Role of β-Chemokines in Suppressing HIV Replication

Fiorenza Cocchi et al. (1) found that three different β-chemokines (RANTES, MIP-1α, and MIP-1β) produced by CD8+ T lymphocytes suppress human immunodeficiency virus (HIV) replication in peripheral blood mononuclear cells (PBMC). Moreover, neutralizing antibodies to all three chemokines eliminate the activity against HIV detected in CD8+ cell supernatants (1). They conclude that these chemokines are responsible for the CD8+ cell anti-HIV activity described in our studies (2–4).

During our attempts to identify CD8+ cell antiviral factors (CAFs) that could mediate CD8+ cell anti-HIV activity, several cytokines—including the interferons α and β, the chemokine IL-8, TGF-β, TNF-α, and the β-chemokines reported by Cocchi et al.—were identified as having antiviral activity (3–5) (see below). None of these cytokines, however, has been present in consistent or sufficient amounts to be CAF.

In addition, neutralizing antibodies to these cytokines have not affected the extent of the anti-HIV activity that we have detected in CD8+ cell culture fluids (3–5).

In our evaluation of the β-chemokines, we found that the concentrations of RANTES, MIP-1α, and MIP-1β in CD8+ cell supernatants as measured by enzyme-linked immunosorbent assay (ELISA) did not correlate with the anti-HIV activity detected in our assays (Fig. 1). Culture fluids with high anti-HIV activity (6) had concentrations of these chemokines from 0.1 to 4 ng/ml, and culture fluids lacking antiviral activity showed similar concentrations. Moreover, none of these three chemokines, even when used together, inhibited HIV-1SF2 replication in purified CD4+ cells at the concentrations found in supernatants of CD8+ cells nor at the concentrations reported by Cocchi et al. (1) (Fig. 2A). This virus is one used in our measurement of CAF antiviral activity (3, 4, 6, 7).

When these chemokines were used with a variety of freshly isolated viruses, different sensitivities were noted (see examples, Fig. 2B to D). Some were highly sensitive to the chemokines (for example, SV), some were resistant, and others showed an intermediate pattern. Cell antiviral factors show antiviral activity against all these viruses. As expected, a mixture of neutralizing antibodies to the three β-chemokines, at quantities similar to those cited (1), did not block the antiviral activity of CD8+ cell supernatants against acute HIV-1SF2 replication.

Table 1. Effect of neutralizing antibodies to β-chemokine on the ability of CAF-containing fluids to suppress HIV-1 replication in CD4+ lymphocytes. A 50% dilution of CAF-containing culture fluid, or the medium control fluid, was left untreated or pretreated with control antibodies (Ab) or a mixture of antibodies specific for each of the three antibodies for 40 min at room temperature before addition to HIV-1SF2-infected CD4+ lymphocytes. Control antibody was nontoxic goat polyclonal (R&D Systems). Anti-chemokine antibody mix consisted of goat polyclonal neutralizing antibodies specific to the human chemokines, RANTES, MIP-1α, and MIP-1β (R&D Systems). Reverse transcriptase (RT) activity shown indicates the average peak of HIV-1SF2 replication (at day 10) in triplicate cultures. Fluids contained β-chemokine levels similar to those shown in Fig. 1. Culture fluids receiving the antibodies to chemokine showed a complete elimination of the chemokines as detected by ELISA (5).
Table 2. Effect of neutralizing antibodies to β-chemokines on CAF-mediated suppression of a β-chemokine-sensitive HIV-1 isolate. As described in Table 1, a 50% dilution of two CAF-containing culture fluids was pretreated with control antibody or with a mixture of neutralizing antibodies to RANTES, MIP-1α, and MIP-1β before addition to HIV-1SF2-infected CD4+ cells. The effect of these treatments on the percentage of suppression of HIV replication by the CAF-containing medium relative to control medium-treated cells is presented. Virus replication in the control culture receiving no CAF was about 150,000 cpm of RT activity per milliliter of culture fluid. The chemokine levels in CAF fluid 1 were 869, 132, and 520 pg/ml for RANTES, MIP-1α, and MIP-1β, respectively. For CAF fluid 2, we selected a CD8+ cell culture supernatant with high chemokine levels, which were 14,161, 14,519, and 11,450 pg/ml for RANTES, MIP-1α, and MIP-1β, respectively. Fluids receiving the anti-chemokine antibodies showed complete elimination of the chemokines as measured by ELISA.

<table>
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<tr>
<th>Antibody</th>
<th>Suppression of HIV production (%)</th>
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<tr>
<td>Control</td>
<td>55</td>
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<td>Neutralizing Ab</td>
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REFERENCES AND NOTES

5. C. E. Mackewicz and J. A. Levy, unpublished observations.
6. CD8+ cell culture supernatants were prepared by our standard procedure (4). In brief, CD8+ cells were obtained from PBMC recovered from asymptomatic HIV-infected individuals. These cells were stimulated for 3 days with antibodies to CD3 attached to immunomagnetic beads. Cells were removed, washed, and cultured for 2 weeks in AIM-V serum-free medium ( Gibco, Gaithersburg, MD) with 1% antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin). Cells were collected every 2 days and stored at -70°C. At the completion of the cell cultivation, the individual fluids recovered were filtered and tested for anti-HIV activity against acute infection of purified normal CD4+ cells with HIV-1SF2. In this CAF assay, 1000 TCID50 of HIV-1SF2 were inoculated into purified CD4+ cells (5 x 104 cells). After 1 hour, cells were trypsinized to remove input virus, and culture fluid was added. The culture fluids were removed every 2 days and assayed for viral reverse transcriptase activity. Fresh control fluid or the CAF-containing fluid mixed with input medium was added to the cultures. Those fluids showing anti-HIV activity (>50% suppression) were pooled, filtered, and used for the assays described in this comment. All CAF-negative fluids that were used showed less than 10% suppression of HIV replication. The CAF+ cell fluids were also tested for antiviral activity with the use of acute infection of the IG5 cell line with HIV-1SF2 (7). The culture fluids were assayed for viral reverse transcriptase activity, and extracts from the cells were examined for luciferase activity (7). Results were compared with those obtained with control culture fluids.
8. The IG5 cell line has a single, stably integrated copy of an HIV LTR linked to a luciferase gene [E. Aguilar-Cordova et al., AIDS Res. Human Retroviruses 10, 295 (1994)] and contains this cell line with HIV-1SF2.

Response: Mackewicz et al. contend that the C-C chemokines RANTES, MIP-1α, and MIP-1β that we have recently identified as major components of the HIV-suppressive soluble activity released by both primary and immortalized CD8+ T cells (1) are not the so-called “CAF” they have investigated in the course of the last 10 years (2). Within 6 to 8 months of the publication of our report (1), a large body of evidence has accumulated that confirms the relevance of chemokines to the physiology of HIV infection. Indeed, a series of new, fundamental advances in our understanding of HIV infection has directly stemmed from the initial connection between HIV and chemokines (3).

It is encouraging that Mackewicz et al. now acknowledge that RANTES, MIP-1α, and MIP-1β can totally suppress HIV infection even at doses as low as 0.05 μg/ml (figure 2B of the comment), in contradiction with their recent statement that “only at high levels (0.5 to 1.0 μg ml-1) do these chemokines show some anti-HIV activity” (4). Some of the other experiments described by Mackewicz et al. (figure 2A and table 1) were performed with T cell line tropic viruses (HIV-1SF2 and HIV-1SF213), which we have previously shown to be insensitive to RANTES, MIP-1α, and MIP-1β (1). This lack of sensitivity is determined by critical changes in the V3 domain of the gp120 envelope glycoprotein (5). The low-level “CAF” activity detected against these isolates—that is, only about 50% RT suppression (table 1 of the comment)—must therefore result from factors other than such chemokines. These other factors may include some of the known HIV-suppressive cytokines produced by CD8+ T cells [the role of most of these factors was not rigorously excluded with the acute infection test, which is performed with T cell line tropic strains, but only with the endogenous trans-well test (6), which is commonly performed with NSI strains] and the C-C chemokine SDF-1 (the newly identified CXCR4-ligand). Nevertheless, it cannot be excluded that other, still unidentified factors (probably cytokines) might also play a role, particularly in a nonspecific manner—for example, by altering T cell activation or metabolism. With regard to results with a “β-chemokine-sensitive” HIV-1 isolate (table 2 of the comment), many other laboratories have already con-
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Editor's Summary