Impaired Humoral Response to Vaccines among HIV-Exposed Uninfected Infants

Beatriz Mariana Abramczuk,1 Tais Nitsch Mazzolla,1 Yara Maria Franco Moreno,1 Tatiane Queiroz Zorzeto,1 Wagner Quintilio,2 Paulo Silva Wolf,2 Maria Heloisa Blotta,3 André Moreno Morcillo,1 Marcos Tadeu Nolasco da Silva,1 and Maria Marlauce dos Santos Vilela1,4

Center for Investigation in Pediatrics, Pediatrics Department, State University of Campinas Medical School, Campinas, São Paulo, Brazil1; Butantan Institute, São Paulo, Brazil2; and Department of Clinical Pathology, State University of Campinas Medical School, Campinas, São Paulo, Brazil3

Received 6 April 2011/Returned for modification 3 June 2011/Accepted 12 July 2011

Little is known about the vaccine protective response for infants born from HIV-infected mothers. We evaluated the antibody response to hepatitis B, tetanus, and diphtheria vaccine in vertically HIV-exposed uninfected infants and compared them to those of control infants not exposed to the virus. The quantitative determination of specific neutralizing antibodies against hepatitis B, diphtheria, and tetanus were performed blindly on serum samples. The results showed that 6.7% of the HIV-exposed uninfected individuals were nonresponders to hepatitis B vaccine (anti-HBs titer, <10 mIU/ml), and 64.4% were very good responders (anti-HBs titer, ≥1,000 mIU/ml), whereas only 3.6% of the nonexposed infants were nonresponders (χ² = 10.93; 1 df). The HIV-exposed uninfected infants showed protective titers for diphtheria and tetanus but lower geometric mean anti-tetanus titers compared to those of the HIV-unexposed infants. Our data point to the necessity of evaluating vaccine immune responses in these children and reinforced that alterations in lymphocyte numbers and functions reported for newborns from HIV-infected mothers interfere with the vaccine response.

The aim of this study was to evaluate the humoral responses to the hepatitis B, diphtheria, and tetanus vaccines in HEU infants and in infants not exposed (NE) to HIV.

MATERIALS AND METHODS

Study population. The HIV-exposed infants were recruited in the Pediatrics Immunodeficiency Out-Patients Unit at the State University of Campinas Clinical Hospital (UNICAMP, Campinas, Brazil). Exposed infants with two undetectable HIV-1 viral loads in RNA PCR assays (with a lower limit of quantification of 50 copies of RNA/ml) were categorized as uninfected infants in accordance with the Brazilian Ministry of Health Guidelines and were included in this study. Infants with congenital or genetic defects were excluded. The NE infants were recruited in Campinas public health centers.

The study protocol was approved by the Committee for Ethics in Research of the State University of Campinas, São Paulo, Brazil.

Vaccination. Hepatitis B vaccine was composed of hepatitis B surface antigen (HBsAg) obtained from DNA-transfected yeast cells (Butang; 10 μg HBsAg with 0.625 mg aluminum hydroxide and 0.05 mg thimerosal). The vaccine is given at birth and at the first and sixth months. DTP/Hib tetravalent vaccine (diphtheria-tetanus-pertussis and Haemophilus influenzae type b) contained four protective units of Bordetella pertussis toxin, two units of diphtheria and tetanus toxoids (1.25 mg of aluminum hydroxide and 0.2 mg of thimerosal), and 10 μg H. influenzae type b capsular polysaccharide conjugated to 20 to 40 μg tetanus toxoid (7). The vaccine is given at 2, 4, and 6 months of life, and a DTP booster is given at 15 months and 4 to 6 years.

The hepatitis B and DTP vaccines were manufactured by the Butantan Institute, São Paulo, Brazil, and the Hib vaccine by Bio-Manguinhos, Rio de Janeiro, Brazil. The infants received the vaccines intramuscularly by following the Brazilian Immunization National Programme.

Blood collection. One milliliter of peripheral blood was collected in EDTA tubes for immunophenotyping and 3 ml in serum-separating tubes to evaluate immune responses to hepatitis B, diphtheria, and tetanus. The collection was done for about 1 month after the third dose from each vaccine.

Quantitative determination of anti-HBs. The quantitative determination of anti-HBs (mIU/ml) was performed blindly on serum samples using a microparticle enzyme immunoassay (MEIA) from Assay Ausab (Abbott Laboratories, Abbott Park, IL). The reliability of the measurements was assessed by the intra-class correlation coefficient (ICC) stratified by sample origin and included...
TABLE 1. Distribution of anti-HBs titers for the HEU and NE infants after the third dose of hepatitis B vaccine

| Group    | Total no. of individuals | No. (%) of individuals with anti-HBs titers of: |
|----------|--------------------------|-----------------------------------------------|
|          |                          | <10 mIU/ml | 10-1,000 mIU/ml | ≤1,000 mIU/ml |
| HEU      | 45                       | 3 (6.7)    | 13 (28.9)       | 29 (64.4)     |
| NE       | 112                      | 4 (3.6)    | 65 (58.0)       | 43 (38.4)     |

lower and upper limits. Seroprotection was defined as an anti-HBs titer of ≥10 IU/ml.

Quantitative determination of tetanus and diphtheria antitoxin. TOBI test. In vivo tests for measuring tetanus and diphtheria antitoxin levels in serum were carried out by a standardized modified toxin-binding inhibition (TOBI) test as described by Hendriksen et al. (13) but employing diphtheria toxoid instead of toxin (19, 31, 32).

Immunophenotyping. Samples of whole blood from the HEU infants were incubated with the anti-human CD3/CD4 fluorescent-conjugated monoclonal antibodies (Beckman Coulter) for 20 min at room temperature. The red blood cells were lysed with ammonium chloride solution (0.15 M NH4Cl, 10 mM KHCO3, 37 mg/liter EDTA 4Na) and washed twice with phosphate-buffered saline. Data were acquired until 10,000 events were recorded in the CD3 gate (Epic XL-MCL flow cytometer; Beckman-Coulter) and analyzed (Expo software; Beckman Coulter). Isotype controls were used to discriminate specific antibody staining.

Statistical considerations. The comparison of vaccine responses between groups was performed by a chi-squared ($\chi^2$) test with a critical $\chi^2$ value of 5.99 (degree of freedom [df], 1) for hepatitis B and by Fisher’s test for diphtheria and tetanus, with a significance level at $P$ (degree of freedom [df], 1) for hepatitis B and by Fisher’s test with a critical $\chi^2$ value of less than 0.05. To evaluate the anti-diphtheria and anti-tetanus titers, the calculation of the geometric mean titers (GMTs) of antibodies was performed on log$_{10}$-transformed data, and we report the antilog of the GMT. For each group, GMTs and 95% confidence intervals (CI) were calculated. For comparisons of the logarithms of the titers, Student’s $t$-test was applied for independent samples. Comparisons manifesting a two-tailed $t$-value of less than 0.05 were considered statistically significant. Correlations between CD4 T-lymphocyte (TCD4) percentages and diphtheria and tetanus titers were performed by Spearman tests.

RESULTS

Study population. Fifty-three HEU (21 female and 32 male) and 112 NE (53 female and 59 male) infants were studied. The anti-HBs titers were determined for 45 HEU and 112 NE infants, and the anti-diphtheria and anti-tetanus titers were determined for 19 HEU and 112 NE infants. Median birth weights for the HEU and the NE groups were 2,940 g (1,585 to 3,520 g) and 3,250 g (2,515 to 4,400 g), respectively. Seven HEU infants were born prematurely (gestational age of <37 weeks), nine were vaginally delivered, and none were breastfed. At the time of the study, the median age for the NE and HEU infants was 7.7 months (6.0 to 11.4 months) and 7.5 months (6.7 to 11.2 months), respectively.

Anti-HBs titers. Table 1 shows anti-HBs titers in serum from both groups after the administration of the third dose of hepatitis B vaccine. In the HEU group, 6.7% of the infants were nonresponders (anti-HBs titer, <10 mIU/ml) and 64.4% were very good responders (anti-HBs titer, ≥1,000 mIU/ml), whereas only 3.6% of the NE infants were nonresponders. There was a significant difference in the number of nonresponding individuals between the HEU and the NE groups ($\chi^2 = 10.93$; df = 1). There was no difference in the CD4 T-lymphocyte percentages between the groups of responders and nonresponders from the HEU group ($P = 0.766$ by Mann-Whitney test).

Diphtheria and tetanus seroprotection. The titers of anti-diphtheria and anti-tetanus antibodies, the mean geometric titers, and 95% CI serum anti-diphtheria and anti-tetanus IgG levels (IU/ml) are shown in Table 2. The percentages of non-responders for diphtheria (<0.1 IU/ml) were 5.3 and 3.6% for the HEU and NE infants, respectively ($P = 0.4$ by Fisher’s exact test). All HEU infants and 98.2% of the NE infants showed protective responses to tetanus ($P = 0.5$ by Fisher’s exact test).

The difference in the GMTs between groups was not significant for anti-diphtheria, but it was significant for anti-tetanus ($P = 0.013$, respectively, each by Student’s $t$-test). There was no correlation between CD4 T-lymphocyte percentages and diphtheria titers or tetanus titers from HEU infants ($P = 0.064$ and 0.085, respectively, each by Spearman’s test).

DISCUSSION

For the HEU infants we observed a nonresponse rate to hepatitis B vaccine of 6.7%, lower geometric mean antitoxin titers to tetanus than those for the NE infants, and normal responses to diphtheria. Our previous results showed that only 0.4% of healthy infants failed to mount seroprotection to hepatitis B 1 month after the completion of the three-dose vaccination schedule (6). In addition, the frequencies of subjects protected against tetanus and diphtheria vaccinated with three doses of DTP were 98.2 and 95.5%, respectively (38). Intrauterine exposure to HIV leads to a poor response to hepatitis B vaccine, even in cases where the newborns do not become infected. Rutstein et al. (28) and Thaithumyanon et al. (35) reported that about 8.0% of HEU infants did not respond to hepatitis B vaccine. As both studies focused on HIV-infected infants, their limitation is that the HEU group was used only for comparisons to the infected infants without an NE infant group. We included, as a control group, age-matched healthy infants not exposed to HIV.

Intrauterine exposure to HIV and ARV interferes with the thymic maturation pathway and selection of T lymphocytes, resulting in lower TCD4$^+$ counts in HEU newborns and infants (4, 8, 12, 24). The HEU infants also presented the enhanced expression of CD40L on activated T lymphocytes (27), higher B-lymphocyte apoptosis levels (20), and alterations in dendritic cells (36) that may interfere with the antigen presentation and immune response to T-dependent antigens.

Moreover, the HEU newborns are known to have lower
naïve TCD4+ cells and T-cell receptor excision circles in cord blood mononuclear cells, probably reflecting impairment in both progenitor cell and thymic function (24). The placentation transfer of HIV particles or proteins is suspected to be the main mechanism that leads to impaired progenitor cell function in HEU infants. Glycoprotein 120 has been described to induce progenitor cell apoptosis and glycoprotein 160 to induce interleukin-3 (IL-3) and IL-6 secretion from cord blood T cells, resulting in the T-cell-mediated stimulation of myelopoiesis, both of which could cause diminished red blood cell and T-cell generation (24).

Unlike what we observed for the anti-HBs response, the percentages of nonresponders to tetanus and diphtheria toxins obtained in this study did not differ between the HEU and the NE infants. However, the HEU infants had lower anti-tetanus GMTs. Interestingly, HIV infection is associated largely with a failure to respond to hepatitis B vaccine but not to diphtheria and tetanus vaccines. However, the HIV-infected children have lower GMTs to diphtheria and tetanus toxoids and are more likely than uninfected persons to lose antibodies within a few years after vaccination (22). Jones et al. (15) observed similar responses to tetanus between HEU and NE infants 2 weeks after a three-dose DTP vaccine scheme (6, 10, and 14 weeks).

The only HEU infant in this study that did not respond with protective titers to diphtheria was a very good responder to anti-HBs and had anti-tetanus titers of 2.01 IU/ml. As the vaccines differ with respect to its components, their immune activation may involve different mechanisms.

Among the HEU infants, we did not observe differences in the percentages of CD4 T lymphocytes between the groups of responders and nonresponders for anti-HBs titers. There also was no correlation between TCD4 percentages and anti-tetanus or anti-diphtheria titers. Gesner et al. (12) reported normal cellular proliferative responses to tetanus and diphtheria antigens for a group of HEU infants with persistent low TCD4 percentages. Taken together, these data indicate that the poor response to anti-HBs and the lower GMTs to tetanus toxoid are more related to functional changes in T lymphocytes and in antigen presentation than to TCD4 counts.

Low responsiveness to measles vaccine in HIV-infected children has been associated with nutritional status rather than with HIV infection (33). This cannot be applied to our findings for the HEU infants concerning hepatitis B and DTP vaccines, as there were no children with a poor general health status in our study. Only two infants were below the weight and the height for their age, but both of them had a protective response to the vaccines.

The poor response to hepatitis B vaccination and the lower tetanus GMTs found for the HEU infants in this study corroborate that intrauterine exposure to HIV and ARV therapy interferes with the response of early vaccination. In accordance with the WHO and the ACIP, the Brazilian Immunization National Programme recommends a booster dose of hepatitis B vaccine at 12 months of age only for HIV-infected children (21). It is important to evaluate the inclusion of this fourth dose of the hepatitis B vaccine for the HEU infants or the anti-HBs serology 1 month after the third dose of hepatitis B vaccine. It also is necessary to study the response to tetanus and diphtheria after the booster doses of DTP vaccination.

**ACKNOWLEDGMENTS**

We are grateful to all of the parents who consented to their infants’ participation in this study. This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), São Paulo, Brazil, grant number 05/52277-3. B.M.A. was the recipient of a FAPESP fellowship (07/57557-6).

This work was performed at the Center for Investigation in Pediatrics, Pediatrics Department, State University of Campinas Medical School, Campinas, São Paulo, Brazil.

**REFERENCES**

1. Abzug, M. J., et al. 2009. Immunogenicity and immunologic memory after hepatitis B virus booster vaccination in HIV-infected children receiving highly active antiretroviral therapy. J. Infect. Dis. 200:935–946.
2. Barret, B., et al. 2003. Combined estimation of tetanus and diphtheria antitoxin in human sera by the in vitro toxin-binding inhibition (ToBi) test. J. Biol. Stand. 31:191–200.
3. Borges-Almeida, E., et al. 2011. The impact of maternal HIV infection on cord blood lymphocyte subsets and cytokine profile in exposed non-infected infants. BMC Infect. Dis. 11:1071–1079.
4. Bunder, M., C. Thorne, and M. L. Newell. 2005. Maternal and infant factors and lymphocyte, CD4 and CD8 cell counts in uninfected children of HIV-infected mothers. AIDS 19:1571–1577.
5. Bunder, M. J., et al. 2005. Haematological parameters of HIV-1-uninfected infants born to HIV-1-infected mothers. Acta Paediatr. 94:1571–1577.
6. Carniel, E. F., et al. 2008. Immunogenicity and safety of combined intradermal recombinant Hepatitis B with BCG vaccines at birth. Vaccine 26:647–652.
7. Clemens, S. C., T. Azevedo, and A. Homma. 2003. Feasibility study of the immunogenicity and safety of a novel DTP/H11001 (PRP-T) Brazilian combination compared to a licensed vaccine in healthy children at 2, 4, and 6 months of age. Rev. Soc. Bras. Med. Trop. 36:321–330.
8. Clerici, M., et al. 2000. T lymphocyte maturation abnormalities in uninfected newborns and children with vertical exposure to HIV. Blood 96:3866–3871.
9. Faye, A., et al. 2007. Characterization of the main placental cytokine profiles from HIV-1-infected pregnant women treated with anti-retroviral drugs in France. Clin. Exp. Immunol. 149:430–439.
10. Feiterna-Sperling, C., et al. 2007. Hematologic effects of maternal antiretroviral therapy and transmission prophylaxis in HIV-1-exposed uninfected newborns. J. Acquir. Immune Defic. Syndr. 45:43–51.
11. Foca, M., et al. 2006. Gender differences in lymphocyte populations, plasma HIV RNA levels, and disease progression in a cohort of children born to women infected with HIV. Pediatrics. 118:146–155.
12. Gesner, M., et al. 1994. Alteration in the proportion of CD4 T lymphocytes in a subgroup of human immunodeficiency virus-exposed-uninfected children. Pediatrics. 95:624–630.
13. Hendriksen, C. F., J. W. van der Gun, and J. G. Kreeftenberg. 1989. Combined estimation of tetanus and diphtheria antitoxin in human sera by the in vitro toxin-binding inhibition (ToBi) test. J. Biol. Stand. 17:191–200.
14. Hygino, J., et al. 2008. Altered immunological reactivity in HIV-1-exposed uninfected neonates. Clin. Immunol. 127:340–347.
15. Jones, C. E., et al. 2011. Maternal HIV infection and antibody responses against vaccine-preventable diseases in uninfected infants. JAMA 305:576–584.
16. Lao-Araya, M., T. Puthanakit, L. Aurpibul, T. Sirisanthana, and V. Sirisanthana. 2007. Antibody response to hepatitis B re-vaccination in HIV-infected children with immune recovery on highly active antiretroviral therapy. Vaccine 25:5324–5329.
17. Le Chenade, J., M. J. Mayaux, C. Guibeneuc-Joyaux, and S. Blanche. 2003. Perinatal antiretroviral treatment and hematopoiesis in HIV-uninfected newborns. AIDS 17:2053–2061.
18. Madhi, S. A., et al. 2005. Immunogenicity and effectiveness of Haemophilus influenzae type b conjugate vaccine in HIV infected and uninfected African children. Vaccine 23:5517–5525.
19. Marcovitz, R., D. C. Matos, R. A. Georgini, and D. Sakauchi. 2002. Potency control of diphtheria component in adsorbed vaccines by in vitro neutralization tests. Biologics 6:105–112.
20. Miyamoto, M., et al. 2010. Low CD4+ T-cell levels and B-cell apoptosis in vertically HIV-exposed noninfected children and adolescents. J. Trop. Pediatr. 56:427–432.
21. Mofenson, L. M., et al. 2009. Guidelines for the Prevention and Treatment of Opportunistic Infections among HIV-exposed and HIV-infected children: recommendations from CDC, the National Institutes of Health, the HIV Medicine Association of the Infectious Diseases Society of America, the Pediatric Infectious Diseases Society, and the American Academy of Pediatrics. MMWR Recomm. Rep. 58:1–166.
22. Moss, W. J., C. J. Clements, and N. A. Halsey. 2003. Immunization of children at risk of infection with human immunodeficiency virus. Bulletin of the World Health Organization. 81:61–70.
23. National Center for Immunization and Respiratory Diseases. 2011. General recommendations on immunization—recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm. Rep. 60:1–64.

24. Nielsen, S. D., et al. 2001. Impaired progenitor cell function in HIV-negative infants of HIV-positive mothers results in decreased thymic output and low CD4 counts. Blood 98:398–404.

25. Ono, E., et al. 2008. Imbalance of naive and memory T lymphocytes with sustained high cellular activation during the first year of life from uninfected children born to HIV-1-infected mothers on HAART. Braz. J. Med. Biol. Res. 41:700–708.

26. Pippi, F., et al. 2008. Serological response to hepatitis B virus vaccine in HIV-infected children in Tanzania. HIV Med. 9:519–525.

27. Romano, M. F., et al. 2006. Increased CD154 expression in uninfected infants born to HIV-positive mothers exposed to antiretroviral prophylaxis. Viral Immunol. 19:363–372.

28. Rutstein, R. M., B. Rudy, C. Codispoti, and B. Watson. 1994. Response to hepatitis B immunization by infants exposed to HIV. AIDS 8:1281–1284.

29. Scott, S., et al. 2007. The influence of HIV-1 exposure and infection on levels of passively acquired antibodies to measles virus in Zambian infants. Clin. Infect. Dis. 45:1417–1424.

30. Siriaksorn, S., T. Puthanakit, T. Sirisanthana, and V. Sirisanthana. 2006. Prevalence of protective antibody against hepatitis B virus in HIV-infected children with immune recovery after highly active antiretroviral therapy. Vaccine 24:3095–3099.

31. Sonobe, M. H., et al. 2007. Determination of low tetanus or diphtheria antitoxin titers in sera by a toxin neutralization assay and a modified toxin-binding inhibition test. Braz. J. Med. Biol. Res. 40:69–76.

32. Souza Matos, D. C. S., et al. 2002. Immunogenicity test of tetanus component in adsorbed vaccines by toxin binding inhibition test. Mem. Inst. Oswaldo Cruz 97:909–913.

33. Tejiokem, M. C., et al. 2007. HIV-infected children living in Central Africa have low persistence of antibodies to vaccines used in the Expanded Program on Immunization. PLoS One 2:e1260.

34. Tejiokem, M. C., et al. 2009. Whole-cell pertussis vaccine induces low antibody levels in human immunodeficiency virus-infected children living in sub-Saharan Africa. Clin. Vaccine Immunol. 16:479–483.

35. Thaithumyanon, P., S. Punnahitananda, P. Praisuwanna, U. Thisyakorn, and K. Ruxrungtham. 2002. Antibody response to hepatitis B immunization in infants born to HIV-infected mothers. J. Med. Assoc. Thai. 85:277–282.

36. Velilla, P. A., et al. 2008. Effect of intrauterine HIV-1 exposure on the frequency and function of uninfected newborns’ dendritic cells. Clin. Immunol. 126:243–250.

37. World Health Organization. 2010. Hepatitis B vaccines: WHO position paper—recommendations. Vaccine 28:589–590.

38. Zorzeto, T. Q., et al. 2009. Immunogenicity of a whole-cell pertussis vaccine with low lipopolysaccharide content in infants. Clin. Vaccine Immunol. 16:544–550.