BAL and Serum IgG Levels in Healthy Asymptomatic HIV-Infected Patients*

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Objectives: To determine if the increased susceptibility to bacterial infection in asymptomatic HIV-infected patients is associated with decreased total IgG or IgG₂ levels in lung epithelial lining fluid.

Background: A decrease in lung IgG levels or subtypes has been proposed as contributing to the increased risk of bacterial lung infections in HIV-infected patients. Previous studies measuring lung lavage IgG concentrations have been inconsistent.

Methods: Twenty-three HIV patients and 25 control subjects underwent BAL. Both patient groups were of similar age, and had similar pulmonary function studies and body mass index. Smokers were equally represented in both groups, and the majority of subjects in both groups were male. Total IgG and IgG₂ levels in lavage fluid were assayed in both cohorts and compared using a two-tailed Student's t test.

Results: The lung lining fluid IgG level in HIV-infected patients was 0.19 ± 0.13 μg/μg of protein (mean ± SD) vs 0.11 ± 0.09 μg/μg of protein in control subjects (p < 0.05). The IgG₂ level in HIV patients was 0.034 ± 0.038 μg/μg of protein and 0.014 ± 0.01 μg/μg of protein in control subjects (p = 0.054). Lavage IgG levels reflected serum IgG values (correlation coefficient, 0.56; p < 0.001) but did not correlate with lung immunoglobulin-producing cells.

Conclusions: The increased susceptibility to bacterial pneumonia in asymptomatic HIV-infected individuals is neither explained by depressed total IgG levels nor a deficiency in IgG₂ levels in the lungs. The strong correlation between serum and lavage IgG levels suggests that lavage IgG derives from serum.

Key words: BAL fluid; HIV infection; immunoglobulin

Abbreviations: BMI = body mass index; DLCO = diffusion capacity of the lung for carbon monoxide; ELF = epithelial lining fluid

A hallmark of the HIV epidemic has been an increase in the frequency of pulmonary infections. The risk of bacterial pneumonia is at least sixfold higher in HIV-infected patients, compared to age-matched control subjects; those who develop pneumonia have a higher mortality. Though CD4 counts can predict an increased risk for pneumonia, HIV-infected patients still develop pneumonia at a much higher frequency when compared to uninfected control subjects. This increased frequency persists even when correction is made for CD4 counts. Additionally, HIV-infected patients become bacteremic with pneumococcal pneumonia more frequently than control subjects and, in general, are predisposed to encapsulated bacterial pathogens. Furthermore, the carriage rates for Streptococcus pneumoniae are the same in HIV-infected patients and control subjects, suggesting a defect in humoral immunity rather than simply an increased exposure to the organism. Although deranged cell-mediated immunity is the hallmark of HIV infection, immunologic defects are more Protean, involving all facets of the host immune response. Indeed, humoral dysfunction is one of the first immunologic sequelae of HIV infection. Included among the humoral defects are depressed specific antibody responses to acute infections and vaccines despite the presence of hypergammaglobulinemia. A major component of the humoral immune system is serum IgG. IgG allows for targeting of antigen for destruction through phagocytosis by macrophages and antibody-dependent cell-mediated cytotoxicity by lympho-
cytes and natural killer cells. Indeed, murine studies show a direct quantitative relationship between protection from pneumococcus and serum level of antcapsular IgG derived from vaccinated patients.8 Since IgG is the primary protective antibody present in the lung, it is reasonable to presume that some of the increased susceptibility to bacterial infections may be due to low IgG levels in epithelial lining fluid (ELF). Both elevated and depressed IgG levels have previously been reported in studies of lavage epithelial lining fluid in HIV-infected patients.9–12 In order to study the effects of HIV infection on lung IgG production, we compared levels of IgG in both serum and BAL fluid from asymptomatic HIV-infected patients and control subjects. IgG2 levels were also evaluated in lavage fluid from both groups. Additionally, we quantified lymphocyte-subset populations to compare with previous studies and to determine if alteration in their composition reflected on BAL IgG levels.

Materials and Methods

Patient selection

Twenty-three HIV-positive patients and 25 control subjects were selected at random from an ongoing HIV lung study project at The Ohio State University. All HIV patients and control subjects completed a standardized questionnaire, underwent spirometry with lung volumes and diffusion capacity of the lung for carbon monoxide (DLCO), chest radiography, phlebotomy, and measurements of body mass index (BMI) and CD4 count. Patients were specifically questioned about respiratory symptoms and smoking status. All subjects were required to be asymptomatic at the time of study. Nonsmokers were defined as those having never smoked or those having quit for at least 2 years. At the time of enrollment, 10 HIV-infected patients were receiving antiretroviral therapy alone, and an additional 8 patients were receiving both antiretrovirals and protease inhibitors. The Institutional Review Board for Human Studies at The Ohio State University approved the study. Informed consent was obtained from all subjects.

BAL

BAL was performed in a standardized manner as previously described.13 Briefly, following local anesthesia with 1% lidocaine, a flexible fiberoptic bronchoscope was introduced into the lower respiratory tract via the nose and wedged in a subsegmental distal bronchus in the right middle lobe or lingula. The BAL was obtained by sequentially instilling and aspirating five 20-mL aliquots of sterile 0.9% saline solution. Recovered fluid was passed through a single layer of sterile surgical gauze to remove particulate matter and mucous. Aliquots were immediately used for differential cell counting and lymphocyte phenotyping. The remaining recovered lavage fluid was stored at −80°C until processed. Aliquots of stored BAL fluid were subsequently concentrated using ultrafilters (Centricon-3 concentrator; molecular weight cutoff, 3,000; Amicon; Beverly, MA). Samples were loaded in 2-mL aliquots into tubes (Centricon-3; Amicon) and spun at 6,500 g for 2 h at 25°C. This resulted in approximately a 20-fold concentration of the samples.

Lavage Cell Analysis

A single operator determined lavage cell counts by direct hemocytometry. Cell differentials were determined using a staining technique (Diff-Quick; Dade Diagnostics of Puerto Rico; Aguada, Puerto Rico) on cytospin preparations. Aliquots of BAL fluid containing approximately 1 million cells were sent to The Ohio State University immunology laboratory for T-lymphocyte counts and subtyping. This was accomplished using a fluorescence-activated cell sortor (FACS; Coulter; Hialeah, FL). Using dual-staining procedures, with directly fluoresceinated antibodies, the relative frequencies of the following phenotypes was determined: CD3+ /CD4+ (T-helper lymphocytes), CD3−/CD8+ (suppressor/cytotoxic T lymphocytes), and S6F1+/CD8+ (activated cytotoxic CD8+ lymphocytes).14,15

Quantification of BAL IgG and Blood IgG Levels

In order to evaluate IgG levels in BAL fluid, IgG levels were measured by using an ultralow-level IgG kit (Kallestad Endplate; Sanofi Diagnostics Pasteur; Chaska, MN) after the method described by Mancini et al.16 The lower detection limit of the kit was 20 μg/mL, and the coefficient of variation was 0.9 to 5.5%. IgG2 levels were measured using a low-level human IgG2 kit (The Binding Site; Birmingham, UK). Briefly, 5-μL aliquots of concentrated BAL fluid (10 μL in the case of IgG2) along with standards were placed in the agarose gel plate wells using the end point diffusion method. Monospecific anti-IgG serum present in the agar gel reacts with IgG present in samples and standards forming a precipitation ring. The ring diameters are squared and compared to plate standards and expressed as micrograms per milliliter of BAL fluid. The lower detection limit of the IgG2 kit was 8 μg/mL, and the coefficient of variation was 1.4 to 5.1%. Blood obtained simultaneously with the lavage fluid was assayed in The Ohio State University immunology laboratory using standard nephelometry methods.

Statistical Analysis

All data were expressed as mean ± SEM. Paired t tests were used to compare populations (Excel 97; Microsoft; Redmond, WA). Statistical significance was defined as a p value < 0.05. Correlations were determined using software package (Excel 97; Microsoft).

Results

Patient Characteristics

In order to compare IgG levels between asymptomatic HIV-infected patients and normal control subjects, patients were randomly selected from an ongoing study of healthy HIV-infected patients at The Ohio State University. All HIV-infected patients were in clinical category A, based on the 1993 Centers for Disease Control and Prevention revised classification of HIV infection.17 The mean ± SD age of the 23 HIV-infected patients was 37 ± 8 years (19 male and 4 female patients). Control subjects were 36 ± 6 years old, the majority of which (n = 23) were men. Fourteen patients in each group were smokers, and 3 patients in each group had a previous history of pneumonia. The mean smoking...
history was 24 ± 22 pack-years in the HIV-positive smokers and 33 ± 21 pack-years in the control subjects who smoked (p = 0.3). The mean CD4 count in the HIV group was 361 ± 214 cells/μL, and 950 ± 386 cells/μL in the control group. BMI (percent predicted) was 103 ± 17% in the HIV patients and 107 ± 25% in control subjects. The FEV1, FVC, total lung capacity, FEV1/FVC, and Dlco per unit of alveolar volume (R) were similar in the two groups (Table 1). Eighteen HIV-infected patients were receiving antiretroviral therapy, and 8 of these patients were additionally receiving protease inhibitors.

Lavage Fluid Characteristics

In order to obtain lavage fluid for IgG determinations and cell analysis, a standardized lavage was performed. BAL recovered an average of 56 mL from HIV patients and 59 mL from control subjects, with a mean of 6 × 10⁶ cells/mL from HIV patients and 5 × 10⁵ cells/mL from control subjects. Macrophages accounted for 93% of lavaged cells in HIV patients and 91% in control subjects. Lymphocytes amounted to 4.3% of cells in HIV patients and 7% in control subjects (Fig 1). Neutrophils accounted for < 2% of total cells in both samples. There was no statistically significant difference in the volume of fluid recovered or in differential cell counts between HIV patients and control subjects.

BAL and Serum IgG Levels

BAL IgG and IgG₂ levels in patients and control subjects were evaluated using the radial immunodiffusion assays as previously described. For IgG₂ estimates, 18 patients and control subjects were available for evaluation. The results were analyzed using three different but well-recognized methods. When referenced to recovered BAL fluid and expression as μg/mL of BAL (Fig 2, bottom left, C), IgG levels in HIV-positive patients had a value of 24.7 ± 23 μg/mL of BAL fluid, while control subjects had values of 11.9 ± 14 μg/mL. This was statistically significant (p < 0.05). IgG₂ levels were 3.78 ± 4.45 μg/mL of BAL fluid in HIV patients (Fig 3, top left, A) and 1.22 ± 0.82 μg/mL of BAL fluid in control subjects (p < 0.05). In order to account for dilutional effects of the instilled fluid, data were also analyzed using fluid protein as a reference (Fig 2, top right, B). When analyzed by this method, the IgG level in HIV BAL was 0.19 ± 0.13 μg/μg of protein and 0.11 ± 0.09 μg/μg of protein in control subjects, which was statistically significant (p < 0.05). IgG₂ levels were 0.034 ± 0.038 μg/μg of protein in HIV patients (Fig 3, top right, B) and 0.014 ± 0.011 μg/μg of protein in control subjects (p = 0.05). Urea was used to determine the dilutional factor present, as previously described by Remmer et al.³¹ Briefly, the IgG level in ELF = IgG level in BAL × serum urea level/BAL urea level. In HIV patients, the BAL IgG level was 2.2 ± 2.0 mg/mL of ELF (Fig 2, bottom right, D) and 1.2 ± 1.4 mg/mL of ELF in control subjects (p = 0.07). IgG₂ levels were 0.37 ± 0.47 mg/mL of ELF in HIV patients (Fig 3, bottom, C) and 0.12 ± 0.09 mg/mL of ELF (p < 0.05). The mean serum IgG level in HIV patients was 17.2 g/L, and 9.45 g/L in control subjects (p < 0.001; Fig 2, top left, A) There was no statistically significant difference in IgG levels between smokers and nonsmokers in the HIV-positive patients and between smokers and nonsmokers in the group as a whole.

Lymphocyte Subpopulations

As previously described in “Materials and Methods,” lymphocyte subpopulations were determined by flow cytometry. Lymphocytes were a proportion-

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Table 1—Pulmonary Function Parameters in Patients and Control Subjects (Percent Predicted)*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HIV-Positive Patients</th>
<th>Control Subjects</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1</td>
<td>91.7 ± 13.1</td>
<td>94.4 ± 11.5</td>
<td>0.45</td>
</tr>
<tr>
<td>FVC</td>
<td>97.5 ± 13.0</td>
<td>101.7 ± 9.4</td>
<td>0.40</td>
</tr>
<tr>
<td>FEV1/FVC</td>
<td>93.9 ± 5.6</td>
<td>93 ± 10.9</td>
<td>0.09</td>
</tr>
<tr>
<td>TLC</td>
<td>103 ± 15.3</td>
<td>103 ± 13.2</td>
<td>0.99</td>
</tr>
<tr>
<td>Dlco/VA (R)</td>
<td>80 ± 13.5</td>
<td>90 ± 14.7</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*Data are provided as mean ± SD. TLC = total lung capacity; Dlco/VA = Dlco per unit of alveolar volume.
ately smaller percentage of the total cells in both HIV and control groups in comparison to historical control groups (4.5% and 7.4%, respectively; Fig 1), and this may be accounted for by the high proportion of smokers in both populations studied. The CD8<sup>+</sup> lymphocyte percentage (suppressor phenotype) and the CD8<sup>+</sup>/S6F1<sup>+</sup> percentage (cytotoxic phenotype) were both higher in HIV-infected individuals than control subjects (p < 0.05). In contrast, the CD4<sup>+</sup> cell percentage was lower in the HIV-infected patients than in control subjects (p < 0.05). Differences in CD19<sup>+</sup> cell percentages between HIV-infected individuals and control subjects were not statistically significant (Fig 4). Overall, the total macrophages per milliliter of BAL fluid was higher in both HIV-infected and control groups when compared with historical controls subjects, but closely follows historical cigarette-smoking control subjects consistent with the predominance of smokers in both groups.

**Comparisons of BAL IgG Levels to Subject Characteristics**

In order to determine whether BAL IgG levels were influenced by alterations in the blood and lung lymphocyte subpopulations, we evaluated correlation coefficients for blood CD4 counts and lung CD4<sup>+</sup>, CD8<sup>+</sup>, and CD19 cell types (Table 2). The strongest relationship was between serum IgG levels and BAL IgG levels...
and BAL IgG levels ($r = 0.56; p < 0.001$). Importantly, lung B-cell numbers (CD19 cells) did not correlate.

**DISCUSSION**

We have demonstrated that BAL IgG levels were statistically higher by two of three methods of determination in HIV-infected individuals in comparison to healthy control subjects. Thus, it is unlikely that a quantitative IgG defect exists in the lung lining fluid of HIV-infected patients. Importantly, a positive correlation was found between serum IgG levels and BAL IgG levels but not between lung B lymphocytes and BAL IgG levels.

Although initial research in HIV infection stressed the importance of T-cell abnormalities, the immunologic derangements are myriad. Defects in humoral immunity are a prominent early finding. Although hypergammaglobulinemia is a prominent feature in the serum, patients are at increased risk of bacterial infection. As IgG is a major component of humoral immunity, we sought to determine if a local deficiency of IgG or the IgG$_2$ subtype in the lung might contribute to the increased risk of infection seen in HIV. In order to minimize confounding variables of depressed immunity, we evaluated a population of healthy asymptomatic HIV-infected individuals and compared them to a control group. Although previous researchers have determined IgG levels in BAL fluid, three of the studies were done on symp-
tomatic patients who were undergoing BAL for suspected pulmonary disease. Only one study evaluated IgG levels in asymptomatic HIV-infected patients, and it found a profound deficit in BAL IgG levels in HIV-infected subjects. The explanation for the apparent discrepancy is not obvious. The study by Twigg et al measured BAL IgG by enzyme-linked immunosorbent assay and only evaluated BAL levels, whereas we used radial immunodiffusion and analyzed both BAL and serum. Our study population was similar in age and blood CD4 count to the study by Twigg et al (eg, 37 years of age vs 35 years of age, and CD4 counts of 361 cells/µL vs 375 cells/µL, respectively). Our study did not have subjects in Centers for Disease Control and Prevention classes B and C, whereas 6 of 16 were so classified in the Twigg et al cohort. Also, the lymphocyte percentage was substantially lower in our study, averaging 4.5% in the HIV patients vs significantly higher lymphocyte percentages in older analyses of HIV-infected individuals. Thus, there may have been differences in lung inflammation between the two reports that explain the differences. However, patients with symptoms and high lymphocyte counts have also been shown to have elevated lung IgG levels.

Our finding that IgG and IgG2 levels were not reduced and indeed were statistically higher by two of three methods of determination in HIV-infected individuals suggests that it is unlikely that local quantitative defects in total IgG or IgG2 levels increase the susceptibility of HIV-infected individuals to bacterial infections. Importantly, our observation is not likely an artifact of the severity of illness in the HIV population that we studied. Our HIV population, which averaged a blood CD4 count of 361 cells/µL, should have a sixfold increased risk of pneumonia, compared to our control population, based on the work of Hirschtick et al in the Pulmonary Complications of HIV Infection Study Group.

It is possible that qualitative defects exist at a local or systemic level that frustrate defenses against bacteria. Earlier studies have documented lower levels of serum IgG2 subclass. IgG2 is the predominant subclass produced in response to pneumococcal polysaccharide antigens. Thus, it is possible that a local qualitative deficiency exists in the lung despite there being normal or elevated total IgG levels in lavage fluid.

It is worth noting that our total cell count, cells per milliliter, and macrophage and lymphocyte percentages in both HIV patients and control subjects closely parallel values found among smokers in the Healthy Study Population. The serum IgG values for our control group closely parallel those in the Healthy Study Population, while HIV patients exhibited characteristic hypergammaglobulinemia. BAL IgG levels in our control group are equivalent to values reported in current and ex-smokers in the Healthy Study Population (11 µg/mL of BAL in our control subjects vs 10 µg/mL and 9.14 µg/mL of BAL in current and ex-smokers, respectively).

Although we did not see a lymphocytic alveolitis in our study population, we did observe the characteristic changes in lymphocyte subpopulations seen with HIV infection of pulmonary cells (Fig 4). The CD8+ cell population was increased and CD4+ cells were decreased. A majority of the CD8+ cells were S6F1+, marking them as activated cytotoxic T cells. They are believed to be involved in attacking HIV-infected cells expressing viral structural proteins.

Table 2—Relationship of BAL IgG Levels to Blood and Lung Factors*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>R</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood CD4</td>
<td>0.27</td>
<td>0.08</td>
</tr>
<tr>
<td>Serum IgG</td>
<td>0.56</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Macrophage, %</td>
<td>0.10</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocyte, %</td>
<td>0.03</td>
<td>NS</td>
</tr>
<tr>
<td>Lung CD4, %</td>
<td>0.17</td>
<td>NS</td>
</tr>
<tr>
<td>Lung CD8, %</td>
<td>0.30</td>
<td>0.04</td>
</tr>
<tr>
<td>Lung CD19, %</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>BMI</td>
<td>0.02</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Both control subject and HIV-positive patient results (n = 48) were compared by simple correlational coefficient (R) to screen for possible relationships. NS = not significant.
No difference in CD19 cell populations was seen between control subjects and HIV-infected patients. This suggests that the elevated IgG levels seen were unlikely to be due to increased local production. In order to determine if the serum IgG level was a factor influencing BAL IgG levels, we compared both data groups. A statistically significant correlation between serum IgG levels and BAL IgG was present ($p < 0.001$). No correlation was found between BAL CD19 cells and BAL IgG levels, again suggesting that the majority of IgG present in ELF was derived from the serum. This is in agreement with the study of Merrill et al,26 which demonstrated a positive correlation between serum IgG$_1$ and IgG$_2$ levels and respective amounts in lavage fluid (note: IgG subclasses 1 and 2 constitute a majority of the Ig in lavage samples). Merrill et al.26 conclude that serum Ig levels determine lavage levels through a transudative mechanism in healthy humans.

The reasons for the elevated serum and lavage IgG levels in asymptomatic HIV-infected patients are not clear. However, if one accepts that lavage IgG levels merely reflect transudation of serum proteins, it is not difficult to explain in the setting of hypergamaglobulinemia seen with HIV infection. Marked B-cell activation is a feature of early HIV infection with in vivo production of elevated amounts of IgG, IgA, and IgD.23 Although many of the antibodies produced are directed against HIV viral epitopes, most reflect a generalized polyclonal B-cell activation. Potential mechanisms for this polyclonal activation may include loss of appropriate CD4 T-cell control or a direct stimulatory effect by HIV-1.27

The finding that combined antiretroviral therapy results in a significant decline in IgG-antibody-secreting cells and a gradual resolution of the hypergamaglobulinemia supports either of these hypotheses.28 More recently, interleukin-15 has been implicated in HIV-1-associated hypergammaglobulinemia. A marked increase in serum IL-15 levels was noted that correlated with serum IgG levels.29 Additionally, the cells are refractory to further stimulation and depressed specific antibody responses are seen, which in part explains the poor response to vaccination.7,30

In conclusion, we have shown that in a healthy population of HIV-infected patients (a majority of whom were smokers) who underwent BAL, total IgG levels and IgG$_2$ levels are higher than in control subjects. This suggests that the immune defects predisposing to bacterial lung infections are unlikely to be due to quantitative IgG abnormalities. It is possible that the CD8$^+$ lymphocytic milieu and deficiency of CD4$^+$ lymphocytes alters alveolar macrophage function to the extent that normal protective mechanisms are impaired.31,32 Further investigation may need to be directed toward defining defects in lung macrophage function or defective specific antibody responses.

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