Macrophage Colony-Stimulating Factor Antagonists Inhibit Replication of HIV-1 in Human Macrophages

Joseph Kutza, Lynne Crim, Steven Feldman, Mark P. Hayes, Marion Gruber, Judy Beeler and Kathleen A. Clouse

*J Immunol* 2000; 164:4955-4960; doi: 10.4049/jimmunol.164.9.4955
http://www.jimmunol.org/content/164/9/4955

**References**
This article cites 31 articles, 12 of which you can access for free at:
http://www.jimmunol.org/content/164/9/4955.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Macrophone Colony-Stimulating Factor Antagonists Inhibit Replication of HIV-1 in Human Macrophages

Joseph Kutza, Lynne Crim, Steven Feldman, Mark P. Hayes, Marion Gruber, Judy Beeler, and Kathleen A. Clouse

Macrophages infected with HIV-1 produce high levels of M-CSF and macrophage-inflammatory protein-1α (MIP-1α). M-CSF facilitates the growth and differentiation of macrophages, while the chemotactic properties of MIP-1α attract both T lymphocytes and macrophages to the site of HIV infection. Studies described in this work indicate M-CSF may function in an autocrine/paracrine manner to sustain HIV replication, and data suggest possible therapeutic strategies for decreasing viral load following HIV infection. We show that macrophage infection with measles virus or respiratory syncytial virus, in contrast to HIV-1, results in production of MIP-1α, but not M-CSF. Thus, M-CSF appears to be specifically produced upon infection of macrophages with HIV-1. Furthermore, addition of M-CSF antagonists to HIV-1-infected macrophages, including anti-M-CSF monoclonal or polyclonal Abs or soluble M-CSF receptors, dramatically inhibited HIV-1 replication and reduced production of MIP-1α. Our results suggest that biologic antagonists for M-CSF may represent novel strategies for inhibiting the spread of HIV-1 by 1) blocking virus replication in macrophages, 2) reducing recruitment of HIV-susceptible T cells and macrophages by MIP-1α, and 3) preventing the establishment and maintenance of infected macrophages as a reservoir for HIV. The Journal of Immunology, 2000, 164: 4955–4960.

Macrophages are critical regulatory and effector cells of the immune response. Their role in innate immunity includes mediation of phagocytosis, as well as release of cytokines and cytotoxic mediators (1). They also facilitate the development of acquired immunity through Ag presentation and release of immunomodulatory cytokines. Although macrophages are immune effectors, they are also susceptible to infection by agents such as bacteria, protozoa, parasites, and viruses (2). Viruses capable of infecting macrophages include several RNA viruses, such as measles virus (MV) (3, 4), respiratory syncytial virus (RSV) (5–7), and HIV-1 (8, 9). Macrophages infected with each of these viruses have shown dysregulated cytokine production, a feature common to virus survival strategies (6, 7, 10, 11).

Previous work from our laboratory has demonstrated that monocytoid-derived macrophages (MDM) produce endogenous M-CSF following entry and replication of HIV-1 (12). Molecular clones of HIV-1 that bind to CD4, but do not enter or replicate in MDM, fail to induce endogenous M-CSF production (12). Others have shown that exogenous addition of this macrophage survival and differentiation factor to cultures of HIV-1-infected MDM results in enhanced HIV-1 replication (13, 14). This may be due in part to enhanced differentiation and to M-CSF-induced increases in monocyte and macrophage cell surface expression of the HIV-1 receptor and coreceptor, CD4 and CCR5 (14, 15). All reports to date indicate that M-CSF plays a major role in the infection of macrophages with HIV-1.

In this study, we find that MDM infected with MV or RSV, unlike those infected with HIV-1, do not show enhanced production of M-CSF, MDM infected with MV or RSV produce significantly lower levels of the proinflammatory cytokines, IL-6 and TNF-α, while MDM infected with each of these three viruses resulted in increased production of the β chemokine, MIP-1α. Thus, the observed cytokine profile is dependent upon the virus type used to infect MDM and M-CSF production is unique to HIV-1-infection. We further show that addition of M-CSF antagonists, including goat polyclonal anti-M-CSF Ab, murine anti-M-CSF mAb, and soluble, dimeric, human M-CSF receptors, to cultures of MDM infected with HIV-1 leads to a dramatic reduction in the level of virus replication. Taken together, our data suggest that M-CSF antagonists warrant consideration for therapeutic development for use in HIV disease to prevent the replication of HIV-1 in cells of the macrophage lineage.

Materials and Methods

Monocyte isolation and culture

PBMC were isolated from blood following leukapheresis of HIV-1-seronegative donors and subsequent density-gradient centrifugation; monocytes were purified by countercurrent centrifugal elutriation, as previously described (12, 16). Elutriated monocytes were >95% viable, as determined by trypan blue exclusion and >90% CD14+, as determined by flow cytometry (FACS) analysis of representative samples. Monocytes were differentiated in culture for 8 days at 37°C in 5% CO2 at a concentration of 4 × 10^5/ml in six-well tissue culture plates (Costar, Cambridge, MA) using DMEM (Life Technologies, Gaithersburg, MD) complete medium containing 10% pooled human serum, 2 mM l-glutamine (Life Technologies), 1 mM sodium pyruvate (Life Technologies), and penicillin (50 U/ml)/streptomycin (50 µg/ml) (Life Technologies) to generate MDM. All reagents used in the isolation and culture of MDM were tested for endotoxin and found to contain less than 0.03 endotoxin U/ml (<3 pg/ml).
Table I. M-CSF production is selective for HIV-1 replication in human MDM

<table>
<thead>
<tr>
<th>Virus</th>
<th>Days</th>
<th>RT (cpm)</th>
<th>MV Titer (<em>log_{10}</em>)</th>
<th>RSV Titer (<em>log_{10}</em>)</th>
<th>None</th>
<th>M-CSF (ng/ml)</th>
<th>TNF-α (pg/ml)</th>
<th>IL-6 (pg/ml)</th>
<th>MIP-1α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1ADA</td>
<td>1</td>
<td>723</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>555</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>749</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>1,925</td>
<td>9.2</td>
<td>*</td>
<td>*</td>
<td>21</td>
<td>35</td>
<td>54</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>7,785</td>
<td>8.0</td>
<td>*</td>
<td>*</td>
<td>35</td>
<td>54</td>
<td>133</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>8,332</td>
<td>10.5</td>
<td>*</td>
<td>*</td>
<td>133</td>
<td>545</td>
<td>133</td>
<td>545</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>10,191</td>
<td>20.0</td>
<td>*</td>
<td>*</td>
<td>670</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measles</td>
<td>1</td>
<td>3.7</td>
<td>11.7</td>
<td>75.9</td>
<td>135.6</td>
<td>445</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.7</td>
<td>7.7</td>
<td>*</td>
<td>123.3</td>
<td>115</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4.7</td>
<td>*</td>
<td>*</td>
<td>151.6</td>
<td>95</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>5.2</td>
<td>*</td>
<td>*</td>
<td>69.3</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5.2</td>
<td>*</td>
<td>*</td>
<td>50.1</td>
<td>52</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>5.2</td>
<td>*</td>
<td>*</td>
<td>26.6</td>
<td>33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>7.2</td>
<td>*</td>
<td>*</td>
<td>28</td>
<td>214</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>6.2</td>
<td>*</td>
<td>*</td>
<td>10.8</td>
<td>356</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSV</td>
<td>1</td>
<td>4.7</td>
<td>*</td>
<td>565</td>
<td>293</td>
<td>&gt;1,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.2</td>
<td>*</td>
<td>124.4</td>
<td>289</td>
<td>700</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>3.7</td>
<td>*</td>
<td>86.4</td>
<td>275</td>
<td>296</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>4.2</td>
<td>*</td>
<td>*</td>
<td>68.1</td>
<td>96</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>4.2</td>
<td>*</td>
<td>*</td>
<td>76.8</td>
<td>44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4.2</td>
<td>*</td>
<td>*</td>
<td>36</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.2</td>
<td>*</td>
<td>*</td>
<td>92.5</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>3.2</td>
<td>*</td>
<td></td>
<td>105</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>58.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>43</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>—</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* ND, not done; +, below the limit of detection. (For the sample dilutions used in these assays, this represents: <4.4 pg/ml TNF-α; <0.7 pg/ml IL-6; <7 pg/ml MIP-1α.)

a MDM differentiated for 8 days were harvested, replated, and infected with HIV-1ADA. Supernatants were collected and cultures provided with fresh medium every 3 days. Supernatants were stored at ~80°C and later analyzed for RT activity and cytokines.

b Cultures were maintained as those infected with HIV-1. Supernatants were analyzed for the presence of cytokines. Cellular lysates prepared at each timepoint from parallel cultures were used to monitor MV and RSV infection of the MDM.

**Virus infection of monocyte-derived macrophages**

**HIV-1.** MDM were harvested by scraping and plated into 24-well tissue culture plates (Nunc, Naperville, IL), at a concentration of 500,000 cells/ml, 1.5 ml/well. After 24–48 h, MDM were infected with HIV-1ADA, as previously described (12). HIV-1ADA was obtained through the AIDS Research and Reference Reagent Program (ARRRP), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), from Dr. H. Gendelman (17, 18), then expanded in human monocytes differentiated for 8 days. HIV-1ADA was added at a concentration of 1 μM MgSO4 (Fisher Scientific, Fair Lawn, NJ) and 50 mM HEPES (BioWhittaker, Walkersville, MD) to a final volume of 100 μl, 1.5 ml/well. Every 3 days thereafter, 80% of the culture medium was replaced. In some experiments, AZT (Sigma, St. Louis, MO) was added at a concentration of 1 μM following virus adsorption and replenished every 3 days. In other experiments, polyclonal goat anti-M-CSF Ab (R&D Systems, Minneapolis, MN), murine anti-M-CSF mAb (R&D Systems), or soluble M-CSF receptors consisting of the extracellular domain of the human M-CSF receptor fused to the Fc portion of human IgG1 (R&D Systems) were added after virus adsorption and replenished every 3 days. The concentration of Ab or soluble receptor added was sufficient to neutralize 100 ng/ml of M-CSF bioactivity, as determined by M-CSF bioassay (data not shown).

MDM cultures infected with HIV-1 were generally maintained in DMEM complete medium, as described above; however, when MDM were infected in parallel with HIV-1, MV, and RSV, 10% inactivated FCS (HyClone, Logan, UT) was used instead of pooled human serum (PHS) to counter the presence of any naturally occurring anti-mesases or anti-RSV Abs in the PHS and used in HIV-1-infected cultures to control for the use of different media components.

A reverse-transcriptase (RT) assay was used to measure the progression of infection in MDM infected with HIV-1. The RT assay used is a 1H-based modification of the methods described by Hoffman (19). Values shown reflect the average of duplicate samples (cpm/25 μl) that differed by not more than 15%.

**MV and RSV.** MDM were harvested and replated as described above. After 24–48 h, MDM were infected with MV or RSV. MV (Edmonston strain) and RSV (A2 strain) were grown in Vero cells (African Green Monkey kidney) cultured in Eagle’s MEM (Mediatech, Herndon, VA) supplemented with 5% FBS (BioWhittaker, Walkersville, MD), 4 mM L-glutamine (BioWhittaker), penicillin (50 U/ml), and streptomycin (50 μg/ml) (Quality Biological, Gaithersburg, MD), fungizone (2.5 μg/ml) (Quality Biological), and gentamicin (0.1 mg/ml) (Quality Biological). MV and RSV were concentrated by pelleting directly from infected tissue culture supernatants and resuspended in serum-free Eagle’s MEM containing 100 mM MgSO4 (Fisher Scientific, Fair Lawn, NJ) and 50 μM HEPES (BioWhittaker), then used to infect MDM at a multiplicity of infection of 0.1 tissue culture-infective dose50 per cell. MDM and culture supernatants were harvested on days 1, 2, 3, 6, 9, 12, 15, and 18 after infection. Viral titers were determined on Vero cell monolayers and end point titers determined by the method of Reed and Muench (20).

**Cytokine assays**

M-CSF bioactivity was determined by measuring the proliferation of the M-CSF-dependent cell line, M-NFS-60, as previously described (12, 21, 22). A Titertek Multiscan 340 plate reader (ICN Biomedicals, Costa Mesa, CA) was used to measure absorbances. The limit of detection for this assay is 1.5 ng M-CSF/ml. Commercial ELISA kits were used to detect the following cytokines: TNF-α, IL-6, and MIP-1α (R&D Systems). The limits of detection for these assays were 4.4, 0.7, and 7 pg/ml, respectively.

**Northern blot analysis of total cellular RNA**

Total RNA was isolated from MDM using Ultraspec total RNA isolation reagent (Biotecx, Houston, TX). RNA was fractionated on 1% agarose gels.
containing formaldehyde and transferred to Duralon-UV membranes (Stratagene, La Jolla, CA). The membranes were hybridized with a 32P-labeled probe specific for M-CSF at 42°C overnight in hybridization buffer (Digene, Silver Spring, MD). The membranes were then washed with 2x SSC containing 0.1% SDS at room temperature, followed by a wash with 0.1x SSC containing 0.1% SDS at 63°C. Blots were exposed to Kodak X-OMAT film at -70°C.

**Results**

**Production of M-CSF is specific to HIV-1-infected MDM**

Several viruses have the capacity to infect human monocytes and macrophages (2). To determine whether production of M-CSF is a global response of MDM following virus infection, two RNA viruses other than HIV-1 were tested for the ability to enhance production of endogenous M-CSF. MDM were infected in parallel with purified preparations of HIV-1ADA, MV (Edmonston strain), or RSV (strain A2). As shown in Table I, all viruses tested were capable of replicating in MDM, albeit with varying kinetics. Titers observed for MV and RSV either exceeded or were consistent with those observed by others upon infection of human macrophages (4, 5, 7). However, only MDM infected with HIV-1 showed enhanced production of M-CSF. MDM infected with MV or RSV showed little or no detectable M-CSF (Table I). In contrast, production of MIP-1a by virus-infected MDM was observed with each virus tested. Production of MIP-1a following infection of MDM with MV or RSV was greatest early in the course of infection, while production by HIV-1-infected MDM paralleled the kinetics of virus replication (Table I). When culture supernatants from the infected MDM were examined for production of proinflammatory cytokines, MDM infected with HIV-1 had no detectable TNF-α or IL-6, while MDM infected with MV or RSV showed significant production of these two cytokines, as previously reported (6, 7, 10, 11). Production of these cytokines was not detected in long-term cultures of uninfected MDM. However, elevated basal production of M-CSF was occasionally observed early after transfer of MDM from medium containing human serum to medium containing FCS (Table I). Taken together, these data suggest that enhanced production of M-CSF is unique to HIV-1-infected MDM.

**Enhanced production of M-CSF protein and mRNA requires HIV-1 replication**

We have previously shown that infection of MDM with HIV-1ADA or HIV-1BAL can induce M-CSF production, while infectious molecular clones of HIV-1 that bind CD4, but fail to enter and replicate in human MDM, do not induce M-CSF protein secretion (12). To determine whether viral entry alone could lead to enhanced M-CSF production, we studied the effects of the nucleoside analogue, AZT, an inhibitor of HIV replication, on M-CSF production in MDM cultures infected with HIV-1. We found that AZT completely inhibited virus replication in the HIV-1-infected MDM cultures (Fig. 1A) and prevented the production of biologically

**FIGURE 1.** AZT inhibits HIV-1 replication in MDM and prevents M-CSF protein production. MDM differentiated for 8 days were harvested, replated, and infected with HIV-1ADA. Supernatants were collected and stored at -80°C, and cultures provided with fresh medium and AZT every 3 days. Supernatant RT activity (A) and M-CSF bioactivity (B) were analyzed. RT activity shown is the mean of duplicate samples of culture supernatant that differed by no more than 15%. M-CSF activity was determined by the M-CSF-dependent M-NFS-60 bioassay. The limit of detection for this assay is 1.5 ng/ml M-CSF.

**FIGURE 2.** AZT inhibits enhanced production of M-CSF mRNA in cultures of HIV-1-infected MDM. MDM differentiated for 8 days were harvested, replated, and infected with HIV-1ADA. Total RNA was extracted at day 15 from uninfected and HIV-infected MDM cultured in the presence and absence of 1 μM AZT. Northern blot analysis was performed using a DNA probe specific for M-CSF RNA.
active M-CSF at the protein (Fig. 1B) and mRNA (Fig. 2) levels. Comparable levels of inhibition were observed in six separate experiments using MDM obtained from different HIV-1-seronegative donors. These results therefore indicate that entry of intact, replication competent HIV-1 alone, without productive infection, does not stimulate M-CSF production.

**M-CSF antagonists inhibit HIV-1 replication in human MDM**

The observations that enhanced M-CSF production is unique to HIV-1-infected MDM and requires active viral replication led us to ask whether blocking the biological activity of M-CSF would inhibit HIV-1 replication in MDM. M-CSF antagonists, including goat polyclonal anti-M-CSF Ab (go M-CSF), murine anti-M-CSF mAb (mo M-CSF), and soluble, dimeric human M-CSF receptors (M-CSF-R:Fc) were added to HIV-1-infected MDM cultures following virus adsorption. Inclusion of these antagonists, which bind to M-CSF and prevent it from interacting with its receptor, resulted in a dramatic decrease in the level of HIV-1 replication (Fig. 3). Data shown are representative of four experiments performed with these antagonists using MDM obtained from different normal donors. The levels of M-CSF antagonist-mediated HIV-1 inhibition observed for all four donors were tested for significance by a one-way ANOVA, followed by the post hoc Tukey test to measure differences among groups. Inhibition observed for the three antagonists on days 21 and 24 postinfection had p values <0.05 and were considered significant. We found no discernible differences between macrophage morphology or viability in HIV-1-infected control cultures or those treated with M-CSF antagonists. There were also no observed changes in the pH of control vs antagonist-treated macrophage cultures, which would accompany changes in cell metabolism and subsequent loss of viability. Control Abs, including purified goat IgG, murine IgG2A, and human IgG1, used in the M-CSF antagonist experiments cause no reduction in HIV-1 replication (data not shown). Thus, M-CSF plays a critical role in the production of HIV-1 by infected MDM.

**Discussion**

In this study, we show that endogenous M-CSF critically regulates HIV-1 replication in human MDM. HIV-1-infected MDM produce M-CSF that can act via an autocrine mechanism (a) to facilitate MDM survival and enhance HIV-1 replication. β chemokines produced by HIV-1-infected MDM (b) attract uninfected monocytes and macrophages to the site. M-CSF acts via a paracrine mechanism (c) to enhance the susceptibility of the infiltrating cells via differentiation and increased expression of CD4 and CCR5. HIV-1 released from infected MDM (d) infects the susceptible cells, thus spreading and prolonging the infection.

M-CSF antagonists diminish MIP-1α production

The kinetics of M-CSF and MIP-1α production parallel HIV replication in MDM. Because antagonists to M-CSF proved to be effective inhibitors of HIV replication, we next determined whether levels of MIP-1α production were concomitantly decreased. As shown in Fig. 4, treatment of HIV-1-infected MDM with levels of M-CSF antagonists that are capable of inhibiting HIV replication (Fig. 3) reduces the level and delays the kinetics of MIP-1α production. These data suggest a critical role for M-CSF antagonists in ablating the recruitment and subsequent infection of human macrophages with HIV-1.
Other in vivo biological effects of M-CSF include regulation of placental function via action on decidual and trophoblastic cells and bone resorption via action on osteoclasts; animal studies indicate M-CSF has antitumor activity and can lower plasma cholesterol levels (23, 24). In vivo and in vitro studies demonstrate that M-CSF plays a major role in HIV-1 replication in human macrophages. Replication of HIV-1 has been associated with increased production of M-CSF in vivo (25), and it has been well documented that in vitro addition of exogenous M-CSF to MDM cultures increases their susceptibility to HIV-1 infection and promotes higher levels of virus replication (13, 14). We have shown that HIV-1-infected MDM produce endogenous M-CSF, which can then facilitate development of macrophages as a long-lived reservoir for HIV-1 (12). As a functional viral reservoir, macrophages can then spread HIV-1 to uninfected macrophages and HIV-susceptible T cells (26).

The β chemokine, MIP-1α, is also produced by MDM in response to infection with HIV-1 (27, 28). MIP-1α is a chemotactic factor that attracts monocytes and T cells (29, 30) and, along with MIP-1β and RANTES, is a natural ligand for the HIV-1 coreceptor CCR5 (31). Thus, in addition to its chemotactic properties, MIP-1α produced by infected cells could bind to CCR5 and reduce the level of HIV binding and entry. However, MIP-1α levels produced in vitro by HIV-1-infected MDM (<10 ng/ml) are substantially lower than the concentrations of CCR5 ligands required to inhibit HIV-1 replication (32, 33), suggesting that the HIV-inhibitory contribution of MIP-1α would be substantially less than its chemotactic effects. Similar to our findings with M-CSF, MIP-1α production by HIV-1-infected MDM is also dependent on viral replication (27, 28), but it is not specific for HIV-1. Intriguingly, it has recently been shown that production of MIP-1α, but not M-CSF, is regulated by the HIV nef protein (34). A role for HIV accessory proteins in the regulation of M-CSF production has yet to be defined and is currently under investigation in our laboratory.

In contrast to MIP-1α, we find that M-CSF production may be a specific response of human MDM to infection with HIV-1 and does not represent a global response of macrophages to virus infection. Furthermore, because M-CSF production enhances and sustains HIV-1 replication in macrophages, it is most likely responsible for maintaining a macrophage viral reservoir. Clinical trials for HIV-infected patients using highly active antiretroviral therapy (HAART) reported a prolonged absence of plasma viremia, followed by a rapid viral rebound upon discontinuation of therapy, suggesting a failure of this approach to eradicate HIV in existing viral reservoirs (35). Recent studies have been conducted using the cytokine, IL-2, in combination with HAART to activate HIV-1-infected T cell reservoirs and render them susceptible to therapy (36). However, other potential reservoirs exist, including the macrophage, which would not be affected by this approach. Development of M-CSF antagonists for treatment of HIV-infected individuals may be helpful in controlling the spread of the virus by targeting the macrophage reservoir for HIV-1. Used in conjunction with HAART therapy, M-CSF antagonists as biologic therapeutics would inhibit HIV infection of macrophages, reduce recruitment of HIV-susceptible T cells and monocytes, and prevent the establishment and maintenance of a macrophage reservoir for HIV.

Acknowledgments

We thank Karen Fields, Valerie Calvert, and Karis Faust for technical assistance with monocyte elutriation and culture. We also thank Indira Hewlett, Ezio Bonvini, and Paris Burd for critical review of the manuscript.

References


