Clinical, Microbiological, and Immunological Characteristics in HIV-Infected Subjects at Risk for Disseminated \textit{Mycobacterium avium} Complex Disease: An AACTG Study

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ABSTRACT

The clinical, microbiologic, and immunologic parameters in HIV-infected subjects first presenting with disseminated \textit{Mycobacterium avium} complex (DMAC) were determined. Four HIV-positive groups not yet on DMAC treatment were enrolled: 19 subjects with CD4 lymphocyte counts $\leq 50/\mu l$ thought to have DMAC on clinical grounds; 18 subjects newly found to have a positive blood culture for MAC; 25 asymptomatic controls (CD4 cell counts $\leq 50$); and 25 asymptomatic controls (CD4 counts 100–250/\mu l). Outcome measures include comparisons between groups for clinical characteristics; results of cultures from blood, marrow, and gastrointestinal and respiratory tracts; immunological markers from staining of marrow and flow cytometry of circulating lymphocytes; and cytokine production of PBMCs. Only 21\% of the 19 patients entered on suspicion of having DMAC grew MAC from blood or marrow. Neither clinical presentation nor laboratory tests differentiated those culture-positive from those culture-negative patients. However, prior PCP or multiple other opportunistic infections were more common in the DMAC group. MAC was isolated from 82\% of marrow and 50\% of blood specimens from the DMAC group. Respiratory or gastrointestinal colonization was present in 36\% of DMAC subjects, but only 5\% of non-DMAC subjects with CD4 counts $<50$ cells/\mu l. CD8$^+$ cells were more frequent in bone marrow, and CD4 cells recognizing MAC antigen were more frequent in blood from DMAC subjects vs. controls. Results suggest an early stage of tissue dissemination preceding persistent bacteremia, and mucosal entry without persistence of colonization. MAC-specific T cell responses apparently develop and persist during DMAC, but are dysfunctional or too infrequent to prevent persistence.

INTRODUCTION

Disseminated infection with \textit{Mycobacterium avium} complex (DMAC) is a late complication of AIDS, associated with very low numbers of CD4-positive lymphocytes.\textsuperscript{1,2} Before highly active antiretroviral therapy (HAART) became available, a high proportion of individuals with CD4-positive lymphocyte counts below 50 cells/\mu l developed DMAC, with risk roughly doubling with each 10 cell/\mu l decrement below 50.\textsuperscript{3} At post-mortem examination, over 50\% of patients with AIDS had extensive tissue involvement with MAC.\textsuperscript{2,4} However, making a diagnosis of DMAC on a clinical basis is difficult, and several

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aspects of the natural history of the infection are still not clear. For example, is the risk for MAC infection associated with the colonization of respiratory and gastrointestinal mucosa? Or does a period of localized or disseminated MAC tissue infection precede the phase of sustained bacteremia?24-6

Despite recognition that disseminated disease develops only after profound CD4+ lymphocyte depletion, the specific immune mechanisms that prevent MAC and are lost in advanced AIDS patients are not defined. Key defense mechanisms against mycobacterial infection include CD4-positive and CD8-positive lymphocytes, macrophages, and various cytokines. Interleukin-γ, interleukin (IL)-2, IL-12, granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor-α (TNF-α) are all believed to enhance the immune response against MAC.7,8 In contrast, IL-6 and TGF-β have been implicated as facilitating MAC growth extracellularly and within macrophages.9,10 Clinical studies to examine these immune effectors in the tissues of subjects at risk for DMAC may increase our understanding of the normal mechanisms of immune protection.

This prospective study was designed to examine several of these issues: (1) identification of clinical predictors for the presence of DMAC, (2) whether tissue infection with MAC occurs prior to sustained bacteremia, and (3) determining immune correlates of risk for DMAC by comparing the immune profiles of DMAC subjects, symptomatic culture-negative subjects, and asymptomatic HIV-positive control groups.

MATERIALS AND METHODS

Study subjects

The study enrolled several types of subjects: one group had CD4+ cell counts below 50 cells/µl plus one or more signs and symptoms suggestive of MAC (chronic unexplained fever, weight loss, elevated alkaline phosphatase, or anemia). Their blood and bone marrow MAC culture status was not known at entry. At analysis, they were divided into Group A—those whose entry cultures grew MAC, and B—those culture negative for DMAC. To ensure adequate numbers of subjects with DMAC, additional subjects newly diagnosed to have MAC by blood culture (but not yet on treatment) were recruited for Group A. Two asymptomatic HIV-infected control groups were also recruited, one having CD4+ cell counts below 50 cells/µl and a second with CD4+ counts between 100 and 250/µl. Group A subjects were culture positive for MAC in blood and/or bone marrow. Group B had signs and/or symptoms suggestive of DMAC and a CD4 count below 50, but had negative entry blood and marrow cultures for MAC. Groups C and D were asymptomatic HIV-infected controls, stratified by CD4 count. Group C serves as the primary control group for the immunopathological analyses because subjects had a CD4 count range similar to the DMAC subjects, yet did not have MAC.

HIV-infected individuals aged ≥18 years were eligible for enrollment if they were receiving either stable or no antiretroviral therapy for ≥6 weeks, had not taken MAC prophylaxis within the prior 6 weeks, and had no clinical or microbiological evidence for acute infection or malignancy. Informed consent was obtained from patients participating in this study, and human experimentation guidelines of the U.S. Department of Health and Human Services and those of the participant institutions were followed throughout.

Clinical evaluation

Subjects' medical histories and physical examinations focused on risk factors and possible signs and or symptoms consistent with MAC. Standard laboratory studies, CD4+ and CD8-positive lymphocyte counts, and plasma HIV-1 concentration were obtained at entry. In addition, the following microbiological and immunological specimens were obtained on the day of entry.

Microbiology

Blood, bone marrow, stool, and respiratory specimens (one from each site) were shipped overnight to the Non-Tuberculous Mycobacterial Reference Laboratory at USC/Children's Hospital of Los Angeles. Blood was collected in SPS or ACD vacutainer tubes. One bone marrow core specimen was placed in sterile nonbacteriostatic saline and a second in 10% formalin. Stool specimens consisted of a rectal swab containing visible fecal material or approximately 0.5 g of stool. Acceptable respiratory specimens included induced sputa, nasopharyngeal wash, bronchial wash, or expectorated sputum.

Blood and bone marrow core specimens were cultured by methods previously described.4,11 Respiratory and stool specimens were processed using the detergent C18-carboxypropylbetaine (CB18).12 Aliquots of the CB18-treated specimen were inoculated directly into a Bectec 12B vial with PANTA supplement and Mitchison 7H11 agar, both in duplicate.3 Stool specimens were first treated with NALC, filtered through a Spin-X II column (Corning CoStar) containing G-50 Sepharose beads, treated with CB18, and inoculated as described for respiratory specimens.

Immunology studies

All were performed in central laboratories using specimens shipped overnight in insulated containers.

Bone marrow immunohistochemistry. These studies were performed in Dr. Luz Bermudez' laboratory. Antibodies were mouse antimouse monoclonals except for rabbit anti-BCG antibody.13,14 IL-12, tumor growth factor-β (TGF-β), interferon-γ (IFN-γ), and TNF-α antibodies were obtained from R&D Systems (Minneapolis, MN). Anti-CD4, anti-CD8, antimacrophage marker (NCL-LN5), goat antirabbit, horse antimouse IgG antibodies, and peroxidase substrates DAB and AEC were from Novacastro Laboratories Ltd. (Newcastle, England).14 Anti-CD56 and anti-CD45ra antibodies were from Pharmingen (San Diego, CA) and rabbit anti-BCG antibody was from Dako (Waco, TX). Immunohistochemical staining procedures were based on the recommended protocol from the Vectastain Elite ABC Kit mouse IgG (Novacastria Laboratories Ltd.). Marrow biopsies were sectioned and prepared as previously described.15-17 Slides were examined at 100x magnification and 20 random fields were scored for the presence of positive cells by at least two investigators, as follows: 1+ = 1–5 cells, 2+ =
Peripheral blood cell surface immunophenotyping by flow cytometry. Peripheral blood mononuclear cells (PBMC) were isolated using CPT blood collection tubes (Becton-Dickinson Biosciences, San Jose, CA). PBMC were processed for six-parameter (four-color) flow cytometric analysis in Dr. Louis Picker’s laboratory, as previously described.22,23 A broad array of CD4, CD8, and γδ cell surface markers was chosen for examination (see Table 1).

Single cell cytokine production following stimulation with PMA/ionomycin and with specific antigens. Pan-T-cell cytokine synthesis-defined subsets were determined by Dr. Picker, as previously described.22 Cytokines measured included IFN-γ, TNF-α, IL-2, and IL-4. For determination of antigen-specific cytokine responses, PBMC were stimulated with or without appropriately titered MAC (crude lysates provided by Dr. L. Bermudez) and cytomegalovirus (CMV; BioWhittaker, Walkersville, MD) antigen preparations, as previously described.23

Induced cytokine synthesis in whole blood cultures. Induced expression of TNF-α and IFN-γ was measured in whole blood culture by Dr. Robert Wallis, as previously described.24 TNF-α was measured in 20 hr cultures, stimulated by Escherichia coli 026:b6 lipopolysaccharide (LPS, Sigma), 100 ng/ml. IFN-γ was measured in the presence and absence of phytohemagglutinin A (PHA, Sigma) 5 μg/ml; candida antigen (CASTA, Greer Labs) 40 μg/ml; M. avium sensitin (MAS, Statens Institute, Copenhagen), or culture filtrate of M. avium LR114 5 μg/ml, prepared as previously described.24

Statistical analysis

Statistical comparisons for categorical data were performed by Chi square and Fisher’s exact tests as appropriate. The Kruskal–Wallis test was employed for continuous measures.25 Pair-wise comparisons have been made for each group vs. control group C (CD4 cells <50/μl) based on Wilcoxon tests. The Bonferroni procedure was used to set a significance level of 0.05/3 = 0.0167 for each individual test to ensure the set of three tests has a significant level of 0.05. However, no adjustment was made for the number of parameters that were evaluated.

Classification and regression tree (CART) analysis, a form of recursive partitioning, was used to explore and identify immunological factors that might differentiate Group A vs. C, B vs. C, and D vs. C.26

RESULTS

Subject characteristics

Eighty-seven subjects were enrolled over a 27-month period at 10 sites. Three sites (Ohio State—28%, UT-Southwestern—28%, Penn—20%) accounted for 76% of enrollees. Subjects were 92% male, 45% were black, and the median age was 37 years. Table 2 displays their baseline characteristics by group. Group A (culture-proven MAC) contains 22 subjects. Four of them were from the group of 19 subjects enrolled because of signs and symptoms compatible with MAC and had MAC isolated from bone marrow (4/4) or blood (1/4) at entry. The other 18 in Group A were enrolled because of a positive blood culture reported prior to study entry. Group B contains the 15 subjects from the “suspected DMAC” group who proved to be culture negative.

The subjects with DMAC (Group A) did not differ from the asymptomatic control Group C (CD4 <50) in gender, age, race, or IV drug use. Roughly half of each group was supposedly taking antiretroviral therapy (41% of Group A and 52% of C). However, the median CD4 cell count in Group A was 7 vs. 22 cells/μl for Group C (p = 0.016), and DMAC subjects were more likely than controls to have a history of prior candidiasis (p = 0.01), lower hemoglobin (p = 0.001), and increased alkaline phosphatase values (45% vs. 4%, p = 0.001).

Groups A and B were compared to determine whether or not clinical data at presentation could discriminate between the two groups. The frequency of individual symptoms (fever, chills, night sweats, diarrhea, weight loss, cachexia), mean log HIV-1 RNA values, frequency of hemoglobin <8 g/dl, thrombocytopenia, elevated serum alkaline phosphatase, or history of antiretroviral therapy did not differ between the two groups. In contrast, 14/22 (64%) Group A subjects had a history of prior Pneumocystis carinii pneumonia vs. only 3/15 (20%) Group B subjects (p = 0.02); similarly, a history of two or more prior opportunistic infections (OIs) was more common in Group A (68%) vs. Group B (40%) subjects.

Microbiology results

Entry bone marrow cultures were positive in 18 of 22 Group A subjects (82%); 10 of these marrow-positive subjects also had positive blood cultures (56%) (Table 3). Of the four subjects whose marrow cultures were negative, one had a positive blood culture and three were negative. These three had been positive in their local site laboratory during study screening and had been granted an entrance exemption for exceeding the

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Table 1. Lymphocyte Cell Surface Markers Measured by Flow Cytometry

<table>
<thead>
<tr>
<th>Specific marker measured</th>
<th>WBC absolute</th>
<th>CD4 absolute and percent</th>
<th>CD8 absolute and percent</th>
<th>HLA-DR (on monocytes) surface density</th>
<th>Gamma/delta absolute and percent</th>
<th>Naive absolute and percent (out of CD4+)</th>
<th>CD27(−) memory/total memory (out of CD4+)</th>
<th>HLA-DR+ absolute and percent (out of CD4+)</th>
<th>CD25 bright+ % (out of CD4+), CD25 Dim+ % (out of CD4+)</th>
<th>Naive absolute and percent (out of CD8+)</th>
<th>CD27(−) memory/total memory (out of CD8+)</th>
<th>CD45RO− memory/total memory (out of CD8+)</th>
<th>CD28(−) CD57+(+)/(total memory (out of CD8+)</th>
<th>HLA-DR+ absolute and percent (out of CD8+)</th>
<th>CD38 MFI (mean fluorescent intensity on CD8− cells)</th>
</tr>
</thead>
</table>
TABLE 2. SUBJECTS’ DEMOGRAPHIC, CLINICAL, AND LABORATORY CHARACTERISTICS

<table>
<thead>
<tr>
<th>Patient group*</th>
<th>Total (n = 87)</th>
<th>A (n = 22)</th>
<th>B (n = 15)</th>
<th>C (n = 25)</th>
<th>D (n = 25)</th>
<th>p-valueb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>80 (92%)</td>
<td>19 (86%)</td>
<td>15 (100%)</td>
<td>24 (96%)</td>
<td>22 (88%)</td>
<td>0.328c</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black nonHispanic</td>
<td>39 (45%)</td>
<td>16 (73%)</td>
<td>6 (40%)</td>
<td>9 (36%)</td>
<td>8 (32%)</td>
<td>0.019c</td>
</tr>
<tr>
<td>Median age</td>
<td>37</td>
<td>37</td>
<td>41</td>
<td>36</td>
<td>37</td>
<td>0.717d</td>
</tr>
<tr>
<td>Prior OIs</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCP</td>
<td>26 (30%)</td>
<td>14 (64%)</td>
<td>3 (20%)</td>
<td>8 (32%)</td>
<td>1 (4%)</td>
<td>0.042c</td>
</tr>
<tr>
<td>Candidiasis</td>
<td>57 (66%)</td>
<td>20 (91%)</td>
<td>12 (80%)</td>
<td>14 (56%)</td>
<td>11 (44%)</td>
<td>0.010c</td>
</tr>
<tr>
<td>Signs/symptoms</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fever</td>
<td>8 (9%)</td>
<td>4 (18%)</td>
<td>3 (20%)</td>
<td>1 (4%)</td>
<td>0 (0%)</td>
<td>0.171c</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>17 (20%)</td>
<td>7 (32%)</td>
<td>5 (33%)</td>
<td>3 (12%)</td>
<td>2 (8%)</td>
<td>0.154c</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>18 (21%)</td>
<td>7 (32%)</td>
<td>8 (53%)</td>
<td>3 (12%)</td>
<td>0 (0%)</td>
<td>0.154c</td>
</tr>
<tr>
<td>CD4 count</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>29</td>
<td>7</td>
<td>23</td>
<td>22</td>
<td>190</td>
<td>0.016d</td>
</tr>
<tr>
<td>≤10</td>
<td>26 (31%)</td>
<td>15 (68%)</td>
<td>6 (40%)</td>
<td>6 (24%)</td>
<td>0 (0%)</td>
<td>0.003c</td>
</tr>
<tr>
<td>Mean log viral load</td>
<td>5.16</td>
<td>5.21</td>
<td>5.23</td>
<td>5.34</td>
<td>4.88</td>
<td>1.000d</td>
</tr>
<tr>
<td>Alkaline phosphatase &gt;1.25 ULN</td>
<td>18 (21%)</td>
<td>10 (45%)</td>
<td>5 (33%)</td>
<td>1 (4%)</td>
<td>2 (8%)</td>
<td>0.001c</td>
</tr>
<tr>
<td>Hemoglobin &lt;8.0 g/dl</td>
<td>5 (6%)</td>
<td>3 (14%)</td>
<td>2 (13%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.001d</td>
</tr>
</tbody>
</table>

*Group A = proven DMAC. Group B = symptomatic, MAC culture-negative. Group C = asymptomatic control, CD4 < 50.
Group D = asymptomatic control, CD4 100-250.

7-day maximum treatment for DMAC prior to entry. They are included in the DMAC group (A) for analysis purposes because they were likely to have the immunological profile that permits the development of DMAC. No control subjects (Groups C and D) had MAC identified in blood or marrow.

Overall, 8/22 Group A subjects were colonized in respiratory and/or gastrointestinal tract (six respiratory, seven gastrointestinal, Table 3). In contrast, only 1/15 and 1/25 in Groups B and C, respectively, were colonized, each in the respiratory tract only, despite CD4 counts <50 cell/µl. None of 25 Group D subjects (CD4 counts 100-250 cell/µl) was colonized.

Bone marrow immunostaining

Subjects with DMAC and asymptomatic controls with CD4 counts below 50 had similar immunologic parameters, including the generally low frequency of cells producing IL-12, TNF-α, and TGF-β. However, significantly more CD8-positive cells (p = 0.011, Kruskal–Wallis test) and a trend toward more CD45RO-staining memory T cells (p < 0.1) were found in marrow specimens from DMAC subjects compared to the low CD4 controls (Group C). Classification and regression tree (CART) analysis of Group A vs. C demonstrated that the combination of positive staining for CD8+ cells, IFN-γ, and CD45RO memory cells differentiated the DMAC group from Group C controls. All 10 subjects who had CD8 greater than a few and IFN-γ greater than none were from Group A, whereas 11 out of 12 subjects who had zero-to-few CD8 cells and CD45RO <10 cells/high-power field were from Group C. This analysis correctly predicted 80% (16/20) of Group A and 71% (17/24) of Group C.

Peripheral blood cell surface immunophenotyping by flow cytometry

Twenty-three cell phenotype markers were analyzed (Table 1). Owing to the low numbers of target cells in Groups A, B,
and C, it was not possible to perform complete flow analysis for some subjects, thus reducing the power to detect statistical differences between groups. Comparing Groups A and C, few differences were found: the absolute number of circulating CD8+ lymphocytes was significantly lower among the DMAC group (A) compared to the Group C controls (p = 0.009, Kruskal-Wallis test), and there was a trend toward a lower absolute number of circulating γδ T cells in the subjects with DMAC (p = 0.024). A trend was found for a higher number and percent of CD8+ HLA-DR+ activated cells in the DMAC group compared to Group C (p = 0.045).

Single cell cytokine production following stimulation with PMA/ionomycin or antigens

Groups A and C had similar numbers of CD4 positive, CD8-negative, and γδ lymphocytes responding to PMA/ionomycin with IFN-γ, IL-2, and IL-4 secretion. There was a trend among TCR-γδ T cells toward diminished TNF-α secretion in the DMAC group (Group A median = 20.6%, Group C = 54.5%, p = 0.1). The DMAC group had significantly more CD4+ cells secreting IFN-γ in response to MAC antigen than did the control Group C (p = 0.006); in contrast, the response to cytomegalovirus (CMV) antigen did not differ between Groups A and C.

Induced cytokine synthesis in whole blood cultures

High levels of TNF-α were produced in LPS-stimulated cultures of blood of all subjects (Table 4). In contrast, the proportion of subjects with detectable responses to the nonspecific T cell stimulus PHA differed among groups, being greatest in Group D. The frequency of this response in Group A (12%) was less than in control Group C (40%, p = 0.08). In contrast, the proportions of MAC antigen responses in these two groups were similar (36% vs. 57%, p = 0.40), even though the level of IFN-γ production was significantly less in Group A.

**DISCUSSION**

Clinical suspicion was a poor predictor of DMAC. Only 4 of the 19 subjects (21%, CI = 6-46%) enrolled because of signs, symptoms, or routine laboratory findings suggestive of DMAC had a positive culture of blood or bone marrow. Had all suspected cases been treated empirically, 79% would have received unnecessary therapy. The infected group (A) appeared to be already highly vulnerable to opportunistic infections associated with profound loss of circulating CD4-positive cells (68% had CD4 cell counts ≤10/μl vs. 40% of the symptomatic but culture-negative Group B). Group A subjects more frequently had prior PCP (64% vs. 20%), candidiasis (91% vs. 56%), and two or more prior OIs (68% vs. 40%) than Group B subjects.

Compared to the asymptomatic control group with CD4 cells below 50/μl, the DMAC group had significantly more candidiasis, lower CD4 cell counts and hemoglobin, and higher alkaline phosphatase values, further indicating their profound degree of functional immunosuppression.

Bone marrow biopsies and blood were obtained for culture from subjects with suspected or recently diagnosed MAC disease to determine whether tissue infection occurs prior to sustained bacteremia. Marrow cultures were more often positive (82%) than concurrent blood cultures (50%) among DMAC subjects, suggesting the greater utility of marrow culture in documenting DMAC. In a prior study comparing MAC burden in blood and bone marrow in HIV-infected subjects, the bacterial concentration in blood did not correlate with that in bone marrow, and the burden in bone marrow was substantially greater than in blood. In the current study, only one MAC blood-culture-positive subject was bone marrow negative. This finding is compatible with a previous report of the infrequent occurrence of transient MAC bacteremia, which perhaps occurs only during the earliest phase of MAC infection. Such episodes may seed bone marrow and other tissues, where MAC burden then steadily increases. Although the MAC tissue burden may be variable at this stage, most patients have substantial infection of the bone marrow, even though blood cultures may not yet be consistently positive. In the final stage of infection, bacteremia becomes sustained and the tissue burden may increase further.

Colonization of gastrointestinal or respiratory tracts was more common among subjects with DMAC than among those whose CD4 counts were below 50 but whose marrow and blood cultures were negative for MAC (36% in Group A vs. 5% in Groups B and C). While the respiratory and gastrointestinal tracts are the likely portals of invasion for MAC, colonization

**Table 4. Induced Cytokine Synthesis in Whole Blood Cultures**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Stimulus</th>
<th>Subject group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>PHA</td>
<td>2/17 (63)</td>
</tr>
<tr>
<td></td>
<td>Candida</td>
<td>1/17 (18)</td>
</tr>
<tr>
<td></td>
<td>Masb</td>
<td>2/14 (105)</td>
</tr>
<tr>
<td></td>
<td>MAC</td>
<td>5/14 (88)</td>
</tr>
<tr>
<td></td>
<td>CMV</td>
<td>2/17 (61)</td>
</tr>
<tr>
<td>TNF-α</td>
<td>LPS</td>
<td>17/17 (4050)</td>
</tr>
</tbody>
</table>

aNNumber of responders/number tested (geometric mean of response, in pg/ml, excluding nonresponders from calculation).

bM. avium sensitin, Statens Institute.
is usually not detected at the time of presentation. These findings indicate that the organisms may attach, invade the mucosa, and enter the bloodstream in a relatively rapid manner, but do not persist as mucosal colonizers.28,29

In situ immunostaining showed that MAC subjects had higher numbers of CD8+ and CD45RO+ cells in bone marrow specimens than did asymptomatic low CD4-count controls. Conversely, the absolute number of circulating CD8+ lymphocytes was significantly lower among the MAC group (A) compared to the Group C controls. This suggests that these immune effector cells were concentrating in infected tissue, but in small numbers and of questionable effectiveness. Both the DMAC Group and low-CD4 controls had a low but similar frequency of cells staining for IL-12, TNF-α, and IFN-γ (cytokines associated with active antimicrobial cellular defense), as well as for TGF-β, a possibly suppressive cytokine. Increased IFN-γ might be expected with the presence of infection but was not observed despite an increased number of bone marrow CD8 T cells in Group A subjects. This observation suggests that the function of CD8+ lymphocytes was impaired, possibly related to the extremely low numbers of CD4 cells in this group. CART analysis indicated that positive staining for CD8+ cells, IFN-γ, and CD45RO memory cells differentiated the DMAC group from Group C controls. All 10 subjects with CD8 more than a few and IFN-γ more than none were from Group A. Production of IFN-γ was present in the bone marrow of the Group A subjects with CD8 cells more than a few, although at a very low level. Cell-associated IL-12 was not increased in the bone marrow samples from DMAC patients. These results agree with recent findings both in vitro and in mice that MAC infection suppresses IL-12 production by macrophages.15,30

Analysis of single T cell cytokine production following nonspecific pan T cell stimulation showed that cells from all patient groups were capable of cytokine production, particularly by CD8 cells. Groups A and C had similar frequencies of CD4-positive, CD8-positive, and γ/δ lymphocytes responding with IFN-γ, IL-2, and IL-4 secretion. The DMAC group had more CD4-positive cells producing IFN-γ in response to MAC antigen than did controls, but no greater production induced by CMV antigen. MAC-specific effector T cells secreting IFN-γ either develop with early infection or are maintained from previous exposure and appear to remain in small numbers at the time of disseminated infection.

The whole-blood culture technique assessment of cytokine production showed that TNF-α was well expressed in response to LPS by cells from all subjects, regardless of clinical group. Subjects in the control groups produced relatively high levels of IFN-γ following stimulation with MAC antigen, although only approximately half of those in either group had responses. Of interest, the proportion of patients in Groups A and C who responded to MAC antigen did not differ statistically, but the magnitude of response was significantly lower in Group A. These low-level MAC IFN-γ responses in some DMAC subjects indicated that very low numbers of antigen-responsive CD4+ T cells persist in these patients, and are consistent with the results of their MAC antigen-induced IFN-γ expression measured by flow cytometry.

HIV-infected individuals with advanced immunosuppression maintain small populations of T cells (especially CD4+ T cells) that produce cytokines in response to MAC antigen. MAC-specific clinical immune recovery and the occurrence of episodes of immune reaction inflammatory syndrome occur relatively rapidly in subjects with DMAC after successful initiation of HAART.31-33 This restoration of effective immunity against MAC infection is consistent with the prompt expansion and functional improvement of the residual MAC-reactive T cells in response to antiretroviral therapy.

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