Chemokines and HIV replication

SIR — Three chemotactic peptides belonging to the β-chemokine group of cytokines, RANTES, MIP-1α, and MIP-1β, inhibit the infection of $CD4^+$ T cells by primary HIV-1 strains[1]. Although all three peptides suppress HIV-1 replication in T cells, they do so with different efficiency, with RANTES being the most active, followed by MIP-1β, then MIP-1α[1]. Here we demonstrate that, in sharp contrast to their observed antiviral effects in T cells, β-chemokines actually stimulate the replication of primary HIV-1 strains in macrophages, another major target of this virus. The magnitude of stimulation is dependent on the cell donor and HIV-1 strain used, and varies for different β-chemokine peptides.

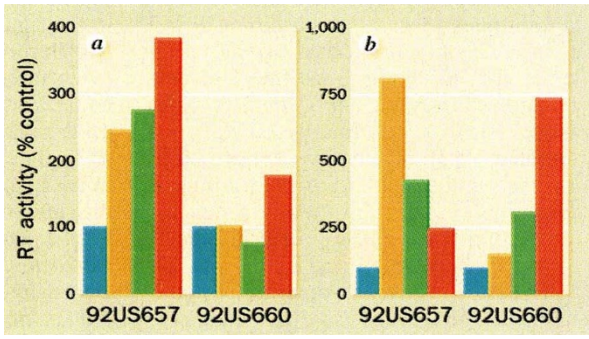
In addition to T lymphocytes, monocyte/macrophages are a rich source of β-chemokines in the body[2]. HIV-1 infection itself upregulates β-chemokine expression in monocytes both *in vitro* and *in vivo*[3]. In the light of their anti-HIV effects on T cells, the release of these peptides by macrophages in response to HIV-1 infection might reflect a defensive manoeuvre of the immune system. To test this hypothesis, we performed experiments to assess the anti-HIV-1 activity of β-chemokine peptides in monocyte cultures, and the effect of chemokine peptides on HIV-1 replication in T lymphocytes.

We used two primary HIV-1 isolates that replicate both in T lymphocytes and monocytes, 92US657 and 92US660 (obtained from NIH AIDS Research and Reference Reagent Program), for infections. We isolated lymphocytes from the same donors as monocytes by passing non-adherent cells through a T-cell enrichment column (R&D, Minneapolis) resulting in a 95% pure $CD4^+$ + $CD8^+$ T-cell population. As expected, RANTES, MIP-1β and MIP-1α (all peptides from PeproTech Inc.) suppress replication of both isolates in T lymphocytes, with a 50% inhibitory concentration (IC_{50}) of 5, 15 and 35 ng ml^{-1}, respectively, in good agreement with previously published results[1]. At 500 ng ml^{-1}, each chemokine inhibited HIV-1 replication by more than 95 %, and this concentration was chosen for testing chemokines' effects on HIV-1 infection of macrophages.

In contrast, we observed an enhancing, rather than an inhibitory, activity of all three β-chemokines on the replication of both HIV-1 strains in macrophage cultures prepared from two donors (see figure). We observed this enhancing effect of β-chemokine peptides throughout the course of infection; data in the figure show results obtained on day 15 post-infection when virus replication reached the peak. This effect is dose-dependent (data not shown) and is not the result of contaminating lipopolysaccharide in chemokine preparations (less than 0.1 ng per μg of peptide) as similar amounts of lipopolysaccharide either have no effect or suppress HIV-1 replication in macrophage cultures (not shown). We obtained similar results (not shown) with another HIV-1 strain, $HIV\text{-}1_{ADA}$, and β-chemokine peptides from a different source (R&D), indicating that the observed phenomenon does not result from peculiarities of the chemokine preparations but rather reflects a general feature of these molecules.

Dragic *et al.*[4] have also observed failure of β-chemokines to inhibit HIV-1 infection of primary monocytes. However, these authors did not detect any stimulatory effect of these peptides on viral replication. The different results could be due to the source of virus used for infection: Dragic *et al.* used recombinant HIV-1 strains, whereas our results were obtained with primary isolates. As shown in the figure, strain differences significantly affect the magnitude of the stimulation. Although the observed stimulatory effect of β-chemokines on HIV-1 replication could be a by-product of their ability to activate macrophages, other mechanisms must be also involved, as MIP-1α is a much more potent stimulatory agent than MIP-1β[5], yet their effect on HIV-1 replication is comparable in most cases (*a* in the figure).

Taken together, these findings suggest that the ability of β-chemokine peptides to either inhibit or stimulate HIV-1 replication is cell-type dependent. Failure of chemokines to inhibit HIV-1 replication in macrophages despite the presence on these cells of CCR-5, a β-chemokine receptor and a co-receptor for HIV-1[4,6], indicates that a different co-receptor which does not bind β-chemokines might be involved in HIV-1 infection of macrophages. Because HIV-1 infection induces β-chemokine expression in monocytes[3], the infected immune system might actually benefit by allowing tempered viral replication in these cells (which are less susceptible to the cytopathic effects of infection than T cells[7]), so that sufficient amounts of the β-chemokines can be produced to inhibit vigorous virus replication in T-lymphocyte populations. On the other hand, high levels of β-chemokine peptides could produce harmful results by enhancing HIV-1 replication in macrophages and/or intensifying virus-induced inflammation, as demonstrated for Coxsackie and influenza viruses[8]. These considerations should be taken into account when considering the use of β-chemokines as anti-HIV therapeutic agents.



Effect of β-chemokine peptides on HIV-1 replication in monocyte cultures. Dark boxes, controls; light hatching, MIP-1α; heavy hatching, MIP-1β; reverse hatching, RANTES. We cultured macrophage cultures prepared from PBMCs of two donors (*a* and *b*) by adherence to plastic[3] for 7 days and then infected them with HIV-1 (2×10^4 c.p.m. of reverse transcriptase (RT) per 10^6 cells in 1 ml medium) in the presence of 500 ng ml^{-1} chemokine β-peptides. We replaced the culture medium every 3 days with fresh medium containing 500 ng ml^{-1} chemokines. Fifteen days after infection, we tested culture supernatants for reverse transcriptase activity. Results are presented as per cent of RT activity in untreated (control) culture supernatants taken as 100%. RT activity for the controls is 5.8×10^5 c.p.m. ml^{-1} for 92US657 and 2.8×10^5 for 92US660. Blue, control; orange, MIP-1α; green, MIP-1β; red, RANTES.

Helena Schmidtmayerova*, Barbara Sherry & Michael Bukrinsky
The Picower Institute for Medical Research, Manhasset, New York 11030, USA
e-mail: *mbukrinsky@picower.edu*

* Also at: Institute of Virology, Slovak Academy of Sciences, 842 46 Bratislava, Slovakia.

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