Interleukin-8 Stimulates Human Immunodeficiency Virus Type 1 Replication and Is a Potential New Target for Antiretroviral Therapy

BRIAN R. LANE,^{1,2} KARIN LORE,³ PAUL J. BOCK,^{1,2} JAN ANDERSSON,⁴ MICHAEL J. COFFEY,⁵
ROBERT M. STRIETER,^{5†} AND DAVID M. MARKOVITZ^{1,2*}

Divisions of Infectious Diseases¹ and Pulmonary and Critical Care Medicine,⁵ Department of Internal Medicine, and Graduate Program in Cellular and Molecular Biology,² University of Michigan Medical Center, Ann Arbor, Michigan 48109-0640, and Department of Microbiology, Pathology and Immunology³ and Department of Medicine, Center for Infectious Medicine,⁴ Karolinska Institutet, Stockholm, Sweden

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Production of the C-X-C chemokines interleukin-8 (IL-8) and growth-regulated oncogene alpha (GRO- α) in macrophages is stimulated by exposure to human immunodeficiency virus type 1 (HIV-1). We have demonstrated previously that GRO- α then stimulates HIV-1 replication in both T lymphocytes and macrophages. Here we demonstrate that IL-8 also stimulates HIV-1 replication in macrophages and T lymphocytes. We further show that increased levels of IL-8 are present in the lymphoid tissue of patients with AIDS. In addition, we demonstrate that compounds which inhibit the actions of IL-8 and GRO- α via their receptors, CXCR1 and CXCR2, also inhibit HIV-1 replication in both T lymphocytes and macrophages, indicating potential therapeutic uses for these compounds in HIV-1 infection and AIDS.

Altered cytokine production by cells exposed to human immunodeficiency virus type 1 (HIV-1) contributes to the systemic symptoms of AIDS (cachexia, anorexia, and malaise) (29), HIV-1-related brain disease (21), and recruitment of immune cells to infected tissue (42). The introduction of highly active antiretroviral therapy (HAART) has dramatically reduced HIV-1 mortality in the United States since 1996 (38). Unfortunately, many patients cannot tolerate therapy, and in others, resistance to the drugs develops (19). Therefore, new viral and cellular targets have been sought for the treatment of HIV-1 infection, either alone or in combination with HAART (24). Cytokines and their receptors are one group of such potential targets for therapy of HIV-1 infections.

In the past few years, it has been shown that the C-C chemokines RANTES, MIP-1 α and MIP-1 β suppress HIV-1 replication (12, 13). The actions of these chemokines are believed to be related to the fact that they are ligands for CCR5, the principal coreceptor used by monocyctotropic isolates of HIV (1, 11, 14, 17, 18). Similarly, SDF-1 α , the only known ligand for CXCR4, the principal coreceptor for T-tropic isolates of HIV, inhibits the replication of CXCR4-using isolates of HIV (X4 HIV) (8, 20, 36). RANTES, MIP-1 α , MIP-1 β , and SDF-1 α inhibit HIV-1 replication both by competing with HIV for binding to CCR5 or CXCR4 and by causing internalization of their respective receptors (2, 3, 41). Interestingly, under some circumstances these same chemokines can actually enhance HIV-1 replication (16, 23, 25, 26, 33, 40, 43). The mechanisms by which RANTES and SDF-1 α can act to augment HIV-1

replication include increasing viral attachment to, and entry into, target cells (16, 23, 26, 43), activating intracellular signaling pathways (23, 25), and augmenting viral gene expression from the HIV-1 long terminal repeat (33).

The role that other chemokines, including two members of the C-X-C chemokine family, interleukin-8 (IL-8) and growth-regulated oncogene alpha (GRO- α), may play in controlling HIV-1 replication and pathogenesis has not been well established. IL-8 has been demonstrated to attract neutrophils and T cells, stimulate monocyte adherence, and mediate angiogenesis by interacting with the C-X-C chemokine receptors CXCR1 and CXCR2 (6, 22, 27, 31, 47). GRO- α was identified initially as a melanoma growth factor and later as a neutrophil chemoattractant (6). GRO- α shares 43% amino acid identity with IL-8 and functions similar to IL-8 by means of its ability to ligate CXCR2 (6). Previous investigations have found either a slight inhibitory effect or no effect of IL-8 on HIV-1 replication (10, 32, 35), and GRO- α was not previously known to have any effect on viral replication. In addition, neither CXCR1 nor CXCR2 has been demonstrated to function as a coreceptor for HIV entry (19).

There is currently great interest in agents that block these same chemokines, or their cognate receptors, for the treatment of a number of illnesses, particularly inflammatory diseases (7). For example, an IL-8-specific monoclonal antibody is currently in use in clinical trials of patients with psoriasis (46). Other drug discovery efforts aimed at these pathways produced SB225002, the first reported potent and selective nonpeptide inhibitor of a chemokine receptor (45). This small molecule inhibitor acts as an antagonist of IL-8 binding to CXCR2 (50% inhibitory concentration = 22 nM), and has >150-fold selectivity over CXCR1 and other chemokine receptors (45).

Several recent findings suggest that interfering with IL-8 and GRO- α function would be an effective therapy for HIV-1 infection. First, elevated levels of both IL-8 and GRO- α are

* Corresponding author. Mailing address: 5220 MSRB III, 1150 W. Medical Center Dr., Ann Arbor, MI 48109-0640. Phone: (734) 647-1786. Fax: (734) 764-0101. E-mail: dmarkov@umich.edu.

† Present address: Department of Medicine, Division of Pulmonary and Critical Care Medicine, UCLA School of Medicine, Los Angeles, CA 90095-1922.

present in the serum and lungs of HIV-1-infected individuals (15, 34, 44). We have recently demonstrated that exposure of MDM to HIV-1 leads to increased IL-8 production, an effect mediated by Tat and the inflammatory cytokine tumor necrosis factor alpha, as well as by gp120 (B. R. Lane et al., submitted for publication). In addition, we have described a novel auto-crine/paracrine loop in which HIV-1 gp120 ligation of CXCR4 on monocyte-derived macrophages (MDM) stimulates the production of GRO- α , and GRO- α further stimulates HIV-1 replication (30a).

We demonstrate here that IL-8 stimulates HIV-1 replication in MDM and T lymphocytes. We also show that increased levels of IL-8 are present in the lymphoid tissue of patients with AIDS. Antibodies that neutralize IL-8 activity, and antibodies that block binding to the receptors CXCR1 and CXCR2, can inhibit HIV-1 replication in macrophages and T cells. Blocking the actions of IL-8 and GRO- α with the small-molecule inhibitor of CXCR2 SB225002 also markedly reduces HIV-1 replication. Thus, we have shown that the autocrine/paracrine loop in which IL-8 and GRO- α participate is a potential target for antiretroviral therapy. Therapeutic compounds currently under development for chemokine-mediated inflammatory disease therefore have the potential to be exploited for the therapy of HIV infection and AIDS.

MATERIALS AND METHODS

Isolation and preparation of human AM, MDM, and peripheral blood lymphocytes (PBL). Alveolar macrophages (AM) were collected by bronchoalveolar lavage of nonsmoking volunteers without lung disease or HIV-1 infection as described previously (30). The recovered bronchoalveolar lavage fluid was centrifuged to collect the cellular portion, and cells were resuspended in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U of penicillin per ml, and 100 μ g of streptomycin per ml (complete DMEM). AM were purified by plastic adherence and cultured 5 days before infection.

Peripheral blood mononuclear cells (PBMC) were collected by venipuncture of healthy volunteers as described previously (30). PBMC contained approximately 20% CD14⁺ monocytes as determined by flow cytometry. To separate the PBMC into subpopulations composed mainly of monocytes or lymphocytes, PBMC were subjected to a plate adherence step for 2 h. Adherent cells were consistently >90% peripheral blood monocytes (PBM) as determined by Diff-Quik analysis and >85% CD14⁺ as determined by flow cytometric staining with a phycoerythrin-conjugated mouse anti-human monoclonal antibody to CD14 (M5E2; PharMingen), as well as >99% viable as determined by trypan blue exclusion. PBM were differentiated into MDM by culture in complete DMEM for up to 2 weeks (3 days in most experiments) prior to infection.

Nonadherent PBMC following the plate adherence step were enriched for lymphocytes and contained less than 2% CD14⁺ monocytes. These monocyte-

FIG. 1. Exogenous IL-8 stimulates HIV-1 replication in AM and MDM. (A) AM were cultured for 5 days before infection with HIV-1_{BaL} and then treated with IL-8 at either 0.5, 5, or 50 ng/ml. Supernatants (25%) were collected twice weekly and replaced with fresh medium. Viral replication was assessed by quantifying the RT activity present in the supernatants at several time points after infection. (B) MDM were treated with IL-8 at the indicated concentrations every 3 days beginning 1 day before infection with HIV-1_{BaL}. Supernatants were analyzed for RT activity 8 days after infection. The increase observed with IL-8 treatment in the range from 5 to 100 ng/ml was determined to be statistically significant relative to no treatment ($P < 0.05$, Student's t test). (C) Antibodies to CXCR1 and CXCR2 block the ability of IL-8 and GRO- α (each at 25 ng/ml) to stimulate HIV-1_{BaL} replication in MDM. Supernatants were analyzed for RT activity 19 days after infection. Data shown are representative of five experiments.

FIG. 2. Exogenous IL-8 stimulates HIV-1 replication in PBL. (A) CD8-depleted PBL were treated with the indicated concentrations of IL-8 and infected with HIV-1_{BaL} (A), HIV-1_{BRU} (B), or HIV-2_{CBL23} (C). Supernatants were collected twice weekly and replenished with fresh medium.

depleted PBMC (PBL) were cultured at 1×10^6 to 2×10^6 /ml in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 U of penicillin per ml, and 100 μ g of streptomycin per ml (complete RPMI). PBL were stimulated with 5 μ g of phytohemagglutinin (PHA; Sigma, St. Louis, Mo.) per ml for 1 to 3 days and then maintained in IL-2 (40 U/ml; Hoffmann-La Roche, Nutley, N.J.). In some experiments, PBL were also depleted of CD8⁺ cells with magnetic Dynabeads M-450 CD8 as instructed by the manufacturer (DynaL, Lake Success, N.Y.).

Preparation of HIV-1 stocks. All of the HIV-1 isolates used in this study were originally obtained from the NIH AIDS Reagent Program. Stocks of HIV-1_{BaL} were prepared by infection of HOS-CD4-CCR5 cells and of HIV-1_{BRU} by infection of CEM-SS cells. For some experiments, viral stocks were prepared by infection of PHA-activated, CD8-depleted PBL; results were identical to those obtained using the cell line-derived viral isolates (data not shown). Other viral stocks were used directly as provided by the NIH AIDS Reagent Program.

HIV-1 infection of MDM and PBL. For each experiment, multiple wells of macrophages or PBL were infected with equal reverse transcriptase (RT) counts of HIV-1 (30×10^6 to 300×10^6 cpm of RT used per 10^5 cells). This amount of CPM of RT activity per cell corresponds to a multiplicity of infection of between 0.001 and 0.01 as determined by titration on HOS-CD4-CCR5 and CEM-SS cell lines. MDM were cultured for 3 days prior to infection, washed after an overnight incubation with the virus, and cultured in complete DMEM. PHA-activated PBL were washed and incubated in complete RPMI plus IL-2 (40 U/ml) and any other treatments overnight. PHA-activated PBL were then spin infected by incubation with HIV-1 for 4 h at 2,500 rpm, washed, and incubated in medium plus IL-2 and other treatments. A portion of the medium (25%) was removed from the MDM and PBL supernatants and replaced twice weekly.

Recombinant human IL-8 and GRO- α , and monoclonal antibodies to CXCR1, CXCR2, IL-8, and GRO- α , were obtained from R&D Systems (Minneapolis, Minn.) and added as indicated in the figure legends. SB225002 was

obtained from Calbiochem (San Diego, Calif.) and reconstituted in dimethyl sulfoxide (DMSO). All other chemicals were obtained from Sigma.

RT assay. Quantification of the RT activity present in the supernatants using a poly (A)-oligo(dT) template primer was used as a measure of HIV-1 replication as previously described (39). RT activity was assayed using ³²P-labeled dTTP incorporated into DNA bound to DE81 paper (Whatman) and quantified using either the Series 400 PhosphorImager and ImageQuant software (Molecular Dynamics, Sunnyvale, Calif.) or a Betascope radioisotope imaging system. RT activity is reported as phosphorimager counts, except in Fig. 1A and Fig. 2A, where activity is reported as beta counts.

Patients and controls. Tissue biopsies from lymph nodes and tonsils were collected from HIV-1-infected patients and snap-frozen. The biopsies from HIV-1-infected patients ($n = 4$) with recent seroconversion were taken within 6 months after verified infection. These patients had a mean viral load of 46,795 HIV-1 RNA copies/ml of plasma and a mean CD4 count of 563/ μ l. Untreated, asymptomatic, chronically HIV-1-infected patients ($n = 5$) had a mean viral load of 12,000 copies/ml and a mean CD4 count of 510/ μ l. Patients treated with ongoing HAART ($n = 2$) had a mean viral load of <50 copies/ml and a mean CD4 count of 960/ μ l. Patients with AIDS ($n = 3$) had a mean viral load of 1,000,000 copies/ml and a mean CD4 count of 54/ μ l. Control tissue was obtained from HIV-1 seronegative healthy adults ($n = 3$) after elective tonsillectomy following institutional review board approval from Huddinge University Hospital, Huddinge, Sweden.

Detection of IL-8 expression by immunohistochemistry. Cryopreserved biopsies embedded in OCT compound (Tissue-TEK; Miles, Elkhart, Ind.) were cut in 8- μ m-thick sections, mounted on HTC glass slides (Novakemi, Stockholm, Sweden), and fixed with 2% formaldehyde (Sigma) in phosphate-buffered saline for 15 min at room temperature. Slides were then washed with balanced salt solution (BSS) (Gibco Ltd., Paisley, United Kingdom) and stored at -20°C for future use.

The staining procedure used to identify IL-8 expression in tissue sections at the single-cell level has previously been described (4, 5). Briefly, endogenous peroxidase was blocked by 1% H₂O₂ in BSS supplemented with 0.01 M HEPES buffer (Gibco) and 0.1% saponin (Riedel de Haen, AG Seelze, Germany). The IL-8-specific monoclonal antibody (NAP-1; 2 μ g/ml; Immunokontakt, Bioggio, Switzerland) was diluted in BSS-saponin and applied overnight at room temperature. After washes in BSS, the sections were incubated with a biotin-labeled secondary antibody (goat anti-mouse immunoglobulin G [IgG]; Caltag Laboratories, South San Francisco, Calif.) for 30 min, followed by incubation with an avidin-biotin-horseradish peroxidase complex (Vectastain; Vector Laboratories). A color reaction was developed by addition of 3'-diaminobenzidine tetrahydrochloride (Vector Laboratories). The sections were counterstained with hematoxylin.

The tissue sections were examined in a Leica RXM microscope (Leica, Wetzlar, Germany) equipped with a 3CCD color camera (DXC-950p; Sony Corporation, Tokyo, Japan). IL-8-expressing cells were identified at the single-cell level due to a characteristic staining pattern of protein localized in the Golgi stacks (4). The specificity of immunofluorescent staining was determined by incubation in the presence of a 10-fold molar excess of recombinant IL-8, which abolished staining. In addition, PBMC stimulated with lipopolysaccharide were used as a positive control for staining. The number of positively stained cells in the total tissue (3×10^6 to 5×10^6 μm^2) was counted manually. As the evaluation of separate positive cells can be difficult due to expected clusters of IL-8-expressing cells, the number of positive cells was sometimes presented as a range.

TABLE 1. IL-8-expressing cells in the lymphoid tissue of HIV-1-infected individuals^a

Category	No. of cells expressing IL-8 in lymphoid tissue					Mean no. of positive cells (<i>P</i> value ^b)
	1	2	3	4	5	
AIDS	14–16	19–24	20–40			22.2
Seronegative controls	0	2–4	4			2.3 (0.026)
Recent HIV-1 seroconverters	0	1	1	10		3.0 (0.034)
Chronic, asymptomatic HIV-1 infection	1–2	1–2	1–4	2–5	2–6	2.6 (0.037)
HAART-treated HIV-1 infection	1	8				4.5 (0.012)

^a The number of cells that stained positively for IL-8 is indicated for patients in each of several clinical categories (described in Materials and Methods).

^b Relative to individuals with AIDS (Student's *t* test). The differences between the other groups were not found to be statistically significant.

FIG. 3. IL-8-expressing cells in lymphoid tissue from HIV-1-infected individuals. IL-8-expressing cells found by intracellular immunohistochemical staining of a lymph node section from an AIDS patient are shown at a magnification of $\times 200$. In the high-power magnification ($\times 630$; inset), the intracellular localization of IL-8 protein to the Golgi-endoplasmic reticulum complex can be seen.

RESULTS

Exogenous IL-8 stimulates HIV-1 replication in MDM and PBL. Previous studies have found increased IL-8 in the context of HIV-1 infection, but none have implicated IL-8 in HIV-1 pathogenesis (10, 15, 28, 34, 37). We have recently demonstrated that exposure of MDM to HIV-1 leads to increased IL-8 production, an effect mediated by Tat and the inflammatory cytokine tumor necrosis factor alpha, as well as by gp 120 (Lane et al., submitted). As certain chemokines (e.g., RANTES, MIP-1 α , MIP-1 β , and SDF-1 α) have been demonstrated to play important roles in HIV-1 pathogenesis, mainly as inhibitors of viral entry (9, 12, 36), we studied the effect of IL-8, and signaling through its receptors CXCR1 and CXCR2, on HIV replication in macrophages and T lymphocytes. Replication of the macrophage-tropic R5 isolate HIV-1_{BaL} in AM was increased by treatment with IL-8 (Fig. 1A). HIV-1_{BaL} replication in MDM was also increased significantly by the addition of IL-8 at doses of between 5 and 100 ng/ml (Fig. 1B). These amounts of IL-8 are most likely physiologic, since they are within the range produced by MDM following exposure to HIV-1 (Lane et al., submitted). We evaluated the addition of IL-8 before infection, after infection, or before and after infection in a number of experiments. Each of these three treatment regimens resulted in an increase in HIV-1 replication,

and we found no significant difference between the regimens in a number of experiments (data not shown). In experiments performed with MDM from 17 different donors, IL-8 increased HIV-1 replication in 15 of the 17 donors (mean, 6.2-fold; range, 0.5- to 50-fold). IL-8 does not appear to confer susceptibility to productive infection with X4 HIV on MDM, as viral replication was not detected when MDM from multiple donors were infected with HIV-1_{BRU} in the presence or absence of exogenous IL-8 (data not shown).

We have recently demonstrated that a second chemokine that can signal through CXCR2, GRO- α , stimulates HIV-1 replication at a range of doses similar to that of IL-8 (30a). We therefore hypothesized that the effect of these chemokines may be mediated by the receptor CXCR2 and possibly by CXCR1, which can also act as a receptor for IL-8. Indeed, the effects of IL-8 and GRO- α on HIV-1 replication were blocked by a combination of antibodies that prevent interaction with CXCR1 and CXCR2 (Fig. 1C). These data indicate that IL-8, which is produced in response to exposure to HIV-1, enhances HIV-1 replication in primary human MDM by acting through its receptors (CXCR1 and CXCR2).

We next investigated whether IL-8 had an effect on HIV-1 replication in CD4⁺ T lymphocytes. Addition of IL-8 in doses exceeding 5 ng/ml stimulated viral replication in PHA-acti-

vated, CD8-depleted PBL (Fig. 2). Replication of the R5 isolate HIV-1_{BaL} in activated PBL was stimulated in 10 of the 11 donors tested (mean, 4.8-fold; range, 0.8- to 19-fold) by the addition of IL-8 (Fig. 2A). IL-8 also stimulated the replication of X4 isolates of HIV, including HIV-1_{BRU} (Fig. 2B) and HIV-2_{CBL23} (Fig. 2C). As observed with infection of MDM, stimulation of HIV-1_{BaL} replication in PBL is mediated through both CXCR1 and CXCR2, as anti-CXCR2 alone partially reduced, and anti-CXCR2 in combination with anti-CXCR1 completely prevented, the stimulatory effect of exogenous IL-8 (data not shown).

IL-8 is increased in the lymphoid tissue of AIDS patients. Previous investigators have demonstrated that levels of IL-8 are increased in the sera and lungs of HIV-1-infected individuals (15, 34, 44). To determine whether IL-8 levels are also increased in the microenvironment in which much of HIV-1 replication occurs in vivo, we evaluated the expression of IL-8 in lymphoid tissue from individuals at various stages of clinical disease progression. Tissue was collected from individuals with AIDS, from those whose infections had been successfully treated with HAART, from those with chronic asymptomatic HIV-1 infection, from recent HIV-1 seroconverters, and from seronegative controls (Table 1). IL-8 was readily detected in the lymphoid tissue by immunohistochemical staining (Fig. 3). Examination of tissue at high power demonstrated that IL-8 is present in the Golgi-endoplasmic reticulum complex within lymphoid cells (Fig. 3). Preincubation of tissue sections with a 10-fold molar excess of IL-8 eliminated the signal, demonstrating the specificity of the immunohistochemical staining (reference 4 and data not shown). A clear increase in the number of IL-8-expressing cells was present in individuals with AIDS compared with individuals in all of the other groups (Table 1). Thus, significantly increased IL-8 expression is seen at a major site of HIV-1 replication, lymphoid tissue, in patients with high viral loads and disease progression.

Anti-IL-8, anti-CXCR1, and anti-CXCR2 antibodies and the small-molecule inhibitor SB225002 inhibit HIV-1 replication in MDM and PBL. As increased levels of IL-8 are present locally at the sites of HIV-1 infection in vivo, and as IL-8 stimulates HIV-1 replication, we hypothesized that inhibition of endogenous IL-8 might reduce HIV-1 replication. Our experiments using exogenous IL-8 indicated that small amounts of IL-8 (5 to 125 ng/ml) are sufficient to stimulate HIV-1 replication (Fig. 1 and 2). These amounts of IL-8 are in the same range as the amount induced by exposure of MDM to HIV-1 and produced constitutively by activated PBL (Lane et al., submitted). To test this hypothesis, we incubated HIV-1-infected cells with antibodies known to antagonize IL-8 function. Addition of an antibody that neutralizes IL-8 activity markedly reduced HIV-1_{BaL} replication in MDM compared with the addition of a mouse IgG control (Fig. 4A). Furthermore, when the IL-8-specific antibody or antibodies that prevent interaction with the IL-8 receptors CXCR1 and CXCR2 were added to HIV-1_{BRU}-infected PBL, viral replication was reduced considerably (Fig. 4B). Control mouse IgG antibody had no effect on HIV-1 replication in PBL.

As antibodies that neutralize the function of IL-8 (Fig. 4A) and GRO- α (30a) and antibodies that prevent interaction with CXCR1 and CXCR2 (Fig. 4B) reduce HIV-1 replication, we hypothesized that inhibition of the pathways by which these

FIG. 4. Depletion of endogenous IL-8 inhibits HIV-1 replication in MDM and PBL. (A) MDM were treated with either mouse IgG1 or an antibody that neutralizes IL-8 activity (each at 20 μ g/ml) and infected with HIV-1_{BaL}. Medium was collected for the RT assay and replenished twice weekly. (B) Immunodepletion of IL-8 or blocking the IL-8 receptors CXCR1 and CXCR2 suppresses HIV-1 replication in CD8-depleted PBL. CD8-depleted PBL were treated with the indicated antibodies (20 μ g/ml) and infected with HIV-1_{BRU}. Medium was collected and replenished twice weekly. Supernatants were analyzed for RT activity on the indicated number of days after infection. Data shown are representative of four experiments.

chemokines signal might serve as a target for anti-HIV drugs. We therefore tested the efficacy of a recently discovered inhibitor of this pathway that is the type of compound potentially more suitable for clinical trials and use. The small-molecule inhibitor of CXCR2 SB225002 significantly inhibited replication of HIV-1_{BaL} in MDM (Fig. 5A) and of HIV-1_{BRU} in PBL (Fig. 5B). The doses of SB225002 used in these experiments did not significantly affect cellular viability, proliferation, and activation of MDM or PBL, as determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay (see legend for Fig. 5). Replication of HIV-1 in MDM and PBL was also significantly diminished by a peptide inhibitor of IL-8 and GRO- α function (data not shown). These data indicate that endogenous IL-8 production and signaling through CXCR1 and CXCR2 play stimulatory roles in HIV-1 replica-

FIG. 5. The small-molecule inhibitor SB225002 inhibits HIV-1 replication in macrophages and T lymphocytes. (A) MDM were treated with 0.01% DMSO (the carrier control) or SB225002 at the doses indicated every 3 days beginning 1 day before infection with HIV-1_{BaL}. The data shown are the mean of the RT activity present in triplicate wells measured on the days indicated. Cellular viability, proliferation, and activation were measured on day 13 by an MTT-based assay and determined to be 0.353 ± 0.036 , 0.382 ± 0.040 , 0.293 ± 0.022 , and 0.276 ± 0.040 for treatment with 0, 25, 100, and 300 nM SB225002, respectively. (B) PHA-activated PBL were treated with DMSO (0.01%) or the indicated doses of SB225002 every 3 days beginning 1 day before infection with HIV-1_{BRU}. HIV-1 replication was assayed by measuring the amount of RT activity in the supernatants on days 3, 6, and 9 after infection. Cellular viability, proliferation, and activation were measured on day 9 by an MTT-based assay and determined to be 0.052 ± 0.017 , 0.031 ± 0.020 , 0.023 ± 0.017 , and 0.046 ± 0.018 for treatment with 0, 100, 300, and 1,000 nM SB225002, respectively. These experiments are representative of six (A) and three (B) independent experiments performed with cells from different donors.

tion in two major targets of infection, MDM and T lymphocytes, and point to the existence of an autocrine/paracrine loop involving IL-8 and HIV-1 replication.

DISCUSSION

The presence of elevated levels of IL-8 in both the sera and lungs of individuals infected with HIV-1 has led several groups to suggest that IL-8 plays a role in HIV-1 pathogenesis, but little evidence has been presented to support these claims (15, 28, 34). Previous investigators found that inhibition of IL-8 had no effect on the activation of macrophages by HIV-1 Tat and that IL-8 had either no effect or a modest inhibitory effect on HIV-1 replication (10, 32, 35). However, we have recently demonstrated that IL-8 is produced by macrophages in response to HIV-1 and by endothelial cells in response to Kaposi's sarcoma-associated herpesvirus and that IL-8 plays an important role in the early angiogenesis of Kaposi's sarcoma (Lane et al., submitted). Here, we demonstrate that IL-8 stimulates HIV-1 replication in both MDM and activated T lymphocytes, findings that are somewhat at odds with those of previous investigators. In contrast to these previous studies, we performed experiments with primary human macrophages and lymphocytes from a large number of individuals and found an effect with amounts of IL-8 that are similar to the amounts of IL-8 found in the sera and lungs of HIV-1-infected individuals. Previous investigators most likely did not detect an effect of IL-8 on HIV-1 replication because the doses used were well above the optimal dose for IL-8 (5 to 125 ng/ml). Our findings that IL-8 expression is increased in the lymphatic microenvironment in AIDS patients and that inhibition of the activity of endogenous IL-8 markedly reduces HIV-1 replication suggest that the role of IL-8 in HIV-1 replication is likely to be biologically relevant. Of further note is the observation that IL-8 stimulates HIV-1 replication in two important target cells of infection, T cells and macrophages. Thus, HIV-1 infection leads to elevated production of IL-8 by MDM, and IL-8 then completes an autocrine/paracrine loop by, in turn, increasing HIV-1 replication in macrophages and lymphocytes. This loop presents an attractive target for antiretroviral therapy.

We have now demonstrated that blocking IL-8 and GRO- α with either antibodies to IL-8, GRO- α , CXCR1, or CXCR2 or the small-molecule inhibitor SB225002 inhibits HIV-1 replication in two important target cells of infection, T lymphocytes and macrophages. SB225002 is a small-molecule inhibitor of chemokine receptor signaling that acts preferentially on CXCR2 (45). We have found that SB225002 is able to inhibit viral replication in both lymphocytes and macrophages, without negatively affecting cellular viability, at doses in the nanomolar range. Thus, inhibitors of CXCR2 signaling may break the vicious cycle in which HIV-1 infection leads to elevated production of IL-8 and GRO- α , which then complete autocrine/paracrine loops by in turn increasing HIV-1 replication. As one IL-8-specific monoclonal antibody (Abgenix) is currently in clinical trials for psoriasis and additional compounds are being developed for the treatment of other inflammatory diseases, it appears attractive to test these agents for use as antiretroviral therapies. Our laboratory findings with SB225002 provide evidence that this or related compounds may be useful as antiretroviral agents as well. The fact that inhibitors of IL-8

and CXCR2 function are already major targets for drug development, along with our findings, which indicate that IL-8 and GRO- α and their receptors may play a role in HIV-1 disease, calls for an exploration of the therapeutic potential of blocking this chemokine axis in HIV infection and AIDS.

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