

## **Macrophage-tropic HIV Induces and Exploits Dendritic Cell Chemotaxis**

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### **Abstract**

Immature dendritic cells (iDCs) express the CC chemokine receptor (CCR)5, which promotes chemotaxis toward the CC chemokines regulated on activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1 $\alpha$ , and MIP-1 $\beta$ . By contrast, mature DCs downregulate CCR5 but upregulate CXC chemokine receptor (CXCR)4, and as a result exhibit enhanced chemotaxis toward stromal cell-derived factor (SDF)-1 $\alpha$ . CCR5 and CXCR4 also function as coreceptors for macrophage-tropic (M-tropic) and T cell-tropic (T-tropic) human immunodeficiency virus (HIV)-1, respectively. Here, we demonstrate chemotaxis of iDCs toward M-tropic (R5) but not T-tropic (X4) HIV-1. Furthermore, preexposure to M-tropic HIV-1 or its recombinant envelope protein prevents migration toward CCR5 ligands. The migration of iDCs toward M-tropic HIV-1 may enhance formation of DC-T cell syncytia, thus promoting viral production and destruction of both DC and T helper lymphocytes. Therefore, disturbance of DC chemotaxis by HIV-1 is likely to contribute to immunosuppression in primary infection and AIDS. In addition, migration of iDCs toward HIV-1 may aid the capture of R5 HIV-1 virions by the abundant DC cell surface protein DC-specific intercellular adhesion molecule (ICAM)3-grabbing nonintegrin (DC-SIGN). HIV-1 bound to DC cell-specific DC-SIGN retains the ability to infect replication-permissive T cells in trans for several days. Consequently, recruitment of DC by HIV-1 could combine with the ability of DC-SIGN to capture and transmit the virus to T cells, and so facilitate dissemination of virus within an infected individual.

**Key words:** dendritic cell • HIV • chemotaxis • chemokine • CCR5

### **Introduction**

The dissemination of HIV-1 and establishment of infection within an individual involves the transfer of virus from mucosal sites of infection to T cell zones in secondary lymphoid organs. How this happens is not certain. Once at these sites, the virus replicates within CD4<sup>+</sup> T helper lymphocytes and macrophages. The immature dendritic cells (iDCs) of the skin and mucosa, Langerhans cells (LCs), have been implicated as the first targets for HIV after sexual contact (1). In the simian immunodeficiency virus/rhesus macaque system, the model that most closely resembles HIV-1 infection in humans, the first cellular targets of infection after vaginal challenge are mucosal DCs (2). The

subsequent appearance of antigen-bearing cells within the T cell areas of draining lymph nodes (2) has led to the suggestion that DCs may act as "Trojan Horses" (3), carrying virus to lymph nodes. The proficiency of DCs in interacting with numerous T cells (4) makes them prime candidates for enhancing viral dissemination (5).

iDCs are located in most nonlymphoid tissues, where they capture and process antigens. After exposure to inflammatory signals, they mature and migrate to secondary lymphoid tissues, where they present antigen to T cells (6). iDCs express CC chemokine receptor (CCR)5, which enables chemotaxis to the CC chemokines regulated on activation, normal T cell expressed and secreted (RANTES), macrophage inflammatory protein (MIP)-1 $\alpha$ , and MIP-1 $\beta$ . Mature DCs downregulate CCR5 but upregulate CXC chemokine receptor (CXCR)4, and show enhanced chemotaxis toward stromal cell-derived factor (SDF)-1 (7). CCR5

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and CXCR4 function as coreceptors for macrophage-tropic (M-tropic) and T cell-tropic (T-tropic) HIV-1, respectively (8, 9). DCs also express CD4 and are susceptible to infection by HIV-1 (3). Here, we demonstrate chemotaxis of iDCs toward M-tropic (R5) but not T-tropic (X4) HIV-1. Furthermore, preexposure to M-tropic HIV-1 or its recombinant envelope protein prevents migration toward CCR5 ligands. These events might play an important role in the recruitment of DCs to mucosal sites of HIV-1 inoculation, and consequently could explain the dissemination of infection.

## Materials and Methods

**Preparation of DCs from Blood and Chemotaxis Assays.** Human DCs were cultured from commercial buffy coats as described previously (7). Chemotaxis assays are described elsewhere (7). In brief,  $5 \times 10^4$  iDCs in 100  $\mu$ l culture medium were added to each 24-well transwell insert (5- $\mu$ m pores; Costar), with 600  $\mu$ l in the lower chamber, with or without chemokine, HIV supernatant, or recombinant gp120. After 2 h incubation at 37°C, the transwell inserts were lifted, and the bases were rinsed twice with 200  $\mu$ l of medium into the lower chamber. Cells in the lower chamber were collected, transferred into 5-ml U-bottomed clear tubes, and fixed with 5% formalin. The number of migrating cells was determined by FACSsort™ (Becton Dickinson) as described previously (7). Results are expressed as the total number of migrating DCs per transwell. Standard deviations are shown for three or four replicate transwells. Data is representative of at least three separate experiments.

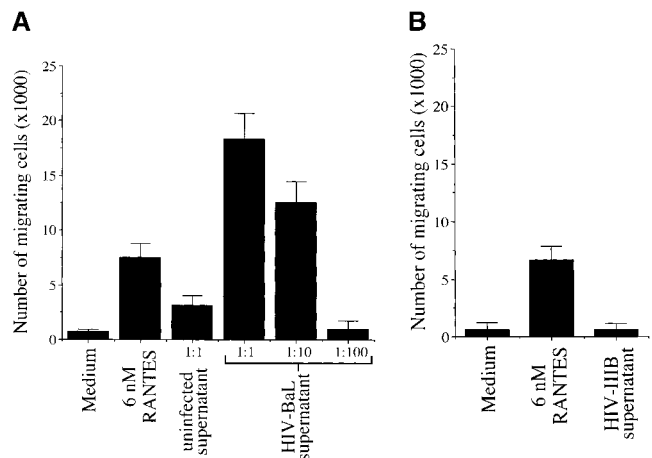
**HIV Supernatants.** Supernatants of HIV-infected and uninfected T cells were separated into concentrate (two times) and filtrate by centrifugation through a 100-kD cutoff centrifuge concentrator (Amicon). In all figures the >100 kD fraction is used as "supernatant." Supernatants were quantified for reverse transcriptase (RT) using the Quant-T-RT assay system (Amersham Pharmacia Biotech). Relative RT units of enzyme by scintillation proximity assay for unconcentrated supernatants from HIV-1<sub>BaL</sub>-infected PM1 cells and HIV-1<sub>IIIIB</sub>-infected H9 cells were  $1.4 \times 10^4$ /ml and  $3.9 \times 10^3$ /ml, respectively (used in Figs. 1 and 4). Supernatants of PM1 cells infected with HIV-1<sub>BaL</sub> or HIV-1<sub>IIIIB</sub> (relative RT units  $2.9 \times 10^3$ /ml and  $3.3 \times 10^3$ /ml) were used in Fig. 2. For immunoprecipitations, 10  $\mu$ g of gp120 Ab 2G12, 10  $\mu$ g of gp120 Ab IgG1b12, and 5  $\mu$ g of gp41 Ab 2F5 (AIDS Reagent Project, National Institute for Biological Standards and Control) were added to 6 ml of BaL viral supernatant or 6 ml of RPMI plus MIP-1 $\beta$  as a control. The solutions were mixed on ice for 30 min before the addition of 20  $\mu$ g protein A sepharose (Sigma-Aldrich) in PBS for 45 min. After 45 min of mixing, the protein A beads were removed by centrifugation. Supernatants were transferred to another tube, and an additional 20  $\mu$ g of protein A sepharose was added. After 45 min, the second protein A beads were removed, and the supernatants were used for chemotaxis assays.

**Recombinant gp120.** DNA encoding gp120 was amplified by PCR from genomic DNA derived from PM1 cells infected with HIV-1<sub>BaL</sub> and HIV-1<sub>ADA</sub> (AIDS Reagent Project, National Institute for Biological Standards and Control). Primers were designed to extend the COOH terminus with a linker and a FLAG epitope (EFGGDYKDDDDKGG). The construct was expressed in Sf9 cells using a p2Bac baculovirus expression system (Invitrogen). Cells infected with recombinant viruses secreting gp120

were screened by Western blotting using both anti-FLAG M2 mAb (Sigma-Aldrich) and anti-gp120 mAb (ARP301; National Institute for Biological Standards and Control). Cell supernatants were loaded onto an anti-FLAG M2 affinity column (Sigma-Aldrich) and eluted with pH 3.5 glycine-HCl according to the manufacturer's instructions. Purified gp120 was checked by SDS-PAGE (both reducing and nonreducing) and behaved as a monomer in gel filtration chromatography. Binding to human CD4 was confirmed by surface plasmon resonance (BIAcore). Stock solutions of gp120 in Tris-HCl pH 8.0 were diluted into RPMI media to the required concentration(s). Tris pH 8.0 in RPMI was used as "medium" control.

## Results

**iDCs Exhibit Chemotaxis toward the Supernatant of T Cells Infected with M-tropic HIV.** Monocyte-derived iDCs were tested in transwell migration assays as described previously (7). These cells exhibit significant chemotaxis toward RANTES and the supernatant from T cells infected with M-tropic HIV-1<sub>BaL</sub>, but not toward the supernatant from T cells infected with T-tropic HIV-1<sub>IIIIB</sub>, or from uninfected cells (Fig. 1). Cell supernatants were separated into concentrate (two times) and filtrate by centrifugation through a 100-kD cutoff centrifuge concentrator (Amicon). In all figures, the >100-kD fraction is used as "supernatant." The >100-kD fraction from HIV-1<sub>BaL</sub>-infected PM1 cells is



**Figure 1.** iDCs migrate toward RANTES and M-tropic HIV culture supernatant but not toward T-tropic HIV or uninfected supernatants. (A) Chemotaxis of  $5 \times 10^4$  immature DCs in a bare transwell migration assay (described in Materials and Methods). The number of cells migrating into the lower transwells ( $\times 1,000$ ) as determined by FACSsort™ (Becton Dickinson) is shown with lower transwells containing medium alone (RPMI 1640), 6 nM RANTES, supernatant from PM1 T cells alone (which express CCR5), and PM1 cells infected with M-tropic HIV-1<sub>BaL</sub>. Cell supernatants were separated into concentrate (two times) and filtrate by centrifugation through a 100-kD cutoff centrifuge concentrator (Amicon). In all figures, the >100-kD fraction is used as "supernatant." The >100-kD fraction from HIV-1<sub>BaL</sub>-infected PM1 cells is highly chemotactic, even when added in a 10-fold dilution; this large molecular mass chemotactic agent is likely HIV-virions/fragments and/or gp120 (see Fig. 3). (B) As in A, except using >100-kD supernatant from H9 T cells infected with the T-tropic HIV-1<sub>IIIIB</sub>. In both graphs, SD from the mean is shown for four experiments, each with two transwells. Results are representative of four experiments

highly chemotactic, even when added in a 10-fold dilution; this large molecular mass chemotactic agent is likely to be HIV-virions/fragments and/or gp120 (see Fig. 3). Migration toward the <100 kD fraction was similar for uninfected and HIV-1<sub>BaL</sub>-infected supernatant, and was only 30% of that toward RANTES (not shown); this small molecular chemotactic agent is presumed to be a T cell-derived chemokine(s).

**Soluble CD4 Increases Chemotaxis toward M-tropic HIV Supernatant.** The binding of CD4 to M-tropic HIV envelope protein (gp120) increases the efficiency of CCR5 binding (10). Addition of soluble CD4 at 10  $\mu\text{g}/\text{ml}$  almost doubled the chemotactic effect of supernatant from cells infected with M-tropic virus, without affecting migration toward supernatant from uninfected cells or RANTES (not shown).

**Anti-HIV Antibodies Remove the Chemotactic Effects of HIV Supernatant.** Immunoprecipitation with anti-HIV antibodies reduced the chemotaxis induced by the supernatant of PM1 cells infected with HIV-1<sub>BaL</sub> to the level induced by the supernatant from PM1 cells infected by HIV-1<sub>IIIb</sub>, suggesting that HIV-1<sub>BaL</sub> virions and/or gp120 induce chemotaxis (Fig. 2).

**iDCs Migrate toward M-tropic gp120.** To confirm that iDCs migrate toward HIV and rule out a requirement for M-tropic virus-induced T cell-derived lymphokines, we examined chemotaxis toward M-tropic recombinant gp120 envelope. iDCs migrate toward M-tropic gp120 from HIV-1<sub>BaL</sub> (Fig. 3 A) and HIV<sub>ADA</sub> (Fig. 3 B). By contrast, chemotaxis was not observed toward recombinant gp120 from T-tropic HIV-1<sub>IIIb</sub> (Fig. 3 C) or recombinant gp120 from the T-tropic strains SF2, MN, W61D, and HXB2 (not shown). Cross-linking of the FLAG-tagged HIV-1<sub>BaL</sub> gp120 with

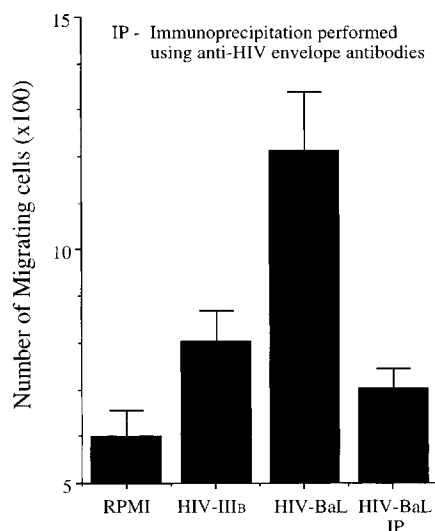
anti-FLAG antibody increased its chemotactic potential by  $\sim 30\%$  (not shown). Treatment of iDCs with anti-CCR5 antibodies prevented migration toward R5 gp120 (Fig. 3 D). Because M-tropic but not T-tropic gp120s induce chemotaxis, and gp120/CCR5 binding induces an intracellular signal indistinguishable from that of a natural chemotactic ligand (11, 12), we conclude that binding of M-tropic gp120 to CCR5 can induce DC chemotaxis.

**Exposure to M-tropic HIV or Its Recombinant Envelope Inhibits the Response of iDCs to Chemokines.** Pretreatment of iDC for 90 min with 6 nM of the CC chemokines RANTES (binds CCR1, 3, 4, and 5), MIP-1 $\alpha$  (binds CCR1 and 5), or MIP-1 $\beta$  (binds CCR5 and 8) substantially diminishes subsequent chemotactic responses to all these chemokines, but not to the CXCR4 ligand SDF-1 (not shown). Pretreatment of iDCs with supernatant of T cells infected with HIV-1<sub>BaL</sub>, but not the supernatant from uninfected cells, likewise reduced responses to these CC chemokines (Fig. 4 A). Conversely, pretreatment of iDCs with CC chemokines reduced subsequent chemotaxis toward HIV-1<sub>BaL</sub> supernatant (not shown). Pretreatment of iDCs with supernatant from T cells infected with T-tropic HIV-1<sub>IIIb</sub> had no effect on migration toward MIP-1 $\alpha$  or RANTES (not shown).

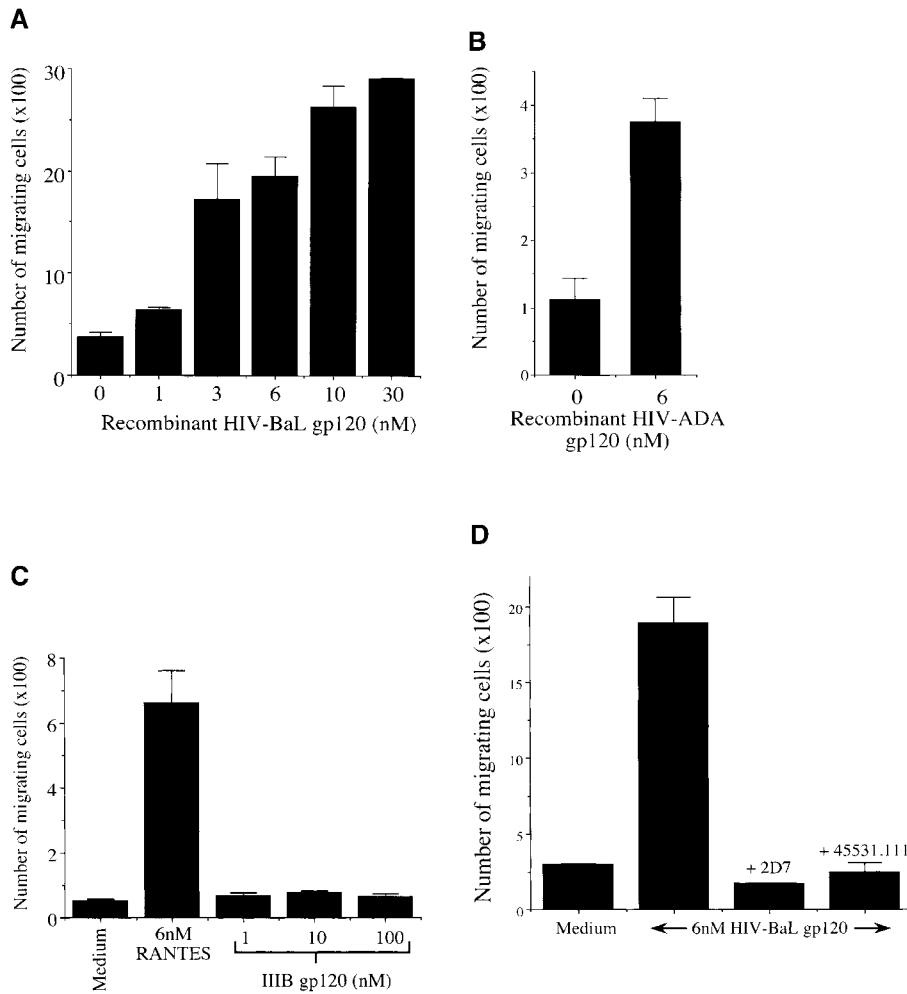
Addition of 3 nM M-tropic HIV-1<sub>BaL</sub> recombinant gp120 to the upper transwell (Fig. 4 B) abolished responses to MIP-1 $\beta$  at concentrations up to 24 nM. In contrast, similar pretreatment with recombinant gp120 from the T-tropic HIV-1<sub>IIIb</sub> (1, 6, 24, and 48 nM) was without effect on migration toward CC chemokines (not shown).

## Discussion

We demonstrate the migration of iDCs toward M-tropic but not T-tropic HIV-1, and substantiate this finding using recombinant envelope (gp120) proteins. These observations have important implications for the establishment, dissemination, and progression of HIV-1 infection within an infected individual. HIV Tat has been reported to induce chemotaxis of DCs and monocytes (13, 14). Our results, which show that the migratory action of HIV-1 is confined to M-tropic HIV virus, indicate that any chemotactic action of Tat is not apparent in the culture supernatant of T cells infected with HIV-1 (Fig. 1 B). We were unable to observe migration of iDCs toward 10 or 100 nM basic Tat peptide (amino acids 31–71; reference 14) or a pool of Tat peptides (amino acids 1–30, 16–45, and 31–71; data not shown). HIV Nef has been reported to induce expression of MIP-1 $\alpha$  and MIP-1 $\beta$  by HIV-1-infected macrophages (15). This induction can mediate the chemotaxis of lymphocytes (15), and presumably iDCs. Although HIV Nef-induced CC chemokine production may be responsible for some CCR5-mediated chemotaxis in our system, we believe this effect to be insignificant compared with the direct effects of gp120 for several reasons: (a) an examination of several viral isolates indicates that the dominant chemotactic element exhibits M-tropism (R5) specificity; (b) addition of 10  $\mu\text{g}/\text{ml}$  soluble CD4, which enhances the



**Figure 2.** HIV envelope antibodies reduce the chemotaxis induced by M-tropic HIV. Immunoprecipitation (IP) of HIV-1<sub>BaL</sub> supernatant reduces the induced chemotaxis to the level of HIV-1<sub>IIIb</sub> supernatant. Similar immunoprecipitation of MIP-1 $\beta$  in RPMI resulted in only a 14% decrease in overall chemotaxis, controlling for general negative effects of either the antibodies or protein A sepharose. SEM is shown for three transwells.

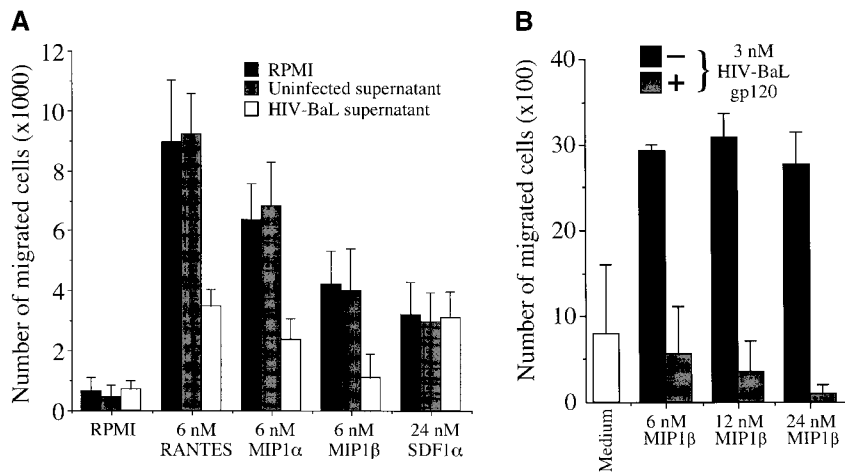


**Figure 3.** iDCs migrate toward recombinant M-tropic but not T-tropic gp120. (A) Chemotaxis of iDCs as described in the legend to Fig. 1 toward baculovirus expressed recombinant M-tropic gp120 from HIV-1<sub>BaL</sub> ( $n = 3$ ). Migration toward HIV-1<sub>BaL</sub> gp120 was observed in 12 experiments. (B) Chemotaxis of iDCs as described in the legend to Fig. 1 toward baculovirus-expressed recombinant M-tropic gp120 from HIV-1<sub>ADA</sub> ( $n = 3$ ). Data is representative of four experiments. (C) Chemotaxis of iDCs as described in the legend to Fig. 1 toward baculovirus-expressed recombinant T-tropic gp120 from HIV-1<sub>IIIIB</sub> gp120 (available from the AIDS Reagent Project, Medical Research Council, UK). Data is representative of three experiments. Binding of gp120s to CD4 was confirmed by surface plasmon resonance (BIAcore). All graphs show SD from the mean for three transwells. Similar results were observed for gp120 from the T-tropic strains SF2, MN, W61D, and HXB2. (D) Addition of 1  $\mu$ g of anti-CCR5 mAb 2D7 (BD Pharmingen) or 45531.111 (R&D Systems) to the transwell insert inhibits migration toward recombinant HIV-1<sub>BaL</sub> gp120. Graph shows the SD from the mean for three replicate transwells. Results are representative of four experiments.

binding of CCR5 to gp120 (10), doubled chemotaxis toward M-tropic viral supernatants without affecting chemotaxis toward CC chemokines (data not shown); (c) the difference in chemotaxis induced by supernatant from uninfected cells and cells infected with T-tropic viruses, including HIV-1<sub>IIIIB</sub> that bears Nef, is negligible (data not shown); (d) immunoprecipitation with anti-HIV envelope antibodies removes the majority of chemotactic activity and reduces it to the level induced by HIV-1<sub>IIIIB</sub> (nef<sup>+</sup>) supernatant; (e) we show the dominant chemotactic agent to be >100 kD; and (f) as recombinant gp120 is active in the nM region (Fig. 3), there is sufficient envelope protein in the viral supernatants to account for all the observed chemotaxis. Supernatants were produced in T cell cultures at  $2\text{--}3 \times 10^6$  cells/ml. Each cell need only produce a total  $10^3$  virions during the several days of infection to put the gp120 concentration (assuming >200 gp120s/virion) in the nM range. Detailed studies of supernatants from HIV-1<sub>HXB3</sub>-infected T cell cultures are relevant (16, 17). It was shown that viral supernatants contained  $10^9\text{--}10^{10}$  physical particles/ml. In freshly isolated viral stocks, the ratio of infectious to noninfectious viral particles ranged from  $1:10^4\text{--}1:10^7$  (16). HIV-1<sub>HXB3</sub> virions spontaneously shed gp120

proteins with a half-life of  $\sim 30$  h (16), and typical viral stocks were found to have >0.5 nM soluble gp120 in addition to virus-associated protein (17). An envelope particle, consisting of a single envelope spike, contains three gp120s. The avidity of such a trimeric interaction with CCR5 is likely to be considerably more than the sum of the individual affinities. In addition, observations in other systems indicate that higher multimerization of a soluble ligand can yield a significantly greater intracellular signal (18). Thus, virions/virion fragments in the supernatants may induce more chemotaxis than the sum of migration induced by their individual component gp120 molecules.

iDCs express CCR5 and migrate toward the CC chemokines RANTES, MIP-1 $\alpha$ , and MIP-1 $\beta$  (7). Preformed stores of RANTES (19) are released by CTLs when they encounter antigen. Activated CTLs also transcriptionally upregulate MIP-1 $\alpha$  and MIP-1 $\beta$  (20). As CTLs are the only cells capable of recognizing MHC I-restricted antigen, this chemokine secretion might facilitate the recruitment of bone marrow-derived APCs, including DCs, into infected areas (21). M-tropic gp120 is also a ligand for CCR5 and can generate an intracellular signal similar to that generated by natural ligands (11, 12). Thus, HIV-1 may exploit the



**Figure 4.** Exposure of iDCs to M-tropic HIV or its recombinant envelope protein inhibits subsequent chemotaxis toward CCR5 ligands but not toward the CXCR4 ligand SDF-1 $\alpha$ . (A) iDCs were incubated for 90 min at 37°C in RPMI medium alone, or in supernatant from uninfected PM1 cells or supernatant from PM1 cells infected with HIV-1<sub>BaL</sub> before use in bare transwell assays, as described in the legend to Fig. 1. Graph shows SD from the mean for six transwells each. (B) Addition of 3 nM M-tropic HIV<sub>BaL</sub> gp120 (described in the legend to Fig. 3 A) to the transwell insert during a transwell assay (as described in the legend to Fig. 1) inhibits chemotaxis of iDCs toward the CCR5 ligand MIP-1 $\beta$ . SD from the mean is shown for three transwells. Data is representative of three separate experiments.

migration of iDCs toward CCR5 ligands, and subsequently to the T cell areas of draining lymph nodes, to aid both the establishment and dissemination of infection.

HIV-1 is almost entirely M-tropic during primary infection (22, 23), and the use of coreceptors other than CCR5 by such viruses is rare (24). The iDCs of the skin and mucosa, LCs, have been implicated as the first targets for HIV after sexual contact (1). In the simian immunodeficiency virus/rhesus macaque system, the model which most closely resembles HIV-1 infection in humans, the first cellular targets of infection after vaginal challenge are mucosal DCs (2). The subsequent appearance of antigen-bearing cells within the T cell areas of draining lymph nodes (2) has led to the suggestion that DCs may act as “Trojan Horses” (3), carrying virus to lymph nodes. Freshly isolated epidermal LCs express CCR5 but not CXCR4 on their surface and fuse with M-tropic but not T-tropic HIV envelopes (25). If LCs are the first targets of sexually transmitted infection, this may explain why transmission is predominantly restricted to M-tropic strains (22, 23) and why the resistance to infection is conferred by CCR5 deletion (26, 27).

The recruitment of iDCs along virion gradients toward infected helper T cells, and vice versa (12, 28, 29), is likely to lead to the active formation of DC–T cell syncytia. Such syncytia have been documented in vivo (30) and contribute to loss of helper T cells (31). Efficient production of HIV-1 requires the synergistic action of the transcription factors Sp1 and nuclear factor (NF)- $\kappa$ B to drive the HIV-1 promoter (32). Unstimulated T cells express Sp1 but little NF- $\kappa$ B (33), and so T cell cultures require stimulation by antigen or mitogen to support HIV replication efficiently. In contrast, DCs constitutively express high levels of all known NF- $\kappa$ B and Rel proteins, but not Sp1 (33). As a result, DC–T cell fusion brings together the factors required for an upregulation of viral transcription, thus transforming these syncytia into explosive factories for HIV virion production. DC–T cell syncytia do not require activation for efficient virion production (31, 33), and may constitute the principle source of HIV virions in vivo.

Recent observations showing that a DC surface protein, DC-specific ICAM3-grabbing nonintegrin (DC-SIGN),

can specifically capture HIV-1 and promote infection in trans (34) are very relevant to the implications of our observations. DC-SIGN, a type II membrane protein with an external mannose-binding, C-type lectin domain, is exclusively and abundantly expressed on the surface of both mature and immature DCs (35). DC-SIGN is known to bind to HIV-1 envelope (36). Geijtenbeek et al. (34) have recently demonstrated that DC-SIGN-expressing cells can retain attached virions in an infectious state for several days and transmit them to replication-permissive T cells (34). Indeed, at low virus titer, infection of CD4/CCR5-expressing cells was not detected without the help of DC-SIGN in trans (34). Sexual transmission of HIV-1 presumably requires a means for small amounts of virus at mucosal sites of inoculation to gain access to cells that are permissive for viral infection. The modulation of iDC chemotaxis by R5 strains of HIV-1 may enhance the binding of virus to DC-SIGN in vivo. HIV-1 may then exploit the subsequent migration of DCs as they mature to gain access to the T cell compartment in lymphoid tissues, where it can replicate and spread freely.

In summary, the recruitment of iDCs toward M-tropic HIV-1 is likely to assist the establishment of infection by either increasing the productive infection of iDCs or by promoting the binding of virions to DC-SIGN. The inhibition of chemotaxis induced by preexposure to M-tropic HIV-1 (Fig. 4) may result in local arrest of iDCs in vivo. The proficiency of DCs in recruiting numerous T cells in lymphoid tissues (4) makes them strong candidates for further dissemination of the virus (5). Indeed, the preferential recruitment and infection of HIV-specific Th cells by antigen-bearing DCs may be instrumental in the preferential loss of these cells in most patients during primary infection (37, 38). Thus, the recruitment of iDCs by M-tropic HIV-1 is likely to promote the establishment, dissemination, and maintenance of HIV-1 infection.

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