Role of the Mononuclear Phagocyte System in the Immunopathogenesis of Human Immunodeficiency Virus Infection and the Acquired Immunodeficiency Syndrome

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In studies aimed at defining monocyte and macrophage function in patients with human immunodeficiency virus (HIV) infection, we found impaired in vivo Fc receptor–specific clearance in 20 of 25 patients with acquired immunodeficiency syndrome (AIDS) and in five of 13 patients with AIDS-related illnesses. The in vivo function of macrophage C3 receptors was also found to be abnormal: AIDS patients had a relatively large release of cells back into the circulation, suggesting failure of macrophage phagocytosis. The antibody-dependent cell-mediated cytotoxicity of circulating mononuclear cells was significantly lower in AIDS patients than in healthy controls. Monocyte nonspecific phagocytosis and surface marker expression were intact. Defective monocyte and macrophage function is an integral part of the immunopathology of AIDS, leading to the failure to control opportunistic pathogens. Whether these defects are due to intrinsic infection of the mononuclear phagocytes with HIV or are secondary to other events in the network of HIV infection remains to be determined.

Observations and experimental data over the past 100 years have demonstrated that peripheral blood monocytes, free and fixed tissue macrophages, and their bone marrow precursors should be grouped together and referred to as either the reticuloendothelial system (RES) or the mononuclear phagocyte system [1]. The bone marrow monoblast is the most immature cell that is still recognizable as part of the RES; it is believed to be derived from a pluripotential stem cell. The monoblast, by cell division, gives rise to two promonocytes, which in turn mature into monocytes and are released into the circulation. Tissue macrophages are derived from both circulating monocytes and local proliferation of macrophages. Tissue macrophages are widely distributed in organs (liver, spleen, lungs, bone marrow, lymph nodes, and nervous system), in the connective tissue (histiocytes), and in the serous cavities (pleural space and peritoneum) [2]. A primary function of these cells is to clear the blood, lymph, and tissues of microbial pathogens and other foreign particles by phagocytosis. Following phagocytosis, most organisms are killed by oxidative mechanisms [2, 3]. Phagocytosis can occur via nonspecific or specific, i.e., immune, mechanisms. Cells of the monocyte-macrophage series also serve a number of other indispensable immunologic functions. These include production and secretion of immunoregulatory molecules, e.g., interleukin 1 (IL-1), and participation in cellular interactions to produce antibodies and cytotoxic lymphocytes [4, 5].

We have been involved in studies on monocyte and macrophage function of patients with acquired immunodeficiency syndrome (AIDS) and other human immunodeficiency virus (HIV) infections in order to elucidate HIV immunopathogenesis [6–10]. Our interest was stimulated by the observation that many
of the organisms causing widespread infection in patients with AIDS [11], such as *Mycobacterium avium*—*Mycobacterium intracellulare* (M. avium complex), *Cryptococcus neoformans*, and *Candida albicans*, are normally contained by RES macrophages [12-14]. Examination of blood smears indicated the presence of atypical circulating monocytes and bone marrow histiocytes [6], and biopsies of the tissues of AIDS patients showed large numbers of opportunistic pathogens. In addition, an increasing number of reports indicate that monocytes and macrophages can be infected with HIV [15-31].

In this paper, we present our data on monocyte and macrophage function in patients with HIV infections and discuss the implications of these findings and data from previously published studies on this topic in reference to the immunopathology of HIV infection.

**Materials and Methods**

**Clearance studies.** The clearance studies were performed as previously described [7, 9]; most of these patients have been reported separately elsewhere [7, 9, 10] but are reviewed in order to integrate those results with our present findings reported in this paper. Blood was obtained by sterile venipuncture into a heparinized syringe. The erythrocytes were isolated by centrifugation, washed three times with cold physiologic saline, and radiolabeled with 51Cr by incubation at 37°C with Na2 slCrO4 (ICN Pharmaceuticals, Irvine, Calif.). The radiolabeled cells were washed twice with cold saline and photometrically standardized to 3.3 x 10⁸ cells/mL.

For the Fc-specific clearance studies, an aliquot of the 51Cr-labeled cells was sensitized by the addition of purified, aggregate-free IgG antibody to Rh0(D). This mixture was incubated at 37°C with Na2 slCrO4 (ICN Pharmaceuticals, Irvine, Calif.). The radiolabeled cells were washed twice with cold saline and photometrically standardized to 3.3 x 10⁶ cells/mL.

For the C3-receptor clearance studies, an aliquot of the 51Cr-labeled cells was mixed for 45 minutes in an ice water bath with IgM cold agglutinin obtained from a patient with chronic idiopathic cold agglutinin disease. Following the incubation, an equal volume of fresh autologous serum (as a source of complement) was added to the mixture and incubated at 18°C for 30 minutes, followed by a 5-minute incubation at 37°C to allow the cold agglutinin to dissociate. The cells were then washed twice in 37°C physiologic saline, resuspended at 3.3 x 10⁹/mL in normal saline, diluted 10-fold with saline, and injected via a butterfly needle through a forearm vein. Nine samples of venous blood were taken over a 2-hour interval, and clearance curves were drawn.

To determine whether in vivo clearance function could be successfully immunomodulated, we treated eight patients with AIDS with γ-interferon (Genentech, San Francisco, Calif.) for 7 days: two with 0.001 mg/[m²-d], two with 0.01 mg/[m²-d], two with 0.1 mg/[m²-d], and two with 1.0 mg/[m²-d].

**Antibody-Dependent Cell-Mediated Cytotoxicity**

These studies have been described in detail elsewhere [8]. Briefly, fresh peripheral blood mononuclear (effector) cells were incubated with chromated chicken red blood cells (CRBC) and rabbit anti-CRBC serum (Cappel Laboratories, Cochranville, Pa.) at six effector-to-target ratios (E:T) ranging from 20:1 to 0.6:1 for 5 hours at 37°C in a 95% air/5% CO₂ incubator. Following this, 100 μL of the supernatant was removed and analyzed for the level of radioactivity. The percentage of cytotoxicity was determined by the following formula:

\[
\text{cytotoxicity (\%) = } \frac{\text{test cpm} - \text{spontaneous release cpm}}{\text{freeze-thaw cpm} - \text{spontaneous release cpm}} \times 100
\]

In a complementary series of experiments, the antibody-dependent cell-mediated cytotoxicity (ADCC) of peripheral blood cells of healthy donors was tested in the presence of 10%-40% serum from AIDS patients.

**Quantitation of monocyte phagocytosis by flow cytometry.** Our method was adapted from Nagel et al. [32]. Heparinized whole blood was added to LeucoPREP tubes (Becton Dickinson, Mount View, Calif.) and centrifuged for 17 minutes at 1,500 x g. Plasma was saved and the mononuclear cells were washed twice in RPMI 1640 medium (GIBCO, Grand Island, N.Y.), counted, and diluted to a final concentration of 2.5 x 10⁶ cells/mL of RPMI 1640.
and 20% plasma; 500 μL of the cell suspension was dispensed in each of four 12 × 75 mm tubes per subject. A fifth tube contained only 500 μL of RPMI 1640 plus 20% plasma. All tubes were placed in a 37°C water bath, and 2.0 μ fluorescent latex beads (Polysciences, Warrington, Pa.) were added at a concentration of 36 beads per cell to three of the tubes containing cells and the tube containing RPMI 1640 and plasma. At different time intervals (10, 30, and 60 minutes), a tube containing beads and cells was removed from the water bath, and phagocytosis was stopped by adding 3 mL of cold phosphate-buffered saline (PBS) containing 0.1% gelatin and 5 mM ethylenediamine tetraacetic acid (EDTA). The tubes containing only cells or only beads were incubated for 60 minutes. Each tube was then washed twice in the PBS/gelatin/EDTA at 400 × g for 10 minutes at 4°C and placed on ice. The cells were resuspended in 500 μL of PBS plus 0.2% NaN₃, and 20 μL of the monoclonal antibody, M-3-phycoerythrin (Becton Dickinson), were added. The tubes were incubated for 30 minutes at 4°C. The cells were washed twice in PBS/gelatin/EDTA and resuspended in 1.0 mL of medium.

Flow cytometry was used to quantitate phagocytosis by the monocytes. Cells were analyzed on an EPICS C (Coulter Electronics, Hialeah, Fla.) fluorescein activated cell sorter equipped with an argon laser (Coherent, Palo Alto, Calif.) operated at 488 nm, 400 mW, and 40 A. Gating was performed on the monocyte population as determined by the M-3 monoclonal antibody (red fluorescence) and forward angle light scatter (size). The tube containing only beads was run as a control, and the gate was adjusted so that no free beads were included. Approximately 10,000 cells were then analyzed for each of the three time-interval tubes at a flow rate of 100 cells/s, and the green fluorescence distribution was displayed as a 256-channel histogram. It was possible to discriminate five populations of monocytes containing phagocytosed microspheres.

Monocyte function and activation of surface marker expression. Flow cytometry was used to detect binding of monoclonal antibodies (MAbs) directed to human monocyte surface markers [33]. We used FcRMAb32 and IV3 (gifts of Dr. C. Anderson) [34, 35], which bind to human leukocyte type I and type II Fc receptors, respectively; H5A4 (gift of Drs. J. T. August) [36], which identifies the α-subunit of the type 3 complement receptor (CR3) and is implicated in the critical phagocyte adhesion function [37]; and Mo3e (gift of Dr. R. Todd) [38], which appears to bind to the surface receptor for migration inhibition factor. This latter MAb shows negligible or very low-level binding to fresh circulating monocytes but strikingly high binding to monocytes "activated" by 20-hour culture with bacterial endotoxin, a phorbol ester, or muramyl dipeptide [38].

In brief, blood was collected in EDTA from volunteer patients with AIDS or ARC not undergoing systemic antimicrobial or other chemotherapy and from healthy volunteer laboratory workers (controls). Mononuclear cell fractions were prepared by centrifugation, washed three times, and divided into two portions. The first was suspended in pH 7.20 MAb buffer and kept at 4°C while aliquots were reacted with proper dilutions of the four above-mentioned test MAbs and IgG1, IgG2a, and IgM isotype-specific controls (Coulter, Inc., Hialeah, Fla.), and then with affinity-purified FITC (fluorescein isothiocyanate)-conjugated Fab fragments of goat antimouse anti-IgG and -IgM (Tago, Inc., Burlingame, Calif.). Aliquots were also reacted with directly conjugated anti-Leu 3, anti-Leu 2, and anti-LeuM3 (Becton Dickinson) to identify proportions of CD4⁺ lymphocytes, CD8⁺ lymphocytes, and monocyte populations, respectively.

The second portion of cells from each subject was suspended in RPMI 1640 medium containing 5% heat-inactivated fetal calf serum and incubated for 60 minutes in 3 mL tissue culture wells at 37°C, after which nonadherent cells were removed. Adherent cells were then cultured at 37°C in this medium for 20 hours with 50 ng/mL of Escherichia coli lipopolysaccharide (LPS, Difco, Detroit, Mich.). Cells were subsequently removed by rinsing and gentle scraping with a policeman after a 20-minute incubation with EDTA-containing MAb buffer [38]. These 20-hour culture cells were then reacted with the same MAbs as described above for the fresh cells. After fluorescent staining, all cells were fixed in 400 x EDTA and analyzed on a Coulter EPICS C flow cytometer within 48 hours.

Results

Clearance studies. Circulating IgG-sensitized erythrocytes undergo attachment to splenic macrophage Fc receptors, triggering phagocytosis and thus removing the erythrocytes from the circulating blood.
MINUTES AFTER INJECTION

Figure 1. Survival of IgG-sensitized, 51Cr-labeled autologous erythrocytes expressed in cpm/mL of blood over time.

For the period studied, the clearance follows first order kinetics, and a clearance half-time can be calculated. Examples of clearance curves of a healthy volunteer and a typical patient with AIDS are shown in figure 1. The clearance half-times for 25 patients with AIDS (20 reported elsewhere [7, 9]), 13 patients with an AIDS-related illness (reported elsewhere [7, 10]), and 17 healthy controls are depicted in figure 2. The mean clearance half-time for the 25 patients with AIDS (mean ± SEM, 71.8 ± 7.1 minutes) was significantly longer than the combined value of the healthy control subjects (29.2 ± 2.2 minutes, P < .001; Wilcoxon’s rank sum test). Further, 20 of 25 patients with AIDS had a clearance half-time longer than that of the upper 95% confidence limit of the 17 healthy controls (x* = 26.0, P < .001).

In patients with AIDS-related illnesses, abnormal clearances were found essentially only in those patients with immune thrombocytopenic purpura (ITP) [10]. Four of five patients with ITP had abnormal clearances as opposed to one of eight patients with other AIDS-related illnesses (P = .03, Fisher’s exact test) (figure 2). Further, the one patient with an abnormal clearance and without ITP developed AIDS [7] shortly thereafter.

In a previous study on Fc-receptor function in AIDS, one patient with AIDS who was receiving γ-interferon at the time of his study had a normal clearance study [7]. Because γ-interferon increases in vitro monocyte Fc-receptor expression [40], this suggested it may improve in vivo Fc-receptor func-

Figure 2. Fc receptor-specific clearance in healthy volunteers, patients with AIDS-related illnesses, and patients with AIDS. The shaded area represents the 95% confidence limit for the controls. The patients with immune thrombocytopenic purpura (ITP) are indicated by open boxes (□).

tion. To test this hypothesis we examined Fc-receptor clearance in six AIDS patients with Kaposi’s sarcoma before and after intravenous γ-interferon. The results, displayed in figure 3, show that with lower doses no improvement in Fc-receptor clearance was seen. With increasing doses, however, a slight improvement was seen, but the study had to be terminated in the patients receiving the largest dose (1 mg/m²) because of drug intolerance.

In order to determine whether the above clearance defect was limited to splenic Fc-receptor function or represented a more global macrophage defect, we studied C3 receptor-specific clearance. (These studies are detailed elsewhere [9].) In terms of functional activity, these receptors are primarily present on hepatic Kupffer cells [39]. Clearance of C3-coated erythrocytes follows a triphasic clearance curve.
There is an initial rapid clearance of the cells from the bloodstream because of their attachment to C3 receptors. The attached cells then are either phagocytosed by the hepatic Kupffer cells or released back into the circulating blood because of cleavage of the complement components. The released cells are not immunologically active and circulate with a half-time equal to that of unsensitized cells. As previously reported [9], figure 4 illustrates the clearance curves of C3-coated erythrocytes for healthy heterosexual men, healthy homosexual men, and patients with AIDS. In heterosexual men, ~50% of the cells were cleared during the initial 10-20 minutes. Only a small portion of the cells were released back into the bloodstream.

Homosexual men had a greater initial clearance of the inoculum than the heterosexual controls but had a small release of cells back into the circulating blood. No differences were seen between the HIV-seropositive and HIV-seronegative homosexual men.

Patients with AIDS had striking differences from these two control groups. They had a relatively large initial clearance of up to 39.4% ± 7.5% of the initial inoculum, with a large release of cells (25.6% ± 3.2%) back into the bloodstream. This release was significantly greater than that seen with either control group (P<.005 vs. heterosexual men, P<.05 vs. homosexual men).

**ADCC assay.** When antibody-sensitized CRBCs are used as targets, adherent cells of the monocyte-macrophage lineage are the effector cells. The attachment of the Fc receptor-positive effector cell to the target is followed by phagocytosis of the CRBC [8] (figure 5). Thus, this assay is an approximate in vitro equivalent of our IgG clearance studies. As previously reported [8] and as shown in table 1, at all six effector-to-target ratios, there was a clear reduction in ADCC activity in AIDS patients as compared to that in healthy heterosexual controls.

**Figure 3.** Effect of intravenous γ-interferon on Fc receptor-specific clearance in patients with AIDS. No significant changes in clearance half-time were seen.

**Figure 4.** Clearance of C3-sensitized, 51Cr-labeled autologous erythrocytes from the circulation for eight healthy heterosexual men, eight healthy homosexual men, and seven patients with AIDS. Values are mean ± SEM at each point. Data taken from [9].

**Figure 5.** Scanning electron micrograph of a monocyte phagocytosing a chicken red blood cell. Original magnification, 10,000 x.
Table 1. Comparison of antibody-dependent cell-mediated cytotoxicity (ADCC) in healthy controls and patients with AIDS.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Mean % cytotoxicity ± SEM for indicated effector-target ratio</th>
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<tr>
<td></td>
<td>20:1</td>
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<tr>
<td>Healthy controls (n = 8)</td>
<td>45.9 ± 3.0</td>
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<tr>
<td>Patients with AIDS (n = 11)</td>
<td>27.0 ± 5.0</td>
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*P value*<.001 <.05 <.05 <.05 <.001 <.05

NOTE. Table adapted from [8].

*P* determined by Student's *t* test.

We next assayed the ADCC activity of mononuclear cells from healthy controls in the presence of either autologous serum or serum from patients with AIDS. No significant difference was seen between autologous serum and 10% AIDS serum. When 25% or 40% AIDS serum was used, however, a clear reduction in ADCC activity was seen [8].

Quantitation of phagocytosis by laser flow cytometry. We next determined whether there was a non-specific as well as a specific defect in monocyte phagocytosis. Phagocytosis was quantitated using fluorescent microspheres and laser flow cytometry in seven healthy controls, six patients with AIDS-related illnesses, and 62 patients with AIDS. Figures 6A, 6B, and 6C show fluorescent-bead adhesion kinetics at 10, 30, and 60 minutes, respectively. At 10 minutes most monocytes adhering to and phagocytosing beads have only one bead (37.9% ± 4.6% in healthy controls, 48.7% ± 14.0% in patients with AIDS-related illnesses, and 35.7% ± 6.8% in patients with AIDS), but by 60 minutes nearly half the cells hold five or more fluorescent beads (50.0% ± 6.4%, 39.6% ± 10.4%, and 52.6% ± 12.1%). This in vitro sequential, nonspecific phagocytic process did not differ significantly among normal controls, patients with AIDS, or those with AIDS-related illnesses.

Monocyte function and activation surface marker expression. To examine whether monocyte surface receptors are altered in vivo in HIV infection, we studied several recently reported monoclonal antibodies directed to human monocytes with specificity for two types of Fc receptors (FcRMAb32 and IV3), the α-subunit of CR3 (H5A4), and a surface determinant that appears after in vitro activation (Mo3e) [33]. These and isotype-specific control monoclonal antibodies were reacted with freshly obtained circulating mononuclear cells from 10 patients with AIDS or AIDS-related illnesses and 10 control subjects, as well as with these patients' and controls' in vitro-activated monocytes. Results of monoclonal antibody binding consistently showed no signifi-
cant difference between patients and normal controls in expression of these receptors and activation markers in either the baseline or activated states [33].

Discussion

A number of investigators have now documented a variety of abnormalities in monocyte function in patients with AIDS. These include defects in monocyte numbers, phagocytosis, secretory products, and monocyte–T cell interactions.

Monocyte numbers. When analyzing immune function, it is logical to enumerate the effector cell population. Using morphologic criteria, most investigators have noted that at least some patients with AIDS have a moderate-to-marked reduction in the number of circulating monocytes [6, 41–44], though the overall mean may not be significantly different from control values [45]. There is one report, however, using the LeuM3 monoclonal antibody and laser flow cytometry, that suggests the number of peripheral blood monocytes is increased [46]. Moreover, using an ox rosette-forming procedure, the number of CD4+ monocytes were found to be reduced from 60%–80% in healthy persons to <20% in AIDS patients, paralleling the decrease in CD4+ lymphocytes [47]. This could not be confirmed, however, with immunofluorescence [48]. A loss of CD4+ monocytes could have important pathogenic implications because the CD4 surface antigen has been recognized as the site of attachment of HIV to the target cell [16].

Bronchoalveolar macrophages have also been quantitated in patients with AIDS. Though only small numbers of patients have been studied, there appears to be a decrease in the relative numbers of macrophages obtained by bronchoalveolar lavage but no difference in absolute numbers [49, 50].

Monocyte chemotaxis, phagocytosis, and intracellular killing. Effective killing of microorganisms by monocytes requires several important steps: migration of the monocytes to the appropriate site, particle recognition and attachment, particle ingestion, and intracellular killing. All of these steps have been studied in patients with AIDS.

Chemotaxis refers to the movement of phagocytic cells towards an infected site in response to a signal. Monocyte chemotaxis in AIDS patients has been shown to be defective in response to a variety of signals, including N-formylmethionylleucylphenylalanine (F-Met-Leu-Phe), lymphocyte-derived chemotactic factor, complement fragment C5a, endotoxin-activated serum, and casein [42, 44, 51, 53]. Nielsen et al. [54], however, found normal monocyte chemotaxis in response to casein and zymosan-activated serum. Defective chemotaxis may be due to an inhibiting factor present in the serum of HIV-infected men that is similar to the HIV transmembrane protein gp 41 [55].

Engulfment of targets can occur by either immune (receptor-specific) or nonimmune (nonspecific) mechanisms. Most studies indicate that immune phagocytosis is impaired in patients with AIDS, while nonimmune mechanisms remain intact. We have shown that both Fc and C3 receptor–mediated clearances are abnormal in AIDS patients (figures 2 and 4), as is in vitro ADCC killing (table 1). We also quantitated nonspecific monocyte phagocytosis of fluorescent latex beads with laser flow cytometry; despite the use of this powerful tool, however, no differences were found among controls, patients with AIDS-related illnesses, and patients with AIDS.

To investigate possible mechanisms of this discrepancy we utilized flow cytometry and monoclonal antibodies against cell surface Fc and complement receptors [33]. Differences in the expression of the Fc receptor have been suggested to exist, for example, in systemic lupus erythematosus and have been used to relate increased Fc-receptor binding of IgG to defective in vivo clearance of IgG-sensitized erythrocytes [34, 56, 57]. No difference in Fc-receptor expression was seen in our patients. Further, the CR3 molecule, critical for phagocyte target adhesion [27], was also present in proportions comparable to those in normal subjects both before and after LPS activation.

Insofar as the monoclonal antibody binding data reveal the structural integrity of several monocyte surface receptors critical to phagocytosis, these data suggest that there may be functional intracellular perturbations in monocytes of patients with AIDS. Alternatively, since our in vivo studies reveal profound defects and the presence of AIDS serum depressed ADCC of normal monocytes, this suggests that a serum factor may be suppressing immune-specific receptor function. A final possibility is that the characteristics of the circulating monocyte may be quite different from those of the tissue macrophages. This may be particularly true in HIV infections because impaired monocyte maturation has been demonstrated [58].

Defects in receptor-specific phagocytosis have also
been described by other investigators. For example, Pinching et al. [51] reported that AIDS patients have impaired Fc-dependent killing of Candida Guilliermondii. In contrast, Roux-Lombard et al. found that phagocytosis of opsonized sheep red blood cells was normal in AIDS patients [59]. Further, there was normal killing of C. albicans, C. neoformans, Aspergillus fumigatus, Thermoascus crustaceus, and Giardia lamblia by AIDS patients' monocytes [54, 60, 61]. Both culture-derived macrophages and bronchoalveolar macrophages from AIDS patients are able to kill G. lamblia [60, 62], but culture-derived macrophages had a decreased ability to kill Toxoplasma gondii [63].

Estevez and colleagues [64] showed that there is normal engulfment of Candida pseudotropicalis but that intracellular killing (lytic activity) was deficient. Since intracellular killing of fungi occurs by oxidative mechanisms, this finding is in conflict with Nielsen et al. [54], who reported that monocyte superoxide generation in patients with AIDS is normal. It is consistent, however, with Roux-Lombard et al. [59], who found decreased superoxide generation.

Because increased tumor burdens are characteristic of patients with AIDS [65, 66], the level of monocyte tumoricidal activity is of interest. Kleinerman et al. [67] found that activated monocytes killing of melanoma target cells was normal. This implies that in vivo immunomodulators could be successful in increasing the antitumor activity of AIDS patients' monocytes [67].

SECRETORY PRODUCTS. Of all the monocyte's secretory products, perhaps the most important is IL-1, which activates lymphocytes and induces interleukin 2 (IL-2) production [68, 69]. Somewhat conflicting results regarding IL-1 secretion in patients with AIDS have been reported. Enk et al. [43] and Lepe-Zuniga et al. [70] have reported that IL-1 production was increased in unstimulated monocytes from AIDS patients compared with that in healthy controls, and production increased further with LPS stimulation. A subset of patients who produced high amounts of IL-1 was identified and found to have higher amounts of serum immunoglobulins, especially antibodies to Epstein-Barr virus, which suggests a possible cofactor role [70]. Fauci [71], however, also found IL-1 production was increased in unstimulated monocytes, but there was no further increase with the usual inducers of IL-1 [72]. Roux-Lombard found no difference between IL-1 production in unstimulated monocytes of AIDS patients and those of controls, but with an increased concentration of concanavalin, he found increased IL-1 production in monocytes of AIDS patients [59]. Alcocer-Varela et al. [73], Ernst et al. [74], and Murray et al. [75] studied IL-1 production in stimulated monocytes and found it to be equal to that in monocytes of control subjects. Moreover, systemic administration of IL-2 to AIDS patients led to a further increase in IL-1 production [74]. In further contrast, Berman et al. [76] found decreased IL-1 production in monocytes of AIDS patients and correlated this with the presence of serum inhibitors of IL-1 production. Finally, Eden and Turino found that bronchoalveolar macrophages from patients with AIDS have an increased capacity to secrete IL-1 [77].

MONOCYTE-T CELL INTERACTIONS. Most T lymphocyte activities require an accessory cell (usually a monocyte or macrophage) to "process" or "present" the antigen or mitogen to the T cell. This process is dependent on the class II antigens of the major histocompatibility gene complex—IIa antigen in the mouse or DR antigen in humans [78]. The numbers of these cells bearing these markers are therefore of interest in patients with AIDS. Heagy et al. [79] used flow cytometry and dual color fluorescence to quantitate the number of HLA-DR⁺ peripheral blood monocytes from patients with AIDS. Only one of eight healthy individuals had <50% HLA-DR⁺ monocytes (72.9% ± 15.1%, mean ± SD). However, four of six patients with AIDS had <50% HLA-DR⁺ monocytes (46.2% ± 22.0%). They also found that incubation of monocytes from AIDS patients in vitro with γ-interferon resulted in an increase in HLA-DR expression to near-normal levels. Significant reductions in class II antigens have also been reported by Roy et al. [80] and Tsang et al. [81].

Similar results were reported by Belsito et al. [82] for Ia⁺ Langerhans' skin cells, which function as the antigen-presenting cells of the skin. Skin biopsies were obtained from the inner aspect of the arm. Control subjects (both healthy normal controls and patients with other infections or malignancies) had 721 ± 13 Ia⁺ cells/mm², while patients with AIDS had 278 ± 33 cells/mm². The fact that two different cell populations (Langerhans' skin cells and blood monocytes) have this defect implies that this is not an artifact and is a significant abnormality in patients with AIDS.

The important question raised by these studies is whether there is also a functional monocyte antigen-presenting defect. Prince et al. [83] assessed the
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anti-Leu4 induced proliferative response of T cells. By mixing experiments they found that decreased T cell responsiveness was secondary to both a monocyte defect and a T cell defect. Similar results were noted by Kirkpatrick et al. [84] with Candida as antigen and by Shannon et al. [85] using the mitogens phytohemagglutinin and pokeweed. Somewhat contradictory findings were reported by Hofmann et al. [86], who found that heavily irradiated peripheral blood mononuclear cells from patients with AIDS were capable of restoring transformation responses to phytohemagglutinin, pokeweed mitogen, or purified protein derivative. Murray et al. [75] also found an intact capacity of AIDS monocytes to act as accessory cells in concanavalin A and phytohemagglutinin-induced T cell proliferation. Thus, it appears that AIDS patients have a monocyte-presenting defect for at least some mitogens and antigens.

One possibility that might explain these differences between patients with HIV infections and healthy subjects is a variance in monocyte activation. The monoclonal antibody Mo3e appears to be a useful marker for monocyte activation; it is minimally expressed on resting cells, and its up-modulation is associated with activation of cellular protein kinase C [38, 87–89]. We found no significant differences, however, in the binding of this monoclonal antibody (nor any of the other antibodies) between healthy control subjects and patients with HIV infections [33].

Another possibility might be that the HIV-infected monocytes could produce a suppressive substance. Lunardi-Iskander et al. reported that adherent cells from patients with AIDS inhibit T cell–colony growth in response to phytohemagglutinin. The precise mechanism was not determined, but the substance(s) decreased production of IL-2 and its receptor [90].

Hypotheses and Future Work

Perhaps the most important question yet to be answered concerns the mechanism of the macrophage dysfunction. The most obvious possibility is that dysfunction is secondary to monocyte/macrophage infection with HIV. A related possibility is that HIV infection of another cell type, e.g., CD4+ lymphocytes, could lead to loss of lymphokines necessary for normal macrophage function or to generation of a monocyte/macrophage serum inhibitory product. Experimental evidence supports both possibilities. First, Murray et al. reported that there is impaired production of γ-interferon in patients with AIDS, correlated with the inability of monocytes from AIDS patients to phagocytose G. lamblia, T. gondii, and Leishmania donovani [60, 91]. This suggests that administration of γ-interferon might correct some of the AIDS abnormalities, but we could not document any action on Fc-receptor function (figure 3), nor could Pennington et al. find a change in monocyte superoxide anion production [92]. Second, a secretory product from murine leukemia viruses inhibits macrophage migration. Further, this substance is antigenically related to an immunosuppressive retroviral protein, P15E [93–96].

Another important question is why some abnormalities are detected in patients with AIDS but not in many patients with AIDS-related illnesses. There are several potential reasons for this. (1) The defect in monocyte function takes a considerable time to develop, and infection has not been present long enough in patients with AIDS-related illnesses. (2) Monocyte defects after HIV infection occur only in a susceptible subpopulation. This could be genetic or due to another as yet unidentified cofactor. (3) The monocyte defects are secondary to the opportunistic pathogens or tumors found in AIDS, either alone or in combination with HIV infection. Thus, they would be considered an epiphenomenon.

Several experimental approaches to the examination of these possibilities are possible. First, monocytes from controls could be isolated and certain functions tested before and after in vitro infection with HIV. Second, a cohort of patients with AIDS-related illnesses could be monitored prospectively with routine monocyte function tests performed to determine whether defects develop over time, especially when patients develop AIDS. Of special interest would be whether any such defects are predictive of which patients eventually develop full-blown AIDS. Another strategy might be to evaluate monocyte function in AIDS patients before and after treatment with an antiretroviral drug such as azidothymidine [97]. If improvements are seen, the data would imply that defective monocyte function is secondary to lymphocyte-monocyte interactions since the dideoxynucleosides fail to inhibit HIV replication in cultured human macrophages but not in cultured human lymphoblastoid cells [98].

In summary, monocytes and macrophages of patients with AIDS demonstrate a wide variety of in vitro and in vivo abnormalities. Since many of the microorganisms causing infections in AIDS are intracellular pathogens that are normally held in check...
by cells of the RES, it is likely that defective mono­
cyte and macrophage function contributes to the multiple occurrence of aggressive infections in AIDS. Further studies of monocyte and macrophage func­
tion in AIDS will contribute to our understanding of the mechanisms of their poor immune response and to possible strategies to restore these patients to their full immune potential.

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