**Interleukin 10 Increases CCR5 Expression and HIV Infection in Human Monocytes**

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**Summary**

The immunosuppressive and antiinflammatory cytokine interleukin (IL) 10 selectively upregulates the expression of the CC chemokine receptors CCR5, 2, and 1 in human monocytes by prolonging their mRNA half-life. IL-10–stimulated monocytes display an increased number of cell surface receptors for, and better chemotactic responsiveness to, relevant agonists than do control cells. In addition, IL-10–stimulated monocytes are more efficiently infected by HIV BaL. This effect was associated to the enhancement of viral entry through CCR5. These data add support to an emerging paradigm in which pro- and antiinflammatory molecules exert reciprocal and opposing influence on chemokine agonist production and receptor expression.

Chemokines are a superfamily of proteins that play a crucial role in immune and inflammatory reactions and in viral infections (1–6). Chemokines can be grouped in two main subfamilies defined as CXC (or α) and CC (or β) according to the spacing of the first two cysteine residues (1, 3). Recently, the new chemokines lymphotactin and fractalkine have been reported and define two additional classes of the chemokine superfamily (1, 3). Inflammatory cytokines (e.g., IL-1, TNF-α, and IL-6) and bacterial products are potent inducers of chemokine production both in vitro and in vivo (1–3). Contrary to this, molecules with immunosuppressive and antiinflammatory activity, such as IL-10 and glucocorticoid hormones, inhibit chemokine production (7, 8).

Chemokines bind to and activate seven-transmembrane domain receptors (1–3, 9–11). Four receptors for the CXC chemokines, named CXCR1–4, and eight for CC chemokines (CCR1–8) have been cloned and characterized in leukocyte populations. With only a few exceptions, chemokine receptors bind multiple chemokines and recently it was shown that some of them can function as entry/fusion cofactors for HIV-1 infection (4–6, 11).

The regulation of expression of chemokine receptors may play a central role in the tuning of the chemokine action, but to date it has been the object of limited attention (12–15). Here we report that the immunosuppressive and antiinflammatory cytokine IL-10 (16–18) selectively upregulates the expression of CC chemokine receptors in human mononuclear phagocytes by increasing the half-life of their mRNA. This unexpected action is functionally relevant for migration and HIV infection. These results are consistent with a novel paradigm of regulation of chemokines and their receptors by pro- and antiinflammatory signals.

**Materials and Methods**

Monocytes. PBMCs were obtained from buffy coats of healthy blood donors. Monocytes were obtained by Ficoll (Biochrom, Berlin, Germany) and Percoll (Pharmacia Biotech AB, Uppsala, Sweden) gradients (19). Purity was >90% as assessed by immunofluorescence and FACS® analysis for cell surface expression of CD14.

FACS® Analysis. Cell staining was performed using monoclonal antibodies followed by FITC–conjugated affinity-purified, isotype-specific goat anti–mouse antibody (Techno-Genetics Turin, Italy). Anti-CD14 (IgG2a; gift of Dr. P. Beverly, Jenner Institute, London, UK) and LS87 5C7 (anti-CCR5; IgG2a) (20) were used. Mouse IgG2a, kappa (UPC10) (Sigma Chemical Co., St. Louis, MO) was used as irrelevant control antibody. In some cases

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results are expressed as relative fluorescence intensity (RFI), calculated according to the formula: RFI = mean fluorescence (sample) − mean fluorescence (control) / mean fluorescence (control).

Chromatases. Monocyte migration was evaluated using a chemotaxis microchamber technique (N euroProbe, Pleasanton, CA) using polycarbonate filters (5 μm pore size; N euroProbe), as previously described (15). Human recombinant monoclonal chemotactic protein (MCP)-1, macrophage inflammatory protein (MIP)-1α and RANTES (regulated on activation, normal T cell expressed and secreted) were obtained as previously described (24). For CXCR4, the concentration of IL-10 was varied in a range of 0.1–10 ng/ml for MCP-1 and MIP-1α, and of AOP-RANTES for 30 min before exposure to IL-10 (Fig. 1 A). Because of the peculiarities of MCP-4 and its relevance as HIV fusion cofactor, subsequent studies were conducted on this receptor. The effect of IL-10 was concentration dependent (effective concentration [EC]50 = 0.3 ± 0.1 ng/ml; 0.015 nM) and fast, already detectable after 30 min and reaching a plateau after 2 h of stimulation, with a maximal increase observed at 10 ng/ml (0.5 nM ) IL-10 (Fig. 1 B and C). The estimated half-life of MCP-4 mRNA was 165 min and was augmented to 260 min (n = 2) after exposure to IL-10 (Fig. 1 D). In contrast, the rate of nuclear transcription of the gene, as investigated by nuclear runoff analysis, was not affected (Fig. 1 E).

Having observed that IL-10 selectively upregulated expression of the CC chemokine receptors CCR1, 2, and 5, it was important to investigate the functional relevance of this enhancement. As shown in Fig. 2, IL-10–treated monocytes responded better to CC chemokines in terms of chemotactic migration (Fig. 2 A) and intracellular calcium transients (data not shown). The effect was best observed when suboptimal agonist concentrations were used (e.g., 1 and 10 ng/ml for MCP-1 and MIP-1α, respectively). At the concentration of 10 ng/ml, IL-10–treated monocytes showed an increase of 237 and 189% in chemotaxis above control values for MCP-1 and MIP-1α, respectively. It is noteworthy that IL-10 pretreatment did not appreciably affect the spontaneous migration of monocytes. In agreement with these results, IL-10 substantially increased the expression of CCR5 evaluated by both cytofluorimetric analysis (Fig. 2 B and C) and by ligand binding assays with radiolabeled MIP-1α (13,864 ± 3,257 and 22,925 ± 3,804 receptors per cell, P < 0.01; with identical affinity 2.6 ± 0.9 and 3.4 ± 0.9 nM for control and treated cells, 20 ng/ml for 20 h, respectively). The effect of IL-10 on CCR5 surface expression was also observed when monocytes were exposed to HIV (Fig. 2 C).

The macrophage-tropic HIV-1 strain BaL (27) was used to investigate whether IL-10–induced upregulation of CC chemokine receptors affected the susceptibility of monocytes to infection. A productive HIV infection was observed in human peripheral blood monocytes that were incubated

**Results**

Incubation of human monocytes with an optimal concentration of IL-10 (10 ng/ml) for 4 h increased the expression of CCR1, 2, and 5 as evaluated by Northern blot analysis (Fig. 1 A). CCR3 and CCR4 are expressed at very low levels in human monocytes (15) and their expression was not induced by IL-10 (data not shown). No major variations in the expression of CXCR2 were detectable in 3 different donors (Fig. 1 A). In one experiment a partial reduction of CCR5 mRNA levels was detected (Fig. 1 A). Because of the peculiarities of CCR5 in terms of ligands and its relevance as HIV fusion cofactor, subsequent studies were conducted on this receptor. The effect of IL-10 was concentration dependent (effective concentration [EC]50 = 0.3 ± 0.1 ng/ml; 0.015 nM) and fast, already detectable after 30 min and reaching a plateau after 2 h of stimulation, with a maximal increase observed at 10 ng/ml (0.5 nM ) IL-10 (Fig. 1 B and C). The estimated half-life of CCR5 mRNA was 165 min and was augmented to 260 min (n = 2) after exposure to IL-10 (Fig. 1 D). In contrast, the rate of nuclear transcription of the gene, as investigated by nuclear runoff analysis, was not affected (Fig. 1 E).

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The macrophage-tropic HIV-1 strain BaL (27) was used to investigate whether IL-10–induced upregulation of CC chemokine receptors affected the susceptibility of monocytes to infection. A productive HIV infection was observed in human peripheral blood monocytes that were incubated
with the virus shortly after isolation. IL-10 caused a clear enhancement of virus multiplication, as previously reported using monocyte-derived macrophages (24). A panel of CC chemokines, including MIP-1α, MIP-1β, and MCP-1 was tested in parallel to RANTES for their capacity to interfere with HIV replication in control and in IL-10–stimulated monocytes. RANTES caused a detectable, although modest, delay in the onset of virus production in untreated monocytes and completely inhibited IL-10–induced upregulation of viral replication (Fig. 2D). In comparison, the other tested chemokines showed a very modest effect on viral replication (not shown). In this regard, the higher potency of RANTES as HIV inhibitor compared to other chemokines has been recently reported (28). In addition, AOP-RANTES, a RANTES mutein with antagonistic activity (21), completely abolished HIV replication in control as well as IL-10–treated monocytes (data not shown).

To validate this hypothesis, we analyzed the kinetics of proviral HIV DNA accumulation in control versus IL-10–stimulated monocytes. Proviral HIV DNA was readily demonstrated 16 h after infection (a time frame compatible with a single round of HIV replication) in IL-10–stimulated, but not control, monocytes, whereas similar signals were observed in control and IL-10–treated cells 40 h after infection (Fig. 3). AOP-RANTES substantially suppressed HIV DNA accumulation in both control and IL-10–stimulated cells (Fig. 3), as a result of interference with viral entry. CCR5 membrane expression, which was already upregulated after 6 h of incubation with IL-10, remained elevated during the subsequent 40 h. HIV infection resulted only in a minor decrease of IL-10–induced CCR5 expression (Fig. 2C).

Discussion

The results presented here show that the potent antiinflammatory and immunosuppressive cytokine IL-10 can upregulate expression of functional CCR1, 2, and 5 receptors in human monocytes. The effect of IL-10 was selective in that CCR3 and 4, which are normally expressed at very low levels, were not induced, and, in one experiment, CXCR4, which is present and functional in monocytes, was slightly decreased. The modulatory action of IL-10 was mediated by prolongation of mRNA half-life. This observation, together with recent findings with prototypic pro- and antiinflammatory molecules (15 and our unpublished...
observatiors, see also below), indicates that receptor mRNA stability is a crucial set point for the action of chemokines.

IL-10 has been shown to have divergent effects on HIV replication in macrophages in vitro, depending on experimental conditions such as cytokine concentrations (24, 29–31). In this study, we found that IL-10 promoted a productive infection of monocytes by the macrophage-tropic HIV BAl strain, an effect that was associated with an increase of viral entry. Since the IL-10-mediated enhancement was inhibited by AOP-RANTES and completely abolished by AOP-RANTES, we infer that upregulation of CCR5 plays a major role in IL-10 enhancement of HIV replication, at least under these experimental conditions. A.S. Fauci has recently observed a transient decrease of circulating HIV virions (viremia) in HIV-infected individuals who were injected intravenously with IL-10 (Fauci, A.S., personal communication). Our results suggest a potential mechanism perhaps contributing to this in vivo effect, i.e., the enhancement of cell surface expression of CCR5 and other chemokine receptors by IL-10 may favor the sequestration and, eventually, the entry of free circulating virions. However, it should be stressed that IL-10 may exert multiple effects on HIV infection, such as the inhibition of HIV replication dependent upon release of proinflammatory cytokines, as previously reported (29), in addition to the effect observed in this study.

The in vivo relevance of IL-10–mediated upregulation of CCR5 in monocytes from sites of inflammation, the regulation of chemokine receptor expression in mucosal tissues, contributing to the dominant role of this fusion coreceptor in primary HIV infection.

In addition to IL-10, we recently found that other molecules with antiinflammatory activity, such as glucocorticoid hormones, upregulate certain CC chemokine receptors (e.g., CCR2; data not shown). These agents concomitantly inhibit chemokine (e.g., M C P-1) production in monocytes (7, 8). Reciprocally, at least certain prototypic primary proinflammatory agents (endotoxin, TNF) induce chemokine production and inhibit receptor expression (references 12, 15 and our unpublished observations). Hence, an emerging paradigm indicates that at least some pro- and antiinflammatory molecules exert reciprocal and opposing influences on chemokine ligand production and receptor expression. This interplay may serve as a negative feedback mechanism and as a means to regulate the efflux of mononuclear phagocytes from sites of inflammation. The regulation of chemokine receptor expression mediated at the level of transcript stability may represent a novel target for pharmacological intervention in inflammatory diseases and viral infections.
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