Lymphocyte-Reactive Autoantibodies in Human Immunodeficiency Virus Type 1—Infected Persons Facilitate the Deletion of CD8 T Cells by Macrophages

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The number of peripheral blood CD8 T cells declines in advanced stages of human immunodeficiency virus (HIV) infection coinciding with the transition from a clinically asymptomatic state of infection to AIDS. Although blood monocytes/macrophages exhibit cytotoxicity for CD4 T cells soon after HIV infection, cytotoxicity against CD8 T cells occurs at the time when HIV infection advances. The cytotoxic reaction is mediated by immunoglobulins that bind to T cells and which can be eluted from them. The immunoglobulins enable macrophages from noninfected persons to destroy healthy T cells in tissue culture. Lymphocyte-reactive autoantibodies (LRAs) occur physiologically as a result of chronic allo- or self-antigen stimulation. Lymphopenic, autoimmune lupus erythematosus patients exhibit LRAs that facilitate the deletion of T cells by macrophages. It is proposed that LRAs represent an immunoregulatory cytotoxic mechanism that is activated after chronic immune stimulation and is engaged by HIV to deplete host lymphocytes.

Human immunodeficiency virus (HIV) induces in its host vigorous humoral and cellular immune reactions [1]. Although neutralizing antibodies may be beneficial in curbing the spread of HIV, there are indications that humoral immune reactions may aid HIV in destroying the host immune system. HIV-specific antibodies form immune complexes with the virus that facilitate the infection of macrophages via receptors for the immunoglobulin Fc portion [2, 3]. By expressing CD4-reactive HIV envelope molecules, such immune complexes facilitate the cellular conjugate formation between cytotoxic macrophages and CD4 T cells, which results in the destruction of CD4 T cells in vitro [4–6] and massive deletion of CD4 T cells in vivo [7].

If (as is widely believed) the loss of CD4 T cells is a major cause of HIV-induced immunodeficiency, this noninfective deletion of CD4 T cells may represent a significant pathogenic mechanism. However, experimental and therapeutic CD4 T cell deletion in mice and humans demonstrates little support for the thesis that CD4 T cell deletion causes immunodeficiency, because adequate immune functions can be maintained at extremely low CD4 T cell levels [8]. What distinguishes the HIV-infected person from experimentally CD4 T cell–depleted individuals is the fact that after HIV infection, nondeleted T cells fail to mount adequate immune reactions [5, 9, 10]. This deficiency applies to both CD4 T and CD8 T cells, and has been attributed to the inability of accessory cells to provide costimulatory assistance [5, 9].

Macrophages from HIV-infected subjects are incapable of up-regulating costimulatory surface molecules of the B7 family [5]. The up-regulation of B7 molecules on accessory cells [11], or the supply of such molecules in a bystander fashion [12], is a prerequisite for the T cell immune response. As macrophages lose their costimulatory capacity in the course of HIV disease, they gain the ability to destroy targeted T cells [5, 6]. Two distinct macrophage subsets have been identified [13, 14]. One subset, which we refer to as M1, can costimulate the T cell response. The second subset, which we refer to as M2, destroys targeted lymphocytes. M1 and M2 cells differ phenotypically and require antagonistic differentiation signals for their development [13, 14].

CD8 T cells have a major role in the body’s defense against noncytopathic viral infection [15]. HIV induces a strong cytotoxic T cell response in its human host [16]. Immediately after the initial viremia, viable T cell–tropic HIV variants are undetectable in blood [17, 18]. The immune system fails, however, to eradicate HIV variants in macrophages. Macrophagetropic HIV variants continue to circulate in the blood and facilitate the later reemergence of T cell–tropic variants [17, 18]. Nontolytic CD8 T cells curb the replication of T cell–tropic and macrophagetropic HIV variants for an extended period of time, during which the patient generally remains asymptomatic. However, the CD8 T cell number eventually declines, and clinical symptoms of AIDS develop [19–22]. Seropositive persons who maintain a high frequency of CD8 T cells keep HIV replication at low levels [19–22]. The quintessential nonpro-
gressor is the HIV-infected chimpanzee [24]. Chimpanzees possess a proportionally larger CD8 T cell compartment than humans. After infecting the chimpanzee, HIV replicates slowly in CD4 T cells unless CD8 T cells are deleted [25].

These observations establish a potentially important link between the development of AIDS in HIV-infected persons and a decline of CD8 T cells. No explanation has been advanced for how CD8 T cells are lost in HIV-1-infected persons.

The present study was designed to determine whether macrophages, which have previously been shown to destroy CD4 T cells in an antibody-dependent cellular cytotoxicity (ADCC) reaction [4–6], can destroy CD8 T cells in this fashion. It is known that HIV infection causes the generation of lymphocyte-reactive autoantibodies (LRAs) [26–32]. The presence of these antibodies correlates with the development of immune deficiency. In one study, most of the 46 subjects who exhibited LRAs but none of 15 seropositive subjects without these antibodies developed AIDS within 30 months of observation [26]. Monoclonal antibody (MAb) analysis identified these autoantibodies as low-affinity, polyspecific immunoglobulin [33].

Materials and Methods

Study subjects. Sixty-four persons in various stages of HIV infection and receiving different treatment regimens and 12 age-matched healthy control donors made repeated contributions to this study. CD4 T cell counts in patient blood was the only clinical disease parameter that was followed. Twenty-five sera from systemic lupus erythematosus (SLE) patients were randomly selected from a serum bank established at the New York Medical College Department of Medicine.

Cell preparation and macrophage cytotoxicity assay. Peripheral blood mononuclear cells (PBMC) were isolated from donor blood by ficoll-hypaque density sedimentation. Washed cells were resuspended in RPMI 1640 (Sigma, St. Louis) containing 5% fetal calf serum (FCS; Sigma) and counted in an ultraplane improved hemacytometer (Neubauer). Since the fraction of monocytes varies substantially in the blood of HIV-infected persons, the monocyte numbers were first determined by flow cytometry [5], and the number of PBMC containing 4 × 10^5 monocytes/0.1 mL RPMI 1640 (with 20% FCS) were plated in 96-well flat-bottom tissue culture plates (Falcon, North Haledon, NJ). The nonadherent (NA) cells were removed 60 min later after the cultures were agitated. After two gentle washes with culture medium, the supernatant was replaced with a suspension of 2.5 × 10^5 NA cells from a healthy donor in 0.1 mL RPMI (with 5% FCS).

NA cells were prepared by culturing 2 × 10^5 PBMC/0.1 mL RPMI 1640 (with 20% FCS) for 45 min and recovering those cells that did not stick to the culture vessel after gentle agitation. The procedure was repeated once to assure that the NA cell fraction contained <1% macrophages. The combination cultures were incubated for 2 days, counted, and washed twice. Live lymphocytes were gated in the flow cytometer and assayed for remaining CD4, CD8, or double-negative phenotypes [5]. The absolute number of surviving cells was calculated, and the percentage of cells killed by the macrophages was determined applying the following formula: Percent deleted cells = (cells cultured with control macrophages – cells cultured with patient macrophages)/cells cultured with control macrophages × 100.

To facilitate the release of cell surface–bound immune complexes, PBMC were incubated overnight at 5 × 10^6 cells/mL in buffered saline at 4°C. The cells were pelleted, and the filtered (0.22 μm; Millipore, Bedford, MA) supernatants were added to the culture medium at a final concentration of 10%.

Flow cytometry. PBMC were phenotyped before and after tissue culture. Lymphocytes and monocytes/macrophages were distinguished by side- and forward-scatter analysis in a flow cytometer (FACScan; Becton Dickinson Immunocytometry Systems, Mountain View, CA) [5]. Cells were stained as previously described [5] with fluorescein isothiocyanate (FITC)–labeled or biotinylated MAbs. The biotin label was revealed using streptavidin-phycocerythrin (Sigma). The following MAbs were used: anti-CD3, anti-CD4 and anti-CD8 OKT3 (OKT4 and OKT8, respectively; Ortho Diagnostic Systems, Raritan, NJ). Immunoglobulin-positive cells were identified through treatment with diluted (1:100) affinity-isolated FITC-conjugated goat anti-human immunoglobulin F(ab)2 antibody (Tago, Burlingame, CA).

Reagents. Recombinant HIV-1 gp120SF2 was provided by K. Steimer (Chiron Biocine, Emeryville, CA) [55, 56]. Serum from a seropositive donor was used as a source of anti-gp120 antibody. Equivalent results were obtained with a monoclonal gp120 V3-specific MAb (clone 447; provided by S. Koenig, Gaithersburg, MD) [6]. Protein G (Pharmacia LKB Biotechnology, via Sigma) was used to separate serum IgG according to the manufacturer’s instructions.

Results

Macrophages from HIV-infected donors destroy in coculture CD4 T cells and CD8 T cells from healthy control donors. In tissue culture, macrophages from HIV-infected persons destroy CD4 T cells from healthy persons. The surface of the phagocytes adsors CD4-reactive immune-complexed HIV envelope antigens, which enable them to form cellular conjugates with CD4 T cells and to destroy them [5, 6]. In cell cultures that first demonstrated this phenomenon [5], the proportional representation of CD8 T cells was consistently found to increase, which was taken as an indication that CD4 T cells rather than CD8 T cells were destroyed by macrophages from seropositive donors. In extending these studies, we determined the absolute numbers of CD4 and CD8 T cells from healthy donors that die in coculture with macrophages from HIV-infected persons. We found that in advancing stages of HIV infection, CD8 T cells, in addition to CD4 T cells, become targets of destruction by macrophages. Figure 1A shows the macrophage destruction of CD8 T cells from a healthy donor as a function of the seropositive macrophage donor’s number of CD4 T cells in the blood. The simultaneous destruction of CD4 T cells is shown for comparison (figure 1B).

Care was taken in these experiments to plate macrophages and lymphocytes in a consistent ratio. We added one macrophage to every 5 lymphocytes. This reflects approximately...
medium of gp120/anti-gp120 immune complexes [6]. If CD8 T cells were to be killed in a bystander fashion, in the presence of exogenous CD4-reactive gp120/immunoglobulin complexes, their destruction should be enhanced. Experiments showed, however, that CD4-reactive immune-complexed HIV envelope molecules enhance the macrophage-dependent destruction of CD4 T cells but not the destruction of CD8 T cells. If anything, they inhibited the destruction of CD8 T cells (figure 3A).

This finding could be interpreted to mean that while bystander kill of CD8 T cells may occur, the exogenous gp120/immunoglobulin complexes may have redirected cytotoxicity to CD4 T cells. To further explore the possibility of bystander kill, we took advantage of results from previous experiments that demonstrated a rapid turnover of CD4-reactive immune complexes on the macrophage surface [6]. Within 12 h after immunoglobulin is removed from the environment, immune complexes virtually disappear from the macrophage surface, and the macrophages no longer target CD4 T cells for destruction. Figure 3B confirms these findings and also demonstrates that macrophages from AIDS patients lose the ability to destroy CD8 T cells within 12 h of preincubation in tissue culture. The administration of exogenous CD4-reactive immune-complexed HIV envelope antigens restores the targeting of CD4 but not CD8 T cells for destruction. Thus, there is evidence that in earlier stages of HIV infection, host macrophages destroy CD4 T cells but not CD8 T cells and that in tissue culture, exogenous

**Figure 1.** Macrophages (MΦ) from HIV-infected donors spontaneously destroy CD4 and CD8 T cells from healthy donors. Macrophages from seropositive and seronegative donors were cocultured with lymphocytes from seronegative persons, and deletion of CD8 (A) and CD4 (B) T cells was determined 2 days later. Results are plotted against macrophage donor CD4 T cell blood count. Individual results were grouped together as indicated (n); average ± SD is shown. Shaded area designates 95% confidence limits.

The ratio in which these cells exist in the blood of healthy donors. The ratio changes, however, in HIV-infected persons with a preferential lymphocyte loss and, consequently, an increase in the proportion of macrophages. Figure 2 depicts an experiment that shows that the cytotoxicity for T cells from AIDS patients increases when the proportion of macrophages is increased.

**CD8 T cells are not destroyed by macrophages in an innocent bystander fashion.** We considered the possibility that macrophages from HIV-infected donors destroy CD8 T cells in a bystander fashion. Previous experiments demonstrated that CD4 T cells are targeted by cytotoxic patient macrophages through CD4-reactive immune-complexed HIV envelope molecules [5, 6]. Once activated by such immune complexes, the macrophages could theoretically destroy innocent bystander CD8 T cells.

Macrophage-mediated deletion of CD4 T cells in tissue culture can be induced or enhanced by the inclusion in the culture of gp120/anti-gp120 immune complexes [6]. If CD8 T cells were to be killed in a bystander fashion, in the presence of exogenous CD4-reactive gp120/immunoglobulin complexes, their destruction should be enhanced. Experiments showed, however, that CD4-reactive immune-complexed HIV envelope molecules enhance the macrophage-dependent destruction of CD4 T cells but not the destruction of CD8 T cells. If anything, they inhibited the destruction of CD8 T cells (figure 3A).

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**Figure 2.** Enhanced macrophage cytotoxicity for T cells at elevated macrophage/lymphocyte ratios. Macrophages from seropositive and seronegative donors were cocultured with lymphocytes from seronegative persons. % deletion was calculated in comparison to lymphocytes cultured in absence of macrophages. Cultures contained macrophages from seropositive donors with CD4 T cell counts <200/µL (○) or seronegative donors (●). Macrophages from 4 HIV-infected donors and 3 noninfected donors were evaluated. Results are averages ± SD.
Figure 3. CD8 T cells are directly destroyed by macrophages from AIDS patients but not as innocent bystanders. **A**, Lymphocytes from healthy persons were cultured with macrophages from AIDS patients (n = 24) with blood CD4 T cell counts of <200 cells/μL, alone (open bars), or in presence of CD4-reactive immune-complexed HIV gp120 (hatched bars). Deletion of CD4 and CD8 T cells was measured 2 days later. Error bars designate SD. HIV gp120 diluted 1 μg/mL with diluted serum from asymptomatic seropositive donor; anti-gp120 (αgp120) diluted 1:1000. **B**, Experiment is identical to (A) except that macrophages were cultured alone for 12 h before lymphocytes and gp120/immunoglobulin complexes were added.

CD4-reactive gp120/immunoglobulin complexes restore this destruction of CD4 T cells but not CD8 T cells. Combining this evidence, it seems reasonable to conclude that macrophages employ ligands for the deletion of CD8 T cells, which are distinct from the CD4-reactive immune-complexed HIV envelopes that facilitate the deletion of CD4 T cells.

**LRAs are produced by individuals with advanced HIV infection.** Autoreactive immunoglobulin have been detected in HIV-infected subjects [26–32]. Although specific antigenic targets have been recognized in individual cases, no specific auto-antigen has been reliably associated with AIDS pathogenesis. The occurrence of T cell–reactive antibodies, however, is a common feature of progressive HIV infection. We have examined the T cells from 62 HIV-infected donors for the presence of LRAs, which are readily detected on the surface of T cells that normally express no immunoglobulin. The cells were treated with FITC-labeled goat anti-human immunoglobulin, and the fluorescence label was assayed by flow cytometry. Figure 4 demonstrates that CD4 and CD8 T cells of HIV-infected subjects express immunoglobulin in a broad range of concentrations that are above the values of T cells from healthy donors. A few individuals express more immunoglobulin on CD4 T cells than on CD8 T cells (figure 4C). The presence of CD4-specific autoantibodies has been noted previously in ~10% of seropositive persons [36].

The possibility that macrophage cytotoxicity for T cells was mediated by LRAs was investigated by testing the capacity of serum LRAs and of antibodies eluted from lymphoid cells of AIDS patients to facilitate the lymphocyte destruction in cultures of PBMC from seronegative donors. Eluates were prepared by incubating PBMC from a patient with high autoantibody expression overnight in cold balanced salt solution. Immunoglobulin released into the supernatant or present in patient serum reacted with healthy lymphocytes (data not shown). In the presence of macrophages, serum from AIDS patients and eluates from lymphoid cells of AIDS patients facilitated the deletion of CD4 and CD8 T cells (figure 5).
Immunoglobulin (Ig) expression by CD4 and CD8 T cells from HIV-infected subjects. Peripheral blood mononuclear cells from seropositive and seronegative donors were incubated with fluorescence-labeled goat anti-human immunoglobulin F(ab)2. The intensity of the reaction by CD4 (A) and CD8 (B) T cells was monitored by flow cytometry and is expressed as function of donor blood CD4 T cell count. Anti-immunoglobulin reactivity of CD8 T cells is plotted in (C) against anti-immunoglobulin reactivity of CD4 T cells to demonstrate that both T cell subsets are subject of immunoglobulin reactivity in HIV-infected subjects. ●, HIV-infected donors; ○, seronegative donors; FI, fluorescence intensity.

To confirm that immunoglobulin and not another factor related to HIV infection is responsible for the observed T cell destruction, we isolated the immunoglobulin fraction from 1 serum sample with high LRA activity and found that this fraction but not the immunoglobulin-negative fraction mediated the deletion of T cells by macrophages (figure 6). Furthermore, LRA are produced independently of HIV infection under conditions of chronic stimulation of the immune system. For example, LRA are produced in individuals with SLE, in which lymphopenia is a characteristic diagnostic factor [37]. Twenty-five sera from SLE patients were randomly selected from a serum bank. Most of the donors produced LRAs at the time of serum collection, as determined in the immunoglobulin-binding assay (figure 7). The serum expression of LRA correlated positively with the capacity of the serum to facilitate macrophage-mediated deletion of T cells (figure 7).

Discussion

This study explored further aspects of the role of accessory cells in the development of HIV-related immunodeficiency. We demonstrate that the HIV-1-infected person may arm monocytes/macrophages with LRAs and enable them to destroy targeted lymphocytes in an ADCC fashion. NK cells, granulocytes, and macrophages are the major ADCC effector cells [38], and they all may delete T cells via LRAs. However, NK cells [39] and granulocytes [40] progressively lose cytotoxic activity after HIV infection, whereas macrophage cytotoxic activity increases [5, 6]. The heightened macrophage cytotoxicity reflects a fundamental change of macrophage function in HIV-infected persons. It occurs in conjunction with declining antigen presentation and costimulatory macrophage function and may contribute decisively to lymphopenia and immune dysfunction.
Figure 6. Macrophage-mediated T cell deletion is mediated by immunoglobulin fraction of serum from AIDS patient. Peripheral blood mononuclear cells from healthy donor were incubated with serum or with fractions thereof in indicated concentrations. Two days later, cells were washed, counted, and phenotyped for T cells expressing CD4 (A) or CD8 (B) coreceptor. Immunoglobulin fraction was prepared by protein G column chromatography. Retained fraction was eluted with buffer volume equivalent to loading serum and extensively dialyzed. Bilirubin was assayed to indicate dilution factor of run through fraction compared with starting serum. Error bars indicate SD of 3 determinations.

Dysfunction. Therefore, the imbalanced macrophage function in HIV-infected subjects warrants consideration.

Immune complex–dependent and antibody-dependent macrophage cytotoxicity can be inhibited by anti-CD16 MAb, implicating CD16 (the low-affinity FcyIIIR [38]) as a mediator of the cytotoxic mechanism [6]. FcyIIIR facilitates immune complex–mediated HIV infection of macrophages [2, 3]. This suggests that CD16 may not only predispose macrophages for the immune complex–mediated and autoantibody-mediated deletion of T cells but may also increase macrophage susceptibility for immune complex–mediated HIV infection. Macrophage CD16 expression and cytotoxic activity can be enhanced by treatment with interleukin (IL)-10 [13, 14], a cytokine that blocks the expression of major histocompatibility complex (MHC) II molecules [41] and B7 molecules [11, 13]. IL-10 abrogates macrophage antigen-presenting and costimulatory activity [11, 12].

Two macrophage subpopulations have been distinguished on the basis of surface antigen phenotypes and their mechanism of generation [13]. We refer to them as M1 and M2 cells. M1 cells express MHC II, up-regulate B7, and are generated in the presence of interferon (IFN)-γ or cAMP. IL-10 blocks their development. M2 cells fail to up-regulate B7 expression, but they express CD16. The development of M2 cells is facilitated by IL-10 and inhibited by IFN-γ or cAMP [13, 14]. It follows that IFN-γ tilts the M1-M2 balance toward costimulatory M1 cells, while IL-10 favors the development of cytotoxic M2 cells. An IL-10 predominance has been described in HIV-infected subjects [14, 42]. An enlarged Th2 cell population has been cited as a potential source of elevated IL-10 levels [42]; however, this proposal has been challenged [43].

Another, less recognized, IL-10 source is the macrophage. Macrophages release IL-10 after HIV infection [44, 45] and after reaction with immune complexes [46]. Monocyte-derived dendritic cells, which are prototypic immunostimulatory accessory cells and potent IL-12 producers, lose their immunostimulatory qualities and promote the production of IL-10 when they mature in the presence of PGE2 [47]. Macrophage PGE2 production is greatly enhanced in HIV-seropositive persons [48]. It is possible that macrophages initiate alterations in the type 1–type 2 cytokine balance rather than becoming mere objects of it after infection with HIV, when they react with
Among the shared features of HIV disease and SLE are B cell hyperreactivity [37, 51, 52], lymphopenia [1, 37], LRAs [1, 37], and immune dysfunction [1, 37]. Central to the immune dysfunction in both diseases is a deficiency at the level of antigen presentation. Comparing PBMC immune functions for HIV-infected donors and SLE patients, Shearer and colleagues [53, 54] observed in both diseases a deficiency in the ability of macrophages to present recall antigen or alloantigen to T cells. Similar to AIDS patients’ monocytes [44, 45], SLE patients’ monocytes deviate from the norm in producing IL-10 [53–55], which may contribute to depressed cellular immunity in both diseases.

In conclusion, the data presented herein help build a case for a special role of macrophages and related cells in promoting HIV disease. By infecting macrophages, HIV may tilt the cytokine balance toward IL-10 predominance and inhibit antigen presentation and costimulation; inhibit the macrophage capacity to destroy intracellular parasites, which cause opportunistic diseases; and enhance the ability of macrophages to destroy targeted lymphocytes. CD4-reactive immune-complexed HIV envelopes initially facilitate the exclusive deletion of CD4 T cells. After the development of LRAs, LRA-armed macrophages additionally delete CD8 T cells. HIV infection of macrophages may, therefore, be of special pathogenic significance. The chimpanzee, which does not replicate macrophagetropic HIV variants [24], and humans who lack receptors for them [58], do not develop AIDS. It is conceivable that HIV was not a deadly human virus before it developed macrophagetropic variants.

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References

3. Homsy J, Meyer M, Tateno M, Clarkson S, Levy JA. The Fc and not CD4 function with other disorders resulting from chronic immune stimulation, such as occurs after chronic alloantigen stimulation in graft-versus-host disease [49] and after chronic self-antigen stimulation in SLE [37]. The excessive depletion of CD4 T cells, which is characteristic of HIV infection, may be partially attributed to a direct cytopathic effect. However, since most of the CD4 T cells that undergo apoptosis in seropositive subjects are not HIV-infected [50], it is likely that an indirect deletion mechanism, such as that which occurs with ADCC killing of CD4 T cells coated with CD4-reactive immune-complexed HIV envelope molecules [5, 6], accounts for a large portion of CD4 T cell deletion.

Figure 7. Sera from patients with systemic lupus erythematosus contain lymphocyte-reactive autoantibodies and facilitate destruction of T cells by macrophages. Reactivity of CD4 (●) and CD8 (○) T cells with LRA in serum of SLE patients was measured and is plotted against macrophage deletion of CD4 and CD8 T cells as facilitated by these sera.


