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J Clin Invest. 1996;**97**(6):1550-1555. <https://doi.org/10.1172/JCI118578>.

Research Article

Decreased antigen (Ag)-specific T cell (TC) proliferation and IL-2 production are detected in all stages of HIV disease. To determine whether dendritic cell dysfunction and/or abnormal cytokine production contribute to HIV-induced immune dysregulation, we studied TC responses to recall Ags (influenza virus and tetanus toxoid) presented by Langerhans cells (LC) in six pairs of HIV-discordant identical twins, and the modulation of these responses by anti-IL-10 (alphaIL-10) mAbs and IL-12. LC from HIV+ twins induced IL-2 comparable to normal LC in cultures containing TC from uninfected twins. In contrast, IL-2 production was markedly decreased in cultures containing TC from HIV+ twins. IL-12 enhanced Ag-specific IL-2 production by TC from two patients with CD4+ counts > 600. In contrast, alphaIL-10 mAbs enhanced IL-2 production in influenza virus-stimulated cultures containing TC from two patients with CD4+ counts < 20. Thus, these findings suggest that immunologic dysfunction of dendritic cells does not contribute to impaired secondary immune responses in HIV+ individuals. Although few patients were studied, partial immune reconstitution in vitro, as demonstrated here, may help to predict those individuals who might benefit from cytokines or antibodies against cytokines as immunotherapy for HIV disease.

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Modulation of T Cell Responses to Recall Antigens Presented by Langerhans Cells in HIV-discordant Identical Twins by Anti-Interleukin (IL)-10 Antibodies and IL-12

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Abstract

Decreased antigen (Ag)-specific T cell (TC) proliferation and IL-2 production are detected in all stages of HIV disease. To determine whether dendritic cell dysfunction and/or abnormal cytokine production contribute to HIV-induced immune dysregulation, we studied TC responses to recall Ags (influenza virus and tetanus toxoid) presented by Langerhans cells (LC) in six pairs of HIV-discordant identical twins, and the modulation of these responses by anti-IL-10 (α IL-10) mAbs and IL-12. LC from HIV⁺ twins induced IL-2 comparable to normal LC in cultures containing TC from uninfected twins. In contrast, IL-2 production was markedly decreased in cultures containing TC from HIV⁺ twins. IL-12 enhanced Ag-specific IL-2 production by TC from two patients with CD4⁺ counts > 600. In contrast, α IL-10 mAbs enhanced IL-2 production in influenza virus-stimulated cultures containing TC from two patients with CD4⁺ counts < 20. Thus, these findings suggest that immunologic dysfunction of dendritic cells does not contribute to impaired secondary immune responses in HIV⁺ individuals. Although few patients were studied, partial immune reconstitution in vitro, as demonstrated here, may help to predict those individuals who might benefit from cytokines or antibodies against cytokines as immunotherapy for HIV disease. (*J. Clin. Invest.* 1996. 97:1550–1555.) **Key words:** dendritic cells • antigen presenting cells • cytokines • immunotherapy • AIDS

Introduction

Dendritic cells (DC)¹ are potent antigen presenting cells (APC) that are critical for the initiation of primary and sec-

ondary immune responses (1, 2). Because DC are targets for HIV (through binding, endocytosis, and/or infection) (3–16), disruption of normal DC function has been proposed as a major factor in immune dysregulation associated with HIV disease (17–20). Few studies, however, have focused on the function of DC obtained from tissues of HIV-seropositive (HIV⁺) individuals (5, 21–24). This is probably because of the difficulty in obtaining adequate numbers of relatively pure populations of DC. In addition, when performing assays of DC immune function that require autologous T cells (TC) (e.g., recall antigen [Ag]-induced responses), abnormal results could be entirely dependent on HIV-induced TC dysfunction. Thus, such tests do not allow for an accurate interpretation of DC function.

To address this problem, functional studies using DC and TC obtained from HIV-discordant identical twins provide an excellent model system. Using such a system, we previously determined that recall Ag presentation by Langerhans cells (LC) was normal in three HIV-infected twins (using uninfected syngeneic TC as responders) (24). LC are potent APC of the epidermis that morphologically, phenotypically, and functionally resemble DC found in blood and other tissues (2). In this report, we extend our earlier observations by examining LC recall Ag presentation and memory TC responses in a larger group of HIV-discordant identical twins. Importantly, this group included individuals in varied clinical disease categories, including two patients with CD4⁺ cell counts < 20.

Cytokine dysregulation has also been postulated as being an important factor in contributing to defective immune responses of HIV-infected individuals (25, 26). As HIV disease progresses in an individual, cytokine production by PBMC stimulated in vitro shifts from a dominant type 1 (e.g., IL-2, IFN- γ) to a dominant type 2 (e.g., IL-4, IL-10) cytokine profile (27–31). Furthermore, it has been shown that defects in Ag-stimulated IL-2 production and in TC proliferation using PBMC from HIV⁺ individuals can be partially reversed in vitro by the addition of rhIL-12 (32, 33), a potent cytokine that promotes type 1 cytokine immune responses, or by neutralizing anti-IL-10 (α IL-10) mAbs (30, 33). It is unclear whether IL-12 and α IL-10 mAbs act directly on TC or indirectly by enhancing APC function. To investigate these issues further, we studied the effects of rhIL-12 and α IL-10 mAbs on recall Ag-stimulated immune responses, using mixtures of TC and LC obtained from HIV-discordant identical twins, as described above. These studies add further insight into the mechanisms of action of IL-12 and α IL-10 mAbs on secondary immune responses and may help to predict those individuals who might benefit from cytokines or antibodies against cytokines as immunotherapy for HIV disease.

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Received for publication 20 October 1995 and accepted in revised form 9 January 1996.

1. *Abbreviations used in this paper:* DC, dendritic cell; LC, Langerhans cell; TC, T cell; Ag, antigen; APC, antigen presenting cell; TET, tetanus toxoid; FLU, influenza A virus.

J. Clin. Invest.

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0021-9738/96/03/1550/06 \$2.00

Volume 97, Number 6, March 1996, 1550–1555

Methods

Patients and clinical evaluation. Six pairs of male monozygotic twins discordant for HIV serology were studied in parallel; i.e., skin and blood were obtained on the same day from an HIV⁺ patient and his HIV-seronegative (HIV⁻) twin brother. Twin pairs were not involved in any other investigative protocols. Current CD4⁺ cell counts and use of antiretroviral medications were recorded. Patients were classified according to the Centers for Disease Control revised (CDC-r) guidelines for staging of HIV-infected adults (34). This protocol was approved by the National Cancer Institute Institutional Review Board, and informed consent was obtained from each individual before entry into the study.

Preparation of cell suspensions. Suction blisters were induced on clinically normal skin from the anterior thighs by vacuum suction and heat. Blister roofs (epidermal sheets) were removed and single-cell suspensions of epidermal cells were prepared by limited trypsinization as previously described (35). Cells were resuspended in RPMI 1640 (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated pooled human AB⁺ serum (Sigma Chemical Co., St. Louis, MO), 100 U/ml penicillin (Gibco Laboratories), 100 µg/ml streptomycin (Gibco Laboratories), 2 mM L-glutamine (Gibco Laboratories), 10 mM Hepes (Gibco Laboratories), and 1 µg/ml indomethacin (Sigma Chemical Co.) (complete medium), and the number of viable cells was determined by trypan blue exclusion. The percentage of LC in each epidermal cell suspension was then determined by anti-HLA-DR mAb staining and flow cytometry, as previously described (24). All epidermal cell suspensions contained keratinocytes (the major subpopulation of cells), LC, melanocytes, and probably rare Merkel cells. As all standard immune functional assays using epidermal cell suspensions are dependent on LC, the term "LC" is used hereafter when discussing epidermal cell suspensions.

Whole blood from HIV⁺ and HIV⁻ individuals was drawn into plastic syringes containing preservative-free heparin sodium (Lypomed, Deerfield, IL). PBMC were separated on lymphocyte separation medium (Organon Teknika Corp., Durham, NC), washed twice in PBS, resuspended in RPMI 1640 supplemented with 5% human AB⁺ serum, and incubated in 75-cm² plastic culture flasks (Costar Corp., Cambridge, MA) for 1 h at 37°C. Nonadherent cells were drawn off and further enriched for CD3⁺ TC by negative selection using an mAb cocktail plus complement (Lympho-Kwik™; One Lambda Inc., Los Angeles, CA) according to the manufacturer's guidelines. Viable TC were resuspended in complete medium at 4 × 10⁶/ml.

In vitro tests for secondary immune responses. The ability of TC from HIV⁺ and HIV⁻ individuals to produce recall Ag-stimulated IL-2 was determined. 2 × 10⁵ TCs were cultured alone or cocultured with the following: tetanus toxoid (TET) at 2 LF/ml (Connaught Laboratories, Willowdale, Ontario, Canada) or influenza virus (FLU) at 1:500 (A/Bangkok RX73 H3N2; Bioqual, Rockville, MD) in the presence or absence of 3 × 10³ autologous or twin (syngeneic) LC. Additional cultures were supplemented with 20 U/ml of rhIL-12 (Genetics Institute, Cambridge, MA) or 5 µg/ml of neutralizing αIL-10 mAbs (JES3-19F1; DNAX Research Institute, Palo Alto, CA). These concentrations had previously been shown to augment Ag-stimulated immune responses in HIV⁺ individuals optimally (30, 32). To prevent IL-2 consumption by the stimulated cells, all culture wells contained 2 µg/ml of the anti-human IL-2 receptor mAb anti-Tac (generously provided by Dr. John Hakimi, Hoffman-La Roche, Nutley, NJ). Cells were incubated in 96-well flat-bottomed culture plates (Costar Corp.) in triplicate in a humidified 5% CO₂ atmosphere at 37°C for 7 d. Culture supernatants were then collected and stored at -20°C until assayed for IL-2 content. IL-2 production was determined by ELISA (R&D Systems, Minneapolis, MN) according to the manufacturer's guidelines. We chose to measure TC IL-2 production (as opposed to TC proliferation) as a marker of TC activation because of previous studies in which we demonstrated this to be the most sensitive method to detect defective immune responses in HIV⁺ individuals (36).

Table I. Clinical Characteristics of the HIV⁺ Twins Studied

No.	Age	CDC-r stage	CD4 ⁺ Cells	Anti retroviral treatment
	yr		No./mm ³	
1	40	B3*	9	None
2	35	C3*	16	d4T [‡]
3	30	B3*	107	ddI [§]
4	44	C2*	270	None
5	31	A1	616	None
6	33	A1	680	None

*AIDS patient by CDC-r guidelines (34); [‡]stavudine; [§]didanosine.

Results

Patients. A summary of the clinical characteristics of the HIV⁺ individuals studied is found in Table I. All patients were men, and the median age was 34 yr (range: 30–44). Median CD4⁺ cell count was 189/mm³ (range: 9–680). Four patients had AIDS as determined by CDC-r criteria (34). Of note, four patients were not receiving antiretroviral therapy at the time they were studied.

The percentage of LC in epidermal cell suspensions and HLA-DR expression by LC is similar in HIV⁺ and HIV⁻ individuals. In clinically uninvolved skin, LC are the only epidermal cells to constitutively express cell surface MHC class II molecules. Thus, the percentage of LC contained within epidermal cell suspensions can be accurately determined by anti-HLA-DR mAb staining followed by flow cytometric analysis. Consistent with our previous findings (24), the percentage of LC in epidermal cell suspensions and HLA-DR expression by LC (as measured by mean fluorescence intensity) was similar in HIV⁺ individuals and their corresponding HIV⁻ twin brothers (mean ± SD: 2.67 ± 1.10% vs 2.69 ± 1.12% and 1,001 ± 283 vs 812 ± 222, respectively).

Recall Ag presentation by LC derived from HIV⁺ individuals is normal. TC derived from all six HIV⁻ twins responded to TET or FLU presented by LC from HIV⁻ (autologous) or HIV⁺ (syngeneic) twins (Fig. 1). Median IL-2 production was similar in both groups (205 pg/ml [range: 8–1,431] vs 204 pg/ml [range: 12–1,393] for TET and 61 pg/ml [range: 0–136] vs 115 pg/ml [range: 0–140] for FLU). Importantly, Ag presentation by LC obtained from two HIV⁺ patients with CD4⁺ cell counts < 20 was normal (HIV⁺ twins No. 1 and 2 in Fig. 1). Of note, one HIV⁻ twin was a nonresponder to FLU and one HIV⁻ twin was a nonresponder to TET (twin No. 3 in Fig. 1 A and No. 5 in Fig. 1 B). By contrast, TC derived from five of six HIV⁺ twins failed to produce or had markedly decreased IL-2 production in Ag-stimulated cultures (Fig. 1). The one exception was HIV⁺ twin No. 5, who had a normal response to FLU (Fig. 1 A). These defective immune responses occurred in cultures containing LC derived from either HIV⁺ (autologous) or HIV⁻ (syngeneic) twins. In fact, no major differences in median IL-2 production were noted in these two groups (3 pg/ml [range: 0–21] vs 5 pg/ml [range: 0–19] for TET and 0 pg/ml [range: 0–80] vs 0 pg/ml [range: 0–46] for FLU). No background IL-2 was produced in TC-LC cocultures in the absence of recall Ags, nor in TC-Ag cultures in the absence of LC (not shown).

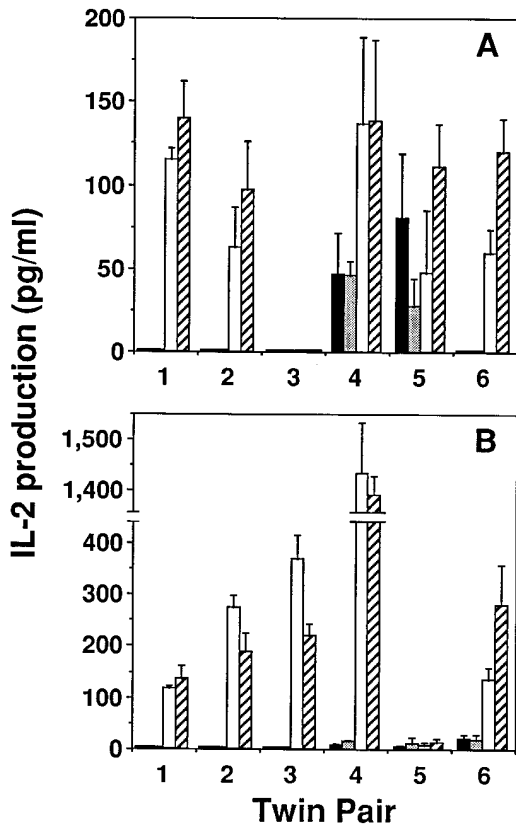


Figure 1. LC from HIV⁺ individuals present recall Ags well to normal syngeneic TC, whereas TC from HIV⁺ individuals fail to respond to recall Ags even in the presence of normal syngeneic LC. ■ TC⁺/LC⁺, ▨ TC⁺/LC⁻, □ TC⁻/LC⁻, ▩ TC⁻/LC⁺. 3×10^3 LC obtained from skin of HIV⁺ and HIV⁻ monozygotic twins were cultured with 2×10^5 autologous or syngeneic TC in the presence of the recall Ags FLU (A) or TET (B). All cultures contained anti-TAC Abs to prevent consumption of IL-2. Supernatants were collected at 7 d, and total IL-2 production was measured by ELISA.

rhIL-12 and α IL-10 mAbs modulate secondary immune responses in vitro in HIV⁺ individuals. rhIL-12 was added to twin TC-LC cocultures in an attempt to augment Ag-specific IL-2 production. In Ag-stimulated cultures, rhIL-12 enhanced IL-2 production in cultures containing TC from HIV⁺ twins with CD4⁺ cell counts > 600 (Fig. 2); in these experiments, rhIL-12 did not exhibit differential effects on cultures containing LC from HIV⁺ twins compared with LC from HIV⁻ twins (Fig. 2). In contrast, the addition of rhIL-12 had no significant effect on IL-2 production in cultures containing TC derived from HIV⁺ twins with CD4⁺ TC counts < 300 (not shown). In cultures containing TC derived from HIV⁻ twins, rhIL-12 slightly enhanced median IL-2 production for FLU-specific (104 pg/ml [range: 0–140] vs. 245 pg/ml [range: 23–416] and TET-specific responses (204 pg/ml [range: 12–1,431] vs 239 pg/ml [range: 130–836]). In all experiments, there was no significant background IL-2 production (i.e., TC-LC cocultures in the absence of Ag) with the addition of rhIL-12 (not shown).

To determine whether endogenous IL-10 production was contributing to defective immune responses in the HIV⁺ twins (in a system devoid of M ϕ), IL-10 activity was blocked by neutralizing α IL-10 mAbs and Ag-stimulated IL-2 production was determined. In cultures containing TC from HIV⁺ twins with

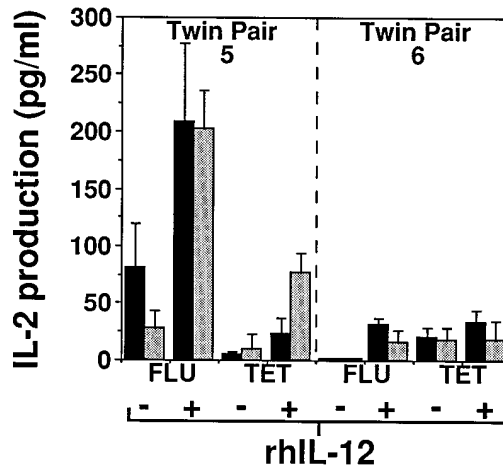


Figure 2. rhIL-12 enhances recall Ag-stimulated IL-2 production by TC obtained from patients in early-stage HIV disease. ■ TC⁺/LC⁺, ▨ TC⁺/LC⁻. 3×10^3 LC obtained from skin of HIV⁺ and HIV⁻ monozygotic twins were cultured with 2×10^5 TC obtained from the HIV⁺ twins in the presence of the recall Ags TET or FLU and in the absence or presence of 20 U/ml of rhIL-12. All cultures contained anti-TAC Abs to prevent consumption of IL-2. Supernatants were collected at 7 d, and total IL-2 production was measured by ELISA.

CD4⁺ cell counts < 20, α IL-10 mAbs enhanced FLU-stimulated IL-2 production, but not TET-stimulated IL-2 production (Fig. 3); in these experiments, α IL-10 mAbs did not exhibit differential effects on cultures containing LC from HIV⁺ twins compared with LC from HIV⁻ twins (Fig. 3). In contrast, the addition of α IL-10 mAbs had no significant effect on Ag-stimulated IL-2 production in cultures containing TC derived from HIV⁺ twins with CD4⁺ TC counts > 100 (not shown). In cultures containing TC derived from HIV⁻ twins, α IL-10 mAbs slightly enhance Ag-specific median IL-2 pro-

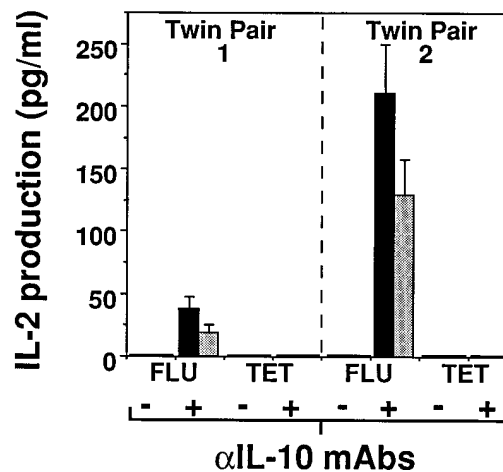


Figure 3. α IL-10 mAbs enhance FLU-stimulated, but not TET-stimulated, IL-2 production by TC obtained from patients with advanced AIDS. ■ TC⁺/LC⁺, ▨ TC⁺/LC⁻. 3×10^3 LC obtained from skin of HIV⁺ and HIV⁻ monozygotic twins were cultured with 2×10^5 TC obtained from the HIV⁺ twins in the presence of the recall Ags TET or FLU and in the absence or presence of 5 mg/ml of neutralizing α IL-10 mAbs. All cultures contained anti-TAC Abs to prevent consumption of IL-2. Supernatants were collected at 7 d and total IL-2 production was measured by ELISA.

duction (104 pg/ml [range: 0–140] vs 142 pg/ml [range: 0–258] for FLU and 204 pg/ml [range: 12–1,431] vs 241 pg/ml [range: 14–986] for TET). In all experiments, there was no significant background IL-2 production (i.e., TC-LC cocultures in the absence of Ag) with the addition of α IL-10 mAbs (not shown).

Discussion

One aim of this study was to evaluate APC function of freshly isolated DC from HIV-infected individuals. We found that LC obtained from clinically uninvolved skin of six HIV⁺ individuals, including two patients with CD4⁺ cell counts < 20, were quantitatively normal, expressed normal levels of HLA-DR, and were able to stimulate secondary immune responses normally using HIV-uninfected syngeneic TC as responders. In a smaller group of HIV-discordant identical twin pairs, we previously demonstrated similar findings (24). Combining results from these two studies (i.e., in nine HIV-discordant identical twin pairs), there have been no detectable defects in the ability of LC to process/present soluble protein Ags. Our study did not specifically address the issue of immunologic function of HIV-infected DC, as previously assessed in *in vitro* systems (4, 7, 13). Thus, our findings may be explained by either the relative infrequency of HIV infection of LC *in vivo* (estimated by one group to occur in \sim 1% of LC [37]), or by a relative inability of HIV to induce defects in APC function in LC. Since LC were not purified from other epidermal cells, it is also possible that cytokines secreted by contaminating keratinocytes may have masked APC dysfunction of LC. Regardless, our *ex vivo* analysis, unique in the use of syngeneic cells obtained from HIV-discordant monozygotic twins, strongly suggests that immunologic dysfunction of DC does not contribute to impaired secondary immune responses in early or late stages of HIV disease.

Twin experiments such as these offer the unique opportunity to study the relative contributions of TC and APC in the generation of secondary immune responses. We are the only group to have studied function of DC in HIV⁺ individuals in this manner. Other investigators, obtaining syngeneic cells derived from HIV-discordant pairs, have used either M ϕ (38–40) or irradiated PBMC (41, 42) as APC. Although relatively few HIV⁺ individuals have been previously studied, most of these patients (i.e., 9 of 11) also had no detectable defects in APC function when using normal syngeneic TC as responders in cell-mixing experiments.

Previously, we detected a defect in the ability of freshly isolated LC from AIDS patients to stimulate allogeneic TC (24). Similarly, Macatonia et al. detected functional defects in CD11c⁻ blood DC (i.e., functionally “immature” DC) isolated from HIV⁺ individuals (22). Thus, the previous findings suggest that there are defects in the ability of DC isolated from HIV⁺ individuals to activate allogeneic TC fully, perhaps by failing to “mature” (i.e., failing to up-regulate costimulatory and adhesion molecule expression) during culture. Phenotypic and functional analysis of cultured LC (analogous to interdigitating DC in lymph nodes) obtained from AIDS patients would be important to study this issue further. In contrast, soluble protein Ag presentation is a function best performed by freshly isolated LC (2), and thus may be less dependent on up-regulation of cell surface expression of accessory molecules. As shown in this report, freshly isolated LC obtained from HIV⁺ individuals are fully competent in performing this function.

IL-12 enhances natural killer cell activity against virus-infected cells and is important in promoting type 1 cytokine immune responses (43, 44). In HIV disease, IL-12 production may be impaired (45, 46, and Chougnat, C., et al., manuscript submitted for publication), and this may therefore lead to impaired natural killer cell activity as well as to expansion of type 2 cytokine immune responses. In previous studies, the addition of rhIL-12 has been shown to restore IL-2 production and proliferation to recall Ags (including immunogenic synthetic HIV peptides) in HIV disease (32, 33). Similarly, in this current report we found that IL-12 enhanced recall Ag-specific IL-2 production in two patients with CD4⁺ cell counts > 600. IL-12 preferentially stimulates Th1 CD4⁺ and CD8⁺ TC (43, 47), which may explain why TC obtained from our four HIV⁺ patients in more advanced disease stages failed to respond to the addition of IL-12. Zhang et al. also found that exogenous IL-12 failed to enhance immune responses significantly in advanced AIDS patients (33).

IL-10 has potent antiviral and immunosuppressive effects. *In vitro*, IL-10 decreases HIV replication in M ϕ (48–54) and DC-TC coculture (54a) systems. In addition, HIV-infected persons have increased levels of constitutive and inducible IL-10 in certain cell populations, e.g. PBMC, M ϕ , and lymph node-derived cells (30, 50, 52, 55). This increase in endogenous IL-10 production may reflect a normal host response designed to decrease viral load. However, a major consequence of high levels of endogenous IL-10 is immunosuppression. *In vitro* treatment with IL-10 decreases APC function of normal DC (56–58) and M ϕ (59, 60). Furthermore, when administered intravenously to normal volunteers, rhIL-10 led to a dose-dependent decrease in TC number and mitogen-induced TC proliferation (61). In previous studies of HIV⁺ patients, α IL-10 mAbs partially reversed defective recall Ag-induced TC proliferation and IL-2 production *in vitro* (30, 33), suggesting that immune defects in these individuals were in part mediated by endogenous IL-10. Although the exact mechanism by which α IL-10 mAbs act to enhance immune responses *in vitro* is unknown, we believe they may act by inhibiting M ϕ -derived IL-10, resulting in enhancement of APC function.

To gain further insight into the effects of blocking IL-10 activity on immune responses in HIV-infected persons, in a system devoid of blood-derived M ϕ , we examined the effects of α IL-10 mAbs on recall Ag-stimulated TC responses. We found that α IL-10 mAbs enhanced FLU-stimulated IL-2 production only from TC obtained from HIV⁺ individuals with CD4⁺ cell counts < 20. This finding suggests that immunosuppression in late-stage HIV disease, but not early-stage disease, is in part mediated by CD8⁺ TC-derived IL-10. This would be consistent with findings which have documented type 2 cytokine production by CD8⁺ TC in AIDS (55, 62). It is also possible that IL-10 was being produced by small numbers of CD4⁺ TC present in these cultures. It is unlikely that epidermal cells (e.g., LC and keratinocytes) were producing significant amounts of IL-10 in our experiments, because epidermal cells isolated from both HIV⁻ and HIV⁺ individuals stimulated normal TC immune responses well in the absence of α IL-10 mAbs. It is unclear exactly why we found disparate responses to α IL-10 mAbs in the FLU- vs TET-stimulated cultures. Since it is known that FLU can infect blood DC and stimulate CD8⁺ TC responses (63), and that IL-10 can down-regulate MHC class I expression (64), perhaps α IL-10 mAbs allowed for more efficient presentation of MHC class I-associated

ated FLU peptides to CD8⁺ TC. This would explain why FLU responses, and not TET (MHC class II-restricted), were enhanced by α IL-10 mAbs. It would also explain why α IL-10 mAbs failed to enhance FLU-stimulated responses in patients with greater CD4⁺/CD8⁺ TC ratios.

Our current study may have therapeutic implications for HIV-infected persons. A major concern of using immunotherapy to enhance cell-mediated immunity in HIV disease, however, is the potential to increase viral replication and thereby worsen overall clinical disease. Thus, this type of immunotherapy would probably need to be combined with antiviral agents, with careful monitoring of immunologic, virologic, and clinical parameters. Although few patients were studied, partial immune reconstitution in vitro, as demonstrated here for α IL-10 mAbs and IL-12, may help to predict those individuals who might benefit from cytokines or antibodies against cytokines as immunotherapy for HIV disease.

Acknowledgments

We thank the twin pairs who volunteered their time and effort to participate in this study, Inga Tokar, Harry Schaefer, and Hideo Asada for expert technical assistance, Betsey Herpin and Robert Walker for assisting with patient recruitment, and Mark C. Udey for critical review of the manuscript.

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