HIV-1 induces IL-10 production in human monocytes via a CD4-independent pathway

Jiaxiang Ji, Gautam K. Sahu, Vivian L. Braciale and Miles W. Cloyd

Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX 77555-1070, USA

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Abstract

In HIV-infected patients, increased levels of IL-10, mainly produced by virally infected monocytes, were reported to be associated with impaired cell-mediated immune responses. In this study, we investigated how HIV-1 induces IL-10 production in human monocytes. We found that CD14 monocytes infected by either HIV-1213 (X4) or HIV-1BaL (R5) produced IL-10, IL-6, tumor necrosis factor- (TNF-), and to a lesser extent, IFN- . However, the capacity of HIV-1 to induce these cytokines was not dependent on virus replication since UV-inactivated HIV-1 induced similar levels of these cytokines. In addition, soluble HIV-1 gp160 could induce CD14 monocytes to produce IL-10 but at lower levels. Cross-linking CD4 molecules (XLCD4) with anti-CD4 mAbs and goat anti-mouse IgG (GAM) resulted in high levels of IL-6, TNF- and IFN- but no IL-10 production by CD14 monocytes. Interestingly, neither anti-CD4 mAbs nor recombinant soluble CD4 (sCD4) receptor could block IL-10 secretion induced by HIV-1213, HIV-1BaL or HIV-1 gp160 in CD14 monocytes, whereas anti-CD4 mAb or sCD4 almost completely blocked the secretion of the other cytokines. Furthermore, HIV-1213 could induce IL-10 mRNA expression in CD14 monocytes while XLCD4 by anti-CD4 mAB and GAM failed to do so. As with IL-10 protein levels, HIV-1213-induced IL-10 mRNA expression in CD14 monocytes could not be inhibited by anti-CD4 mAB or sCD4. Taken together, HIV-1 binding to CD14 monocytes can induce CD4-independent IL-10 production at both mRNA and protein levels. This finding suggests that HIV induces the immunosuppressive IL-10 production in monocytes and is not dependent on CD4 molecules and that interference with HIV entry through CD4 molecules may have no impact on counteracting the effects of IL-10 during HIV infection.

Introduction

HIV can infect key cells of the immune system, including CD4 T cells, blood monocytes, dendritic cells (DCs) and tissue macrophages, but the numbers of such cells infected by and replicating HIV at any given time in infected individuals are extremely small (1, 2). Thus, host responses and/or indirect effects of HIV replication are considered to be most important for HIVs ability to undermine the immune system. Impaired cytokine regulation, which appears even before the development of profound CD4 lymphopenia and disease progression (3, 4), is thought to contribute to the immunologic deficiencies in HIV infection (5). It has been suggested that in HIV-infected patients, IL-10 may cause detrimental effects by contributing to decreased production of Th1 cytokines such as IL-2 and IL-12, and by inhibiting antigen-presenting cell function and cell-mediated immune responses (6–13). In particular, IL-10 gene expression in lymph nodes has been demonstrated to be sustained from the onset of infection (14, 15), and increased levels of IL-10 were produced by PBMCs from HIV+ individuals compared with PBMCs from uninfected individuals (6). Furthermore, PBMCs from patients with low CD4 T cell counts (<200 mm–3) secreted 3-fold more IL-10 in response to polyclonal activators than did PBMCs from patients with high CD4 T cell counts (>500 mm–3), suggesting an association of IL-10 with disease progression (16). Phenotypic analyses of freshly isolated cytokine-secreting cells demonstrated that CD14 macrophages/monocytes were the dominant source of IL-10 in HIV-infected individuals (17). In vitro, HIV infection rapidly and transiently induces IL-10 production in monocytes (16). Infection of macrophages resulted in IL-10 mRNA expression within 3–12 h, with detection of biologically active IL-10 in the culture supernatants as early as 12 h post-infection (15). Although IL-10 has been studied extensively during HIV infection, the mechanism of IL-10...
induction by HIV is presently undefined. It is known that most CD4+ T cells that possess HIV DNA are resting cells which are abortively infected and only possess un-integrated HIV DNA (18, 19). The frequencies of monocytes or macrophages actively replicating HIV are even lower than those for CD4+ T cells replicating HIV (~1 in 10^5 total CD4+ cells) (1, 2), and thus it may be possible that virus binding is enough to induce IL-10 production in monocytes. Entry of primary HIV into CD4+ host cells, including monocytes, involves the binding of gp120 envelope glycoprotein to the CD4 molecule (20, 21), with subsequent binding of a specific chemokine receptor as an obligate second receptor (e.g. CCR5 and CXCR4 for HIV-1) (22, 23). It has been shown that interaction of gp120 with PBMCs (whole-virus or gp120 molecules) can induce production of cytokines such as IL-6, tumor necrosis factor-α (TNF-α), IFN-α and IFN-γ (24-28). However, it has not yet been addressed whether HIV binding per se signals the cells to produce IL-10. In this study, we compared the abilities of HIV-1, gp160 or cross-linking CD4 molecules (XLCD4) by anti-CD4 mAbs/goat anti-mouse IgG (GAM) to induce IL-10 production in monocytes. To test whether CD4 molecules are involved in IL-10 production, we examined the blocking effects of anti-CD4 mAb and recombinant soluble CD4 (scCD4) receptor on HIV-1- or gp160-induced IL-10 production in CD14+ monocytes. The role of CD4 molecules was evaluated in induction of IL-10 in comparison to induction of other cytokines (e.g. IL-6, TNF-α and IFN-γ).

Methods
Antibodies and other reagents
Unless specified, all mAbs specific for human cell surface molecules, cytokines and recombinant cytokines were obtained from BD Biosciences (San Diego, CA, USA). For cell purification or depletion of specific cell population, purified mAbs specific for CD3, CD4, CD8, CD14, CD19, CD56 and glycoporphin A were used. For FACS analysis, FITC- or PE-conjugated mAbs specific for CD4, CD8, CD14, CD19 and CD56 were used. FITC- or PE-labeled mouse IgG1, IgG2a and IgG2b isotype controls were applied as needed. For ELISA, pairs of mAbs (purified and biotinylated) specific for IL-6, IL-10, TNF-α and IFN-γ and the corresponding recombinant cytokines were used. For cross-linking CD4 molecules (XLCD4), purified anti-CD4 mAb and GAM (Sigma, St Louis, MO, USA) were used. FITC- or PE-conjugated mAbs were used for CD4, CD8, CD14, CD19 and CD56 were used. For blocking HIV-1 binding to CD4 molecules, anti-CD4 mAb (clone: RPA-T4, NA/LE, binding to domain 1 of CD4 molecules), OKT4 (binding to domain 3 of CD4 molecules, American Type Culture Collection, Gaithersburg, MD, USA) and recombinant sCD4 (1-3 domains) (ImmunoDiagnostics, Inc., Woburn, MA, USA) were used. Mouse IgG controls were applied as needed.

Isolation of PBMCs and depletion of indicated cell population
Blood was obtained from HIV-negative healthy human volunteers. PBMCs were isolated from heparinized venous blood by centrifugation through Lymphocyte Separation Medium (Mediatech Inc., Herndon, VA, USA) and washed twice with PBS. The cell pellet was re-suspended in RPMI 1640 medium (GIBCO, Carlsbad, CA, USA) supplemented with 10% human AB serum, penicillin-streptomycin and L-glutamine. PBMCs were depleted of CD4+, CD8+, CD14+ (monocyte), CD19+ (B) and CD56+ (NK) cells by using the corresponding mAbs in conjunction with GAM-coated beads (Dynal, Lake Success, NY, USA). Briefly, PBMCs were incubated with appropriate amounts of anti-CD4, anti-CD8, anti-CD19 or anti-CD56 antibody for 30 min on ice. After two washes, cells were incubated with GAM-coated beads for 30 min at 4°C with gentle shaking. The supernatants were recovered after the magnetic bead-positive cells were removed using a magnetic separator. The percentage of the depleted cell population after separation is usually <0.1%, as analyzed by flow cytometry.

Purification of CD14+ monocytes
CD14+ monocytes were purified from PBMCs by negative selection using magnetic beads (Dynal). Briefly, PBMCs were incubated with appropriate amounts of antibody cocktail (anti-CD3, anti-CD16, anti-CD19, anti-CD56 and anti-glycophorin A) for 30 min on ice. After incubation with GAM-coated beads for 30 min at 4°C with gentle shaking, the supernatant was recovered after the magnetic bead-positive cells were removed using a magnetic separator. The purified cell preparations usually contained 92-95% CD14+ monocytes, as analyzed by flow cytometry.

HIV-1 strains and native gp160 protein
X4 virus HIV-1213 was isolated from PBMCs of infected individuals as described (29), and propagated in a human T-cell leukemic cell line (CEM) cells. R5 virus HIV-1Bal. was propagated in another human T lymphoblastic cell line (VB). Virus was collected when the infected cells were ~100% HIV antigen positive and concentrated by ultracentrifugation. Uninfected CEM or VB culture supernatants were collected with or without concentration as mock control. Monocytes were infected with HIV-1 at a multiplicity of infection equal to 1. In some experiments, HIV-1213 was UV inactivated before infection as described (29). UV-inactivated virus did not productively infect monocytes as determined by PCR for HIV-1 gag region and p24 ELISA. HIV-1213 gp160 was purified using affinity chromatography from SF-9 cells infected with gp160-expressing baculovirus by Bio-Molecular Tech., Inc. (Frederick, MD, USA). All the HIV-1 and gp160 preparations were tested and found free of bacterial endotoxin.

Cross-linking CD4 molecules
Cells were incubated with mouse anti-human CD4 mAb (2 µg per 10^6 cells) for 30 min on ice. After three washes, cells were cultured in 96-well plates coated with GAM. For the coating of GAM to the plates, 50 µl of GAM in coating buffer (100 µg ml^-1 in 30 mmol l^-1 NaHCO3, 15 mmol l^-1 Na2CO3, pH 9.6) was added to each well, incubated at 4°C overnight and washed five times with PBS.

Measurement of cytokines by ELISA
After HIV-1 infection or treatment with gp160, cell culture supernatants were collected after overnight incubation for
TNF-α and 72 h for IL-6, IL-10 and IFN-γ. Specific ELISAs were performed using pairs of mAbs and recombinant mouse IL-6, IL-10, TNF-α and IFN-γ (BD Biosciences). The ELISA assay sensitivities for IL-6, IL-10, TNF-α and IFN-γ were 4, 8, 8 and 4 pg ml⁻¹, respectively.

Detection of IL-10 mRNAs by RNase protection assay

Total RNAs were extracted from CD14⁺ monocytes after overnight HIV infection or XLCD4 using a Micro RNA isolation kit (Stratagene, La Jolla, CA, USA). RNase protection assay (RPA) was conducted using RiboQuant multiprobe RPA kits together with the human cytokine template-2 (hCK-2) set (BD Biosciences). Briefly, anti-sense cRNA probes were transcribed via T7 RNA polymerase in the presence of [α-³²P]UTP (3000 Ci mmol⁻¹; ICN Biomedical, Inc., Irvine, CA, USA). After heating the preparations briefly at 90°C to denature the RNA, the labeled probes were incubated with 2-μg portions of total RNA samples in hybridization buffer at 45°C for 16 h. Annealed products were digested with an RNase A–RNase T1 mixture for 1 h at 30°C. The protected fragments were precipitated, dried, re-suspended in loading buffer and denatured for 3 min at 90°C before electrophoresis on 4.75% acrylamide gels (BIO-RAD, Hercules, CA, USA). The gels were dried for 1 h at 70°C before exposure to Kodak X-AR film at −70°C. The identity of each protected fragment was established by analyzing its migration distance using a standard curve of migration distance versus the log nucleotide length for each undigested probe. The quantity of a given mRNA species in the original RNA sample was determined based on the signal densities as determined with an AlphaImager 2200 optical densitometer (Alpha Innotech Corp., San Leandro, CA, USA) for the appropriately sized, protected probe fragment bands. Sample loading was normalized to the amount of the housekeeping gene coding for ribosomal protein L32.

Statistical analysis

Differences between experimental groups were examined using the Student’s t test. A difference in mean values was considered significant when \( P < 0.05 \) or very significant when \( P < 0.01 \).

Results

CD14⁺ monocytes are the major source of IL-10 in PBMCs after HIV-1213 infection or gp160 treatment in vitro

Th2, Th0, CD8⁺ T cells and monocytes can produce IL-10 after their activation or stimulation. Hagiwara et al. (17) reported that CD14⁺ macrophages/monocytes in PBMCs removed from HIV-infected individuals are the dominant source of IL-10. However, the frequency of IL-10-producing CD4⁺ T cells increases in patients at advanced stages of HIV disease (30, 31). None of these studies demonstrated that HIV itself directly induced the IL-10 because HIV⁺ patients also possess other opportunistic agents. To determine the source of IL-10 after HIV direct infection of PBMCs, we depleted CD4⁺, CD8⁺, CD14⁺, C19⁺ or CD56⁺ cells from PBMCs in vitro before infection and measured the subsequent IL-10 secretion. As determined by ELISA, a high level of IL-10 production was seen for HIV-1213-infected PBMCs but no cytokines were detected in uninfected mock control (Fig. 1A). HIV-1 213-infected PBMCs depleted of CD14⁺ cells produced 10-fold lower levels of IL-10 than whole PBMCs (Fig. 1A). However, there was no significant change in IL-10 production from PBMCs depleted of CD8⁺, CD19⁺ or CD56⁺ cells (Fig. 1A).
Interestingly, there was a significant increase in IL-10 production in CD4+–depleted PBMCs (Fig. 1A). Similarly, no IL-10 production was detected in CD4+–depleted PBMCs treated with HIV-1213 gp160 although gp160 treatment induced much less IL-10 production in PBMCs as compared with HIV-1 infection (Fig. 1B). These results indicate CD4+ monocytes are required for most of the IL-10 production in HIV-1-infected PBMCs. Furthermore, we examined the effects of HIV-1 infection or gp160 treatment on IL-10 production in purified CD14+ monocytes. As shown in Table 1, CD14+ monocytes alone produced IL-10 after HIV-1213 infection or HIV-1213 gp160 treatment.

No IL-10 induction by XLCD4 with anti-CD4 antibody and GAM in CD14+ monocytes

CD4 molecules are the primary receptors for HIV-1 and bind HIV-1 gp120/160 with high affinity. To address the question as to whether signals through CD4 molecules are sufficient to induce IL-10 production, we compared the effects of XLCD4 with anti-CD4 mAb and GAM, HIV-1 infection and gp160 treatment on IL-10 production in CD14+ monocytes. As shown in Table 1, infection by HIV-1213 or HIV-1BaL induced IL-10 and other cytokines, such as IL-6, TNF-α and low levels of IFN-γ production in CD14+ monocytes, while XLCD4 with anti-CD4 antibodies and GAM failed to induce IL-10 production. However, XLCD4 with anti-CD4 mAb and GAM in CD14+ monocytes still induced IL-6, TNF-α and IFN-γ production. UV-inactivated HIV-1213 induced similar levels of tested cytokines as HIV-1 indicating that replication was not required. Furthermore, HIV-1213 gp160 induced similar levels of IL-6, TNF-α and IFN-γ but lower levels of IL-10 production in CD14+ monocytes as compared with HIV-1213. Of note, marginal or no cytokines were detected in medium control, uninfected mock control, CD4 molecule ligation with anti-CD4 antibody alone or GAM control. As detected by RPA, HIV-1213 infection, but not XLCD4 with anti-CD4 mAb and GAM, resulted in IL-10 mRNA expression (Fig. 2A and C). RPA did not detect IL-10 mRNA expression in HIV-1213 gp160-treated CD14+ monocytes (data not shown).

Anti-CD4 antibody did not inhibit the capacity of CD4+ monocytes to produce IL-10 after HIV-1 infection or gp160 treatment

Anti-CD4 mAb against domain 1 of CD4 molecules blocks the interaction of HIV-1 gp160/gp120 and CD4 molecules, and thus inhibits HIV-1 infectivity (27, 32). To determine whether IL-10 induction by HIV-1 needs the involvement of CD4 molecules, we pre-treated the CD14+ monocytes with anti-CD4 mAb to block the site on CD4 molecules for HIV-1 gp120 binding and measured the cytokines after HIV-1 infection and gp160 treatment. As shown in Table 2, anti-CD4 mAb (clone RPA) against domain 1 of CD4 molecules almost completely inhibited IL-6, TNF-α and IFN-γ secretion induced by HIV-1 infection (HIV-1213 or HIV-1BaL) or HIV-1213 gp160 treatment, whereas it did not block IL-10 production. As expected, anti-CD4 mAb (clone OKT4) against domain 4 of CD4 molecules did not block the secretion of any tested cytokines induced by HIV-1 or HIV-1213 gp160. Furthermore, anti-CD4 mAb against domain 1 of CD4 molecules did not block IL-10 mRNA expression induced by HIV-1213 infection (Fig. 2B and D). Anti-CD4 mAb (clone RPA or OKT4) alone, however, did not induce any cytokines from CD14+ monocytes (data not shown).

Recombinant sCD4 receptor did not inhibit HIV-1-induced IL-10 production in CD14+ monocytes

Recombinant sCD4 receptor binds HIV-1 gp120 and neutralizes HIV-1 infectivity in CD4+ cells (27, 33). To test whether recombinant sCD4 receptor inhibits the capacities of HIV-1 or gp160 to induce IL-10 in CD14+ monocytes, we incubated HIV-1 or gp160 in the presence of sCD4 receptor before infection or treatment of CD14+ monocytes and then determined the IL-10 expression and secretion. As shown in Table 3, similar to anti-CD4 mAb, sCD4 receptor almost completely inhibited IL-6, TNF-α and IFN-γ secretion induced by HIV-1 infection (HIV-1213 or HIV-1BaL) or HIV-1213 gp160 treatment. However, sCD4 receptor did not inhibit the IL-10 induction by the infection with HIV-1213 or HIV-1BaL, or by HIV-1213 gp160 treatment. Furthermore, sCD4 did not block IL-10 mRNA expression induced by HIV-1 infection (Fig. 2B and D). Of note, sCD4 itself did not induce any cytokines from CD14+ monocytes (data not shown).

Discussion

In this study, we showed that HIV-1 or gp160 could directly induce IL-10 production in monocytes, and this occurred through interactions independent of CD4 molecules. In both instances, IL-10 induction was not inhibited either by mAb against CD4 or by sCD4. However, the same anti-CD4 mAb or sCD4 treatment almost completely inhibited production of other cytokines such as IL-6, TNF-α and IFN-γ. These results suggest that CD4 binding is necessary for induction of some cytokines (e.g. IL-6, TNF-α and IFN-γ) but not for induction of all cytokines (e.g. IL-10) in monocytes following HIV-1 infection or gp160 treatment.
In HIV infection, the likelihood of CD4 engagement occurring in vivo is extremely high, especially through the binding of circulatory and cell-associated gp120/gp160 with or without their induced antibodies (34–36). Interestingly, we found that cytokine profiles induced via CD4 bound by CD4 mAb and GAM, HIV-1 and gp160 in monocytes are quite different (Table 1 and Fig. 2). XLCD4 with anti-CD4 mAb and GAM failed to induce IL-10 although it induced IL-6, TNF-α and IFN-γ in monocytes. In contrast, both HIV-1 and gp160 could induce IL-10 as well as IL-6, TNF-α and IFN-γ. These results suggest that signals via CD4 molecules are not necessary for IL-10 induction and that other regions of gp160 unrelated to the CD4 binding may be involved. In support of this proposal, either anti-CD4 mAb or sCD4 could not block the IL-10 mRNA expression and secretion induced by HIV-1 or gp160 in monocytes (Tables 2–3 and Fig. 2). In addition, we could not find anti-CXCR4/CXCR5 or anti-CXCR4/CXCR5 blocked IL-10 production after HIV-1 exposure, suggesting no involvement of chemokine co-receptor (data not shown). However, consistent with previous reports (24, 25, 27), both anti-CD4 mAb and sCD4 could almost completely inhibit HIV-1- or gp160-induced other cytokines such as IL-6, TNF-α and IFN-γ (Tables 2–3), confirming that induction of these cytokines requires signals via CD4 molecules.

In addition to CD4 molecules and/or chemokine receptors, monocytes possess several other HIV-1-binding proteins that may play roles in IL-10 production after HIV-1 exposure. Recently, DC-SIGN has been demonstrated on DCs as the receptor which captures HIV-1 and transmits the virus to CD4 T cells (37, 38). In human blood, a small cell population (~0.01–0.04% of total PBMCs) expresses DC-SIGN and represents a subset of precursor DCs (39). These blood DC-SIGN+ DCs co-express CD14 and are able to capture HIV-1 through binding of gp120 to DC-SIGN (39). Therefore, it is reasonable to determine whether DC-SIGN plays a role in IL-10 induction by HIV-1 in monocytes. However, in our hands, anti-DC-SIGN antibodies did not block IL-10 induction in HIV-1-infected or gp160-treated monocytes (data not shown) arguing against this possibility. Furthermore, IL-10 induction by HIV-1 cannot be attributed to virus binding to other mannose receptors as described (40) because we could not find whether sugars such as mannan and D-mannose blocked IL-10 production (data not shown). Identification of the molecule(s) or receptor which binds HIV-1/gp160 and signals IL-10 production is an obvious future goal.

We demonstrated that CD14+ monocytes are the major source of HIV-1- or gp160-induced IL-10 production in vitro (Fig. 1). This is consistent with the report in HIV-infected

Fig. 2. Effect of anti-CD4 mAb and sCD4 on IL-10 mRNA expression in HIV-1-infected CD14+ monocytes. Freshly purified CD14+ monocytes were infected with HIV-1213 (multiplicity of infection: 1) or signaled by cross-linking CD4 molecules with anti-CD4 mAb and GAM for overnight. Total RNA of monocytes, freshly purified CD14+ monocytes were incubated with anti-CD4 mAb (clone RPA, 25 μg ml⁻¹) or mouse IgG control for 30 min on ice before infection with HIV-1213. To block the CD4-binding structures in HIV-1, prior to infection HIV-1213 was incubated with or without recombinant sCD4 (25 μg ml⁻¹) for 2 h on ice. After HIV-1 infection for overnight, total RNA of monocytes was extracted and RPA was performed. Results of a representative assay are shown (A) and quantities of each IL-10 mRNA detected in anti-CD4 mAb-blocking and sCD4-blocking experiments were normalized and expressed relative to the amount of L32 (D).
Table 2. Effects of anti-CD4 mAbs on production of cytokines in CD14+ monocytes infected with HIV-1 or treated with gp160

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<tr>
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<th>IL-10</th>
<th>IL-6</th>
<th>TNF-α</th>
<th>IFN-γ</th>
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<tr>
<td>Mock</td>
<td>&lt;8</td>
<td>10 ± 2</td>
<td>23 ± 3</td>
<td>&lt;4</td>
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<tr>
<td>HIV-1213</td>
<td>532 ± 33</td>
<td>6591 ± 443</td>
<td>574 ± 24</td>
<td>60 ± 6</td>
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<tr>
<td>+ IgG control</td>
<td>521 ± 43</td>
<td>6789 ± 624</td>
<td>561 ± 21</td>
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<td>+ anti-CD4 (RPA)</td>
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<td>5831 ± 512</td>
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<tr>
<td>+ anti-CD4 (RPA)</td>
<td>450 ± 42</td>
<td>261 ± 25**</td>
<td>39 ± 5**</td>
<td>&lt;4**</td>
</tr>
<tr>
<td>+ anti-CD4 (OKT4)</td>
<td>462 ± 51</td>
<td>5893 ± 605</td>
<td>463 ± 6</td>
<td>50 ± 3</td>
</tr>
<tr>
<td>Medium</td>
<td>&lt;8</td>
<td>18 ± 2</td>
<td>35 ± 3</td>
<td>&lt;4</td>
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<tr>
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<td>6709 ± 587</td>
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Freshly purified CD14+ monocytes were incubated with anti-CD4 mAbs (25 μg ml⁻¹) or mouse IgG control for 30 min on ice, and then infected with HIV-1213 or HIV-1gal (multiplicity of infection: 1) or treated with HIV-1213 gp160 (10 μg ml⁻¹). Cell culture supernatants collected overnight (TNF-α) or at 72 h (IL-6, IL-10 and IFN-γ) were tested for indicated cytokines by a specific ELISA. Two anti-CD4 mAbs (25 μg ml⁻¹) recombinant sCD4 (25 μg ml⁻¹) were added at 30 min on ice, and then infected with HIV-1213 or HIV-1gal (multiplicity of infection: 1) or treated with HIV-1213 gp160 (10 μg ml⁻¹). Cell culture supernatants collected overnight (TNF-α) or at 72 h (IL-6, IL-10 and IFN-γ) were tested for indicated cytokines by ELISA. Results represent the mean ± SD of three experiments. Asterisks indicate a statistically significant difference (**P < 0.01) compared with the IgG controls.

Table 3. Effects of recombinant sCD4 receptor on production of cytokines in CD14+ monocytes infected with HIV-1 or treated with gp160

<table>
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<tr>
<th></th>
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<tr>
<td>Mock</td>
<td>&lt;8</td>
<td>11 ± 2</td>
<td>20 ± 2</td>
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<td>554 ± 27</td>
<td>73 ± 7</td>
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<tr>
<td>+ sCD4</td>
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<td>183 ± 31**</td>
<td>38 ± 21**</td>
<td>&lt;4**</td>
</tr>
<tr>
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<td>5678 ± 560</td>
<td>447 ± 31</td>
<td>49 ± 5</td>
</tr>
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<td>+ sCD4</td>
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<td>6594 ± 543</td>
<td>711 ± 56</td>
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<td>+ sCD4</td>
<td>44 ± 6</td>
<td>167 ± 27**</td>
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CD4-independent IL-10 induction by HIV-1 effects on production of cytokines in CD14+ monocytes infected with HIV-1 or treated with gp160.

Table 2. Effects of anti-CD4 mAbs on production of cytokines in CD14+ monocytes infected with HIV-1 or treated with gp160.

Table 3. Effects of recombinant sCD4 receptor on production of cytokines in CD14+ monocytes infected with HIV-1 or treated with gp160.

Patients in vivo (17). However, even depleted of CD14+ cells, HIV-1-infected or gp160-treated PBMCs still produced a little IL-10 (Fig. 1), suggesting that another IL-10-producing cell population exists in addition to CD14+ cells. Although it has been reported that the frequency of IL-10-producing CD4+ cells is increased in patients at advanced stages of HIV disease (30, 31), we could not find any IL-10 produced by purified CD4+ cells after HIV-1 infection or gp160 treatment (data not shown). Interestingly, PBMCs depleted of CD4+ cells produced higher levels of IL-10 as compared with whole PBMC population (Fig. 1). One reason for this may be that CD4+ cells, which express higher levels of CD4 molecules, may compete with monocytes to bind HIV-1 or gp160. Therefore, when CD4+ cells were depleted from the PBMCs, the competition was relieved. In addition, we failed to find any cell populations such as B cells, CD8+ cells and NK cells producing IL-10 after being signaled by HIV-1 or gp160 (data not shown). It has been shown that in peripheral blood, most monocytes (>85%) express high levels of CD14 and little or no CD16 (41), but a minor subset (5–15%) co-expresses low levels of CD14 and high CD16, called CD14＋CD16⁺ monocytes (42, 43), which are more susceptible to HIV-1 infection (44). It remains to be determined whether such CD14＋CD16⁺ monocytes remain in CD14-depleted PBMCs and account for the minor source of IL-10 in HIV-1-infected monocytes in vitro. The high levels of viremia at advanced stages of HIV-1 disease when CD4+ T cells are markedly depleted suggests that other cells may be responsible for viral replication in vivo (45). Monocytes express highCCR5 and low but functional CXCR4 HIV-1 co-receptor and support replication of X4 HIV-1 as well as R5 HIV-1 strains (45–48). In view of the coincidence between the emergence of X4 HIV strains and the high levels of IL-10 in the serum of patients at advanced stages of HIV-1 disease (45) and the fact that extensive studies on IL-10 production in HIV-1-infected monocytes have been done using R5 HIV-1, we investigated the role of CD4 molecules in induction of IL-10 production in monocytes using X4 as well as R5 virus. The present study has demonstrated that HIV-1 (HIV-1213 or HIV-1gal) and envelope protein HIV-1213 gp160 both induce IL-10 production in monocytes in a CD4-independent manner (Tables 2–3 and Fig. 2). However, we found that CD4-independent IL-10 induction applied to other X4 virus strains such as HIV-1C, HIV-1AC-1 and their derived gp160s, and other R5 virus strain HIV-1gal, although different virus strains induced different levels of IL-10 production (data not shown). Therefore, it appears that CD4-independent IL-10 induction is not restricted to certain HIV-1 strains. Similar to reports in which induction of IL-1, IL-6, TNF-α and IFN-γ did not require active infection with HIV (25, 49), induction of IL-10 and other cytokines (e.g. IL-6, TNF-α and IFN-γ) by monocytes does not require active infection with HIV-1 since gp160 and UV-inactivated HIV-1 caused the production of these tested cytokines (Fig. 1 and Tables 1–3). It has been reported that HIV-1 proteins such as envelope protein gp41 (50, 51), gp120 (9) and regulatory proteins Nef (50) and Tat (50, 52) induce IL-10 production in monocytes in vitro. It remains unclear whether a common receptor or multiple different receptors are signaled by different components of HIV-1 for IL-10 induction. It has been demonstrated that IL-10 inhibits IL-6, TNF-α and IFN-γ production in activated monocytes (13). We were able to detect that the levels of IL-6, TNF-α and IFN-γ were up-regulated in HIV-1-infected monocytes after addition of neutralizing anti-IL-10 mAbs (data not shown). However, IL-6, TNF-α and IFN-γ co-exited with IL-10 in HIV-1-infected or gp160-treated monocytes (Tables 1–3), indicating a very complex cytokine network. From the virological perspective, IL-10 predominantly inhibits HIV-1 replication in monocytes (53, 54). In contrast, other cytokines such as IL-6 (55), TNF-α...
(56, 57) and IFN-γ or their combinations (58) (e.g. TNF-α and IFN-γ) have been associated with the induction or augmentation of HIV-1 expression. The most intriguing aspect concerning the mechanisms of cytokine induction was the observation that IL-10 induction appears to be independent of CD4 molecules, while IL-6, TNF-α and IFN-γ induction required interaction between CD4–HIV-1/gp160 (Tables 2–3). The reasons for this are not clear. In other infectious states caused by EBV or schistosomes, IL-10 may provide pathogen-infected cells a mechanism of escape from immune surveillance and thus establish a chronic carrier state (59, 60). In this context, interference with HIV entry through CD4 molecules may have no impact on HIV-induced IL-10 production and on counteracting the effect of IL-10 during HIV infection. It is not known how HIV induces IL-10 production in monocytes and once this is completely revealed, it could possibly lead to new therapy.

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