No Difference in Antibody Responses to Tetanus Vaccine Among HIV-Exposed and -Unexposed Infants in Botswana

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ABSTRACT
Background: In Botswana, more than 10% of HIV-exposed, uninfected infants (HEU) are hospitalized or die in the first 6 months of life, largely due to infectious causes. Vaccine responses can act as a marker of the immune response to infectious antigens. Previous studies of antibody responses to vaccines in HEU have had conflicting results. We compared antibody titers to tetanus vaccine between HEU and HIV-unexposed infants (HUU), and explored whether tetanus antibody titers predicted risk of hospitalization in the first 2 years of life among HEU.

Methods: 443 HIV-infected and 451 HIV-uninfected mothers and their 453 HEU / 457 HUU live-born infants were followed in a prospective observational study in Botswana (“Tshipidi”). Quantitative tetanus toxoid IgG was measured in plasma samples from 18-month-old infants. Geometric mean antibody titers (GMT) were compared between HEU and HUU infants, and between HEU infants who were or were not hospitalized by age 2.

Results: Plasma was available at 18 months for 39 HEU and 42 HUU infants. Within this subset, there were 15 hospitalizations (12 in HEU) [RR of hospitalization among HEU = 1.34 (p=0.009)]. 73% of hospitalizations overall, and 81% in HEU, were due to infection (primarily pneumonia/bronchiolitis and gastroenteritis). Among infants who had received 3 or 4 doses of tetanus vaccine by 18 months, there were no significant differences in tetanus GMT between HEU and HUU. Among HEU who had received 3 or 4 doses of tetanus vaccine by 18 months, there were no significant differences in tetanus GMT between infants who were hospitalized and infants who were not.

Conclusion: In this small sample of infants from Botswana, we did not identify differences in antibody responses to tetanus vaccine between HEU and HUU. Although HEU demonstrated an increased risk of hospitalization, response to tetanus vaccine did not appear to be a significant predictor of morbidity. It is possible that cell-mediated immune defects play a larger role than humoral immune defects in the increased susceptibility to infection among HEU.

INTRODUCTION
Nearly 1.5 million HIV-exposed, uninfected (HEU) infants are born each year. HEU infants have an increased incidence of infectious morbidity and mortality compared to their HIV-unexposed (HUU) peers. In Botswana, more than 10% of HEU infants are hospitalized or die by 6 months of age; HEU infants account for 46% of under-2 mortality in the country. Vaccine responses provide a good model for evaluating the immune status of the host. Decreased responses to a vaccine antigen could be indicative of global immune dysfunction. Previous studies of vaccine responses in HEU infants have shown conflicting results. Some studies have shown decreased T-cell responses to BCG vaccine, decreased cellular and humoral immunity to the tetanus vaccine, and low antibody titers and avidity to the measles and Haemophilus influenzae vaccines. Yet other studies have shown comparable or even increased antibody titers to vaccine antigens.

Infants in Botswana receive tetanus vaccine at 6, 10, and 14 weeks of age. We hypothesized that tetanus responses would be lower in 18-month-old HEU compared with HUU infants. We also hypothesized that low tetanus titers would be predictive of hospitalization by age 2 in HEU infants.

METHODS
443 HIV-infected, antiretroviral-treated and 451 HIV-uninfected mothers and their infants were followed from 2010-2012 by the Tshipidi study in Botswana. Peripheral venous blood was collected from infants at birth and at 1, 6, and 18 months of age. Plasma was isolated on-site and cryopreserved until needed. Quantitative tetanus antibody was measured in 18-month plasma samples using the Tetanus Toxoid IgG ELISA assay (IBL America). Standards with a range from 0.1-5.0 IU/mL were included in each experiment. Results for each sample were compared to a standard curve and expressed in International Units (IU/mL). Statistical analyses were performed using Prism software version 7.03 (GraphPad, La Jolla, CA). Geometric mean titers were compared between groups using t-tests with statistical significance defined as p < 0.05.

RESULTS

Figure 1. Rate of hospitalization in the first 24 months of life. Among 39 HEU and 44 HUU infants, 12 HEU (30.8%) and 3 HUU (9.5%) infants were hospitalized.

Figure 2. Tetanus IgG antibody titer at 18 months among 39 HEU infants who were or were not hospitalized, according to number of tetanus vaccine doses received. NS, not significant.

Table 1. Indication for hospitalization by age 2 years among HEU and HUU infants.

<table>
<thead>
<tr>
<th>Indication</th>
<th>HEU (n=39)</th>
<th>HUU (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of total hospitalizations</td>
<td>11 (10 infants)</td>
<td>3 (3 infants)</td>
</tr>
<tr>
<td>Pneumonia/bronchiolitis</td>
<td>6 (46.2%)</td>
<td>0</td>
</tr>
<tr>
<td>Gastroenteritis with dehydration</td>
<td>4 (30.8%)</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>Paraffin ingestion</td>
<td>2 (15.4%)</td>
<td>0</td>
</tr>
<tr>
<td>Meconium aspiration</td>
<td>1 (7.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Orthopedic fracture</td>
<td>0</td>
<td>3 (33.3%)</td>
</tr>
<tr>
<td>Failure to thrive</td>
<td>0</td>
<td>3 (33.3%)</td>
</tr>
</tbody>
</table>

SUMMARY
• HEU infants were more likely than HUU infants to require hospitalization in the first 24 months of life.
• The majority of hospitalizations in HEU infants were for infectious indications.

CONCLUSIONS
• HEU infants demonstrate higher rates of infectious morbidity and mortality in the first two years of life.
• Antibody responses to tetanus vaccine do not appear to be a predictor of infectious morbidity among HEU infants in this cohort.
• It is possible that cell-mediated immune defects play a larger role than humoral immune defects in the increased susceptibility to infection among HEU infants.

FUTURE DIRECTIONS
• We are measuring cell-mediated vaccine responses to tetanus and BCG vaccine using dual-color fluorospect (LI-2 and IFNγ) on cryopreserved PBMCs from HEU and HUU infants in the Tshipidi cohort.
• We will determine cytokine profiles for all HEU infants in the Tshipidi cohort, and analyze vaccine responses and clinical outcomes according to CMV infection status.
• We will assess whether early CMV infection affects DNA methylation profiles in HEU and HUU immune cells.

ACKNOWLEDGEMENTS
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