The kinetics of the immune response to the 23-valent pneumococcal polysaccharide vaccine (PPV) were studied in 38 children who received bone marrow transplants (BMTs). Anti-pneumococcal antibody concentrations increased 1 and 3 months after vaccination for all 5 serotypes tested, but, in 21 children, the vaccine was not adequately immunogenic. Children vaccinated >18 months after receiving a BMT had a 4.2-fold increased odds of poor response (P = .06). Antibody concentrations returned close to baseline levels 9 months after vaccination. Avidity declined significantly as early as 1 month after vaccination and remained low thereafter. Antibody concentration responses to PPV were superior among 9 healthy control children (P = .001); 37 of 38 children with a BMT elicited adequate, persistent immune responses to Haemophilus influenzae conjugate vaccine. Immune responses to PPV in children with a BMT are suboptimal, short lived, and associated with declining avidity. The different kinetics of antibody concentration and avidity indicate that both markers should be used for evaluating pneumococcal vaccines in this high-risk population.

Effective prevention of infection from Streptococcus pneumoniae in children who are recipients of bone marrow transplants (BMTs) is considered a priority because these children are at increased risk of infection for 3–5 years after transplantation. However, the protectiveness of the licensed 23-valent pneumococcal polysaccharide vaccine (PPV) in this population is uncertain. Other studies of adult recipients of BMTs have shown various results for PPV immunogenicity [1–3].

It has been argued recently that antibody avidity, the strength of the interaction in the antigen-antibody binding, highly correlates with antibody function [4] and should be taken into consideration for the evaluation of immune responses to vaccination [5, 6]. However, there are no data on the avidity of PPV antibody responses in child recipients of BMTs.

To thoroughly evaluate pneumococcal vaccination in children with BMTs, we studied the kinetics of concentration, isotype pattern, and avidity of PPV immune responses in such subjects up to 12 months after vaccination. For comparison, we also evaluated the immune responses to Haemophilus influenzae–tetanus conjugate polysaccharide vaccine (PRP-T) in the same children, to study differences between thymus-dependent and -independent responses in this setting.

Subjects and Methods

Study population. Thirty-eight children (24 boys) were recruited from the Bone Marrow Transplantation Unit, Aghia Sophia Children’s Hospital, Athens. BMT indications included malignancy (n = 20),  β-thalassemia (n = 15), severe combined immunodeficiency (n = 1), Job’s syndrome (n = 1), and osteopetrosis (n = 1). BMT was autologous in 10 children and allogeneic in 25; 3 children received grafts from matched, unrelated donors. No children had been vaccinated previously with PPV or PRP-T or had chronic graft-versus-host disease. One child had had a splenectomy. Thirty-four children were receiving penicillin prophylaxis. We also obtained data on 1-month responses to PPV for 9 healthy control subjects of similar ages.
### Table 1. Geometric mean concentration and avidity index at baseline and 1-month responses to 23-valent pneumococcal polysaccharide (PPV) and *Haemophilus influenzae*-tetanus conjugate (PRP-T) vaccine in children with bone marrow transplants (BMTs) and in controls.

<table>
<thead>
<tr>
<th>Antibody response</th>
<th>Children with BMTs</th>
<th>Controls</th>
<th>Children with BMTs</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1-month</td>
<td>Baseline</td>
<td>1-month</td>
</tr>
<tr>
<td>Pneumococcal serotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.6 (0.1-2.4)</td>
<td>1.0 (0.1-9.0)</td>
<td>0.1 (0.1-0.4)</td>
<td>1.3 (0.3-5.5)</td>
</tr>
<tr>
<td>6B</td>
<td>0.3 (0.1-2.3)</td>
<td>0.4 (0.1-6.6)</td>
<td>1.5 (0.4-17)</td>
<td>7.7 (0.8-40)</td>
</tr>
<tr>
<td>14</td>
<td>1.1 (0.2-7.6)</td>
<td>1.9 (0.2-25)</td>
<td>0.6 (0.1-5.4)</td>
<td>2.8 (0.1-9.6)</td>
</tr>
<tr>
<td>19F</td>
<td>0.6 (0.1-10)</td>
<td>0.9 (0.1-7.1)</td>
<td>0.1 (0.1-0.8)</td>
<td>0.6 (0.1-2.6)</td>
</tr>
<tr>
<td>23F</td>
<td>0.3 (0.1-6.3)</td>
<td>0.5 (0.1-18)</td>
<td>1.0 (0.2-3.1)</td>
<td>5.7 (0.5-38)</td>
</tr>
<tr>
<td>Polyribosylribitol phosphate</td>
<td>1.7 (0.7-3.5)</td>
<td>8.2 (0.3-76)</td>
<td>NP</td>
<td>NP</td>
</tr>
</tbody>
</table>

**NOTE.** Data are geometric means (ranges); antibody concentrations are in µg/mL. NP, not pertinent (comparisons were made between children with BMTs and control children for pneumococcal antibodies and between PPV and PRP-T responses in BMT recipients).

- *a* Fold-increase between baseline and 1 month is greater than any of the respective fold-increases for any pneumococcal serotype (*P* < .05 by Wilcoxon signed rank test).
- *b* Fold-increase between baseline and 1 month is greater than the respective fold-increase in BMT recipients (*P* < .05 by Mann-Whitney *U* test).
- *c* Fold-increase between baseline and 1 month is greater than any of the respective fold-increases for any pneumococcal serotype (*P* = .001 by Wilcoxon signed rank test).

### Results

**Vaccination schedule.** All children received 1 subcutaneous dose of PPV (Pneu-Immune 23; Lederle Laboratories, Pearl River, NY). All BMT recipients were vaccinated subsequently with PRP-T vaccine (ActHib; Pasteur Mérieux, Lyon, France). Blood samples were collected before and 1, 3, 6, 9, and 12 months after vaccination. Serum samples were separated, frozen, and stored (−20°C) until tested.

**Antibody concentrations.** IgG antibodies for pneumococcal serotypes (PS) 3, 6B, 14, 19F, and 23F were measured by use of a modified ELISA [7]. Absorption of serum antibodies to the common cell wall polysaccharide was accomplished by incubation (30 min at 37°C) of the test serum (1:50) with cell wall polysaccharide antigen (10 µg/mL; Statens Seruminstitut, Copenhagen). For determination of IgG1 and IgG2 subclass PS-antibodies specific for 14 and 19F, monoclonal mouse anti-human IgG1 and IgG2 were added (clones HP 6070 and HP 6002, respectively; Zymed Laboratories, San Francisco), followed by alkaline phosphatase-conjugated goat anti-mouse IgG antibody (Jackson Immunoresearch Laboratories, West Grove, PA). Results (in µg/mL) were calculated on the officially assigned values of the 89-SF reference serum (donated by C. Frash, Food and Drug Administration, Bethesda). Polyribosylribitol phosphate (PRP) IgG and subclasses were measured by using *H. influenzae* type b oligosaccharide-human serum albumin conjugate (HBOHA; Wyeth-Lederle Vaccines and Pediatrics, West Henrietta, NY) as capture antigen and Center for Biologics Evaluation and Research (Bethesda, MD) standard lot 1983 as reference serum.

**Avidity.** Relative antibody avidity was determined by a modified elution ELISA [8]. A serum dilution chosen to give an absorbance of 1.0 was loaded onto microtiter plates coated with 10 µg/mL of polysaccharide or 1 µg/mL HBOHA and left for 2 h at 37°C. Ammonium thiocyanate (NH₄SCN; Sigma, St. Louis), a chaotropic compound that interferes with the antigen-antibody reaction, was added subsequently, in concentrations ranging from 0.25 M to 4 M, and left for 15 min at 37°C. Addition of NH₄SCN does not affect the amount of polysaccharide bound to the plate. The remainder of the assay was done following the pneumococcal or PRP-specific ELISAs. The shift in the binding curve due to NH₄SCN correlates to antigen-antibody binding strength. The avidity index (AI) corresponds to the NH₄SCN concentration required to give a 50% reduction in absorbance at 492 nm. The interassay coefficient of variation was 5.5%–12.7% for pneumococcal and 5.5% for PRP ELISAs.

**Statistical methods.** Comparisons of continuous measures used the Wilcoxon signed rank test and, for paired measurements, the Mann-Whitney *U* test. An adequate response was defined as a >2-fold rise in antibody concentration. A patient was considered to be a “poor responder” if he or she had an adequate response in <3 of 10 measurements, 1 and 3 months after vaccination (5 PS-specific responses were measured at each of the 2 time points). Univariate and multivariate logistic regressions examined candidate predictors of poor response (age, sex, BMT type, underlying diagnosis, time of vaccination, low IgG2, CD4:CD8 ratio, and CD3 T cell and CD19 B cell counts). Linear regressions evaluated predictors of antibody concentration and avidity changes. Analyses were conducted using SPSS software, version 8.0 (SPSS, Chicago); *P* values are 2-tailed.

**Results**

**PS-specific immune responses.** Median age of the BMT recipients in this study was 86 months (interquartile range [IQR], 49–158), compared with 91 months (IQR, 61–139) for control subjects. Median time from BMT to vaccination was 22 months (IQR, 15–31). Median (IQR) CD3 and CD19 cell counts and CD4:CD8 ratio were 1500 cells/mm³ (1250–2045), 682 cells/mm³ (394–753), and 1.1 (0.7–1.4), respectively. Ten children with BMTs had low serum IgG2 levels.

Only 6 of the 38 BMT recipients responded to ≥3 serotypes 1 month after vaccination, compared with 6 of 9 control subjects (risk ratio, 0.24; *P* = .001). The fold-increase in antibody concentration at 1 month was more prominent among controls than among BMT recipients for all 5 serotypes (table 1). Twenty-one children with BMTs were poor responders (<3 adequate PS-specific responses of a total of 10 tested at 1 and 3 months). Furthermore, in children with BMTs, antibody re-
responses were short lived, and average concentrations returned close to baseline 9 months after vaccination (figure 1A).

Both IgG1 and IgG2 increased significantly at 1 month in BMT recipients. IgG2 was the predominant isotype (geometric mean [μg/mL], 1.48 vs. 0.31 for PS14; 0.87 vs. 0.14 for 19F). Persistent elevations over baseline were seen only for PS14-IgG2 ($P = .0003$ at 6 months; $P = .06$ at 12 months).

Ten (77%) of 13 children vaccinated <18 months after receiving a BMT responded poorly, compared with 11 (44%) of 25 children vaccinated later (odds ratio [OR], 4.24; $P = .06$).

Figure 1. Changes in geometric mean anti-pneumococcal serotypes (PS; ●) and anti-polysylribotol phosphate (PRP) IgG antibody concentration (▲) (A) and avidity (B) over time for up to 12 months after vaccination. *Significantly different ($P < .05$), compared with baseline values; +, significantly increased ($P < .05$), compared with 1-month values.
We found no difference in antibody response between autotransplant and allotransplant recipients (OR, 0.75; \( P = .70 \)). No other significant associations with demographic, clinical, and immunologic parameters were seen.

**Avidity of PS-specific antibodies.** There were modest correlations between the baseline AIs of the 5 serotypes (7/10 correlation coefficients were \( >0.3 \), with \( P < .10 \)). Baseline avidity was unrelated to age, time of vaccination, sex, or immunologic parameters \( (P > .10 \) for all correlation coefficients). AIs decreased significantly after vaccination for all 5 serotypes. Avidity stabilized at the new levels as early as 1 month after vaccination, and there was no statistically significant change for any of the 5 serotypes between 1- and 12-month values (figure 1B). No demographic, clinical, or immunologic parameters correlated with avidity changes.

The AIs for PS serotypes 3 and 23F were significantly higher at baseline in controls than in BMT recipients \( (P = .04 \) and \( P = .01 \), respectively). Among controls, the AIs for 2 of 5 serotypes decreased significantly at 1 month (table 1).

**PRP-specific immune responses.** All but 6 children achieved titers \( >1 \mu g/mL \) at 1 month; all but 1 achieved such levels at 3 months. Titers were maintained 12 months after vaccination. The fold-increase versus baseline was significantly larger for PRP than for PPV \( (P < .01 \) for all time points). Vaccination induced mostly IgG1 subclass responses. Although avidity decreased immediately after vaccination, it increased subsequently \( (P = .02 \) for 6 vs. 3 months, \( P = .03 \) for 12 vs. 6 months; figure 1B).

**Predictors of changes in PS-antibody concentration and avidity over time.** The 12-month PS-specific IgG concentration could be predicted for all 5 serotypes from 3-month concentrations alone (adjusted \( R^2 = 0.55, 0.27, 0.59, 0.16, \) and 0.50 for serotypes 3, 6B, 14, 19F, and 23F, respectively; \( P < .001, P = .02, P < .001, P = .08, \) and \( P = .002 \), respectively). Knowledge of the baseline and 1-month concentrations improved the prediction (adjusted \( R^2 = 0.67, 0.62, 0.62, 0.44, \) and 0.57, respectively; \( P < .03 \) for all models).

Avidity at 12 months after vaccination could be moderately well predicted from 3-month values alone for serotypes 3, 6B, 14, and 19F (adjusted \( R^2 = 0.33, 0.25, 0.71, \) and 0.42, respectively; \( P = .02, P = .03, P < .001, \) and \( P = .007 \), respectively), but not for serotype 23F \( (P = .54) \). Knowledge of the baseline avidity was generally not predictive of avidity at 12 months.

Baseline IgG concentrations generally did not correlate with baseline avidity. Similarly, IgG concentrations 12 months after vaccination did not correlate with the respective AIs. When all serotypes were considered, a larger decline in avidity correlated modestly with a larger increase in antibody concentration in the same period \( (r = -.22, P = .004) \).

**Discussion**

This is the first study to document the kinetics of the immune response, including IgG subclasses and avidity, to the 23-valent PPV in children who have received BMTs. Despite a limited sample size, our study suggests that these antibody responses are inadequate in this high-risk population. Criteria of an adequate immune response to PPV have not been clearly defined. Musher et al. [9] considered as responders those subjects who mounted a \( >2\) -fold increase in half the tested serotypes. With these criteria, 9% of normal subjects are poor responders. Despite more lenient criteria, most of our patients did not respond. The only other study that has been done in a similar population [2] reported 50% PPV immunogenicity in children vaccinated 1–2 years after receiving a transplant, whereas all children who were vaccinated later responded. This overestimate is probably due to lack of absorption of the nonspecific and, probably, nonprotective cell wall polysaccharide antibodies [10].

The lapse of time after BMT may predict the response. Seventy-seven percent of children vaccinated \( <18 \) months after transplantation were poor responders; however, vaccination after this period induced adequate responses in 56%, which suggests a maturation delay in the response to PPV after BMT [2]. However the IgG2 predominance suggests that this delay does not involve impaired immunoglobulin class switching. Modest associations with other parameters, such as low serum IgG2 or CD4:CD8 ratio, could have been missed because of small sample size.

Increases in antibody concentrations were short lived and disappeared within 6 months. Twelve-month levels could be predicted by the early titers and, on average, they had returned to baseline. Rubins et al. [6] reported that both young and elderly adults maintain high antibody levels for 16 months after immunization. Revaccination is recommended every 3–4 years in adults; however, according to our data, for children who have had BMTs, this period should be much shorter.

In contrast, PRP-T immunogenicity, isotype pattern, and kinetics of antibody concentration were satisfactory and similar to what is known for healthy infants receiving routine PRP-T vaccination. This finding provides further evidence for the improved immunogenicity of conjugate polysaccharide vaccines in subjects unable to respond to polysaccharide antigens.

Avidity measurements offer complementary information. High-avidity pneumococcal antibodies have better opsonizing capacity in vitro and offer better protection to mice [4]. PPV administration resulted in an early and significant decrease in AI, and there was no avidity maturation afterwards, as in the case with PRP-T. To our knowledge, this is the first study to report a detrimental effect in avidity after receipt of PPV. Two other studies [6, 11] reported no significant changes in AI in young and elderly adults after PPV administration. We also observed avidity decreases after PPV was given to 9 healthy children, which suggests a possible age-related phenomenon.

The kinetics of antibody concentration and avidity revealed that 6–12 months after PPV vaccination, antibody levels had returned to baseline, whereas avidity was lower than at baseline. Since antibody avidity may become biologically more impor-
tant at low serum antibody concentrations [12], it is possible that, during that period, children with BMTs are more susceptible to pneumococcal disease.

PPV is recommended for administration at 6 months after BMT to cover the period of highest risk for invasive disease [13]. However, the immune responses generated by PPV in this setting are poor, short lived, and associated with decreased antibody avidity, and children vaccinated so early after BMT are more likely to be poor responders. Although repeated measurements of antibody concentrations and frequent revaccination may be an option, alternative vaccines are urgently needed for children receiving BMTs. Pneumococcal conjugate vaccines have shown improved immunogenicity in subjects unable to respond to PPV [14] and may establish immunologic memory, but no data are available for their immunogenicity in children who have had BMTs. In our study population, immune responses to the conjugate PRP-T vaccine were adequate, long lasting, and associated with avidity maturation. The different kinetics of antibody concentration and avidity between a thymus-dependent and a thymus-independent polysaccharide vaccine among children receiving BMTs, in association with evidence that avidity is a surrogate of the induction of immunologic memory for conjugate vaccines [15], suggest that both markers should be used in evaluating conjugate pneumococcal vaccines in this setting.

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References