Whole-Cell Pertussis Vaccine Induces Low Antibody Levels in Human Immunodeficiency Virus-Infected Children Living in Sub-Saharan Africa

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The WHO recommendations for the immunization of children infected with human immunodeficiency virus (HIV) differ slightly from the guidelines for uninfected children. The introduction of antiretroviral therapy for HIV-infected infants should considerably prolong their life expectancy. The question of the response to the whole-cell pertussis (wP) vaccine should now be addressed, particularly in countries in which pertussis remains endemic. To evaluate the persistence of antibodies to the wP vaccine in HIV-infected and uninfected children who had previously received this vaccine in routine clinical practice, we conducted a cross-sectional study of children aged 18 to 36 months, born to HIV-infected mothers and living in Cameroon or the Central African Republic. We tested blood samples for antibodies to the wP vaccine and for antibodies to diphtheria and tetanus toxoids (D and T, respectively) in the context of the use of a combined DTwP vaccine. We enrolled 50 HIV-infected children and 78 uninfected, HIV-exposed children in the study. A lower proportion of HIV-infected children than uninfected children had antibodies against the antigens tested for all valences of the DTwP vaccine. Agglutinin levels were substantially lower in HIV-infected than in HIV-exposed but uninfected children (30.0% versus 55.1%, respectively; P = 0.005). We also observed a high risk of low antibody levels in response to the DTwP vaccine in HIV-infected children with severe immunodeficiency (CD4 T-cell level, <25%). The concentrations of antibodies induced by the DTwP vaccine were lower in HIV-infected children than in uninfected children. This study supports the need for a booster dose of the DTwP vaccine in order to maintain high antibody levels in HIV-infected children.

There are almost 2 million children under the age of 15 years living with human immunodeficiency virus (HIV) in sub-Saharan Africa, according to the UNAIDS (http://www.unaids.org). Without appropriate antiretroviral therapy (ART), these children experience progressive immune depression. They are hypersusceptible to infectious diseases, although infection by some pathogens may be prevented by immunization. The World Health Organization (WHO) recommendations for the immunization of HIV-infected children differ slightly from the general guidelines for non-HIV-infected children (13). The use of vaccines for HIV-infected children raises questions about the capacity of these children to display a response to the vaccine. The most frequent combination of vaccines used by the Expanded Program on Immunization (EPI) comprises diphtheria and tetanus toxoids and inactivated whole-cell Bordetella pertussis adsorbed onto an aluminum salt (DTwP vaccine). This combined vaccine is scheduled for immunization at the ages of 6, 10, and 14 weeks, and a first booster dose between the ages of 15 and 18 months is recommended. Pertussis (whooping cough) is a highly communicable respiratory tract infection caused by the bacterium Bordetella pertussis. The disease remains a serious health concern, especially for infants and young children. According to the WHO, in 2003 an estimated 17.6 million cases, leading to 279,000 deaths, occurred worldwide, and 90% of cases affected young children living in developing countries (2). Pertussis spreads easily from adolescents and adults to children, through droplets produced during coughing and sneezing, and many children who contract pertussis have coughing spells that last from 4 to 8 weeks. Complications are most likely for young infants, the most common and generally fatal complication being bacterial pneumonia with severe respiratory distress. Early treatment with antibiotics (macrolides), if available, may slightly reduce the severity of the illness, but above all, it is important to stop transmission. Prevention involves immunization, and different vaccines, based on whole-cell pertussis (wP) and acellular pertussis (aP) vaccines, are currently available. The vaccines are effective at preventing severe clinical disease in infancy and have a significant impact on the circulation of B. pertussis. In resource-constrained settings, the combined DTwP vaccine is used due

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to its low price. Without booster doses, older children and adults may experience waning immunity and may serve as reservoirs for the transmission of bacteria (22, 23). Therefore, in industrialized countries, additional booster doses in the form of an aP vaccine are now recommended (8, 12).

In this study, we evaluated the persistence of antibodies induced by the wP vaccine in HIV-infected and HIV-exposed but uninfected children born to HIV-infected mothers and living in Central Africa. These children had previously received a three-dose regimen of the combined DTwP vaccine as part of a routine clinical practice. In addition, we assessed the levels of antibodies to the diphtheria and tetanus toxoid valences of the combined DTwP vaccine in these children.

MATERIALS AND METHODS

We conducted a cross-sectional study with HIV-infected children and HIV-exposed, uninfected children, born to HIV-infected mothers, living in Cameroon or in the Central African Republic. All parents or legal guardians gave their written informed consent. The study was approved by the National Ethics Committees in Cameroon and the Central African Republic. The study was conducted in accordance with the Declaration of Helsinki. The detailed study design has been reported previously (21). Briefly, children were included in the study if they had received at least a primary vaccine series of three doses of the DTwP vaccine, as certified by their immunization card. Two different combined DTwP vaccines were used in routine clinical practices in the two countries during the study period: one (SII Triple Antigen) produced by the Serum Institute of India (Pune, India) and the other (DTCOg) by Sanofi Pasteur (Marcy l’Etoile, France).

Blood samples were collected and processed at the Centre Pasteur in Yaoundé, Cameroon, and at the Institut Pasteur in Bangui, Central African Republic. One serum sample was collected from each child between the ages of 18 and 36 months and was frozen at −20°C for antibody testing. Antibodies to pertussis bodies to pertussis toxin (PT). Antibodies to diphtheria and tetanus toxoids were measured on site. Some serum samples were sent to the French National Reference Center for Pertussis at the Institut Pasteur in Paris, France, for the measurement of agglutinins (AGG) and antibodies to pertussis toxin (PT). Antibodies to diphtheria and tetanus toxoids were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Diphtheria ELISA IgG test kit and Tetanus ELISA IgG test kit; Genzyme Virotech, Rüsselsheim, Germany). Sera with antibody concentrations greater than or equal to 100 mIU/ml were considered to be positive. For the wP valence, sera were analyzed by a standardized ELISA to quantify antibodies to PT, which is specific to B. pertussis infection (19). Assay cutoffs were set at four times the minimum level of detection, corresponding to 8 ELISA units (EU)/ml for antibodies to PT. Sera were considered positive if they had levels above 25 EU/ml. Antibodies to PT did not last for more than 2 years after vaccination with a wP vaccine, and we considered titers of antibodies to PT of ≥100 EU/ml in the absence of a booster dose within the 2 years before sampling as indicative of a recent infection with B. pertussis (10). AGG, i.e., antibodies that agglutinate bacteria, directed mostly against fimbrial antigens, were detected using the microagglutination test (17). Positive results were defined as values equal to or greater than the value of the first dilution tested (1/20 dilution).

Moreover, complete blood counts, tests for proteinemia, and tests for HIV type 1 (HIV-1) infection of children, including assessment of the HIV-1 load and lymphocyte subpopulation counts, were carried out. In Cameroon, the plasma HIV-1 RNA load (viral load [VL]) was quantified by a commercial assay (Versant bDNA HIV kit, version 3.0; Bayer Diagnostics, Emeryville, CA) according to the manufacturer’s instructions. The threshold for quantification was 50 HIV-1 RNA copies/ml. In Bangui, the plasma HIV-1 RNA levels were determined by real-time TaqMan reverse transcription-PCR with the protocol established by the ANRS Working Group for Viral Quantification (18). The limit for quantification was 400 HIV-1 RNA copies/ml. CD4 T cells were counted using a fluorescence-activated cell sorter (FACSscan) flow cytometer (Becton Dickinson Biosciences, San Jose, CA).

The chi-square test or Fisher’s exact test, as appropriate, was used to compare categorical variables between HIV-infected and HIV-exposed but uninfected children. The proportions of HIV-infected and HIV-exposed, uninfected children with antibodies to PT and with AGG above the defined threshold of detection were reported. Univariate logistic regression analyses were used to assess the effects of covariates on the odds ratios (OR) of low levels of antibody to each valence of the DTwP vaccine (using AGG for the wP valence). Com-positive categorical variables were created to evaluate the effects of HIV infection: (i) HIV status combined with the percentage of CD4 T cells (HIV-exposed but uninfected or HIV-infected children and ≥25% CD4 T cells; HIV-infected children and <25% CD4 T cells); (ii) HIV status combined with VL (HIV-exposed but uninfected or HIV-infected children and a VL of <10,000 copies/ml; HIV-infected children and a VL of ≥10,000 copies/ml); (iii) HIV status combined with the duration of ART (HIV-exposed but uninfected children or HIV-infected children and ≥6 months of ART; HIV-infected children and <6 months of ART or no ART). Uninfected children who had been exposed to HIV perinatally were considered the reference group in this study. All statistical analyses were performed using STATA, version 8.0 (Stata Corp., College Station, TX), with a significance level of 5%.

RESULTS

We analyzed 128 children, 50 of whom were HIV infected while 78 were HIV exposed but uninfected. Very few children (7 infected and 12 not infected with HIV) received a booster dose of the DTwP vaccine in a routine clinical practice. The two groups of children were last immunized at similar times (mean ages, 19.7 months for the HIV-exposed group and 17.4 months for the HIV-exposed but uninfected group). The proportion of HIV-infected children that tested positive for AGG was lower than that of HIV-exposed, uninfected children (Table 1). However, the proportion of HIV-infected children positive for antibodies to PT was similar to that of HIV-exposed, uninfected children. Between the two countries, the proportion of children with detectable AGG did not differ, but there was a significant difference in the proportions with antibodies to PT (13.6% in the Central African Republic versus 40.6% in Cameroon; P = 0.001).

Univariate logistic regression was used to analyze the levels of antibody to each valence of the DTwP vaccine (Table 2). There was a significant association between a short duration of ART or no ART and low levels of antibodies to the DTwP vaccine. Severe immunodeficiency (<25% CD4 T cells; VL ≥10,000 copies) was also associated with low levels of antibodies to the DTwP vaccine. Only covariates linked to HIV status were statistically significant for the diphtheria and tetanus toxoid valences. The covariates linked to HIV status (ART, percentage of CD4 T cells, and VL) and to the time elapsed after the last immunization were statistically significant for the wP valence.

| Antibody | No. of doses | HIV-infected children | HIV-exposed, uninfected children |
|----------|--------------|-----------------------|-------------------------------|
|          | No. tested   | No. (%) with positive responses | No. tested | No. (%) with positive responses |
| AGG      | 3            | 43 (23.3)             | 97 (55.1) | 0.005 |
|          | 4            | 7 (71.4)              | 12 (75.0) | 1.0   |
| Anti-PT  | 3            | 43 (25.3)             | 66 (50.8) | 0.42  |
|          | 4            | 7 (42.9)              | 12 (25.0) | 0.6   |

TABLE 2. Proportions of children who tested positive for AGG and of those with antibodies to PT after three or four doses of whole-cell pertussis vaccine in relation to HIV status

a By the chi square test or Fisher’s exact test as appropriate.
b In a study by Grimprel et al. (10) in which four doses of the vaccine were given, 70 to 90% of HIV-exposed, uninfected children had AGG, while 30 to 40% had anti-PT antibodies.
the high anti-PT titers may correlate with exposure to *B. pertussis*. The two HIV-exposed, uninfected children with persistent coughs had low titers of antibodies to PT, and their coughs were not likely to be linked to pertussis. Six other children had elevated antibodies to PT (>100 EU/ml); of these, four tested negative for AGG (one HIV-infected child and three HIV-exposed but uninfected children) and two tested positive for AGG (both HIV exposed but uninfected). No children were reported to have had a persistent cough at the time of blood collection. Acute pertussis infection was
probable for the six children discussed above, based on their levels of antibodies to PT and the time after the last immunization. It is difficult to differentiate between antibodies following infection and antibodies resulting from vaccination, since no culture or PCR was performed to document the microorganism responsible for the persistent cough.

**DISCUSSION**

Combination DTwP vaccines have been used successfully for several years. The first priority of the EPI is to achieve at least 90% coverage with three doses of DTwP vaccine for infants. However, according to the WHO, in 2004 only 44 countries (27%) had reached the threshold of 80% coverage with three doses; moreover, only one sub-Saharan African country achieved >80% coverage (2). As reported in our study, the booster dose is not performed in routine clinical practices in most developing countries.

It is well known that wP vaccines are difficult to produce reproducibly and that their efficacies may differ according to the manufacturer (7). For instance, the efficacy of a DTwP vaccine with a three-dose regimen (at 2, 4, and 6 months) was estimated to be around 50% (95% CI, 37.0 to 57.6%) in a study performed in Sweden using a DTwP vaccine produced by Connaught Laboratories in the United States (11) and around 36% (95% CI, 14.2 to 52.1%) in a study performed in Italy with the same vaccine (9). However, in Senegal, the efficacy with the same schedule was calculated to be 91% (95% CI, 81 to 96%) with a DTwP vaccine produced by Sanofi Pasteur, France (20). No data relating to the SII vaccine are available, whereas several studies report that the DTCq vaccine from Sanofi Pasteur induces a strong humoral response (3, 15, 20). We were not able to analyze the data in relation to the vaccine manufacturers in our study, but differences in vaccines attributed to the vaccine manufacturer certainly need further investigation, using a three-dose-regimen in 6, 10, and 14 weeks.

We showed that at around 18 months after the last vaccination, only 19.2% of HIV-positive children and 48.2% of HIV-exposed, uninfected children possessed detectable antibodies after three doses of wP vaccine. The antibody response was lower in HIV-infected children than in HIV-exposed, uninfected children. However, neither the type nor the level of antibodies against the wP vaccine correlated clearly with efficacy, and these results may therefore not reflect a risk of severe clinical disease (7). Historical comparisons indicate that the practice of primary vaccination before the age of 6 months without a booster immunization is not optimal (16). Therefore, a fourth dose at the age of 18 months is recommended by the WHO and is given in most industrialized countries as a booster in the form of an aP vaccine. A four-dose regimen (with one booster dose) and a five-dose regimen (with two booster doses), even with a less effective vaccine, have satisfactorily controlled whooping cough in the United States (7).

*B. pertussis* infection seems to be uncommon among HIV-infected children and adults (1, 5, 6, 14), but infection by *B. pertussis* is rarely considered and is difficult to diagnose biologically. A paucisymptomatic or an atypical form of pertussis in HIV-infected children may be misdiagnosed. Intracellular *B. pertussis* has been demonstrated in macrophages by using bronchoalveolar lavage specimens from HIV-infected patients (4). The patients were paucisymptomatic, and other opportunistic infections were associated with the severity of their respiratory symptoms. In this study, cases of prolonged coughing were reported for almost one-fifth of HIV-infected children; however, these instances were not confirmed microbiologically.

To conclude, it is not clear whether HIV-infected children may be at greater risk of severe pertussis, or at risk of more frequent pertussis disease, than HIV-exposed, uninfected children. However, the former have lower titers of antibody to wP vaccine antigens and may serve as reservoirs for pertussis transmission in the population, since asymptomatic carriers have been reported. Therefore, the introduction of a fourth dose of pertussis vaccine should be considered a priority for all children living in countries in which pertussis is still a major health problem. To make this measure effective, these countries should extend the free delivery of the EPI vaccine to children above the age of 1 year. In the mid-term, monitoring the antibody response after a four-dose regimen will aid in the introduction of adequate recommendations for supplementary vaccine doses during the teenage years in the sub-Saharan population.

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