Antibody Response to *Haemophilus influenzae* Type b Vaccine in Relation to the Number of CD4 T Lymphocytes in Adults Infected with Human Immunodeficiency Virus

Frank P. Kroon, Jaap T. van Dissel, Ger T. Rijkers, Jerry Labadie, and Ralph van Furth

A prospective study of antibody production by adults infected with human immunodeficiency virus (HIV) after vaccination with tetanus toxoid--conjugated *Haemophilus influenzae* type b (Hib) vaccine was conducted. Concentrations of antibodies to the two immunogenic components of the vaccine (i.e., polyribosylribitol phosphate [PRP] and tetanus toxin) were determined. Individuals were divided into three groups according to the CD4 T lymphocyte count: group 1, \( \leq 100 \times 10^6/L \); group 2, \( 101-300 \times 10^6/L \); and group 3, \( > 300 \times 10^6/L \). After vaccination, concentrations of IgM and IgG antibodies to PRP were significantly lower in group 1 than in the other patient groups and controls. A CD4 T lymphocyte count of \( < 100 \times 10^6/L \) and an impaired proliferative response of lymphocytes to monoclonal antibody to CD3 were independently associated with a less than threefold increase in concentrations of IgG antibody to PRP. Analysis of IgG subclasses demonstrated that the production of IgG1 antibodies was predominantly affected. Postvaccination concentrations of antibody to tetanus toxin were significantly lower in group 1 than in group 3 and controls. Both prevaccination and postvaccination concentrations of antibody to tetanus toxin were not correlated with the magnitude of the response of antibody to PRP. We conclude that HIV-infected individuals with CD4 T lymphocyte counts of \( < 100 \times 10^6/L \) demonstrate an impaired antibody response after vaccination with conjugated Hib vaccine.

Impaired cellular immunity is the main cause of opportunistic infections in HIV-infected individuals. However, diminished humoral immunity has also been demonstrated in vitro [1–3] and in vivo [4], in particular with respect to T lymphocyte--dependent antigens [5, 6]. The clinical importance of impaired humoral immunity in HIV-infected individuals is underscored by an increased incidence of infections due to microorganisms like *Streptococcus pneumoniae* [7, 8] and *Haemophilus influenzae* [9–11]. It has been estimated that infections caused by *H. influenzae* occur five to 40 times more frequently in HIV-infected adults than in the general population [10–13]. One-third of the cases of invasive *H. influenzae* infections in HIV-infected adults are caused by *H. influenzae* type b (Hib) [11]. Invasive infections caused by Hib are potentially preventable by antibodies to the bacterial capsular polysaccharide antigens. To improve immunity to Hib, vaccination of HIV-infected individuals could be an option.

The aim of the present study was to investigate the antibody response of HIV-infected adults after vaccination with Hib vaccine conjugated with tetanus toxoid as carrier protein and to relate this response to the number of CD4 T lymphocytes and the stage of HIV disease.

**Materials and Methods**

**Study Populations**

HIV-seropositive adults from the Infectious Diseases Outpatient Clinic of the University Hospital Leiden (Leiden, the Netherlands) were eligible for this study when they were free of active opportunistic infections. They were invited by letter to participate, and 54 of 59 patients agreed. Ten healthy HIV-negative members of the hospital staff served as controls. Informed consent was obtained from all participants.
Vaccination

Between November 1993 and April 1994, all subjects were immunized with a single dose of conjugated Hib vaccine (Act-HIB, Pasteur Mérieux Sérum et Vaccins, Lyon, France). This vaccine, which is used in the national vaccination program for children in the Netherlands, contains polyribosylribitolphosphate (PRP) covalent bound to tetanus toxoid as carrier protein. Each dose of PRP–tetanus toxoid contains 10 μg of polysaccharide and 25 μg of tetanus toxoid; this vaccine contains no adjuvant. The vaccine was injected into the deltoid or quadriceps muscle; hemophiliacs received a subcutaneous injection.

Laboratory Investigations

Blood samples taken just before vaccination (day 0) and 30 days thereafter were used to determine antibody concentrations, WBC count, differential blood cell count, lymphocyte subsets, and proliferative responses of lymphocytes. Serum samples for antibody assays were stored at −20°C until all specimens had been collected, coded, and tested in a blinded fashion. Serum samples from a single individual were tested simultaneously.

Measurement of Antibodies

Concentrations of antibodies specific to PRP were determined by ELISA as previously described [14]. The antibody concentration was calculated by determining the ratio of parallel running lines of dilutions of both the reference serum and the sample under study. We used a reference hyperimmune plasma pool that contained 32.5 μg of antibody/mL [15] and 27.2 μg of IgG antibody to PRP/mL, 4.05 μg of IgG1 antibody to PRP/mL, and 9.48 μg of IgG2 antibody to PRP/mL (D. Ambrosino, unpublished data); the concentrations of antibodies in this reference hyperimmune plasma pool were assigned to be 100 arbitrary units (AU)/mL each for IgM antibody to PRP, IgG antibody to PRP, IgG1 antibody to PRP, and IgG2 antibody to PRP. The lowest limit of detection was 0.5 AU/mL. 2-Mercaptoethanol treatment of sera indicated that IgM antibodies to tetanus toxoid did not interfere with detection of IgG subclass antibodies (data not shown).

Antibodies to tetanus toxoid were measured by the toxin-binding inhibition test as described elsewhere [16]. This test is based on inhibition of the binding of toxin to horse antitoxin–coated ELISA plates by preincubation of toxin with twofold dilutions of the serum under investigation. The lowest limit of detection of antitoxins is 0.01 IU/mL. For analysis of data, the concentration in sera without detectable antibodies was arbitrarily set at 0.005 IU/mL; concentrations of >16 IU/mL were arbitrarily set at 32 IU/mL.

Determination of Lymphocyte Subsets and Lymphocyte Reactivity

The lymphocyte subset counts and the proliferative response of lymphocytes to phytohemagglutinin, monoclonal antibodies to both CD2 and CD28 (CD2/CD28), and monoclonal antibody to CD3 were assessed by the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam) as previously described [17].

Analysis of Data

For analysis of data, patients were divided into three groups on the basis of their CD4+ cell counts (see under Results). Univariate analysis of variance was performed. In case of a significant difference between group means (P < .05), a multiple comparison procedure was used to determine which group means were significantly different from each other (Bonferroni test).

To identify factors predictive of an adequate humoral immune response (arbitrarily defined as a threefold or more increase in concentration of antibody to PRP after vaccination), 17 variables were analyzed; the variables included age, gender, antiretroviral therapy, route of HIV infection, Centers for Disease Control and Prevention (CDC) classification of AIDS [18], prevaccination concentrations of IgM and IgG antibodies to PRP, pre- and postvaccination concentrations of antibody to tetanus toxoid, and immunologic parameters (the absolute number of CD4+ CD8+, and B lymphocytes; CD4-to-CD8 cell ratio; and the lymphocyte proliferative responses to phytohemagglutinin, monoclonal antibodies to CD2/CD28, and monoclonal antibody to CD3). The power of statistical analysis of factors like gender and route of HIV infection was limited because of the small numbers of females and individuals with nonhomosexual transmission of HIV. In the analysis, the mean of the immunologic parameters found for days 0 and 30 was used. Those variables showing an univariate association with an adequate response (P < .10) were further analyzed by stepwise multiple logistic regression to identify factors independently predictive of an adequate humoral immune response in HIV-infected individuals. Continuous variables showing a bivariate association with postvaccination antibody concentrations were further analyzed by stepwise multiple linear regression.

To investigate whether the carrier molecule of PRP (i.e., tetanus toxoid) elicited a humoral immune response that might have influenced the response to PRP, a similar analysis of pre- and postvaccination antibodies to tetanus toxoid and the interaction between PRP and tetanus toxoid was performed.

Results

Study Population

Five female and 49 male HIV-infected individuals were enrolled in the study. The mean age of HIV-infected individuals was 40 years (range, 16–65 years) (table 1). The 10 healthy controls had a mean age of 36 years (range, 26–43 years). Values for the immunologic parameters are summarized in table 2. According the CDC classification of AIDS in 1987...
Table 1.  Age, sex, CD4+ T lymphocyte count, and CDC classification of 54 HIV-infected individuals and 10 healthy controls given conjugated Hib vaccine.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No. of subjects</th>
<th>Mean age in y (range)</th>
<th>Male-to-female ratio</th>
<th>Mean CD4+ T lymphocyte count in 10^6/L (range)</th>
<th>CDC stage of AIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-infected</td>
<td>54</td>
<td>40 (16–65)</td>
<td>49/5</td>
<td>221 (1–930)</td>
<td>16 38</td>
</tr>
<tr>
<td>CD4+ T lymphocyte count (10^6/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤100 (group 1)</td>
<td>21</td>
<td>40 (28–65)</td>
<td>18/3</td>
<td>19 (1–100)</td>
<td>1 20</td>
</tr>
<tr>
<td>101–300 (group 2)</td>
<td>16</td>
<td>39 (16–50)</td>
<td>15/1</td>
<td>203 (110–360)</td>
<td>1 15</td>
</tr>
<tr>
<td>&gt;300 (group 3)</td>
<td>17</td>
<td>40 (23–60)</td>
<td>16/1</td>
<td>499 (280–930)</td>
<td>14 3</td>
</tr>
<tr>
<td>Healthy non-HIV-infected</td>
<td>10</td>
<td>36 (26–43)</td>
<td>6/4</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

NOTE.  CDC = Centers for Disease Control and Prevention; Hib = Haemophilus influenzae type b; NA = not applicable.

[18], 16 individuals were classified as CDC group II, and 38 were classified as group IV. Forty individuals acquired HIV infection by homosexual contact, and nine acquired HIV infection by heterosexual or bisexual contact; five individuals were hemophiliacs who acquired HIV infection via blood-derived products. Twenty-one HIV-infected individuals had CD4+ T lymphocyte counts of ≤100 x 10^6/L (group 1), 16 had CD4+ T lymphocyte counts of 101–300 x 10^6/L (group 2), and 17 had CD4+ T lymphocyte counts of >300 x 10^6/L (group 3).

All individuals in group 1 were treated with antiretroviral drugs (monotherapy with zidovudine [AZT], 9; monotherapy with didanosine [ddI], 3; combination therapy with AZT and ddI, 9). In group 2, five individuals did not take antiretroviral therapy, but 11 patients did (AZT monotherapy, 5; combination therapy with AZT and ddI, 4; combination therapy with AZT and zalcitabine, 2). Only three patients in group 3 took antiretroviral therapy (AZT monotherapy).

Adverse Reactions to Vaccination

Besides mild soreness at the site of injection that was reported by some individuals, the vaccination was well tolerated. The numbers of CD4+ T lymphocytes in patients did not differ before vaccination and 30 days thereafter (P > .1). During the first 2 months after vaccination, none of the individuals died.

Response to Immunization

Antibodies to PRP.  Prevaccination concentrations of IgM antibody to PRP were significantly lower in group 1 than in group 2; postvaccination concentrations of IgM antibody to PRP were significantly lower in group 1 than in the other groups of patients and controls (table 3). The increase in concentrations of IgM antibody to PRP was significantly lower in group 1 than in controls. Prevaccination concentrations of IgG antibody to PRP did not differ significantly between the various groups. The postvaccination concentrations of IgG antibody to PRP and the increase in concentrations of IgG antibody to PRP were significantly lower in group 1 than in the other groups of patients and controls (table 3). Significantly fewer individuals in group 1 than in the other groups of patients and controls had a threefold or more rise in concentrations of IgG antibody to PRP (figure 1).

Table 2.  Immunologic parameters for 54 HIV-seropositive individuals who were given conjugated Hib vaccine.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value for indicated group*</th>
<th>Normal value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>CD4+ T lymphocyte count (10^6/L)</td>
<td>10 (2–90)</td>
<td>205 (110–275)</td>
</tr>
<tr>
<td>CD8+ lymphocyte count (10^6/L)</td>
<td>400 (50–1,250)</td>
<td>900 (600–2,300)</td>
</tr>
<tr>
<td>CD4+/CD8+ cell ratio</td>
<td>0.04 (0.01–0.24)</td>
<td>0.19 (0.1–0.44)</td>
</tr>
<tr>
<td>B lymphocyte count (10^6/L)</td>
<td>50 (50–500)</td>
<td>75 (50–250)</td>
</tr>
<tr>
<td>Lymphocyte proliferative response (cpm)</td>
<td>90 (35–1,055)</td>
<td>585 (40–2,075)</td>
</tr>
<tr>
<td>Anti-CD3</td>
<td>300 (60–8,300)</td>
<td>3,100 (400–11,300)</td>
</tr>
<tr>
<td>PHA</td>
<td>2,950 (200–16,150)</td>
<td>12,200 (2,850–20,200)</td>
</tr>
</tbody>
</table>

NOTE.  Anti-CD3 = monoclonal antibody to CD3; Hib = Haemophilus influenzae type b; anti-CD2/CD28 = monoclonal antibodies to both CD2 and CD28; PHA = phytohemagglutinin.

* Group 1 (21 individuals), CD4+ T lymphocyte counts of ≤100 x 10^6/L; group 2 (16 individuals), CD4+ lymphocyte counts of 101–300 x 10^6/L; and group 3 (17 individuals), CD4+ lymphocyte counts of >300 x 10^6/L. Values for immunologic parameters are the median of values determined on day 0 and day 30, with ranges shown in parentheses.
Table 3. Concentrations of antibodies to PRP and tetanus toxin in HIV-infected individuals and healthy controls who received conjugated Hib vaccine.

<table>
<thead>
<tr>
<th>Antibody, time</th>
<th>Group 1*</th>
<th>Group 2*</th>
<th>Group 3*</th>
<th>Controls*</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM to PRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevac</td>
<td>4.3</td>
<td>10.0</td>
<td>6.8</td>
<td>6.5</td>
</tr>
<tr>
<td>Fold increase</td>
<td>1.6</td>
<td>3.2</td>
<td>3.9</td>
<td>5.3</td>
</tr>
<tr>
<td>IgG to PRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevac</td>
<td>12.3</td>
<td>15.5</td>
<td>12.3</td>
<td>15.5</td>
</tr>
<tr>
<td>Fold increase</td>
<td>2.8</td>
<td>11.2</td>
<td>10.7</td>
<td>12.2</td>
</tr>
<tr>
<td>IgG1 to PRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevac</td>
<td>23.2</td>
<td>35.1</td>
<td>31.2</td>
<td>17.6</td>
</tr>
<tr>
<td>Fold increase</td>
<td>1.4</td>
<td>5.2</td>
<td>3.8</td>
<td>8.7</td>
</tr>
<tr>
<td>IgG2 to PRP</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevac</td>
<td>7.6</td>
<td>13.1</td>
<td>6.8</td>
<td>20.3</td>
</tr>
<tr>
<td>Fold increase</td>
<td>1.8</td>
<td>2.7</td>
<td>3.7</td>
<td>3.0</td>
</tr>
<tr>
<td>Antibody to tetanus toxin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prevac</td>
<td>0.8</td>
<td>1.5</td>
<td>2.7</td>
<td>4.0</td>
</tr>
<tr>
<td>Fold increase</td>
<td>1.4</td>
<td>3.5</td>
<td>5.8</td>
<td>9.6</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

NOTE. Concentrations of antibodies are expressed as geometric means (95% CI). Hib = Haemophilus influenzae type b; PRP = polyribosylribitolphosphate.

* Group 1, 21 individuals with CD4+ T lymphocyte counts of <100 × 10^6/L; group 2, 16 individuals with CD4+ T lymphocyte counts of 101–300 × 10^6/L; group 3, 17 individuals with CD4+ T lymphocyte counts of >300 × 10^6/L; controls, 10 healthy non-HIV-infected individuals.

Analysis of IgG subclass antibodies to PRP (table 3) showed that prevaccination concentrations of IgG1 antibody to PRP were significantly higher in group 2 than in controls; postvaccination concentrations of IgG1 antibody to PRP in group 1 were significantly lower than those in the other patient groups and controls, and the increase in concentrations of IgG1 antibody to PRP was lower in group 1 than in controls. Significantly fewer individuals in group 1 than in the other groups of patients and controls had a threefold or more rise in concentrations of IgG1 antibody to PRP (figure 1). Prevaccination concentrations of IgG2 antibody to PRP did not differ significantly between the various groups. Postvaccination concentrations of IgG2 antibody to PRP were lower in all three groups of patients than in controls, although this difference was not significant. The increase in concentrations of IgG2 antibody to PRP was similar in all groups.

In a bivariate analysis, postvaccination concentrations of IgM and IgG antibodies to PRP were associated with the proliferative response of lymphocytes to monoclonal antibody to CD3 and with the number of CD4+ lymphocytes; postvaccination concentrations of IgG1 antibody to PRP were associated with the number of CD4+ lymphocytes. The increase in concentrations of IgM, IgG, and IgG1 antibodies to PRP was associated only with the number of CD4+ lymphocytes. In a multiple linear regression analysis, the postvaccination concentration of antibody to tetanus toxin was the only factor signifi-
impaired immune response after immunization with conjugated Hib vaccine [21–23]. Our results indicate that a CD4+ lymphocyte count of >100 x 10^6/L is needed for an adequate antibody response.

Healthy children immunized with a conjugated Hib vaccine respond mainly with IgG1 antibody formation [24]. In healthy adults vaccinated with conjugated Hib vaccine, IgG2 antibodies to PRP also contribute substantially to the IgG antibody response [25, 26]. In healthy adults vaccinated with nonconjugated Hib polysaccharide vaccine, the antibody response is dominated by IgG2, a general characteristic of T lymphocyte–independent type 2 antigens; after vaccination with protein-conjugated Hib polysaccharide vaccine (which is a T lymphocyte–dependent antigen) the antibodies to PRP are mainly IgG1, while variable but significant amounts of IgG2 are produced as well. Our finding that in HIV-infected adults with low CD4^+ lymphocyte counts IgG1 antibody production is more seriously affected than the IgG2 response suggests different degrees of T lymphocyte dependency of the antibody response to conjugated Hib vaccine.

Our study showed that the tetanus toxoid component of the vaccine induced antibodies to tetanus toxin in a T lymphocyte–dependent fashion. We did not find a correlation between pre-vaccination immunity to tetanus toxin and the antibody response to PRP. Other investigators [27, 28] reported a beneficial effect of carrier priming with regard to the immunogenicity of polysaccharide protein–conjugated Hib vaccines in animals and in healthy immunocompetent individuals.

In adults, the response to Hib vaccination can be considered as a secondary antibody response because during life immunity has developed due to regular contact with Hib and/or cross-reacting antigens of Escherichia coli K 100 [29]; this contact sustains a certain degree of humoral immunity. In a previous study of HIV-infected patients [6], we demonstrated that the secondary antibody response to T lymphocyte–dependent vaccines (i.e., diphtheria toxoid, tetanus toxoid, and poliovirus vaccines) was impaired in individuals with CD4^+ T lymphocyte counts of <300 x 10^6/L, most prominently in patients with CD4^+ T lymphocyte counts of <100 x 10^6/mL.

In HIV-infected individuals, infections with Hib predominantly occur in patients with advanced HIV disease [9]. The mechanisms by which adult patients with HIV infection seem to lose adequate protection against Hib infections are unknown. In children, concentrations of antibody protective against Hib polysaccharide vaccine was compared with Hib polysaccharide vaccines in animals after vaccination. and in healthy immunocompetent individuals.

Discussion

The main conclusion to be drawn from this study is that vaccination with T lymphocyte–dependent conjugated Hib vaccine is associated with an impaired antibody response in HIV-infected adults with low CD4^+ T lymphocyte counts. This conclusion is based on the low postvaccination concentrations of antibody to PRP and the small number of individuals with CD4^+ T lymphocyte counts of <100 x 10^6/L who exhibited a threefold or more increase in antibody levels.

The T lymphocyte dependence of the antibody response following vaccination with conjugated Hib vaccine was demonstrated in an earlier study of HIV-infected adults with different stages of the disease [19]; diphtheria toxoid–conjugated Hib polysaccharide vaccine was compared with Hib polysaccharide vaccine. It was shown that the increase in concentrations of antibody to PRP after vaccination with the conjugated vaccine was correlated with the number of CD4^+ lymphocytes in the group of patients with CD4^+ T lymphocyte counts of <200 x 10^6/L, while no correlation was found for individuals with higher CD4^+ T lymphocyte counts. Another study [20] showed that antibody formation in adults who recently acquired HIV infection (of whom 60% had CD4^+ cell counts of ≥500 x 10^6/L) was normal following vaccination. It has also been demonstrated that children with advanced HIV disease elicit an

![Figure 1. Percentage of individuals with a threefold or more increase in concentrations of IgM, IgG, IgG1, and IgG2 antibodies to polyribosylribitolphosphate after vaccination with conjugated Haemophilus influenzae type b vaccine.](https://academic.oup.com/cid/article-abstract/25/3/600/291344)
their role in T lymphocyte–dependent antibody production, contribute to the cellular host defense against Hib infection is not known. In vitro nonconjugated PRP, despite being a T lymphocyte–independent antigen, is capable of inducing both IL-2 receptor expression and proliferation of CD4+ lymphocytes [33]. In this sense, progressive loss of functional CD4+ lymphocytes in patients with AIDS could pose an additional risk factor for Hib disease.

Assuming that antibodies to Hib play an important role in protection of HIV-infected individuals against severe Hib infection, it has been tentatively advocated that HIV-infected individuals should be vaccinated against Hib infection [18, 34]. However, it is still uncertain whether vaccination might increase HIV load and enhance progression of HIV disease [35]; this issue should be addressed in vaccination policies for these patients. In this study, the number of CD4+ T lymphocytes was not affected by vaccination, and in a recently conducted study with conjugated pneumococcal vaccine and polysaccharide vaccine [36], we did not observe an increase in HIV type 1 load. When vaccination is considered, we would advise administration of the vaccine as early as possible during the course of HIV infection, preferably before CD4+ lymphocyte counts drop to <100 × 10^9/L. The presently used antiretroviral regimens containing protease inhibitors, which can increase the number of CD4+ T lymphocytes, may have favorable effects on the antibody response following immunization. This issue is now under investigation.

Acknowledgments

The authors thank Ms. W. Dorama, Ms. J. Noomen, and Ms. A. Stevenhagen for assisting in data collection and the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam) where the lymphocyte subset analyses were performed.

References