Interleukin-10 inhibits initial reverse transcription of human immunodeficiency virus type 1 and mediates a virostatic latent state in primary blood-derived human macrophages in vitro

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Interleukin-10 (IL-10), a product of T lymphocytes, B cells and macrophages, participates in Th-2 immune responses and modulates macrophage functions including possible interactions with pathogens. We have found that Chinese hamster ovary cell-derived human recombinant (hr) IL-10 inhibits human immunodeficiency virus type 1 strains Ada and Ba-L (HIV-1Ada and HIV-1Ba-L) replication in primary tissue culture-derived macrophages in a dose-dependent manner. Inhibition by IL-10 treatment (> 5 U/ml) was effective 72 h before or 24 h after infection and cytokine activity blocked by anti-hrIL-10 antibody (19F1), or lost after heat inactivation of IL-10. Viral production was measured by determining p24 and reverse transcriptase levels while reverse transcription kinetics for the long terminal repeat (LTR) and gag were assessed at timed intervals after infection and quantified by 32P end-labelling. IL-10 inhibited early steps of infection without modulating cell surface CD4+ levels. The onset of LTR reverse transcription was delayed by 4 to 8 h and the number of LTR transcripts was decreased by 77% at 24 h and by 87% at 48 h after infection. IL-10 effects were reversible; after cytokine washout, cells treated before infection showed lower levels of virus compared with those treated after infection. IL-10 biological activity was confirmed in three virus-independent assays. These results demonstrate IL-10 decreases HIV-1 reverse transcription upon macrophage infection and subsequently mediates viral latency in vitro. Therefore, IL-10 may be involved in the effective control of HIV-1-infected macrophages in vivo.

Introduction

AIDS pathogenesis involves sustained growth of human immunodeficiency virus type 1 (HIV-1) within CD4+ macrophages and T lymphocytes (Gartner et al., 1986; Gendelman et al., 1989; Popovic & Gartner, 1987; Schuitemaker et al., 1991). The continued presence of a majority of HIV-1 DNA-positive infected cells not producing viral RNA has been described recently in peripheral blood and lymph nodes of AIDS patients from early to late stages of the disease. The mechanisms regulating this viral latency are poorly understood as are cellular factors such as cytokines that may influence variations in viral tropism for different host cells in asymptomatic and symptomatic patients (Mikovits et al., 1992; Pantaleo et al., 1993; Embretson et al., 1993). Cytokines regulate cellular interactions as well as HIV-1 growth in lymphocytes and macrophages in vitro (Poli & Fauci, 1992; Montaner et al., 1993), and may contribute to the control of viral expression in latently infected monocytes which have been shown to express virus upon activation (Mikovits et al., 1992). During AIDS pathogenesis there is a progressive dysregulation of pro-inflammatory cytokines and a loss of cell-mediated responses suggesting a role for cytokines throughout the disease (Fauci, 1993; Fan et al., 1993; Trauger et al., 1993). In addition, a prognostic increase of humoral inducers, Th-2-type cytokines such as interleukin-10 (IL-10), has been proposed as a marker of end-stage disease (Clerici & Shearer, 1993; Clerici et al., 1994).

IL-10 is produced by both Th-1 and Th-2 clones in addition to macrophages and B cells. IL-10 is associated with the negative regulation of IL-12 and interferon-γ (IFN-γ) and the development of a Th-2-type immune response (Del Prete et al., 1993; D'Andrea et al., 1993). IL-10 effects on macrophage functions include inhibition of cytotoxicity, oxidative burst, nitric oxide and cytokine production [IL-6, tumour necrosis factor-α (TNF-α and IL-1β)], and inducing the expression of IL-1ra and FcγR1 (de Waal Malefyt et al., 1991, 1992; te Velde et al., 1992; Gazzinelli et al., 1992; Ralph et al., 1992; Oswald et al., 1992). Therefore, the study of IL-10 and its regulation of HIV-1 in vitro is of interest in understanding the possible mechanisms which regulate HIV replication at different stages of AIDS. In this report, we show that hrIL-10 inhibits HIV-1 replication in tissue culture.
differentiated macrophages in a reversible manner and decreases initial reverse transcription, without down-regulating CD4.

Methods

Isolation and culture of peripheral blood mononuclear cells and lymphocytes.

Human peripheral blood mononuclear cells were isolated from healthy donors as described (Collin et al., 1993); in short, Ficoll Hypaque-isolated mononuclear cells were incubated for 1 h in 2 % gelatin-coated plates. Adherent cells (macrophages), demonstrated to be > 94 % CD14+ by fluorescence-activated cell sorter (FACS) analysis were cultivated in 5 % pooled human serum for 48 h before transfer to either a 24-well plate (Nunc Inc.) at a density of 5 × 10^5 cells/well (1 ml total volume), or 6-well plates (Nunc Inc.) at 1:5 × 10^5 cells/well (3 ml total volume). Non-adherent lymphoid cells (PBLs) were stimulated with 20 % fetal calf serum (FCS) and subsequently maintained with 10 % FCS and 80 ng/ml IL-2 (Pharmacia) in T 25 flasks at a density of 1 × 10^6 cells/ml (5 ml total volume).

HIV strains. Macrophage-tropic virus stocks HIV-1ADA (Gendelman et al., 1988) and HIV-1mo-L (Gartner et al., 1986) were grown and titrated in macrophages as described (Collin & Gordon, 1994), to 1.9 × 10^6 TCID_50 and 2.7 × 10^6 TCID_50 respectively. T cell-tropic virus stock HIV-1inl (Popovic et al., 1984) was grown in C8166 cells and titrated to 4.4 × 10^6 TCID_50 in PBLs.

Cytokine treatment and HIV infection of target cells. Chinese hamster ovary (CHO) cell-derived hrIL-10 (1.5 × 10^6 U/ml) and neutralizing monoclonal antibody (MAb) 19F1 were kindly provided by Dr K. Moore and Dr R. de Waal Malefyt (DNAX). HrIL-10 titration studies were performed in triplicate cultures of macrophages and PBLs from the same donor with decreasing concentrations of hrIL-10 (< 25 pg endotoxin per μg of protein, LPS-Limulus tested) starting from 500 U/ml. The cytokine was added to 3-day-old macrophage cultures for 72 h, leaving an equal portion of untreated cells for 24 h post-infection (p.i.) treatment, and HIV-infected controls. HIV-1ADA (m.o.i. of 0.12) was added to treated and untreated macrophages (6-day-old), whereas HIV-1inl (m.o.i. of 0.03) was added only to treated PBLs and its control triplicates. Medium and hrIL-10 were replenished every 3 days until 8 days p.i., at which time all treated cells were washed, and medium without any hrIL-10 was replaced thereafter every 3 days until 16 days in macrophages. Culture supernatants were stored at −70 °C until assayed. Growth curves of HIV-1ADA (m.o.i. of 0.12) and HIV-1mo-L (m.o.i. of 0.04) in triplicate macrophage cultures were analysed in 6-well plates. hrIL-10 (50 U/ml) was added 72 h before or 24 h after challenge with HIV-1. Control cultures included uninfected or infected cells with 3 μg/ml 19F1 MAb plus hrIL-10, 3 μg/ml 19F1 MAb alone, or heat-inactivated hrIL-10 (95 °C for 30 min). Medium and hrIL-10 were replaced every 3 days for 16 days.

Growth curves for recovery experiments were performed in triplicate macrophage cultures in 24-well plates with an identical design to those described above; however, these were infected with threefold dilutions of HIV-1e3m (m.o.i. of 0.62, 0.2, 0.069 and 0.023) respectively. hrIL-10 and fresh medium were added every 3 days until 7 days p.i., at which time all treated cells were washed, and medium without any hrIL-10 was replaced thereafter every 3 days until 14 days p.i. Supernatants were collected every 2 days and stored at −70 °C.

Analysis of viral reverse transcription kinetics by PCR. Macrophages were cultured in 48-well plates (2 × 10^5 cells/well) and treated for 72 h with 10-fold dilutions of hrIL-10 (starting at 200 U/ml) or with a fixed concentration of hrIL-10 (50 U/ml) before infection with HIV-1ADA (m.o.i. of 0.12). Cells treated with dilutions of hrIL-10 (200 U/ml, 20 U/ml, 2 U/ml) were lysed at 0, 2, 4, 8, 12, 16, 20, 24 and 48 h, while cells treated with 50 U/ml were lysed at 0, 2, 4, 8, 12, 16, 20, 24, 36, 48, 60 and 72 h p.i. in a buffer containing 100 mM-KCl, 20 mM-Tris pH 8.4, 500 μg/ml protease K and 0.2 % (v/v) NP40. PCR primer sequences used for LTR, gag, and human β-globin gene (DNA control) and the PCR assay were as described (Collin & Gordon, 1994), using a Programmable Thermal Controller (M.J. Research Inc.).

Quantitative PCR. This was performed as described (Collin & Gordon, 1994), comparing experimental samples with known amounts of HXB2 DNA (NIH AIDS reagent program). The proviral DNA was excised by XbaI digestion and titrated into macrophage cell lysate. HXB2 titration was confirmed by comparison with an LTR PCR product from known dilutions of ACH-2 cells (Clouse et al., 1989) which contain one HIV-1 DNA copy per cell. PCR products were measured by incorporation of [α-^32P]ATP (Amersham) via end-labelling of the 3’ primer. Triplicate samples of amplified fragments were resolved on a 2% agarose gel and dissolved in scintillant for liquid scintillation spectrometry (LS5000 CE; Beckman Instruments). Experimental values were interpolated from a curve fitted to HXB2 DNA titration values using a hyperbolic function (Sigma Plot, Jandel Scientific).

Detection of CD4, FcyR1 and HLA-DR/II/D4 in macrophages. Macrophages were treated with hrIL-10 (50 U/ml) for 24 h and assayed for CD4, CD64 and MHCII expression by FACS analysis (Becton Dickinson & Co.). ADP 318/C4120 Mouse IgG1 MAb (MRC AIDS-directed Programme) against CD4, 10.1 mouse IgG1 against CD64 (FcR1), and 52 mouse IgG1 against MCHII DR/D4 (both 10-1 and 52 kindly provided by Dr Nancy Hogg, Imperial Cancer Research Fund, London, U.K.) were used. MOPC-21 (Sigma) mouse IgG1 was used in both hrIL-10-treated and untreated samples as an isotype-matched antibody control. Fluorescein-isothiocyanate-conjugated rabbit anti-mouse Ig (RAM-FITC) (Serotec Ltd) was used to detect bound antibody.

Reverse transcriptase (RT) activity assays. These were performed as described (Montaner et al., 1993). In brief, 5 μl of culture supernatants were added to 50 μl of a mixture containing poly(A), oligo(dT) (Pharmacia), MgCl_2, and ^3P-labelled dTTP (Amersham) and incubated at 37 °C. Three μl of the mixture were spotted in duplicate onto two different DE81 papers, dried and washed in 2 × SSC buffer and 95 % ethanol. One paper was then dried, cut and assayed by scintillation counting while the other was analysed by phosphorImaging (Molecular Dynamics). This assay is less sensitive (by 10-fold) in comparison to the ‘in-house’ p24 assay described below.

HIV ELISA. Supernatants were tested for the p24 antigen using an in-house-adapted ELISA as described (Montaner et al., 1993). Selected macrophage-tropic viral supernatants were tested further with a Coulter HIV-1 p24 kit (Bedfordshire). The sensitivity for macrophage-tropic strains between the two ELISA assays differs by a factor of 100.

Results

Inhibition of HIV-1 replication in macrophages by IL-10

The effect of IL-10 on HIV-1 replication was determined using a range (500 to 0.005 U/ml) of cytokine concentrations added 72 h before or 24 h after virus challenge at a defined m.o.i. In three independent initial experiments, hrIL-10 inhibited virus replication in both HIV-1ADA and HIV-1mo-L-infected macrophages compared with untreated controls, but did not affect HIV growth in PBLs infected with HIV-1inl under identical conditions. Dose
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Response titrations demonstrated that concentrations of hrIL-10 higher than 5 U/ml inhibited extracellular p24 and RT production in macrophages treated with cytokine for 72 h prior to, or 24 h after, challenge with HIV-1AD₂₃ (m.o.i. of 0.12) and subsequently maintained with hrIL-10 (Fig. 1). Anti-IL-10 neutralizing MAb 19F1 as well as heat inactivation of cytokine were used to confirm IL-10 specificity of the anti-HIV effect, excluding LPS or non-specific effects (Fig. 2). The use of 19F1 alone as a control reproducibly resulted in more virus production than infected controls suggesting an inhibition of endogenous IL-10 within infected macrophages (not shown). The morphological assessment of macrophages in these experiments confirmed that continued hrIL-10 treatment of macrophages for 14 days after HIV-1AD₂₃ infection prevented virus-induced syncytia formation and cytolysis while preserving a rounded macrophage morphology (Fig. 3).

IL-10 delays and inhibits HIV-1 reverse transcription kinetics without modulating surface CD4 levels

In analysing potential mechanisms of anti-HIV action of IL-10 we determined whether the cytokine down-regulated the receptor for HIV-1, CD4, on macrophages. FACS analysis demonstrated that treatment with 50 U/ml IL-10 72 h before infection did not alter the levels of surface CD4 on macrophages (Fig. 4).

We examined further steps in the infection cycle by comparing the reverse transcription kinetics in IL-10-treated and control macrophages. We measured early reverse transcription steps using PCR amplification of the provirus LTR (Fig. 5a, b) and gag signal at timed intervals after infection. Our results demonstrate a reproducible 4 to 8 h delay (three independent experiments) of the appearance of provirus DNA in hrIL-10-treated macrophages compared with untreated controls. The HIV LTR signal appeared between 8 to 12 h in untreated controls while appearing between 12 to 16 h in hrIL-10-treated macrophages. In addition, the HIV LTR signal did not increase during the 72 h period after infection (longest examined) of hrIL-10-treated cultures, in contrast to untreated controls, indicating a sustained inhibition of virus production and spread. The HIV LTR signal, indicating the completion of late stages in reverse transcription, was barely detectable in hrIL-10-treated macrophages (not shown). However, HIV-1AD₂₃ recovery experiments in hrIL-10-treated macrophages, described below, indicate that a degree of reverse transcription must have been completed after entry.

To quantify this observation we tested different concentrations of hrIL-10 in duplicate over a 48 h time-course p.i. Inhibition of the proviral HIV-1 LTR signal increased from 77% at 24 h p.i. (Fig. 5c), to 87% at 48 h (not shown) confirming a continued inhibition by hrIL-10. These results suggest an anti-HIV inhibitory mechanism by decreasing the successful completion of virus entry between the stages of binding of the CD4 receptor and the start of reverse transcription. Further investigation of the specific site of action (fusion, accessible nucleotide pools, cellular enzymes, etc.) between these steps will be necessary.

IL-10 acts as a virostatic modulator in macrophages

In determining the effects of IL-10 (50 U/ml) in infected macrophages we examined the recovery of virus from infected cultures after cessation of treatment. To assess whether pretreatment with IL-10 decreased the amount
Fig. 2. HrIL-10 inhibits HIV-1 RT production in macrophages. The figure shows a composite of a phosphorImager exposure of DE81 papers spotted with 3 µl of extracellular supernatants derived from duplicate 16-day growth patterns of HIV-1ADA (m.o.i. of 0.12). Representative growth patterns are shown for infected untreated control, cultures treated with 50 U/ml hrIL-10 72 h before infection and 24 h after infection and HIV-1ADA-infected cultures treated with heat-inactivated (H.I.) IL-10 or IL-10 plus MAb 19F1.

Fig. 3. Phase contrast micrographs to illustrate the morphological effects of 50 U/ml hrIL-10 in macrophages [14 days p.i. with HIV-1ADA (m.o.i. of 0.12)] in 6-well plates. Untreated uninfected (a) and infected (c) controls are shown as references for infected cultures treated with hrIL-10 (b) and hrIL-10 plus neutralizing MAb 19F1. Arrows show characteristic cytolysis and giant cell formation in macrophages owing to HIV infection. IL-10 alone (without HIV infection) showed characteristic rounded morphology (not shown).
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Fig. 4. HrIL-10 does not modulate cell surface CD4+ levels in macrophages. FACS analysis for CD4+ fluorescence in macrophages after 72 h pre-infection treatment with 50 U/ml hrIL-10 illustrating a lack of modulation by the cytokine. Untreated (left panel) and hrIL-10-treated (middle panel) CD4+ fluorescence shifts (clear peaks) are compared with non-specific background fluorescence labelling by an isotype-matched MAb (opaque peaks). The overlap of both untreated and hrIL-10-treated specific CD4+ fluorescence shifts is illustrated in the right panel.

of latently established virus we compared the recovery of p24 antigen from cultures treated with cytokine before and after infection, as described with decreasing amounts of HIV-1. Discontinuation of treatment at day 7 after infection of both groups was followed by a further 7 days of culture in which recovery of virus production was measured (Fig. 6). As expected, recovery of virus production was proportional to the initial m.o.i. within each particular treatment group. However recovery between treatment groups differed although identical m.o.i. values were used at the time of infection. The recovery from cultures treated before infection yielded consistently lower levels of virus than those which had been treated p.i. These results suggest that (i) preinfection treatment decreases virus load in macrophages upon infection, and (ii) IL-10 acts as a mediator of a virostatic latent state after infection.

Virus-independent assays measuring IL-10 biological activity in macrophages

To confirm the bioactivity of IL-10 and its neutralization by the MAb 19F1, we tested described morphological, phenotypic, and functional effects of this cytokine in macrophages. IL-10-induced morphological modulation was characterized by rounding of cells compared with untreated controls (not shown). Accordingly, anti-HIV effects and morphological effects corresponded to the same concentrations of IL-10 as described above. FACS analysis confirmed an increase of FcγR1 surface expression, and a down-regulation of MHCII (not shown), both of which have been reported as IL-10 effects on macrophages (Fan et al., 1993; de Waal Malefyt et al., 1992). Functional activity of IL-10 was also established by inhibition of mRNA IL-1β production in macrophages after LPS stimulation (not shown), an inhibition blocked by MAb 19F1. These results confirmed that we were using a biologically active IL-10 as measured by known criteria.

Discussion

We have previously presented a defined model system to study macrophages and corresponding PBLs from normal individual donors upon challenge with different macrophage- and T cell-tropic HIV-1 strains (Montaner et al., 1993). In the present study we demonstrate that IL-10 can promote a virostatic latent state within macrophages by inhibiting virus replication in the continued presence of IL-10. To determine the effect of IL-10 on virus growth in macrophages we treated cells from 72 h before or 24 h after infection with decreasing concentrations of cytokine. Concentrations higher than 5 U/ml IL-10 inhibited HIV-1AAd in macrophages in both treatment protocols (Fig. 1). The specificity of the inhibition by IL-10 was confirmed by the use of a neutralizing MAb to IL-10 (19F1) and by heat inactivation (Fig. 2). In addition, the use of 19F1 alone resulted in a higher virus output suggesting an inhibition by endogenously produced IL-10 within infected cultures. Titrations of IL-10 with or without virus showed a positive correlation between concentrations of IL-10 required to induce morphological changes (rounded cell morphology) with those to inhibit HIV-1 growth in macrophages. This rounded morphology was maintained throughout the inhibition of HIV-1, contrasting with giant cell formation and cytolysis present in untreated infected controls (Fig. 3).

In determining potential anti-HIV mechanisms of action by IL-10 we demonstrate that CD4 expression
Fig. 5. HrIL-10 delays/reduces entry/early reverse transcription in macrophages without modulating membrane-associated CD4+ levels. The figure shows one of three independent time sequences over 72 h p.i. by HIV-1AD A (m.o.i. of 0·12). (a) Two double-loaded 2% agarose gels with PCR products for HIV LTR (540 bp) and human ß-globin control (238 bp) after HIV infection in untreated macrophages. (b) Similar format, but for macrophages treated with 50 U/ml hrIL-10 (72h before HIV infection). (c) Quantification of the copy number per 1000 cells of HIV-1 LTR 24 h p.i. with HIV-1AD A (m.o.i. of 0·12) at three concentrations of hrIL-10 (2, 20 and 200 U/ml). Representative experiments are shown.

was not affected (Fig. 4) but that there was a delay and reduction of the initial quantity of reverse transcription after infection, delayed by 4 to 8 h and reduced by 77% at 24 h p.i. (Fig. 5). Later steps of reverse transcription such as gag expression were barely detectable in the IL-10-treated cells owing to the low LTR signal and the inherently inefficient completion of reverse transcription in untreated macrophages described by Collin & Gordon (1994). Therefore, IL-10 can decrease the amount of virus successfully infecting macrophages in addition to inhibiting virus production within infected macrophages.

Interestingly, we have observed that IL-10 decreases endocytic uptake of dextran, a fluid phase marker, by macrophages (manuscript in preparation). This related effect potentially provides a mechanism by which viral entry could be delayed and subsequently reduced without down-regulating CD4+ or totally inhibiting infection. Alternatively, other mechanisms that might delay and inhibit reverse transcription include down-modulation of other membrane molecules needed for binding, or inhibition of cellular components involved in virus fusion, uncoating or reverse transcription. This effect is in contrast to that of other Th-2-like cytokines such as IL-4 (not shown) and IL-13 (Montaner et al., 1993), which inhibit virus production independent of effects on the total initial amount of DNA measured at the onset of reverse transcription (LTR). However, IL-4 has been reported to inhibit the completion of reverse transcription (Schuitemaker et al., 1992b). Further experiments are needed to characterize the specific mechanism by which IL-10 may decrease initial reverse transcription after virus binding.

The anti-HIV effect of IL-10 in macrophages depended on the continued presence of cytokine as shown by virus recovery experiments after cytokine washout from cultures previously p24 antigen- and RT-free (Fig. 6). Furthermore we demonstrated a lower recovery in pretreated compared with post-treated cultures although they were infected at an identical multiplicity, suggesting that IL-10 pretreatment decreases the amount of successfully infected macrophages.

IL-10 may also inhibit the efferent stage of the HIV-1 life-cycle since it inhibits TNF-α and IL-1β production in macrophages, both of which are produced after HIV-1 infection and subsequently up-regulate virus production.

Fig. 6. HIV-1 is recoverable from IL-10-treated macrophages after washout of cytokine. P24 antigen levels (extracellular) 14 days p.i. are shown for different m.o.i. (0·62, 0·2, 0·069 and 0·023) in triplicate cultures. Results are derived from hrIL-10-treated macrophage cultures (72 h pre-infection and 24 h after infection) maintained with cytokine for 7 days p.i. and without cytokine for a further 7 days. Solid bars indicate control groups, and shaded bars, (■) and (□) correspond to cultures treated pre-infection and post-infection, respectively. Data are presented in ng/ml as the mean (±S.D.) of triplicate assays for each m.o.i. and treatment used.
in macrophages (de Waal Malefyt et al., 1992; Herbein et al., 1994; Poli & Fauci, 1992). This mechanism is further suggested by the recent study showing that neutralization of endogenous TNF-α or IL-1β by antibody blocks HIV-1 replication in PBMCs (Kinter et al., 1994) and our observation in which IL-10 inhibits virus production if added 24 h after infection (Fig. 1 and 2). Therefore, in addition to decreasing initial viral infection, IL-10 may contribute to the maintenance of post-integration HIV latency by its inhibition of monocyte IL-6, TNF-α and IL-1β production.

Taken together these results implicate IL-10 as a candidate mediator of HIV-1 latency within macrophages in vivo. Although the asymptomatic period of AIDS is characterized by partially maintained Th-1 (IL-12, IFN-γ) responses and a predominance of isolation of macrophage-tropic HIV isolates (obtained from activated PBMC cultures), we cannot rule out a possible role of IL-10 during this period in mediating macrophage viral latency. Recent patient-derived PBMC studies have shown either no difference or increased IL-10 secretion patterns (endogenous and upon activation) in HIV-infected cultures in relation to disease progression, even though both reports describe increased IL-10 levels in infected versus uninfected patients at all times tested (Chehimi et al., 1994; Clerici et al., 1994). Additionally, preliminary in vivo data suggest that there is no increase in IL-10 mRNA expression in lymph nodes and peripheral blood during progression to AIDS (Graziosi et al., 1994). Taken together, the data to date are diverse in relation to the role of IL-10 throughout AIDS pathogenesis. Yet, it is interesting to note current opinions postulating that during end-stage disease the loss of cell-mediated responses is concurrent with an increase of Th-2 cytokines such as IL-10, and is further characterized by an increase of lymphocyte-tropic isolates (Clerici & Shearer, 1993; Clerici et al., 1994; Schuitemaker et al., 1992a). Further experiments should address the potential effects of cytokines such as IL-10, IL-13 and IL-4 (all Th-2-like anti-HIV in macrophages) in determining viral tropism in vivo.

IL-10 should be added to other reported inhibitors of HIV in macrophages such as IFN-α, IFN-β and both Th-1 (IFN-γ, IL-13) and Th-2 (IL-4, IL-13) cytokines as potential mediators of viral latency in macrophages in vivo (Montaner et al., 1993; Poli & Fauci, 1992; Kazazi et al., 1992; Schuitemaker et al., 1992b). IL-4 and IFN-γ have also been reported to up-regulate HIV-1 within macrophages, yet undefined factors such as differences in culture conditions, timing of treatments, cytokine doses used, cell differentiation and donor variation have been proposed in explaining these variable effects.

In conclusion, IL-10 provides a possible mechanism by which activated T lymphocytes and macrophages can suppress HIV replication within macrophages in lymphoid tissue and inhibit the dissemination of monocytotropic variants.

Note. At the time of revision we learned of two recent studies relating to HIV-1 infection by IL-10 in monocyte cell lines (Saville et al., 1994) and primary cells (Weissman et al., 1994). Weissman’s study supports an anti-infection IL-10 inhibitory mechanism via blockage of endogenous TNF-α and IL-6 in primary macrophages.

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