Cutting Edge: Dichotomous Effects of $\beta$ -Chemokines on HIV Replication in Monocytes and Monocyte-Derived Macrophages

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The role of β-chemokines in the pathogenesis of HIV disease remains undefined. Given the potent capacities of these proteins to attract mononuclear cells to inflammatory sites, such as lymph nodes of patients with HIV disease, the effects of exposure of monocytes and monocyte-derived macrophages to β-chemokines before HIV infection were compared with their effects when added either simultaneously with or after HIV infection. In this system, HIV replication was substantially increased in cells that had been exposed to β-chemokines before HIV infection. These effects were pertussis toxin sensitive. By contrast, HIV replication was inhibited in cells that had been exposed to β-chemokines either simultaneously with or after HIV infection. These effects were not pertussis toxin sensitive. In view of this potent capacity of β-chemokines to stimulate HIV replication, treatments approaches for HIV disease based on the apparent inhibitory activity of these proteins on viral replication should be undertaken with caution. The Journal of Immunology, 1998, 160: 3091–3095.

The interactions among chemokine receptors, chemokines, and HIV are likely to be critical to the pathogenesis of HIV disease. While it has been established that chemokine receptors are essential coreceptors with the CD4 receptor for HIV-1 entry into susceptible cells, the physiologic role of chemokine receptors per se in the pathogenesis of HIV disease is yet to be established. In general, most monocytotropic HIV-1 strains use CCR5 to enter macrophages and primary CD4 T lymphocytes (1), whereas T-tropic HIV-1 strains use CXC chemokine receptor 4 to enter primary CD4 T lymphocytes and T cell lines (2). The ligands for CCR5 (RANTES, MIP-1α, and MIP-1β) and CXC chemokine receptor 4 (stromal-derived-factor-1) have been demonstrated to inhibit HIV entry into CD4 T cells and PBMCs, as well as monocytic and CD4 T cell lines (3–6). Given these inhibitory effects, increased production of these proteins has been suggested to be a protective host immune response against HIV infection and disease progression (3). However, there are conflicting reports regarding the influence of β-chemokines on HIV-1 replication in MDM and tissue macrophages, with enhancement, inhibition, or no effect reported (7–10). The effects of β-chemokines on HIV replication in monocytes have not previously been reported.

We have previously demonstrated that β-chemokine expression is strongly enhanced in lymph nodes of patients with HIV disease (11). Thus, cells recruited to HIV lymph nodes are likely to interact with β-chemokines before exposure to the virus given that the primary physiologic role of β-chemokines is to direct the traffic of mononuclear cells to sites of inflammation (12). However, studies to date have examined the influence of β-chemokines only when added simultaneously with and/or after HIV infection. Accordingly, we analyzed the effects of timing of exposure to β-chemokines on HIV replication in monocytes and MDM using an in vitro system modeled on this in vivo scenario. β-Chemokine exposure produced dichotomous effects on HIV replication in this system.

Materials and Methods

Isolation of monocytes

Monocytes were isolated from PBMCs of uninfected healthy HIV-seronegative donors by countercurrent elutriation and anti-CD3 complement-dependent lysis as previously described (13). Monocytes were cultured at a density of $1 \times 10^6$ cells/ml either for 48 h at 37°C before HIV infection or for 7 days before infection to permit differentiation into MDM. All media, viral stock, and reagents were endotoxin free as assessed by the Limulus amebocyte lysate assay (Sigma Chemical Co., St. Louis, MO). Flow cytometry analysis revealed these cultures to have $<1\%$ CD3 contamination. Purity was further confirmed by the inability of T-tropic HIV strains such as HIV-1 PNL4-3 to grow in these cultures.

HIV infection of monocytes and MDM and p24 Ag assay

The laboratory monocytotropic strain HIV-1_dal, obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, was used in this study. Cells were inoculated with cell-free HIV isolates at $1 \times 10^5$ cpm/ml of room temperature activity ($10^5$ TCID$_{50}$ (tissue culture-infective dose)/ml on PBMCs and at a multiplicity of infection of 0.02/cell).
and allowed to adsorb for 4 h before complete aspiration of medium, washing, and addition of fresh medium. Media was replaced every 3 days and supernatants were collected, stored at 2\(^\circ\)C, and batched for HIV p24 Ag quantitation by ELISA (Organon Teknika, Durham, NC). Results above 12 pg/ml were considered positive. Results were expressed either as raw data or as a fold change of the untreated control culture according to the following formulae:

- When p24 Ag was increased in treated cultures:
  \[
  \text{fold change} = \frac{\text{p24 Ag treated}}{\text{p24 Ag control}} - 1
  \]
- When p24 Ag was decreased in treated cultures:
  \[
  \text{fold change} = -\left(\frac{\text{p24 Ag control}}{\text{p24 Ag treated}} - 1\right)
  \]

Chemokine and pertussis toxin treatment

Recombinant human RANTES, MIP-1\(\alpha\), or MIP-1\(\beta\) (R&D Systems, Minneapolis, MN) was added to cells either before, simultaneously with, or after HIV infection in increasing concentrations from 0.1 to 500 ng/ml. In the preinfection treatment experiments, cell cultures were exposed to chemokines for 48 h. In monocyte cultures, chemokines were added to cells on the day of isolation, and the cells were subsequently infected on day 2. In MDM cultures, chemokines were added on day 5 after plastic adherence, and cells were infected on day 7. Before HIV infection, cell cultures were then washed three times, replenished with fresh media not containing added chemokines, and then infected with HIV. In the simultaneous infection treatment experiments, cell cultures were treated with chemokines during the 4-h period of HIV infection only. In the postinfection treatment experiments, chemokines were initially added to the cultures immediately after the inoculum was washed off and then every 3 days thereafter with each media change.

Before chemokine treatment cells were exposed to 500 ng/ml of pertussis toxin (Calbiochem, San Diego, CA) for 12 h. Cells were treated with pertussis toxin once only except for cells that were treated with \(\beta\)-chemokines following HIV infection, where pertussis toxin was applied every 3 days with fresh media.

Results and Discussion

By varying the timing of chemokine exposure, enhancement or suppression of HIV-1 replication in monocytes and MDM was demonstrated (Fig. 1). When cells were exposed to \(\beta\)-chemokines before HIV infection, subsequent HIV replication was enhanced. All three \(\beta\)-chemokines tested enhanced HIV-1 replication in both monocytes and MDM, which ranged from 140 to 800% of untreated control cultures. The three \(\beta\)-chemokines stimulated HIV-1 replication to comparable levels in monocytes although MIP-1\(\alpha\) caused greater increases in HIV replication in MDM than did either RANTES or MIP-1\(\beta\). By contrast, addition of \(\beta\)-chemokines either simultaneously with or after HIV infection inhibited subsequent viral replication. Under these conditions, all three chemokines inhibited HIV-1 replication to a comparable degree. Inhibition ranged from 20 to 88% compared with untreated controls.

These effects persisted for up to 14 days in culture. While maximal
inhibition was achieved when chemokines were added both simultaneously with and after HIV infection, they were negated by pretreatment with β-chemokines (data not shown).

These dichotomous effects were concentration dependent (Fig. 2). The enhancement of HIV replication in cells pretreated with β-chemokines was seen across a broad concentration range from 10 to 500 ng/ml. Conversely, the inhibitory effects of β-chemokines were observed only at concentrations of 100 ng/ml and above. In vitro and in vivo studies suggest that chemokines regulate leukocyte migration at concentrations in the 0.1 to 100 ng/ml range (14). Above or below these levels, leukocyte chemotaxis or adhesion in vitro is abrogated. We were unable to demonstrate significant inhibition at concentrations of <100 ng/ml as has been reported by other laboratories (15). While concordant results were observed in both monocytes and MDM, monocytes were generally more susceptible to both the stimulatory and inhibitory effects of β-chemokines. As expected, IL-8, an α-chemokine, had no effect on HIV replication (data not shown).

We analyzed endogenous production of β-chemokines in our endotoxin-free system. We observed that while nonspecific stimuli such as monocyte isolation and adherence to plastic resulted in transient induction of β-chemokines, HIV infection was associated with sustained induction of these proteins. All three chemokines were induced at levels between 5 and 10 ng/ml, which was four to six times that found in uninfected control cultures. However, unlike other laboratories (15, 16), we were unable to demonstrate enhanced HIV replication following inhibition of endogenous β-chemokines by neutralizing Abs (data not shown).

Our data conflict with two previous reports. The first demonstrated enhancement of HIV replication in day 7 MDM that had been exposed to β-chemokines simultaneously with and after HIV infection (7). Differences in viral inoculum, viral strain, and culture conditions may have contributed to these conflicting results. The viral inoculum used in this previous study was five times lower than that used in our study. The increased numbers of uninfected cells in the culture system immediately following HIV inoculation would thus be exposed to β-chemokines before HIV infection. Our data would suggest, therefore, that HIV replication in these cells would be subsequently increased. Indeed, when we repeated the experiment using a viral inoculum of 0.004 multiplicity of infection/cell, five times lower than the inoculum used in our initial experiments, enhancement of HIV replication was observed.
even when β-chemokines were added simultaneously with and after HIV infection. A second study reported insensitivity of day 14 MDM to the effects of β-chemokines (21). We have shown here and in other work (M. Kelly, unpublished observations) that the sensitivity to β-chemokines decreases as the monocyte matures following adherence to plastic. These observations broadly correlate with the alterations in permissiveness to HIV infection by differentiating monocytes (H. Naif, unpublished observations) and changes in CCR5 surface expression which increases in the early stages of monocyte differentiation (22). However, preliminary work suggests that CCR5 expression is not altered by 48 h of chemokine treatment or chemokine withdrawal at physiologic concentrations.

The mechanisms producing the dichotomous effects of β-chemokines on HIV replication in monocytes and MDM have not been established. As has been previously reported (5), we found that the inhibitory effects of β-chemokines when applied either simultaneously with (Fig. 3) or after (data not shown) HIV infection were insensitive to pertussis-toxin and therefore do not involve signaling through pertussis toxin-sensitive G proteins. However, the stimulatory effects of β-chemokines were dependent on cell signaling events via pertussis toxin-sensitive G protein-linked pathways. While the downstream signaling events in this system have not been elucidated, β-chemokines may stimulate a number of intracellular mechanisms that may lead to increased HIV replication. β-Chemokines have been demonstrated to provide costimulatory signals for human T lymphocytes (17, 18), and MIP-1α has been demonstrated to increase proliferation and cytokine production of murine tissue macrophages (19). While the effects of β-chemokines on human monocytes and MDM are unknown, activation of these cells by β-chemokines may be expected to increase subsequent HIV replication as has been shown for other stimuli, such as TNF-α (20). Furthermore, β-chemokines may upregulate nuclear transcription factors, such as nuclear transcription factor κB, that may, in turn, augment HIV transcription. TGF-β, IFN-γ, IL-4, and IL-13 also have dichotomous effects on HIV replication in vitro (23, 24). Some of these effects are concentration dependent and vary according to the timing of HIV infection. However, the exact mechanisms of their effects have not been established.

It is likely that mononuclear cells recruited to lymph nodes and other inflammatory sites during HIV infection do so under the influence of β-chemokines. The data presented here demonstrating increased HIV replication in pretreated monocytes and MDM suggest that mononuclear cells arriving in lymph nodes may be rendered more susceptible to HIV infection by prior exposure to β-chemokines during emigration. Conversely, cells may be exposed to HIV in the presence of β-chemokines, which may limit HIV infection by preventing viral entry into susceptible cells. However, in the experiments presented here and in other work (3, 8) significant inhibition was observed only at high concentrations of β-chemokines, which may be above those achieved in vivo.

Preliminary work in our laboratory suggests that CD4 T lymphocytes are also susceptible to the enhancing effects of β-chemokines on HIV-1 replication observed in monocytes and MDM. Furthermore, primary clinical isolates have also demonstrated similar sensitivity as HIV-1_Day 1, to the dichotomous effects of β-chemokines (M. Kelly, unpublished observations).

The data presented here suggest that β-chemokines have dichotomous and opposing effects on HIV replication. Because variation in experimental conditions such as the size of the inoculum, degree of maturation of the target cell, and other unidentified host factors alter the in vitro effects observed further studies are required to determine which of these effects are dominant in vivo. Therefore,

until the exact role of β-chemokines in HIV disease has been established, treatment approaches with these proteins or pharmacologic analogues should proceed only with caution.

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References


