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 PBMC 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. 2, B 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 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

<table>
<thead>
<tr>
<th>Antibody treatment</th>
<th>RT activity (x 1000 cpm/ml)</th>
</tr>
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<tbody>
<tr>
<td>Control fluid</td>
<td>1952</td>
</tr>
<tr>
<td>Control Ab</td>
<td>1740</td>
</tr>
<tr>
<td>Chemokine-Ab mix</td>
<td>1881</td>
</tr>
</tbody>
</table>

Legend:

- **RT** = Reverse transcriptase
- **CAF** = Chemokine-Containing Fluid
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 689, 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>
<thead>
<tr>
<th>Antibody</th>
<th>Suppression of HIV production (%)</th>
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<tbody>
<tr>
<td>Neutralizing Ab</td>
<td>CAF 1</td>
</tr>
<tr>
<td>Control</td>
<td>55</td>
</tr>
<tr>
<td>50% CAF</td>
<td>46</td>
</tr>
</tbody>
</table>

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 immune magnetic beads. Cells were removed, washed, and cultured for 2 weeks in AIM-V serum-free medium (Gibco, Gaithersburg, MD) with 1% antioxidants (100 μlimentin and 100 μg/ml propamidine). Fluids 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, 100 TCID₅₀ of HIV-1SF2 were inoculated into purified CD4⁺ cells (8 x 10⁶ 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 or without serum 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 anti-viral activity with the use of acute infection of the 1G5 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.

In summary, some β-chemokines exhibit anti-HIV activity in vitro against certain primary isolates. However, as observed with interferons, IL-8, TGFβ, and TNFα, these cytokines are not primarily responsible for the noncytotoxic antiviral activity we observed with CD8⁺ cells (3). Production of CAF is highest in asymptomatic individuals and decreases with progression to disease (3). RANTES, MIP-1α, and MIP-1β are not present in higher concentrations in CD8⁺ cell culture fluids from HIV-infected individuals who are long-term survivors as compared with those fluids from individuals in whom the disease is progressing (5). They do not show the broad antiviral activity of CAF. Moreover, they do not appear to suppress HIV transcription, as do CAF and CD8⁺ cells when they are added to infected CD4⁺ cells (7). Nevertheless, recent observations about the β-chemokines (9) further support the value of studying natural immune factors against HIV infection.

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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 0.005 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-1SF13), 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—this 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-X-C chemokine SDF-1 (the newly identified CXCRA4-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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