Normal IgG and Impaired IgM Responses to Polysaccharide Vaccines in Asplenic Patients

Deborah C. Molrine, George R. Siber, Yecheskel Samra, Drora Samra Shevy, Kristin MacDonald, Rocco Cieri, and Donna M. Ambrosino

Asplenic patients are at increased risk for life-threatening infections with polysaccharide-encapsulated organisms, and reports of responses to polysaccharide vaccines have been conflicting. Thirty-six asplenic patients and 15 healthy controls were immunized with pneumococcal, Haemophilus influenzae type b (Hib), and meningococcal vaccines. Antibody concentrations to Hib and pneumococcal serotypes 14 and 18C were measured by ELISA. IgG antibody responses to all three antigens were similar in asplenic patients and controls at 28 days following immunization. In contrast, asplenic patients had significantly lower IgM concentrations in response to Hib (P < .05) and to both pneumococcal serotypes 14 (P < .005) and 18C (P < .001). IgA anti-Hib antibody was also lower in the asplenic group, as was total anti-Hib antibody measured by RIA. These results document that IgG responses to polysaccharide vaccines are normal in asplenic patients. The impaired IgM responses of these patients may explain conflicting reports from studies that measured only total antibody-binding concentrations.

Asplenic patients are at increased risk for infections with Streptococcus pneumoniae, Haemophilus influenzae type b (Hib), and Neisseria meningitidis [1–3]. The Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC) recommends that 23-valent pneumococcal vaccine be administered to individuals with anatomic or functional asplenia. Hib conjugate and 4-valent meningococcal vaccines are also recommended for these high-risk patients [4, 5].

Previous reports have been conflicting on the effect of splenectomy on immune responses to polysaccharide antigens. Findings from various studies have shown normal or impaired serum opsonic activity and antibody responses to pneumococcal immunization in splenectomized individuals compared with nonsplenectomized controls [6–10].

To further document the effect of splenectomy on responses to polysaccharide vaccines, we report isotype-specific antibody responses of patients who are asplenic secondary to trauma compared with those of healthy controls. This study was conducted in 1980–1981 in Israel, but assays were not conducted until now for class-specific responses to capsular polysaccharides. It has recently been recognized that results from older pneumococcal assays were affected by C polysaccharide that contaminated pneumococcal antigen preparations [11]. In this study, we used C polysaccharide–absorbed ELISA determinations for pneumococcal IgG and IgM specific assays. In addition, class-specific antibodies directed to Hib polysaccharide were measured.

Materials and Methods

Study population and immunizations. Thirty-six individuals who had undergone splenectomy as a result of trauma and 15 healthy nonsplenectomized controls were enrolled. Splenectomized subjects and controls were immunized simultaneously with three nonconjugated polysaccharide vaccines. Hib capsular polysaccharide vaccine (0.5 mL containing 50 µg of capsular polysaccharide [Merck Sharp & Dohme, West Point, PA]) was mixed in the same syringe with bivalent meningococcal vaccine (0.5 mL containing 50 µg of each capsular polysaccharide A and C [Connaught Laboratories, Swiftwater, PA]) and administered in the right deltoid muscle. Concurrently, 14-valent pneumococcal vaccine (0.5 mL containing 50 µg of capsular polysaccharide of types 1, 2, 3, 4, 6A, 7F, 8, 9N, 12, 14, 18C, 19F, 23F, and 25 [Merck, Sharp & Dohme]) was administered in the left deltoid muscle. Of 36 asplenic subjects, 4 were females and 32 were males (ages, 6–54 years; mean, 27). The male predominance in the asplenic group reflects the inclusion of soldiers splenectomized after gunshot wounds. The interval from time of splenectomy to immunization was documented for 35 of the 36 subjects (mean, 6 years; range, 3 months to 12 years). Only 2 individuals were immunized at an interval of <3 years (3 and 6 months). The control group consisted of 15 healthy volunteers: 8 were female and 7 were male (ages, 17–53 years; mean, 28). Sera
were obtained before immunization, at 1 and 4 weeks, and at 6 months after immunization and stored at −20°C until assayed.

**Antibody assays.** IgG and IgM antibody concentrations to two pneumococcal capsular polysaccharides contained in the vaccine were measured at 7, 28, and 180 days following immunization by cell wall polysaccharide (CPS)–absorbed ELISA, as previously described [11]. Serotypes 14 and 18C were selected for measurement because they were known to produce reliable responses following immunization. Sera were diluted in PBS-Tween solution containing CPS at 2.5 µg/mL (pneumococcal absorbent pool lot B; provided by Porter Anderson, University of Rochester, Rochester, NY) and incubated for 30 min. Goat anti-human IgG or IgM alkaline phosphatase conjugate (Caltag Laboratories, S. San Francisco) was added (depending on which immunoglobulin was being measured), followed by phosphatase substrate. A reference plasma pool PSAB-89SF (Food and Drug Administration [FDA], Bethesda, MD) was used with assigned values for serotypes 14 and 18C of 27.8 and 4.46 µg/mL, respectively, for IgG, and 1.19 and 1.27 µg/mL, respectively, for IgM.

Total binding anti-Hib antibody was measured by RIA using a standard FDA protocol with tritiated polyribosylribitol phosphate (provided by Porter Anderson), as previously described [12]. IgG, IgM, and IgA anti-Hib antibody concentrations were measured by ELISA using Hib oligosaccharide coupled to human albumin (provided by Porter Anderson) [12] and standardized using a serum reference pool with assigned values of 27.2 µg/mL for Hib IgM, 1.62 µg/mL for Hib IgG, and 2.39 µg/mL for Hib IgA. All serum samples for a given time point from both groups were run in the same assays to minimize effects due to interassay variation.

**Statistics.** Logarithms of antibody concentrations were used for statistical calculations. Antibody concentrations below the assay limit of sensitivity were assigned values equal to one-half the lower limit. The geometric mean of the average is reported for group comparisons. Comparisons of geometric means of antibody concentrations were performed for normally distributed data by using the two-tailed t test and for non-normally distributed data by the Mann-Whitney rank sum test.

### Results

**IgG-specific anti-capsular polysaccharide antibody concentrations.** Geometric mean IgG antibody concentrations to Hib capsular polysaccharide were similar for asplenic and control groups at peak concentrations measured 28 days following immunization (table 1, figure 1). At the earlier time point of 7 days after immunization, the geometric mean IgG anti-Hib antibody of the control group was 14.4 µg/mL versus 8.02 µg/mL for the splenectomized group (P = .032).

Geometric mean IgG antibody concentrations to the capsular polysaccharides of pneumococcal serotypes 14 and 18C were also similar for both groups before immunization and at 7 and 28 days after immunization (figure 1). Although the control group had higher antibody concentrations at these time points than the asplenic group, none of these differences were statistically significant (table 1). IgG antibody concentrations to both serotypes were also measured at 180 days following immunization and were not statistically different. Geometric mean IgG antibody concentrations to types 14 and 18C for asplenic subjects were 20.1 µg/mL and 4.2 µg/mL, respectively, versus 30.6 µg/mL and 5.2 µg/mL, respectively, for controls.

**IgM specific anti-capsular polysaccharide antibody concentrations.** In contrast to the IgG results, IgM-specific antibody concentrations of persons in the asplenic group were lower than those of the control group for all three antigens. Geometric mean IgM antibody concentrations to Hib capsular polysaccharide were significantly lower in the asplenic group than in the control group at both 7 days (P = .022) and 28 days (P = .048) following immunization (table 1). Similar results were observed for pneumococcal-specific IgM antibodies as the splencotomized group demonstrated significantly decreased antibody concentrations for type 14 and type 18C before immunization (P < .05), 7 days following immunization (P < .001), and 28 days later (P < .005) (table 1). The significant difference

### Table 1. Antibody responses to Haemophilus influenzae type b (Hib) and pneumococcal capsular polysaccharides.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Type 14</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 28</th>
<th>Type 18C</th>
<th>Day 0</th>
<th>Day 7</th>
<th>Day 28</th>
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<tr>
<td></td>
<td></td>
<td>Asplenic</td>
<td>Controls</td>
<td></td>
<td></td>
<td>Asplenic</td>
<td>Controls</td>
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<td>Hib</td>
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<tr>
<td>Total</td>
<td>2.69 (0.256–28.3)</td>
<td>7.39 (0.850–64.3)</td>
<td>23.9 (2.76–208)</td>
<td>3.55 (0.385–32.7)</td>
<td>20.3 (2.16–190)</td>
<td>40.2 (4.40–368)</td>
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<tr>
<td>IgG</td>
<td>4.82 (0.597–38.8)</td>
<td>8.02 (1.60–40.2)</td>
<td>19.7 (2.83–137)</td>
<td>7.56 (2.04–28.0)</td>
<td>14.4 (2.98–69.2)</td>
<td>19.5 (4.30–88.4)</td>
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<tr>
<td>IgM</td>
<td>1.13 (0.158–8.11)</td>
<td>1.13 (0.179–7.16)</td>
<td>1.60 (0.158–16.1)</td>
<td>1.68 (0.276–10.2)</td>
<td>2.18 (0.523–9.08)</td>
<td>3.18 (0.609–16.6)</td>
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<tr>
<td>IgA</td>
<td>0.774 (0.183–3.28)</td>
<td>1.17 (0.286–4.74)</td>
<td>2.97 (0.421–21.0)</td>
<td>3.98 (0.192–5.60)</td>
<td>5.22 (0.421–21.0)</td>
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<td>Pneumococcal</td>
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<tr>
<td>Type 14</td>
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<tr>
<td>IgG</td>
<td>5.55 (0.425–72.6)</td>
<td>7.69 (0.605–97.9)</td>
<td>25.2 (2.44–260)</td>
<td>6.54 (0.229–187)</td>
<td>10.9 (0.691–173)</td>
<td>39.7 (2.85–552)</td>
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<tr>
<td>IgM</td>
<td>0.955 (0.225–4.06)</td>
<td>1.12 (0.369–3.37)</td>
<td>2.83 (0.529–15.1)</td>
<td>2.63 (0.336–20.5)</td>
<td>2.65 (0.778–9.03)</td>
<td>7.19 (1.39–37.3)</td>
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<tr>
<td>Type 18C</td>
<td></td>
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<tr>
<td>IgG</td>
<td>2.08 (0.132–32.6)</td>
<td>3.02 (0.280–32.5)</td>
<td>6.85 (1.40–33.5)</td>
<td>3.31 (0.521–21.1)</td>
<td>5.35 (1.46–19.6)</td>
<td>10.9 (3.49–33.8)</td>
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<tr>
<td>IgM</td>
<td>0.898 (0.157–5.89)</td>
<td>0.802 (0.155–4.16)</td>
<td>1.78 (0.260–12.2)</td>
<td>1.79 (0.279–11.5)</td>
<td>2.32 (0.366–14.7)</td>
<td>7.25 (1.24–42.4)</td>
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</table>

**NOTE.** Data are geometric mean antibody concentrations in µg/mL (95% confidence interval). P ≤ .005, .05; .001 by t test for normally distributed data or Mann-Whitney rank sum test for non-normally distributed data comparing antibody concentrations of asplenic patients to those of healthy controls.
IgM and IgG antibody responses to capsular polysaccharides of *Haemophilus influenzae* type b and pneumococcal (Pneumo) serotypes 14 and 18C before (day 0) and after (days 7 and 28) immunization with polysaccharide vaccines. ● represents geometric mean antibody concentrations from 15 non-splenectomized persons (controls); ○ represents geometric mean antibody concentrations from 36 persons who underwent splenectomy secondary to trauma. * Statistically significant between groups (P < .05) by t test for parametric analysis and by Mann-Whitney rank sum test for nonparametric analysis.

In serotype 18C IgM antibody was maintained over a long time period with a geometric mean of 0.88 μg/mL for asplenic persons compared with 2.1 μg/mL for controls at 6 months after immunization (P = .02).

IgA-specific anti-Hib antibody concentrations. IgA responses were measured to only the capsular polysaccharide of Hib. The geometric mean IgA antibody concentration to Hib was significantly lower in the asplenic group than in the control group at both 7 days (P < .001) and 28 days (P = .014) following immunization.

Total binding anti-Hib antibody concentrations. Total anti-Hib antibody as measured by RIA was significantly lower for asplenic subjects than controls at 7 days following immunization (7.39 vs. 20.3 μg/mL, P < .005). The geometric mean antibody titer of the asplenic group was also lower at 28 days after immunization, although this difference was not statistically significant (P = .135; table 1). Geometric mean total anti-Hib antibody concentrations did not differ between the groups before immunization.

**Discussion**

We immunized 36 asplenic patients with pneumococcal, meningococcal, and Hib polysaccharide vaccines and measured IgG, IgM, and IgA responses to three capsular polysaccharide antigens. Asplenic patients had significantly lower IgM con-
centrations to all three antigens at 7 and 28 days following immunization compared with those of healthy controls. IgA responses to Hib vaccine were also determined, and asplenic subjects had significantly lower IgA anti-Hib antibody at both 7 and 28 days following immunization. In contrast, IgG concentrations to all three antigens were similar for splenectomized patients and healthy controls at 28 days following immunization. Of note, asplenic patients had significantly lower IgG anti-Hib concentrations at 7 days after immunization, suggesting that the kinetics of the IgG response may be different, although by the time of peak antibody concentrations, IgG-specific responses were not significantly different from those of controls. The mechanisms for these findings are not clear and may not be specific for polysaccharide vaccines. Total serum IgM concentrations have been reported to be lower in asplenic patients, although other studies report normal total IgM concentrations [3, 8]. The spleen is considered to be the predominant organ for producing IgM antigen-specific responses [8]. Recent data suggest that lymphoid germinal centers are not the only sites responsible for the maturation of humoral immune responses; affinity maturation of serum antibody continues in the bone marrow compartment [13]. These observations are consistent with our findings and offer an explanation for normal IgG responses in individuals with asplenia.

Our results of isotype-specific responses to polysaccharide vaccines may explain conflicting previous reports on responses of asplenic patients to polysaccharide vaccines. Some of the previous studies used total binding assays to assess anti-pneumococcal antibody concentrations [6, 8, 10]. The impaired total antibody responses for asplenic patients in some of the studies may have been due to the lower antigen-specific IgM antibody responses, which contributed significantly to the total binding results. This finding was noted in our study, as geometric mean total anti-Hib antibody concentration was lower for asplenic subjects than for controls. Furthermore, total anti-Hib binding antibody measured by RIA did not equal the sum of IgG, IgM and IgA antibody concentrations measured by ELISA. The sum of ELISA concentrations for Hib was less than the total binding antibody concentration measured by RIA at 28 days following immunization for controls but was equivalent in the asplenic group (table 1). Possible explanations for these differences include differential contribution of isotypes to the RIA binding assay as well as the value assignment of standards for ELISA IgG, IgM, and IgA assays. If IgM antibody is relatively more efficient at binding and/or precipitating the antigen-antibody complex, RIA results for patients producing less IgM antibody would appear lower than what ELISA IgM differences would predict.

Other assay differences may also explain results that conflict with previous reports. The one study that noted impaired pneumococcal IgG responses for asplenic patients used an ELISA that did not absorb antibody to CPS polysaccharide [7]. The present study used the FDA-CDC standard CPS-absorbed ELISA and documented that the predominant IgG response to polysaccharide vaccines is normal in asplenic individuals.

We also recently used the CPS absorbed ELISA to assess responses to pneumococcal polysaccharide vaccine in Hodgkin’s disease patients, of whom 84% were asplenic. These patients had received treatment 2–24 years previously, and their IgG responses to 23-valent polysaccharide vaccine were similar to those of healthy controls [12].

IgG antibody is the predominant isotype-specific response following immunization and is used to predict protective responses to pneumococcal vaccine [14]. In our study, IgG antibody responses to polysaccharide vaccines administered following splenectomy were similar to those of healthy controls and thus were predicted to be protective for this high-risk group.

The current ACIP recommendation for persons with functional or anatomic asplenia is pneumococcal reimmunization once for these individuals at highest risk for serious disease. Persons with asplenia who are ≥10 years old at the time of reimmunization should be reimmunized if it has been ≥5 years since initial pneumococcal immunization; reimmunization at 3 years after receipt of initial pneumococcal vaccine should be considered for children who will be ≤10 years at the time of reimmunization [5]. ACIP does not routinely recommend the administration of more than one reimmunization due to the lack of safety data. However, we are concerned that asplenic individuals would be at risk for serious disease as antibody concentrations decline over time [14]. This would be of particular concern for the youngest asplenic patients who would not receive immunization beyond 5 years of age, when IgG responses are mediocre or poor [15]. We therefore suggest reimmunization every 5 years with pneumococcal (and probably meningococcal) polysaccharide vaccine for all asplenic patients, as findings from our study support their ability to elicit normal IgG antibody responses.

References