

Sensitivity of human immunodeficiency virus infection to various α , β and γ chemokines

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Examination of a large panel of chemokines indicates that in addition to RANTES, MIP-1 α and MIP-1 β , the β -chemokine MCP-2 and, to a lesser extent, the γ -chemokine lymphotactin also show anti-human immunodeficiency virus (HIV) activity in cell culture. The amount of chemokine needed to suppress HIV replication by $\geq 50\%$ was generally greater (≥ 250 ng/ml) than that required for inhibition of virus infection by RANTES, MIP-1 α and MIP-1 β . The β -chemokine MCP-3 was found to enhance the replication of both non-syncytium-inducing (NSI) and syncytium-inducing (SI) viruses at high concentrations (0.5–5 μ g/ml). In contrast to a previous report, macrophage-derived chemokine was not found to inhibit HIV replication of either NSI or SI viruses, but at low concentrations enhanced NSI virus replication. When small amounts of RANTES or MCP-2 were added together with high concentrations of non-inhibitory chemokines, the anti-HIV effects were countered. Information on chemokines that affect HIV infection could be useful for future therapeutic strategies.

The β -chemokines RANTES, MIP-1 α and MIP-1 β have been shown to inhibit the infection of cultured peripheral blood mononuclear cells (PBMC) by non-syncytium-inducing (NSI) isolates of HIV (Cocchi *et al.*, 1995). This finding, along with the identification of the receptor for stromal-derived factor-1 as an HIV-1 coreceptor (Feng *et al.*, 1996), led to observations that several chemokine receptors can act as coreceptors for HIV infection (reviewed by Berger, 1997). Recently, macrophage-derived chemokine (MDC) (Pal *et al.*, 1997) and monocyte chemotactic protein-2 (MCP-2) (Gong *et al.*, 1998) have been identified as other β -chemokines that can block HIV infection. These chemokines can exert their anti-HIV activity either by interfering with the binding of the virus

to its coreceptor or by down-regulating the receptor expression on the cell surface (Trkola *et al.*, 1998).

Our present studies with a large panel of chemokines indicate that most had no effect on HIV infection. RANTES, MIP-1 α and MIP-1 β , as well as MCP-2, and to a lesser extent, lymphotactin, significantly inhibited replication of certain primary isolates of HIV-1. MCP-3 and MDC enhanced virus replication. In addition, we found that the chemokine-mediated antiviral effect of RANTES and MCP-2 could be prevented, in part, by the addition of another chemokine with different biological activity.

For these studies CD4⁺ cells from seronegative donors were isolated from Ficoll–Hypaque-separated PBMC using anti-CD4 antibody-coupled immunomagnetic beads (Dynal) (Mackewicz *et al.*, 1991). More than 95% were CD4⁺/CD3⁺ cells (less than 1% CD8⁺ cells) as assessed by flow cytometry (Landay *et al.*, 1993). Cell cultures were grown in RPMI 1640 medium (BioWhittaker) supplemented with 10% heat-inactivated (56 °C, 30 min) foetal calf serum (Gemini Bioproducts), 100 U/ml recombinant IL-2 (Glaxo-Wellcome), 2 mM L-glutamine and 1% antibiotics.

The four primary HIV-1 isolates used (designated SV, NB, EM and ALA-22) were obtained from clinically healthy HIV-infected subjects. The biological phenotype of SV, NB and ALA-22 was non-syncytium-inducing as determined in MT-2 cells. These viruses have been cultivated in the laboratory for less than 2 months and only in PBMC. Their infection is blocked by RANTES, MIP-1 α and MIP-1 β . The SF2 strain of HIV-1, isolated from a patient with candidiasis (Levy *et al.*, 1984), and the primary isolate, EM, have a tropism for T-cell lines *in vitro*. They are syncytium-inducing (SI), non-macrophage-tropic viruses resistant to the inhibitory effects of the β -chemokines MIP-1 α , MIP-1 β and RANTES (Mackewicz *et al.*, 1996). The TCID₅₀ of each virus was determined in PBMC as described (McDougal *et al.*, 1985).

The purified CD4⁺ T-cells were stimulated with phytohaemagglutinin (PHA, 3 μ g/ml; Sigma) for 3 days, washed and infected with 10–40 TCID₅₀ of HIV-1 per 10⁶ cells as described (Barker *et al.*, 1998). After incubation for 2 h, cells were washed, and plated at 5 \times 10⁵ cells per well in duplicate or triplicate in 48-well plates (Becton Dickinson). Varying concentrations of recombinant chemokines were added and the cultures were

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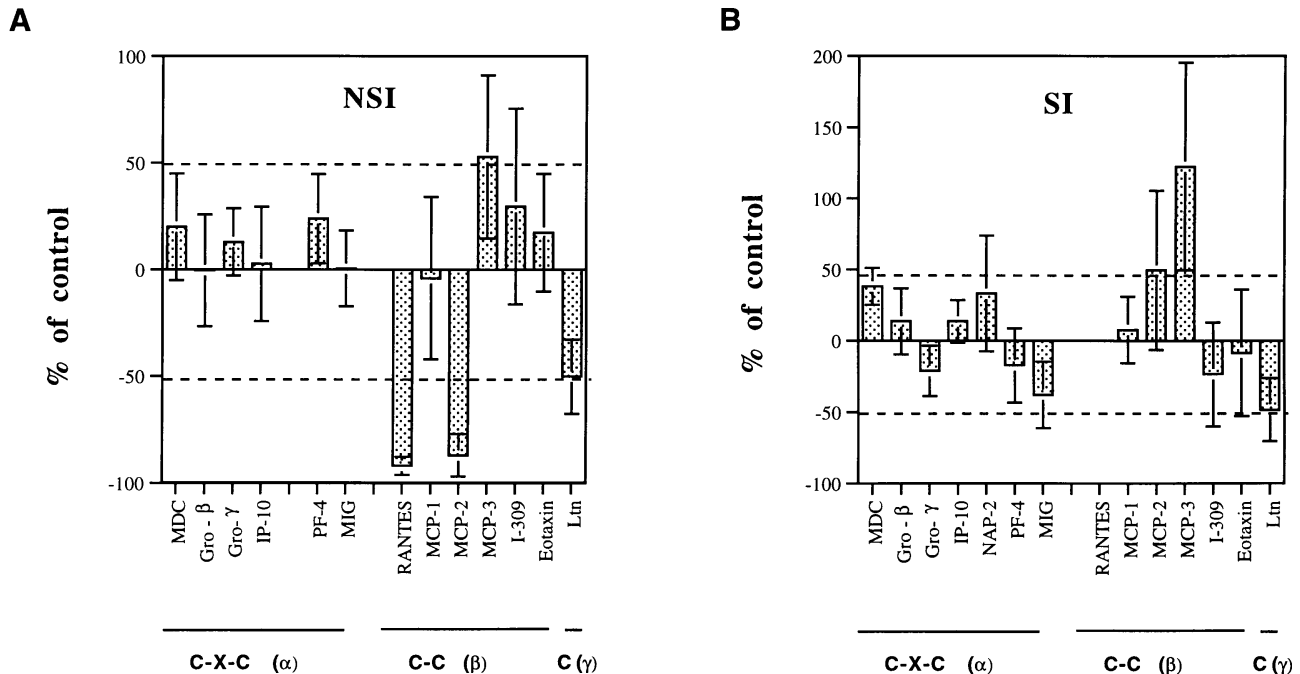


Fig. 1. Effect of chemokines on HIV-1 replication in CD4⁺ lymphocytes. Non-syctium-inducing (NSI) viruses (SV, ALA-22, NB) (A) or syctium-inducing (SI) virus isolates (EM, SF2) (B) were inoculated onto PHA-stimulated CD4⁺ T-cells at an input of 10–40 TCID₅₀ per 10⁶ cells. Two hours after infection, cells were washed and plated at about 10⁶ cells per well in duplicate wells of a 48-well plate in the presence of different concentrations of chemokines. Results obtained using 0.5–1 µg/ml of chemokines are shown (with the exception of MCP-1 with NSI and SI strains, and MCP-3 with SI strains: 5 µg/ml was used). Cultures were maintained for 6–10 days and were replenished with fresh chemokine-containing medium every 2–3 days. Virus replication was measured in the collected supernatants by RT assays (Hoffman *et al.*, 1985). The effect of each chemokine on virus replication is shown as a percentage relative to that obtained with infected CD4⁺ T-cells inoculated with virus alone. The results are representative of at least two separate experiments per chemokine, with at least two different NSI or SI isolates. The results were confirmed with CD4⁺ cells from different donors. Suppression or enhancement was considered present if virus replication was reduced or increased by ≥ 50%, respectively. Suppression by RANTES was observed with some NSI viruses at 5–50 ng/ml; MIP-1α, MIP-1β and MCP-2 generally inhibited NSI virus replication at ≥ 100 ng/ml (data not shown). Lymphotactin suppressed NSI viruses sometimes at 1 µg/ml and consistently suppressed both NSI and SI viruses at 2 µg/ml. Enhancement of NSI and SI viruses by MCP-3 was sometimes observed at 200–2000 ng/ml; it was consistently noted at 5 µg/ml. With MDC, enhancement was observed with 10–100 ng/ml. In some experiments, depending on the donor CD4⁺ cells used, enhanced HIV replication of NSI strains of HIV was observed at ≥ 1 µg/ml eotaxin and ≥ 3 µg/ml MCP-1. Sometimes inhibition of HIV replication was observed with Gro-β at ≥ 100 ng/ml. NAP-2 did not show any effect with NSI strains in one experiment. IL-8, interleukin 8; Gro, growth-related oncogene; IP10, interferon-inducible protein 10; NAP-2, neutrophil-attracting peptide 2; PF-4, platelet factor 4; MIG, monokine induced by γ-interferon; RANTES, regulated on activation normal T-cell expressed and secreted; MIP, macrophage inflammatory protein; MCP, monocyte chemotactic protein; MDC, macrophage-derived chemokine.

passed every 2–3 days for up to 1 week. Cultures were replenished with fresh medium and chemokine at each passage. The culture fluids were monitored for virus replication by measurement of reverse transcriptase (RT) activity (Hoffman *et al.*, 1985).

The recombinant chemokines studied were used at concentrations ranging from 50 ng to 5000 ng/ml and were provided by Michael Luther, Glaxo-Wellcome. All were obtained from R&D Systems, except for NAP-2, MIG, PF4 and lymphotactin, which were obtained from Peprotech. The recombinant MDC proteins evaluated came from both R&D Systems and Peprotech. The range of concentrations of chemokines tested for anti-HIV activity corresponded approximately to 0.5–100 ED₅₀ of chemotactic activity (as noted by the company providing the reagents). Results were analysed for statistical significance by the Mann–Whitney U-test.

Fig. 1 shows the effect of 0.5–5 µg/ml of a large panel of commercially available recombinant chemokines. For most of the cytokines, the effect did not vary over a large range of concentrations (50 ng–5 µg). They caused a < 20% difference in HIV replication when compared with the control-infected culture, regardless of the virus used (Fig. 1). Besides RANTES, MIP-1α and MIP-1β, only MCP-2 and lymphotactin produced an inhibitory effect within the range of concentrations tested (Fig. 2). With MCP-2 and NSI viruses, up to a 90% decrease in virus replication was observed with ≥ 1 µg/ml, and 70% with ≥ 0.25 µg/ml (Figs 1 and 2). No substantial inhibitory effect of this chemokine on SI viruses (Fig. 2) was noted. Lymphotactin at a concentration of 1 µg/ml or more reduced the replication of both NSI and SI viruses by at least 50% (Fig. 2). In some experiments, Gro-β inhibited infection by SI viruses, but this finding was not consistently seen.

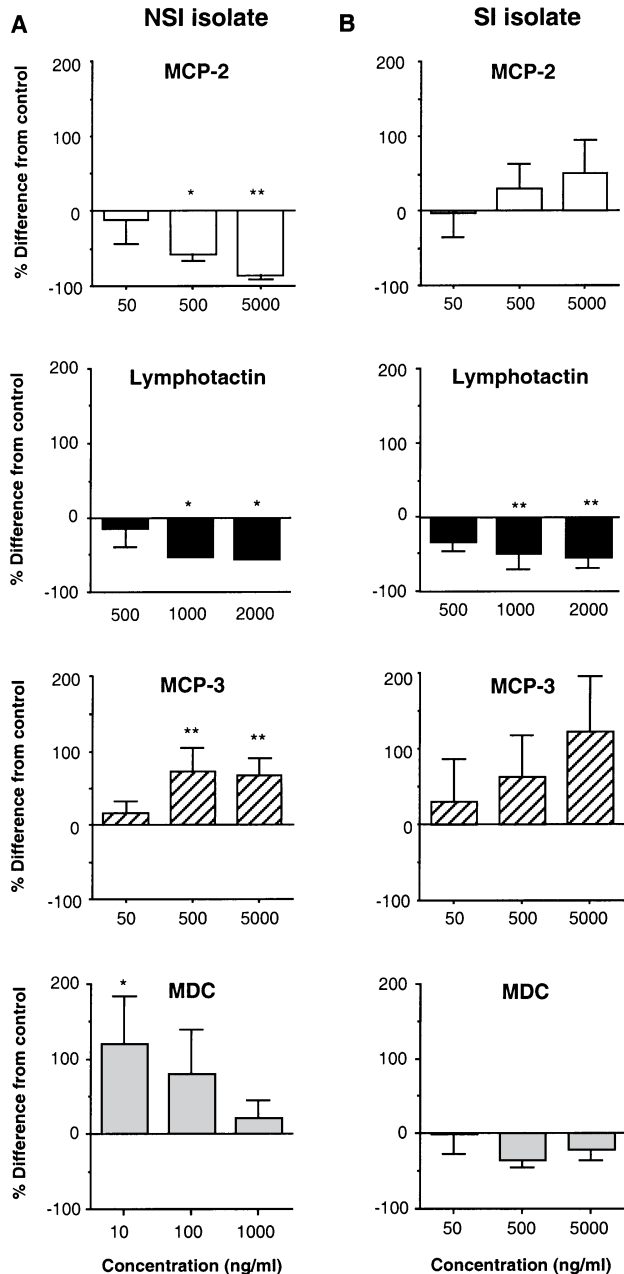


Fig. 2. Effect of MCP-2, lymphotactin, MCP-3 and MDC on HIV replication. PHA-stimulated CD4⁺ lymphocytes were inoculated with either the SV NSI isolate (A) or the SF2 SI isolate (B) at 10–20 TCID₅₀ per 10⁶ cells. After 2 h, cells were washed and plated at 5 × 10⁵ cells per well in triplicate in a 48-well plate in the presence of different concentrations (ng/ml) of recombinant MCP-2, lymphotactin, MCP-3 and MDC. Cultures were maintained for 7 days and replenished with fresh chemokine-containing medium every 2–3 days. Virus replication was measured in the collected culture supernatants by RT assays (Hoffman *et al.*, 1985). The number of replicates in the control and chemokine-treated group varied between 3–7 and 2–4 samples, respectively. The results reflect the extent of virus replication at day 7 expressed as a percentage relative to that obtained from control-infected CD4⁺ cells not receiving the chemokine. Virus replication in control cultures ranged between 100 × 10⁵ and 300 × 10⁵ c.p.m./ml of RT activity. The results are representative of five separate experiments, three different NSI viruses (SV, NB and ALA-22) and two SI isolates (EM, SF2). **, *P* ≤ 0.01; **P* ≤ 0.05.

In contrast to the above observations, the β -chemokine MCP-3 consistently enhanced replication of both NSI and SI viruses when used at high concentrations (5 μ g/ml) and at a lower concentration (0.5 μ g/ml) with the NSI viruses evaluated (Figs 1 and 2). This effect was statistically significant for NSI viruses (*P* < 0.01) and close to significant for SI viruses (5 μ g/ml; *P* < 0.08). MCP-1, a β -chemokine related to MCP (Adams & Lloyd, 1997), showed no substantial effect on replication of either NSI or SI isolates (Fig. 1). An enhancement of HIV infection was sometimes noted with MCP-1 and eotaxin with NSI isolates (Fig. 1). Surprisingly, MDC, at low concentrations, was also found to have an enhancing effect on the replication of NSI (*P*, 0.05), but no significant effect on SI isolates. As much as 200% increases in HIV replication were observed at 10 ng/ml MDC (Fig. 2). The variations in effects of chemokines on HIV replication (Figs 1 and 2) probably reflect differences in the CD4⁺ cells used. In contrast to one report (Kinter *et al.*, 1998) we did not see enhancement of SI isolates by RANTES, MIP-1 α and MIP-1 β , but we did not pretreat cells with the chemokines nor use suboptimal concentrations of virus.

Several chemokines share receptors (Adams & Lloyd, 1997) and therefore may compete for the same receptor. By other mechanisms, the effect of one chemokine may dominate over another. In evaluating this possibility, RANTES at a concentration (5 ng/ml) that suppressed replication of NSI viruses (SV or NB) by 70% was mixed with MCP-3 (1000 ng/ml); the inhibition of virus production was abrogated. In another study, MCP-3 (2000 ng/ml) prevented inhibition of virus replication by a low concentration of RANTES (20 ng/ml). At higher concentrations of RANTES (80 ng/ml) this effect of MCP-3 was not observed. In addition, whereas MCP-2 (500–1000 ng/ml) inhibited replication of the NSI virus, ALA-22, the addition of MCP-1 (1000 ng/ml) prevented this activity.

These studies indicate that, in addition to the chemokines already known to have anti-HIV properties, MCP-2 and to a lesser extent, lymphotactin, showed consistent inhibition of HIV replication (Figs 1 and 2). As with MIP-1 α , MIP-1 β and RANTES, the effect of MCP-2 (at \geq 1 μ g/ml) was limited to NSI isolates. These results support findings recently reported (Gong *et al.*, 1998). Lymphotactin was effective in reducing virus replication of both NSI and SI isolates. However, high doses (\geq 1 μ g) of lymphotactin were necessary to show a substantial (55%) decrease in virus production (Fig. 2).

CCR5 and CXCR4 are the primary coreceptors for macrophage-tropic and T-cell-tropic HIV-1 strains, respectively (for a review see Berger, 1997). In addition, certain virus strains can use the CC-chemokine receptors CCR-1, CCR2b and CCR3 (Berger, 1997; Frade *et al.*, 1997). All the MCP recognize CCR2b (the chemokine receptor to which CCR5 is most closely related: 76% identity) (Rucker *et al.*, 1996), whereas CCR3 serves as a receptor for RANTES, MIP-1 α , MIP-1 β , eotaxin, MCP-2, MCP-3 and MCP-4 (Adams & Lloyd, 1997). Nevertheless, despite the overlap of receptor

usage among the MCPs, only MCP-2, probably because it can bind to CCR5 (Gong *et al.*, 1998), showed a reproducible inhibitory effect on replication of the NSI isolates evaluated (Figs 1 and 2).

Our results also indicated that, at certain concentrations, MCP-3 and MDC can enhance virus replication in CD4⁺ cells (Figs 1 and 2). These findings contrast with a previous report showing inhibitory activity of MCP-3 on replication of SI isolates of HIV-1 in PBMC (Schols *et al.*, 1997), and MDC on both NSI and SI HIV isolates (Pal *et al.*, 1997). We did not detect any inhibition of HIV production by either of these chemokines. Recently, MDC was shown to be a ligand for CCR-4 but not CCR-1, CCR-3 or CCR-5 (Yoshida *et al.*, 1998). Thus, MDC would not be expected to compete for HIV binding to a virus coreceptor. However, these different observations may be due to the experimental conditions used (e.g. PBMC instead of CD4⁺ cells), the various concentrations of chemokine evaluated or the source of the chemokine. Nevertheless, the recombinant MDC we used came from two sources (R&D Systems and Peprotech) and had chemotactic activity. The reported antiviral activity of MDC has also not been confirmed by other studies in which recombinant MDC was used (Lee *et al.*, 1998). The maturation of MDC, resulting in NH₂-terminal modification, could influence its receptor binding and activation profile. This possibility could account for the conflicting results obtained with the recombinant MDC versus the purified natural product originally used (Pal *et al.*, 1997).

The mechanisms underlying chemokine-mediated anti-HIV activity may involve competition for receptor occupancy and/or down-regulation of the receptor (Trkola *et al.*, 1998). Conceivably, MCP-2 could exert its inhibitory activity on NSI isolates by competitive binding to CCR5, which does not occur with the other MCPs; lymphotactin-mediated inhibition of NSI and SI strains could involve mechanisms different from competition for the receptors. In terms of enhancement, the increase in HIV replication observed with the addition of some chemokines (Figs 1 and 2) may result from upregulation of certain virus receptors as reported recently (Dolei *et al.*, 1998). These possibilities are under study.

Chemokines may have opposite regulatory effects and therefore specific combinations of chemokines could result in qualitatively different effects on HIV replication than that observed when single chemokines are used alone. In this regard, we have found that chemokines, devoid of any inhibitory activity on HIV, may affect inhibition induced by other chemokines. In particular, the concentration of RANTES or MCP-2 required to produce a certain level of inhibition must be increased when these chemokines are present in combination with MCP-3 and MCP-1, respectively.

This study presents further information on the potential role of chemokines in blocking HIV infection. Most chemokines do not appear to substantially affect the levels of HIV replication in CD4⁺ T-cells. Moreover, levels of those

chemokines that inhibit HIV infection were higher than expected under normal physiological conditions. Nevertheless, we cannot exclude the possibility that these chemokines play some role in HIV pathogenesis, perhaps via inflammatory responses or by interfering with HIV adaptation to certain coreceptors. Further understanding of these cytokines might assist in the development of novel antiviral strategies that are capable of blocking infection by both macrophage-tropic and T-cell line-tropic isolates.

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