Proliferative and Cytokine Responses of Human T Lymphocytes Isolated from Human Immunodeficiency Virus–Infected Patients to the Major Surface Glycoprotein of Pneumocystis carinii

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The current study examined the proliferative capacity and cytokine secretion pattern of peripheral blood mononuclear cells (PBMC) from human immunodeficiency virus type 1 (HIV-1)–infected patients in response to the major surface glycoprotein (MSG) of Pneumocystis carinii. PBMC from AIDS patients with <200 CD4 cells/mL had significantly less proliferative responses to MSG than did healthy controls. Cytokine analysis indicated that interferon-γ secreted in response to MSG was also significantly less. There was no significant difference in interleukin-4 levels following incubation with MSG between any of the groups; however, all the HIV-infected patients had slightly elevated levels. When the CDC class C3 patients who had a previous episode of P. carinii pneumonia were compared with those who had not had a previous episode, there was a significant increase in the proliferative response to MSG and in interleukin-4 secretion. CDC class C3 patients who had a previous episode of P. carinii pneumonia showed a predominately Th2 response to MSG.

Pneumocystis carinii pneumonia is the most common and often fatal opportunistic infection in patients infected with human immunodeficiency virus type 1 (HIV-1) [1]. It is estimated that 80% of patients with AIDS not receiving anti-P. carinii prophylaxis ultimately develop P. carinii pneumonia during the course of their disease and, even in patients receiving prophylaxis, recurrent episodes of P. carinii pneumonia are relatively common [2]. Cell-mediated immunity is believed to be the major mechanism by which the host controls P. carinii infection [3]. A variety of cell-mediated functions, including the production of cytokines and different T cell subsets, have been implicated in this protective role [3].

Investigation into the host cellular immune response to P. carinii has focused on the major surface glycoprotein (MSG), which is thought to play a crucial role in host-pathogen interactions. MSG is readily identified on the surface of all life-cycle stages of P. carinii [4] and appears to play a role in host-parasite interaction by mediating attachment of the organism to alveolar cells [5]. MSG is highly immunogenic and elicits both humoral and cellular immune responses [3, 4].

The immune response to infectious agents is often characterized by a dominance of either cell-mediated or humoral type effector mechanisms. Two classes of T helper (Th) cells have been described: Th1 cells, which produce interleukin (IL)-2 and interferon (IFN)-γ, favor cell-mediated immunity and delayed type hypersensitivity [6]; Th2 cells, which produce IL-4, IL-5, and IL-10, favor humoral responses [6]. During many strong immune responses, these two effector pathways appear to be exclusive: Th1 and Th2 cells are mutually inhibitory or self-stimulatory. Since immune dysfunction is central in AIDS, it is not surprising that cytokines play an important role in the replication and pathogenesis of HIV infection. It has been demonstrated

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that HIV-infected asymptomatic subjects initially had a good T cell response to HIV peptides [7]. However, in the later stages of disease, T cell proliferation in response to recall antigens is impaired [8] and IL-2 and IFN-γ production are decreased while IL-4 production is increased, reflecting the involvement of Th2-like cells in the later stages of HIV infection [9]. Recent studies have indicated that Th2 cell-associated IL-4 may contribute to the immune dysregulation and progression of HIV-induced disease [7].

Here we investigated the proliferative capacity and cytokine secretion pattern of mononuclear cells from HIV–1–infected patients at different stages of the disease in response to MSG from P. carinii.

Materials and Methods

Subjects. Peripheral blood mononuclear cells (PBMC) were obtained by ficoll-hypaque gradient separation from peripheral blood of healthy volunteers and HIV-infected persons. The 12 healthy controls (4 women, 8 men) were 21–52 years old. The patients, who attended or received medical care at the University of Cincinnati AIDS Treatment Center or the HIV Clinic at the Cincinnati Veterans Affairs Medical Center (7 women, 46 men), were 22–60 years old. According to the Centers for Disease Control and Prevention criteria [10], on the basis of CD4 lymphocyte counts, there were 14 patients with CD4 cell counts >500/mL, 13 with 200–500/mL, and 26 with <200/mL. Patients who had a CD4 cell count of <200/mL or a previous episode of P. carinii pneumonia received chemophrophylaxis, usually trimethoprim-sulfamethoxazole. The CD4 cell counts were obtained on the same day that patients’ blood was obtained for use in this study.

Antigen preparation. Due to the limited supply of human-derived P. carinii available for laboratory studies, organisms isolated from rats are commonly being used in its place. P. carinii were isolated from the lungs of corticosteroid-treated Lewis rats as previously described [3]. To control for possible contaminating lung tissue remaining in the P. carinii preparation (lung control), Lewis rats, housed in a protected environment to prevent exposure to P. carinii, were also treated with corticosteroids. Lungs from these rats were processed in the exact manner as described for P. carinii–infected lungs. For the lung control animals, the absence of antibodies to whole P. carinii and MSG was confirmed by Western blot analysis of sera prior to the removal of the lungs. MSG was purified from organisms by high-performance liquid chromatography. MSG was isolated on the basis of size using a Macrosphere GPC 150 column (Alltech Associates, Deerfield, IL) under isocratic conditions in 0.1 M KH2PO4 (pH 7.0), 0.2 M NaCl as previously described [3]. MSG migrated as a band with a molecular mass of 120 kDa on SDS-PAGE gels under reducing conditions. The purity of the preparation was shown by Coomassie blue staining, reactivity with MSG-specific monoclonal antibodies, and by the absence of endotoxin as confirmed by the limulus amebocyte assay (<0.125 U/mL; Whittaker Bioproducts, Walkersville, MD). The protein concentration of the lung control was determined and adjusted to the same level as for MSG.

Lymphocyte proliferation assays. One hundred microliters of PBMC, at 10⁶ cells/mL in RPMI 1640 (containing 2 mM l-gluta-
mine, 100 U/mL penicillin, 100 µg/mL streptomycin, and 10% heat-inactivated fetal calf serum [RPMI complete]), were plated in triplicate in flat-bottom 96-well plates. Cells were cultured at 37°C with 5% CO₂ for 5 days in medium alone or medium supplemented with ConA (10 µg/well), lung control (1 µg/well), or MSG (1 µg/well). Cells were pulsed with 1 µCi of [³²P]thymidine per well (2 Ci/mmol; New England Nuclear, Boston) for 18 h prior to harvest onto glass fiber filter strips with an automatic multisample cell harvester. The samples were then counted in a liquid scintillation counter. The data were expressed as the mean counts per min of triplicate cultures.

Cytokine assays. PBMC (10⁶ cells/mL) were cultured for 3 days in RPMI complete alone or supplemented with MSG (10 µg/mL) or lung control (10 µg/mL). Supernatants of stimulated or nonstimulated cells were harvested and stored at –70°C until assayed for cytokines. IL-4 and IFN-γ were determined by specific commercial ELISA (Genzyme, Boston) exactly as specified by the manufacturer. Detection limits were 45 pg/mL for IL-4 and 100 pg/mL for IFN-γ.

Statistical analysis. Statistical significance of the observed differences between groups was evaluated using the nonparametric two-sided Wilcoxon-Mann-Whitney rank tests by INSTAT (GraphPad Software for Science; San Diego). Significance was accepted when the P value was <.05.

Results

Our earlier studies have shown a blastogenic response to whole P. carinii organisms by human PBMC from HIV-infected patients [11]. These experiments specifically investigated the proliferative response to MSG by PBMC isolated from healthy donors and from HIV-infected persons at all stages of disease. Table 1 shows that there was a significant decrease in the proliferative response of PBMC from AIDS patients with <200 CD4 cells/mL to MSG compared with the response of PBMC from healthy controls. There was no significant difference observed in the blastogenic response between patients with >500 CD4 cells/mL or 200–500/mL and healthy controls. These data suggest that proliferative response by PBMC to MSG is dependent on the amount of circulating CD4 cells. Our findings using the mitogen ConA showed similar results. The magnitude of the response to the mitogen was directly related to the number of CD4 cells (table 1). However, the significant proliferative responses to ConA by PBMC isolated from patients (compared with cells incubated in medium alone) demonstrated that the cells were capable of responding and proliferating. Last, as shown previously [11], there was no significant proliferative response by any of the groups to the lung control (data not shown).

We next examined whether MSG induced IFN-γ secretion from PBMC isolated from HIV-infected subjects. Table 1 shows that there was a significant decrease in the amount of IFN-γ secreted in response to MSG stimulation by PBMC isolated from patients with <200 CD4 cells/mL. There was no difference between healthy controls and individuals with >500 or 200–500 CD4 cells/mL in the levels of IFN-γ secreted. No
significant IFN-γ secretion was observed following incubation with lung control (data not shown).

We then investigated whether there was a difference in the levels of IL-4 secreted by PBMC in response to MSG and whether this was observed as HIV disease progressed. Table 1 shows that there was no significant difference between any of the groups in the amount of IL-4 secreted. Of interest, in comparison with healthy controls, all of the patients with HIV infection had elevated levels of IL-4 following stimulation with MSG; those with <200 CD4 cells/mL demonstrated the highest levels of IL-4. No IL-4 was detected following incubation with the lung control (data not shown).

In these experiments, we examined whether there were differences in the proliferative response to MSG by PBMC from CDC class C3 patients (<200 CD4 cells/mL, AIDS-defining illness) who had had an episode of documented *P. carinii* pneumonia versus those who had not. As shown in Table 2, those patients who had had previous *P. carinii* pneumonia (n = 6) had significantly increased blastogenic responses to MSG compared with patients who had not had such an episode (n = 8). The increased proliferative response of patients who had previous episodes of *P. carinii* pneumonia was not due to increased numbers of CD4 cells; in fact, these patients had significantly lower CD4 cell counts than did those who had not had previous *P. carinii* pneumonia (60 ± 43 vs. 121 ± 62/mL; *P < .05*).

Table 2 also demonstrates the differences in cytokine secretion in response to MSG within the CDC class C3 patients between those individuals who had a history of *P. carinii* pneumonia and those who did not. Those with previous *P. carinii* pneumonia secreted significantly elevated levels of IL-4 in response to MSG than those without previous *P. carinii* pneumonia. In contrast, there was no significant difference in the level of IFN-γ secreted in response to MSG from CDC class C3 patients regardless of whether or not they had previously had *P. carinii* pneumonia. These data suggest that in the CDC class C3 patients who had *P. carinii* pneumonia, there was a predominately humoral immune response to MSG.

### Discussion

The results presented here have shown that there was a significant reduction in the proliferative response to MSG by HIV-infected patients who had <200 CD4 cells/mL. These data are in agreement with several reports demonstrating impaired proliferative responses of cells isolated from HIV-infected persons to recall antigens, pokeweed mitogen, and CD3-mediated stimulation [8]. It has been suggested that HIV infection causes a selective defect in T cell responses to specific antigens and, from the data presented here, this also appears to be the case for MSG.

In contrast, CDC class C3 patients who had recovered from a previous episode of *P. carinii* pneumonia had significantly higher proliferative responses to MSG than class C3 patients who never had pneumonia. Even though patients with *P. carinii* pneumonia had fewer CD4 cells, they appeared to retain sufficient numbers of memory CD4 cells to mount a more vigorous response. Cayota et al. [8] reported similar results in proliferative experiments using isolated CD4 cell subsets from symp-
tomatic AIDS patients. Naive cells were found to display a defect in proliferative capacity while memory cells had a normal proliferative response. A recent report by Waldrop et al. [12] demonstrated that CD4 cells from HIV-infected patients that were responsive to cytomegalovirus were dramatically increased in ~40% of the patients compared with control subjects, including those with relatively advanced disease. Thus, the examination of protective immunity to common pathogens within the realm of HIV infection may not be dependent on increasing the overall number of T cells but on increasing memory T cells reactive with such pathogens.

Several studies, which measured mitogen-stimulated cytokine production by PBMC of HIV patients, have shown that there is a decrease in IFN-γ and IL-2 (Th1 cytokines) production and an increase in IL-4 and IL-10 (Th2 cytokines) with progression of the disease [13]. In the current report, there was a significant decrease in the amount of secreted IFN-γ in response to MSG by HIV patients with <200 CD4 cells/mL. Moreover, there was an increase in IL-4 production within these groups in response to MSG stimulation. Thus, MSG, a specific T cell antigen of \textit{P. carinii}, elicits a similar pattern of immunologic responses seen with recall antigens in HIV-infected persons.

This report is one of the first to directly compare the responses to a recall antigen, MSG, of PBMC isolated from CDC class C3 patients who have had an episode of \textit{P. carinii} pneumonia with responses of those who have not had the infection. Patients who had \textit{P. carinii} pneumonia had a significant increase in their IL-4 production in response to MSG compared with those who had not had \textit{P. carinii} pneumonia. It is therefore possible that in these CDC class C3 patients who had \textit{P. carinii} pneumonia, a predominately Th2 (humoral) response was generated in response to MSG. We have recently reported, using CD4 cells isolated from rats with previous exposure to \textit{P. carinii}, that upon restimulation of these cells with MSG, IL-4 was increased in response to MSG. We have recently reported, using CD4 cells isolated from rats with previous exposure to \textit{P. carinii}, that upon restimulation of these cells with MSG, IL-4 was increased in response to MSG. We have recently reported, using CD4 cells isolated from rats with previous exposure to \textit{P. carinii}, that upon restimulation of these cells with MSG, IL-4 was increased in response to MSG.

References


