Roles for CXC Chemokine Ligands 10 and 11 in Recruiting CD4+ T Cells to HIV-1-Infected Monocyte-Derived Macrophages, Dendritic Cells, and Lymph Nodes

John F. Foley, Cheng-Rong Yu, Rikki Solow, Maureen Yacobucci, Keith W. C. Peden and Joshua M. Farber


http://www.jimmunol.org/content/174/8/4892

This article cites 60 articles, 34 of which you can access for free at: http://www.jimmunol.org/content/174/8/4892.full#ref-list-1

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

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

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
Roles for CXC Chemokine Ligands 10 and 11 in Recruiting CD4+ T Cells to HIV-1-Infected Monocyte-Derived Macrophages, Dendritic Cells, and Lymph Nodes

John F. Foley,* Cheng-Rong Yu,* Rikki Solow,2† Maureen Yacobucci, † Keith W. C. Peden,3† and Joshua M. Farber3*‡

We investigated roles for chemoattractants in dissemination of HIV-1 by examining the induction of T cell-active chemokines in HIV-1-infected human monocyte-derived macrophages and dendritic cells. Of the 12 chemokines analyzed, mRNAs for two, CXCL10 and CXCL11, ligands for the chemokine receptor CXCR3, were up-regulated in both cell types upon infection by HIV-1. Induction of these chemokine genes in infected cultures was dependent on both viral entry and reverse transcriptase activity, but not on the HIV-1 envelope glycoprotein. Conditioned medium from infected cells was chemotactic for freshly isolated human CD4+ T cells, and chemotaxis was abolished by pretreatment with an Ab against CXCR3. A lymph node from an HIV-1-infected individual expressed CXCL10 and CXCL11 mRNAs in the paracortex, including venules, as detected by in situ hybridization, whereas neither mRNA was detected after highly active antiretroviral therapy. Because CCR5 on CD4+ T cells is found predominantly on cells that also express CXCR3, these data implicate CXCL10 and CXCL11 in the recruitment of susceptible T cells to HIV-1-infected lymph nodes, macrophages, and dendritic cells. This recruitment might enhance the sequestration of T cells in infected lymphoid organs and the spread of infection between cells, contributing to the immunopathology of AIDS. The Journal of Immunology, 2005, 174: 4892–4900.

The ability of HIV-1 to take advantage of immune responses that ordinarily contribute to host defense is critical in the pathogenesis of AIDS (reviewed in Ref. 1). One component of host defense, the chemokine system, has been appreciated to have a major role in HIV disease, following the discoveries that CCL3, CCL4, and CCL5 could suppress infection by some strains of HIV-1 and that CXCR4 and CCR5 were the major coreceptors that, along with CD4, are necessary for HIV-1 to enter cells (reviewed in Ref. 2). The use, by HIV-1, of chemokine receptors as coreceptors is a striking example of viral exploitation of the immune system. We considered whether the chemokines' physiological role as leukocyte chemoattractants might also contribute to disseminating HIV infection. Our initial question was whether infection of dendritic cells and macrophages by HIV-1 might induce chemokines that could recruit susceptible CD4+ T cells and thereby facilitate the infection of T cell targets. We focused on dendritic cells and macrophages because they are APCs that are infected by macrophage (M)-tropic strains of HIV-1 (3, 4) and are postulated to serve, respectively, as targets of initial infection that can carry virus to lymphoid organs (5–7) and as viral reservoirs (8, 9), and because their interaction with CD4+ T cells can be exploited by HIV-1 to spread infection. Dendritic cells can also transmit infection without becoming infected themselves (10).

Most studies of chemokines in AIDS have focused on the ligands for CCR5 and CXCR4, either on their suppressive effects in vitro or on their possible role in inhibiting viral transmission or disease progression in humans (reviewed in Ref. 2). Some studies, like ours, have addressed potential roles for chemokines in recruiting target cells to infected cells/sites, possibly contributing to the spread of infection (11–19) and/or to inflammatory damage, particularly in the brain (20–23). In most of these studies, HIV infection or viral products have been reported to induce CC chemokines (11–16, 20), whose direct effects on infection of T cells by CCR5-using (R5) viruses would be inhibitory. Some studies have revealed induction of CC and CXC chemokines as the result of broad screens for changes in gene activity related to infection of cells or monkeys with HIV-1 or SIV, respectively (19, 24). We were interested in the induction in HIV-1-infected APCs specifically of ligands for chemokine receptors expressed on CCR5+CD4+ T cells. Such ligands would be able to recruit, to foci of infection, T cells made susceptible to HIV-1 by their expression of CCR5 (and CXCR4) and by their receiving activating signals from the infected APCs.

In the experiments described below, we analyzed monocyte-derived macrophages (MDM) and dendritic cells (MDDC) for the expression of 12 chemokines after infection by HIV-1. Our data showed dramatic induction of CXCL10 and CXCL11, but none of the other chemokines examined, as a direct and early result of HIV-1 infection. CXCL10 and CXCL11 are chemokines inducible by type I and type II IFNs (25–27). With CXCL9, they share the receptor CXCR3, which is expressed on subsets of T cells, NK cells, and B cells (28). For CD4+ T cells, CXCR3 is expressed

*Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892; and 1Laboratory of Retrovirus Research, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892

Received for publication January 13, 2004. Accepted for publication February 3, 2005.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported in part by the National Institutes of Health Intramural AIDS Targeted Antiviral Program and the National Vaccine Program Office.

2 Deceased January 2, 2002.

3 Address correspondence and reprint requests to Dr. Joshua M. Farber, Laboratory of Molecular Immunology, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Building 10, Room 11N228, MSC 1888, Bethesda, MD 20892. E-mail address: joshua_farber@nih.gov; or Dr. Keith W. C. Peden, Laboratory of Retrovirus Research, Center for Biologies Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892. E-mail address: peden@cbcr.fda.gov

Copyright © 2005 by The American Association of Immunologists, Inc.
preferentially on Th1 cells (29). Our additional experiments suggested that CXCL10 and CXCL11 might contribute not only to bringing susceptible T cells to infected APCs, but also to the recruitment and retention of T cells to lymph nodes in HIV-infected individuals. Together, our results reveal a component of the innate immune system induced by HIV-1 infection that can be exploited by the virus to the detriment of the host.

Materials and Methods

Cell culture

Elutriated monocytes, lymphocytes, and PBMC were obtained from healthy donors by the Department of Transfusion Medicine, National Institutes of Health, under a protocol approved by the institutional review board. Macrophages were derived by culturing monocytes for 1 wk in IMDM containing 10% human serum type AB (Sigma-Aldrich), 50 ng/ml recombinant human GM-CSF (R&D Systems), 100 μg/ml gentamicin sulfonate, and 1 mM sodium pyruvate (Invitrogen Life Technologies). Dendritic cells were derived by culturing monocytes for 1 wk in RPMI 1640 containing 10% FBS, 50 ng/ml recombinant human GM-CSF, 1000 U/ml recombinant human IL-4 (R&D Systems), 100 μg/ml gentamicin sulfonate, and 1 mM nonessential amino acids, and 1 mM sodium pyruvate (30). The parent and the CXCR3-expressing 300-19 mouse pre-B cell lines were gifts from B. Moser (Theodor Kocher Institute, University of Bern, Bern, Switzerland). These cells were cultured in DMEM containing 10% FBS and 55 μM 2-ME with the addition of 1 mg/ml G418 (Invitrogen Life Technologies) for the CXCR3-expressing cells.

Preparation of viral stocks and viral infections

Viral stocks were prepared after transfection of 293T cells with infectious molecular clones (12 μg) using PolyFect reagent (Qiagen) as suggested by the vendor. The DNAs were pLAI (31), encoding a CXCR4-using (X4) virus, and pAD (32), encoding an R5 and M-tropic virus. Hybrid viruses were constructed between LAI and the M-tropic AD, JR-CSF, and YU-2 by cloning the envelope genes of the latter viruses into pNL4-3 (HIV-1 LAI/AD, LAI/JR-CSF, and LAI/YU-2, respectively). After transfection, the medium was collected at 24 and 48 h, and the amount of virus was determined by its reverse transcriptase (RT) activity (33). To obtain high titer stocks, virus was pelleted at 35,000 rpm for 45 min in an SW41 rotor at 4°C and resuspended in medium overnight at 4°C. For infection of MDM in six-well plates, 10 7 cells/ml in RPMI 1640 containing 10% FBS and 1 mM sodium pyruvate (Invitrogen Life Technologies) was used for infection. After overnight incubation at 37°C in 5% CO2, the cells were harvested and analyzed by real-time PCR for levels of CXCR3 mRNA.

Assays for chemokines

Levels of CXCL10 and CXCL11 were determined by ELISA using Ab pairs and reagents from R&D Systems and recombinant CXCL10 and CXCL11 from PeproTech. Levels of CCL2 and CCL3 were determined using the appropriate Quantikine colorimetric sandwich ELISA kits (R&D Systems).

Chemotaxis assays

Chemotaxis assays were performed using the Transwell system with 6.5-mm diameter membranes containing 3.0-μm pores (Corning Costar) as previously described (37). After overnight incubation at 37°C in 5% CO2, cells migrating into the lower wells were harvested and counted using a hemocytometer. Primary CD4+ T cells were isolated by negative selection from buffy coats using the RosetteSep human CD4+ T cell enrichment mixture (StemCell Technologies). Isolated CD4+ T cells were resuspended at 105 cells/ml in chemotaxis medium and preincubated for 2 h at 37°C with anti-CXCR3 (BD Pharmingen) or control IgG1 (R&D Systems) at 25 μg/ml. After centrifugation, cells were resuspended at 107 cells/ml in conditioned medium from uninfected MDM, containing anti-CXCR3 or control IgG1, and used for chemotaxis assays as described above with conditioned medium from uninfected or HIV-1-infected MDM in the lower wells. For each sample tested, results from duplicate or triplicate wells were averaged and displayed as the mean number of cells migrated per well ± SEM.

Results

We were interested in analyzing HIV-1-infected MDDC and MDM for the expression of ligands for the major chemokine receptors found on CCR5+CD4+ T cells that might be relevant for recruiting these cells within infected tissues. From our work (36, 40; K. Song, R. L. Rabin, and J. M. Farber, unpublished observations) and that from other laboratories (28, 41), these receptors include primarily CXCR3, CCR4, CCR1, CCR2, CCR4, (CCR5), and CCR6. MDDC and MDM were cultured uninfected or exposed to the T cell line (TCL)-tropic HIV-1 X4 strain, HIV-1_LAI, or the M-tropic R5 strain, HIV-1 AD. In contrast to M-tropic viruses, the TCL-tropic X4 strains of HIV-1 do not replicate efficiently in MDDC (3) or MDM (42). Three days after adding virus, RNA was harvested and analyzed by real-time PCR for levels of chemokine mRNAs and, for normalization, of the mRNA for GAPDH. The data are presented as the fold increases in chemokine mRNAs in virus-exposed vs unexposed cells (Fig. 1). For both MDDC and MDM, significant increases in the levels of mRNAs for CXCL10 and CXCL11 were induced after culture with HIV-1 AD, but not with HIV-1_LAI.

For the other chemokine mRNAs examined, including that for CXCL9, the third known CXCR3 agonist, no significant increases were induced in either cell type by either strain of virus. In addition, because of previous reports (12, 20), we measured CCL2 and CCL3 in our MDM and MDDC supernatants and found no increases after HIV-1 infection (data not shown).
The induction of CXCL10 and CXCL11 mRNAs by HIV-1 AD was consistent with the ability of M-tropic R5 (but not TCL-tropic) viruses to replicate in immature MDDC and in MDM. Because the tropism of HIV-1 strains is dependent primarily on the envelope glycoprotein (Env), we sought to strengthen the correlation between tropism and chemokine induction by infecting MDM with chimeric viruses containing the \( \text{env} \) gene of HIV-1 AD as well as the \( \text{env} \) genes from the M-tropic R5 strains, HIV-1 YU-2 and HIV-1 JR-CSF, on an HIV-1 LAI background. All these chimeric viruses induced CXCL10 and CXCL11 mRNAs in MDM similarly to HIV-1AD (data not shown).

We determined the time course of induction of CXCL10 and CXCL11 mRNAs after infection of cultures of MDDC (Fig. 2A) and MDM (Fig. 2B) with HIV-1 AD. Significant induction of chemokine mRNAs was delayed in cultures of MDDC (day 3) compared with MDM (days 1–2). This difference correlated with the difference in the kinetics of accumulation of viral mRNA, which showed its initial rise on day 3 in the MDDC (Fig. 2A) compared with an earlier rise, between days 1 and 2, in the MDM cultures (Fig. 2B). In additional experiments with HIV-1 AD-infected MDM, we measured RT activity in the infected cell culture medium as well as CXCL10 and CXCL11 mRNA levels in the infected cells and found a correlation between the extent of viral replication, which differed among donors, and the strength of induction of both chemokine mRNAs in the cell cultures (data not shown). For cultures of MDDC and MDM, levels of CXCL10 and CXCL11 mRNAs fell with time. Because the fold induction of chemokine mRNAs was generally higher for MDM compared with MDDC, we focused on MDM in the studies shown below. It is important to note that levels of induction in MDM and MDDC varied among donors, although the patterns were consistent.

To examine the requirements for induction of chemokine mRNAs, we measured levels of CXCL10 and CXCL11 mRNAs 3 days after infection of MDM cultures with HIV-1 AD in the presence or the absence of either the anti-human CD4 Ab RPA T4 or the RT inhibitor ZDV. Preventing receptor-mediated viral entry by blocking CD4 or inhibiting viral RT with ZDV abolished the induction of CXCL10 and CXCL11 (Fig. 3A). A number of investigators have reported the induction of chemokines, including CXCL10, in a variety of cell types by gp120 (14, 15, 22). We addressed the requirement for viral gp120 in the induction of CXCL10 and CXCL11 mRNAs (Fig. 3A). A number of investigators have reported the induction of chemokines, including CXCL10, in a variety of cell types by gp120 (14, 15, 22). We addressed the requirement for viral gp120 in the induction of CXCL10 and CXCL11 mRNAs (Fig. 3A). A number of investigators have reported the induction of chemokines, including CXCL10, in a variety of cell types by gp120 (14, 15, 22). We addressed the requirement for viral gp120 in the induction of CXCL10 and CXCL11 mRNAs (Fig. 3A). A number of investigators have reported the induction of chemokines, including CXCL10, in a variety of cell types by gp120 (14, 15, 22). We addressed the requirement for viral gp120 in the induction of CXCL10 and CXCL11 mRNAs (Fig. 3A).
viruses were able to induce mRNAs for CXCL10 and CXCL11 (data not shown). Induction of CXCL10 and CXCL11 mRNAs by the Vif and MA.Vpr mutant viruses were diminished relative to wild-type virus, probably because these mutants are not able to produce efficient spreading infections.

We then asked whether induction of the chemokine mRNAs was directly dependent on viral infection or was due to induction by virus of a secreted factor that was, in turn, responsible for activation of the chemokine genes. CXCL10 and CXCL11 are immediate early genes in response to IFNs (26, 43) and would be induced together, these data establish that induction of CXCL10 and CXCL11 mRNAs was a direct effect of, and required, viral infection, but did not require gp120/41 or virally induced secreted factors, such as type I IFNs.

To analyze the production of CXCL10/11 proteins by infected MDM and MDDC, culture medium from infected or uninfected cells was collected on successive days and assayed by ELISA. Although there was donor-to-donor variability, infected MDM (Fig. 4A) could secrete CXCL10 at concentrations expected to be biologically active as early as 1 day after infection and peaking on day 3 at >10 ng/ml, whereas the level in medium from uninfected cells was undetectable (<50 pg/ml). CXCL11 was also detected in culture medium from infected MDDC, although usually at lower levels than detected for MDM (data not shown). Surprisingly, although mRNAs for CXCL10 and CXCL11 were induced in comparable extents, no detectable CXCL11 (<50 pg/ml) was found in culture medium from these or other (data not shown) cultures of MDM. By spiking infected cultures with recombinant CXCL10 or

**FIGURE 3.** Induction of CXCL10 and CXCL11 mRNAs in HIV-1-infected MDM is dependent on viral replication, but not gp120. A, MDM were left uninfected or were infected with HIV-1AD alone, in the presence of ZDV, or in the presence of an anti-hCD4 Ab. Three days postinfection, fold increases in CXCL10 mRNA (■) and CXCL11 mRNA (□) were determined in infected cells relative to levels in uninfected cells, as described in Fig. 1. The mean fold increases ± SEM in mRNAs from two experiments are shown. B, MDM were left uninfected or were infected for 48 h with HIV-1 pseudotyped with VSV-G. Results shown are the mean fold increases in CXCL10 (■) and CXCL11 (□) mRNAs ± SEM for experiments and are representative of four experiments. C, MDM were cultured for 6 and 24 h (in the absence or the presence of ZDV) with conditioned medium from infected MDM in which chemokine induction had been demonstrated (not shown) or from noninfected MDM as a control. Results shown are the mean fold increases ± SEM in CXCL10 mRNA, relative to control cells, for duplicate cultures and are representative of four experiments (for some of which, cells were harvested at >24 h). D, MDM were left uninfected or were infected with HIV-1AD in the absence or the presence of a neutralizing mAb against human IFN-αβ receptor chain 2 (clone MMHAR-2; 15 μg/ml) or isotype control (IgG2α; 15 μg/ml) for 4 days, with fresh medium and Abs added each day. On day 4, uninfected MDM were treated for 6 h with recombinant human IFN-α (250 U/ml), after which cells were harvested, and RNA isolation and real-time PCR analysis were performed as described in Fig. 1. Data shown are the mean fold increases in CXCL10 (■) and CXCL11 (□) mRNAs ± SEM in infected MDM (AD) and IFN-α-treated MDM (IFNα), either alone (−) or with isotype control Ab (IgG2α) or anti-IFN-αβ receptor chain 2 neutralizing Ab (αIFNα), relative to uninfected, untreated MDM. All infections, Ab incubations, and IFN-α treatments were performed in duplicate. These data are representative of three donors.
CXCL10, we found that although levels of the former were unchanged or increased over time, levels of CXCL11 fell from 3 ng/ml at the time of addition to undetectable on day 3 (data not shown), so our ability to measure CXCL10 and not CXCL11 may be explained by differences in the stabilities of the chemokines in these cultures.

To establish that the CXCL10 secreted by HIV-1\textsubscript{AD}-infected MDM was biologically active, we performed cell migration experiments using the supernatants assayed for CXCL10 (Fig. 4A) and a mouse pre-B cell line that had been transfected to express CXCR3. Supernatants from infected, but not uninfected, MDM showed chemotactic activity for CXCR3-expressing cells, but not the parent nontransfected cells, and supernatant activities were proportional to the levels of CXCL10 measured by ELISA (Fig. 4B).

It was of greater importance to demonstrate activity on primary T cells. Migration assays were performed using freshly isolated human CD4\textsuperscript{+} T cells that were resuspended in medium from uninfected cultures of MDM before being placed in the upper wells of a Transwell apparatus above supernatants from HIV-1\textsubscript{AD}-infected MDM in which we had measured the CXCL10 concentration at 2.2 ng/ml (data not shown). To determine the contribution of CXCR3 ligands to chemotaxis, CD4\textsuperscript{+} T cells were preincubated with either anti-human CXCR3 (IgG1 isotype) or an IgG1 isotype control. Significantly more CD4\textsuperscript{+} T cells migrated to the culture medium from HIV-1\textsubscript{AD}-infected MDM than to the medium from the uninfected cells, and this enhanced migration was eliminated by treatment with the CXCR3-neutralizing Ab (Fig. 4C).

These data demonstrate that the HIV-1\textsubscript{AD} infected MDM produced chemotactic factor(s) for resting CD4\textsuperscript{+} T cells and that these factor(s) were CXCR3 ligands.

To support possible roles for CXCL10/11 during HIV disease, we investigated the expression of CXCL10/11 mRNAs in lymph nodes taken from an HIV-1-infected individual before and 10 wk after the initiation of highly active antiretroviral therapy (HAART).\textsuperscript{4} The mRNAs for CXCL10 (Fig. 5, A and B) and CXCL11 (Fig. 5, D and E) were expressed in the T cell regions of the lymph node removed before HAART was initiated. This lymph node contained large amounts of viral RNA, and not unexpectedly, immunohistochemistry showed that the T cell regions contained large numbers of macrophages and dendritic cells (J. M. Orenstein, unpublished observation). In contrast, after 10 wk of HAART, a lymph node from the same individual, although still hyperplastic, was now negative for CXCL10/11 mRNAs (Fig. 5, C and F). Correspondingly, little viral RNA was found in the lymph node removed after therapy (J. M. Orenstein, unpublished observation). Close inspection of the tissue sections from the node taken before HAART revealed linear distributions for some of the hybridization signal. This was due to grains overlying venules within the T cell zones (Fig. 5, G-I). Together these data showed that mRNAs for CXCL10/11 were expressed in a lymph node taken during active HIV-1 infection, but not in a node taken from the same individual once viral replication had been suppressed. The mRNAs for both chemokines were expressed in similar patterns (within macrophage- and dendritic cell-rich T cell zones), including in association with paracortical venules.

**Discussion**

Our initial question was whether the infection of dendritic cells and macrophages by HIV-1 might induce chemokines that could recruit susceptible CD4\textsuperscript{+} T cells, particularly CCR5\textsuperscript{+} cells, and thereby facilitate the infection of T cell targets and dissemination.

\textsuperscript{4} Abbreviations used in this paper: HAART, highly active antiretroviral therapy; MT, retrovirus; macrophage tropic; MDCC, monocyte-derived dendritic cell; MDM, monocyte-derived macrophage; NDV, Newcastle disease virus; RT, reverse transcriptase; TCL, T cell line tropic; VSV, vesicular stomatitis virus; ZDV, zidovudine.
of the virus. We focused, therefore, on ligands for chemokine receptors expressed on a significant proportion of CCR5<sup>+</sup>CD4<sup>+</sup> T cells. Of the 12 chemokine genes whose expression we analyzed, only two, CXCL10 and CXCL11, were significantly induced in MDDC and MDM by HIV-1 infection. We found that induction of these two genes required viral infection by M-tropic viruses, but not the HIV-1 envelope glycoprotein, and was not mediated by secreted factors. We showed that HIV-1-infected MDM secreted active CXCL10 within 1 day and that all HIV-1-induced chemotactic activity from MDM for CD4<sup>+</sup> T cells was due to CXCR3 ligands. Finally, we showed that the genes for both CXCL10 and CXCL11 were expressed in lymph node during active infection, but not after initiation of effective therapy, and that expression was not only within paracortical T cell regions, but also along venules.

Although investigators have reported the induction of a variety of CC chemokines in monocytes/macrophages by HIV-1 (11–13, 20), we did not find that any of these CC chemokine genes were up-regulated. Some investigators have reported induction of chemokines in monocyte/macrophages (14, 15) and astrocytes (22) by gp120 or extracellular Tat (23). As noted above, the induction we found for CXCL10 and CXCL11 required viral infection, could occur with pseudotyped virus lacking gp120/41, and was not due to secreted factors.

Discrepancies between our studies and those that showed induction of a number of CC chemokines by HIV-1 may be due, at least in part, to technical differences, including 1) the use of different culture conditions to derive macrophages from peripheral blood monocytes, including, for example, using M-CSF instead of GM-CSF (11–14, 20, 21); 2) the use of different viruses, including HIV-1<sub>Ada</sub> and HIV-1<sub>Ba-L</sub>, whereas we used HIV-1<sub>AD</sub> (11–13, 20, 21); and 3) the use of lower levels of inoculating virus than were used in our study (12, 20, 21). We believe that the final point is particularly germane. Low inocula of virus might produce infections with very different kinetics, obscuring early events that require synchronous infection and allowing for changes to occur in culture conditions leading to the accumulation of additional chemokines. The early induction of CXCL10 and CXCL11 raised the possibility that these chemokines might themselves induce additional factors. However, in experiments not shown, 6 days of treatment with CXCL10 and CXCL11, alone and in combination, failed to induce mRNAs for chemokines CCL2–5 in MDM.

Three recent reports showed induction of CXCL10 (along with other chemokines that were not induced in our experiments) by HIV infection of PBMC (16), MDM (21), or MDDC (19). Wetzels et al. (16) did not identify the mononuclear cells responsible for production of CXCL10 or whether chemokine induction was a direct or an indirect effect of the virus, although they found that induction of CXCL10 requires active viral infection. Poluektova et al. (21) showed induction of CXCL10 and CXCL9 in MDM, but did not investigate the requirements for chemokine induction. Izmailova et al. (19) showed the induction of multiple chemokines in a chip-based screen of gene expression, including CXCL10, CXCL9, and CCL8 by HIV infection of MDDC in vitro, which they attributed to the activity of Tat. Interpretation of these data was complicated, however, by the lack of correspondence in the time courses of accumulation of Tat and chemokines and by the use of an adenovirus vector to express Tat in MDDC, which, based on the data discussed below (44), might have influenced expression of the CXCL10 gene.

The novel findings in our study include that CXCL11 can be induced in cells by infection with HIV-1; that, in contrast with
other chemokines, CXCL10 and CXCL11 are induced in a quantitatively significant, selective, and direct fashion early in HIV-1 infection of MDDC and MDM; that the principal chemotactic activity produced specifically by infected MDM in vitro is due to ligands for CXCR3; and that CXCL10 and CXCL11 can be expressed in lymph nodes from an HIV-1-infected individual, are associated with active viral replication, and involve venules as well as T cell regions.

Compared with the data for CXCL10 and the third CXCR3 ligand, CXCL9, published information on CXCL11 is limited. CXCL11 was described initially as being highly inducible in astrocytes and monocytes by both IFN-β and IFN-γ (26, 27) and to be the most potent of the CXCR3 ligands (27). In its responsiveness to both classes of IFNs, CXCL11 is similar to CXCL10 and differs from CXCL9 (25). The similarities in the regulation of CXCL11 and CXCL10 are consistent with the shared elements and structure of the genes’ promoters, which differ from those of CXCL9 (45).

In contrast with the limited investigation of CXCL11, there are many examples of the induction of CXCL10 by viruses and viral surrogates, the first being our report that murine CXCL10 could be induced by dsRNA in the presence of cycloheximide (46). Data from the CXCL10 knockout mouse support a role for CXCL10 in antiviral host defense (47). A partial list of viruses that induce murine and/or human CXCL10 in cells in culture include paramyxoviridae (Newcastle disease virus (NDV) (48), Sendai virus (49), and measles virus (50)) and adenoviruses (44). In the case of NDV, irradiated virus was able to induce CXCL10, and induction did not require new protein synthesis (48), whereas the induction of CXCL10 by adenovirus vectors was a response to the viral capsid (44). Studies of virus-induced signals have shown that NDV can induce the CXCL10 gene directly, in an IFN-independent fashion, through IFN regulatory factor-3 (51). Together, these data establish that activation of the CXCL10 gene is part of the immediate, innate response to a range of viral infections, consistent with our findings for induction of CXCL10 by HIV-1. By inference, this would also apply to our findings for the CXCL11 gene based on the shared organization of the two genes’ promoters.

A role for these proteins as the chemokines most closely associated with the antiviral response is supported by their impressive induction both by virus directly and by IFN-α/β, which distinguishes them from other chemokines, including some of those reported by others to be induced by HIV-1 infection. Even the related, third CXCR3 ligand, CXCL9, which was reported recently to be induced by HIV-1 (19, 21), is highly dependent for its induction on IFN-γ both in vitro (52) and in vivo (53), so that induction in the absence of cells producing IFN-γ would be expected to be minimal. In line with our real-time PCR data, when we used the conditioned medium from infected vs uninfected MDM in a chemotaxis assay with CD4+ T cells, all HIV-1-induced chemotactic activity could be blocked by Ab neutralization of CXCR3, indicating that none of the non-CXCR3 chemokines was contributing. The attraction of CD4+ T cells expressing CXCR3 would thereby recruit cells expressing CCR5, given that CCR5+CD4+ T cells are generally found within the CXCR3+ subset (28, 36). In the absence of significant induction of CCR5 ligands (which can block infection), the CXCR3 ligands induced in MDM and MDDC by HIV-1 would serve to recruit CD4+ T cells into an environment in which virus could be efficiently transmitted from cell to cell.

The expression of the genes for CXCL10 and CXCL11 in an HIV-1-infected lymph node suggests that these chemokines may have a role in pathogenesis and/or host defense in AIDS. Conversely, the disappearance of CXCL10/11 expression after HAART suggests that the down-regulation of these genes may be important for some of the restorative effects of therapy on immune homeostasis. We have shown that CXCL10 is made by macrophages and dendritic cells in inflamed tonsils (36), and we presume that the signal in the T cell regions of the lymph node not associated with vessels is made by these cells. Based on our in vitro data and the loss of expression after HAART, it is possible that some of the signal is from infected cells. However, in the absence of opportunistic infection, the number of infected macrophages or dendritic cells at any given time is likely to be small (54). It is more likely that most of the signal is the indirect effect of cytokines such as IFN-γ and TNF-α on macrophages and/or dendritic cells. This supposition is supported by our finding that this tissue also expresses CXCL9 (36), which, as shown and discussed above, is not induced in vitro by HIV-1, but is highly inducible by IFN-γ. This also would explain the expression of CXCL10 and CXCL11 along venules, because although endothelial cells have been shown to express these chemokines in response to cytokines (55, 56), not surprisingly, we did not see HIV-1 replication along vessels in this lymph node (data not shown).

Our finding that CXCL10 and CXCL11 are expressed along paracortical venules suggests that these chemokines may be involved in recruiting memory T cells to infected lymph nodes from the blood. CXCR3 has been shown to be able to mediate adhesion of T cells to endothelial cells under conditions of flow in response to CXCL10 (57), and CXCL11 has been shown to be a particularly potent chemokine in mediating transendothelial migration (56). It is noteworthy that just as CXCL10 and CXCL11 were, and CXCL9 was not, induced by infection with HIV-1 in vitro, CXCL10 and CXCL11 were expressed in an identical pattern in vivo, which differed from the pattern that we saw for CXCL9 (36). These observations are analogous to those by Zhao et al. (58), who reported that CXCL10 and CXCL11 were both expressed by vessels in endomyocardial biopsies from human cardiac allografts, which was not the case for CXCL9. To our knowledge, our study is the first demonstration of the expression of chemokines by venules of HIV-1-infected lymph node, providing candidates mediating the enhanced recruitment of T cells to these tissues.

Investigations of the rapid rise in CD4+ (and CD8+) T cells after initiation of HAART revealed that a major contributor to peripheral lymphopenia during active viral replication in AIDS is the preferential redistribution of T cells to lymphoid organs (59–61). Recruitment and retention of T cells to infected lymph nodes will have two potentially deleterious consequences: 1) the disrupted circulation of T cells, leading to both lymphopenia in the peripheral blood and inability of T cells to respond at the appropriate tissue sites; and 2) the prolonged exposure of susceptible T cells to high concentrations of cell-associated virus in the activating environment of the lymph node, which has been shown to be the major site of ongoing viral replication (62). In light of these considerations, the expression of CXCL10 and CXCL11 in the paracortex and on paracortical vessels may contribute directly to CD4+ T cell infection and immunopathology in AIDS.

Acknowledgments

We thank Dr. Michael Polis (National Institute of Allergy and Infectious Diseases, National Institutes of Health) for patients’ lymph node samples, Prof. Jan Orenstein (George Washington University Medical Center) for sharing his data and for helpful consultation, Dr. Jeffrey Smith (University of California-Los Angeles School of Medicine) for providing the CXCL11 clone, Dr. Michael Emerman (Fred Hutchinson Cancer Research Center) for the pL.VSV-G clone, and Dr. Bernhard Moser (Theodor-Kocher Institute) for the CXCR3-expressing cell line.

Disclosures

The authors have no financial conflict of interest.
15. Choe, W., D. J. Volsky, and M. J. Potash. 2001. Induction of rapid and extensive chemokine expression or ligand binding.
17. Choe, W., D. J. Volsky, and M. J. Potash. 2001. Induction of rapid and extensive chemokine expression or ligand binding.


