T Lymphocyte Responses to Mycobacterial Antigen in AIDS Patients with Disseminated Mycobacterium avium-Mycobacterium intracellulare Infection*

Henry W. Murray, M.D.; Donna A. Scavuzzo, M.D.; Sotiros D. Chaparas; and Richard B. Roberts, M.D.

Patients with acquired immunodeficiency syndrome (AIDS) who have Mycobacterium avium-Mycobacterium intracellulare (MAI) infection typically have widely disseminated disease, often fail to respond to multi-drug chemotherapeutic regimens, and show little or no inflammatory tissue response. To determine if this clinicopathologic state correlates with in vitro lymphocyte responses to specific antigen, peripheral blood mononuclear cells from 18 patients with AIDS who had MAI bacillemia were stimulated with either particulate (heat-killed bacille Calmette Guérin [BCG]) or soluble (M intracellulare) mycobacterial antigens. In comparison to reactive cells from healthy control subjects testing positive with purified protein derivative of tuberculin (PPD) or from MAI-colonized (non-AIDS) control subjects, cells from 16 (89 percent) patients with AIDS essentially failed to show any antigen-induced proliferative activity or secretion of \( \gamma \)-interferon; however, in two patients, antigen-stimulated proliferation or \( \gamma \)-interferon production was modest but within the range of responses of normal healthy control subjects. Thus, although an occasional patient with AIDS can develop disseminated MAI infection despite the presence of antigen-reactive cells in vitro, most MAI-infected patients with AIDS display a striking defect in responsiveness to both particulate and soluble mycobacterial antigens. Since treatment with \( \gamma \)-interferon activates the mononuclear phagocyte in vivo, these results suggest a rationale for a trial of \( \gamma \)-interferon therapy in patients with AIDS who have disseminated MAI infection.

Prior to 1981, disseminated infections caused by the Mycobacterium avium-Mycobacterium intracellulare complex (MAI) were rare and occurred almost exclusively in patients receiving prolonged and intensive immunosuppressive therapy; however, with the advent of the acquired immunodeficiency syndrome (AIDS), MAI infections have become commonplace, reflecting the prominence in AIDS of infections caused by pathogens against which a successful host defense is primarily T cell-dependent. In patients with AIDS, MAI infection is often accompanied by persistent bacillemia and positive cultures from multiple sites, and most patients either fail to respond or promptly relapse despite treatment with a four-drug or five-drug regimen. Upon organ biopsy or at autopsy, characteristic findings in these patients include little or no inflammatory or granulomatous changes and overwhelming numbers of intracellular mycobacteria in the tissues. Thus, both clinical and histopathologic observations strongly suggest the absence of any effective T cell-dependent, macrophage-mediated immune response to MAI in patients with AIDS. In this report, we provide the in vitro correlate for these clinicopathologic findings by demonstrating that in response to specific mycobacterial antigen, T cells from virtually all patients with AIDS who have disseminated MAI infection display a striking defect in both proliferative activity and the capacity to secrete \( \gamma \)-interferon, a key macrophage-activating lymphokine.

**Materials and Methods**

**Patients and Control Subjects**

The 18 patients studied fulfilled the standard criteria of the Center for Disease Control for the diagnosis of AIDS, and all had positive blood cultures for MAI at the time of study or within the previous four weeks. The ages of the patients ranged from 24 to 49 years, and 16 were homosexual men. Of the two women studied, one was an intravenous drug abuser, and the other was a recipient of a transfusion. Infection with MAI was the first manifestation of AIDS in two patients; the others had developed one or more opportunistic infections \((n = 14)\) or Kaposi's sarcoma \((n = 2)\) 1 to 19 months prior to the diagnosis of disseminated MAI infection. The four positive control subjects consisted of (a) two healthy individuals testing positive with purified protein derivative of tuberculin (PPD), and (b) two non-AIDS patients with chronic obstructive pulmonary disease and positive sputum cultures for MAI presumed to reflect respiratory tract colonization. Three healthy PPD-negative laboratory personnel served as negative control subjects.

**Mycobacterial Antigens**

Viable, Pasteur-strain bacille Calmette-Guérin (BCG) was ob-
Lymphocyte Enumeration, Proliferation, and γ-Interferon Generation

Peripheral blood helper T cells were identified by the use of the monoclonal antibodies, OKT4 and OKT8, in a standard indirect immunofluorescence assay.24 Peripheral blood mononuclear cells were separated by the Ficoll-Hypaque technique and cultivated at 37°C in 95 percent air with 5 percent carbon dioxide in RPMI 1640 medium containing antibiotics and 15 percent heat-inactivated normal heterologous human serum.25 Proliferative activity was determined using standard techniques26 by the incorporation of tritiated-thymidine in triplicate cultures of 0.5×10^6 cells after three days of stimulation with concanavalin A (ConA) (15μg/ml)27 or after six days of stimulation with either HKBCG (1, 10, and 100×10^6/ml) or various dilutions of MI antigen (1:25, 1:50, 1:100, and 1:500). Results are expressed as net counts per minute (counts per minute of stimulated cultures minus counts per minute of unstimulated cultures). For γ-interferon generation,28,29 mononuclear cells (1.5×10^6 per 0.5 ml) were stimulated for 48 hours with either concanavalin A (15μg/ml),30 HKBCG (0.5, 5.0, and 50×10^6/ml), or MI antigen (1:10, 1:50, and 1:100 dilutions). The resulting culture supernatants were tested for γ-interferon activity using a radioimmunoassay test kit (Centocor) which specifically measures γ-interferon.30 Gamma-interferon activity was measured against a National Institutes of Health (NIH) standard for γ-interferon (Gg 23-901-530, Biological Response Modifiers Program) and is expressed as NIH units.31 The results in Figures 1 and 2 indicate optimal (peak) responses to the various concentrations of the two antigens used. For most control subjects and patients, optimal proliferative and γ-interferon-producing responses were achieved using 5 to 10×10^6 HKBCG organisms per milliliter and a 1:100 dilution of MI antigen (not shown).

RESULTS

Control Responses to Mycobacterial Antigen

As indicated in Figures 1 and 2, mononuclear cells from the PPD-positive and MAI-colonized controls (group A) readily proliferated and secreted γ-inter-
feron in response to stimulation with both HKBCG and M1 antigen; however, these responses were quite variable. Cells from PPD-negative control subjects (group B) showed little or no proliferation or γ-interferon generation, indicating the specificity of the responses of the positive control subjects.

Patients with AIDS

As a group, mononuclear cells from the 18 patients with AIDS who had MAI bacillemia (group C) displayed essentially no response to any concentration of either mycobacterial antigen, and mean values for proliferative activity and γ-interferon production were equal to or lower than those of the PPD-negative control subjects (Fig 1 and 2); however, seven of 13 patients with AIDS secreted normal levels of γ-interferon in response to stimulation with concanavalin A consistent with our prior observations using nonspecific mitogens. 10,11 Analysis of data from individual patients showed that in response to HKBCG, no patient with AIDS who was tested displayed either proliferative activity or γ-interferon production in the normal range (eg, ≥7,065 counts per minute or ≥25 units/ml; see legends to Fig 1 and 2); and in both assays, the responses of most patients with AIDS were barely detectable. Individual responses to M1 antigen were largely similar; however, cells from one (9 percent) of 11 tested patients showed normal proliferative activity (4,483 counts per minute) and one (7 percent) of 15 patients showed γ-interferon production (17 units/ml) which was in the normal range (eg, ≥14 units/ml) (see legends to Fig 1 and 2). To test responses to another specific but nonmycobacterial antigen, cells from eight of the patients with AIDS who were seropositive to cytomegalovirus (serum complement fixation titer ≥1:8) were stimulated with cytomegalovirus antigen. 11 Seven of the eight showed no proliferation (<500 counts per minute) or γ-interferon production (<10 units/ml) in response to this viral antigen (data not shown), whereas cells from healthy seropositive control subjects show 10,500 counts per minute or more and 100 units/ml or more, respectively. 11 One patient's cells displayed low but measurable values to cytomegalovirus antigen, and he was the one patient with AIDS who showed normal M1 antigen-induced proliferative activity.

Lymphocyte Counts

Peripheral blood T cell subsets were enumerated for all 18 patients, and each showed grossly abnormal values. The absolute number of T4⁺ (helper) cells was markedly reduced below the lower limit of normal (328/cu mm), 11 ten patients had 2 to 25 T4⁺ cells per cubic millimeter, six had 30/cu mm to 70/cu mm, and two had >100/cu mm (110/cu mm and 174/cu mm). The mean number of T4⁺ cells per cubic millimeter for the group with AIDS was 40±11 vs 853±53 for healthy individuals. 11 In addition, in all 18 patients, the T4/T8 cell ratio was less than 0.55 (normal range, 0.84 to 3.4), with a mean of 0.15±0.04 (normal, 1.6±0.1). 11 Although deficient numbers of T4⁺ cells probably explained the failure to respond to mycobacterial antigen, 11,12 there was no apparent correlation between T4⁺ cell number and function. The two patients whose cells displayed either normal proliferative or γ-interferon-generating activity had 35 and 18 T4⁺ cells per cubic millimeter, respectively; and, conversely, the two patients with more than 100 T4⁺ cells per cubic millimeter both showed less than 500 counts per minute and less than 5 units/ml in the proliferation and γ-interferon assays, respectively.

Discussion

The failure of peripheral blood mononuclear cells to respond to stimulation with a previously encountered, specific microbial antigen is characteristic of patients with fully established AIDS and opportunistic infections. 10,11,12,13 In such patients, this defect has been demonstrated using a number of diverse microbial antigens (tetanus toxoid; cytomegalovirus; Herpes simplex virus; Toxoplasma gondii; Candida 11,12,13,19) in assays which have measured T cell proliferation, interleukin 2 secretion, and γ-interferon production. 10,11,12,13 On the basis of the results of this study in which cells from 16 (89 percent) of 18 patients with MAI bacillemia showed essentially no response, both particulate and soluble mycobacterial antigen can now be added to the list of specific microbial antigens to which T cells from infected patients with AIDS typically fail to respond; however, cells from two patients showed either proliferative or γ-interferon-generating responses, which, although modest, were considered to be normal, given the broad range of control responses. While there is little doubt that successful host defense against mycobacteria is primarily dependent on T cells, 10,12 the results with these latter two patients indicate that an occasional patient with AIDS may nevertheless develop disseminated MAI infection despite antigen-reactive cells in vitro; however, in such patients the level of T cell reactivity as judged by γ-interferon secretion is low, and if the direct macrophage-activating effects of γ-interferon 10,12,84 are important in defense against mycobacteria, 85,86 this level is still apparently inadequate to control or suppress systemic MAI infection. To test this possibility, we have proceeded to initiate a trial of recombinant γ-interferon therapy in patients with AIDS in whom MAI bacillemia persists despite a multi-drug chemotherapeutic regimen. 87 Given that (1) AIDS mononuclear phagocytes including alveolar macrophages are fully responsive to recombinant γ-interferon in vitro; 10,12,88 (2) the recombinant γ-interferon-stimulated
monocyte can act against mycobacteria;"\(^{38}\) (3) experimental \(M\ intracellulare\) infection responds in vivo; to treatment with recombinant \(\gamma\)-interferon;"\(^{39}\) (4) exogenous recombinant \(\gamma\)-interferon activates the human monocyte in vivo (patients with cancer);"\(^{38}\) lepromatous leprosy,\(^{44}\) and AIDS,\(^{46}\) including the induction of activity against mycobacteria (\(M\ leprae\)); and, finally, (5) as demonstrated herein, that AIDS T cells fail to secrete mycobacterial antigen-induced \(\gamma\)-interferon, such a trial appears to be warranted.

REFERENCES

8. Masur H. \(M\) avium-intracellulare: another scourge for individuals with the acquired immunodeficiency syndrome. JAMA 1982; 2483013
15. Cunningham AL, Marigan TC. \(L\) ev-3 + T cells produce \(\gamma\)-interferon in patients with recurrent herpes labialis. J Immunol 1984; 132:197
25. Edwards CK, Hedegaard HB, Zlotnik A, Gangadharam PR, Johnston RB, Pabst MJ. Chronic infection due to \(M\) intracellulare in mice: association with macrophage release of prostaglandin \(E_2\) and reversal by injection of indomethacin, muramyl dipeptide, or interferon-\(\gamma\). J Immunol 1986; 136:1820
26. Book CAA, Steele J, Fraher L, Barker S, Kamali R, O’Riordan J. Vitamin \(D_3\) gamma interferon, and control of proliferation of \(M\) tuberculosis by human monocytes. Immunology 1986; 57:159