Decreased Serum Opsonic Activity against *Streptococcus pneumoniae* in Human Immunodeficiency Virus–Infected Ugandan Adults

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Type-specific immunoglobulin G (IgG) to pneumococcal capsular polysaccharide (CPS) and opsonic activity against *Streptococcus pneumoniae* were evaluated in serum samples from 36 Ugandan adults with community-acquired pneumonia and 58 asymptomatic Ugandan adults with or without human immunodeficiency virus type 1 (HIV-1) infection. The levels of serum IgG to CPS were significantly higher in HIV-1–infected subjects than in HIV-uninfected subjects. Serum samples from HIV-1–infected subjects that had lower IgG titers demonstrated higher opsonic activity against type 3 (titers of 7) and type 9 (titers of 7–11) pneumococcal strains. Plasma HIV-1 load also correlated inversely with serum opsonic activity against these strains, and peripheral blood CD4+ lymphocyte numbers also tended to correlate with serum opsonic activity in asymptomatic HIV-1–infected adults. Our findings suggest that the opsonic activity of type-specific IgG is impaired in the serum of HIV-1–infected African adults, which may expose them to a serious risk of invasive pneumococcal infections.

More than two-thirds of the 40 million people living with HIV in the world reside in sub-Saharan Africa [1]. *Streptococcus pneumoniae* causes invasive infections in HIV-infected subjects in this area [2–5]. Furthermore, we, as well as other investigators, have documented that *S. pneumoniae* is the most common pathogen causing community-acquired pneumonia (CAP) in Ugandan and Kenyan adults living with HIV [6, 7]. A substantial activation and dysfunction of B cells that are associated with HIV infection may predispose these individuals to invasive pneumococcal infections [8–11].

The host defense against pneumococcal pneumonia depends largely on alveolar macrophages and the opsonization of specific IgG to pneumococcal capsular polysaccharide (CPS) and complement, followed by phagocytic killing [12, 13]. The phagocytic system in HIV-infected individuals has been a subject of extensive investigation, and several functional defects in phagocytic cells in such persons have been reported [14–16]. The mechanisms of impaired humoral immunity to *S. pneumoniae* in HIV-infected individuals, however, remain unclear.

We hypothesize that an impaired host defense against invasive pneumococcal infection could be attributed to a decreased type-specific IgG response or to a decrease in opsonic activity of type-specific IgG in the circulating serum of HIV-infected subjects. To further explore this hypothesis, we examined the levels of type-specific IgG, as well as the opsonic activity against *S. pneumoniae,*
in serum samples from HIV-infected adults. We report here a decrease in serum opsonic activity against *S. pneumoniae*, despite increased levels of type-specific IgG, in HIV-infected Ugandan adults.

**METHODS**

**Subjects and serum samples.** From November 1998 through March 2001, 36 patients with CAP were enrolled in our study on the day of admission to Mulago Hospital, Makerere University (Kampala, Uganda), after written informed consent had been obtained [6]. The diagnostic criteria for CAP have been described elsewhere [6, 17, 18]. Of these patients, 29 (13 men and 16 women) were infected with HIV-1, and 7 (6 men and 1 woman) were not HIV infected. From July through October 1998, 35 asymptomatic HIV-1–infected Ugandan adults (10 men and 25 women) and 23 asymptomatic HIV-uninfected Ugandan adults (17 men and 6 women) were randomly enrolled at the Joint Clinical Research Centre (Kampala) after providing informed consent [19]. Serum samples were obtained from these subjects at the time of enrollment and stored at −80°C. Neither the HIV-1–infected patients with CAP nor the asymptomatic HIV-infected subjects had received any antiretroviral therapy. HIV-1 serostatus and CD4+ lymphocyte counts in peripheral blood were determined at the time of enrollment [6, 19]. For the 35 asymptomatic HIV-1–infected subjects, plasma HIV RNA loads were quantified as described elsewhere [19]. The mean \( \log_{10} \) plasma HIV RNA load (± SD) of these subjects was 4.4 ± 1.0 copies/mL. The protocols of these 2 studies were reviewed and approved by the Ugandan AIDS Research Committee and the National Council for Science and Technology of Uganda. Frozen serum samples were shipped to Japan in temperature-controlled (liquid nitrogen) thermal containers. Control immune serum was obtained from healthy volunteers who had been immunized with the 23-valent pneumococcal polysaccharide vaccine.

**Measurement of total IgG and serotype-specific IgG.** Total IgG levels in serum were measured by laser nephelometry using purified human IgG and goat antibody to human IgG (Cappel). During the period 1998–2002, we determined the serotype of pneumococcal strains isolated from patients with CAP in Uganda. Among 29 strains, 3 strains of type 3 and 5 strains of type 9 were identified (authors’ unpublished data). We therefore decided to determine the levels of type-specific IgG to type 3 and type 9 CPS (because these are the major serotypes) by ELISA, according to a method reported elsewhere [20, 21]. Ninety-six-well flat-bottom microtiter plates were coated with bicarbonate buffer containing type 3 CPS (50 \( \mu \)g/mL) or type 9 CPS (5 \( \mu \)g/mL) and incubated overnight at 4°C. Twofold serially diluted preabsorbed serum with cell-wall polysaccharide (CWPS; Statens Serum Institute, Copenhagen, Denmark) was added to the antigen-coated plates, and the plates were incubated for 30 min at room temperature [20]. After the plates were washed, alkaline phosphatase–conjugated anti–human IgG (Biosource), diluted 1:2000, was added to each well, and the plates were incubated for 30 min at room temperature. The reaction was developed by \( p \)-nitrophenyl phosphate (Sigma Chemicals), and the optical density at 405 nm was read. The end-point titers were expressed as the reciprocal log, of the final dilution, giving an optical density at 405 nm of >0.016 for type 3 CPS and >0.130 for type 9 CPS.

**Chemiluminescence (CL) assay and opsonophagocytic killing assay.** Luminol-enhanced CL was measured as described elsewhere [22]. *S. pneumoniae* serotypes 3 (strain P97-182) and 9 (strain P20-049), both isolated from Ugandan patients with CAP, were used for this assay. Human polymorphonuclear leukocytes (PMNLs) were isolated from the peripheral blood of a healthy, HIV-uninfected volunteer and suspended in Hanks’ balanced salt solution buffer with \( Ca^{2+} \) and \( Mg^{2+} \) (HBSS+). Baby rabbit complement (Cedarlane Laboratories) was used as a complement source. The reaction mixtures contained human PMNLs (5 × 10⁵ cells), bacterial suspension (5 × 10⁶ cfu), 0.1 mg of luminol (5-amino-2,3-dihydro-1,4-phthallazinedione; Sigma), 25 \( \mu \)L of complement, and 25 \( \mu \)L of heat-inactivated test serum or heat-inactivated control immune serum (CS) in a total volume of 500 \( \mu \)L of HBSS+ containing 1% gelatin. After the reaction mixtures had been allowed to equilibrate at 37°C for 10 min, the bacterial suspension, complement, and heat-inactivated test serum or the CS were added to activate the system. The light emission was recorded continuously for 90 min with a 6-channel Biolumat LB9505 luminometer (Berthold).

The phagocytic killing assay for the killing of type 9 *S. pneumoniae* by human PMNLs was simultaneously performed in the same manner as the CL assay, using 7% rabbit blood agar, as described elsewhere [23]. The percentage of survival (mean ± SD of 4 tests) was calculated as follows: (bacterial load after incubation/bacterial load before incubation) × 100. No significant bacterial killing was found in samples containing PMNLs alone (94.8% ± 7.4%), PMNLs and CS (81.8% ± 15.9%), or PMNLs and complement (90.5% ± 13.7%). In contrast, a significant level of bacterial killing was found in samples containing PMNLs, CS, and complement (13.8% ± 2.9%). The CL response peaked and the light emission reached 8.21 × 10⁶ cpm in samples containing PMNLs, CS, and complement at 40 min after incubation. However, a minimal increase in light emission was found in samples containing PMNLs and CS (1.96 × 10⁴ cpm), PMNLs and complement (1.76 × 10⁵ cpm), and PMNLs alone (7.49 × 10⁴ cpm) at 40 min after incubation. These results indicate that a correlation exists between opsonophagocytic killing and the luminol-enhanced CL response. We, therefore, used the CL ratio (the ratio
of the peak CL value in the presence of test serum to the peak CL value in the presence of the CS) to evaluate serum opsonic activity. Because of the limited volume of stored serum, 2 samples from HIV-1–infected asymptomatic subjects and 15 samples from HIV-uninfected asymptomatic subjects were not available for testing of opsonic activity against type 3 strains.

**Statistical analysis.** The StatView statistical package (version 5.0) and SPSS software (version 10) were used for data analysis. All data were expressed as mean ± SD. Levels of serum IgG, levels of serum IgG to CPS, and serum opsonic activity were compared between the 2 groups using the unpaired Student’s t test or the Mann-Whitney U test. The subject’s age and peripheral blood CD4+ lymphocyte count, levels of serum IgG, and bacterial killing in the opsonophagocytic assay were analyzed by 1-way analysis of variance and by multiple comparison methods, including the Bonferroni-Dunn and Scheffé tests. Levels of serum IgG to CPS and serum opsonic activity among patients with CAP and asymptomatic subjects with or without HIV infection were analyzed by the Kruskal-Wallis test and Turkey’s multiple comparison. The significance of the correlations was estimated using Spearman’s rank correlation. Data were considered to be statistically significant when \( P \) was <.05.

**RESULTS**

**Total IgG and type-specific IgG.** The clinical and laboratory characteristics of 64 HIV-1–infected and 30 HIV-uninfected subjects are shown in table 1. Among 29 HIV-1–infected patients with CAP, 7 patients were found to be infected with *S. pneumoniae*, whereas no pneumococcal pneumonia was found among 7 HIV-uninfected patients with CAP. The peripheral blood CD4+ lymphocyte count of patients with CAP was significantly lower than that of asymptomatic subjects in the HIV-infected group \( (P = .002) \) and in the HIV–1–infected group \( (P < .001) \). Decreased CD4+ lymphocyte counts, however, have been found in patients with CAP during the acute phase, regardless of HIV status [24]. The total serum IgG level among HIV-1–infected subjects was significantly lower than that among HIV-uninfected subjects \( (38.20 ± 8.74 \text{ vs. } 19.95 ± 4.14 \text{ mg/mL; } P < .001) \), which confirms previous reports of B cell activation in such individuals [8–11]. No significant difference, however, was found in total levels of serum IgG between patients with CAP and asymptomatic subjects, regardless of HIV serostatus (table 1). The titers of IgG to type 3 and type 9 strains in serum were significantly higher among the 64 HIV-1–infected subjects than among the 30 HIV-uninfected subjects \( (8.34 ± 0.93 \text{ vs. } 7.07 ± 1.02 \text{ for type 3 and } 10.13 ± 1.32 \text{ vs. } 7.50 ± 1.01 \text{ for type 9; } P < .001, \text{ for either type}) \); total levels of serum IgG were significantly correlated with levels of serum IgG to type 3 CPS \( (r = 0.684; P < .0001) \) and type 9 CPS \( (r = 0.716; P < .0001) \) among the 64 HIV-1–infected subjects.

**Serum opsonic activity.** The opsonic activity in serum samples from 8 HIV-1–infected subjects with type-specific IgG titers of 7 for the type 3 strain was significantly higher than that in samples from 55 HIV-1–infected subjects with type-specific IgG titers of 8–10 (table 2; \( P < .001 \)). The opsonic activity in serum samples from 56 HIV-1–infected subjects with

**Table 1. Clinical characteristics and laboratory data for 64 subjects with community-acquired pneumonia (CAP) and 30 asymptomatic (AS) subjects in Uganda.**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-1–infected subjects</th>
<th>HIV-uninfected subjects</th>
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<tbody>
<tr>
<td></td>
<td>CAP ((n = 29))</td>
<td>AS ((n = 35))</td>
</tr>
<tr>
<td></td>
<td>CAP ((n = 7))</td>
<td>AS ((n = 23))</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>38.0 ± 8.3</td>
<td>34.2 ± 6.7</td>
</tr>
<tr>
<td>Peripheral blood CD4+ cell count, mean cells/µL ± SD</td>
<td>151 ± 138( ^{a} )</td>
<td>471 ± 295</td>
</tr>
<tr>
<td>Serum IgG level, mean mg/mL ± SD</td>
<td>38.0 ± 8.4( ^{c} )</td>
<td>38.0 ± 9.1( ^{c} )</td>
</tr>
<tr>
<td></td>
<td>19.9 ± 3.8</td>
<td>20.0 ± 4.3</td>
</tr>
</tbody>
</table>

\( ^{a} P < .0001, \text{ vs. HIV-infected or HIV-uninfected AS subjects; } P = .012, \text{ vs. HIV-infected patients with CAP.} \)

\( ^{b} P = .002, \text{ vs. HIV-uninfected AS subjects.} \)

\( ^{c} P < .001, \text{ vs. HIV-infected patients with CAP or HIV-uninfected AS subjects.} \)

**Table 2. Comparisons of opsonic activity against type 3 and type 9 *Streptococcus pneumoniae* in serum samples containing different levels of type-specific IgG from HIV-1–infected subjects in Uganda.**

<table>
<thead>
<tr>
<th>Type-specific IgG titer</th>
<th>Type 3 ((n = 63))</th>
<th>Type 9 ((n = 64))</th>
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<tbody>
<tr>
<td></td>
<td>No. of subjects</td>
<td>Opsonic activity, mean ± SD</td>
</tr>
<tr>
<td>7</td>
<td>8</td>
<td>1.15 ± 0.34( ^{a} )</td>
</tr>
<tr>
<td>8</td>
<td>28</td>
<td>0.88 ± 0.38</td>
</tr>
<tr>
<td>9</td>
<td>21</td>
<td>0.99 ± 0.37</td>
</tr>
<tr>
<td>10</td>
<td>6</td>
<td>0.82 ± 0.26</td>
</tr>
<tr>
<td>11</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>12</td>
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<td>13</td>
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</table>

\( ^{a} P < .001, \text{ for subjects with IgG titers of 7 vs. subjects with IgG titers of 8–10.} \)

\( ^{b} P < .001, \text{ for subjects with IgG titers of 12 and 13 vs. subjects with IgG titers of 7–11.} \)
type-specific IgG titers of 7–11 for the type 9 strain was also significantly higher than that in serum samples from 8 HIV-1–infected subjects with type-specific IgG titers of 12 and 13 (P<.001). In contrast, no significant difference was found in opsonic activity against the type 3 strain in serum from 30 HIV-uninfected subjects with different titers of type 3–specific IgG (P = .589, by the Kruskal-Wallis test). Similarly, no significant difference in opsonic activity against the type 9 strain was found in serum from 30 HIV-uninfected subjects with different titers of type 3–specific IgG (P = .757).

More interestingly, a significant correlation between the peripheral blood CD4+ lymphocyte count and serum opsonic activity against the type 3 strain was found for asymptomatic HIV-1–infected subjects, as shown in figure 1A (r = 0.354; P = .043). A similar relationship between these 2 parameters was found for the type 9 strain, although the correlation was not statistically significant (figure 1B; r = 0.282; P = .101). Because an inverse correlation between peripheral blood CD4+ lymphocyte counts and plasma HIV-1 RNA loads already has been demonstrated in asymptomatic HIV-infected Ugandan adults [19], we compared serum opsonic activity with the plasma virus load in such subjects. We found an inverse correlation between the plasma virus load and serum opsonic activity against the type 3 strain (figure 1C; r = −0.298; P = .092) and the type 9 strain (figure 1D; r = −0.414; P = .016).

Serum opsonic activity in patients with CAP with or without HIV infection. A significant decrease in serum opsonic activity against the type 9 strain was found among 64 HIV-1–infected subjects, compared with 30 HIV-uninfected subjects (1.09 ± 0.52 vs. 1.73 ± 0.53; P < .001). In contrast, the levels of serum opsonic activity against the type 3 strain were slightly lower among 63 HIV-infected subjects than among 15 HIV-uninfected subjects (0.94 ± 0.37 vs. 1.04 ± 0.34; P = .286), although the difference was not statistically significant. Furthermore, a significant decrease in serum opsonic activity against the type 3 strain was found among HIV-1–infected patients with CAP, compared with activity levels for asymptomatic HIV-1–infected subjects (figure 2C), whereas no significant difference was noted in the serum levels of IgG to type 3 CPS between these 2 groups (figure 2A). Serum opsonic activity against the type 9 strain for HIV-infected patients with CAP was also significantly lower than that for asymptomatic HIV-infected sub-

Figure 1. Correlation between peripheral blood CD4+ lymphocyte count and serum opsonic activity against type 3 (A) and type 9 (B) Streptococcus pneumoniae and correlation between plasma HIV-1 RNA load and serum opsonic activity against type 3 (C) and type 9 (D) strains among HIV-1–infected Ugandan adults.
Figure 2. Comparison of levels of serum IgG to type 3 pneumococcal capsular polysaccharide (CPS) (A) and type 9 CPS (B) and serum opsonic activity against type 3 (C) and type 9 (D) *Streptococcus pneumoniae* among patients with community-acquired pneumonia (CAP) and asymptomatic subjects (AS) with (HIV+) or without (HIV−) HIV infection. *P < .05; **P < .01; ***P < .001, by Kruskal-Wallis test with Turkey’s multiple comparison.

jects (figure 2D), although no significant difference was noted in the levels of serum IgG to type 9 CPS between these 2 groups (figure 2B). No significant differences were observed in serum opsonic activity against type 3 and type 9 strains and in serum levels of IgG to type 3 and type 9 CPS between HIV-uninfected patients with CAP and asymptomatic HIV-uninfected subjects (figure 2C and 2D).

**DISCUSSION**

An increase in levels of type-specific IgG in serum from HIV-infected subjects, compared with levels typical of HIV-uninfected subjects, was found. A significant correlation was also found between levels of type-specific IgG and total IgG in serum from HIV-infected subjects. In contrast, previous studies have reported that levels of serum IgG to CPS in HIV-1–infected subjects were significantly lower than those in HIV-uninfected subjects [25, 26] or that no difference was found in levels of type-specific IgG between HIV-1–infected and HIV-uninfected subjects [27].

Two studies recently reported that the avidity of type-specific IgG affects both in vitro opsonic activity and in vivo protective activity and that this response is critical in pneumococcal infection [28, 29]. These observations, however, have been largely confined to HIV-uninfected subjects. In the present study, serum samples from HIV-infected subjects that contained lower type-specific IgG titers (titers of 7 for type 3 and of 7–11 for type 9) had higher levels of opsonic activity against the type 3 and type 9 strains (table 2). These data, together with the observation of increased serum levels of type-specific IgG in HIV-infected subjects, who are susceptible to invasive pneumococcal infection, may suggest that these subjects have specific functional abnormalities of type-specific IgG. As a result, we examined the avidity of type 9–specific IgG in a limited number of serum samples from HIV-1–infected and HIV-uninfected Ugandan adults, using a method based on the dissociation of antibody-antigen complexes in the presence of 0.6 mol/L sodium thiocyanate [30]. A significant correlation between the 2 parameters was found in a group of 6 asymptomatic HIV-uninfected subjects (r = 0.81; P = .04), which is consistent with reports published elsewhere [28, 29]. In contrast, no correlation was found between the avidity levels of type-specific IgG and opsonic activity in a group of 7 asymptomatic HIV-infected subjects (r = 0.58; P = .33). These data suggest that
the function of IgG to CPS in the serum of HIV-infected subjects may be impaired.

Interestingly, we found a significant correlation between peripheral blood CD4+ lymphocyte counts and serum opsonic activity against the type 3 strain among HIV-1–infected subjects (figure 1A). A similar relationship was found between peripheral blood CD4+ lymphocyte counts and serum opsonic activity against the type 9 strain in HIV-1–infected subjects (figure 1B). Furthermore, the HIV-1 load correlated inversely with serum opsonic activity in asymptomatic HIV-infected subjects (figure 1C and 1D). These data suggest that HIV-1 itself may inhibit serum opsonic activity against S. pneumoniae. The interference of HIV with type-specific IgG and complement, therefore, will require further investigation. We also found a significantly decreased level of opsonic activity in serum from HIV-1–infected patients with CAP, compared with asymptomatic HIV-1–infected subjects (figure 2C and 2D). Because a transient increase in HIV RNA levels is found during the acute phase of bacterial pneumonia in HIV-infected adults [31], the increased virus load might suppress opsonic activity in serum from HIV-1–infected CAP patients at the time of admission. These data strongly support previous findings that indicate that the risk for bacterial pneumonia is highest among HIV-infected subjects with CD4+ lymphocyte counts of <200 cells/μL in African countries, as well as industrialized countries [6, 32], and demonstrate a new finding, that a defect in immunity is a mechanism in invasive pneumococcal infections in these subjects.

A recent study reported that the use of double absorption improved the correlation between antibody concentration and opsonic activity against type 4 and type 19F pneumococcal strains [33]. The notable exceptions, however, were type 3 and type 14 CPS, in which double absorption had a minimal effect on antibody concentrations. We therefore examined whether double absorption might lead to an improvement in opsonic activity against the type 9 strain, using serum samples from 6 HIV-1–infected subjects. We found that absorption of the cross-reactive antibodies with CWPS and 22F CPS had no effect on the opsonic activity against type 9 strain, compared with absorption with CWPS alone (data not shown).

In conclusion, we report decreased levels of serum opsonic activity against S. pneumoniae, despite increased levels of serum IgG to type 3 or type 9 CPS, among HIV-infected Ugandan adults. Plasma HIV-1 loads correlated inversely with levels of opsonic activity, whereas peripheral blood CD4+ lymphocyte counts tended to correlate directly with serum opsonic activity, against the type 3 and type 9 strains of S. pneumoniae among asymptomatic HIV-1–infected subjects. These data suggest that defective serum opsonic activity plays an important role in invasive pneumococcal infections in HIV-infected African adults. Further investigations will be required to clarify the specific functional abnormalities of type-specific IgG in the serum of HIV-1–infected African adults.

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References


