The relationship between Mycobacterium avium complex (MAC) bacteremia and proinflammatory cytokine and human immunodeficiency virus type 1 (HIV-1) RNA levels in AIDS was investigated. During a prospective study, blood samples were drawn monthly for mycobacterial cultures. Sera were available at baseline and onset of MAC bacteremia from 20 cases and at corresponding times from 19 controls. Mean interleukin-6 (IL-6) levels were 154% greater at the time of MAC bacteremia in cases than in controls. The IL-6 levels correlated with body temperature, serum tumor necrosis factor (TNF-α) levels, and alkaline phosphatase levels ($P = .004$ for each). Although TNF-α levels tended to rise more in MAC patients than in controls, the difference was not significant. However, among both cases and controls, serum TNF-α levels rose significantly from baseline to the time of last sample, irrespective of MAC infection ($P = .015$). Bacteremia was not associated with increased serum HIV-1 RNA levels. Thus, early MAC bacteremia is associated with increases in serum IL-6 levels, while TNF-α levels rise over time during advanced AIDS.

Disseminated Mycobacterium avium complex (MAC) disease is common during advanced AIDS. Cellular expression of tumor necrosis factor-α (TNF-α) is induced by mycobacterial proteins in vitro [1] and during tuberculosis in human immunodeficiency virus (HIV)—infected persons [2]. In addition, immune activating events such as febrile illnesses and immunizations cause transient increases in plasma HIV RNA. Cytokines that promote HIV expression in vitro, such as TNF-α and interleukin-6 (IL-6), may enhance HIV replication [3]. Among HIV-infected patients, active viral replication drives progressive CD4 cell depletion [4]. Current treatment strategies focus on minimizing HIV replication. The present study investigated whether early MAC bacteremia was associated with changes in serum IL-6, TNF-α, and HIV RNA levels in patients with AIDS.

Materials and Methods

Patients. Patients previously enrolled in a prospective clinical trial of clarithromycin prophylaxis in AIDS were studied [5]. Patients with $<100$ CD4$^+$ cells/mm$^3$ and baseline blood cultures negative for MAC were randomized to twice daily clarithromycin or placebo. Only patients from the placebo arm were included in the present analysis. Blood was drawn monthly for MAC surveillance cultures, and sera were sampled every 16 weeks. All patients with adequate stored sera and for whom matched controls could be identified were included. We identified 20 patients who developed MAC bacteremia (cases). A control was selected for each (except 1 patient who was a control for 2 cases). Cases and controls were
matched for baseline CD4⁺ cell count and time from baseline to last serum sample.

**Antiretroviral use.** Many patients received antiretroviral agents (zidovudine, didanosine, zalcitabine, stavudine, or foscarinet). These drugs were included in the analysis only if prescribed for at least 1 week before the pertinent serum sample. Antiretrovirsals started after baseline but stopped at least 1 week before the pertinent sampling were not included in the analysis.

**Sample processing.** Whole blood was collected, and serum was separated by centrifugation and stored at −70°C. Sera from some time points were not available, and plasma samples were unavailable.

**Cytokine assays.** Sera were analyzed for TNF-α (Medgenix, Stillwater, MN) and IL-6 (R&D Systems, Minneapolis) in a blinded manner. The lower limit of IL-6 detection was 0.15 pg/mL. Lower values were censored to 0.15 pg/mL. The lower limit of TNF-α detection was 16 pg/mL. All results exceeded this level.

**HIV RNA analysis.** Sera were assayed for HIV RNA in a blinded manner using the branched-chain DNA method (Chiron) [6].

**Mycobacterial cultures.** MAC was isolated from blood using 7H11 agar or 7H12 broth (or both) by a radiometric method.

**Statistical analysis.** Nominal and ordinal variables were compared by χ², and interval and ratio variables were assessed by Student’s t test or Pearson’s correlation coefficient. For multiple linear regression, entry and exit probabilities of .05 and .10 were used, respectively. All P values were based on two-tailed tests. Subjects with missing data were excluded from individual analyses.

**Results**

Between November 1992 and July 1993, 682 patients with blood cultures negative for mycobacteria at baseline participated in a study of clarithromycin prophylaxis for disseminated MAC infection [5]. Of 334 randomized to placebo, 53 developed disseminated MAC infection, as defined by a positive blood culture. Twenty of these 53 patients, for whom adequate serum samples were available, were included in these analyses, as were 19 matched controls. None received specific MAC therapy during the study, including times when samples for the present analyses were collected. Of the 39 subjects, 90% were male, mean weight at baseline was 150 ± 5 lbs, age was 38.2 ± 1.1 years, CD4⁺ cell number was 23.1 ± 3.6 cells/mm³, CD4⁺ cell percentage was 3.3% ± 0.5%, CD8⁺ cell number was 530 ± 59 cells/mm³, and CD8⁺ cell percentage was 61.5% ± 2.0%. Groups did not differ with respect to sex or weight or CD4 or CD8 cell counts or percentages (P > .05), although cases were slightly younger (P = .046).

For MAC cases, time from baseline to last serum sample (at or following onset of MAC bacteremia) ranged from 64 to 474 days (median, 250). Time from first positive MAC culture to last sample ranged from 0 (both obtained the same day) to 4 months. This interval was 0, 1, 2, 3, and 4 months for 6, 10, 1, 2, and 1 cases(s), respectively. For controls, time between samples was similar, ranging from 19 to 484 days (median, 253).

Cases and controls had similar IL-6 and TNF-α levels at baseline (table 1). However, MAC bacteremia was associated with elevated IL-6 levels (P = .03). Similarly, when cases and controls were compared based on calculated differences between individual final and baseline IL-6 levels (to control for interpatient variability), the change in IL-6 levels remained significant (P = .027).

In contrast, although TNF-α levels tended to rise more among cases, the increase specifically associated with MAC bacteremia was not significant (table 1). Levels of TNF-α were higher among both groups at final sample time (42.5 ± 5.2 pg/mL) compared with baseline (28.4 ± 2.4 pg/mL), regardless of bacteremia (P = .015). There was no correlation between TNF-α levels and duration of storage (not shown).

The HIV RNA levels varied widely among the 39 subjects at all times, from <500 copies/mL to 4,266,000 copies/mL. Assays using serum have been shown to yield HIV RNA values 38% lower than plasma levels [7]. Mean baseline log HIV RNA level was similar in cases and controls (table 1), and HIV RNA levels at the time of bacteremia in cases did not differ from levels in control patients. To control for interpatient variability, the change over time in individual patients was calculated based on the first and last sample. This difference was not significant.

We further examined the relationship between clinical characteristics and MAC bacteremia. Other than a trend toward higher mean body temperatures (99.2 ± 0.4°F vs. 98.3 ± 0.2°F, P = .059) and lower hemoglobin levels (11.1 ± 0.6 vs. 12.4 ± 0.4 g/dL, P = .069) in cases versus controls, respectively, MAC bacteremia was not associated with changes in white blood, platelet, CD4, or CD8 cell counts or percentages, serum alkaline phosphatase, triglycerides, or body weight (P > .05).

**Table 1.** Comparison of serum levels of HIV RNA, tumor necrosis factor-α (TNF-α), and interleukin-6 (IL-6) in MAC cases and controls.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>MAC cases</th>
<th>Controls</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6 level (pg/mL)</td>
<td>Baseline sample</td>
<td>4.2 (0.6)</td>
<td>5.6 (2.3)</td>
</tr>
<tr>
<td></td>
<td>Last sample*</td>
<td>13.7 (3.6)</td>
<td>5.4 (1.0)</td>
</tr>
<tr>
<td></td>
<td>Last minus baseline</td>
<td>9.6 (3.6)</td>
<td>−0.2 (2.4)</td>
</tr>
<tr>
<td>TNF-α level (pg/mL)</td>
<td>Baseline sample</td>
<td>28.2 (3.9)</td>
<td>28.6 (3.0)</td>
</tr>
<tr>
<td></td>
<td>Last sample</td>
<td>45.4 (7.8)</td>
<td>39.7 (6.5)</td>
</tr>
<tr>
<td></td>
<td>Last minus baseline</td>
<td>17.8 (7.0)</td>
<td>11.5 (6.5)</td>
</tr>
<tr>
<td>Log₁₀ HIV RNA (copies/mL)</td>
<td>Baseline sample</td>
<td>4.47 (0.16)</td>
<td>4.42 (0.17)</td>
</tr>
<tr>
<td></td>
<td>Last sample</td>
<td>4.46 (0.12)</td>
<td>4.40 (0.20)</td>
</tr>
<tr>
<td></td>
<td>Last minus baseline</td>
<td>−0.01 (0.14)</td>
<td>−0.02 (0.12)</td>
</tr>
</tbody>
</table>

NOTE. MAC, M. avium complex infections. Mean values are shown (SE). * Last sample = sample at or following onset of MAC bacteremia in cases and corresponding sample from controls.
In addition to baseline and time of MAC bacteremia, samples from intermediate time points were available from some patients, yielding a total of 53 sera from cases and 54 from controls. Analysis of these 107 samples (including baseline and final samples) revealed that serum IL-6 correlated with alkaline phosphatase (r = .41, P < .001) and body temperature (r = .38, P < .001). For TNF-α, weaker correlations were observed with triglycerides (r = .25, P = .011) and hemoglobin (r = −.23, P = .022). There was a strong correlation between IL-6 levels and TNF-α levels (r = .38, P < .001). Neither cytokine correlated with log HIV RNA.

We repeated the above analyses after controlling for antiretroviral use. At baseline, 19 (95%) cases and 17 (89.5%) controls were receiving at least one antiretroviral agent, for a mean of 1.10 (±0.10) and 1.16 (±0.14) agents per case and control patient, respectively. At the time of final sample, 5 patients in each group had started at least one new agent. In addition, some drugs received at baseline were discontinued, so that at study end controls and cases were receiving a mean of 1.0 ± 0.2 and 0.8 ± 0.1 agents, respectively.

When the 10 patients who started new agents were compared with the other 29 patients, there was no difference in IL-6, TNF-α, or log HIV RNA levels from baseline to final sample (P > .05 for each). Although the number of patients was small, among the 20 MAC case patients, there were no significant differences in changes in IL-6, TNF-α, or log HIV RNA levels among the 5 patients who had new antiretrovirals added compared with the 15 who did not.

Infectious or neoplastic complications other than MAC bacteremia could influence cytokine or HIV-1 RNA levels. However, such events were equally distributed between groups. At least one major infectious or neoplastic disease was diagnosed after collection of baseline but before last serum samples in 9 case and 9 control patients, and included cytomegalovirus retinitis (7), Pneumocystis carinii pneumonia (4), Kaposi’s sarcoma (3), nonretinal cytomegalovirus disease (2), bacterial pneumonia (2), and lymphoma, pancreatitis, and intravenous catheter infection (1 each). All non-MAC infections except 2 were diagnosed at least 1 month prior to MAC bacteremia (1 in a case, 1 in a control). When these 2 subjects were excluded from analysis, final IL-6 levels remained higher in cases (14.4 ± 3.7 pg/mL) than controls (5.6 ± 1.0 pg/mL, P = .031), and final temperatures remained higher in cases (99.5 ± 0.4°F) than controls (98.3 ± 0.2°F, P = .04).

Discussion

This study demonstrates that early MAC bacteremia is associated with increased serum IL-6 but not TNF-α or HIV RNA levels. These findings were unexpected given the strong association between tuberculosis and increased expression of both proinflammatory cytokines and HIV [2, 8, 9]. In addition, a previous study demonstrated elevated TNF-α levels among patients with disseminated MAC infection [10]. Unlike the previous report, we identified patients from a prospective, randomized study which lasted for over a year [5]. Of interest, in this prospective study, serum TNF-α levels rose in all patients irrespective of MAC disease, suggesting that TNF-α expression increases over time in HIV disease as has been suggested by some [11] but not all [12] earlier cross-sectional studies.

The cytokines TNF-α and IL-6 participate in the host immune response to various intracellular pathogens, including mycobacteria. Increased production of TNF-α has been demonstrated during tuberculosis [1, 8], and serum TNF-α levels fall when HIV-infected adults with active tuberculosis receive effective therapy [13]. While the mechanisms involved are not well understood, exposure of peripheral blood mononuclear cells from healthy, tuberculin-positive volunteers to live Mycobacterium tuberculosis induces transcription of TNF-α and IL-6 mRNA. Similarly, mononuclear cells from patients with active pulmonary tuberculosis demonstrate elevated cytokine mRNA levels and increased production of TNF-α in response to exogenous stimuli [8]. During mycobacterial infection, TNF-α may be involved in immune pathways that result in fever and tissue necrosis, inhibit mycobacterial growth, and promote granuloma formation. Furthermore, TNF-α facilitates killing of intracellular MAC, while both TNF-α and IL-6 production may correlate with increased survival of MAC-infected macrophages [14].

The physiologic effects of TNF-α and IL-6 occur largely in the local tissue microenvironment at sites of infection. In the present study, activation of cytokine or HIV-1 expression may have been greater in the tissues during MAC bacteremia but was not reflected by circulating levels. Our findings of changes in IL-6 but not TNF-α or HIV-1 RNA levels may also reflect stage of MAC disease. In the present study, MAC bacteremia was probably diagnosed before extensive lymphoreticular organ involvement. This was predicted by the study design, which involved monthly blood cultures, regardless of symptoms, and is supported by the minimal clinical abnormalities specifically associated with bacteremia. This differs from the usual stage of diagnosis, by which time patients typically have high-grade fever and anemia. A report describing increased TNF-α levels during MAC involved such clinically overt disease [10].

In contrast to the present study, Havlir et al. [15] observed increased circulating HIV-1 RNA levels during early MAC bacteremia. Elevated IL-6 levels may precede HIV-1 RNA increases in bacteremic patients. It is possible that, in the present study, increased HIV-1 RNA levels would have been observed at later times.

Improved culture methods and newer macrolide antibiotics have influenced the approach to MAC infections in patients with AIDS [5]. We have shown that onset of MAC bacteremia may be associated with minimal clinical or laboratory evidence of disease, increased IL-6 levels, and little evidence of TNF-α or HIV RNA changes. The opportunity for early preemptive therapy at the time of minimal pathogenic effect should abort subsequent clinical disease and complications. However, in the
study by Pierce et al., surveillance blood cultures were performed monthly and patients were offered standard therapy when cultures became positive [5]. Despite this approach, mortality was greater among placebo recipients.

This study offered the opportunity to examine cytokine and HIV RNA levels during early MAC infection. If enhanced cytokine-induced HIV expression underlies the mortality seen in persons with progressive disseminated MAC infection, early prevention or treatment of MAC may prevent the cascade of events that accelerate the course of HIV-1 infection.

Acknowledgments

We thank Mark Pierce for creative input and Niki Webb and Daniel Georges for technical assistance.

References