Dysregulated Production of Interleukin-10 (IL-10) and IL-12 by Peripheral Blood Lymphocytes from Human Immunodeficiency Virus-Infected Individuals Is Associated with Altered Proliferative Responses to Recall Antigens

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The loss of immune function following infection with human immunodeficiency virus (HIV) may result from altered production of immunoregulatory cytokines such as interleukin-10 (IL-10) and IL-12. In this study, we analyzed IL-10 and IL-12 production by mitogen-stimulated peripheral blood mononuclear cells (PBMC) from HIV+ individuals and correlated their levels with proliferative responses to the recall antigens HIV p25 and influenza virus. We report two distinct groups of HIV+ patients. One group produced small amounts of IL-10, had PBMC that proliferated in response to recall antigens, and demonstrated enhanced recall antigen-induced proliferation upon addition of anti-IL-10 antibodies and/or IL-12. Conversely, the second group produced high levels of IL-10, had PBMC that failed to proliferate to recall antigens, and did not demonstrate enhanced proliferation upon addition of anti-IL-10 antibodies and/or IL-12. Mitogen-stimulated PBMC from both groups produced significantly lower levels of IL-12 than did those from HIV− controls. Analysis of the source of the IL-10-producing cell subset in PBMC demonstrated that in HIV+ individuals, IL-10 is produced by monocytes, while in HIV− controls, it is produced by both T cells and monocytes. Taken together, our results suggest that monocytes from HIV+ individuals secrete decreased amounts of IL-12, a Th1-type cytokine, which may lead to the development of Th2-type responses characterized by high IL-10 secretion and immune dysfunction.

Infection of CD4+ T cells and monocyte/macrophages with human immunodeficiency virus (HIV) results in a progressive loss of effective immune responses (3, 44). HIV employs a variety of mechanisms to undermine the effectiveness of the host immune system, including dysregulation of cytokines which influence its replication. HIV enhances the transcription of cytokines that act to stimulate virus replication, namely, interleukin-4 (IL-4), IL-6, granulocyte-monocyte colony-stimulating factor (GM-CSF), and tumor necrosis factor alpha (TNF-α) (3, 27, 38, 39, 45). In addition, HIV causes decreased expression of IL-2, which may result in suppression of protective cell-mediated immune responses (CMIR) and further increases in virus load (3, 10, 26, 44).

The role of the immunoregulatory cytokines IL-10 and IL-12 in HIV-induced immunodeficiency is not well understood. IL-10 is a pleiotropic molecule that inhibits CMIR by suppressing T helper cell type 1 (Th1) responses (5, 19, 43a, 49). It has been associated with the immunopathogenesis of a number of diseases, including septic shock, lymphoproliferative disorders, and autoimmune diseases (32, 33, 40, 50). Natural killer (NK) cell stimulatory factor, or IL-12, is a heterodimeric disulfide-linked polypeptide composed of a heavy chain (p40) and a light chain (p35) (8, 53). The heavy chain is inducible following stimulation, but IL-12 biological activity is limited to the dimeric polypeptide (8, 53). IL-12 enhances the proliferation and cytotoxic activity of T cells and NK cells and stimulates gamma interferon (IFN-γ) production (8, 28, 29, 53). IL-12 has been implicated in protective immune responses to a number of parasitic infections and in antitumor activities (2, 9, 36).

IL-12 induces differentiation of naïve T helper cells into cells with the Th1 phenotype, whereas IL-4 and IL-10 generally induce development of Th2-type cells (30, 37, 43, 51, 53). Selective induction of Th1 or Th2 responses by pathogens leads to the development of cell-mediated and humoral immune responses, respectively (30, 37, 43, 51, 53). In HIV infection, it has been suggested that sequential loss of immune responses to recall antigens, alloantigens, and mitogens is associated with diminished Th1-type (IL-2 and IFN-γ) and enhanced Th2-type (IL-4) cytokine production (10, 11, 13, 26). These observations led to the suggestion that a Th1-Th2 switch may be a feature of progression from HIV infection to AIDS (13–15). Since IL-10 and IL-12 mediate humoral immune responses and CMIR, respectively (17, 30, 37, 43, 51, 53), HIV infection may disrupt the balance between IL-10 and IL-12 production, resulting in loss of immune competence. We have previously shown that unstimulated peripheral blood mononuclear cells (PBMC) from HIV+ individuals exhibit IL-10 up-regulation that is inversely correlated with IFN-γ expression (20). In the present studies, we provide evidence that IL-10 production by phytohemagglutinin (PHA)-stimulated HIV+ PBMC is correlated with a lack of responsiveness to recall antigens and that diminished IL-12 secretion by monocytes in HIV+ individuals may contribute to the development of Th2-type responses and immune dysfunction. Furthermore, we demonstrate that IL-10 is produced predominantly by monocytes and not by T cells in the PBMC of HIV+ individuals.
Isolation and culture of PBMC. Blood was obtained for mononuclear cell isolation from healthy adult volunteers and HIV-1 individuals with CD4+ T-cell counts ranging from 88 to 517 cells/mm³. Blood samples were collected following approval of the protocol by the Ethics Review Committee of the Ottawa General Hospital, University of Ottawa, Ottawa, Canada. Clinical information for each of the HIV-1 patients, including CD4+ and CD8+ T-cell counts, was obtained. All patients were Epstein-Barr virus seropositive but had no clinical manifestations of infectious mononucleosis or Kaposi's lymphomas. None of the patients had clinical evidence of bacterial or fungal infection at the time of specimen collection. Cell proliferation experiments were performed with PBMC from 39 HIV-1 patients. CD4+ counts were available for 34 of these patients. We used a newly developed assay for Disease Controlling Immunology to stage to identify the level of AIDS disease in this population (6). One patient was in stage I, 21 were in stage II, and 10 were in stage III. The mean CD4+ T-cell count was 272 per mm³ (median, 258; range, 88 to 517). All HIV-1 individuals had recently been vaccinated against influenza virus, whereas all HIV-1 controls were unvaccinated. Standard methods were used for cell preparation and fractionation (16). In brief, PBMC were isolated by density gradient centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). The cell layer, consisting mainly of mononuclear cells, was collected and washed three times in phosphate-buffered saline (PBS). PBMC were resuspended in complete Iscove's modified Dulbecco's medium (IMDM; Sigma Chemical Company, St. Louis, Mo.) at a concentration of 2 × 10⁶ cells/ml.

Culture medium and reagents. All cells were cultured in IMDM (Sigma) supplemented with 10% fetal calf serum (GIBCO Bioculture Laboratories, Grand Island, N.Y.), 100 U of penicillin per ml, 100 μg of streptomycin per ml, 10 mM HEPEs (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid), and 2 mM glutamine. HIV antigens p25 and p120 were kindly provided by the National Immunology Laboratory, IL. AIDS Research and Reference Reagents Program, Bethesda, Md., and Chiron Corporation, Emeryville, Calif. Influenza virus antigen (heat-inactivated strain A/Hong Kong/1/68) was generously provided by Earl Brown, University of Ottawa, Ottawa, Ontario, Canada. Anti-CD3 antibodies were reagents supplied from the OKT-3 hybridoma (American Type Culture Collection [ATCC], Rockville, Md.). IL-12 was generously provided by Maurice Gaty, Hofmann La Roche, Nutley, N.J. IL-10 and neutralizing anti-IL-10 antibodies were obtained from R&D Systems, Minneapolis, Minn.

Culture supernatants. To determine the ability of PBMC from HIV-1 individuals to produce IL-10, the cells were cultured at a concentration of 2 × 10⁶ cells/ml in 24-well tissue culture plates (Falcon Labware, Oxnard, Calif.). PBMC were stimulated with PMA-GIBCO (at a final dilution of 1:50), anti-CD3 antibodies (ATCC) at a final dilution of 1:200, and pokeweed mitogen (PWM; GIBCO) at a final dilution of 1:100. The supernatants were harvested after 24, 48, and 72 h and frozen at −70°C. Supernatants were thawed once at the time of analysis of cytokine production by enzyme-linked immunosorbent assay (ELISA). One thawing of the supernatants did not influence the measured cytokine concentration.

Measurement of IL-10. IL-10 was measured by ELISA with a two-site monoclonal antibody (MAb) assay that recognizes distinct epitopes, as described previously (1). Briefly, the plates (Nunc Immunomodules) were coated overnight at 4°C with purified anti-IL-10 MAB JES5-9D7 (rat immunoglobulin G1 [IgG1], obtained from PharMingen, San Diego, Calif.) at a concentration of 3 μg/ml in the coating buffer (0.1 M NaHCO₃ [pH 8.2]). The plates were washed with PBS-10% FCS and blocked with PBS-0.5% bovine serum albumin for 1 h. PBMC were incubated in 200 μl of IMDM with 10% FCS at 10⁶ cells/ml for 48 h. The plates were washed with PBS-0.1% Tween 20 and blocked with PBS–10% FCS. The wells were washed three times in phosphate-buffered saline (PBS). PBMC were stimulated with PHA-M (GIBCO) at a final dilution of 1:50, anti-CD3 antibodies (ATCC) at a final dilution of 1:200, and pokeweed mitogen (PWM; GIBCO) at a final dilution of 1:100. The supernatants were harvested after 24, 48, 72 h and frozen at −70°C. Supernatants were thawed once at the time of analysis of cytokine production by enzyme-linked immunosorbent assay (ELISA). One thawing of the supernatants did not influence the measured cytokine concentration.

Measurement of IL-12. IL-12 was measured by sandwich ELISA with two different monoclonal antibodies (MAb) which recognize distinct epitopes, as described previously (1). Briefly, the plates (Nunc Immunomodules) were coated with 2.5 μg of 2A1 antibody (rat anti-human IL-12 p70; Hoffmann-La Roche) in a coating buffer of sodium carbonate, 0.035 M sodium bicarbonate [pH 9.6] overnight at 4°C. The wells were blocked with 0.5% bovine serum albumin in PBS. The wells were washed at room temperature for 3 h with culture supernatants to be analyzed for IL-12. IL-12 was secreted by PBMC stimulated with PMA-GIBCO (10 ng/ml) in a co-culture of 10⁶ human CD4+ T cells and 5 × 10⁵ cells (in the case of PHA and anti-CD3 antibodies) or 7 days (for influenza virus and p25 antigen) in a final volume of 200 μl in triplicate in 96-well plates (Becton Dickinson Labware, Lincoln Park, N.J.). The cells were stimulated in the presence or absence of neutralizing anti-IL-10 antibodies (R&D Systems; 5 μg/ml) and/or IL-12 (Hoffmann-La Roche; 170 U/ml). Cells were pulsed with 0.5 μC of [³H]thymidine (Amersham, Arlington Heights, Ill.) and cultured for a further 16 h, followed by cell harvesting and measurement of [³H]thymidine incorporation.

The SI was calculated as the ratio of [³H]thymidine incorporation (counts per minute [cpm]) by PBMC stimulated in the presence of antigen or mitogen to that of PBMC cultured in the absence of antigen or mitogen.

Depletion of T and B cells from PBMC. T and B cells were depleted from the PBMC with anti-CD2 and anti-CD19 antibody-coated immunomoles, respectively (Dynal, Lake Success, N.Y.), essentially as described by the manufacturer. PBMC were incubated with immunomoles at a ratio of 10:1 at 4°C with gentle rotation for 40 min. The cells attached to the immunomoles were washed once and separated with a magnetic field. The remaining unbound cells were washed and analyzed for contaminating T or B cells by flow cytometric analysis with fluorescent isothiocyanate (FITC)-conjugated anti-CD3 antibody (Becton Dickinson) and phycoerythrin-conjugated anti-CD20 antibody (Becton Dickinson), respectively. Anti-CD2- and anti-CD19-depleted PBMC contained less than 2% T cells and B cells, respectively. In addition, T-cell-depleted PBMC did not proliferate in response to either PHA or anti-CD3 antibodies. Similarly, B-cell-depleted PBMC did not proliferate in response to anti-IgM-conjugated Sepharose beads (Bio-Rad Laboratories, Richmond, Calif.), further confirming the complete removal of target cells by the immunomoles (data not shown).

Depletion of monocytes. PBMC were depleted of monocytes by using anti-CD14-coated immunomoles (Dynal) as described above for T- and B-cell depletion. CD14-negative PBMC contained less than 2% monocytes, as determined by flow cytometric analysis for contaminating monocytes with phycoerythrin-conjugated anti-CD14 antibody (Becton Dickinson). Alternatively, monocytes and NK cells were removed by incubating with anti-IgG1-coated antibody (IgG1; Sigma) overnight at 4°C. Briefly, PBMC (3 × 10⁶ cells/ml) were incubated with 0.05 M IgG1 in serum-free medium at room temperature for 35 min. IgG1 was neutralized by the addition of FCS at a final concentration of 10%. The cells were washed three times and assayed for T-cell proliferation by thymidine incorporation in response to PHA and anti-CD3 antibodies. IgG1 treatment did not influence T-cell proliferation, as [³H]thymidine incorporation was found to be comparable to that of untreated PBMC (data not shown).

Statistical analysis. Means were compared by the two-tailed Student’s t test. The results were expressed as mean ± standard error of the mean (SEM).

RESULTS

IL-10 production following stimulation of PBMC with mitogens. To measure IL-10 production by PBMC following mitogenic stimulation, PBMC from 65 HIV+ individuals were cultured with either PHA or anti-CD3 antibodies, and supernatants collected after 48 h were harvested for IL-10 production. HIV+ individuals were classified as either low IL-10 producers or those that produced levels of IL-10 comparable to those of HIV− controls (IL-10 producers). Low IL-10 producers (n = 38) were those whose PBMC produced less than a threefold increase in levels of IL-10 following PHA stimulation compared with the level produced by unstimulated PBMC. The lower levels of IL-10 secreted by HIV+ low IL-10 producers were not due to altered kinetics, as IL-10 amounts were small when measured after 24, 48, and 72 h of culture (data not shown). PBMC of HIV+ IL-10 producers (n = 27) exhibited a greater than threefold increase in IL-10 production (Fig. 1). Similar results were obtained following stimulation of PBMC with anti-CD3 antibodies, although IL-10 production was low relative to that induced by PHA (Fig. 1). Following mitogenic stimulation, PBMC from HIV− controls (n = 25) secreted threefold-higher levels of IL-10 than unstimulated PBMC (Fig. 1).

Differential proliferation of HIV+ PBMC from low IL-10 producers and IL-10 producers to recall antigens. Since IL-10 inhibits IFN-γ and IL-2 synthesis by T cells and alters the expression of costimulatory molecules on antigen-presenting cells (17, 19–22) or alters IL-10 production by PBMC of HIV+ individuals may affect their proliferative responses to recall antigens. Therefore, we analyzed the proliferation of PBMC from 39 of the above-mentioned 65 HIV+ individuals to a panel of recall antigens, including HIV gp120,
HIVp25 (p25), and influenza virus (FLU). A twofold increase in thymidine incorporation (SI of >2) following addition of the recall antigen was considered a positive response. PBMC from 90% of the HIV1 individuals did not proliferate in response to gp120 (data not shown). Distinct patterns of proliferative responses to the recall antigens p25 and FLU by PBMC from HIV1 low IL-10 producers and IL-10 producers were observed. PBMC from low IL-10 producers (n = 17) proliferated in response to p25 and FLU antigens, whereas PBMC from IL-10 producers (n = 22) did not (Fig. 2A, 2B, and 3). The proliferative responses of five representative low IL-10 producers and five IL-10 producers are shown in Fig. 2A and B, respectively. Thymidine incorporation by PBMC from IL-10 producers did not exceed 1,000 cpm following antigenic stimulation (Fig. 2B). PBMC from HIV1 individuals (n = 10) did not proliferate in response to p25 and FLU antigens; the results for PBMC from one representative normal individual are shown in Fig. 2B. Differences in proliferative responses to FLU antigen between HIV− and HIV+ individuals may have been due to differences in the vaccination rate. To ensure comparability between HIV− and HIV+ individuals, PBMC from one HIV− individual were always included with those from HIV+ individuals in each proliferation assay. The correlation between IL-10 production status and proliferation of PBMC in response to p25 antigen is shown in Fig. 3. A similar correlation between IL-10 production status and proliferation of PBMC in response to FLU antigen was observed (data not shown). CD4+ T-cell counts were higher in low IL-10 producers than in IL-10 producers (305 ± 273 per mm3 [range, 88 to 517] versus 217 ± 101 per mm3 [range, 104 to 475]; P = 0.045). No correlation between proliferative responses and IL-10 production by PHA-stimulated PBMC was observed for HIV1 controls.

Anti-IL-10 antibodies enhance the proliferative responses of PBMC from low IL-10 producers to HIV p25 antigen. To determine whether the proliferative responses to recall antigens were affected by endogenously produced IL-10, the proliferation of HIV+ PBMC in response to p25 in the presence and absence of anti-IL-10 antibodies was measured. PBMC from low IL-10 producers demonstrated various degrees of proliferation enhancement in the presence of anti-IL-10 antibodies (Fig. 4), whereas PBMC from IL-10 producers remained unresponsive (data not shown). The proliferative responses of PBMC from five representative individuals from the low IL-10 producer subset of HIV+ patients are shown in Fig. 4. Enhanced proliferation was not due to a nonspecific Fc receptor-mediated stimulatory effect, as anti-IL-4 antibodies and isotype-matched control antibodies did not enhance proliferation (data not shown). Anti-IL-10 antibodies did not alter the proliferative response of PBMC from HIV+ IL-10 producers even when added at a high concentration (25 µg/ml) (data not shown). Again, thymidine incorporation by PBMC from HIV+ IL-10 producers did not exceed 1,000 cpm. Similar results were obtained with FLU as the recall antigen (data not shown). PBMC from HIV− individuals (n = 10) did not proliferate in response to either FLU or p25 in the presence or absence of anti-IL-10 antibodies. The proliferative responses of PBMC from one such individual are shown in Fig. 4.
Monocytes represent the major source of IL-10 production in HIV+ PBMC. HIV infection has been proposed to cause an expansion of Th2-like cells (13, 14), which could constitute a major source of IL-10. To analyze IL-10 production by various cell types, PBMC from six HIV+ individuals and six HIV- controls were depleted of either B cells, T cells, or monocytes and stimulated with PHA. Following T-cell depletion, IL-10 production was abolished in PBMC from HIV- controls, whereas in PBMC from HIV+ individuals, no significant change in IL-10 production was observed (Fig. 5). Monocyte depletion with l-LME abrogated IL-10 production in PBMC from both HIV- and HIV+ individuals (Fig. 5). B-cell depletion did not alter IL-10 production in PBMC from either HIV- or HIV+ individuals (Fig. 5). These results suggest that in HIV+ individuals, IL-10 is mainly produced by monocytes, whereas in HIV- controls, IL-10 is produced by both T cells and monocytes. PHA-stimulated, monocyte-depleted PBMC from HIV+ individuals require the presence of T cells to produce IL-10, whereas PHA-stimulated, monocyte-depleted PBMC from HIV+ individuals do not.

Depletion of T cells by anti-CD2 antibody-coated Sepharose immunobeads, depletion of B cells by anti-CD19 antibody-coated Sepharose immunobeads, and depletion of monocytes by l-LME were complete and specific, as described in Materials and Methods. However, the presence of trace amounts of IL-10 produced by contaminating CD2+ T cells and B cells in these experiments cannot be excluded.

Impaired production of IL-12 by HIV+ PBMC. IL-10 dysregulation may be associated with altered production of IL-12 (17–19, 43a, 49). To measure IL-12 production by monocytes and B cells, PBMC were stimulated with PWM, and supernatants were collected after 48 h of culture and analyzed for the presence of IL-12. PBMC from HIV+ individuals (n = 9) produced significantly lower levels of IL-12 (16.1 ± 10.1 pg/ml) than did those from HIV- controls (n = 12; 352 ± 15.7 pg/ml; P < 0.001). The lower levels of IL-12 were not due to altered kinetics, as the levels of IL-12 in supernatants were low following 24, 48, and 72 h of culture (data not shown). PBMC from HIV+ individuals were able to respond to PWM, since PWM-induced IL-10 production was comparable to that in HIV- controls (HIV-, 93.6 ± 34.7 pg/ml; HIV+, 58.5 ± 9.6 pg/ml). Similar results were obtained following PHA and anti-CD3 antibody stimulation: HIV+ PBMC did not produce IL-12 beyond basal unstimulated levels, whereas HIV- PBMC produced high levels of IL-12 (data not shown). Since IL-12 is not produced by T cells (8, 53), the increased IL-12 production observed following stimulation of HIV- PBMC with PHA or anti-CD3 antibodies may have been due to stimulation of monocytes and/or B cells. Thus, HIV infection results in impaired IL-12 production by PBMC, while IL-10 production following PWM stimulation was comparable to that of HIV- controls.

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IL-12 enhances proliferation of PBMC from low IL-10 producers to HIV p25 antigen. To determine whether exogenous IL-12 could restore proliferation of HIV⁺ PBMC to recall antigens, PBMC were stimulated with HIV p25 antigen in the presence or absence of IL-12. IL-12 enhanced the proliferation of PBMC from low IL-10 producers only (Fig. 4). The proliferative responses of PBMC from IL-10 producers and normal individuals were not altered (data not shown), even when high concentrations of IL-12 (500 U/ml) and/or high concentrations of anti-IL-10 antibodies (25 μg/ml) were added to the cultures (data not shown). Anti-IL-10 antibodies synergized with IL-12 in enhancing the proliferation of PBMC from low-IL-10-producing HIV⁺ individuals (data not shown).

DISCUSSION

In this study, we have demonstrated an association of abnormal patterns of IL-10 and IL-12 secretion by HIV⁺ PBMC with alterations in proliferative responses to recall antigens. PBMC from HIV⁺ individuals producing low levels of IL-10 proliferated in response to HIV p25 antigen, and their proliferation could be enhanced by the addition of anti-IL-10 antibodies and/or IL-12. Conversely, PBMC from HIV⁺ IL-10 producers failed to proliferate in response to HIV p25. These results are in agreement with an earlier report suggesting that IL-10 production by PHA-stimulated HIV⁺ PBMC is correlated with disease progression (15). Since a sequential loss of responsiveness to recall antigens, alloantigens, and mitogens has also been correlated with disease progression (15), low-IL-10-producing individuals in our study may be in an early stage of HIV infection and may progress to a more advanced stage of disease characterized by higher IL-10 production and unresponsiveness to recall antigens. The present cross-sectional study of asymptomatic HIV⁺ individuals does not address the question of stability of IL-10-producing phenotype over time or its association with clinical or virological markers of disease progression. Longitudinal studies to address these issues are under way.

PBMC from HIV⁺ low IL-10 producers exhibit IL-10- and IL-12-mediated regulatory pathways, as their proliferative responses could be enhanced by the addition of anti-IL-10 antibodies and/or IL-12. The mechanism through which HIV⁺ IL-10 producers lose their ability to respond to recall antigens, even in the presence of anti-IL-10 antibodies, is unclear. The relevant T-cell subsets in this group of individuals may have become tolerant to IL-10 after long-term exposure since, in a previous study, we reported spontaneous production of IL-10 by unstimulated HIV⁺ PBMC (20). IL-10 has been shown to induce tolerance through inhibition of Th1-type cytokines (19, 43a), possibly through altered expression of costimulatory molecules, such as major histocompatibility complex (MHC) class II molecules and/or B7/B7I (19, 21, 22, 43a). IL-10 has also been shown to induce immune tolerance in vivo if administered during the induction phase of the immune response (24, 25). The reasons underlying the failure of IL-12 to enhance proliferation in the IL-10-producing subset of cells from HIV⁺ individuals is also not clear. Their PBMC may have lost IL-12 receptors or developed defects in IL-12 receptor-induced cytokines or signaling molecules.

Various groups of investigators have reported differing levels of IL-10 production by HIV⁺ PBMC following stimulation with PHA (4, 23, 34, 41, 48). Different stimulation protocols, stages of disease, concurrent infections, and administration of antiviral medications may have contributed to the observed variability. A number of mechanisms to explain the loss of IL-10 production by the low-IL-10-producing subset may be operative. IL-10-producing Th2-type cells that support viral replication (41) may have been prematurely lost. Alternatively, HIV⁺ individuals may exhibit a deficiency of IL-10-inductive cytokines. IL-10 production in monocytes is regulated by TNF-α (54), whereas IL-12 regulates IL-10 production by T cells (unpublished data). Altered production of TNF-α or IL-12 may thus have affected the level of IL-10 production by HIV⁺ PBMC.

Stimulation of HIV⁺ PBMC with either PWM, PHA, or anti-CD3 antibodies did not induce IL-12 production above that produced by unstimulated PBMC. These results confirm previous observations of impaired IL-12 production by HIV⁺ PBMC (7, 12). Our results do not distinguish between a direct inhibitory effect of HIV on IL-12 production and an indirect effect through counterregulatory cytokines such as IL-10 and transforming growth factor beta (17). Addition of exogenous IL-10 in vitro inhibited IL-12 production by PBMC (unpublished observations), indicating a possible role for IL-10 in altered IL-12 production, at least in HIV⁺ IL-10 producers.

Cytokines secreted by monocytes/macrophages following exposure to antigens or pathogens may play a vital role in driving naive T-helper (Th0) cells to develop into cells with distinct Th1 and Th2 phenotypes (37, 42, 43, 47). IL-12 and IFN-γ have been shown to induce the development of Th1-type cells (37, 42, 43), while IL-1, IL-4, and IL-10 induce the development of Th2-type cells (40, 42, 50, 51). IL-12 and IFN-α production has been shown to be defective in monocytes from HIV-infected patients (7, 12, 31). Our results indicate that in HIV⁺ PBMC, IL-10 is mainly produced by monocytes, in contrast to HIV⁺ PBMC, in which IL-10 is produced by both T cells and monocytes. IL-12 production was significantly reduced in both subsets of HIV⁺ individuals, irrespective of their IL-10 producer status. A similar increase in IL-10 production and decrease in IL-12 production were observed in promonocytic cell lines infected with HIV in vitro (unpublished observations). Studies by other groups have demonstrated that monocytes from HIV⁺ individuals produce either normal or increased amounts of IL-1, IL-6, TNF-α, and GM-CSF (46), which have been shown to enhance HIV replication (3, 27, 35, 38, 39, 45). Taken together, these findings indicate that HIV may employ monocytes to systematically enhance its own replication, inhibit the development of Th1-type responses, and favor the emergence of Th2-type responses to pathogens which normally induce Th1-type responses.

The role of T-helper subsets and cytokine patterns in HIV infection has recently been addressed (4, 23, 34, 41, 48). Two groups of investigators have suggested that progression to AIDS is associated with an alteration in T-helper-cell phenotype, from a Th1 to a Th2 phenotype (13–15), or from a Th1 to a Th0 phenotype (41, 48). In our studies, correlation of low IL-10 production with preserved proliferative responses and of relatively high IL-10 production with loss of proliferative responses indicates a predominance of Th2-type responses in HIV disease progression. Further experiments are needed to more clearly define the cytokine secretion patterns of HIV⁺ T-cell and monocyte subsets at the single-cell level and to investigate the critical role of monocytes in the pathogenesis of AIDS.

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