Opposite Effects of IL-10 on the Ability of Dendritic Cells and Macrophages to Replicate Primary CXCR4-Dependent HIV-1 Strains

Petronela Ancuta, Youssef Bakri, Nicolas Chomont, Hakim Hocini, Dana Gabuzda and Nicole Haeffner-Cavaillon

*J Immunol* 2001; 166:4244-4253; doi: 10.4049/jimmunol.166.6.4244
http://www.jimmunol.org/content/166/6/4244

**References**  This article cites 74 articles, 32 of which you can access for free at: http://www.jimmunol.org/content/166/6/4244.full#ref-list-1

**Subscription**  Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**  Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**  Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Opposite Effects of IL-10 on the Ability of Dendritic Cells and Macrophages to Replicate Primary CXCR4-Dependent HIV-1 Strains

Petronela Ancuta,* Youssef Bakri,** Nicolas Chomont,* Hakim Hocini,* Dana Gabuzda,§ and Nicole Haeflner-Cavaillon*†

We investigated the effect of IL-10 on replication of primary CXCR4-dependent (X4) HIV-1 strains by monocyte-derived dendritic cells (DCs) and macrophages (MΦs). MΦs efficiently replicated CXCR4-dependent HIV-1 (X4 HIV-1) strains NDK and VN44, whereas low levels of p24 were detected in supernatants of infected DCs. IL-10 significantly increased X4 HIV-1 replication by DCs but blocked viral production by MΦs as determined by p24 levels and semiquantitative nested PCR. IL-10 up-regulated CXCR4 mRNA and protein expression on DCs and MΦs, suggesting that IL-10 enhances virus entry in DCs but blocks an entry and/or postentry step in MΦs. The effect of IL-10 on the ability of DCs and MΦs to transmit virus to autologous CD4+ T lymphocytes was investigated in coculture experiments. DCs exhibited a greater ability than did MΦs to transmit a vigorous infection to CD4+ T cells despite their very low replication capacity. IL-10 had no effect on HIV-1 replication in DC:T cell cocultures but markedly decreased viral production in MΦ:T cell cocultures. These results demonstrate that IL-10 has opposite effects on the replication of primary X4 HIV-1 strains by DCs and MΦs. IL-10 increases X4-HIV-1 replication in DCs but does not alter their capacity to transmit virus to CD4+ T lymphocytes. These findings suggest that increased levels of IL-10 observed in HIV-1-infected patients with disease progression may favor the replication of X4 HIV-1 strains in vivo.

Strains of HIV-1 isolated from recently infected individuals are predominantly macrophage (MΦ)1-tropic, nonsyncytium inducing, and use CCR5 as a coreceptor in combination with CD4 (1). Later in the course of HIV-1 disease, the virus frequently expands its coreceptor use to include CXC chemokine receptor (CXCR) 4, CCR3, CCR2, and other coreceptors in addition to CCR5, resulting in a selective advantage for the virus (2). The emergence of CXCR4-dependent HIV-1 (X4 HIV-1) strains precedes a more rapid decline in CD4+ T cell counts and progression to AIDS, suggesting that X4 strains contribute to AIDS pathogenesis (3). Furthermore, an early acquisition of CXCR4 usage predicts a poor prognosis (1, 4). Because the rate of HIV turnover in vivo is high (5, 6) and a small number of amino acid mutations in gp160 envelope glycoprotein is sufficient to change viral tropism (7, 8), it is not clear why CXCR4-dependent viruses do not emerge earlier.

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. After several controversial reports, it is now established that dendritic cells (DCs) and MΦs play an important role in the natural history of HIV-1 infection. DCs and MΦs are assumed to be the first targets of viral infection in the genital or rectal mucosa (9, 10) and serve as long-term reservoirs in chronically HIV-1-infected patients (11–15). Tissue MΦs represent a highly productive source of HIV during opportunistic infections at advanced stages of HIV disease (15), whereas DCs exhibit a high capacity to transmit virus to CD4+ T lymphocytes, even in the absence of DC infection per se (16–19). DCs and MΦs express CCR5 and are highly susceptible to infection by CCR5-dependent HIV-1 (R5 HIV-1) strains (16, 20–23). DCs and MΦs also express functional CXCR4 HIV-1 coreceptor and support replication of primary X4 HIV-1 strains (24, 25). However, laboratory-adapted X4 HIV-1 strains such as IIIB appear to be blocked during postentry steps in MΦs (26). Interestingly, exposure to bacterial products renders MΦs highly susceptible to X4 HIV-1 strains in vitro (27). Similarly, it has been reported that maturation of DCs is associated with up-regulation of CXCR4 expression and a higher susceptibility of DC to infection with X4 HIV-1 strains (28, 29). These observations raise the possibility that DCs and MΦs may be involved in promoting the transition from an R5 to X4 HIV-1 phenotype during the natural course of HIV infection.

Several studies have reported a Th1/Th2 shift in the cytokine pattern from production of IL-2/IFN-γ toward IL-4/IL-10 during the course of HIV-1 infection (30). A shift from R5 to X4 HIV-1 variants has also been associated with disease progression (1). Based on these observations, it has been suggested that...
Th2 cytokines may have an important role in the evolution of HIV-1 tropism (31). The cytokine environment may determine susceptibility to HIV infection in part by the regulation of HIV-1 coreceptor expression (32). IL-4 down-regulates CCR5 but up-regulates CXCR4 expression on CD4+ T cells and favors high replication of X4 HIV-1 strains (33). IL-4 can also increase HIV-1 replication in DC:T cell cocultures by stimulating T cell proliferation (34). IL-4 treatment renders DCs highly susceptible to infection with X4 HIV-1 strains because of CXCR4 up-regulation (25). In contrast, IL-4 diminishes CCR5 expression and replication of R5 HIV-1 strains by MФs (32, 35). These observations suggest that IL-4 may play a role in the switch of HIV-1 strains from an R5 to X4 phenotype in vivo (36).

Increased levels of IL-10 in serum of HIV-1-infected patients have been reported to coincide with a dramatic decrease in CD4+ T cells counts and progression to AIDS (37, 38). The role of IL-10 in the emergence of X4 HIV-1 strains in vivo is unknown. It has been reported that IL-10 up-regulates CCR5 expression on monocyte (Mo)/MФ but blocks R5 HIV-1 replication at a postentry step (32). In contrast, IL-10 decreases both CCR5 expression and R5 HIV-1 strain replication in CD4+ T lymphocytes (39). Studies on the effect of IL-10 on CXCR4 expression on CD4+ T lymphocytes have reported contradictory results (39, 40).

In view of the coincidence between the emergence of X4 HIV-1 strains and the high levels of IL-10 in the serum of patients at advanced stages of HIV-1 disease, we investigated the effect of IL-10 on the capacity of Mo-derived MФs and DCs to replicate primary X4 HIV-1 strains. We also examined the effect of IL-10 on X4 HIV-1 replication in MФ/DC and T cell cocultures, which more closely mimic the microenvironment of lymphoid tissues. In this study, we show that IL-10 inhibits replication of X4 HIV-1 strains in MФs but significantly increases viral replication in DCs. We also show that IL-10 decreases X4 HIV-1 replication in Mo:T cell cocultures, but has no inhibitory effect on viral replication in DC:T cell cocultures. These results suggest that IL-10 may favor the emergence of X4 HIV-1 strains at advanced stages of HIV disease.

Materials and Methods

Reagents and Abs

Human rM-CSF, GM-CSF, IL-4, and IL-10 were purchased from R&D System Europe (Oxon, U.K.). Anti-CD14 (My4)-FITC, anti-CD16-PE/Cy5, anti-CD11b-PE, anti-CD80-FITC, anti-CD86-FITC, anti-CD83-PE mAbs were from Immunotech (Beckenm Coulter, Villepinte, France); anti-CCR5-PE, anti-CXCR4-PE anti-HLA-DR-FITC, anti-CD33-PE, anti-CD4-PE, and anti-CD3-PE mAbs were from BD Becton Dickinson (Le Pont de Claix, France). Anti-CD40-FITC mAb was from Calbiochem-Novabiochem (San Diego, CA). Anti-CD1a-PE, Anti-CD1a-PE mAbs were purchased from Tebu (Le Perray, France).

Purification of CD4+ T lymphocytes

PBMCs were isolated from the buffy coats of healthy donors by Ficoll gradient (Eurobio, Les Ulis, France). T lymphocytes were isolated from PBMC by E-rosette formation using 2-aminoethylisothiouronium bromide (Eurobio, Les Ulis, France). T lymphocytes were isolated from PBMC by using Dynabeads M-450 CD8 (Dynal France, Le Perray, France).

Flow cytometry analysis

The expression of membrane Ags by the different cell subsets was analyzed by flow cytometry using three-color direct immunofluorescence. After incubation with the different mAbs for 30 min at 4°C, cells were washed twice with PBS containing azide (0.01%), BSA (0.2%), and fixed using a 1% formaldehyde PBS buffer. An aliquot of cells fixed with a 4% formaldehyde PBS buffer was subsequently stained with fluorochrome anti-CXCR4 mAbs in the presence of a saponin buffer. Stained cells were analyzed using a FACSCalibur flow cytometer (BD Becton Dickinson) and the CellQuest software. Subsequent gating according to light-scattering properties and CD33 expression permitted us to identify Mo-derived cells.

Detection of CXCR4 mRNA

RT-PCR was performed using a modified RT-PCR protocol previously described by Trujillo et al. (44). Total RNA was isolated using RNaseasy mini Kit (Qiagen, Courtaboeuf, France). Extraction columns were dried under vacuum, and RNA samples were resuspended in 20 µl of diethylpyrocarbonate water. A series of increasing amounts of cDNA (5 µl, 2.5 µl, and 1 µl) from the cDNA reaction mixture were subjected to PCR amplification using Taq polymerase (Roche Diagnostic Systems, Meylan, France) in an automated PTC 200 Thermal Cycler (Corbett Research, Erithrocide H; Appligene, Strasbourg, France). After 32 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 40 s (CXCR4), or 55°C for 60 s (GAPDH), and extension at 72°C for 40 s, followed by a final extension at 72°C for 15 min. The following primers were used for CXCR4: 5’-GTT ACC ACC TGG AGG GAG TTC A-3’ and 5’-CAG ATG AAT GTA CAC TCT GC-3’. For GAPDH, 5’-TGG AGG GTA GCA GAC GAC CCC-3’ and 5’-CAG ATG AAT GTA CAC TCT GC-3’. PCR products (15 µl) were visualized under UV transillumination by ethidium bromide staining after electrophoresis on a 2% agarose gel.

HIV strains and infections

X4 HIV-1ndK and HIV-1v44 strains (45, 46) were kindly provided by Dr. Françoise Barré-Sinoussi (Pasteur Institute, France). After 6 days of differentiation, MФs and DCs obtained in the presence or absence of IL-10 were incubated with 4 or 20 ng/ml of DNase-treated viral suspension for 12 h. Heat-inactivated virus (1 h at 56°C) was used as a negative control. Cells were then washed three times, counted, and further cultured in RPMI 1640 medium 10% FCS containing 10% human recombinant cytokines (42). In each experiment, MФs and DCs were cocultured with autologous CD4+ T lymphocytes and were harvested every 3 days and appropriate cytokines were then added together with fresh medium. The kinetics of viral production was followed by sequential measurement of p24 in duplicate supernatants using the NIH-1 p24 core profile ELISA kit (NEN Life Sciences Products, France).
Detection of HIV-1 DNA

HIV-1 DNA was detected by nested PCR as described (29). Briefly, MΦs and DCs were exposed to 4 or 20 ng/ml/10^6 cells HIV-1NDK or HIV-1N4NL and analyzed for the expression of Pol HIV-1 DNA products 24 h later. Cells were then washed and lysed in a buffer containing 10 mM Tris-HCl (pH 8.3), 50 mM NaCl, 0.5% Tween 20 (Bio-Rad, Hercules, CA), 0.5% Nonidet (Sigma, St. Louis, MO) and proteinase K (20 μg/ml Boehringer, Mannheim, Germany). Cell lysates (10^6 cells/ml) were incubated for 1 h at 56°C, and proteinase K was then inactivated at 90°C for 10 min.

Relative amounts of virus in different samples were estimated by endpoint dilutions of the lysates in lysed HIV-1 CEM cells (1 × 10^6/ml). Serially diluted samples (30 μl) were added to 0.5 μl of each primer, 25 mM dNTP, Taq DNA polymerase (Roche Diagnostic Systems) in 50 μl final volume. After 5 min at 94°C, 35 cycles were performed in an automated DNA Thermal Cycler (Crocodile III; Appligene), each consisting of 1 cycle at 94°C, 1 min at 55°C, and 1 min at 72°C. Pol primers were P3 (5'-TGG GAA GTT CAA TTA GGA ATA CCA C-3) and P4 (5'-CTT CTA TCC ATG TAT TGG T-3) (Perkin-Elmer, Foster City, CA).

For the nested PCR, 2 μl of amplified products were submitted to another 35 cycles of amplification under the same conditions using internal primers P5 (5'-ATC AGT AAC AGT ACT GGA TGT G-3) and P6 (5'-GGAT AGA TAA CTA TGT CTG GAT T-3) (Perkin-Elmer). PCR sensitivity was 1 copy/3 × 10^3 cells as determined relative to serial dilutions of 8E5/LAV cells (1 copy HIV/cell) in HIV-1 CEM cells. The approximate number of DNA copies was determined relative to the standard curve obtained with 8E5 cells. PCR was also performed with β-globin primers PCO4 (5'-CAG TTC ATC ATC CAC GAT CAC C-3) and GH2 (5'-GAA GGT CAC AGG ACA GTG AC-3) (Perkin-Elmer) as amplification and DNA content controls. Nested PCR products (15 μl) were visualized under UV transillumination by ethidium bromide staining after electrophoresis on a 2% agarose gel.

RANTES production

RANTES production by MΦs, MΦ/IL-10s, DCs, and DC/IL-10s was quantitated in supernatants from HIV-1X4NL-infected cells (2 × 10^3 cells/ml) at day 3 postinfection by ELISA (Medigenix Diagnostic, Fleurus, Belgium).

Results

Effect of IL-10 on Mo differentiation into MΦs or DCs

MΦs were differentiated into MΦs and DCs in the presence or absence of IL-10. At day 6, the expression of cell surface molecules specific for Mo/MΦ (CD14 and CD16) and for DC (CD1a and CD83) lineage as well as molecules involved in Ag presentation (CD40, CD80, CD86, HLA-DR) and phagocytosis (CD11b, CD11c) was assessed by flow cytometry analysis (Table I).

Cells cultured in the presence of M-CSF (MΦs) were adherent and exhibited typical MΦ morphology (Fig. 1A). MΦs expressed high levels of CD14, CD16, CD11b, and CD11c, low levels of CD40, HLA-DR, and a low percentage expressed CD80, CD86, and CD1a molecules (Table I). As expected, MΦs were CD83^- (Table I). Mo differentiated in the presence of M-CSF plus IL-10 (MΦ/IL-10) were adherent and exhibited a morphology similar to that of MΦs (Fig. 1B). Phenotypic analysis indicated that MΦ/IL-10 did not express CD80, CD86, CD1a, and CD83 markers but expressed similar levels of CD14, CD16, HLA-DR, and CD11c, and lower levels of CD11b and CD40, compared with MΦs (Table I).

When MΦs were cultured in the presence of GM-CSF plus IL-4, they differentiated into DCs that exhibited a characteristic irregular shape, formed multiple nonadherent colonies (Fig. 1C), and acquired the expression of CD1a in parallel to a progressive loss of expression of CD14 (Table I) (42, 48, 49). They were CD83^low and CD86^low and a minority were CD16^+. DCs expressed higher levels of CD80, CD40, HLA-DR, and CD11b, but lower levels of CD11c, compared with MΦs (Table I). The combination of GM-CSF plus IL-4 plus IL-10 induced Mo differentiation into adherent cells (DC/IL-10) that exhibited a ramified morphology (Fig. 1D). DC/IL-10 expressed similar levels of CD40, CD86, CD11b, and CD11c compared with DCs. The expression of HLA-DR molecules by DCs was significantly increased by IL-10: mean fluorescence intensity (MFI) 103.4 ± 35.8 for DCs and 170 ± 51.5 for DC/IL-10 (p = 0.008, Student’s t test). DC/IL-10 expressed low levels of CD1a and did not express CD83 or CD80 (Table I). Phenotypic analysis showed that DC/IL-10s are more closely related to DCs than to MΦs, despite their expression of CD16 and CD14 markers (Table I). No cellular proliferation was detected in MΦs, MΦ/IL-10s, DCs, and DC/IL-10 cultures using a [3H]thymidine uptake assay, and IL-10 did not induce loss of cell viability as determined by trypan blue staining (data not shown).

Replication of primary X4 HIV-1NDK strain in MΦs and DCs

We examined the ability of MΦs and DCs to support productive infection with the primary X4 HIV-1NDK strain. As shown in Fig. 2, MΦs infected with HIV-1NDK replicated the virus to high levels, with p24 levels reaching 13 792 pg/ml at day 12 postinfection. In contrast, low levels of viral replication occurred in DC cultures infected with HIV-1NDK, with p24 levels reaching only 40 pg/ml at day 12 postinfection (Fig. 2). To test the ability of MΦs and DCs to transmit virus to CD4^+ T lymphocytes, HIV-1NDK-infected MΦs and DCs were cocultured with PHA-stimulated autologous CD4^+ T cells. The results indicated a significant increase in HIV-1NDK replication in DC:T cell cocultures (1088-fold higher compared with DC alone), with p24 levels reaching 43,520 pg/ml at day 12 postinfection (Fig. 2). This increased viral replication was strictly dependent on physical contact between the two cell types, as determined by using a transwell system (data not shown). No significant change in viral production was observed when MΦs were cocultured with CD4^+ T cells (13 792 pg/ml vs 14 544 pg/ml)
ml, at day 12 postinfection) (Fig. 2). However, MΦ transmission of virus to PHA-stimulated CD4⁺ T cells could be detected when they were infected with lower doses of HIV-1 NDK (4 ng/ml instead of 20 ng/ml) or with R5 HIV-1 strains (data not shown). These results indicate that despite very low levels of viral replication, DCs transmitted the virus to CD4⁺ T cells more efficiently than MΦs, which can support high levels of virus replication in the absence of T cell cocultures.

**IL-10 inhibits replication of primary X4 HIV-1 strains in MΦs**

We investigated the effect of IL-10 on viral production by MΦs infected with HIV-1 NDK (20 ng/ml/10⁶ cells). IL-10 was present in the cell cultures during the differentiation and infection periods. The results in Fig. 3A show that IL-10 inhibited viral replication by MΦs. The p24 levels decreased from 3.49 log in the absence of IL-10 to 0.04 log in the presence of IL-10, at day 12 postinfection. Parallel experiments were performed using another primary X4 HIV-1 strain, VN44 (20 ng/ml/10⁶ cells). IL-10 treatment also inhibited replication of HIV-1 VN44 in MΦs (data not shown). We also examined the HIV DNA content in HIV-1 NDK-infected MΦs and MΦ/IL-10s at 24 h postinfection. We detected 10⁴ copies of Pol HIV-1 DNA/10⁶ cells in MΦs and 10² DNA copies/10⁶ cells in MΦ/IL-10s (Fig. 3B), indicating that MΦ/IL-10s harbored 2 log less HIV DNA copies compared with MΦs. A similar inhibitory effect of IL-10 on HIV DNA copy number was observed when MΦs were infected with HIV-1 VN44 (data not shown).

**IL-10 regulation of CD4 and CXCR4 expression in MΦs**

To determine whether IL-10 decreased X4 HIV-1 replication and viral DNA content in MΦs by diminishing their permissiveness to virus entry, we examined the expression of CD4 and CXCR4 molecules on MΦs and MΦ/IL-10s. Flow cytometry analysis showed that MΦs expressed both CD4 (82%, MFI 26) and CXCR4 (92%,...
NDK (20 ng/ml/10^6 cells) in the presence or absence of IL-10 (10 ng/ml) were infected with HIV-1NDK (20 ng/ml/10^6 cells) for 12 h. At day 6 of differentiation, MΦ/IL-10s were obtained as described in Materials and Methods. MΦ/IL-10s, MΦ/IL-10 plus AD were stained with anti-CXCR4-PE mAbs (right: surface intracellular). The expression of CXCR4 molecules was determined by flow cytometry analysis. Dot plot analyses from one experiment representative of five independent experiments performed on cells from different donors are shown.

MΦ 53). IL-10 induced up-regulation of both CD4 (96%, MFI 39) and CXCR4 (99%, MFI 128) (Fig. 4). The discrepancy between the up-regulation of CD4/CXCR4 expression on MΦ/IL-10s and inhibition of viral replication suggests that reduced viral replication in MΦ/IL-10s may result from either a postentry block and/or an effect of IL-10 on biochemical properties of CXCR4.

To gain insights into the mechanism of CXCR4 regulation by IL-10 in MΦs, we analyzed CXCR4 mRNA and protein expression in MΦs and MΦ/IL-10s, by RT-PCR and flow cytometry analysis, respectively. The results indicated an increase in CXCR4 mRNA expression in MΦ/IL-10s compared with MΦs (Fig. 5A). Treatment of MΦ/IL-10s with actinomycin D (AD) (1 μg/ml) diminished CXCR4 mRNA levels (Fig. 5A) but induced only a slight decrease in intracellular protein expression (Fig. 5B). Cell surface expression of CXCR4 was similar in MΦ/IL-10s in the presence or absence of AD (Fig. 5B). These results suggest that IL-10 regulates CXCR4 expression in MΦs at the translation or posttranslation level rather than at the transcription level.

**IL-10 increases replication of primary X4 HIV-1 strains in DCs**

We then investigated the effect of IL-10 on HIV-1NDK replication by DCs. Viral production was assessed in culture supernatants of DCs cultured and infected in the presence or absence of IL-10 (10 ng/ml) with HIV-1NDK (20 ng/ml/10^6 cells). Surprisingly, in contrast to the inhibitory effect of IL-10 on HIV-1NDK replication by MΦs, we observed that IL-10 significantly increased viral production by DCs (1.80 log vs 2.99 log, at day 12 postinfection) (Fig. 6A). Similar experiments were performed using another primary X4 HIV-1 strain, VN44 (20 ng/ml/10^6 cells). IL-10 inhibition of viral replication in MΦ/IL-10 (data not shown). These results together with the preceding experiments demonstrate that IL-10 has opposite effects on the replication of X4 HIV-1 strains in MΦs and
DCs. We next determined the HIV DNA content in HIV-1 NDK-infected DCs and DC/IL-10s. We detected $10^2$ copies of Pol HIV DNA/10^6 cells in DCs and $10^3$ Pol HIV DNA copies/10^6 cells in DC/IL-10 (Fig. 6B), indicating that DC/IL-10 harbored 1 log more HIV DNA copies compared with DCs. A similar effect of IL-10 on viral DNA copy number was observed when DCs were infected with HIV-1 VN44 (data not shown). These results indicate that IL-10-mediated stimulatory effects on viral replication are associated with increased viral entry and/or reverse transcription.

DCs. We next determined the HIV DNA content in HIV-1 NDK-infected DCs and DC/IL-10s. We detected $10^2$ copies of Pol HIV DNA/10^6 cells in DCs and $10^3$ Pol HIV DNA copies/10^6 cells in DC/IL-10 (Fig. 6B), indicating that DC/IL-10 harbored 1 log more HIV DNA copies compared with DCs. A similar effect of IL-10 on viral DNA copy number was observed when DCs were infected with HIV-1 VN44 (data not shown). These results indicate that IL-10-mediated stimulatory effects on viral replication are associated with increased viral entry and/or reverse transcription.

**FIGURE 6.** Stimulatory effect of IL-10 on HIV-1 NDK replication and viral DNA content in DCs. DCs were differentiated and infected with HIV-1 NDK (20 ng/ml/10^6 cells) in the presence or absence of IL-10 (10 ng/ml) (see Materials and Methods). Each infection was performed in duplicate. Culture supernatants were collected every 3 days and duplicates were pooled and assessed for p24 Ag. A, Values are the mean ± SD of three experiments performed with cells from different donors. DCs and DC/IL-10s were then infected with HIV-1 NDK (20 ng/ml/10^6 cells) for 12 h. At 24 h postinfection, relative Pol HIV DNA content was assessed by nested PCR in limiting dilution of infected cell lysates in uninfected CEM cell lysates. B, β-Globin DNA level was assessed and used as a control for DNA content. The PCR sensitivity was 1 copy/3 × 10^6 cells, as determined in parallel experiments by serial dilutions of 8E5/LAV cells (1 copy HIV DNA/cell) in HIV-1 CEM cells. Results of one of three similar experiments performed are presented.

IL-10 regulation of CD4 and CXCR4 expression in DCs

To determine whether IL-10 increased X4 HIV-1 replication and viral DNA content in DC by increasing their susceptibility to virus entry, we examined the expression of CD4 and CXCR4 molecules in DC cultured in the presence or absence of IL-10. DC expressed high levels of CD4 (95%, MFI 50) and only a low percentage of cells were CXCR4+ (43%, MFI 15) (Fig. 7). IL-10 had no effect on CD4 expression (97%, MFI 50) but significantly enhanced the expression of CXCR4 molecules was determined by flow cytometry analysis.

**FIGURE 7.** Effects of IL-10 on CD4 and CXCR4 expression by DC. DC and DC/IL-10 were stained with anti-CD4-PE and anti-CXCR4-PE mAbs. The expression of CD4 (left) and CXCR4 (right) on DC and DC/IL-10 was determined by flow cytometry analysis. Dot plot analyses from 1 representative experiment of 10 independent experiments performed are shown.

**Table II.** RANTES production by MΦ, MΦ/IL-10, DC, and DC/IL-10 infected with HIV-1 NDK

<table>
<thead>
<tr>
<th>Expt</th>
<th>MΦ</th>
<th>MΦ/IL-10</th>
<th>DC</th>
<th>DC/IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>549*</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>38</td>
<td>304</td>
</tr>
<tr>
<td>3</td>
<td>6.2</td>
<td>0</td>
<td>117</td>
<td>623</td>
</tr>
</tbody>
</table>

* RANTES production (pg/ml) by HIV-1 NDK-infected cells was detected in the culture supernatants at day 3 postinfection using an ELISA detection Kit (Medgenix Diagnostic). Values represented are for pooled culture supernatants from triplicate wells. Data obtained on cells provided from three different donors are presented.
expression of CXCR4 (Fig. 7). Thus, the percentage of CXCR4^+ cells increased from 38.8 ± 12.2% in the absence of IL-10 (DCs) to 62.9 ± 15.6% in the presence of IL-10 (DC/IL-10) (p < 0.05, Student’s t test).

In parallel experiments, we analyzed CXCR4 mRNA and protein expression in DCs and DC/IL-10s. The results indicated an increase of CXCR4 mRNA expression in DC/IL-10s compared with DCs (Fig. 8A). Treatment of DC/IL-10s with AD (1 μg/mL) importantly diminished CXCR4 mRNA expression (Fig. 8A) and induced a significant decrease in intracellular protein CXCR4 expression (Fig. 8B). Cell surface CXCR4 expression was slightly decreased in DC/IL-10s treated with AD compared with DC/IL-10 (Fig. 8B). These results suggest that IL-10 regulates CXCR4 expression on DCs at the transcription as well as at the posttranscription level.

Production of RANTES by HIV-1_{NDK} infected MΦs, MΦ/IL-10s, DCs, and DC/IL-10s

We assessed the production of RANTES in culture supernatants of MΦs, MΦ/IL-10s, DCs, and DC/IL-10s infected with HIV-1_{NDK} at day 3 postinfection. We detected in two experiments of three a production of RANTES by HIV-1_{NDK}-infected DCs and more significantly we observed in all experiments a high production of this β-chemokine in DC/IL-10 culture supernatants (Table II). These results suggest that RANTES induces cellular activation via CCR5, thus, favoring X4 HIV-1 strains replication by DC/IL-10. Of note, CCR5 expression is also increased on DCs cultured in the presence of IL-10 (data not shown).

Effect of IL-10 on HIV-1_{NDK} replication in MΦ:T cell and DC:T cell cocultures

We tested the effects of IL-10 on the replication of HIV-1_{NDK} in cocultures of MΦ:T cells and DC:T cells. MΦs, MΦ/IL-10s, DCs, and DC/IL-10s were infected with HIV-1_{NDK} and then cocultured with PHA-stimulated autologous CD4^+ T cells. The results in Fig. 9A show that IL-10 efficiently decreased HIV-1_{NDK} replication in MΦ:T cell cocultures (3, 3 log vs 1 log at day 12 postinfection). In contrast, IL-10 had no significant effect on HIV-1_{NDK} replication in DC:T cell cocultures (5, 2 log vs 5, 3 log at day 12 postinfection) (Fig. 9B). In control experiments, we tested the effect of IL-10 and IL-10 plus IL-4 on viral replication by CD4^+ T cells. IL-10 had no significant effect on the level of HIV-1_{NDK} replication by CD4^+ T cells compared with lymphocytes cultured in medium alone (4, 8 log vs 4, 9 log at day 8 postinfection). In contrast, the addition of IL-10 plus IL-4 increased viral production by CD4^+ T cells compared with IL-10-treated cells (4, 9 vs 5, 3 log at day 9 postinfection) (data not shown). These findings demonstrate that IL-10 decreases replication of X4 HIV-1 strains in MΦ:T cell cocultures but has no significant effect on viral replication in DC:T cell cocultures.

Discussion

A Th2 cytokine pattern has been associated with acquisition of the X4 HIV-1 phenotype in infected patients (36). The effect of Th2 cytokines on the replication of X4 HIV-1 strains by primary cells has not been fully elucidated. IL-10 is known to skew the Th1/Th2 balance to a Th2 profile by selectively blocking IL-12 synthesis in DC (50). The frequency of IL-10-producing CD4^+ T cells (51–54) and IL-10 production by in vitro stimulated PBMC are increased in patients at advanced stages of HIV-1 disease (55). Serum levels of IL-10 also have been reported to be increased in HIV-infected patients with disease progression (37). In vitro experiments demonstrated that HIV-1 infection up-regulates IL-10 expression in Mo/MΦ (56). Many reports have demonstrated that IL-10 decreases the replication of MΦ-tropic HIV-1 strains in Mo/MΦ (35, 43, 56–62) despite up-regulation of CCR5 expression (32, 63). In contrast, IL-10 at lower concentrations can act in synergy with several cytokines to enhance viral replication by cells of the monocytic lineage (64, 65).

In the present study, we tested the effect of IL-10 on the susceptibility of MΦs and DCs to infection with primary X4 HIV-1 strains and found that IL-10 significantly increased replication of X4 HIV-1 strains in DCs but abolished viral replication in MΦs. To mimic the IL-10 microenvironment present in HIV-infected patients at late stages of disease, we performed experiments in the continuous presence of IL-10 during cell differentiation as well as during the infection and postinfection periods. The morphology and phenotype of MΦ/IL-10 was similar to that of MΦs. In contrast, DC/IL-10s were morphologically distinct from DCs. DC/IL-10s were adherent and exhibited a ramified morphology whereas DC exhibited an irregular shape and formed multiple nonadherent colonies. DC/IL-10s also exhibited phenotypic differences compared with DCs including higher levels of HLA-DR, lower levels of CD1a, and lack of CD80 and CD83 expression. DC/IL-10s were CD14^-CD16^+ compared with DCs, which express low levels of these molecules. Previous studies reported that IL-10 blocks the differentiation of Mos into DCs but promotes their maturation to MΦs, based on the observation that these cells exhibited higher anti-bacterial activity (66) and lower Ag presenting capacities (67). However, we previously demonstrated that the phenotype and the function of DC/IL-10 (CD14^-CD16^+) remains more closely related to DCs than to MΦs (68). Consistent with this conclusion, Buellens et al. (69) suggested that IL-10 only prevents the maturation of DCs triggered by inflammatory events (e.g., LPS). In
contrast, when DC maturation involves CD40-dependent interactions with T cells, the selective inhibition of IL-12 synthesis by IL-10 would favor the development of Th2-type responses (69). Consistent with these findings, we and others suggest that Mos differentiated in the presence of GM-CSF plus IL-4 plus IL-10 exhibit phenotypic and functional DC-like characteristics (68, 70).

When MΦs and DCs were cultured without IL-10, MΦs efficiently replicated X4 HIV-1NDK, whereas low levels of viral replication were detected in infected DCs. The expression of CXCR4 was higher on MΦs than on DCs, suggesting that MΦs may be more permissive for CXCR4-dependent viral entry. These results are in agreement with previous studies that demonstrated the expression of functional CXCR4 HIV coreceptor on MΦs and DCs and the ability of these cells to be productively infected by primary X4 HIV-1 strains (24, 25).

IL-10 significantly increased replication of HIV-1NDK by DCs at a concentration that abolished viral replication by MΦs. HIV DNA copy number directly correlated with changes in HIV p24 levels in both MΦ/IL-10 and DC/IL-10, compared with MΦs and DCs, respectively. The inhibition of viral replication in MΦs was not due to an effect of IL-10 on cell viability, because the long-term viability of MΦ/IL-10 was slightly increased compared with that of MΦs. We further demonstrated that the opposite effects of IL-10 on viral replication by MΦs and DCs were not restricted to the HIV-1NDK strain, because similar results were obtained with another primary X4 HIV-1 strain, VN44. The opposite effects of IL-10 on viral replication by MΦs and DCs were not due to differential regulation of CD4/CXCR4 expression, because the expression of these molecules was up-regulated by IL-10 in both cell types. Furthermore, IL-10 up-regulated CXCR4 mRNA expression on both cell types.

Treatment of MΦs with AD partially decreased CXCR4 mRNA levels in MΦ/IL-10 without inducing a significant decrease in CXCR4 protein expression. This finding may be explained in part by a high stability of CXCR4 mRNA in MΦ/IL-10. These results also indicate that in MΦs, IL-10-induced increase of surface CXCR4 expression is not directly correlated with CXCR4 mRNA synthesis and/or membrane turnover. IL-10 has been shown to decrease fluid phase pinocytosis and mannose receptor-mediated uptake in MΦs (71). Thus, one possibility is that IL-10 may enhance surface CXCR4 expression by inducing protein accumulation because of a decreased membrane turnover. Recently, Lapham et al. (72) reported that CXCR4 monomers but not high m.w. CXCR4 species can associate efficiently with CD4 and mediate entry of X4 HIV strains. Taken together, these observations raise the possibility that the inhibitory effect of IL-10 on X4 HIV-1 strain replication by MΦs may be a consequence of an alteration in the biochemical properties of CXCR4 molecules or their ability to associate with CD4. This hypothesis remains to be investigated. In contrast to MΦs, AD treatment of DC/IL-10 nearly abolished CXCR4 mRNA expression in addition to diminishing protein expression, suggesting that IL-10 regulates CXCR4 expression at the transcription level. However, the enhancing effect of IL-10 on CXCR4 expression may not be the only mechanism responsible for increased X4 HIV-1 replication by DC/IL-10. It is possible that IL-10 may have indirect effects on the efficiency of viral entry. Kinter et al. (73) reported that RANTES mediates enhancement of X4 HIV-1 strain replication by increasing the colocalization of CD4 and CXCR4 on primary CD4+ T cells. Therefore, we tested the production of RANTES by X4 HIV-1-infected MΦs, MΦ/IL-10, DCs, and DC/IL-10 and found that IL-10 induced high levels of RANTES production in DCs but not in MΦs. Together, these findings raise the possibility that IL-10 may favor CXCR4/CD4 association and entry of X4 HIV-1 strains in DCs as a consequence of cellular activation induced by RANTES via CCR5. It is also possible that IL-10 may have additional effects on early postentry events in the viral life cycle. These hypotheses remain to be investigated. Taken together, our results suggest that viral replication in MΦs and DCs is dependent on distinct cellular factors or pathways differentially regulated by IL-10. Furthermore, using similar culture conditions, we have observed that IL-10 decreased R5 HIV-1 strain replication by both DCs and MΦs. Thus, replication of X4 and R5 HIV-1 strains in DCs is differentially regulated (unpublished observations).

In vivo, both MΦs and DCs interact with T cells in the microenvironment of lymphoid tissue. We demonstrated that DCs but not MΦs exhibit an extraordinary capacity to transmit virus to CD4+ T cells, consistent with previous studies (16, 17, 28, 74, 75). In DC:T cell cocultures, viral production increased 1088-fold compared with that in DCs alone. In contrast, no significant increase was detected in viral production in MΦ:T cell cocultures compared with MΦs alone. In the presence of IL-10, we found complete inhibition of viral production in MΦ:T cell cocultures. IL-10 did not inhibit HIV-1NDK replication in CD4+ T cells alone, in agreement with Patterson et al. (39). Therefore, the inhibition of HIV-1NDK replication in MΦ/IL-10:T cell cocultures by IL-10 is likely to be a consequence of an inhibitory effect on viral replication in MΦs. Of note, IL-10 did not alter the capacity of DCs to transmit virus to CD4+ T cells because viral replication in DC/IL-10:T cell cocultures was similar to that in DC:T cell cocultures. This finding is consistent with our previous study demonstrating that Mos differentiated in the presence of GM-CSF plus IL-4 plus IL-10 exhibit DC-like functional characteristics (68, 70). In contrast to our findings, Weissman et al. (34) demonstrated the capacity of IL-10 to block the replication of the laboratory adapted HIV-1INN strain in DC:T cell cocultures. Possible explanations for this discrepancy include differences in cell culture methods used to generate DCs, and differences between primary vs laboratory-adapted X4 HIV-1 strains.

In conclusion, our results demonstrate that IL-10 inhibits the replication of primary X4 HIV-1 strains in MΦs and MΦ:T cell cocultures but significantly increases viral replication in DCs without affecting viral production in DC:T cell cocultures. The inhibitory effect of IL-10 on viral replication by MΦs could occur during entry and/or postentry steps. In contrast, IL-10 may enhance the susceptibility of DCs to infection with X4 HIV-1 strains during entry and possibly other early steps in the virus life cycle. To our knowledge, this is the first demonstration that IL-10 differentially regulates X4 HIV-1 replication by MΦs and DCs. The molecular mechanisms for this differential effect remain to be determined. Our studies suggest that IL-10 may stimulate the ability of DCs to propagate X4 HIV-1 strains in vivo and support the hypothesis that IL-10 may favor the replication of X4-HIV-1 strains as HIV-infected individuals progress to AIDS.

Acknowledgments
We thank Dr. Françoise Barré-Sinoussi (Pasteur Institute, Paris, France) for providing us X4 HIV-1NDK and HIV-1INN, strains. We also thank Dr. Michel Goldman (Faculty of Medicine, Brussels, Belgium) for critical review of the manuscript and Michel Paing (Broussais Hospital, Paris, France) for editorial assistance.

References


from human immunodeficiency virus-infected individuals studied in relation to IL-10 and prostaglandin E2 production. Blood 89:570.


