Regulation of CC Chemokine Receptor 5 and CD4 Expression and Human Immunodeficiency Virus Type 1 Replication in Human Macrophages and Microglia by T Helper Type 2 Cytokines

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Macrophages, microglia, and other mononuclear phagocytes serve as cellular reservoirs for viral persistence in patients with acquired immunodeficiency syndrome. To understand host mechanisms that affect human immunodeficiency virus type 1 (HIV-1) pathogenesis by modulating expression of coreceptors, cytokine regulation of CC chemokine receptor 5 (CCR5) and CD4 expression on monocytes, monocyte-derived macrophages (MDMs), and microglia was investigated. Interleukin (IL)–4 and IL-10 enhanced the entry and replication of HIV-1 in microglia through up-regulation of CD4 and CCR5 expression, respectively. IL-4 stimulated HIV-1 replication in MDMs but down-regulated CD4 and CCR5 expression and inhibited virus entry, whereas IL-10 had the opposite effects. Thus, mechanisms independent of CCR5 and CD4 expression levels are involved in pathways that regulate HIV-1 replication in MDMs. CCR5 up-regulation by IL-10 was associated with increased migration of microglia in response to macrophage inflammatory protein–1β. These findings suggest that increased production of T helper type 2 cytokines in the later stages of disease can enhance virus entry and replication in mononuclear phagocytes and facilitate chemotactic migration.

Macrophages, microglia, and other mononuclear phagocytes (MPs) are targets for human immunodeficiency virus type 1 (HIV-1) infection and represent a major cellular reservoir for viral persistence in the central nervous system (CNS) and other tissues, including lymph node, spleen, lung, and bone marrow [1–4]. Macrophage-tropic HIV-1 strains initiate primary infection and are present during all stages of HIV-1 disease. Infected monocytes serve as a vehicle for dissemination of virus to the CNS and other target organs [1–3]. Persistent infection of macrophages and microglia can lead to the development of HIV-1–related clinical complications, including encephalopathy, pneumonitis, and bone marrow dysfunction [2–5]. HIV-1 replication in MPs is influenced by immunoregulatory cytokines [6, 7]. Virus production in MPs can be stimulated by some cytokines, such as macrophage colony–stimulating factor, tumor necrosis factor (TNF)–α, and interleukin (IL)–6, whereas other cytokines, such as interferon (IFN)–α and IFN-γ, exert a suppressive effect on HIV-1 replication [6, 7]. Mechanisms underlying the effects of cytokines on HIV-1 replication in MPs are not well defined.

Chemokine receptors are G protein–coupled 7–transmembrane-segment receptors that have diverse and important roles in many biological processes, including immune responses and inflammatory reactions, hematopoiesis, angiogenesis, and CNS development [8]. Several chemokine receptors are used, together with CD4, as coreceptors for HIV-1 entry into target cells. CCR5 and CXCR4 are the major coreceptors for macrophage-tropic and T cell line–tropic HIV-1 strains, respectively [9, 10]. CCR5 is the major coreceptor for HIV-1 infection of macrophages and microglia [11–13]. CXCR4 is also expressed on macrophages and microglia and can support efficient replication by a subset of primary CXCR4-using viruses [14, 15]. CCR5 and CXCR4 are differentially expressed on various cell types, and their expression can be regulated by a variety of stimuli, including inflammatory cytokines [16–22]. Studies of genetic polymorphisms in CCR5, particularly the CCR5 Δ32 mutation (32-bp deletion in the CCR5 gene), suggest that the levels of CCR5 expression determine cellular susceptibility to HIV-1 infection and can affect disease progression in vivo [23]. The level of CD4 expression is also a critical determinant of cellular susceptibility to HIV-1 infection [10, 24]. CCR5 expression is up-regulated on macrophages and microglia in the CNS of patients with HIV-1 encephalitis and HIV-1–associated dementia [25–27].
Previous studies have examined the regulation of CCR5 and CD4 expression on blood-derived monocytes, monocyte-derived macrophages (MDMs), and dendritic cells [16–20], but factors that regulate the expression of CCR5 and CD4 on human microglia have not been defined.

Cytokines produced in response to HIV-1 infection serve as important mediators of virus-host interactions and influence disease pathogenesis [6, 7]. IL-1β, IL-6, IL-10, and TNF-α are elevated in the serum, lymphoid tissues, cerebrospinal fluid, and/or brain in AIDS patients [6, 7, 28–35]. An imbalance in production of Th1 and Th2 cytokines (e.g., impaired production of IL-4 and/or IL-10) has been associated with HIV-1 disease progression [6, 7, 36, 37]. To understand host factors that affect HIV-1 pathogenesis by modulating expression of HIV-1 coreceptors on MPs in the CNS and other tissue reservoirs, we investigated the regulation of CCR5 and CD4 expression and HIV-1 replication in monocytes, MDMs, and microglia.

Materials and Methods

Reagents and antibodies. Recombinant human IL-1β, IL-4, IL-6, IL-10, IFN-γ, TNF-α, macrophage inflammatory protein (MIP)–1β, and RANTES were purchased from R&D Systems. Recombinant human IL-2 was from Collaborative Biomedical Products. Lipopolysaccharide (LPS) and dexamethasone were from Sigma. Phytoerythrin-conjugated antibodies to CCR5 (2D7), CXCR4 (12G5), CD4, CD14, and CD145 were from BD Pharmingen. Fluorescein isothiocyanate–conjugated anti-CD11b was from Coulter-Immunochem. Fluorescein isothiocyanate–conjugated anti-CD11c and anti-CD68 were from Dako. All of these antibodies are mouse monoclonal antibodies. Biotinylated Ricipus communis agglutinin (RCA–1) was purchased from Vector Laboratories.

Preparation and culture of monocytes and MDMs. Human peripheral blood mononuclear cells were prepared from buffy coats of blood from healthy HIV–1–negative donors by density gradient centrifugation on Ficoll-Paque (Pharmacia Biotech). Monocytes were purified from peripheral blood mononuclear cells by plastic adherence for 1 h at 37°C or by magnetic separation with anti-CD14–conjugated microbeads (Miltenyi Biotec) to >98% purity, as determined by staining with CD14 and CD11b monoclonal antibodies. Purified cells were cultured in RPMI 1640 medium supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, and 10% (vol/vol) heat-inactivated fetal bovine serum (Mediatech) at 2 × 10^5 to 5 × 10^5 cells/mL. MDMs were obtained by culture of monocytes in the same medium in the presence of 40 U/mL granulocyte-macrophage colony–stimulating factor for 7 days. Granulocyte-macrophage colony–stimulating factor was then removed from the culture medium.

Preparation and culture of microglia. Primary human fetal brain cultures (HBCs), which contain a mixture of astrocytes, neurons, and microglia, were prepared by incubation of cells from 16–20-week-old abortuses in high-glucose Dulbecco’s modified Eagle medium (Life Technologies) supplemented with 2 mM L-glutamine, 100 U/mL penicillin, 100 mg/mL streptomycin, 1 mM sodium pyruvate, and 10% (vol/vol) heat-inactivated calf serum (Hyclone), as described elsewhere [38]. Microglia were purified after 2–4 weeks of incubation by 3–5 cycles of gentle shaking and washing, followed by plastic adherence for 1 h at 37°C, as described elsewhere [39]. The purity of microglia obtained by this method was >95%, as determined by staining with anti-CD45. Purified microglia were maintained in 50% astrocyte-conditioned medium and used within 1 week. To obtain microglia-enriched HBCs, microglia were added back to HBCs from the same donor in 24- or 48-well plates.

Flow cytometry. To block nonspecific binding, cells were first incubated with 5% (vol/vol) mouse serum and 50 μg/mL normal human IgG in staining buffer (2.5% [vol/vol] fetal bovine serum, 0.02% [wt/vol] NaN4, in PBS) at 4°C for 10 min. For staining of cell-surface antigens, cells were incubated at 4°C for 30 min with antibodies specific to chemokine receptors (CCR5 or CXCR4) or CD antigens (CD4, CD11b, CD11c, CD14, or CD45). In some experiments, cells were incubated with 7-aminocaproic acid for 10 min to exclude nonviable cells from analysis. Cells were then fixed with PBS containing 1% (wt/vol) paraformaldehyde. To identify microglia in HBCs, fixed cells were further incubated with biotinylated RCA-1 at room temperature for 30 min and then stained with Cy-chrome–conjugated streptavidin at room temperature for 30 min. For intracellular staining of CD68, cells (either unfixed or fixed) were treated with Cytofix/Cytoperm solution (BD Pharmingen) and then incubated with fluorescein isothiocyanato-Cy-chrome–conjugated anti-CD68 monoclonal antibody in Perm/Wash solution (BD Pharmingen). Cells were then fixed with PBS containing 1% (wt/vol) paraformaldehyde. To identify microglia in HBCs, fixed cells were further incubated with biotinylated RCA-1 at room temperature for 30 min and then stained with Cy-chrome–conjugated streptavidin at room temperature for 30 min. For intracellular staining of CD68, cells (either unfixed or fixed) were treated with Cytofix/Cytoperm solution (BD Pharmingen) and then incubated with fluorescein isothiocyanato-Cy-chrome–conjugated anti-CD68 monoclonal antibody in Perm/Wash solution (BD Pharmingen) at room temperature for 30 min according to the manufacturer’s protocol. Stained cells were then analyzed with a FACScan (Becton Dickinson) or an EPICS XL (Beckman Coulter) flow cytometer.

Detection of CCR5 mRNA transcripts by reverse-transcriptase polymerase chain reaction (RT-PCR). Total RNA was extracted from microglia-enriched HBCs treated with the indicated stimuli for 16 h with use of Trizol Reagent (Life Technologies) and then treated with RNase-free DNase I (Boehringer Mannheim) to remove any contaminating DNA. Purified RNA (0.5 μg) was used for cDNA synthesis with Superscript II RNase H– RT (Life Technologies) and random hexamer primers (Promega). One-tenth of the reaction was used as a template for PCR amplification with AmpliTaq DNA polymerase (Perkin-Elmer) and the CCR5 primer pair (5′-ACCCATGGCAAATTCCATGG-3′ and 5′-TCTGAGGACGCTCTTGCTGGA-3′), as described elsewhere [40], for 40 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min. PCR products were fractionated on a 2% agarose gel that contained ethidium bromide and were visualized by transillumination. Glyceraldehyde-3-phosphate dehydrogenase-3A (5′-ACCCATGGCATAATTCCATGG-3′ and 5′-TTCTCATAGCAGGGACTAGG-3′) was amplified in parallel to control for the amount of total RNA used in PCR reactions.

Production of HIV-1 and recombinant viruses. HIV-1JR-FL stocks were prepared from the supernatants of infected PM1 cells and quantitated for RT activity by incorporation of [3H]dTTTP into an artificial poly(A)(dT)15 template, as described elsewhere [15]. Replication-defective recombinant green fluorescent protein (GFP) reporter viruses were generated as described elsewhere [38]. Briefly, 293T cells were cotransfected with the env–deficient pNL4-3env–GFP plasmid and pSVIIenv–JR-FL by use of the calcium phosphate method. The pNL4-3env–GFP plasmid encodes full-length
NL4-3 HIV-1 proviral DNA with a frame shift in envelope and expresses GFP in place of nef. pSVIIIenv–JR-FL expresses the envelope protein from HIV-1JR-FL.

**HIV-1 replication assays.** MDMs and microglia-enriched HBCs incubated in the presence or absence of IL-4 or IL-10 were infected by incubation with equivalent amounts of virus stocks (10,000–25,000 Hcpm RT units of HIV-1 per milliliter) in the presence of 2 μg/mL polybrene. MDMs prepared from monocytes purified by magnetic separation with anti-CD14–conjugated microbeads were used in these experiments. After 16 h of incubation at 37°C, cultures were washed twice with PBS, and fresh medium, with or without IL-4 or IL-10, was added. At the indicated time points (twice per week), one-half of the culture medium was removed and used for the quantitation of RT activity. Fresh medium, with or without IL-4 or IL-10, was added back into the culture at each medium change.

**Single-cycle HIV-1 infection assays.** MDMs and microglia-enriched HBCs were infected by incubation with equivalent amounts of replication-deficient recombinant GFP reporter virus stocks (20,000–50,000 Hcpm RT units of HIV-1 per milliliter) in the presence of 2 μg/mL polybrene. MDMs prepared from monocytes purified by magnetic separation with anti-CD14–conjugated microbeads were used in these experiments. After 16 h of incubation at 37°C, cultures were washed twice with PBS and then incubated in fresh medium, with or without IL-4 or IL-10. After an additional 4–6 days in culture, cells were collected, stained with phycoerythrin-conjugated anti-CD45 (microglia-enriched HBCs), and analyzed with a FACScan or an EPICS XL flow cytometer. Microglia were identified by gating on CD45+ cells.

**Calcium mobilization assays.** Cells (2 × 10⁵ to 10 × 10⁵) were resuspended in 1 mL of calcium influx (CI) buffer (Hanks’ buffered saline solution, 10 mM HEPES [pH 7.2], 0.1% [wt/vol] bovine serum albumin) and incubated with 2–5 μM Fura-2/AM (Molecular Probes) for 30 min at 37°C. Cells were then washed twice with PBS and resuspended in 2 mL of CI buffer. Calcium flux measurements in response to MIP-1β were then taken at excitation wavelengths of 340 and 380 nm and reported as a ratio of measurements at 340 nm to measurements at 380 nm.

**Chemotaxis assays.** Migration of microglia was examined by use of a 48-well microchamber (Neuro Probe). In brief, MIP-1β and RANTES were diluted in serum-free Dulbecco’s modified Eagle medium/F12 medium (Life Technologies) containing 0.5% (wt/vol) bovine serum albumin and then placed in the lower wells of the chamber. Cells (5 × 10⁶ to 2 × 10⁷ cells/mL) were then placed in the upper compartment of the chamber. The upper and lower wells of the chamber were separated by a 5-μm polycarbonate filter (Neuro Probe). The chamber was incubated for 60 min at 37°C, after which the filter was removed, and cells on the upper surface were scraped off, fixed, and stained with a Diff-Quik kit (Dade Behring). Cell migration was measured by counting the number of cells adherent to the lower surface of the filter in at least 4 high-power magnification fields (×400) by light microscopy. Each condition was tested at least in duplicate. Results are shown as the chemotaxis index, which represents the ratio between cells migrated in response to chemokines and cells migrated in the presence of medium alone (background migration).

**Statistical analysis.** Statistical analysis was done with 1-way analysis of variance (ANOVA) or Student’s t test. P < .05 was considered to be statistically significant.

**Results**

**Phenotypic characterization of MDMs and microglia.** MDMs and microglia were prepared from peripheral blood monocytes and primary HBCs, respectively, and morphology and immuno-phenotypes were characterized. The morphologic characteristics of MDMs and microglia were distinct (figure 1). MDMs exhibited typical macrophage morphology and were tightly adherent. Microglia were ameboid, rounded or irregular, and loosely adherent and exhibited heterogeneous morphology, which is consistent with the findings of previous studies [41]. The levels of myeloid markers (CD11b, CD11c, CD14, and CD68), CD4, and CD45 on microglia were similar or slightly lower than levels on MDMs (table 1 and figure 2). As expected, MDMs and micro-

![MDMs and Microglia](image_url)

**Figure 1.** Morphology of monocyte-derived macrophages (MDMs) and microglia. Monocytes were prepared from peripheral blood mononuclear cells by magnetic separation with anti-CD14–conjugated microbeads and cultured for 7 days to obtain MDMs. Microglia were prepared by gentle shaking of primary human fetal brain cultures incubated for 2–4 weeks and further purified by plastic adherence. Cells shown are representative of cultures obtained from 3 different donors. Original magnification, ×100.
Table 1. Immunophenotype of monocyte-derived macrophages (MDMs) and microglia.

<table>
<thead>
<tr>
<th>Monoclonal antibody</th>
<th>MDMs</th>
<th>Microglia</th>
</tr>
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<tbody>
<tr>
<td>CD1a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CD4</td>
<td>+ or ++</td>
<td>+</td>
</tr>
<tr>
<td>CD11b</td>
<td>+ or ++</td>
<td>+</td>
</tr>
<tr>
<td>CD11c</td>
<td>+ or ++</td>
<td>+</td>
</tr>
<tr>
<td>CD14</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>CD45</td>
<td>+++</td>
<td>++ or +++</td>
</tr>
<tr>
<td>CD68</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD83</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CD86</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>+++</td>
<td>++ or +++</td>
</tr>
</tbody>
</table>

NOTE. The no. of molecules expressed on the cell surface (except CD68, which is expressed intracellularly) was quantitated as the specific staining index (SSI), calculated by dividing the mean fluorescence intensity (MFI) of cells labeled with specific monoclonal antibodies by the MFI of cells labeled with isotype-matched control IgG. –, SSI < 1.5; +, 1.5 ≤ SSI < 2; +, 2 ≤ SSI < 5; ++, 5 ≤ SSI < 50; ++++, 50 ≤ SSI < 500; +++, SSI ≥ 500. Data are from 3 independent experiments involving cells obtained from different donors.

MDMs expressed higher levels of major histocompatibility complex class II (HLA-DR) and CD86 than did microglia, which is consistent with the findings of previous studies [42]. Expression of CD4, CD11b, CD11c, and CD45 was significantly higher on MDMs than on microglia in some experiments, but this finding was donor dependent. In human fetal brain-cell cultures, microglia were identified according to forward/side-scatter versus side/side-scatter profiles and by double staining of CD68 and RCA-1 (figure 2A and 2B). Like purified microglia (figure 2F), all RCA-1^highCD68^+ cells were CD45^+, as shown by 3-color flow cytometry analysis. In addition, all CD45^+ cells were RCA-1^highCD68^+. Therefore, CD45 was also used as a marker to identify microglia in human fetal brain-cell cultures.

Regulation of CCR5 and CD4 on monocytes, MDMs, and microglia. Previous studies have shown that levels of CCR5 expression determine cellular susceptibility to HIV-1 infection and can affect disease progression in vivo [23]. Low levels of CD4 on the cell surface of MDMs and microglia may restrict infection by certain HIV-1 strains [10, 24]. We therefore examined the regulation of CCR5 and CD4 on monocytes, MDMs, and microglia in response to various stimuli (tables 2 and 3 and figures 3 and 4). Cells were treated with a panel of cytokines or LPS for 48 h, followed by flow cytometry analysis to examine CCR5 and CD4 expression on the cell surface. Cytokines and LPS were used at concentrations similar to those used in previous studies [16–22]. As was found by Albright et al. [43], CCR5 was expressed on only 50%–80% of microglia, with some variability observed among different donors. However, distinct subpopulations of microglia could not be identified on the basis of CCR5 expression. The relative changes in cell-surface expression of CCR5 and CD4 were calculated according to the mean fluorescence intensity of cells stained with antibodies specific to CCR5 or CD4 and according to isotype control, as described elsewhere [21]. Data from at least 5 different donors of each cell type are summarized in tables 2 and 3. No differences in CCR5 and CD4 expression were observed between monocytes or MDMs purified by plastic adherence and those purified by anti-CD14–conjugated microbeads. Compared with monocytes and MDMs, microglia expressed lower levels of CCR5 and CD4 on the cell surface (figures 3 and 4). Among the stimuli examined, IL-10 up-regulated CCR5 expression on monocytes by 75% and on MDMs by 60% (P < .05, by 1-way ANOVA) (table 2). IL-2, IL-6, and IL-10 up-regulated CCR5 expression on microglia by 31%, 32%, and 57%, respectively (P < .05,
by 1-way ANOVA). IL-4 had no significant effect on CCR5 expression on microglia but down-regulated CCR5 expression on monocytes and MDMs by 54% and 29%, respectively (P < .05, by 1-way ANOVA). Other cytokines, LPS, and dexamethasone down-regulated or had no effect on CCR5 expression on monocytes, MDMs, and microglia. Thus, the regulation of CCR5 expression on monocytes, MDMs, and microglia was generally similar.

The regulation of CD4 expression on microglia was markedly different than that for monocytes and MDMs (table 3). IL-4 up-regulated CD4 expression on microglia by 67% but down-regulated CD4 expression on monocytes and MDMs by 57% and 55%, respectively (P < .05, by 1-way ANOVA) (figures 3 and 4). IL-6 also up-regulated CD4 expression on microglia by 32% (P < .05, by 1-way ANOVA). In contrast to IL-4, IL-10 up-regulated CD4 expression on MDMs by 34% (P < .05, by 1-way ANOVA), IL-1β, IFN-γ, TNF-α, and LPS down-regulated CD4 expression on monocytes and MDMs by 54%–97% (P < .05, by 1-way ANOVA) but had no significant effect on CD4 expression on microglia. Other stimuli exhibited minor effects on CD4 expression on monocytes, MDMs, and microglia. Thus, regulation of CD4 expression on MPs was highly cell dependent.

Regulation of CCR5 mRNA expression on microglia. Previous studies have demonstrated that IL-10 up-regulates CCR5 cell-surface expression on monocytes by increasing mRNA transcription or stability [17, 18], whereas LPS down-regulates CCR5 expression on MDMs, independent of de novo protein synthesis [20]. To examine the mechanisms underlying the regulation of CCR5 cell-surface expression on microglia, CCR5 mRNA expression in microglia was analyzed by RT-PCR (figure 5). Equivalent levels of glyceraldehyde-3-phosphate dehydrogenase mRNA were detected, demonstrating that equivalent amounts of total RNA were present in all samples. IL-10 up-regulated CCR5 mRNA expression in microglia, which was consistent with the effect of this cytokine on CCR5 cell-surface protein expression (table 2). In contrast, IL-2 up-regulated CCR5 cell-surface protein expression (table 2) but had no effect on CCR5 mRNA expression (figure 5). Surprisingly, LPS up-regulated CCR5 mRNA expression (figure 5) but down-regulated CCR5 cell-surface protein expression (table 2). Thus, cytokines and LPS regulate CCR5 cell-surface protein expression by several different mechanisms, depending on the stimulus.

IL-4 and IL-10 enhance HIV-1 replication in microglia but have opposite effects in MDMs. Previous studies suggest that levels of IL-4 and IL-10 are increased in the serum and/or tissues of AIDS patients with advanced disease and may play important roles in the pathogenesis of HIV-1 [28, 29, 36, 37]. Furthermore, levels of IL-10 are higher in the cerebrospinal fluid and brains of AIDS patients with HIV-1–associated dementia [32–34]. We investigated the effects of IL-4 and IL-10 on HIV-1 replication in MDMs and microglia with the CCR5-tropic HIV-1JR-FL, which was isolated from brain tissue [44] (figure 6). IL-4 and IL-10 enhanced HIV-1JR-FL replication in microglia to 158%–180% and 221%–339% of levels in untreated cultures, respectively (P < .05, by Student’s t test). IL-4 also enhanced HIV-1JR-FL replication in MDMs to 520%–1701% of levels in untreated cultures. In contrast, IL-10 inhibited HIV-1JR-FL replication in MDMs to 23%–36% of levels in untreated cultures (P < .05, by Student’s t test). The enhancement of HIV-1 replication in microglia by IL-4 and IL-10 is consistent with the regulatory effects of these cytokines on CD4 and CCR5 expression. In contrast, an inverse relationship was found between the effects of IL-4 and IL-10 on HIV-1 replication in MDMs and the effects on CD4 and CCR5 expression (table 4). These findings suggest that IL-4 and IL-10 enhance the entry and replica-

Table 2. Change in cell-surface expression of CCR5 on monocytes, monocyte-derived macrophages (MDMs), and microglia, in response to stimulation by cytokines, lipopolysaccharide (LPS), or dexamethasone.

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>Monocytes</th>
<th>MDMs</th>
<th>Microglia</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β, 20 ng/mL</td>
<td>101 ± 7</td>
<td>85 ± 8</td>
<td>113 ± 10</td>
</tr>
<tr>
<td>IL-2, 100 U/mL</td>
<td>102 ± 6</td>
<td>90 ± 11</td>
<td>131 ± 7*</td>
</tr>
<tr>
<td>IL-4, 20 ng/mL</td>
<td>46 ± 6*</td>
<td>71 ± 8*</td>
<td>100 ± 11</td>
</tr>
<tr>
<td>IL-6, 20 ng/mL</td>
<td>121 ± 9</td>
<td>124 ± 8*</td>
<td>132 ± 5*</td>
</tr>
<tr>
<td>IL-10, 20 ng/mL</td>
<td>175 ± 14*</td>
<td>160 ± 11</td>
<td>157 ± 10*</td>
</tr>
<tr>
<td>IFN-γ, 1000 U/mL</td>
<td>80 ± 11</td>
<td>62 ± 13*</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>TNF-α, 20 ng/mL</td>
<td>76 ± 7*</td>
<td>78 ± 8*</td>
<td>76 ± 7*</td>
</tr>
<tr>
<td>LPS, 100 ng/mL</td>
<td>64 ± 10*</td>
<td>49 ± 9*</td>
<td>79 ± 14</td>
</tr>
<tr>
<td>Dexamethasone, 10−6 M</td>
<td>93 ± 11</td>
<td>100 ± 9</td>
<td>86 ± 6*</td>
</tr>
</tbody>
</table>

NOTE. In each individual experiment, relative changes in cell-surface expression of CCR5 were calculated as [(MFIfα− MFIcon)/(MFIst− MFIst−)] × 100. MFIst− and MFIcon are mean fluorescence intensity (MFI) of stimulated cells labeled with specific monoclonal antibodies to CCR5 and with isotype-matched control IgG, respectively. MFIst− and MFIcon are MFI of unstimulated cells. Data are mean ± SE of 5–10 independent experiments involving cells obtained from different donors. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

* P < .05, compared with medium control, by 1-way analysis of variance.

Table 3. Change in cell-surface expression of CD4 on monocytes, monocyte-derived macrophages (MDMs), and microglia, in response to stimulation by cytokines, lipopolysaccharide (LPS), or dexamethasone.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Monocytes</th>
<th>MDMs</th>
<th>Microglia</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β, 20 ng/mL</td>
<td>27 ± 15*</td>
<td>38 ± 13*</td>
<td>111 ± 12</td>
</tr>
<tr>
<td>IL-2, 100 U/mL</td>
<td>97 ± 6</td>
<td>94 ± 10</td>
<td>114 ± 8</td>
</tr>
<tr>
<td>IL-4, 20 ng/mL</td>
<td>43 ± 10*</td>
<td>45 ± 5*</td>
<td>167 ± 16*</td>
</tr>
<tr>
<td>IL-6, 20 ng/mL</td>
<td>104 ± 8</td>
<td>107 ± 8</td>
<td>132 ± 10*</td>
</tr>
<tr>
<td>IL-10, 20 ng/mL</td>
<td>98 ± 19</td>
<td>134 ± 6*</td>
<td>120 ± 11</td>
</tr>
<tr>
<td>IFN-γ, 1000 U/mL</td>
<td>46 ± 6*</td>
<td>24 ± 5*</td>
<td>111 ± 11</td>
</tr>
<tr>
<td>TNF-α, 20 ng/mL</td>
<td>26 ± 13*</td>
<td>20 ± 6*</td>
<td>106 ± 10</td>
</tr>
<tr>
<td>LPS, 100 ng/mL</td>
<td>3 ± 2*</td>
<td>16 ± 6*</td>
<td>86 ± 18</td>
</tr>
<tr>
<td>Dexamethasone, 10−6 M</td>
<td>126 ± 34</td>
<td>114 ± 20</td>
<td>118 ± 7*</td>
</tr>
</tbody>
</table>

NOTE. In each individual experiment, relative changes in cell-surface expression of CD4 were calculated as [(MFIfα− MFIcon)/(MFIst− MFIst−)] × 100. MFIst− and MFIcon are mean fluorescence intensity (MFI) of stimulated cells labeled with specific monoclonal antibodies to CD4 and with isotype-matched control IgG, respectively. MFIst− and MFIcon are MFI of unstimulated cells. Data are mean ± SE of 3–7 independent experiments involving cells obtained from different donors. IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

* P < .05, compared with medium control, by 1-way analysis of variance.
tion of CCR5-tropic HIV-1 in microglia through up-regulation of CD4 and CCR5 expression, respectively, whereas factors other than levels of CD4 and CCR5 expression are involved in the pathways that regulate viral replication in MDMs.

**Regulation of HIV-1 entry into MDMs and microglia by IL-4 and IL-10.** To determine whether the effects of IL-4 and IL-10 on HIV-1 replication in MDMs and microglia were due to effects on virus entry, single-cycle infection assays were done with a replication-defective recombinant GFP reporter virus pseudotyped with the HIV-1RFL envelope (figure 7). Recombinant virus without envelope (pcDNA3 vector alone) was used as a negative control. The efficiency of viral infection (percentage of GFP-positive cells) was determined by flow cytometry analysis. As was seen with CD4 and CCR5 regulation in MDMs, IL-4 inhibited infection of MDMs by HIV-1RFL-pseudotyped virus by 49%, whereas IL-10 enhanced infection of MDMs by 985% \((P < .05, \text{by Student’s } t \text{ test})\) (figure 7). Therefore, IL-4 inhibited and IL-10 enhanced HIV-1 entry during a single cycle of infection. IL-4 and IL-10 markedly enhanced infection of microglia with HIV-1RFL-pseudotyped virus by 187% and 588%, respectively \((P < .05, \text{by Student’s } t \text{ test})\) (figure 7), which was consistent with the stimulatory effects of these cytokines on CD4 and CCR5 expression on microglia. These results suggest that IL-4 and IL-10 can influence HIV-1 entry into MDMs and microglia through regulation of CD4 and CCR5 expression. However, the effects of these cytokines on virus entry do not necessarily correlate with effects on viral replication (table 4).

**MIP-1β-induced calcium mobilization and chemotaxis of microglia.** Increased numbers of MPs have been observed adjacent to blood vessels and within inflammatory lesions in the brains of AIDS patients [3–5]. Furthermore, enhanced production of MIP-1α, MIP-1β, and RANTES has been detected in the CNS of AIDS patients [27, 45–47]. We therefore examined whether CCR5 up-regulation by IL-10 might potentiate the chemotactic response of microglia to CCR5 ligands. MIP-1β–induced calcium mobilization in microglia was measured. Microglia demonstrated a robust response to a very low dose of MIP-1β (figure 8A), despite the presence of very low levels of CCR5 on the cell surface (figure 4). IL-10 had no significant effect on the magnitude of calcium influx. Microglia migrated in response to RANTES but exhibited no significant chemotactic response to MIP-1β in most donors (figure 8B), which may reflect the low levels of surface CCR5 or in vitro culture conditions. The addition of IL-10 enhanced MIP-1β–but not RANTES–induced chemotactic migration (figure 8B). This finding may reflect the use of other chemokine receptors by RANTES, including CCR1 and CCR3, which are also expressed on microglia (data not shown). These results suggest that very low levels of CCR5 on the cell surface of microglia are sufficient to mediate robust calcium influx signaling but not chemotaxis in response to MIP-1β. The enhancement of MIP-1β–induced chemotaxis by IL-10 suggests that increased levels of IL-10 in the blood and brains of AIDS patients may facilitate chemotactic migration of MPs into or within the CNS by up-regulating CCR5.

**Discussion**

In this study, we demonstrated enhancement of CCR5-tropic HIV-1 entry and replication in microglia by IL-4 and IL-10 through up-regulation of CD4 and CCR5 expression, respectively. IL-4 also stimulated HIV-1 replication in MDMs but down-regulated CD4 and CCR5 expression and inhibited viral entry, whereas IL-10 had the opposite effects. Thus, factors other than levels of CCR5 and CD4 expression are involved in the pathways that regulate viral replication in MDMs (table 4). We further demonstrated that up-regulation of CCR5 expression by IL-10 potentiates the migration of microglia in response to MIP-1β, as has been shown for monocytes [17, 18]. Together, these findings suggest that increased production of Th2 cytokines in the later stages of disease can influence HIV-1 pathogenesis by enhancing entry and/or replication of HIV-1 in MPs and facilitating chemotactic migration into or within the CNS.

Microglia are derived from either monocytes or hematopoietic progenitor cells that differentiate into microglia on entry into the CNS [4, 48–52]. Microglia exhibit heterogeneous morphology and immunophenotype that depend on location in the brain and the differentiation and activation states of the cell [48–52]. The myeloid marker CD14 was readily detected on microglia in our culture system, in contrast to the findings of some previous studies [49, 50]. CD14 expression has also been detected on microglia in some other studies, and its expression appears to be labile and can be up-regulated, depending on the cell culture conditions [51, 52]. These discrepancies might result from the use of different preparations of cells (freshly isolated vs. cultured), techniques for staining (immunohistochemistry vs. flow cytometry), or particular clones of anti-CD14 monoclonal antibodies for staining or from differences in cell culture conditions. We found that microglia expressed significantly lower levels of HLA-DR and CD86 than did MDMs, which is consistent with the findings of previous studies [42]. Previous studies also suggest that, although microglia, monocytes, and macrophages are related cell types that belong to the MP lineage, they exhibit many phenotypic and functional differences [39, 42, 48–52]. The differences we observed in the regulation

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**Figure 3.** Regulation of CCR5 and CD4 expression on monocytes and monocyte-derived macrophages (MDMs). Monocytes and MDMs were cultured for 48 h in medium alone or in the presence of various stimuli. Cells were then collected and stained for flow cytometry. Blue lines, cells stained with isotype control monoclonal antibody; red lines and green lines, unstimulated cells and stimulated cells, respectively, stained with anti-CCR5 or anti-CD4 monoclonal antibodies. Results shown are from 3–5 independent experiments involving cells obtained from different donors. IL, interleukin; LPS, lipopolysaccharide. X-axis, relative fluorescence intensity; Y-axis, no. of cells.
of CCR5 and CD4 expression and HIV-1 replication reinforces such a view and suggests that results obtained from experiments involving monocytes and MDMs cannot necessarily be extrapolated to microglia.

Previous studies suggest that the levels of CCR5 expression determine cellular susceptibility to HIV-1 infection and can affect disease progression in vivo [23]. In addition, low levels of CD4 on the cell surface of MPs may restrict infection by certain HIV-1 strains [10, 24]. We examined the regulation of CCR5 and CD4 expression on monocytes, MDMs, and microglia by a panel of cytokines and LPS. The regulation of CCR5 expression on monocytes, MDMs, and microglia was generally similar, whereas regulation of CD4 expression on microglia was markedly different from that for monocytes and MDMs. Thus, regulation of CD4 expression on MPs appears to be highly cell dependent. The down-regulation of CCR5 expression by IL-4, TNF-α, and LPS; the up-regulation of CCR5 expression by IL-10; and the down-regulation of CD4 expression by IL-1β, IFN-γ, TNF-α, and LPS on monocytes and MDMs shown in our study are consistent with the findings of previous reports [16–20]. Down-regulation of CD4 expression by IL-4 on monocytes and MDMs and up-regulation of CCR5 expression on MDMs by IL-6 have not been reported elsewhere. We found that regulation of CCR5 and CD4 expression on monocytes and MDMs by cytokines and LPS was generally similar, but there were some notable exceptions. In particular, IL-10 up-regulated CD4 expression on MDMs but not on monocytes. One limitation of our study is that only single concentrations of each cytokine were used. Previous studies suggest that lower doses might have similar effects [16–22].

We showed that IL-10 up-regulated CCR5 mRNA expression in microglia, suggesting that the up-regulation of surface CCR5 expression was due to increased CCR5 mRNA transcription and/or stability, which is consistent with the results of previous studies involving monocytes [17, 18]. LPS also up-regulated CCR5 mRNA in microglia but down-regulated surface CCR5 protein expression, possibly because of effects on intracellular traffick-
ing (i.e., enhanced internalization and reduced recycling), as has been observed elsewhere in MDMs [20]. Thus, various stimuli have different mechanisms for regulation of CCR5 expression on MP.

IL-4 exhibited dichotomous effects on HIV-1 replication in MDMs. IL-4 inhibited HIV-1 entry into MDMs through down-regulation of CCR5 and CD4 expression, but a net increase in viral replication resulted. This may reflect a stimulatory effect on viral gene transcription, because previous studies suggest that IL-4 can stimulate HIV-1 long terminal repeat–driven gene expression through the nuclear factor–κB activation pathway [53, 54]. The stimulatory effect of IL-4 on HIV-1 replication in MDMs demonstrated in our study is consistent with the findings of some previous studies [55–57] but contradictory to the findings of other studies [16, 53, 54, 58, 59]. These contradictory results may reflect differences in the concentration of cytokines used, timing of cytokine exposure, cell preparation and culture methods, and virus strains used. For example, Schuite-maker et al. [53] demonstrated that IL-4 has inhibitory effects on HIV-1 replication in MDMs treated with IL-4 for 5 days before but not after HIV-1 infection. It is possible that inhibitory effects of IL-4 on HIV-1 entry predominated over stimulatory effects that occur at later stages of the virus life cycle.

The inhibitory effects of IL-10 on HIV-1 replication in MDMs demonstrated in our study are consistent with the results of previous studies [60–66]. In contrast, some studies reported that IL-10 can enhance HIV-1 replication in MDMs and monocytes [17, 67, 68]. These discrepancies likely can be explained by dose-dependent effects. Low doses of IL-10 (<0.1 ng/mL) have been shown to inhibit HIV-1 replication, whereas higher doses (>2 ng/mL) can enhance replication [63, 67]. For example, Sozzani et al. [17] showed that IL-10 up-regulates CCR5 expression and enhances HIV-1 replication in monocytes. Those investigators used 0.1 ng/mL IL-10, whereas we used 20 ng/mL. We found that IL-10 enhanced HIV-1 entry into MDMs and microglia but did not have inhibitory effects on post-entry steps before HIV-1 long terminal repeat–driven gene expression in single-cycle infection assays (figure 7). One possibility is that IL-10 may inhibit HIV-1 replication in MDMs by interfering

**Table 4.** Summary of effects of interleukin (IL)—4 and IL-10 on monocyte-derived macrophages (MDMs) and microglia.

<table>
<thead>
<tr>
<th>Cytokine, cell type</th>
<th>Change in expression of HIV-1 coreceptor</th>
<th>Change in HIV-1 activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCR5</td>
<td>CD4</td>
</tr>
<tr>
<td>IL-4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDMs</td>
<td>†</td>
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</tr>
<tr>
<td>Microglia</td>
<td>NC</td>
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</tr>
<tr>
<td>IL-10</td>
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<td>MDMs</td>
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</tr>
<tr>
<td>Microglia</td>
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<td>NC</td>
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</tbody>
</table>

NOTE. HIV-1, human immunodeficiency virus type 1; NC, no statistically significant change; †, down-regulation or inhibition; ††, up-regulation or enhancement.
with viral protein processing and virus assembly [64] or by other unknown mechanisms, rather than by inhibiting long terminal repeat–driven gene expression [65], and that these inhibitory effects predominate over enhancing effects on virus entry.

CCR5 expression on macrophages and microglia is up-regulated in the brains of AIDS patients with HIV-1–associated dementia [25–27]. Among various cytokines that we examined, we found that only IL-2, IL-6, and IL-10 up-regulated CCR5 expression on microglia. IL-10 was the most potent up-regulator of CCR5. Previous studies have demonstrated elevated levels of IL-6 and IL-10 in the cerebrospinal fluid and brain tissue of AIDS patients with HIV-1–associated dementia, whereas IL-2 and IL-4 were not elevated [30–35]. However, IL-6 was shown to inhibit HIV-1 replication in microglia in human fetal brain-cell cultures [69]. Together, these findings suggest that IL-10 may contribute to disease progression in the CNS in the late stages of AIDS by up-regulating CCR5 expression and enhancing the entry and replication of CCR5-tropic HIV-1 strains in microglia. Up to 4 ng/mL IL-10 has been detected in the cerebrospinal fluid of AIDS patients [34], which is within the effective dose range of IL-10 shown to up-regulate CCR5 expression on MPs in vitro ([EC]₅₀ = 0.3 ± 0.1 ng/mL) [17]. Local concentrations in some areas of brain tissue might be as high as those used in the present study because of local cytokine production by microglia and astrocytes. Calcium signaling was not enhanced by IL-10, suggesting that very low levels of CCR5 on the cell surface are sufficient to mediate a robust signaling response. However, the up-regulation of CCR5 expression by IL-10 on monocytes and microglia was associated with an enhanced chemotactic response to MIP-1β [17, 18] (figure 8). These studies suggest that elevated levels of IL-10 in blood and brain may facilitate the migration of MPs into or within the CNS in response to β-chemokines by up-regulating with viral protein processing and virus assembly [64] or by other unknown mechanisms, rather than by inhibiting long terminal repeat–driven gene expression [65], and that these inhibitory effects predominate over enhancing effects on virus entry.

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CCR5 expression. Increased infiltration of MPs may then promote disease progression through production of HIV-1 virions and viral proteins, as well as other neurotoxic factors [3–5].

We demonstrated that IL-10 enhances HIV-1 replication in microglia but inhibits HIV-1 replication in MDMs. These findings suggest that IL-10 may have dichotomous effects on HIV-1 replication in MPs, depending on the cell type or tissue microenvironment, and the results also raise the possibility that IL-10 may enhance replication of CCR5-tropic HIV-1 strains in the CNS while enhancing entry into but suppressing replication of these strains in other tissue compartments. To mimic in vivo conditions, human fetal brain-cell cultures, which contain neurons and astrocytes in addition to microglia, were used for studies of HIV-1 infection. We cannot exclude the possibility that the presence of other soluble factors in human fetal brain-cell cultures may account for the opposing effects of IL-10 on HIV-1 replication in MDMs and microglia that were observed in these in vitro studies. Divergent effects could also be explained by the different maturation stages of MDMs and microglia. Further studies are required to determine the role of IL-10 in HIV-1 replication within different tissue microenvironments in vivo.

In conclusion, we demonstrated the regulation of CCR5 and CD4 expression on MPs by cytokines, including IL-4 and IL-10, and correlated this with susceptibility of microglia to HIV-1 infection. We found that IL-4 and IL-10 enhance the entry and replication of CCR5-tropic HIV-1 in microglia through up-regulation of expression of CD4 and CCR5, respectively, whereas factors other than levels of CD4 and CCR5 expression are involved in the pathways that regulate HIV-1 replication in MDMs. Thus, the effects of IL-4 and IL-10 on HIV-1 replication in MPs are more complicated than the regulatory effects of these cytokines on the levels of CCR5 and CD4 expression. We also demonstrated that up-regulation of CCR5 expression by IL-10 potentiates the migration of microglia in response to MIP-1β. These findings suggest that increased production of Th2 cytokines in the later stages of disease can influence HIV-1 pathogenesis by enhancing entry into and/or replication of HIV-1 in MPs and facilitating chemotactic migration into or within the CNS.

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