Lung Fluid Immunoglobulin from HIV-Infected Subjects Has Impaired Opsonic Function against Pneumococci


Background. The incidence of pneumococcal pneumonia is greatly increased among human immunodeficiency virus (HIV)–infected subjects, compared with among non–HIV-infected subjects. Lung fluid levels of immunoglobulin G (IgG) specific for pneumococcal capsular polysaccharide are not reduced in HIV-infected subjects; therefore, we examined immunoglobulin subtypes and compared lung fluid IgG opsonic function in HIV-infected subjects with that in healthy subjects.

Methods. Bronchoalveolar lavage (BAL) fluid and serum samples were collected from 23 HIV-infected and 26 uninfected subjects. None of the subjects were receiving highly active antiretroviral therapy, and none had received pneumococcal vaccination. Pneumococcal capsule–specific IgG levels in serum and BAL fluid were measured by enzyme-linked immunosorbent assay, and IgG was concentrated from 40 mL of BAL fluid. Opsonization and opsonophagocytosis of pneumococci with serum, BAL fluid, and BAL IgG were compared between HIV-infected subjects and healthy subjects.

Results. The effect of type 1 pneumococcal capsular polysaccharide–specific IgG in opsonizing of pneumococci was significantly less using both serum and BAL IgG from HIV-infected subjects, compared with serum and BAL IgG from healthy subjects (mean level, 8.9 fluorescence units [95% confidence interval, 8.1–9.7 fluorescence units] vs. 12.1 fluorescence units [95% confidence interval, 9.7–15.2 fluorescence units]; P = .002 for lung BAL IgG). The opsonophagocytosis of pneumococci observed using BAL IgG from HIV-infected subjects was significantly less than that observed using BAL IgG from healthy subjects (37 fluorescence units per ng of IgG [95% confidence interval, 25–53 fluorescence units per ng of IgG] vs. 127 fluorescence units per ng of IgG [95% confidence interval, 109–145 fluorescence units per ng of IgG]; P < .001).

Conclusion. HIV infection is associated with decreased antipneumococcal opsonic function in BAL fluid and serum.

Streptococcus pneumoniae is the most common cause of pneumonia worldwide [1] and is also a major cause of invasive disease syndromes, such as bacteremia and meningitis, and mucosal syndromes, such as otitis media and sinusitis [2]. The burdens of pneumonia [3] and bacterial meningitis [4] are particularly high in Africa, where the incidence and severity of these infections are greatly increased among patients who are also infected with HIV [5, 6].

Pulmonary and systemic defense against pneumococcal infection are independently regulated and differentially altered in HIV infection. In brief, immunity against capsulate bacteria, such as pneumococcus, is critically dependent on immunoglobulin [7] and complement [8] coating the bacteria (i.e., opsonization), followed by phagocytosis by tissue macrophages and neutrophils. General systemic immune defects in HIV infection that are associated with impaired pneumococcal defense include CD4+ lymphocyte depletion, decreased dendritic cell function, increased numbers of activated circulating B cells producing nonspecific IgG,
loss of splenic and lymph node architecture, and decreased reticulo-endothelial clearance of opsonized particles [9]. The overall effect in HIV-infected adults is that there are lower levels of circulating specific antipneumococcal IgG [10] and decreased vaccine responses [11]; in addition, serum samples from HIV-infected subjects show lower serum bacterial killing [12] and oxidative burst [13] using neutrophil-based assays than do serum samples from seronegative subjects.

The pulmonary mucosal surface, however, does not exhibit the same immune defects in HIV-infected subjects as it does in systemic defense. Bronchoalveolar lavage (BAL) studies have demonstrated pulmonary CD8+ lymphocytosis [14] and a pro-inflammatory cytokine milieu, including increased IFN-γ [15]. Alveolar macrophages are the predominant pulmonary phagocyte and require opsonizing immunoglobulin for ingestion of S. pneumoniae [7]. We showed no defect in the ingestion of opsonized S. pneumoniae by alveolar macrophages from HIV-infected adults [16]. Pulmonary mucosal immunoglobulin responses to protein antigen are locally regulated and independent of serum levels [17], and mucosal immunity is relatively preserved, compared with systemic immunity, in HIV-infected adults [18, 19].

Previously, we studied pneumococcal capsular polysaccharide (Pn) antibody concentrations in BAL fluid from HIV-infected and healthy adults. Levels of 23-valent pneumococcal capsular polysaccharide vaccine (PPV)–specific IgG in BAL fluid from HIV-infected adults were not significantly different from those in BAL fluid from control subjects, and they were shown to be highest in a group of HIV-infected patients with recent invasive pneumococcal disease, suggesting that specific responses to infection were present [20]. In this set of studies, we have examined IgA levels, IgG subtypes, and immunoglobulin function. We measured IgA to examine the contribution of IgA to immunoglobulin levels in BAL fluid, and we measured IgG subtypes to test the hypothesis that there might be insufficient pertinent IgG in the alveolar milieu. In particular, IgG2 is important in polysaccharide responses, is dependent on IFN-γ levels [21], and has been shown to be altered in HIV-infected subjects [22]. BAL IgG function has not previously been compared using BAL samples from HIV-infected adults and uninfected control subjects because of the lack of sensitivity of current opsonophagocytic assays [23] and the dilution inherent in BAL. In this study, we used protein G column purification as a means of concentrating BAL IgG, coupled with a sensitive flow cytometric assay [8], to test the hypothesis that there might be impaired antipneumococcal opsonic function in BAL fluid from HIV-infected subjects. We confirmed our findings using a second opsonic assay to measure binding and uptake of labeled pneumococci by human monocyte-derived macrophages.

**METHODS**

**Subjects and samples.** Adult volunteers with no recent illness and normal chest radiograph findings were recruited by advertisement and gave written informed consent to bronchoscopic examination with lavage, HIV testing, and sample storage. This study was given ethical approval by the Liverpool School of Tropical Medicine Research Ethics Committee and the College of Medicine Research Ethics Committee of the University of Malawi (Blantyre, Malawi).

**Bronchoscopic examination and BAL.** Bronchoscopic examination with lavage was performed as previously described [16]. In brief, after administration of topical anaesthesia, four 50-mL aliquots of sterile normal saline at 37°C were instilled and removed from a subsegmental bronchus of the right middle lobe using gentle hand suction. BAL was placed in siliconized glass containers on ice and was spun within 30 min to remove the cell pellet, and the supernatant fluid was stored at −80°C.

**IgG affinity purification.** A protein G column was washed with binding buffer (5 mL of 0.1M Na acetate; pH 5.0) in accordance with the manufacturer’s instructions (Pierce Biotechnology) before diluted BAL fluid (40 mL, diluted 1:1 with binding buffer) was passed unassisted through the column. The loaded column was then washed, and bound IgG was eluted using elution buffer (0.1 M glycine HCl; pH 2.5). The pH of eluted fluid was immediately increased by adding 100 µL of 1M TrisCl (pH 7.4) to each milliliter of fluid eluted from the column. BAL IgG was concentrated from eluted fluid using a Biomax-5 micropore spin column (Millipore) according to the manufacturer’s instructions. In brief, a 2-spin process allowed clean, concentrated BAL IgG (confirmed by protein gel electrophoresis) from 40 mL of BAL fluid to be stored in a final volume of 3 mL. Neat BAL fluid could not be concentrated in this manner because of the viscosity of BAL surfactant and proteins other than IgG. Samples were stored at −80°C.

**ELISA measurement of total and specific IgG.** Total IgG levels in serum, BAL fluid, and concentrated BAL samples were measured using a sandwich ELISA, as previously described [20]. In brief, antihuman IgG (Dako) was used as trap antibody; samples were diluted at 1:60,000 for serum, 1:500 for BAL fluid, and 1:1000 for concentrated BAL IgG; and alkaline phosphatase conjugated anti IgG (Sigma) was the detection antibody. O-phenylenediamine dihydrochloride substrate was used (100 µL of 0.1 mg per mL of O-phenylenediamine dihydrochloride in 1M diethanolamine and 0.5 mmol/L MgCl2; pH 9.8) with 50 µL of 5M H2SO4 to stop the reaction, and the plate was read at 492 nm (Optimax Tunable Microplate Reader; Molecular Devices).

Pn type 1 (Pn1)–specific IgG was measured using a standard capture ELISA [24]. In brief, all samples and standards were preadsorbed using C-polysaccharide (Statens Seruminstitut) and capsular serotype 22F polysaccharide (ATCC) [25]. Plates
were incubated overnight with Pn1 (ATCC) and then incubated with blocking buffer (5% fetal calf serum in PBS) before sample loading. Serum samples were diluted 1:200 in PBS, but concentrated BAL IgG and neat BAL fluid were not diluted. A serial dilution of Pneumococcal Standard Serum, lot 89-S (US Food and Drug Administration) was used as the standard. Plates were incubated for 2 h at room temperature, washed, and dried. A total of 100 μL of alkaline phosphatase–conjugated anti-IgG (Sigma) was added, and the assay was completed exactly as in the total IgG assay described above. We measured 23-valent PPV–specific IgG, IgA, IgG1, and IgG2 in BAL fluid using the same capture ELISA as the Pn1 assay described above, but replacing Pn1 with 23-valent PPV and the appropriate detection antibody, as described elsewhere [20].

**Bacterial stocks.** *S. pneumoniae* of capsular type 1 (ATCC 6301; strain SSISP 1/1; Statens Seruminstitut) was chosen because of the high incidence of this type in the study population [26]. Mid-log broth was spun to a pellet, and bacteria was resuspended briefly in PBS. A Quellung reaction was performed using type 1–specific rabbit serum (Statens Seruminstitut) to confirm adequate bacterial encapsulation. In flow cytometry experiments, fresh stock bacteria were used immediately. In experiments requiring labeled bacteria, the bacterial pellet was resuspended in stock fluorescein isothiocyanate (FITC) solution (1 mg/mL) for 1 h at 4°C in the dark on a shaker plate. The bacteria were then washed in PBS and used immediately.

**Antibody-binding flow cytometry assay.** The 100-μL aliquots of bacteria, prepared as described above, were spun to a pellet (13,000 rpm for 2 min) and resuspended in 100 μL of diluted serum (diluted 1:10, 1:100, or 1:1000 in RPMI), 100 μL of BAL fluid, 100 μL of concentrated BAL IgG (diluted 1:10 in RPMI), or 100 μL of RPMI alone. Resuspended bacteria were rotated for 40 min at 37°C with 5% CO₂, washed twice in cold PBS, and resuspended in 200 μL of 4% formaldehyde. Fixed bacteria were washed twice, resuspended vigorously, and incubated for 20 min in the dark at room temperature with 100 μL of 1:20 diluted FITC conjugated antihuman IgG (Sigma). Finally, the samples were washed in PBS and resuspended for analysis. Flow cytometry settings were determined using control samples and were set constant during the recording of mean linear fluorescence (MLF) in the FITC channel for 10,000 gated events from each sample.

**Pneumococcal attachment and phagocytosis assay.** A fluorimetric assay was performed using opsonized pneumococci and human monocytes in a manner similar to that used in our previous study involving mycobacteria [27]. In brief, 5 × 10⁵ monocytes were allowed to adhere to flat-bottom plates overnight and were washed. FITC-labeled type 1 pneumococci opsonized using serum (1:10 dilution) or BAL IgG (1:6 dilution) at a multiplicity of infection of 100 were added to monocyte-containing wells. Control wells included monocytes alone and labeled bacteria with no monocytes. All wells were incubated for 30 min and then washed gently 3 times before being read on a fluorimeter at 320 nm (Gemini EM; Molecular Devices). Trypan blue was then added to quench the extracellular FITC, and the plates were immediately read again at 320 nm. The prequench fluorescence reading in test wells, minus the background obtained from cell-free control wells, measured the sum of bound and internalized bacteria. Postquench fluorescence (minus the background) measured internalized pneumococci. Data were expressed both as fluorescence units and as a corrected value per nanogram of IgG in the Pn1-specific ELISA.

**Statistics.** The data from HIV-infected subjects and healthy subjects for each assay were compared using geometric means with confidence intervals and the rank sum (nonparametric) test (Stata 9; StataCorp).

**RESULTS**

**Subjects and samples.** A total of 49 subjects (28 of whom were men) were recruited to the study. All subjects were Malawians, 23 had HIV infection, and none were being treated with antiretroviral therapy. None of the subjects had received PPV or conjugate vaccine, and none of the subjects were cigarette smokers. Demographic and clinical details for the subjects are summarized in table 1 by HIV infection status. There were no significant differences between the groups with respect to age, sex distribution, or incidence of previous invasive pneumococcal disease (i.e., bacteraemia or meningitis; range, 4 months to 3 years prior to study), but the HIV-infected group had lower CD4⁺ cell counts than did healthy subjects. Serotype data were not available regarding the recorded disease episodes, but Pn1-specific IgG levels were not different in serum samples or BAL fluid between subjects with and subjects without a history of pneumococcal disease. No subject had an adverse reaction to bronchoscopic examination, all BAL volumes were >50 mL, and there was no difference in the BAL return between HIV-infected subjects and healthy subjects (mean value, 125 mls vs. 122 mls; P = .68).

**IgG levels are higher in BAL fluid from HIV-infected subjects than in BAL fluid from control subjects.** Total IgG and Pn1-specific IgG concentrations are shown in the upper and lower panels of figure 1 for BAL fluid, IgG extracted from BAL fluid, and serum. The concentration of IgG from BAL fluid resulted in ~10-fold increases in the concentration of both total IgG and Pn1-specific IgG. HIV-infected subjects had a higher level of total IgG than did healthy subjects both in BAL (P < .001, by rank-sum test) and in concentrated BAL IgG solution (P < .001, by rank-sum test). HIV-infected subjects had higher levels of Pn1-specific IgG than did healthy subjects in concentrated BAL IgG (P < .001, by rank-sum test) and serum (P < .001, by rank-sum test). There was an inverse correlation of...
BAL Pn1-specific IgG with CD4+ cell count ($r = -0.31; P = .05$).

Pn1-specific IgG values measured in neat BAL fluid were below the lower limit of detection of the assay (10 ng/mL) in 25 of 49 subjects; therefore, we compared 23-valent pneumococcal capsule–specific IgG and IgG subtypes 1 and 2 with extrapolated values where needed (14 subjects had IgG values <10 ng/mL). Pn-specific IgG in BAL fluid was higher in BAL fluid from HIV-infected subjects than in BAL fluid from healthy subjects (mean Pn-specific IgG level, 103 ng/mL vs. 18 ng/mL; $P = .004$, by rank-sum test). Pn-specific IgG1 was higher in BAL fluid from HIV-infected subjects than in BAL fluid from healthy subjects (mean Pn-specific IgG1 level, 20 ng/mL vs. 11 ng/mL; $P = .002$, by rank-sum test). Pn-specific IgG2 was also higher in BAL fluid from HIV-infected subjects than in BAL fluid from healthy subjects (mean Pn-specific IgG2 level, 25 ng/mL vs. 9 ng/mL; $P = .004$, by rank-sum test). The Pn-specific IgG1 to IgG2 ratio was not different when compared between HIV-infected and healthy subjects (IgG1:IgG2, 2.1 in HIV-infected subjects vs. 1.8 in control subjects; $P = .3$). Levels of Pn-specific IgA in BAL fluid were not significantly different between HIV-infected subjects and control subjects (mean Pn-specific IgA level, 13 ng/mL vs. 7.6 ng/mL; $P = .06$).

Levels of BAL IgG binding to bacteria are lower in HIV-infected subjects than in control subjects. The geometric MLF of bacteria labeled with FITC-conjugated antihuman IgG for serum diluted 1:1000, 1:100, or 1:10 showed a dose response with increasing MLF values resulting from opsonization with increasing concentrations of serum. Opsonization of bacteria using neat BAL fluid gave very low MLF values, below those achieved with 1:100 diluted serum, but opsonization using concentrated BAL IgG gave MLF values that were between 1:10 and 1:100 dilutions of serum. MLF values for serum diluted 1:10 and concentrated BAL IgG were compared by HIV status (figure 2) and showed lower values of IgG binding to bacteria in samples from HIV-infected subjects than in samples from healthy subjects (for serum diluted 1:10, $P = .002$, by rank sum test; for concentrated BAL IgG, $P = .002$, by rank-sum test). The IgG binding resulting from opsonization with BAL IgG from HIV-infected subjects was lower than that with BAL IgG from healthy subjects, despite containing 4 times the amount of serotype 1 IgG (figure 1); therefore, when the MLF value obtained was expressed per nanogram of Pn1-specific IgG measured by ELISA, differences between healthy subjects and HIV-infected subjects were even more pronounced. Samples from HIV-negative subjects had a mean MLF value of 17 fluorescence units per nanogram of IgG, compared with 4 fluorescence units per ng of IgG in HIV-positive subjects ($P < .001$, by rank-sum test). There was no significant correlation between IgG binding and CD4+ cell count.

Opsonophagocytosis with BAL IgG is lower in HIV-infected patients than in control subjects. The binding and internalization of opsonized bacteria in vitro are shown by opsonic fluid and HIV status in figure 3. There was a significant difference in binding and internalization (prequench) between HIV-infected and healthy subjects, both when BAL IgG was compared directly and after correction for concentration of Pn1–specific immunoglobulin (3.7 fluorescence units per ng of IgG [95% CI, 2.5–5.3 fluorescence units per ng of IgG] vs. 12.7 fluorescence units per ng of IgG [95% CI, 10.9–14.5 fluorescence units per ng of IgG]; $P < .001$, by rank-sum test). Postquench data (figure 3) also showed a significant difference between HIV-positive subjects and healthy subjects when corrected for IgG concentration (1.5 fluorescence units per ng of IgG [95% CI, 1.0–2.3 fluorescence units per ng of IgG] vs. 4.9 fluorescence units per ng of IgG [95% CI, 4.4–5.4 fluorescence units per ng of IgG]; $P < .001$, by rank-sum test). There was no significant difference by HIV status with respect to the percentage of bound bacteria internalized, and there was no significant correlation between CD4+ cell count and either bacterial binding or internalization in this assay.

**DISCUSSION**

We have demonstrated impaired bacterial binding of Pn1-specific IgG and impaired opsonophagocytosis of pneumococci in

**Table 1. Demographic and clinical characteristics of 49 study subjects, by HIV infection status.**

<table>
<thead>
<tr>
<th>Variable</th>
<th>HIV-positive subjects ($n = 23$)</th>
<th>HIV-negative subjects ($n = 26$)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, M:F</td>
<td>14:9</td>
<td>14:12</td>
<td>NS</td>
</tr>
<tr>
<td>Age, mean years ± SD</td>
<td>32.4 ± 8.4</td>
<td>28.7 ± 9.7</td>
<td>NS</td>
</tr>
<tr>
<td>Previous hospital admission with proven pneumococcal disease</td>
<td>7</td>
<td>2</td>
<td>NS</td>
</tr>
<tr>
<td>CD4+ cell count, median cells/µL (range)</td>
<td>212 (7–620)</td>
<td>795 (476–1342)</td>
<td>.001</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td>0</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

**NOTE.** NS, not significant.
HIV-infected subjects, compared with healthy subjects, using IgG from both BAL fluid and serum samples. Concentrations of Pn1-specific IgG in concentrated BAL IgG and serum were higher in HIV-infected subjects than in healthy subjects but, despite this difference, opsonic function per volume of opsonic fluid was lower in HIV-infected patients than in healthy subjects. A corrected value of opsonic function per nanogram of measured Pn1-specific IgG showed a significantly lower function in HIV-infected subjects, compared with healthy subjects, in 2 different functional assays.

The major strength of this study is the direct comparison of antipneumococcal immunoglobulin function using both BAL fluid and serum samples from both HIV-infected subjects and healthy subjects. Previous studies of antipneumococcal immunoglobulin function have not been able to compare IgG function using BAL fluid because of the lack of sensitivity of opsonophagocytic assays of capsulate pneumococci [23]. We increased the concentration of BAL IgG to solve this problem and then coupled this method with 2 previously published sensitive opsonic assays, using both a relevant monocyte phagocytic model [27] and a nonphagocytic immunoglobulin-binding method [8]. We have previously described an increased antipneumococcal immunoglobulin concentration in BAL fluid from HIV-infected adults, compared with the antipneumococcal capsular polysaccharide (Pn1)–specific IgG in bronchoalveolar lavage (BAL) fluid, concentrated BAL IgG, and serum samples from HIV-infected (HIV pos) and healthy (HIV neg) subjects. Total IgG levels were higher in HIV-infected subjects (n = 23) than in healthy subjects (n = 28) in BAL fluid (geometric mean IgG level, 10.1 μg/mL [95% CI, 6.4–15.8 μg/mL] vs. 3.0 μg/mL [95% CI, 2.5–3.6 μg/mL]; P < .001, by rank-sum test) and in concentrated BAL IgG (geometric mean IgG level, 174.6 μg/mL [95% CI, 123–249 μg/mL] vs. 57 μg/mL [95% CI, 40–80 μg/mL]; P < .001, by rank-sum test). Levels of Pn1-specific IgG in neat BAL fluid were at the lower limit of detection for this ELISA assay, but a significantly higher level was seen in HIV-infected subjects than in healthy subjects in both concentrated BAL IgG (298 ng/mL [95% CI, 205–432 ng/mL] vs. 99 ng/mL [95% CI, 89–111 ng/mL]; P < .001, by rank-sum test) and serum (2950 ng/mL [95% CI, 2335–3728 ng/mL] vs. 1608 ng/mL [95% CI, 1218–2122 ng/mL]; P < .001, by rank-sum test). The graph is plotted using a linear scale, with the box plot indicating 25th–75th centiles and the median value and the whiskers indicating the 5th and 95th centiles. Outlying values are not shown.
Figure 3. Binding, internalization, and percent internalization of type 1 pneumococci in vitro after opsonization with serum or bronchoalveolar lavage (BAL) IgG. Fully capsulated mid-log phase type 1 pneumococci were labeled using FITC and were opsonized using diluted serum (1:10) or concentrated BAL IgG (diluted 1:6). Labeled, opsonized bacteria were allowed to adhere to human monocytes, and excess bacteria were washed off. Fluorimetric measurements were recorded after removal of background values obtained using the binding of labeled opsonized bacteria to cell-free wells. Data are presented for opsonization using serum and BAL IgG, the latter being shown both as absolute values and expressed per nanogram of type 1 pneumococcal capsular polysaccharide (Pn1)–specific IgG measured by ELISA in the same sample. A, Fluorimetric measurements of total cell-associated bacteria, by HIV-infection status of the donor. Opsonization with fluids from HIV-infected subjects resulted in less bacterial binding than did opsonization with fluids from healthy subjects (HIV-infected serum vs. healthy serum, 10.5 vs. 12.5 fluorescence units; P = .006; HIV-infected BAL IgG vs. healthy BAL IgG, 11 vs. 12.5 fluorescence units; P = .02). Data expressed as fluorescence units per nanogram of Pn1 IgG showed a highly significant difference (HIV-infected subjects vs. healthy subjects, 3.7 vs. 12.7 fluorescence units per ng of Pn1 IgG; P < .001). B, Fluorimetric measurements obtained after quenching the extracellular signal using trypan blue. There was no significant difference in absolute values when fluorimetry was compared by HIV-infection status, but HIV-infected subjects showed significantly less bacterial internalization per nanogram of Pn1 IgG (HIV-infected BAL IgG vs. healthy BAL IgG, 1.6 vs. 4.9 fluorescence units per ng of Pn1 IgG; P < .001). Both graphs are plotted using a linear scale, with the box plot indicating 25th–75th centiles and the median value, whiskers indicating 5th and 95th centiles, and asterisks indicating outlying values.

The implications of this study are that not all immunoglobulin concentration in BAL fluid from healthy subjects [20], and have also demonstrated that HIV infection does not, in itself, have a general inhibitory effect on macrophage binding or uptake of S. pneumoniae [16] or Mycobacterium tuberculosis [27]. The data from experiments involving type 1 pneumococcus in this study may be extended to other types, because 23-valent capsular polysaccharide IgG concentrations showed the same pattern, and other published data show that different pneumococcal capsular serotypes elicit a similar response to vaccination and disease [28]. The monocytic model used here is more representative of the stable situation in healthy lungs [29] than are neutrophil assays, which model the conditions pertaining in acute inflammation [30].

Direct comparisons of immunoglobulin function in serum and BAL fluid are made difficult by the dilution of lung fluid that results during BAL. Published data have shown that extracellular lung fluid was diluted 1:60 during BAL by using a comparison of urea concentrations in BAL fluid and serum and assuming equilibrium between undisturbed extracellular lung fluid and serum [31]. The observation in this study that BAL fluid levels of Pn1-specific IgG were ~100 times lower than those measured in serum samples suggests that native extracellular lung fluid IgG levels may be similar to those measured in serum.

A possible explanation for the impaired IgG function described here could be that HIV-infected subjects have limited IgG diversity, compared with healthy subjects. HIV gp120 binding of specific B cell receptors causes clonal deletion of B cells in HIV infection [32]. A selective depletion of VH3 genes coding for IgG with pneumococcal opsonizing properties has been described in HIV-infected adults [33]. HIV infection is also likely to alter other mucosal responses to pneumococcal infection, however, including the immunoglobulin response to protein antigens [34] and CD4-dependent cell-mediated immunity [35]. Immunoglobulins directed to surface protein antigens are protective against pneumococcal infection by several mechanisms, including alteration of complement deposition [36] and reduction in pneumococcal carriage [37]; therefore, the extent of impaired mucosal immunity in patients with HIV infection is likely to be greater than that described in this study.

The implications of this study are that not all immunoglobulin specific to Pn are protective against pneumococcal disease and that a similar impairment in IgG function is seen in both BAL fluid and serum samples from HIV-infected subjects. The impairment of BAL IgG function is likely to be a contributing factor to the excess of pneumococcal disease seen in HIV-infected subjects.

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Potential conflicts of interest. All authors: no conflicts.

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