A Case-Control Study to Investigate Serological Correlates of Clinical Failure of 23-Valent Pneumococcal Polysaccharide Vaccine in HIV-1–Infected Ugandan Adults

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We have investigated the association between the concentration of anti–polysaccharide pneumococcal capsule–specific (anti-PS) immunoglobulin G and the killing activity, in serum, in invasive pneumococcal disease (IPD) events and response to 23-valent polysaccharide vaccine in human immunodeficiency virus (HIV)–infected Ugandans. Case patients with IPD had lower concentrations of anti-PS IgG before and after vaccination and before the IPD event (P<.01 for 5 [i.e., 4, 9V, 14, 18C, and 19F] of 6 serotypes assessed). After vaccination, case patients were less likely than were control subjects to develop detectable serum killing activity against the 2 serotypes tested—for 19F, this activity was detected in 16% of case patients versus 37% of control subjects (P = .08); for 23F, it was detected in 11% of case patients versus 40% of control subjects (P = .02). Thus, absolute concentration of anti-PS IgG and an attenuated response to polysaccharide are associated with risk of IPD in HIV-infected adults.

Streptococcus pneumoniae is a frequent and serious infection in HIV-1–infected adults in Africa [1]. When control of invasive pneumococcal disease (IPD) by 23-valent pneumococcal polysaccharide vaccine (PPV) was investigated in a randomized controlled clinical trial (RCT) in HIV-infected Ugandans [2], it was found to be ineffective—there was an excess of pneumococcal-related events in vaccine recipients. We hypothesized that PPV not only failed to induce a protective anti-pneumococcal immune response but in some cases had exacerbated the preexisting immune defects that increase susceptibility of HIV-infected adults to pneumococcal disease [2].

Pretrial immunogenicity studies had found a suboptimal but significant response to vaccination [3], in agreement with reports of results in other HIV-1–infected groups [4–6]. After the trial, we investigated the relationship of clinical events to, and the impact of vaccination on, the level and function of anti-pneumococcal antibodies.

Subjects, materials, and methods. The RCT of PPV has been described in detail elsewhere [2]. Thirty-one first-IPD events were diagnosed during the course of the RCT and during the 8 months immediately after the end of the trial. Four control subjects were matched to each case patient, on the basis of age (in blocks of 5 years), sex, vaccination status, and CD4 T cell–count strata (in blocks of 50 cells/mm³). When >4 appropriate control subjects were identified, the individual with enrollment date closest to that of the case patient was chosen. A prevaccine serum sample and a 1-month-postvaccine serum sample were available from each case patient and control subject. For measurements before the IPD event, the serum sample used was that collected on the date closest to the IPD event when the case patient was well, and not either <1 week or >3 months before the IPD event; the serum samples from the control subjects were chosen to match, as closely as possible, the time elapsed since study enrollment, within the same time frame that was used for the case patient. For the nonfatal cases, a serum sample taken 1 month after the IPD event was available. The clinical work was performed in accordance with the UK Medical Research Council Guidelines for good clinical practice in clinical trials and had ethical approval from the Ugandan Ministry of Health, The Ugandan Virus Research Institute Institutional Research Board (IRB), and the Liverpool School of Tropical Medicine IRB, Liverpool, United Kingdom. The study participants gave signed informed consent in their local language.

Quantitative measurements of anti–polysaccharide pneumococcal-capsule–specific IgG (anti-PS) were performed, as described elsewhere [7], for serotypes 4, 9V, 14, 18C, 19F, and 23F, which are 6 of the 19 serotypes responsible for the 31 IPD episodes in the present study and for which absolute values, in micrograms per milliliter, are calculable in terms of the stan-

The efficacy of a 23-valent polysaccharide vaccine against invasive pneumococcal disease (IPD) was assessed in a routine vaccination program in Australia. The vaccine was found to be efficacious in preventing IPD among vaccinated individuals, with a reduction in IPD cases by 48% among vaccinated children aged 2–5 years and 37% among vaccinated adults aged 65 years and older. These findings support the use of the 23-valent polysaccharide vaccine in routine vaccination programs to prevent IPD in children and adults.

**Results**. Suitable matches were available for all the 31 case patients. Unmatched baseline parameters were similarly distributed: median (range) CD4 T cell count was 199 cells/mm³ (3–929 cells/mm³) in case patients and 187 cells/mm³ (3–1012 cells/mm³) in control subjects; and 30 ± 6.5 in control subjects; and mean ± SD time between vaccination and IPD-event serum sample was 191 ± 199 days for case patients and 199 ± 170 days for control subjects. Nineteen of the case patients had been vaccinated, and, 20 individuals (46%) overall had CD4 T cell counts >200 when they were vaccinated.

For serotypes 4, 9C, 18C, and 19F, concentrations of anti-PS IgG before vaccination were significantly lower in the case patients than in the control subjects (P = .01) (figure 1). For all serotypes except 23F, a significant rise (P < .01) in anti-PS IgG was demonstrable in vaccine recipients overall; however, in the control-subject serum samples, the highest absolute postvaccine levels (table 1). For 5 of the serotypes, the postvaccine geometric mean concentration (GMC) of anti-PS IgG was higher in the control subjects than in the case patients.

Before vaccination, measurable killing of serotypes 19F and 23F was present in none of the prevaccine case patient serum samples and in 5 (7%) and 7 (8%), respectively, of the prevaccine control-subject serum samples; the maximum titers for these 2 serotypes were 1:32 and 1:124, respectively. After vaccination, measurement of killing of serotypes 19F and 23F was present in 3 (16%) and 2 (11%) (P < .01 for change; McNemar’s χ² = 9.8), respectively, of the case-patient serum samples and 28 (37%) and 30 (40%) (P < .01 for change; McNemar’s χ² = 23), respectively, of the control-subject serum samples. The production of measurable killing in response to vaccination was more likely in the control subjects than in the case patients (table 1). No changes in measurable killing of serotypes 19F and 23F were noted in the placebo recipients; measurable killing, at any time point, was present in none of the case patients and in 3 (6%) [maximum titer, 1:32] and 7 (15%) [maximum titer, 1:64], respectively, of the control subjects.

Serum samples from case patients predated the IPD event by a median of 37 days (range, 12–65 days). Concentrations of anti-PS IgG against all serotypes except 23F were significantly lower in the case patients than in the control subjects (figure 1). None of the pre-IPD-event serum samples from case patients were able to kill type 19F or 23F pneumococci, whereas 8 (7%) [titers, 1:8–1:32] and 12 (10%) [titers, 1:8–1:128], respectively, of the pre-IPD-event serum samples from control subjects were able to do so.

The 6 serotypes used in the assays accounted for 10 IPD events: 3 were type 4, 1 was type 9V, 1 was type 14, 1 was type 18C, 2 were type 19F, and 2 were type 23F. In these 10 case-control quintuplets, the pre-IPD-event level of anti-PS IgG against a given disease-causing serotype was <0.35 μg/mL in 3 of 10 case patients and in 5 of 40 in the matched control subjects. Thus, there was a borderline association between this level of anti-PS IgG and disease (P = .07, conditional logistic regression) and a positive and negative predictive value of 83% and 38%, respectively, for protection in this population; when a cutoff of 1 μg/mL was used, these values were 73% and 60%, respectively.

For the 28 post-IPD-event serum samples available, only 8 cases of IPD were caused by serotypes measured in the EIA. Peri-IPD-event disease fold rises were 0.7–1.0, compared with vaccine fold rises of 0.5–2.9 in the 5 vaccine recipients. No relationship between vaccine fold rise and disease fold rise was discernible. In the 2 cases caused by serotype 19F and in the 2 cases caused by 23F, measurable killing of the serotype was not detected in any pre-event or post-IPD-event serum samples. Two serotype 7–caused IPD events, 1 serotype 5–caused IPD event, and 1 serotype 10–caused IPD event developed measurable post-IPD-event killing of serotype 19F. Measurable killing was not detected in any of the remaining 20 post-IPD-event serum samples available for analysis.

Concentrations of anti-PS IgG showed strong interserotypic correlation (Pearson correlation coefficient [r] > 0.75) in all comparisons, except for serotype 14, which had no correlation with the other serotypes. Moreover, anti-PS IgG against 19F and 23F correlated weakly with opsonophagocytic activity (for 19F, Spearman’s ρ = 0.75).
Figure 1. Box-and-whisker plots of prevaccine (A), postvaccine (B), and pre-IPD-event (C) concentrations of anti-PS IgG, in all individuals (both vaccine and placebo recipients), for 6 representative serotypes, by case patients (n = 31) and control subjects (n = 124). Boxes represent the interquartile range; whiskers extend to the upper and lower adjacent value, to a maximum of 1.5× interquartile range; circles represent values outside this range. #, Prevaccine concentrations significantly lower (P ≤ .01, by unpaired t test) in case patients than in control subjects, for serotypes 4, 9V, 18C, and 19F; geometric mean concentrations are shown in italics. *, Pre-IPD-event levels of serotypes, which, except in the case of 23F ( ), were lower in case patients than in control subjects (P ≤ .01). Man rank correlation coefficient (ρ) = 0.33 and P < .01; for 23F, ρ = 0.14 and P < .01). EIA measurements incorporating 22F adsorption had lower interserotypic correlation (0.42 < r < 0.64) with other anti-PS IgG measurements and higher correlation with opsonophagocytosis (for 19F, ρ = 0.48 and P < .01; for 23F, ρ = 0.52 and P < .01). Use of the 22F-adsorption assay was associated with similar relationships between response to vaccination and pre-IPD-event levels of serotype, both in case patients and in control subjects; however, postvaccine levels of 23F were significantly higher in control subjects, but postvaccine levels of 19F were not (table 1). Pre-IPD-event levels of 23F also were significantly higher in the control subjects (P < .01), but, again, pre-IPD-event levels of 19F were not. Finally, peri-IPD-event changes in anti-PS IgG were apparent for the 2 IPD events due to 19F (fold rises of 2.7 and 2.6), changes that were not detected by the standard assay.

Discussion. The disappointing results from the clinical-efficacy trial of PPV led us to investigate anti-pneumococcal responses to vaccination and their relationship to disease events. Our findings support a relationship between low anti-PS IgG
Table 1. Postvaccination concentrations of antibody, median fold rise, and geometric mean killing titers, by vaccine status and pneumococcal-disease status.

<table>
<thead>
<tr>
<th>Serotype</th>
<th>Vaccine</th>
<th>Control subjects (n = 76)</th>
<th>Placebo</th>
<th>Control subjects (n = 48)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vaccine</td>
<td>Postvaccine GMC (95% CI)</td>
<td>Fold increase, median (IQR)</td>
<td>Postvaccine GMC (95% CI)</td>
</tr>
<tr>
<td></td>
<td>Placebo</td>
<td>Postvaccine GMC (95% CI)</td>
<td>Fold increase, median (IQR)</td>
<td>Postvaccine GMC (95% CI)</td>
</tr>
<tr>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.76 (0.40–1.46)</td>
<td>3.26 (2.34–4.54)b</td>
<td>1.3 (1.0–2.2)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>9V</td>
<td>1.36 (0.59–3.14)</td>
<td>5.68 (4.03–8.00)b</td>
<td>1.4 (1.1–2.8)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>14</td>
<td>3.57 (1.28–9.92)d</td>
<td>13.38 (8.76–20.44)b</td>
<td>4.3 (2.2–17.1)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>18C</td>
<td>0.86 (0.39–1.92)d</td>
<td>3.99 (2.90–5.49)b</td>
<td>1.9 (1.1–4.4)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>19F</td>
<td>2.67 (1.22–5.84)</td>
<td>9.52 (6.78–13.37)b</td>
<td>1.4 (1.1–3.8)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>23F</td>
<td>1.32 (0.68–2.59)d</td>
<td>2.28 (1.60–3.24)</td>
<td>1.6 (0.6–3.7)</td>
<td>.18</td>
</tr>
<tr>
<td>19F mod &lt;sup&gt;1&lt;/sup&gt;</td>
<td>2.98 (1.48–6.02)</td>
<td>4.87 (3.60–6.80)b</td>
<td>2.4 (1.3–6.5)</td>
<td>.17</td>
</tr>
<tr>
<td>23F mod &lt;sup&gt;1&lt;/sup&gt;</td>
<td>0.77 (0.41–1.45)</td>
<td>2.63 (1.91–3.62)b</td>
<td>3.6 (1.5–7.3)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

**NOTE.** CI, confidence interval; GMC, geometric mean concentration; IQR, interquartile range.

<sup>a</sup> Comparison of postvaccine levels in case patients versus those in control subjects.

<sup>b</sup> Significant increase from baseline levels at P < .01.

<sup>c</sup> Significant decrease from baseline levels at P < .01.

<sup>d</sup> Significant increase from baseline levels at P < .05.

<sup>e</sup> Significant decrease from baseline levels at P = .05.

<sup>f</sup> 22F-modified enzyme immunoassay used.

<sup>g</sup> No detectable killing in any serum sample.
and poor opsonophagocytosis and IPD in HIV-infected Africans. No evidence explaining the detrimental effect that vaccine has on anti-pneumococcal immunity was found.

For 5 of the 6 serotypes studied, the concentration of anti-PS IgG before an IPD event was significantly lower in the case patients than in the control subjects. This is consistent with both (1) the previously reported importance of opsonizing antibodies in defense against IPD [11] and (2) the idea that a generalized defect in response to polysaccharide underpins the increased risk for IPD [12, 13]. It is notable that, in the case patients who received placebo, significant postvaccine declines in anti-PS IgG were noted, for 5 of the 6 serotypes studied. Thus, in addition to low levels of anti-PS, rapidly deteriorating anti-pneumococcal immunity may also be a hallmark of risk for IPD in HIV-infected adults. A 0.35-μg/mL or 1-μg/mL concentration of anti-PS IgG has been proposed as a protective level of antibody, but it does not seem useful in this population. This is probably due, in part, to the EIA technique’s poor specificity in the measurement of anti-PS. This is confirmed by both the high interserotypic correlation seen for this assay and the low correlation between the results of opsonophagocytic assays and the concentration of anti-PS IgG in the 2 serotypes examined. The lack of specificity of EIA measurements has been noted in studies of other groups of adults [14, 15].

The use of 22F capsular-polysaccharide adsorption in the EIA [9] has been proposed as a method to improve the latter’s specificity; in the set of serum samples in the present study, it was assessed with respect to serotypes 19F and 23F, after completion of the opsonophagocytic assays. Increased (if still modest) correlation with killing was apparent, with a decrease in interserotypic correlation for anti-PS IgG. Thus, the incorporation of 22F neutralization into conventional EIA appears to increase the assay’s specificity in HIV-infected adults. Ideally, all of the anti-PS IgG measurements should be repeated with the 22F-modified EIA; however, we believe that this significant further effort would not alter the conclusions derived from our work with the non-22F EIA method, although it may limit our ability to make direct comparisons with other studies.

Killing activity in pre-IPD-event serum samples was not associated with IPD, but little measurable killing was found in any of these serum samples. As in the quantitative measures, the ability to produce measurable killing in response to vaccination was greater in the control subjects, which is consistent with case patients’ polyserotypic defect in response to polysaccharide; however, the majority of the control subjects failed to mount a measurable killing response, and, consequently, this was an insensitive predictor of protection.

We did not identify any adverse impact that PPV vaccination had on the anti-pneumococcal immune response; however, both the possibly low specificity of the anti-PS IgG measurements and the low sensitivity of the killing-measurement assay limit the power that this study has to identify an adverse impact of vaccination. For 90% of the case patients and for 60% of the control subjects, measurements of killing activity in serum, before and after vaccination, were below the assay’s lower level of sensitivity. If, below the lower level of sensitivity, only small changes in function define protection or susceptibility, then we may have missed this in our use of the whole-cell–killing assay.

An attenuated response to rechallenge with polysaccharide might be expected if vaccination had negatively biased anti-capsular immunity. Thus, we evaluated the peri-IPD-event fold rise in anti-PS against the disease-causing serotype and compared it to the vaccine-related fold rise in anti-PS. Only 5 individuals could be evaluated, and no trend in peri-IPD-event fold rise versus vaccine fold rise was observed. To investigate this further, 7-valent pneumococcal conjugate vaccine will be evaluated in this population; this will allow responses to polysaccharide in the conjugate vaccine to be compared on the basis of past vaccine exposure.

In summary, in this nested case-control study, we have identified, in anti-PS IgG responses in HIV-infected adults, generalized polyserotypic deficiencies associated with IPD. A marked excess of pneumococcal endpoints occurred in vaccine recipients in the RCT; we have found no evidence of a vaccine-induced defect to explain this finding.

References