ex-er-ba-tion) [ex- + L. acer harsh] increase in the severity of a disease or any of its symptoms.

**Ex-er-e-sis** (ex-e-rè-sis) [Gr. "a taking out"] exeresis.

**Ex-a-gi-go** (ek-sal-go) trademark for a preparation of hydromorphone hydrochloride.

**Ex-ter-tion** (ex-tor-tion) a muscle that turns a part outward.

**Extracerebrum** (ek-stral-ka-sishun) a feeling of extreme elation, often associated with delusions of grandeur.

**Ex-meta-zine** (ek-sa-met-a-zin) HMPAO, hexamethylpropylamine oxide, a neutral lipophilic compound that traverses the blood-brain barrier and localizes in the brain; complexed with technetium-99m, it is used for imaging of cerebral regional blood flow in the detection of altered regional perfusion in stroke, identification of Alzheimer disease, evaluation of epilepsy, and diagnosis of brain death. The same complex can also be used to label autologous leukocytes for diagnostic studies of intra-abdominal inflammatory lesions and bowel disease. See table at toctanum.
EXHIBIT 225
common sense

noun [U]

US /ˈkæm·ənˈsens/

the ability to use good judgment in making decisions and to live in a reasonable and safe way

(Definition of common sense from the Cambridge Academic Content Dictionary © Cambridge University Press)

EXAMPLES of common sense

Some theories in physics contain direct paradoxes in view of common sense and 'folk physics'.

From the Cambridge English Corpus

The final three chapters discuss the relationship between religion, morality and civilization, religious language and truth, and religious knowledge and common sense.

From the Cambridge English Corpus

These examples are from the Cambridge English Corpus and from sources on the web. Any opinions in the examples do not represent the opinion of the Cambridge Dictionary editors or of Cambridge University Press or its licensors.

More examples
EXHIBIT 226
safe adjective

\sāf \safer; safest

Definition of safe (Entry 1 of 2)

1 : free from harm or risk : UNHURT

2 a : secure from threat of danger, harm, or loss
   b : successful at getting to a base in baseball without being put out

3 : affording safety or security from danger, risk, or difficulty

4 obsolete, of mental or moral faculties : HEALTHY, (SEE <A HREF="/DICTIONARY/">(SEE {DXT" CLASS = 'MW_T_DXT'><SPAN CLASS='TEXT-UPPERCASE'>sense I) II

5 a : not threatening danger : HARMLESS
   b : unlikely to produce controversy or contradiction

6 a : not likely to take risks : CAUTIOUS
   b : TRUSTWORTHY, RELIABLE
EXHIBIT 227
unsafe adjective

un·safe 1 \ən-ˈsāf\
unsafest

Definition of unsafe

: not safe: such as

a  : able or likely to cause harm, damage, or loss
   // water that is unsafe to drink
   // unsafe driving habits
   // unsafe levels of lead

b  : not giving protection from danger, harm, or loss
   // an unsafe vehicle
   // unsafe working conditions

c  : not protected from danger, harm, or loss
   // feeling frightened and unsafe

d  : likely to take risks: not careful
   // unsafe drivers
dangerous adjective

dan·ger·ous l \\dän-je-rəs \; \dän-jərəs, -zhərəs \\

Definition of dangerous

1 : involving possible injury, pain, harm, or loss : characterized by danger
   **// a dangerous job**

2 : able or likely to inflict injury or harm
   **// a dangerous man**

Other Words from dangerous

Synonyms & Antonyms

Choose the Right Synonym

More Example Sentences

Learn More about dangerous
EXHIBIT 229
Defining the scientific method

The rise of ‘omics’ methods and data-driven research presents new possibilities for discovery but also stimulates disagreement over how science should be conducted and even how it should be defined.

Modern biological research methods are powerful tools in biologists’ arsenal for investigating biology. But is the ability of these methods to amass extraordinary amounts of data altering the nature of scientific inquiry?

As schoolchildren we are taught that the scientific method involves a question and suggested explanation (hypothesis) based on observation, followed by the careful design and execution of controlled experiments, and finally validation, refinement or rejection of this hypothesis. Developed by thinkers including Bacon, Descartes and Pierce, this methodology has been credited with much of science’s success. Modern philosophers such as Feyerabend have argued that this is not how most science is conducted, but up to now most modern scientists have subscribed to the hypothesis-centric scientific method.

Scientists’ defense of this methodology has often been vigorous, likely owing to the historic success of predictive hypothesis-driven mechanistic theories in physics, the dangers inherent in ‘fishing expeditions’ and the likelihood of false correlations based on data from improperly designed experiments. For example, The Human Genome Project was considered by many at the time to be a serious break with the notion that proper biological research must be hypothesis-driven. But the project proceeded because others successfully argued that it would yield information vital for understanding human biology.

Methodological developments are now making it possible to obtain massive amounts of ‘omics’ data on a variety of biological constituents. These immense datasets allow biologists to generate useful predictions (for example, gene-finding and function or protein structure and function) using machine learning and statistics that do not take into account the underlying mechanisms that dictate design and function—considerations that would form the basis of a traditional hypothesis.

Now that the bias against data-driven investigation has weakened, the desire to simplify ‘omics’ data reuse has led to the establishment of minimal information requirements for different types of primary data. The hope is that this will allow new analyses and predictions using aggregated data from disparate experiments.

Last summer, the editor-in-chief of Wired, Chris Anderson, went so far as to argue that biology is too complex for hypotheses and models, and that the classical scientific method is dead. Instead, he called for these methods to be replaced by powerful correlative analyses of massive amounts of data gathered by new technologies similar to how Google Translate relies on only correlative analyses of documents on the internet.

This generated quite a response from the scientific community with California Institute of Technology physicist Sean Carroll arguing in Edge that “hypotheses aren’t simply useful tools in some potentially outdated vision of science; they are the whole point. Theory is understanding, and understanding our world is what science is all about.”

Is the generation of parts lists and correlations in the absence of functional models science? Based on the often accepted definition of the scientific method, the answer would be a qualified no. But not everyone would agree. Carroll’s colleague, David Goodstein, previously stated in a Thesis article in Nature Physics that “science, it turns out, is whatever scientists do.” A philosopher would find this to be a circular and unfulfilling argument, but it is likely that many biologists who are more interested in the practical outcomes of their methods than their philosophical underpinnings would agree with this sentiment.

But the rise of methodologies that generate massive amounts of data does not dictate that biology should be data-driven. In a return to hypothesis-driven research, systems biologists are attempting to use the same ‘omics’ methods to generate data for use in quantitative biological models. Hypotheses are needed before data collection because model-driven quantitative analyses require rich dynamic data collected under defined conditions and stimuli.

So where does this leave us? It is likely that the high complexity of biology will actually make full biological understanding by purely correlative analysis impossible. This method works for Google because language has simple rules and low complexity. Biology has neither constraint. Correlations in large datasets may be able to provide some useful answers, but not all of them.

But ‘omics’ data can provide information on the size and composition of biological entities and thus determine the boundaries of the problem at hand. Biologists can then proceed to investigate function using classical hypothesis-driven experiments. It is still unclear whether even this marriage of the two methods will deliver a complete understanding of biology, but it arguably has a better chance than either method on its own.

Philosophers are free to argue whether one method is science and the other is not. Ultimately the public who funds the work and the biologists who conduct it want results that will materially impact the quality of life regardless of what the method is called.
EXHIBIT 230
Definition of unscientific in English:

**unscientific**

Translate unscientific into Spanish

Pronunciation /ˌʌnˈsɪən tɪfɪk/ /ˈɑːnsərən tɪfɪk/ 🎧

**ADJECTIVE**

1. Not in accordance with scientific principles or methodology.
   ‘our whole approach is hopelessly unscientific’
   + More example sentences + Synonyms

2. Lacking knowledge of or interest in science.
   ‘we seem to be an unscientific nation’
   + More example sentences + Synonyms

Pronunciation 🎧

unscientific /ˌʌnˈsɪən tɪfɪk/ /ˈɑːnsərən tɪfɪk/ 🎧
EXHIBIT 231
Control group

Control group, the standard to which comparisons are made in an experiment. Many experiments are designed to include a control group and one or more experimental groups; in fact, some scholars reserve the term experiment for study designs that include a control group. Ideally, the control group and the experimental groups are identical in every way except that the experimental groups are subjected to treatments or interventions believed to have an effect on the outcome of interest while the control group is not. Inclusion of a control group greatly strengthens researchers’ ability to draw conclusions from a study. Indeed, only in the presence of a control group can a researcher determine whether a treatment under investigation truly has a significant effect on an experimental group, and the possibility of making an erroneous conclusion is reduced. See also scientific method.

A typical use of a control group is in an experiment in which the effect of a treatment is unknown and comparisons between the control group and the experimental group are used to measure the effect of the treatment. For instance, in a pharmaceutical study to determine the effectiveness of a new drug on the treatment of migraines, the experimental group will be administered the new drug and the control group will be administered a placebo (a drug that is inert, or assumed to have no effect). Each group is then given the same questionnaire and asked to rate the effectiveness of the drug in relieving symptoms. If the new drug is effective, the experimental group is expected to have a significantly better response to it than the control group. Another possible design is to include several experimental groups, each of which is given a different dosage of the new drug, plus one control group. In this design, the analyst will compare results from each of the experimental groups to the control group. This type of experiment allows the researcher to determine not only if the drug is effective but also the effectiveness of different dosages. In the absence of a control group, the researcher’s ability to draw conclusions about the new drug is greatly weakened, due to the placebo effect and other threats to validity. Comparisons between the experimental groups with different dosages can be made without including a control group, but there is no way to know if any of the dosages of the new drug are more or less effective than the placebo.

It is important that every aspect of the experimental environment be as alike as possible for all subjects in the experiment. If conditions are different for the experimental and control groups, it is impossible to know whether differences between groups are actually due to the difference in treatments or to the difference in environment. For example, in the new migraine drug study, it would be a poor study design to administer the
questionnaire to the experimental group in a hospital setting while asking the control group to complete it at home. Such a study could lead to a misleading conclusion, because differences in responses between the experimental and control groups could have been due to the effect of the drug or could have been due to the conditions under which the data were collected. For instance, perhaps the experimental group received better instructions or was more motivated by being in the hospital setting to give accurate responses than the control group.

In non-laboratory and nonclinical experiments, such as field experiments in ecology or economics, even well-designed experiments are subject to numerous and complex variables that cannot always be managed across the control group and experimental groups. Randomization, in which individuals or groups of individuals are randomly assigned to the treatment and control groups, is an important tool to eliminate selection bias and can aid in disentangling the effects of the experimental treatment from other confounding factors. Appropriate sample sizes are also important.

A control group study can be managed in two different ways. In a single-blind study, the researcher will know whether a particular subject is in the control group, but the subject will not know. In a double-blind study, neither the subject nor the researcher will know which treatment the subject is receiving. In many cases, a double-blind study is preferable to a single-blind study, since the researcher cannot inadvertently affect the results or their interpretation by treating a control subject differently from an experimental subject.

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