Maternal HIV Infection and Antibody Responses Against Vaccine-Preventable Diseases in Uninfected Infants

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INFECTIOUS DISEASES ACCOUNT FOR nearly 6 million deaths worldwide annually in children younger than 5 years.1 Immunization against vaccine-preventable infections therefore remains essential to achieving Millennium Development Goal 4, which is to reduce childhood mortality by two-thirds.2 Before acquisition of immunity, infants are protected by maternal IgG transferred across the placenta. Maternal antibody levels, immunization, infection, and infant gestational age can influence the efficiency of this process.3-7 Although maternal antibody is essential to protect the infant in the first months of life, maternal-specific antibody can also interfere with the infant’s own response to vaccination.8

The high prevalence of maternal human immunodeficiency virus (HIV) in many parts of the resource-poor world, coupled with successful programs to reduce mother-to-child transmission of HIV, has led to increasing numbers of HIV-exposed infants who are not HIV-infected themselves (ie, HIV-exposed infants).9 These infants and children represent a vulnerable group with increased rates of lower respiratory tract infection and meningitis and up to

Context Altered immune responses might contribute to the high morbidity and mortality observed in human immunodeficiency virus (HIV)–exposed uninfected infants.

Objective To study the association of maternal HIV infection with maternal- and infant-specific antibody levels to Haemophilus influenzae type b (Hib), pneumococcus, Bordetella pertussis antigens, tetanus toxoid, and hepatitis B surface antigen.

Design, Setting, and Participants A community-based cohort study in Khayelitsha, Western Cape Province, South Africa, between March 3, 2009, and April 28, 2010, of 109 HIV-infected and uninfected women and their infants. Serum samples from 104 women and 100 infants were collected at birth and samples from 93 infants were collected at 16 weeks.

Main Outcome Measure Level of specific antibody in mother–infant pairs at delivery and in infants at 16 weeks, determined by enzyme-linked immunosorbent assays.

Results At birth, HIV-exposed uninfected infants (n=46) had lower levels of specific antibodies than unexposed infants (n=54) did to Hib (0.37 [interquartile range {IQR}, 0.22-0.67] mg/L vs 1.02 [IQR, 0.34-3.79] mg/L; P<.001), pertussis (16.07 [IQR, 8.87-30.43] Food and Drug Administration [FDA] U/mL vs 36.11 [IQR, 20.41-76.28] FDA U/mL; P<.001), pneumococcus (17.24 [IQR, 11.33-40.25] mg/L vs 31.97 [IQR, 18.58-61.80] mg/L; P=.02), and tetanus (0.08 [IQR, 0.03-0.39] IU/mL vs 0.24 [IQR, 0.08-0.92] IU/mL; P=.006). Compared with HIV-uninfected women (n=58), HIV-infected women (n=46) had lower specific antibody levels to Hib (0.67 [IQR, 0.16-1.54] mg/L vs 1.34 [IQR, 0.15-4.82] mg/L; P=.009) and pneumococcus (33.47 [IQR, 4.03-69.43] mg/L vs 50.84 [IQR, 7.40-118.00] mg/L; P=.03); however, no differences were observed for antipertussis or antitetanus antibodies. HIV-exposed uninfected infants (n=38) compared with HIV-unexposed infants (n=55) had robust antibody responses following vaccination, with higher antibody responses to pertussis (270.1 [IQR, 84.4-355.0] FDA U/mL vs 91.7 [IQR, 27.9-168.4] FDA U/mL; P=.006) and pneumococcus (47.32 [IQR, 32.56-77.80] mg/L vs 14.77 [IQR, 11.06-41.08] mg/L; P=.001).

Conclusion Among South African infants, antenatal HIV exposure was associated with lower specific antibody responses in exposed uninfected infants compared with unexposed infants at birth, but with robust responses following routine vaccination.

JAMA. 2011;305(6):576-584

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These antibodies. A number of factors are likely to contribute to this increased vulnerability, including socioeconomic factors, but immunological phenomena might also be important.

To design appropriate interventions for these vulnerable infants, it is important to understand how maternal HIV infection influences infant susceptibility to common pathogens. We therefore studied the association of maternal HIV infection with maternal- and infant-specific antibody levels. Because absolute levels of antibody that associate with protection against infection are poorly defined for a number of specific antibodies, we assessed how maternal HIV affects both the magnitude and putative protective levels of these antibodies.

METHODS

Study Setting

The study was conducted between March 3, 2009, and April 28, 2010, in a community health center in Khayelitsha, Western Cape Province, South Africa, a rapidly expanding urban informal settlement. In this context, all women are offered voluntary counseling and testing for HIV at antenatal care registration; the participation is consistently close to 100%. In 2009, the HIV prevalence among women attending antenatal clinics was 32%, with reported vertical transmission of 3.3%. During the study period, the Prevention of Mother to Child Transmission program consisted of dual therapy for mothers and infants, starting with the administration of zidovudine at 28 or more weeks’ gestation, then zidovudine for 1 month to the infant and a single dose of nevirapine to both mother and infant. Mothers were eligible for highly active antiretroviral treatment if their CD4 count was less than 200 cells/µL. Exclusive infant feeding options were encouraged and mothers were provided with free formula for 6 months if they chose exclusive formula feeding.

The study was approved by the Universities of Cape Town and Stellenbosch, South Africa, and the National Health Service Research Ethics Committee, England. Our study was nested in a cohort study investigating the influence of maternal HIV and mycobacterial infection on infant immune responses to BCG vaccination. The BCG vaccination (Danish strain 1331, Statens Serum Institute, intradermal vaccine) was delayed until 6 weeks of age to allow for determination of infant HIV infection and to avoid BCG vaccination of HIV-infected infants and vaccine adverse events. Infants received all other routine vaccines according to the South African Expanded Program on Immunization schedule: oral polio vaccine (Sanofi Pasteur, Lyon, France) at birth; combination diphtheria, tetanus toxoid, and pertussis vaccine, and Haemophilus influenzae type b vaccine (DTP-Hib; Sanofi Pasteur); hepatitis B (Heber Biotec, Havana, Cuba); and oral polio vaccine at 6, 10, and 14 weeks. From July 2009, pneumococcal 7-valent conjugate (Wyeth, Andover, Massachusetts) and rotavirus vaccinations (GlaxoSmithKline, Rixensart, Belgium) were administered at 6 and 14 weeks, and diphtheria, tetanus toxoid, and acellular pertussis vaccine combined with inactivated polio vaccine and Hib (DTaP-IPV/Hib; Sanofi Pasteur) replaced the DTP-Hib vaccine. Tetanus immunization is not routinely administered to pregnant women in this setting; therefore, no vaccines were administered to participating mothers.

Eligibility

Women were eligible if they had delivered a healthy infant at the Site B Maternal Obstetric Unit within the previous 24 hours, knew the result of the HIV test at antenatal care registration, and were willing and able to provide written informed consent for themselves and their infant. Mothers were excluded if they were younger than 18 years (2 women), planning to move away during the study period (8 women), did not intend to return to the routine Site B baby clinic for ongoing care (15 women), or had evidence of active tuberculosis or were on tuberculosis treatment (1 woman), or had a current household or other close tuberculosis contact (1 woman). Infants weighing less than 2.5 kg or estimated at less than 36 weeks’ gestation (8 infants), with acute illness (1 infant), or part of a twin birth (2 infants) were excluded. Consecutive eligible women were enrolled irrespective of their HIV status. Once sufficient numbers of HIV-uninfected women were reached (approximately 50% of the sample), HIV-infected women were consecutively enrolled. A study nurse obtained written informed consent in the participants’ home language.

Study Measures

A venous blood sample was collected from the mother and infant within 24 hours of delivery and transported to the laboratory within 4 hours. All infants had a further venous blood sample collected at 16 weeks. Mothers who tested negative for HIV during pregnancy had a rapid HIV test (Abbott Determine HIV-1/2, Toyko, Japan) at enrollment with pretest and posttest counseling to confirm their HIV status. The HIV-exposed infants had an HIV polymerase chain reaction (Amplicor HIV-1 DNA kit, version 1.5; Roche Molecular Systems Inc, Branchburg, New Jersey) performed at ages 4 and 16 weeks. Infant vaccination status was verified from vaccination cards (“Road to Health” records). Serum was separated and stored at −80°C for analysis by standard commercial enzyme-linked immunosorbent assays by researchers blinded to maternal HIV infection status and personal information.

Laboratory Assays

Hib capsular polysaccharide and pneumococcal capsular polysaccharide specific IgG were measured using VaccZyme Human Anti-Hib and Anti-PCP Enzyme Immunoassay kits (MK016 and MK012, The Binding Site Ltd, Birmingham, England). Microwells in the pneumococcal assay were supplied precoated with pneumococcal
capsular polysaccharide antigens 1-5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, 33F, and incorporated C-polysaccharide antibody absorption, which confers limited protection against pneumococcal infection. Specific IgG to Bordetella pertussis (pertussis) and tetanus toxoid were measured using SERION enzyme-linked immunosorbent assays classic kits (ESR120G and ESR108G Serion Immunodiagnostica GmbH, Würzburg, Germany). Hepatitis B surface antigen was measured using an Axsym HBsAg (V2) kit (Abbott, Wiesbaden, Germany) in a fully automated system. All reagents, including controls, were supplied with the commercial kits and manufacturer's instructions were followed.

Anti-Hib antibody titers of more than 1.0 mg/L were regarded as protective; pertussis titers of more than 30 Food and Drug Administration (FDA) U/mL were regarded as positive (defined by the manufacturer); tetanus antibody levels were classified as providing sufficient protection if more than 0.1 IU/mL; and more than 10 mLU/mL was regarded as seropositive and protective against hepatitis B infection. No level of protective immunity has been established for a collective response to multiple pneumococcal serotypes.

Data Management and Statistical Analysis

Statistical analyses were completed using SPSS version 18 (SPSS Inc, Chicago, Illinois) and GraphPad Prism version 5.0a (GraphPad Software Inc, La Jolla, California). Two-sided $P<.05$ was considered significant. All comparisons were prespecified except for the comparison of infants who had not received all vaccinations, which was post hoc.

The magnitude of specific antibody response between groups was compared using the unpaired $t$ test when data were normally distributed; interquartile ranges (IQRs) are shown. When the distribution was nonnormal, data were log transformed; the unpaired $t$ test was used if resulting distributions were normal; and the Mann-Whitney test was used for nonnormal data. Simple correlations were assessed using Pearson or Spearman correlation in the case of normal or nonnormal distribution, respectively. A multiple linear regression model was used to assess the relationship between the magnitude of maternal and infant Hib, pertussis, pneumococcal, and tetanus responses at delivery in relation to maternal HIV status, treating maternal age, gravidity, and household type (informal structure or brick house), a proxy for socioeconomic status in this community, as covariates. Infant sex and birth weight were used as additional covariates in analyses of infant responses at birth. All independent variables were entered into the model simultaneously (forced entry method). Proportions were compared using the Fisher exact test; if any cell contained a value of zero, 1.0 was added to all cells before testing was performed. Hepatitis B specific antibody data had a binomial distribution and therefore only the proportion of participants with seroprotective levels of hepatitis B-specific antibody results were analyzed. Placental transfer was defined as the ratio of infant-to-mother specific IgG concentration at birth. Missing data were excluded from analysis. We did not adjust for multiple comparisons.

Sample size was determined for the cohort study; this study was powered to investigate differences between antibody responses in HIV-exposed and HIV-unexposed infants of at least 30%, with the prespecified hypothesis that the magnitude of responses would be lower in HIV-exposed infants.

RESULTS

Participant Characteristics

Of 120 eligible mother-infant pairs, 11 mothers declined to participate; therefore, 109 maternal-infant pairs were enrolled (91% participation rate). Of these pairs, 47 mothers (43%) were infected with HIV and 62 (57%) were uninfected. All women testing negative for HIV at their antenatal care registration had a further repeat negative HIV test at delivery. Samples were collected from 105 mothers (96% of the maternal sample; 47 were infected and 58 were uninfected with HIV) at delivery, and from 101 infants (93% of the infant sample; 47 were exposed and 54 were unexposed to HIV) at birth. Sample volumes were insufficient for 4 women and 8 infants. One infant (1%) was determined to be infected with HIV at 4 weeks and was referred for rapid initiation of antiretroviral treatment (mother-infant pair subsequently was excluded from analysis). Follow-up samples were available for 94 infants (87%; 38 were exposed and 55 were unexposed to HIV) at a mean postnatal age of 16.4 weeks (SD, 1.7). One late follow-up sample was excluded from the analysis (collected at 28 weeks after birth). The final analysis was based on samples from 104 women and 100 infants collected at birth and samples from 93 infants collected at 16 weeks.

Characteristics of the study cohort are shown in Table 1. All HIV-infected women chose exclusive formula replacement feeding. The mean (SD) CD4 count among the HIV-infected women was 474 (252) cells/µL and the median (IQR) viral load was 800 (357-6000) copies/mL. Seven women had CD4 counts of less than 200 cells/µL; 3 of these were taking highly active antiretroviral treatment at enrollment and 4 were referred to commence highly active antiretroviral treatment following delivery.

Infant-Specific Antibody Responses at Birth

At birth, HIV-exposed uninfected infants had significantly lower specific antibody levels compared with unexposed infants to Hib (0.37 [IQR, 0.22-0.67] mg/L vs 1.02 [IQR, 0.34-3.79] mg/L; $P<.001$), pertussis (16.07 [IQR, 8.87-30.43] FDA U/mL vs 36.11 [IQR, 20.41-76.28] FDA U/mL; $P<.001$), pneumococcus (17.24 [IQR, 11.33-40.25] mg/L vs 31.97 [IQR, 18.58-61.80] mg/L; $P=.02$), and teta-
nus (0.08 [IQR, 0.03-0.39] IU/mL vs 0.24 [IQR, 0.08-0.92] IU/mL; \( P = .006 \)) (Figure 1).

These lower levels observed in HIV-exposed infants at birth corresponded with a lower proportion of HIV-exposed infants with levels considered to be protective against Hib (17% vs 52%; \( P < .001 \)), pertussis (24% vs 37%; \( P = .001 \)), tetanus (43% vs 74%; \( P = .002 \)), and hepatitis B (21% vs 54%; \( P = .01 \)).

In a multiple linear regression model for factors associated with magnitude of specific antibody response at birth, HIV exposure remained associated with reduced Hib titers (unstandardized regression coefficient \( b = 0.49; \) SE, 0.12; \( P < .001 \)), pertussis \((b = 0.38; \) SE, 0.08; \( P < .001 \)), pneumococcus \((b = 0.24; \) SE, 0.10; \( P = .01 \)), and tetanus \((b = 0.52; \) SE, 0.16; \( P = .002 \)) levels (eTable 1, available at http://www.jama.com). There was no association with maternal age, gravidity, housing structure, infant sex or birth weight for Hib, pneumococcus, and tetanus levels, but increased maternal age was associated with higher pertussis-specific antibody titers \((b = 0.02; \) SE, 0.01; \( P = .03 \)) (eTable 1).

Maternal-Specific Antibody Responses

To investigate the mechanisms associated with infant response, we measured specific maternal antibody levels in parallel. HIV-infected women had lower specific antibody levels than HIV-exposed women to Hib \((0.67 [IQR, 0.16-1.54] \text{mg/mL vs 1.34 [IQR, 0.15-4.82]} \text{mg/mL; \( P = .009 \)}) and pneumococcus \((33.47 [IQR, 4.03-69.43] \text{mg/mL vs 50.84 [IQR, 7.40-118.00]} \text{mg/mL; \( P = .03 \)}). No differences were observed for pertussis \((22.07 [IQR, 12.48-29.67] \text{FDA U/mL vs 23.64 [IQR, 12.87-54.68]} \text{FDA U/mL; \( P = .26 \)}) or tetanus \((0.09 [IQR, 0.03-0.33] \text{IU/mL vs 0.15 [IQR, 0.06-0.67]} \text{IU/mL; \( P = .12 \)}) between HIV-infected and uninfected women. In a multiple regression model for factors associated with level of maternal-specific antibody response, maternal HIV infection remained associated with low Hib and pneumococcal antibody levels; however, there was no significant association with maternal age, gravidity, or housing structure for any of the specific antibody responses (eTable 1).

HIV-infected women were less likely to have anti-Hib antibodies levels considered to be protective (35% vs 59%; \( P = .02 \)). The proportion of women with protective antibody levels against pertussis (24% vs 38%; \( P = .14 \)), tetanus (47% vs 64%; \( P = .11 \)), or hepatitis B (26% vs 33%; \( P = .52 \)) was similar in HIV-infected and HIV-uninfected women. The overall proportion of all women with protective antibody levels was low for pertussis (32%), tetanus (41%), and hepatitis B (30%).

In HIV-infected women, CD4 count was positively correlated with the level of antibody to pertussis \((r = 0.31; \) \( P = .04 \)), pneumococcus \((r = 0.33; \) \( P = .03 \)), and tetanus \((r = 0.37; \) \( P = .01 \)), but not with Hib \((r = -0.07; \) \( P = .63 \)) (eTable 2). There was no correlation between maternal HIV viral load and any specific antibody level (eTable 2).

In HIV-infected women and their infants, the correlation between maternal- and infant-specific antibody responses were statistically significant for Hib \((r = 0.91; \) \( P < .001 \)), pertussis \((r = 0.78; \) \( P < .001 \)), pneumococcus \((r = 0.86; \) \( P < .001 \)), and tetanus \((r = 0.95; \) \( P < .001 \)). In HIV-negative women, the correlation between maternal and infant responses were also statistically significant for Hib \((r = 0.95; \) \( P < .001 \)), pertussis \((r = 0.89; \) \( P < .001 \)), pneumococcus \((r = 0.80; \) \( P < .001 \)), and tetanus \((r = 0.93; \) \( P < .001 \)).

Association of Maternal HIV With Placental Transfer of Specific Antibody

The proportion of maternal-specific antibody transferred across the placenta to infants was significantly reduced among HIV-infected women and their infants. Using infant:maternal anti-

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Table 1. Characteristics of HIV-Infected and HIV-Uninfected Women and Their Uninfected Infants

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HIV-Infected Women and Exposed Infants (n = 46 at Birth)</th>
<th>HIV-Uninfected Women and Unexposed Infants (n = 54 at Birth)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age, median (IQR), y</td>
<td>27.0 (24.0-31.3)</td>
<td>24.0 (20.0-27.5)</td>
<td>.002b</td>
</tr>
<tr>
<td>Maternal primigravity</td>
<td>10 (21)</td>
<td>28 (45)</td>
<td>.01b</td>
</tr>
<tr>
<td>Female infant sex</td>
<td>25 (57)</td>
<td>33 (57)</td>
<td>.68b</td>
</tr>
<tr>
<td>Infant delivered by normal vaginal delivery</td>
<td>46 (100)</td>
<td>54 (100)</td>
<td>.99</td>
</tr>
<tr>
<td>Birth weight, mean (SD), kg</td>
<td>3.16 (0.35)</td>
<td>3.23 (0.44)</td>
<td>.38c</td>
</tr>
<tr>
<td>Weight at 16 wks, mean (SD), kg</td>
<td>6.81 (0.93)</td>
<td>6.60 (0.93)</td>
<td>.29c</td>
</tr>
<tr>
<td>Exclusive breast feeding at birtha</td>
<td>0</td>
<td>54 (100)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Exclusive breast feeding at 16 wksb</td>
<td>0</td>
<td>23 (42)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Household lives in informal structurec</td>
<td>36 (78)</td>
<td>34 (54)</td>
<td>.02b</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.
aMann-Whitney U test.
bFisher exact test.
cTest.
dWeight at 16 weeks available for all infants followed up to 16 weeks (38 HIV-exposed infants and 55 HIV-unexposed infants).
eNo breast feeding was reported at any study visit for HIV-exposed infants.
fAn informal structure is a shack constructed of materials such as wood and corrugated iron.
body ratios as a proxy for placental transfer, HIV-infected women had significant reductions in placental transfer of 23% for Hib, 40% for pertussis, and 27% for tetanus-specific antibodies compared with HIV-uninfected women, with a trend toward a reduction in placental transfer of pneumococcal specific antibodies (Table 2).

Among HIV-infected women, there was no association between maternal CD4 count or viral load and placental transfer (eTable 2).

Specific Vaccine-Induced Antibody Responses in Infants at 16 Weeks

In stratified analysis for infants who had received 1, 2, or 3 doses of DTP-Hib vaccine (n=6, 22, and 65, respectively), there was no difference in antibody levels between infants who had received 1 or 2 doses (eTable 3); these groups were therefore combined for further analysis. Similarly, data were combined for infants who had received 1 or 2 doses of pneumococcal capsular polysaccharide (n=15 and 34, respectively). There was no statistical difference in the proportion of HIV-exposed and HIV-unexposed infants who received fewer than 3 doses of DTP-Hib vaccine (25% vs 16%; P=0.31) or fewer than 2 doses of pneumococcal capsular polysaccharide (20% vs 49%; P=0.06) before the 16-week sampling.

Despite initially lower titers at birth, HIV-exposed uninfected infants mounted robust responses following vaccination. In the group that received all 3 scheduled doses of DTP-Hib vaccine, HIV-exposed infants had significantly higher responses to pertussis (270.1 [IQR, 84.4-355.0] FDA U/mL vs 91.7 [IQR, 27.9-168.4] FDA U/mL; P=0.006) than unexposed infants did (Figure 2), but had similar responses to Hib and tetanus. HIV-exposed infants also had higher levels of pneumococcal specific antibody than HIV-unexposed infants did (47.32 [IQR, 32.56-77.80] mg/L vs 14.77 [IQR, 11.06-41.08] mg/L; P=0.001). Among infants who had received only 1 or 2 doses of DTP-Hib vaccine, responses were higher in the HIV-exposed infants than unexposed infants to Hib (6.46 [IQR, 1.74-9.29] mg/L vs

Figure 1. Specific Antibody Titers in Uninfected HIV-Exposed and HIV-Unexposed Infants at Birth

HIV indicates human immunodeficiency virus; FDA, Food and Drug Administration. Specific antibodies to Haemophilus influenzae type b (Hib), Bordetella pertussis antigens, pneumococcus, and tetanus toxoid from serum samples collected within 24 hours of birth were nonpreferentially analyzed on available sample volume by commercially available enzyme-linked immunosorbent assays. Horizontal lines indicate median response. The Mann-Whitney U test was used to compare antibody levels at birth between HIV-exposed and HIV-unexposed infants.

Table 2. Influence of Maternal HIV Infection on Placental Antibody Transfer

<table>
<thead>
<tr>
<th>Specific Antibody</th>
<th>HIV-Infected Mother–Exposed Uninfected Infant Pairs</th>
<th>HIV-Uninfected Mother–Unexposed Infant Pairs</th>
<th>Reduction, %b</th>
<th>P Valuec</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemophilus influenzae type b</td>
<td>0.57 (0.46-0.79)</td>
<td>0.74 (0.61-1.00)</td>
<td>23</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bordetella pertussis</td>
<td>0.91 (0.61-2.00)</td>
<td>1.51 (1.15-2.06)</td>
<td>40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>0.62 (0.41-0.77)</td>
<td>0.73 (0.53-0.94)</td>
<td>15</td>
<td>.05</td>
</tr>
<tr>
<td>Tetanus toxoid</td>
<td>0.65 (0.60-1.2)</td>
<td>1.30 (1.03-1.86)</td>
<td>27</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.

aPlacental transfer of antibody from mother to infant is expressed as a ratio of infant/maternal specific IgG concentration at birth.

bPercentage reduction in placental transfer between HIV-infected and HIV-uninfected women; calculated as the ratio of the placental transfer from HIV-infected women/placental transfer from HIV-uninfected women, subtracted from 100.

cMann-Whitney U test.
0.54 [IQR, 0.24-4.10] mg/L; \( P = .02 \), pertussis (81.16 [IQR, 38.64-195.40] IU/mL vs 11.60 [IQR, 5.30-39.42] IU/mL; \( P < .001 \)), and tetanus (1.86 [IQR, 0.51-2.21] IU/mL vs 0.50 [IQR, 0.10-0.93] IU/mL; \( P = .01 \)) (Figure 2).

The fold increase in antibody level before and after vaccination was significantly higher in the HIV-exposed infants than in the HIV-unexposed infants for Hib (21.15-fold increase [IQR, 6.84-118.40] vs 2.97-fold increase [IQR, 0.71-16.69]; \( P = .007 \)), pertussis (9.51-fold increase [IQR, 2.80-24.25] vs 2.16-fold increase [IQR, 0.41-6.84]; \( P = .002 \)), and pneumococcus (2.06-fold increase [IQR, 0.96-5.70] vs 0.31-fold increase [IQR, 0.26-1.04]; \( P < .001 \)). There was no difference in the fold-increase at pre-vaccination and postvaccination between the 2 groups for tetanus-specific responses (14-fold increase [IQR, 3.26-116.20] vs 12-fold increase [IQR, 2.8136.35]; \( P = .54 \)).

FIGURE 3 shows prevaccination and postvaccination antibody levels for individual infants. Infants with the lowest levels of anti-Hib, pertussis, pneumococcal, and tetanus-specific antibodies showed the greatest vaccine responses at 16 weeks. HIV exposure was associated with a greater magnitude of change between birth and 16 weeks.

**Figure 2.** Specific Antibody Titers in Uninfected HIV-Exposed and HIV-Unexposed Infants at 16 Weeks

HIV indicates human immunodeficiency virus; FDA, Food and Drug Administration. Specific antibodies to *Haemophilus influenzae* type b (Hib), *Bordetella* pertussis antigens, pneumococcus, and tetanus toxoid from serum samples collected at 16 weeks were analyzed by enzyme-linked immunosorbent assays. Three vaccine doses indicate when the vaccine schedule was complete with vaccines administered at 6, 10, and 14 weeks; and 1 or 2 vaccine doses indicate when the schedule was incomplete (except pneumococcal vaccination, for which only 2 doses were scheduled before 16 weeks). Samples were collected from 93 infants at 16 weeks (38 HIV-exposed infants and 55 HIV-unexposed infants) and antibody levels were nonpreferentially completed on available sample volume. The number of samples analyzed for each exposure group in each vaccine dose group is indicated. Horizontal lines indicate median response. For anti-Hib IgG, \( P = .70 \) for uninfected HIV-exposed infants vs HIV-unexposed infants at 3 vaccine doses; \( P = .02 \) for uninfected HIV-exposed infants vs HIV-unexposed infants at 1 or 2 vaccine doses; \( P = .21 \) for uninfected HIV-exposed infants at 3 vaccine doses vs 1 or 2 vaccine doses; and \( P = .001 \) for HIV-unexposed infants at 3 vaccine doses vs 1 or 2 vaccine doses. For antipertussis IgG, \( P = .006 \) for uninfected HIV-exposed infants vs HIV-unexposed infants at 3 vaccine doses; \( P < .001 \) for uninfected HIV-exposed infants vs HIV-unexposed infants at 1 or 2 vaccine doses; \( P = .09 \) for uninfected HIV-exposed infants at 3 vaccine doses vs 1 or 2 vaccine doses; and \( P < .001 \) for HIV-unexposed infants at 3 vaccine doses vs 1 or 2 vaccine doses. For antipneumococcal IgG, \( P = .001 \) for uninfected HIV-exposed infants vs HIV-unexposed infants. For antitetanus IgG, \( P = .71 \) for uninfected HIV-exposed infants vs HIV-unexposed infants at 3 vaccine doses; \( P = .01 \) for uninfected HIV-exposed infants vs HIV-unexposed infants at 1 or 2 vaccine doses; \( P = .43 \) for uninfected HIV-exposed infants at 3 vaccine doses vs 1 or 2 vaccine doses; and \( P < .001 \) for HIV-unexposed infants at 3 vaccine doses vs 1 or 2 vaccine doses.
tussis vaccine and Hib (DTP-Hib) or 2 doses of pneumococcal conjugate vaccine for whom paired samples were available.

Our data also highlight low levels of specific antibody in HIV-uninfected women and tetanus. Results are shown for infants who received 3 doses of combination diphtheria, tetanus toxoid, and pertussis vaccine and Hib (DTP-Hib) or 2 doses of pneumococcal conjugate vaccine for whom paired samples were available.

To our knowledge, we present the most comprehensive study to date evaluating the association of maternal HIV infection and maternal-specific antibody levels and infant antibody responses to routine World Health Organization Expanded Program on Immunization vaccines. We demonstrate that HIV-exposed uninfected infants have lower specific antibody levels at birth than their non–HIV-exposed peers. Similarly, a smaller proportion of these infants have levels deemed to be protective. We show that this is due to a combination of factors: lower antibody titers to Hib and pneumococcus in HIV-infected pregnant women and reduced transplacental transfer of Hib, pertussis, pneumococcal capsular protein, and tetanus. Responses to routine vaccines are not fully understood. However, a plausible explanation is that maternal antibodies inhibit infant responses to vaccination. Our findings are consistent with 2 studies in HIV-infected women from Kenya, indicating that maternal HIV is associated with lower tetanus and measles-specific antibody in cord blood and also with reduced placental antibody transfer. Maternal tetanus-specific antibody levels are lower among HIV-infected women in some studies; inconsistencies observed may be due to differences in vaccination practice during pregnancy.

Although it is known that measles, Hib, and pneumococcal vaccine responses are reduced in children infected with HIV, there is a paucity of studies investigating the influence of infant HIV exposure (in the absence of infection) on responses to vaccines. We observed an increased vaccine response in HIV-exposed infants to pertussis and pneumococcus compared with HIV-unexposed infants following completion of the immunization schedule. This can be explained by the lower maternally derived antibody levels at birth. Conversely, higher levels of maternal antibody among HIV-unexposed infants at birth corresponded with lower responses postvaccination. Other studies have also reported that maternal antibodies can inhibit infant response to measles, tetanus, whole cell pertussis, and Hib vaccines; this effect varies considerably between different vaccines and studies.

The mechanisms through which maternal antibodies inhibit infant responses to vaccination are not fully understood. However, a plausible explanation is that maternal antibodies mask or hide vaccine antigenic epitopes, preventing recognition and binding by infant B cells; a key determinant of infant responses appears to be the maternal antibody-to-vaccine antigen ratio.

HIV-exposed infants who had missed doses of vaccine before sampling at 16 weeks had higher antibody responses than HIV-unexposed infants to Hib and tetanus, as well as pertussis and pneumococcus. An explanation for this observation is that higher maternal antibodies observed among HIV-unexposed infants may influence the response to the first dose of vaccine but not to subsequent doses. A study in Finland reported a similar effect; infants with high levels of maternally derived antibody had lower anti-Hib antibody after the first dose of Hib vaccination, but not after the second dose.

A limitation of our study is enrollment at a single center with a modest number of mother–infant pairs. Sampling was however consecutive and representative of women and infants accessing care in this community setting. We did not have data on maternal vaccination history, due to limitations in recall and documentation. Vaccination records in this setting are typi-
cally available for young children only. Women in our study groups had statistically different but clinically comparable ages; therefore, similar maternal vaccination history between groups could be inferred based on the date of the introduction of the universal Expanded Program on Immunization schedule in South Africa (1973).

Although antibody levels can be used to indicate potential susceptibility to infection, some uncertainty remains regarding the functional relevance of a single so-called protective level. In addition, protective levels for collective response to multiple pneumococcal serotypes are unclear and there is a paucity of evidence for defining protective levels for other antibodies such as pertussis. Functional assays may give a better assessment of the ability of the immune system to effectively clear a pathogen. Further work to address this aspect is ongoing.

We were unable to correlate antibody levels with long-term vaccine responses or clinical outcomes in the women or infants. However, our data contribute to a potential explanation for the higher morbidity and mortality observed among African HIV-exposed infants. For example, the lower observed pneumococcal-specific antibody among HIV-exposed infants before vaccination might be associated with increased severity of pneumonia observed in this group of infants. Our data highlight the need for larger prospective studies to determine whether the lower antibody levels in HIV-exposed infants at birth translate into increased morbidity from vaccine-preventable infections.

Our study results also support the evaluation of novel maternal and neonatal immunization strategies to augment specific antibody responses and potentially prevent infections in infants in early life, particularly in HIV-exposed infants. In view of similar deficiencies also observed in the non-HIV-exposed group, benefits may exist for these infants too.

The implementation of vaccination programs in pregnancy, although resulting in decreased infant and maternal morbidity, is challenging because immunization in pregnancy may impair infant responses to vaccination as a result of increased maternal antibody. Evaluation of pneumococcal or pertussis vaccination strategies during pregnancy, or before pregnancy, in settings with high prevalence of HIV however may benefit both mother and child. An alternative and feasible strategy is neonatal vaccination. For example, neonatal pertussis vaccination is safe and results in early antibody responses; however, responses to Hib and hepatitis B vaccines may be affected. The timing of neonatal vaccinations therefore needs to be carefully considered. We recommend evaluation of both maternal and neonatal vaccination strategies, as each has merits and challenges.

In conclusion, our study describes specific antibody responses in mother-infant pairs with and without maternal HIV infection before and after infant vaccination and elucidates mechanisms for reduced responses in HIV-exposed uninfected infants early in life. A significant percentage of non-HIV-infected women also showed insufficient protection. Larger prospective studies are needed to ascertain the relationship between these observed immune responses and clinical end points. Targeted vaccination strategies may be required in HIV-infected women and their infants.

Author Contributions: Dr Jones 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. Drs Kampmann and Hesseling contributed equally to this article.

Study concept and design: Jones, Esser, Kampmann, Hesseling.

Acquisition of data: Jones, Naidoo, De Beer, Hesseling.

Analysis and interpretation of data: Jones, Kampmann, Hesseling.

Drafting of the manuscript: Jones, Kampmann, Hesseling.

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Statistical analysis: Jones, Hesseling.

Obtained funding: Jones, Kampmann, Hesseling.

Administrative, technical, or material support: Jones, Naidoo, De Beer, Kampmann, Hesseling.

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Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest and none were reported.

Funding/Support: This work was supported by the European Society for Pediatric Infectious Diseases (Dr Jones), the Thrasher Research Foundation (Dr Jones), the Wellcome Trust (Dr Kampmann), National Institute for Health Research, England (Dr Kampmann), and Elizabeth Glaser Pediatric AIDS Foundation (Dr Hesseling).

Role of the Sponsors: The funding agencies had no role in the design or conduct of the study, in the collection, management, analysis, or interpretation of the data, or in the preparation, review, or approval of the manuscript.

Online-Only Material: eTables 1, 2, and 3 are available at http://www.jama.com.

Additional Contributions: We thank the study nurses, Barbara Magwewegwe, RN, and Nonkonzo Dziba, RN, and study counselor, Eunice Mhlamba (Desmond Tutu TB Center, Stellenbosch University, Cape Town, South Africa), who ably assisted with the acquisition of data, and David Schriger, MD, MPH (Los Angeles Emergency Medicine Center, University of California, Los Angeles), for graphical assistance. Miss Magwewegwe, Dziba, and Mhlamba are salaried employees who received no special financial compensation for their contributions. Dr Schriger received no financial compensation for his contribution.

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