HIV-1 Infection Does Not Impair Human Alveolar Macrophage Phagocytic Function Unless Combined With Cigarette Smoking*

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Objective: Macrophages are an important reservoir for the HIV and contribute to innate lung defense by their ability to phagocytose, digest, and process invading pathogens. We hypothesized that HIV-1 infection may lead to a defect in the phagocytic activity of alveolar macrophages.

Design: In order to test this hypothesis, the phagocytic activity of alveolar macrophages from asymptomatic HIV-1 seropositive subjects was compared to healthy seronegative control subjects. Macrophages from one cohort were fed with Escherichia coli and from another cohort with opsonized sheep RBCs (SRBCs), and the phagocytic index was determined at different time intervals.

Setting: A tertiary-care, urban, university-based referral center.

Participants: Asymptomatic HIV-1 seropositive subjects and healthy seropositive control subjects recruited from local community.

Results: No differences were found in the phagocytic activity between alveolar macrophages from the first cohort of eight seropositive and nine seronegative subjects. Although not statistically significant, there was a trend toward a lower phagocytic activity of HIV-positive smokers compared to HIV-positive nonsmokers. Opsonized phagocytic capacity (using opsonized SRBCs) was further analyzed in a second set of five HIV-positive subjects and five healthy control subjects. Whereas HIV status did not affect opsonized SRBC uptake, a history of smoking was associated with a statistically significant depression in phagocytic index.

Conclusions: Although there is no significant impairment of phagocytic capacity in HIV-positive subjects compared to HIV-negative control subjects, cigarette smoking produces a significant depression in phagocytic activity that is amplified in HIV-positive smokers.

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Key words: HIV; macrophage; smoking

Abbreviations: PBS = phosphate-buffered saline solution; SRBC = sheep RBC

Alveolar macrophages reside in the alveolar space and account for > 85% of the total cells in this compartment.1 They play an important role in the pulmonary defense against microorganisms and are strategically placed to protect the large surface of the respiratory epithelium. Alveolar macrophages can induce specific immune responses, including antigen presentation to T cells, and contribute significantly to the innate immunity of the lung. Besides their secretory functions, alveolar macrophages are highly phagocytic cells with the capability to engulf and digest microorganisms such as viruses and bacteria or other particles, eg, RBCs, apoptotic bodies, and cellular debris.2

Nonproliferating macrophages such as alveolar macrophages are a known target for HIV-1, and a productive infection of the virus can occur independently of cellular DNA synthesis.3,4 Macrophages are considered a persistent and productive reservoir for HIV-1 in vitro and may provide the prominent source of increasing viremia in the latter stages of HIV-1
disease. In this regard, pulmonary opportunistic infections, such as Pneumocystis carinii and Mycobacterium avium complex, can dramatically increase the production of virus by lung macrophages in lymph nodes, as well as in the alveolar compartment.

Since HIV-1 is able to “take over” central regulatory elements of the infected cells such as the nuclear factor-κB pathway, it is likely that HIV-1–infected macrophages become functionally impaired. Among the defects that have been described for monocytes/macrophages in HIV-1 infection are reduced secretion of interleukin-1 activity after stimulation with lipopolysaccharide, impaired chemotaxis, as well as Fc-receptor function. Alveolar macrophages were shown to have defective mannose receptor-mediated binding and phagocytosis of P carinii, as well as a defective function against Toxoplasma gondii. However, few studies specifically address the question of the functional state of alveolar macrophages in asymptomatic HIV-1 infection. Musher at al. showed increased phagocytic capacity by HIV-infected alveolar macrophages but depressed phagocytic capacity especially in AIDS patients who smoke and acquire pneumonia. However, in a work by Gordon et al. alveolar macrophages from HIV-positive and HIV-negative subjects showed no significant difference in binding to or internalization of either Streptococcus pneumoniae or coagulase-negative staphylococci. In the present research, alveolar macrophages obtained by BAL from asymptomatic healthy HIV-1–seronegative and HIV-1–seropositive donors were analyzed for their capacity to phagocytose fluorescein-labeled Escherichia coli bacteria and IgG-opsonized sheep RBCs (SRBCs).

MATERIALS AND METHODS

Study Subjects

Over a period of 1 year, HIV-seronegative and asymptomatic HIV-seropositive individuals without evidence for active pulmonary disease were prospectively recruited. Demographic characteristics recorded for all participants included age, sex, and smoking status. Additionally, the peripheral CD4+ T-lymphocyte counts and viral load were determined for the HIV-seropositive individuals.

The capacity to phagocytose E coli bacteria was studied in nine HIV-1–seronegative (control group) and eight HIV-1–seropositive individuals (HIV-1 group). The demographic characteristics of the individual study groups are provided in Table 1. The cell differential of both groups showed no significant differences in the total cell count and the relative composition of the individual cell types (Table 2). Bacteria phagocytosed permacrophase in subjects over time are presented in Table 3. To study the phagocytosis of opsonized SRBCs, 10 HIV-1–seropositive individuals classified as 4 smokers and 6 nonsmokers were recruited. Demographic characteristics are shown in Table 4.

BAL Fluid-Derived Macrophages

Bronchoscopy with BAL was performed as previously described. Briefly, after informed consent, subjects underwent standard bronchoscopy with BAL consisting of sequentially instilling and aspirating sterile saline solution in five 20-mL aliquots into the right middle or lingular bronchus from the wedged position. Recovered lavage fluid was passed through a single layer of sterile surgical gauze to remove mucus and particulate matter. Fluid aliquots were immediately taken for cell counting and preparation of macrophages. The macrophages obtained by BAL were centrifuged at 1,500 revolutions per minute, 4°C for 10 min, washed with cold phosphate-buffered saline solution (PBS) [Bio Whittaker; Walkersville, MD], and then resuspended at 1 × 10^5 per treatment in cold RPMI 1640 (Bio Whittaker).

Phagocytosis Assay

Fluorescein-labeled E coli bacteria were purchased from Molecular Probes Inc. (Eugene, OR), added to alveolar macrophages in a ratio of 10^6 bacteria/10^5 macrophages in suspension in RPMI 1640. At time zero, the cells were pelleted by low-speed centrifugation (200g) to increase contact between bacteria and phagocytes. The samples were prepared in duplicate and incubated for different time intervals (0 min, 30 min, 1 h) at 37°C before fixation in 1% paraformaldehyde. The phagocytosis of fluorescein-labeled E coli was quantified using computer-based image analysis; 3 μL of trypan blue stain (Gibco BRL; Grand Island, NY) was added to 10 μL of the cell sample to quench any free floating and adherent bacteria. The cell suspension was then placed on a microscope slide with a cover slip. Bright-field photographs and fluorescent photographs were taken and analyzed using Image Pro-plus computer program (Media Cybernetics; Silver Spring, MD). The software estimated the ratio of bacteria phagocytosed per cell based on the amount of green fluorescence per cell normalized to the amount of fluorescence per one bacterium.

Alternatively IgG-coated sheep RBCs (SRBCs) were used as a

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age, yr</th>
<th>Male Gender, No.</th>
<th>Smoker, No.</th>
<th>Viral Load RNA by Polymerase Chain Reaction, Copies/mL</th>
<th>Peripheral CD4+ T-Lymphocytes, Cells/μL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 9)</td>
<td>31.4 ± 3.0</td>
<td>5</td>
<td>5</td>
<td>Not available</td>
<td>Not available</td>
</tr>
<tr>
<td>HIV-1 positive (n = 8)</td>
<td>38.9 ± 3.0</td>
<td>7</td>
<td>3</td>
<td>96,994 ± 58,486</td>
<td>1,209 ± 479</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM unless otherwise indicated.
target for the phagocytes. SRBCs (Colorado Serum; Denver, CO) were washed in PBS and labeled overnight with 0.1 ng/mL fluorescein in PBS at 4°C. Fluorescein-labeled cells were then washed in PBS and incubated with a subagglutinating dose of rabbit anti-SRBC IgG (Diamedix; Miami, FL) at 37°C for 1 h. Unbound IgG was removed by washing the cells with PBS. SRBCs (1 × 10⁶ cells) were added to alveolar macrophages (1 × 10⁵ cells) in suspension, and the cells were pelleted by low-speed centrifugation to increase contact between SRBCs and phagocytes. The samples were prepared in duplicate and incubated for different time intervals (0 min, 10 min, 20 min, 30 min) at 37°C to study phagocytosis. The cells were subjected to brief hypotonic lysis with water to get rid of externally bound RBCs before fixation in 1% paraformaldehyde. The cell preparations were then mounted on slides to be viewed under a fluorescence microscope. Phagocytosis was measured by counting the total number of RBCs ingested by 200 cells. For both the E coli studies and the opsonized SRBC studies, the phagocytic activity was plotted as phagocytic index (particles/cell × phagocytic cells/100).

Statistics

Statistics were performed using SigmaStat statistical software (SPSS; Chicago, IL). Comparisons of groups for statistical difference were done using the Student t test after passing the normality and the equal variance test. In cases where the equal variance test failed, the Mann Whitney rank-sum test was applied. Simple linear regression analysis was done to describe the dependent variable as a function of possible independent variables.

Results

Phagocytosis of E coli by HIV and Normal Alveolar Macrophages

Freshly harvested alveolar macrophages from both study groups (Tables 1, 2) were analyzed at different time points (0 min, 30 min, 60 min) for the ability to ingest E coli bacteria. The phagocytic index showed no significant difference between the control subjects (n = 9) and the HIV-positive individuals (n = 8) at any time point (phagocytic index at 30 min, 1.201 ± 249 vs 1.703 ± 651; at 60 min, 2.243 ± 349 vs 2.531 ± 921, respectively) [p = not significant; Fig. 1]. In addition, there was no correlation between the phagocytic activity of alveolar macrophages from the eight individuals with HIV infection and their CD4 counts (R² = 0.058) or viral load (R² = 0.005) despite a range in the CD4 counts and the viral loads within the HIV-positive study subjects (Table 1). When analyzed by the numbers of bacteria phagocytosed per cell at the same time points, there again was no difference between control subjects and HIV-positive individuals (Table 3).

Since cigarette smoking has been previously shown to have an effect on phagocytic function,¹⁶–¹⁸ we also analyzed the data from the perspective of smoking status. As outlined in Figure 2, top, A, although smoking status did not significantly impact phagocytic activity of the cohort of control subjects

Table 2—BAL Fluid Cell Differentials in the E coli Phagocytosis Study Population*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cell Count, × 10⁶</th>
<th>Macrophages, %</th>
<th>Lymphocytes, %</th>
<th>Neutrophils, %</th>
<th>Eosinophils, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal (n = 9)</td>
<td>2.6 ± 0.9</td>
<td>90.7 ± 3.8</td>
<td>7.6 ± 3.8</td>
<td>1.7 ± 0.3</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>HIV-1 positive (n = 8)</td>
<td>1.0 ± 0.4</td>
<td>92.3 ± 1.7</td>
<td>2.7 ± 1.0</td>
<td>5.0 ± 1.8</td>
<td>1.0 ± 0.7</td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM.

Table 3—Bacteria Phagocytosed per Macrophage in Subjects Over Time*

<table>
<thead>
<tr>
<th>Groups</th>
<th>Time, min</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>30</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control subjects (n = 9)</td>
<td>0.0 ± 0.3</td>
<td>15.3 ± 2.7</td>
<td>26.0 ± 3.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HIV positive (n = 8)</td>
<td>1.5 ± 0.7</td>
<td>19.0 ± 6.9</td>
<td>31.8 ± 9.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Data are presented as mean ± SEM.

Figure 1. Comparison of HIV-positive individuals and normal control subjects for the phagocytosis of E coli. The capacity to phagocytose E coli was assessed for alveolar macrophages from HIV-1-seropositive donors (HIV-1, dark bars) and seronegative donors (Normal, open bars). Freshly harvested macrophages were incubated with fluoresceinated heat-killed E coli for the time points shown. Phagocytic index was determined as outlined in "Materials and Methods." Bars represent the mean ± SEM. The differences between the two groups were not statistically different.
Phagocytosis of Opsonized SRBC

Opsonized phagocytosis is critical to lung defense. We have previously noted that individuals with HIV-1 infection are not deficient in the potential to opsonize pathogens based on the IgG levels in lung lavage fluid. We therefore tested a second set of individuals with HIV-1 infection (Table 4) and control subjects for the ability of their lung macrophages to phagocytose opsonized SRBCs. Using IgG-opsonized SRBCs as a target, we again confirmed no detectable difference in phagocytic index between HIV-positive individuals (n = 5) and normal control subjects (n = 5) [Fig 3] or when expressed as numbers of SRBCs ingested per phagocytic cell over time (Table 5). However, when the data were reanalyzed by smoking status alone, smokers had a statistically significant depression in their phagocytic index at all time points, p < 0.05 (at 10 min, 135 ± 13 vs 78 ± 16; at 20 min, 305 ± 27 vs 132 ± 33; at 30 min, 437 ± 34 vs 233 ± 42; and at 60 min, 644 ± 56 vs 401 ± 80 for nonsmokers vs smokers, respectively) [Fig 4, top, A]. Subset analysis (Fig 4, bottom, B) once again suggested that the combination of HIV and smoking had a more profound effect on phagocytosis than either HIV or smoking.

Discussion

The HIV type 1 infects not only lymphocytic but also monocytic cells such as alveolar macrophages. The infection with the virus predisposes the lung to both community-acquired and opportunistic infectious agents. The appearance of pulmonary infections in the late stage of the disease coincides with increased replication of the virus by lung macrophages. Counting for > 85%, the alveolar macrophages represent the most prominent cell type in BAL fluid, which is widely used to characterize the cellular components that populate the large compartment of the alveoli and the airways. In addition to the impairment of the adaptive immune system, a

Table 4—Characteristics of the Opsonized Phagocytosis Study Group*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normal</th>
<th>HIV-1 Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects, No.</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Age, yr</td>
<td>26.6 ± 1.9</td>
<td>39.4 ± 4.7</td>
</tr>
<tr>
<td>Smoker, No.</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Blood CD4, cells/µL</td>
<td>Not available</td>
<td>594 ± 455†</td>
</tr>
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</table>

*Data are presented as mean ± SEM unless otherwise indicated.
†Data are not available in one subject; data are from four of five subjects.
functional defect of alveolar macrophages, in particular in their capability to phagocytose, would further explain the vulnerability of the lung in patients with HIV.

Feeding alveolar macrophages with *E. coli* bacteria did not result in a significant difference of the phagocytic activity of alveolar macrophages from HIV-1 seropositive subjects, when compared to normal, seronegative control subjects. Moreover, there was no correlation between the phagocytic activity and parameters of infection with HIV-1 such as the peripheral CD4 positive lymphocyte count or the viral load of the patients. However, we found that macrophages from HIV-positive smokers had a significantly lower phagocytic activity compared to HIV-positive nonsmokers. This was true for both model targets chosen, *E. coli* and IgG-opsonized SRBCs.

Interestingly, it has been shown that the incidence of bacterial pneumonia, lung damage/emphysema, and lung burden of HIV is increased in HIV-seropositive smokers. These findings suggest that immune defects exist in smokers with HIV infection. It has been shown that cigarette smoking among HIV-positive individuals is associated with a marked depression in both the percentage and absolute numbers of lung lymphocytes. Lung CD4+ and lung CD8+ cell numbers were suppressed by smoking, and lung CD4+/CD8+ cell ratios trended

### Table 5—SRBCs Phagocytosed Per Macrophage Over Time*

<table>
<thead>
<tr>
<th></th>
<th>Time, min</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>20</td>
<td>30</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control subjects (n = 5)</td>
<td>0.0 ± 0.3</td>
<td>3.7 ± 0.3</td>
<td>5.5 ± 0.2</td>
<td>8.3 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>HIV positive (n = 5)</td>
<td>2.2 ± 0.3</td>
<td>3.6 ± 0.6</td>
<td>4.8 ± 0.9</td>
<td>6.1 ± 0.8</td>
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</table>

*Data are presented as mean ± SEM.
toward lower values in smokers. Smokers with HIV infection had increased numbers of alveolar macrophages recovered by BAL and showed suppressed spontaneous production of the proinflammatory cytokines interleukin-1β and tumor necrosis factor-α. In addition, Twigg and coworkers have shown that smoking decreases alveolar macrophage accessory cell function in individuals with HIV infection. Furthermore, cigarette smoking has been shown to represent the single strongest independent risk factor for invasive pneumococcal disease in immunocompetent adults. These experiments suggest that cigarette smoking may have a more profound effect on the function of macrophages than HIV status alone. Furthermore, since the combination of HIV infection and smoking showed trends toward a greater depression in phagocytic activity than either factor alone, the present data support the need for larger studies to confirm this effect and delve into the specific mechanisms.

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