Cytokines are essential mediators of the immune response. Major cytokines secreted by T helper cells are broadly categorized as type 1 or type 2, which are important for cell-mediated and humoral immunity, respectively. Although it has been postulated that the progression from human immunodeficiency virus (HIV) infection to AIDS may be associated with a bias toward a type 2 cytokine profile [1, 2], intense research in this area by various methodologies have so far produced inconclusive results. However, findings on cytokine production after mitogenic stimulation, conducted by most studies, may not reliably predict the response of lymphocytes to specific antigens that have relevance to progression to AIDS. On the other hand, in vitro stimulation of lymphocytes by a recall antigen leads mainly to cytokine production by memory T cell precursors that had been precommitted to a Th1 or Th2 pattern years earlier [3]. Such response can differ from that of new effector cells in subsequent infection [4]. We attempted to bypass these limitations by studying patients with active mycobacterial diseases. In these persons, who are currently stimulated in vivo, most of the circulating antigen-specific lymphocytes represent effector cells that have been differentiated at the site of infection or the draining lymph node and are in transit to and from lymphoid organs. The results of such a study should more accurately reflect the influence of HIV infection on the pattern of cytokine response in these AIDS-defining diseases.

The association between mycobacterial diseases and HIV infection has been well documented [5]. Tuberculosis often occurs early in the course of HIV infection, when cellular immunity is relatively intact [6], in contrast to Mycobacterium avium (MAC) diseases, which are found at more advanced stages of AIDS [7]. The influence of cytokine response profiles on disease manifestations has previously been established for several mycobacterial infections. A distinct dichotomy has been found in leprosy, in which a type 1 (interferon-γ [IFN-γ]) response predominates in tuberculoid leprosy, while a type 2 (interleukin-4 [IL-4]) response is associated with the more severe lepromatous leprosy lesions [8]. It is also well accepted that the defense against tuberculosis depends in part on the capacity of specific T cells to confine the mycobacteria in a localized inflammatory granuloma, mediated mostly by IFN-γ [9]. An enhanced and predominant type 1 cytokine response was found in tuberculous pleuritis [10, 11], in which the bacteria are effectively contained. Strong evidence exists that individuals with IFN-γ–receptor deficiency are particularly susceptible to mycobacterial diseases [12, 13], although it is still not clear if and how IFN-γ plays a role in mediating the actual killing of intracellular mycobacteria in human macrophages [14, 15]. A clear association between a type 2 profile and severe or disseminated human mycobacterial disease other than leprosy has never been established [16, 17]. Nevertheless, in a murine model, progression of tuber-
culous granuloma to necrotic foci and pneumonia was associated with a shift from type 1 to type 0 (mixed) cytokine pattern in the lung tissue [18]. In this report, we examine the interrelationships among HIV infection, cytokine profile, and clinical manifestation of mycobacterial diseases.

Materials and Methods

Patient population. Peripheral blood samples from 29 patients at Grady Memorial Hospital (Atlanta) who had active mycobacterial diseases were obtained within the first week of antimycobacterial treatments, except for 1 sample that was collected 1 year after initiation of therapy. All patients were clinically ill and had cultures positive for mycobacteria from sputum, peripheral blood, or biopsy tissues. Seventeen patients had concomitant AIDS; the remaining 12 patients were seronegative for HIV. Among the HIV-infected group, 10 were infected with Mycobacterium tuberculosis, and their CD4 cell counts ranged from 17 to 638 cells/mm$^3$ (mean, 144). The other 7 patients, who presented with MAC disease, had CD4 cell counts of 5–307 cells/mm$^3$ (mean, 87). Only 1 of the 17 HIV-infected patients had previously received antiretroviral drug (zidovudine). Her HIV virus load (77,000 copies/mL) was near the median of the nontreated group (89,000 copies/mL). None of the HIV-infected patients had a history of mycobacterial diseases or a positive purified protein derivative (PPD) skin test prior to HIV seroconversion. Within the HIV-seronegative group, 6 patients were infected with M. tuberculosis and 6 with M. avium. All MAC-infected patients without HIV infection were culture-positive for M. avium, with positive radiologic findings (mostly infiltrates), and they had concordant clinical signs and symptoms consistent with mycobacterial diseases, as defined by the American Lung Association. There was no history suggestive of immunodeficiency. The ages of the HIV-negative MAC patients ranged from 7 to 50 years (median, 20), which is lower than that of the HIV-positive group, with a range of 18–57 years (median, 37; $P = .04$, Kruskal-Wallis analysis).

Cell preparation. Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood by density centrifugation (Leucoprep; Becton Dickinson, San Jose, CA). Washed cells were cultured at 37°C in 5% CO$_2$ at a density of 10$^6$ PBMC/mL in complete medium consisting of RPMI 1640 (Life Technologies Gibco BRL, Grand Island, NY), gentamycin (80 mg/mL; Sigma, St. Louis), and 10% heat-inactivated fetal calf serum (Atlas Biological, Atlanta). Duplicate cultures were stimulated with 1 μg/mL phytohemagglutinin (PHA; Sigma) or 10 μg/mL tuberculin PPD (Connaught Laboratories, Swiftwater, PA). Unstimulated cells served as controls to determine constitutive cytokine production. After 48 h, culture supernatants were lyophilized and stored at −70°C to be assayed for cytokines by ELISA. The cells were used to enumerate IFN-γ-secreting cells in an ELISPOT assay.

ELISA. IFN-γ and IL-4 in culture medium were determined by ELISAs. Ninety-six well polystyrene plates (Immulon II; Dynatech, Chantilly, VA) were coated with 100 μL of mouse anti-human IL-4 monoclonal antibody (10 μg/mL; Genzyme, Cambridge, MA) or mouse anti-human IFN-γ monoclonal antibody (2 μg/mL clone B-B1; BioSource International, Camarillo, CA) in sterile PBS at 4°C overnight. Uncoated wells served as control blanks. All wells were blocked with PBS containing 0.5% gelatin (Sigma) for 1 h before use. Lyophilized culture supernatant, reconstituted to 1/10 of the original volume, was added to duplicate wells. Serially diluted recombinant human IL-4 and IFN-γ (R&D Systems, Minneapolis) were used to establish standard curves. After 2 h at 37°C, plates were washed with PBS−0.05% Tween. They were probed sequentially, with extensive washing between steps, with polyclonal rabbit anti–human IL-4 (Genzyme) or rabbit anti–human IFN-γ (Calbiochem, San Diego) antibodies at 1:1000 dilution for 2 h at 37°C and goat anti–rabbit IgG conjugated to horseradish peroxidase, at 1:1000 dilution for 1 h at 37°C. The plates were developed by the addition of enzyme substrate (20 mg of o-phenylenediamine with 15 μL of H$_2$O$_2$ in 50 mL of 0.5 M citrate buffer pH 6.0). The enzymatic reaction was stopped after 15 min at room temperature in the dark by adding 50 μL of 4 N sulfuric acid. Optical density values measured at 490 nm were converted to cytokine concentrations based on the standard curves. The lowest concentrations detectable were 4 pg/mL for IL-4 and 100 pg/mL for IFN-γ. Since the samples were concentrated 10-fold by lyophilization before the test, the effective sensitivities of the assay system were 0.4 pg/mL for IL-4 and 10 pg/mL for IFN-γ per milliliter of the original culture medium.

ELISPOT assays. IFN-γ–secreting cells were counted in an ELISPOT assay, adapted from the methodology previously described by Czeckinska et al. [19]. Nitrocellulose 96-well plates (Millititer HA; Millipore, Bedford, MA) were coated overnight at 4°C with 1 μg of mouse anti–human IFN-γ monoclonal antibody (clone B-B1; BioSource) in 50 μL of sterile PBS well per well. The plates were washed and blocked with 1% gelatin in PBS for 1 h at 37°C before use. Susensions containing 0.5 × 10$^6$ or 0.25 × 10$^6$ cells in 200 μL of complete culture medium (see cell preparation section) supplemented with 10 μg/mL PPD were added to individual wells and incubated for 20 h at 37°C in 5% CO$_2$. After the cells were removed by a PBS wash, 100 μL of polyclonal rabbit anti–human IFN-γ antibodies (Calbiochem) were added at 1 μg/mL in PBS containing 1% skim milk and 0.05% Tween 20. Following overnight incubation at 4°C, the plates were extensively washed with PBS−0.05% Tween 20, then were probed with horseradish peroxidase–labeled goat anti rabbit IgG (Sigma) at 1:1000 dilution for 1 h at room temperature. Spots representing IFN-γ–secreting cells were developed with enzyme substrate solution, composed of 10 mg of 3-amino-9-ethylcarbazole (Sigma) in 30 mL of 0.1 M sodium acetate (pH 5.0) and 30 μL of H$_2$O$_2$.

HIV virus load quantitation. The HIV RNA in the plasma of the HIV-infected patients was assayed by the NASBA (Organon Teknika, Bxtel, The Netherlands) technique following the manufacturer’s instructions.

Statistical analysis. χ$^2$ tests of independence and Fisher’s exact tests were used to analyze categorical variables. Since the HIV-infected population was not homogeneous with respect to CD4 cell counts and other immune parameters, continuous variables (e.g., total cytokine quantities) generated from individuals were not normally distributed. In such cases, nonparametric measures (median and quartiles) were used in descriptive statistics. Kruskal-Wallis one-way analysis of variance was used to analyze dependency between two parameters. Standard parametric statistics were used for normally distributed data.

Results

Peripheral blood specimens obtained from 29 patients with active M. tuberculosis or MAC diseases were tested for in vitro
**Table 1.** IFN-γ and IL-4 production in purified protein derivative–stimulated peripheral blood mononuclear cells from patients with active mycobacterial diseases, with or without HIV infection.

<table>
<thead>
<tr>
<th>Cytokine production</th>
<th>All mycobacteria</th>
<th>M. tuberculosis</th>
<th>MAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 26</td>
<td>n = 14</td>
<td>n = 12</td>
</tr>
<tr>
<td>IFN-γ and IL-4 (type 0)</td>
<td>HIV⁺</td>
<td>HIV⁺</td>
<td>HIV⁺</td>
</tr>
<tr>
<td>IFN-γ only (type 1)</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>IL4 only (type 2)</td>
<td>7</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td><em>P</em> for IFN-γ</td>
<td>.027</td>
<td>.505</td>
<td>.015</td>
</tr>
<tr>
<td><em>P</em> for IL-4</td>
<td>.438</td>
<td>.260</td>
<td>1.00</td>
</tr>
</tbody>
</table>

NOTE: +, positive; −, negative. χ² tests for homogeneity.

Cytokine production. On the basis of ELISA results of PBMC culture medium after 48 h of PPD stimulation, the cytokine profiles were defined as type 0 when both IFN-γ and IL-4 were produced, type 1 for production of IFN-γ and no detectable amounts of IL-4, and type 2 for production of IL-4 and no detectable amounts of IFN-γ [2]. The cells from 3 patients (2 HIV-infected, 1 HIV-seronegative) did not survive during the culture and were excluded from analysis.

**Cytokine profiles in mycobacterial diseases.** There was no constitutive cytokine production detected in the unstimulated cells. In response to PPD stimulation, the dominant cytokine pattern produced (table 1) by the HIV-infected group was type 2 (7/15, 47%), followed by type 0 (5/15, 33%). In contrast, among the HIV-seronegative patients, the cytokine profile was either type 0 (6/11, 55%) or type 1 (5/11, 45%). It is notable that the type 2 profile was observed only in the HIV-infected population. The difference in the cytokine profiles between the 2 populations was statistically significant (*P* < .05, χ² test).

**Patients with MAC disease.** When the patient population was stratified according to the mycobacterial species (M. tuberculosis vs. M. avium), a distinct dichotomy was observed. Within the group with MAC disease, all of the HIV-seronegative patients responded to PPD stimulation with IFN-γ production (figure 1A), demonstrating either type 0 (4/6, 67%) or type 1 (2/6, 33%) profiles. By comparison, 5 of the 6 HIV-infected persons (83%) had a type 2 cytokine pattern and produced only IL-4. The difference in profile distribution (table 1) between HIV-infected and HIV-seronegative patients (*P* = .016, χ² test) can be attributed entirely to a reduction in the number of those producing IFN-γ (*P* = .015, Fisher’s exact test), since no increase in the number of those producing IL-4 was observed (*P* = 1.00, Fisher’s exact test).

As summarized in table 2, the amount of IL-4 produced by the HIV-infected patients (median, 5.2 pg/mL) was actually lower than that of the HIV-seronegative patients (median, 8.8 pg/mL) who produced this cytokine (*P* = .048). Only 1 patient among the HIV-infected persons with concomitant MAC diseases responded by producing IFN-γ. Unlike the other MAC patients, this individual had a history of pulmonary tuberculosis and had received 1 year of antituberculosis therapy prior to the current episode of MAC infection. Her IFN-γ production (870 pg/mL) was more comparable to that of the tuberculosis patients (median, 1050 pg/mL) and higher than that of all other MAC patients (range, 22–727 pg/mL), probably reflecting the activation of memory cells from her past tuberculosis infection.

Clinically, 2 of the 5 patients with MAC infections who responded with a type 2 cytokine profile had bacteremia; all 7 patients producing IFN-γ (with either type 1 or type 0 profiles) had localized pulmonary disease.

**Patients with M. tuberculosis disease.** Among the 14 patients with active tuberculosis, the differences in cytokine profiles between HIV-infected and uninfected persons were less marked. There was a shift in profile from mainly type 1 (3/5, 60%) in HIV-seronegative patients to type 0 (5/9, 56%) and type 2 (2/9, 22%) in the HIV-infected patients (table 1). The difference was due mainly to an increase in the number of persons who produced IL-4 (2/5 vs. 7/9; figure 1B), although it was not statistically significant in this small sample (*P* = .26, Fisher’s exact test). Restricting the analysis to only those who produced IL-4 (table 2), the amount of IL-4 secreted by the PBMC of the HIV-infected patients (median, 4.4 pg/mL) was generally lower than that of the HIV-seronegative population (median, 12.0 pg/mL). With regard to IFN-γ production, the proportion of IFN-γ producers (7/9) in the HIV-infected population was only slightly lower than that of HIV-seronegative patients (5/5). The quantities of IFN-γ generated by the HIV-infected persons (median, 72.7 pg/mL) was again lower than that generated by the HIV-seronegative patients (median, 1050 pg/mL).

All 5 HIV-seronegative patients (with type 1 and 2 with type 0 cytokine patterns) had pulmonary tuberculosis. Of the HIV-infected persons, all 4 with either type 1 or type 2 patterns had localized disease, while 4 of 5 patients with type 0 profile presented with extrapulmonary tuberculosis.

**Frequency of IFN-γ–secreting cells.** Additional information on the IFN-γ response was obtained by using the ELISPOT assays, which counted the number of IFN-γ–secreting cells (IFN-γSC) after in vitro PPD stimulation of PBMC. The median value for 5 of 6 HIV-infected tuberculosis patients (1 patient not tested) was 324 IFN-γSC/10⁶ PBMC (25th–75th percentile, 96–520), lower than that of the 5 HIV-seronegative tuberculosis patients tested (median, 624/10⁶ PBMC; 25th–75th percentile, 240–720). The difference, however, was not statistically significant due to the small sample size (*P* = .35, Kruskal-Wallis one-way analysis of variance). Similar comparison in the MAC-infected population was not attempted because only 1 HIV-infected MAC patient produced IFN-γ. The amount of IFN-γ produced per secreting cell in the HIV-infected persons (mean, 27.0 ± 17.3 pg/cell) was similar to that of the HIV-seronegative patients (mean, 28.3 ± 18.0 pg/cell), indicating no down-regulation of IFN-γ at the single-cell level. Overall, patients with MAC diseases produced less IFN-γ and...
Figure 1. IL-4 and IFN-γ produced after purified protein derivative stimulation of peripheral blood mononuclear cells from patients with (A) active MAC diseases or (B) active tuberculosis.

Table 2. Amounts of IFN-γ and IL-4 produced by peripheral blood mononuclear cells of patients with M. tuberculosis or M. avium complex (MAC) infection who responded to in vitro purified protein derivative stimulation.

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>M. tuberculosis</th>
<th>MAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV+</td>
<td>HIV−</td>
</tr>
<tr>
<td>IFN-γ*</td>
<td>72.7</td>
<td>1050.0</td>
</tr>
<tr>
<td></td>
<td>(25.0–800.0)</td>
<td>(220.0–1515.1)</td>
</tr>
<tr>
<td>IL-4*</td>
<td>4.4</td>
<td>12.0</td>
</tr>
<tr>
<td></td>
<td>(3.1–12.0)</td>
<td>(9.0–15.1)</td>
</tr>
</tbody>
</table>

NOTE. Data are median values (25th–75th percentiles).

* Picogram of cytokine in 1 mL of original cell culture medium. P determined by Kruskal-Wallis 1-way analysis of variance.

had a lower number of secreting cells than did the tuberculosis patients, irrespective of HIV infection status. Nevertheless, the average amount of IFN-γ secreted per responding cell in the patients with MAC diseases (34.5 ± 7.4 pg/cell) was not significantly different from that of the tuberculosis patients (P = .58, Kruskal-Wallis analysis of variance). Since the PPD used in the experiment was derived from M. tuberculosis, the MAC patients may have recognized fewer epitopes on PPD than did the tuberculosis patients, resulting in a small number of activated lymphocyte clones.

Correlation between cytokine responses and T cell counts. HIV infection is characterized by a reduction in CD4+ T cells that is generally ascribed to the immunodeficiency. Abnormalities in the CD8+ T cell numbers are also common. Since both of these cells can produce IFN-γ and IL-4, we questioned whether the differences in cytokine profile might be directly related to the cell count of either subset in HIV-infected patients. The CD4+ and CD8+ T cell counts of 15 HIV-infected patients with active mycobacterial diseases were compared...
among the three cytokine profiles (figure 2). Kruskal-Wallis analysis of variance by rank sum detected no significant difference in the distributions of either cell subset \((P = .75\) and \(.18)\). When similar analyses were repeated using IFN-\(\gamma\) or IL-4 production as the independent variates (table 3), again no statistically significant correlation was found, suggesting that the cell numbers of neither cell population directly determined whether the patient responded by producing either cytokine. However, among those who produced IFN-\(\gamma\) (figure 3), there was a direct correlation between the amount of the cytokine produced and the number of CD4\(^+\) T cells (Pearson’s correlation coefficient = \(.92, .59 < R < .98)\). No similar relationship was detected between IFN-\(\gamma\) and CD8\(^+\) T cell counts \((R = .27, -.54 < R < .82)\), or when IL-4 production was analyzed (data not shown).

**Correlation between cytokine responses and HIV virus load.** Plasma aliquots from the peripheral blood samples used for the cytokine study were available for 14 of the 15 HIV-infected patients for virus load assays. The quantity of HIV RNA copies per milliliter of plasma was compared among individuals who responded to PPD stimulation with the three cytokine profiles (figure 4). No significant difference was detected by Kruskal-Wallis rank sum analysis \((P = .5)\). The median virus load in those who produced IFN-\(\gamma\) \((38,500\) copies/mL) was similar to that in those who did not \((49,000\) copies/mL, \(P = .46)\). The median virus load in those who produced IL-4 was 49,000 copies/mL. Virus load data from 2 of the 3 HIV-infected patients who did not have an IL-4 response were available: 1 was below the detectable level of the assay \((4000\) copies/mL); the other was at 1.5 million copies/mL.

**Table 3.** CD4 and CD8 T cell counts in patients with IFN-\(\gamma\) and IL-4 cytokine responses to stimulation by purified protein derivative.

<table>
<thead>
<tr>
<th>Cell counts/mm(^3)</th>
<th>IFN-(\gamma) Producers</th>
<th>IFN-(\gamma) Nonproducers</th>
<th>IFN-(\gamma) (P)</th>
<th>IL-4 Producers</th>
<th>IL-4 Nonproducers</th>
<th>IL-4 (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD4(^+)</td>
<td>46 (25–135)</td>
<td>96 (24–307)</td>
<td>(.48)</td>
<td>67 (26–160)</td>
<td>54 (17–128)</td>
<td>(.50)</td>
</tr>
<tr>
<td>CD8(^+)</td>
<td>352 (204–729)</td>
<td>484 (232–740)</td>
<td>(.73)</td>
<td>470 (177–571)</td>
<td>905 (232–1014)</td>
<td>(.10)</td>
</tr>
</tbody>
</table>

NOTE. Data are median values (25th–75th percentiles). \(P\) determined by Kruskal-Wallis 1-way analysis of variance by rank-sum between producers and nonproducers.

**Comparison between PPD and mitogen stimulation.** Since mitogenic stimulation induces cytokine production from cells other than those specific for the mycobacterial antigen, we compared the cytokine profiles that resulted from PPD or PHA.
Figure 3. Correlation between IFN-γ production by peripheral blood mononuclear cells and CD4+ and CD8+ cell counts in HIV-infected patients who responded to stimulation by purified protein derivative.

Discussion

Clerici and Shearer [1, 2] proposed in 1994 that a shift from type 1 to type 2 cytokine phenotypes is a critical step in the progression of HIV disease. Studies by others have generally failed to show a clear switch in constitutive or mitogenic-stimulated cytokine production [20], although a subtle type 1 to type 0 shift in response to recall antigens was reported by Maggi et al. [21]. The diverse findings may be due in part to differences in experimental design [22]. Cytokines produced after strong mitogenic stimulation more likely reflect the intrinsic potential of the lymphocytes. In comparison, specific antigenic stimulations are accompanied by regulatory signals from the presenting cells and the microenvironment [23, 24]. Moreover, different T cell subsets differ in their threshold activation requirements. Thus, various stimuli may differentially activate particular subsets of T cells, resulting in different cytokine profiles in the same individuals, as previously described in a mouse model of experimental tuberculosis [25].

In the current study, we have found that PPD and PHA induced different cytokine response profiles in 50% of the HIV-infected patients with mycobacterial disease. In such cases, the mitogen stimulated both IFN-γ and IL-4, while PPD stimulated only one type of cytokine production. One could assume that cytokine profile shifts occur first at the level of antigen-induced responses and can vary among different antigens before the shift can be detected with mitogenic stimulations. We believe that the response to antigen-specific stimulation should have more clinical relevance in these individuals.

There are several additional major and unique advantages provided by the mycobacterial model we used. As previously mentioned, in testing specimens from patients with active diseases, we were able to study effector cells recently differentiated under the current immunologic conditions of the patient, in contrast to long-term memory cells committed previously. By using a cross-reacting antigen shared by two mycobacteria of different virulence, which usually cause diseases at two different stages of AIDS, we can gain insight into the change of cytokine profiles associated with HIV disease progression. In fact, the manifestation of the two mycobacterial diseases might have served as markers for the different degrees of deterioration of the host specific immunity to these microorganisms.
In patients with active mycobacterial diseases, the most clear-cut influence of HIV infection on cytokine production in response to PPD stimulation is the decreased IFN-\(\gamma\) production; the effect on the IL-4 production is more subtle. Our data demonstrated that the alteration can be qualitative or quantitative, depending on the individuals and the mycobacterial species involved. The absence of IFN-\(\gamma\) response is strongly associated with MAC diseases. This qualitative deficit is not related to the CD4 cell counts. Thus, MAC patients with 100–300 CD4\(^+\) cells/mm\(^3\) were likely to have a type 2 cytokine profile, while tuberculosis patients with cell counts as low as 20 were making detectable amounts of IFN-\(\gamma\). In comparison, almost all of the tuberculosis patients produced detectable amounts of IFN-\(\gamma\) in response to PPD stimulation, irrespective of HIV infection status; however, a possible qualitative difference in IL-4 production was detected. Only 2 of the 5 HIV-negative tuberculosis patients responded to PPD by producing IL-4, whereas 7 of the 9 in the HIV-infected group did so. The methodology we used could not differentiate whether the same set of immune cells was responsible for both IFN-\(\gamma\) and IL-4 production or whether a different set of cells producing IL-4 was activated in the HIV-coinfected patients. Since flow cytometric analysis of intracellular cytokines has suggested that coproduction of IFN-\(\gamma\) and IL-4 is rare at the single-cell level [26, 27], it is likely that an additional set of IL-4-producing cells was involved.

HIV infections also affect cytokine production quantitatively. While most HIV-infected tuberculosis patients can respond to PPD by producing IFN-\(\gamma\), we found that the amounts of the cytokine produced were directly related to the CD4 cell counts of the individuals. The reduced magnitude of IFN-\(\gamma\) response in HIV-infected patients with low CD4 cell counts was consistent with the previous findings by Lin et al. [17] and Zhang et al. [28]. Our ELISPOT results further suggest

**Table 4.** Characteristics of patients with type 2 cytokine responses to stimulation by purified protein derivative (PPD) or phytohemagglutinin (PHA).

<table>
<thead>
<tr>
<th>Species, site of infection</th>
<th>No. of CD4 cells/mm(^3)</th>
<th>Cytokine production* in response to stimulation by</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PPD</td>
<td>PHA</td>
</tr>
<tr>
<td><strong>M. avium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Disseminated (blood)</td>
<td>29</td>
<td>&lt;10</td>
<td>8.5</td>
</tr>
<tr>
<td>Disseminated (blood)</td>
<td>96</td>
<td>&lt;10</td>
<td>5.2</td>
</tr>
<tr>
<td>Localized (pulmonary)</td>
<td>17</td>
<td>&lt;10</td>
<td>6.3</td>
</tr>
<tr>
<td>Localized (pulmonary)</td>
<td>24</td>
<td>&lt;10</td>
<td>3.5</td>
</tr>
<tr>
<td>Localized (pulmonary)</td>
<td>307</td>
<td>&lt;10</td>
<td>4.3</td>
</tr>
<tr>
<td><strong>M. tuberculosis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Localized (pulmonary)</td>
<td>178</td>
<td>&lt;10</td>
<td>12.0</td>
</tr>
<tr>
<td>Localized (pulmonary)</td>
<td>638</td>
<td>&lt;10</td>
<td>5.0</td>
</tr>
</tbody>
</table>

NOTE. ND, not done.

* Picograms of cytokine produced by stimulated peripheral blood mononuclear cells in 1 mL of original culture medium.
that the reduction does not occur at the single-cell level. Since we do not know the quantitative threshold required for IFN-γ function in the in vivo microenvironment, it is difficult to predict the clinical significance of this quantitative deficit. In general, the amounts of both IFN-γ and IL-4 produced by PBMC that responded to PPD stimulation were decreased in the HIV-infected population. That the levels of reduction often did not reach statistical significance was most likely due to small sample sizes. The nonparametric statistical tests we used on data that were not normally distributed also have a higher probability of type II error (false acceptance of the null hypothesis).

In our study population, 5 of the 6 HIV-infected MAC patients had type 2 cytokine responses to PPD. Two of these patients presented with disseminated diseases, in contrast to none of the 6 HIV-seronegative patients, who all had type 1 or type 0 cytokine profiles. To our knowledge, this is the first report associating HIV infections to a type 2 cytokine profile and severe MAC disease. Similar association was not detected in patients with tuberculosis, which is caused by a more virulent mycobacterium. Of interest, all of our disseminated M. tuberculosis cases were found in HIV-infected patients with a type 0 cytokine profile, which corresponds to the dominant cytokine pattern in the lung tissue during the necrotic and pneumonic stages in a murine tuberculosis model [17]. The only HIV-infected MAC patient who responded with IFN-γ production after PPD stimulation (figure 1) had a history of treated pulmonary tuberculosis. This might reflect a particular subgroup of HIV-infected individuals who acquired some immunity against MAC from previous exposure to tuberculosis, as recently suggested by Horsburgh et al. [29]. However, in this particular case, the immunity did not protect the patient from developing a localized pulmonary disease.

Our results indicate that in the presence of HIV infection, the IFN-γ response to mycobacterial infection is depressed qualitatively or quantitatively among patients with active mycobacterial diseases. The quantitative deficit is probably due to the depletion of T helper cells; the mechanism for the qualitative change remains unexplained. Given the important role of IFN-γ in antimycobacterial immune defense, the reduction is likely to contribute to the increased susceptibility of HIV-infected patients to mycobacterial diseases. Our results also suggest that HIV infection may lead to an increase in the relative prominence of IL-4 in patients with mycobacterial diseases. Compared with the HIV-negative population with mycobacterial diseases, the number of IL-4 producers among tuberculosis patients was elevated, whereas in the MAC patients, the influence of IL-4 might have been enhanced by the absence of IFN-γ. However, the roles of IL-4 in these mycobacterial diseases remain to be defined. Since it is generally accepted that tuberculosis and MAC diseases occur at sequential stages of immune deficiency, our data suggest that there is a progressive shift of cytokine patterns associated with HIV infection. From mainly a type 1 response profile in the HIV-seronegative persons, the profile shifts to type 0 in HIV-infected patients, accompanied by enhanced susceptibility to tuberculosis, then further progresses to type 2 when the usually avirulent MAC becomes a major cause of disease.

Acknowledgment

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References