Preterm Infants’ T Cell Responses to Inactivated Poliovirus Vaccine

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Background. The antigen-specific T cell responses of preterm infants to immunization are not well understood. The aim of the present study was to compare the T cell responses of preterm infants after inactivated poliovirus vaccination with those of term infants.

Methods. We prospectively enrolled 2-month-old preterm (gestational age, <33 weeks) and term (gestational age, ≥37 weeks) infants to receive 3 doses of diphtheria-tetanus toxoids–acellular pertussis–hepatitis B virus–inactivated poliovirus vaccine. Whole blood and peripheral blood mononuclear cells (PBMCs) were stimulated with poliovirus vaccine, and memory T cell activation was analyzed by flow cytometry and lymphoproliferation, respectively. Levels of poliovirus neutralizing antibodies were measured in serum.

Results. We enrolled 33 preterm and 50 term infants. Preterm infants had fewer circulating CD4+CD45RO+ memory cells activated by staphylococcus enterotoxin B at 2 (P <.005) and 7 (P <.015) months of age. After immunization, preterm and term infants had comparable frequencies of poliovirus-specific CD4+CD45RO+CD69+IFN-γ+ memory T cells (P = .05). PBMCs from preterm infants had diminished poliovirus-specific lymphoproliferation (P <.001). Although all infants developed seroprotective poliovirus antibody titers, serotype 1 titers were lower among preterm infants (P <.03).

Conclusions. Preterm infants develop poliovirus-specific T cell responses that are comparable to those of term infants. However, they demonstrate nonspecific and poliovirus-specific functional T cell limitations, suggesting that investigations into whether T cell differences remain as preterm infants mature are warranted.

The American Academy of Pediatrics (AAP) and the Advisory Committee on Immunization Practices (ACIP) recommend vaccination of very preterm infants when they attain the appropriate chronological age to protect them against infectious diseases. Preterm infants are more susceptible to infections and severe disease because their immune systems are immature [1]. Although both T and B cell responses are necessary for prevention of clinical disease and most studies have found that preterm infants generally produce antibody responses above minimum protective levels after vaccination, the T cell responses of preterm infants to immunization have not been well investigated.

Most studies evaluating the immune response of preterm infants to immunization have been limited to comparisons of antibody levels. In general, vaccination of preterm infants with vaccines against tetanus, Haemophilus influenzae type B (Hib), poliovirus serotypes 1 and 2 [2], measles-mumps-rubella and varicella [3], pneumococcus [4, 5], and meningococcus C [6, 7] has yielded protective antibody titers. Decreased titers relative to those in term infants have been measured in other studies of preterm infant responses to poliovirus serotypes 2 [6] and 3 [2, 6], acellular pertussis [8], and...
Hib when given as part of the combined diphtheria-tetanus toxoids–acellular pertussis–Hib (DTaP-Hib) [9] or DTaP–hepatitis B virus–inactivated poliovirus/Hib (DTaP-HB-IPV/Hib) [10] vaccine. Some studies have likewise revealed lower titers of antibodies to HBV in preterm infants [10–14], although others have reported comparable antibody levels after HBV vaccination when administration of the first dose was delayed [14–16]. A recent study found that Hib titers adequately persisted into the fifth year of life in preterm infants [17], although other long-term follow-up studies have noted that differences in antibody titers between preterm and term infants persist for many years [18, 19], raising the possibility that former preterm status may result in an impaired immunologic response to vaccines that persists at least through early childhood.

Because adequate antibody production by B cells depends on T cell help, disparities in antibody responses between preterm and term infants may reflect developmental differences in T cell immunity. Relatively little is known about T cell responses to vaccines in early life. Studies have found that term infants produce T cell responses to vaccines, including pertussis [20–22], tetanus [23, 24], bacille Calmette-Guérin [25], HBV [26, 27], measles [28], and influenza [29] vaccines. Preterm infants vaccinated against influenza have a lower T cell proliferative response to influenza virus antigen than do immunized term infants [29]. Neonatal T cell responses for many vaccine antigens are diminished, compared with those in adults. Both oral poliovirus vaccine (OPV) and inactivated poliovirus vaccine (IPV) induce cellular immunity in infants and adults [30]; however, term infants produce lower levels of poliovirus-specific interferon-γ (IFN-γ) after 4 doses of OPV than do immunized adults [31].

Whether T cell responses to vaccination in preterm infants are similar to those in term infants is not well understood. The primary aim of the present study was to compare the T cell responses of preterm infants to those of term infants after the primary series of IPV given as part of a combination vaccine. Because studies have also found that very low-birth-weight infants appear to have an increased incidence of adverse events—notably cardiorespiratory events after vaccination in a neonatal intensive care unit (NICU) [32, 33]—the secondary aim of this study was to describe potential vaccine-related adverse events during the 30 days after each of 3 doses.

**METHODS**

**Subjects**

This was an open-label prospective study in which we enrolled healthy preterm (defined as gestational age of ≤33 weeks) and term (defined as gestational age of ≥37 weeks) infants from the outpatient clinics within Northern California Kaiser Permanente between January 2006 and November 2007 who were up to 10 weeks of age at the time of their 2-month well-baby appointment to receive a dose of DTaP-IPV-HBV vaccine (Pediarix; GlaxoSmithKline) at 2, 4, and 6 months of age, as well as concomitant pneumococcal (Prevnar; Wyeth) and Hib (ActHib; Sanofi Pasteur) conjugate vaccines. We obtained blood before initial immunization at 2 months of age (prevaccination) and again 1 month after the third dose of DTaP-IPV-HBV, at 7 months of age (postvaccination). We limited recruitment of preterm infants to those of ≤33 weeks’ gestational age because we wished to focus on a younger preterm population while still achieving our target sample size. Infants who had severe neurologic damage, genetic disorders, a history of immunosuppressive medications were excluded from study participation. The institutional review boards at Northern California Kaiser Permanente, Stanford University School of Medicine, and the Food and Drug Administration approved this study. Written informed consent was obtained from the parents or guardians of all infants before any study-related activities were conducted.

**Assays for T Cell Immune Responses**

Because of limitations in the amounts of blood obtained, we did not perform every assay for every infant.

**Cytokine flow cytometry assay.** All cytokine flow cytometry assays were performed as described elsewhere [34]. Aliquots of 200 μL of fresh heparinized whole blood were stimulated with 2.2 × 10^3–4.4 × 10^3 plaque-forming units of Sabin OPV serotype 3 strain (GenBank accession no. AY184221), uninfected Vero cell lysate, phosphate-buffered saline (PBS), or 2 μg of staphylococcus enterotoxin B (SEB) (Sigma). Costimulatory monoclonal antibodies to CD28 and CD49d (BD Immunocytometry Systems), and phycoerythrin-conjugated anti–IFN-γ, fluorescein isothiocyanate–conjugated anti–CD4, allophycocyanin-conjugated anti-CD69 (BD Immunocytometry Systems), and phycoerythrin-conju-
Neutralizing Antibody Titers

Titers of neutralizing antibodies against poliovirus serotypes 1, 2, and 3 were measured in serum obtained before immunization and 1 month after the third dose, using serum that had been frozen at \(-80^\circ\text{C}\). A microneutralization test was performed in HEp-2c cells, using 100 tissue culture infective doses of reference viruses as recommended by the World Health Organization [37].

**Statistical Methods**

Pre- and postvaccination T cell responses were compared using the paired \(t\) test, whereas preterm and term postvaccination T cell responses and geometric mean titers (GMTs) were compared using the unpaired \(t\) test. We analyzed GMTs in 2 groups: the first group included all infants regardless of whether passive maternal antibodies were present at 2 months, and the second group was a subset of the first and excluded infants who had detectable antibody titers before receipt of the first vaccine dose at 2 months of age. The Fisher exact statistic was used to compare the proportion of infants with a SI of \(\geq 3\) or <3. Pearson correlation coefficients were calculated using Prism software (version 5.0a; GraphPad).

**Postvaccination Adverse Events**

We used the electronic medical records in the Kaiser Permanente Medical Care Program to capture the prespecified medically attended events (MAEs) of fever, seizures, rash, swelling, and irritability by evaluating all infants for the presence of text-string diagnoses corresponding to these outcomes in the electronic records, as well as all emergency department visits and hospitalizations within a 30-day postvaccination risk window. We compared the frequency of MAEs between preterm and term infants during the 30-day postvaccination period using the Fisher exact statistic. We compared selected MAEs among preterm infants during the risk period (postvaccination days 0 to 30) to the number of MAEs that occurred during the control period (postvaccination days 31–60) using the self-controlled case series approach [38]. We used Stata software (version 8.2; StataCorp) for all analyses.

**RESULTS**

We enrolled 33 preterm and 50 term infants. Sixteen infants (4 preterm and 12 term) did not complete the study for the following reasons: inability to obtain blood (\(n = 3\)), withdrew because of parental request (\(n = 4\)), lost to follow-up (\(n = 8\)), and loss of health plan coverage (\(n = 1\)). Among those who completed the study, there were no differences in the age at which the infants were first vaccinated, sex, or age at postimmunization visit (Table 1). Samples from 5 additional infants (2 preterm and 3 term) were not included because of insufficient volume, the samples not being received within 48 h of the blood draw, or invalid assay. Valid postvaccination results were thus obtained from 27 preterm (82%) and 35 term (70%) infants.

As shown in Table 2, at both 2 and 7 months of age preterm and term infants had similar frequencies of circulating CD4+ T cells; however, 7-month-old preterm infants had fewer circulating CD4+CD69+ memory T cells than did 7-month-old term infants (\(P = .005\)). In response to stimulation with SEB, preterm infants had lower mean frequencies of CD4+CD69+IFN-\(\gamma\) T cells than did term infants at both 2 months (\(P = .015\)) and 7 months (\(P = .005\)) of age (Table 2).

To assess vaccine-specific responses, we evaluated poliovirus type 3–specific memory T cell responses. Both preterm and term infants developed a significant increase in poliovirus-specific CD4+CD69+IFN-\(\gamma\) T cell frequencies relative to prevaccination responses, with increases in poliovirus-spe-

**Table 1. Infant Characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Preterm ((n = 33))</th>
<th>Term ((n = 50))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age, weeks</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>31.3 ± 1.4</td>
<td>39.5 ± 1.0</td>
</tr>
<tr>
<td>Range</td>
<td>28–33.6</td>
<td>37–41.9</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>52</td>
<td>48</td>
</tr>
<tr>
<td>Age at first vaccination, mean ± SD, weeks</td>
<td>8.9 ± 0.9</td>
<td>8.8 ± 1.0</td>
</tr>
<tr>
<td>Age at postvaccination analysis, mean ± SD, weeks</td>
<td>32.1 ± 2.3</td>
<td>31.8 ± 2.5</td>
</tr>
</tbody>
</table>

**NOTE.** SD, standard deviation.

\(a\) Excludes 16 infants (4 preterm and 12 term) who did not complete the study. See the main text for details.
cific CD4+CD45RO+CD69+ and CD4+CD45RO+CD69+IFN-γ memory T cell responses (Figure 1). We then compared the poliovirus type 3–specific T cell responses between preterm and term infants. Comparison of poliovirus-specific T cell responses after 3 doses of vaccine revealed that preterm and term infants developed comparable mean frequencies of poliovirus-specific CD4+CD69+ (P = .81), CD4+CD69+IFN-γ (P = .49), CD4+CD45RO+CD69+ (P = .33), and CD4+CD45RO+CD69+IFN-γ+ (P = .79) memory T cell responses (Figure 2).

To test for differences in the poliovirus proliferative responses, we assayed poliovirus type 3–specific cell-mediated immunity. Compared with baseline, PBMCs from preterm infants had significantly more poliovirus-specific proliferation after 3 doses of IPV (mean SI, 1.2 for prevaccination vs 2.9 for postvaccination; P = .009). After vaccination, however, preterm infants were significantly less likely to have a positive SI (≥3) than were term infants (9/15 [60%] vs 18/22 [82%]; P = .003), and mean poliovirus type 3–specific proliferation after vaccination was significantly lower in preterm infants than in term infants (mean SI, 2.9 vs 5.7; P < .001) (Figure 3).

We next assessed the neutralizing antibody response to all 3 poliovirus serotypes after vaccination. All infants with serum available for testing (n = 22 for preterm and n = 28 for term) had a seroprotective antibody response (≥1:8) to all 3 poliovirus serotypes. There were no significant differences in GMTs to poliovirus serotypes 2 and 3 between the 2 groups. In contrast, the GMT to poliovirus serotype 1 was significantly lower in both the group of all preterm infants (GMT, 1338 [95% confidence interval [CI], 852–2103] for preterm vs 2609 [95% CI, 1711–3980] for term; P = .03) and the subset of preterm infants without passive maternal antibodies at baseline (GMT, 1070 [95% CI, 477–2404] for preterm vs 3940 [95% CI, 2308–6724] for term; P = .005) (Table 3). There was no correlation between the GMT to poliovirus serotype 3 and the frequency of poliovirus type 3–specific responder T cells for either preterm or term infants. There was also no correlation between the GMT to poliovirus serotype 3 and the SI response for term infants. Among preterm infants, however, the GMT to poliovirus serotype 3 and the SI response were significantly correlated (Pearson correlation coefficient, 0.54; P = .02).

To compare the safety of this combination vaccine between term and preterm infants, we analyzed adverse events during the 30 days after each dose. During the 30 days after vaccination, there were no MAEs for fever, seizure, or swelling for either preterm or term infants after any vaccine dose. Self-controlled case series analyses revealed no increase in MAEs among preterm infants for fever (0 during the risk period and 4 during the control period; P = .13), rash (1 during risk and 11 during control; P = .006), or irritability (2 during risk and 1 during control; P > .99). Similar self-controlled analyses among term infants did not indicate any increased risk of fever, rash, or irritability (data not shown). During the 30 days after any dose, diagnoses included 1 rash per 98 doses for preterm and 6 rashes per 149 doses for term infants (P = .25), as well as 2 cases of irritability per 98 doses for preterm and 0 cases of irritability for term infants (P = .16). There were a total of 9 emergency department visits (4 per 98 doses for preterm and 5 per 149 doses for term; P = .74) during the 30 days after any poliovirus vaccine dose. There were no hospitalizations within 30 days for either group.

**DISCUSSION**

In support of current ACIP and AAP recommendations to routinely vaccinate preterm infants at the appropriate chronological age, this study demonstrates not only that preterm infants develop seroprotective antibody levels for all 3 poliovirus serotypes administered in a combination vaccine but also that preterm infants respond with poliovirus–specific responder T cells and lymphoproliferation. A previous study reported the development of poliovirus cellular immunity after either OPV or IPV administration among infants born at term [30], whereas another study reported that T cells from Gambian

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**Table 2. Unstimulated and Staphylococcus Enterotoxin B (SEB)–Stimulated Circulating Memory T Cell Frequencies in Preterm and Term Infants**

<table>
<thead>
<tr>
<th>Age, cell type</th>
<th>Preterm</th>
<th>Term</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ cells</td>
<td>53.41 ± 9.25 (n = 22)</td>
<td>49.07 ± 8.32 (n = 46)</td>
<td>.06</td>
</tr>
<tr>
<td>CD4+CD45RO+CD69+ cells</td>
<td>3.98 ± 1.56 (n = 22)</td>
<td>4.71 ± 2.17 (n = 46)</td>
<td>.17</td>
</tr>
<tr>
<td>SEB-stimulated CD4+CD69+IFN-γ+ cells</td>
<td>0.28 ± 0.25 (n = 23)</td>
<td>0.48 ± 0.42 (n = 46)</td>
<td>.015</td>
</tr>
<tr>
<td>7 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ cells</td>
<td>51.05 ± 8.43 (n = 27)</td>
<td>48.04 ± 8.79 (n = 35)</td>
<td>.18</td>
</tr>
<tr>
<td>CD4+CD45RO+CD69+ cells</td>
<td>5.89 ± 2.82 (n = 27)</td>
<td>7.97 ± 2.75 (n = 35)</td>
<td>.005</td>
</tr>
<tr>
<td>SEB-stimulated CD4+CD69+IFN-γ+ cells</td>
<td>0.38 ± 0.45 (n = 27)</td>
<td>0.63 ± 0.48 (n = 35)</td>
<td>.05</td>
</tr>
</tbody>
</table>

**Note.** Data are mean percentages ± standard deviations. Boldface type indicates statistically significant differences. IFN-γ, interferon-γ.
Figure 1. Generation of a poliovirus serotype 3–specific T cell response by preterm and term infants after vaccination. Bars represent grouped mean percentages of CD4+ and memory (CD45RO+) T cells that expressed CD69 and produced interferon-γ (CD69+IFN-γ+) in preterm (A) and term (B) infants. Error bars represent standard errors of the mean. *P* values were calculated using the paired *t* test.
infants immunized with OPV did respond but had a decreased IFN-γ response to all 3 poliovirus serotypes, compared with T cells from immunized adults [31]. The present study establishes that preterm infants, similar to term infants, generate a poliovirus type 3–specific T cell response and that the frequency of circulating poliovirus–specific responder T cells is comparable between preterm and term infants. This study provides reassuring evidence for the current poliovirus vaccination recommendation for preterm infants.

The findings of this study, however, revealed differences between the T cell profiles of term and preterm infants, with preterm infants having significantly fewer circulating CD4+CD45RO+ memory T cells than term infants at 7 months of age. Comparisons to other studies evaluating the immunological profile of preterm infants are challenging, because limited data exist and most previous studies examined lymphocytes from cord blood and/or used different methods [39–41]. Berrington et al [42] recently reported the results of assays of lymphocytes from preterm and term infants in which similar methods and sampling times were used. Compared with Berrington and colleagues’ study, our study demonstrated similar overall frequencies of circulating CD4+ T cells for 2- and 7-month-old preterm and term infants. Unlike their results, we did not detect significant differences in the frequencies of circulating CD4+ T cells between preterm and term infants at 2 and 7 months of age, but we did observe significantly lower frequencies of CD4+CD45RO+ T cells in preterm than in term infants at 7 months of age. Given that the mean gestational ages of the preterm populations in these 2 studies differed (31.3 weeks for the present study vs 26.5 weeks for the study of Berrington and colleagues), we speculate that these subtle differences may relate to immune maturation, especially considering the small size of these studies and individual factors (such as environmental stressors) that could influence the rate at which the cells of premature infants mature. In general, both studies illustrate differences in circulating T cell populations between term and preterm infants.

The results of the present study further indicate that T cells from preterm infants do not respond as strongly to stimulation with SEB as do T cells from term infants. SEB activates T cells by cross-linking the variable region of the β chain (Vβ) on the T cell receptor [43]. These diminished responses may represent
variations in the T cell receptors of preterm infants, but additional evaluation is needed to understand these findings mechanistically. Our demonstration of lower frequencies of circulating memory T cells and diminished SEB responses in preterm infants than in similarly aged term infants suggests that preterm infants, at least early in life, may have nonspecific functional deficits in their T cells.

Interestingly, the present study also revealed antigen-specific T cell deficiencies in preterm infants. Despite the demonstration of equivalent frequencies of poliovirus type 3–specific responder memory T cells, preterm infants had lower poliovirus type 3–specific lymphoproliferation than did term infants. Unlike responder cell frequency measurements, which represent a short stimulation period, cell-mediated proliferation takes place over several days (allowing stimulated T cells to clonally expand) and depends on the production of essential cytokines (IFN-γ and interleukins 12 and 15) [44]. Recent studies have suggested that antigen-presenting cells (APCs) in term infants may be less capable than those in adults of producing the vital cytokines needed for T cell clonal expansion [35]. The finding of decreased PBMC proliferation despite the presence of adequate responder memory T cells suggests that although T cells from preterm infants can appropriately respond to initial poliovirus stimulation, they possess a functional deficiency during the secondary T cell expansion phase, perhaps because of a diminished ability to produce key cytokines. It will be important to assess in follow-up studies whether such early-life deficiencies in preterm infants are sustained as these infants age and whether the persistence of the memory response to vaccination is affected. The real question is whether any clinical implications spring from such differences.

Finally, although preterm infants in this study developed seroprotective titers to all 3 poliovirus serotypes, the GMT to poliovirus serotype 1 was significantly lower in preterm infants. Previous studies have noted decreased titers of antibody to poliovirus serotypes 2 [6] and 3 [2, 6] in preterm infants, and the GMTs to poliovirus serotypes 2 and 3 in the present study were also lower in the subset of preterm infants who did not have passively acquired maternal antibodies, although the difference did not quite reach statistical significance. Furthermore, poliovirus serotype 3–specific antibody titers and lymphoproliferation were correlated in preterm infants. There are a number of potential mechanisms by which a decreased humoral response could be related to decreased lymphoproliferation in preterm infants. First, impaired functioning of helper T cells in preterm infants could result in decreased humoral responses. Alternatively, because APCs play an essential role in initiating and modulating T and B cell responses [45], decreased humoral responses might be expected if preterm infants have limitations in the function of APCs. This is particularly intriguing in light of our speculation about a possible role for APC deficiencies in decreased cell-mediated proliferation in preterm infants. A third hypothesis involves alterations in the expression of the CD40 ligand (CD40-L) on the T cells of preterm infants. The interaction between CD40-L and dendritic and B cells is important to the humoral response, and term newborns have decreased expression of CD40-L on T cells compared with adults [46]. Whether preterm and term infants have equivalent expression of CD40-L is unknown, but it is possible that preterm infants with deficiencies involving CD40-L that could result in a diminished humoral response to vaccination. Given the number of potential mechanisms by which decreased antibody titers could occur, it is apparent that a better understanding of the innate and adaptive immune responses to vaccines in preterm infants is needed.

One limitation of the present study is that our preterm population mainly reflected infants who were healthy enough to be discharged from the NICU before receiving their 2-month
vaccinations. Whether extremely premature infants who are still in the NICU at the time of their 2-month vaccinations have T cell responses comparable to those of the premature population studied here is unknown. Also, because receipt of immunosuppressive medications was an exclusion criterion, this study did not evaluate whether ongoing steroid use further affects the immune responses of preterm infants. Finally, our failure to observe differences in the frequencies of adverse events between preterm and term infants may have resulted from the study being underpowered to detect such differences, given the small sample size.

In summary, in support of current immunization recommendations, we found that preterm infants vaccinated with IPV as part of a licensed combination vaccine develop both T cell responses to poliovirus serotype 3 that are comparable to those of term infants and seroprotective antibody titers to all 3 poliovirus serotypes. No differences in adverse events after immunization were seen between preterm and term infants within 30 days of each immunization. Preterm infants demonstrate both nonspecific and poliovirus-specific functional T cell limitations, necessitating additional studies to assess whether these deficiencies have clinical implications.

Acknowledgments

This study was conducted as part of the Centers for Disease Control and Prevention–supported Clinical Immunization Safety Assessment Network. We thank the children and their parents at Northern California Kaiser Permanente who participated in this study. We are also grateful to Dr Rima Hana Wakim for providing assistance with laboratory assays, to both Dr David Schnurr and the California Department of Health for providing the OPV serotype 3 vaccine strain, and to Dr Devasena Gnanashanmugam for generously allowing us to use the OPV serotype 3 strain provided to her.

References