Journal of NeuroVirology, 8: 599–610, 2002
 ISSN 1355–0284/02 $12.00+.00
DOI: 10.1080/13550280290100923

Taylor & Francis
Taylor & Francis Group

The macrophage response to HIV-1: Intracellular control of X4 virus replication accompanied by activation of chemokine and cytokine synthesis

Iqbal H Chowdhury, Galina Bentsman, Wonkyu Choe, Mary Jane Potash, and David J Volsky

Molecular Virology Division, St. Luke's–Roosevelt Hospital Center, College of Physicians and Surgeons, Columbia University, New York, New York, USA

During human immunodeficiency virus (HIV)-1 infection, T lymphocytes and macrophages play dual roles. They are the primary targets for virus replication, but they are also primary effector cells in acquired and innate immunity, respectively. The authors are now investigating how these roles come together in the response of human monocyte-derived macrophages (MDM) to certain HIV-1. The authors and others have previously shown that MDM permit entry of some X4 virus strains, but control viral replication intracellularly. In the present study, viral DNA synthesis, entry into the nucleus, and transcription to RNA were all observed in X4 virus–infected MDM. MDM arrested HIV-1 replication prior to expression of mature capsid antigen p24 and production of cell-free infectious viral particles. Cell-associated transmissible HIV-1 was detected by cocultivation of infected MDM and susceptible T lymphocytes. A second protective response of MDM to specific R5 as well as X4 HIV-1 was identified in rapid and extensive secretion of tumor necrosis factor-β, macrophage inflammatory protein-1β, and RANTES. These findings support the view that MDM act aggressively to control HIV-1 replication: X4 strains by severely limiting the progeny virus production and R5 strains by producing β-chemokines competent to block virus entry into target cells. Optimizing these innate immune responses offers another means to control HIV-1 infection in the human host. *Journal of NeuroVirology* (2002) **8,** 599–610.

Keywords: AIDS pathogenesis; antiviral immunity; host range; macrophage

Introduction

Macrophages, unlike CD4-bearing T lymphocytes, control the outcome of their human immunodeficiency virus (HIV)-1 infection. T cells are susceptible to all known HIV-1 strains, but macrophages resist productive infection by certain HIV-1. Such host range can be determined at the level of surface receptors for virus (Weiss, 1982). Both T lymphocytes and macrophages carry CD4, the major receptor for HIV-1 (Capon and Ward, 1991). Two principal coreceptors for HIV-1 have also been identified. CCR5, a μ-chemokine receptor, is required by cells for replication of R5 virus strains previously known as macrophage-tropic, non–syncytium-inducing, or slow-low (Alkhatib *et al*, 1996; Choe *et al*, 1996; Deng *et al*, 1996; Dragic *et al*, 1996). CXCR4, an μ-chemokine receptor, is required for infection by lymphotropic, syncytium-inducing, or rapid-high HIV-1, now termed X4 (Feng *et al*, 1996). Many primary strains of HIV-1 can use more than one coreceptor (Deng *et al*, 1996; Bjorndal *et al*, 1997; Bleul *et al*, 1997; Bazan *et al*, 1998; Zhang *et al*, 1998). In early studies, viral host range in many HIV-1 clones was shown to map to the envelope glycoprotein that mediates entry into cells (O'Brien *et al*, 1990; York-Higgins *et al*, 1990; Shioda *et al*, 1991). These observations suggest that the susceptibility of human cells

Address correspondence to Mary Jane Potash, Molecular Virology Division, St. Luke's–Roosevelt Hospital Center, 432 West 58th Street, New York, NY 10019, USA. E-mail: mjp6@columbia.edu

The authors thank Ilene Totillo for help in preparing the manuscript. This work was supported by Public Health Service grants HL-43629 from the National Heart, Lung, and Blood Institute and NS-31492 and NS-39191 from the National Institute for Neurological Disorders and Stroke.

Received 22 January 2002; revised 1 April 2002; accepted 24 May 2002.

to HIV-1 is determined at the stage of virus entry, depending upon the presence or absence of a required viral receptor (Alkhatib *et al*, 1996; Deng *et al*, 1996).

This prediction was confirmed with regard to the pivotal role played by CCR5 in the replication of R5 HIV-1. Many transformed human cells lack CCR5, but once CCR5 is expressed via transfection, they acquire susceptibility to R5 virus replication (Choe *et al*, 1996; Deng *et al*, 1996; Dragic *et al*, 1996; Cheng-Mayer *et al*, 1997). Primary T lymphocytes and macrophages both display CCR5, in part as a function of differentiation (Bleul *et al*, 1997; Naif *et al*, 1998), consistent with their susceptibility to R5 HIV-1. However, susceptibility to X4 HIV-1 does not follow such a clear course. Even in the target cell lines specifically engineered to determine the correlation between receptor utilization and virus replication, there are discrepancies between efficient recognition of CXCR4 and virus expression (Cheng-Mayer *et al*, 1997). Such discrepancies are most apparent in the restricted infection of macrophages by X4 viruses, which were originally defined as incapable of replication in macrophages (O'Brien *et al*, 1990; York-Higgins *et al*, 1990; Shioda *et al*, 1991). Previously, we showed that X4 HIV-1 can bind and fuse with primary macrophages, but fail at later stages of infection (Potash *et al*, 1992). Corroboration was provided in reports that macrophage carry CXCR4 (McKnight *et al*, 1997; Zaitseva *et al*, 1997; Yi *et al*, 1998) and that CXCR4 and CD4 mediate fusion between primary macrophages and X4 HIV-1 (Bazan *et al*, 1998; Schmidtmayerova *et al*, 1998). Finally, primary X4 viruses have been identified that productively infect macrophages (Simmons *et al*, 1998), potentially as a result of a lower requirement for cell surface CXCR4 (Tokunaga *et al*, 2001), in contrast to the bulk of previous work (O'Brien *et al*, 1990; Shioda *et al*, 1991; Schmidtmayerova *et al*, 1992, 1998; Huang *et al*, 1993; Mori *et al*, 1993; Fouchier *et al*, 1994). We and other investigators reported viral DNA synthesis in X4 HIV-1 infected macrophages (Schmidtmayerova *et al*, 1992, 1998; Huang *et al*, 1993; Mori *et al*, 1993). Thus, macrophages impose restriction upon X4 virus replication intracellularly, implying a macrophage-specific cell function, different from receptor display, that arrests the HIV-1 life cycle.

In addition, binding of X4 HIV-1 and its envelope glycoprotein gp120 induces several different responses in macrophages, some of which may be protective. For example, laboratory-adapted X4 HIV-1 and their gp120 were shown to induce Ca^{2+} uptake (Liu *et al*, 2000), secretion of unidentified neurotoxins (Giulian *et al*, 1993), apoptosis (Zheng *et al*, 1999), and membrane tumor necrosis factor-μ (TNF-μ)–driven induction of apoptosis in neighboring cells (Herbein *et al*, 1998). One of the first studies in the area indicated that HIV-1 activation of macrophages to interleukin (IL)-1μ and TNF-μ secretion does not require virus replication (Merrill *et al*, 1989), a key attribute of the various X4 HIV-1 responses (O'Brien *et al*, 1990; Shioda *et al*, 1991; Schmidtmayerova *et al*, 1992, 1998; Huang *et al*, 1993; Mori *et al*, 1993; Fouchier *et al*, 1994). Our recent work demonstrated that macrophages respond to certain R5 and X4 HIV-1 by secretion of abundant TNF-μ and μ-chemokines (Choe *et al*, 2001), all of which are proposed to function during HIV-1 pathogenesis and control. Thus, macrophages engage in characteristic innate immune responses of secretion of proinflammatory cytokines TNF-μ and IL-1μ, as well as other physiologically relevant factors by exposure to HIV-1, independent of productive virus infection.

The present work had two goals. First, with the long-term goal of defining antiviral immunity to X4 HIV-1 in macrophages, we chose first to identify the site of the restriction in the HIV-1 life cycle. We adopted this course because of relative ease of conducting well-defined assays of viral DNA, RNA, and protein expression in contrast to a search within the rather large body of cellular antiviral elements. Second, turning to extracellular products of macrophages, we proposed to extend our recent study to more closely define the time course of chemokine and cytokine synthesis after macrophage activation by HIV-1. This study reports two distinct and powerful responses of macrophages with the potential to limit the spread of HIV-1 in the human host.

Results

Monocyte-derived macrophages resist productive infection by X4 HIV-1

We first used the levels of extracellular p24 to rank different X4 HIV-1 species during replication in monocyte-derived macrophages (MDM) for the productivity of infection. Six X4 viruses were tested for replication in MDM derived from 14 different donors (Table 1). The R5 recombinant virus, NLHXADA-GP (Westervelt *et al*, 1991), used as a positive control, productively infected MDM from all donors, yielding 25 to 130 ng p24 per ml culture supernatant. The six X4 viruses synthesized little p24, with the most productive, NDK and HTLV-III_B, reaching 2% to 9% the levels attained by NLHXADA-GP in the same batch of MDM, consistent with other reports (Collman *et al*, 1989; Ho *et al*, 1992; Hirsch *et al*, 1996). Thus, many X4 HIV-1 strains produce low levels of p24 in MDM, confirming that by this criterion they can be distinguished from R5 HIV-1 strains.

MDM permit viral DNA synthesis and nuclear transport during X4 HIV-1 infection

Our goal is to determine the stage in the viral life cycle at which MDM impose restriction upon X4 virus replication. We compared synthesis of strong stop DNA, the first product of reverse transcription, and *gag* region DNA in NDK- and

Table 1 Replication of X4 viral clones and strains in MDM determined by p24 expression

		X4					
Experiment	*R5 GP*	*Z6*	*PG*	*NL4-3*	*NIT-A*	*IIIB*	*NDK*
1	80,650	29	74	—	—	—	—
2	53,310	—	75	696	—	—	—
3	63,180	—	135	45	—	—	—
4	24,885	—	—	44	—	—	—
5	30,600	—	—	—	271	557	—
6	45,500	—	—	—	105	—	—
7	55,400	—	—	—	42	—	—
8	131,000	—	—	—	—	—	2265
9	48,300	—	—	—	—	—	1270
10	73,200	—	—	—	—	—	2085
11	46,200	—	—	17	1055	—	1945
12	46,000	—	—	—	217	4030	2890
13	55,000	—	—	—	—	—	863
14	48,300	—	—	—	—	—	1217

Note. MDM were infected with NLHXADA-GP at 0.25 to 0.5 pg p24 per cell and with various X4 strains at 1 pg p24 per cell and evaluated at the peak of GP infection (12 to 14 days) for p24 levels in culture supernatants.

NLHXADA-GP–infected MDM by quantitative polymerase chain reaction (PCR) (Figure 1). Note that lysates of NLHXADA-GP–infected cells were diluted when appropriate to generate signals that fall within the standard curve of amplification of 8E5 cell DNA; the dilution factor is indicated in Figure 1. The absence of detectable PCR products at 0 h indicates that there was no contaminating viral DNA present in viral stocks. Twenty-four hours after infection, the levels of strong stop and *gag* DNA were similar in NDK- and NLHXADA-GP–infected MDM, indicating similar efficiencies of viral entry and reverse transcription, which is consistent with the results of previous studies (Schmidtmayerova *et al*, 1992, 1998; Huang *et al*, 1993). Viral DNA copies increased in both populations of cells and the ratio of incomplete to completed DNA molecules was similar in both populations of cells, suggesting that the completion of reverse transcription of NDK and NLHXADA-GP were comparable. However, the rate of increase in DNA burden was lower in NDK- than in NLHXADA-GP–infected cells. These data rule out the possibility that viral entry leading to reverse transcription within 24 h is the major limiting factor in NDK infection of MDM. It should be noted that other X4 viruses, for instance NL4-3, failed to synthesize detectable DNA in infected macropahges (data not shown), consistent with a major block to replication at virus entry as previously described (Fouchier *et al*, 1994).

We repeated the infections and *gag* amplification and used densitometry to determine DNA copy number (Figure 2). The viral DNA burden increased in NLHXADA-GP–infected MDM, reaching about 1800 copies per 10,000 cells 18 days after infection. DNA burden increased in NDK-infected MDM more slowly, reaching a peak of about 300 copies per 10,000 cells at the end of observation. The difference between viral DNA burdens in cells infected by NDK and NLHXADA-GP was insufficient to account for the magnitude of differences in the production of p24 core antigen (Table 1). Therefore, we continued analysis of post–DNA synthesis phases during HIV-1 replication in MDM.

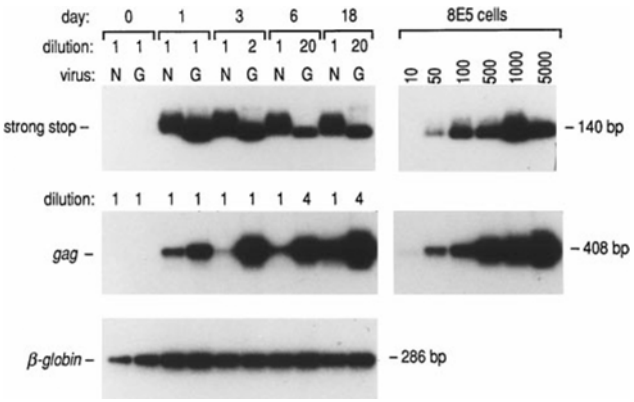

Figure 1 Forms of viral DNA present in MDM infected with R5 or X4 HIV-1. MDM were infected and at the indicated times after infection cells were harvested for amplification of viral DNA by PCR as described in Materials and Methods. The products of 10,000 cells or a 2-fold or 20-fold dilution of their product were electrophoresed. A standard curve of amplification of viral DNA in graded numbers of 8E5 cells was run in parallel for comparison. N indicates NDK-infected cells, G indicates NLHXADA-GP–infected cells.

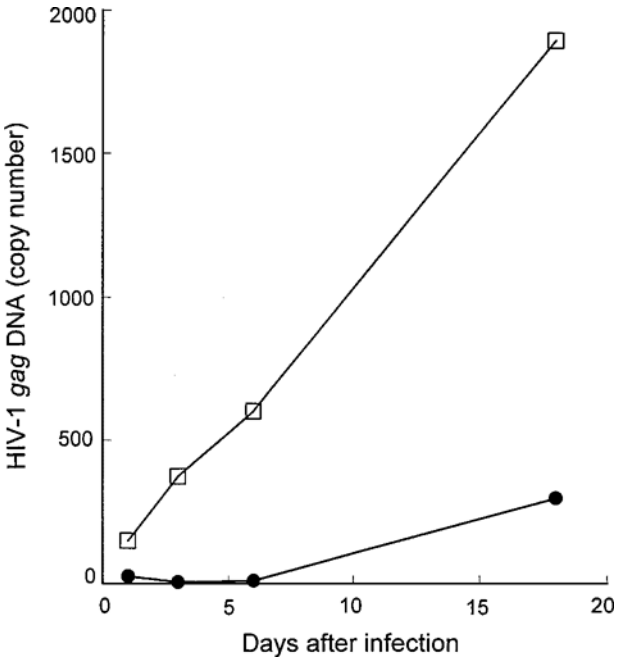

Figure 2 Viral DNA burden in MDM infected with R5 or X4 HIV-1. MDM were infected and viral DNA was analyzed as described in Materials and Methods. The signal from the autoradiogram was subjected to densitometry and the viral DNA copy per 10,000 cells was calculated. Open squares indicate PCR products present in NLHXADA-GP–infected cells, closed circles indicate products in NDK-infected cells.

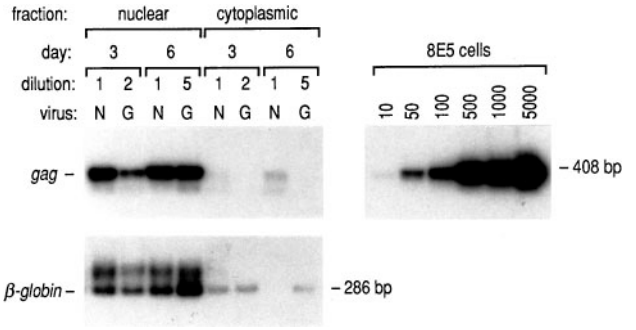

Figure 3 Viral DNA localization in MDM infected with R5 or X4 HIV-1. MDM were infected and 3 or 6 days after infection, as indicated, were harvested and fractionated into nuclear and cytoplasmic; lysates were standardized and were subjected to PCR amplification of HIV-1 *gag* DNA as described in Materials and Methods. N indicates NDK-infected cells, G indicates NLHXADA-GP–infected cells.

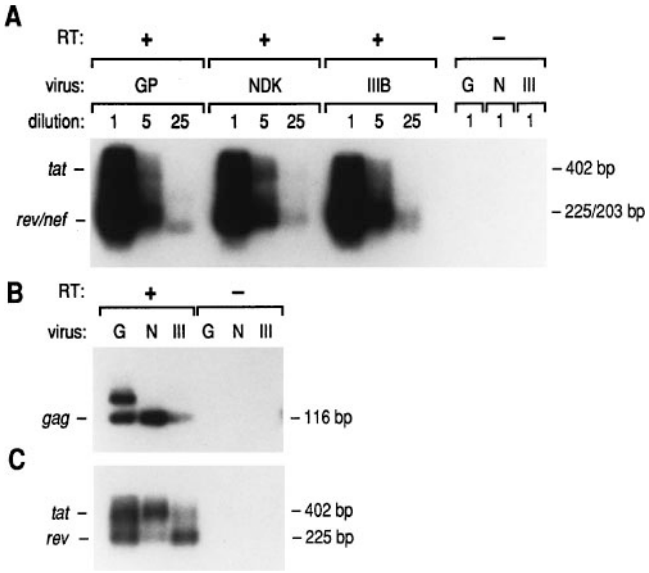

Figure 4 Forms of viral RNA present in MDM infected with R5 or X4 HIV-1. MDM were infected and RNA was isolated as described in Materials and Methods. RNA was incubated with (+) or without (○) reverse transcriptase for synthesis of cDNA and both pools were then subjected to amplification using the primers which amplify *tat, rev*, and *nef* doubly spliced mRNAs (panels **A** and **C**), or *gag* unspliced mRNA (panel **B**) as described in Klotman *et al* (1991). Cells from the same experiment were used for amplification shown in panels **B** and **C**.

To determine whether the block to X4 virus replication occurred during nuclear entry of viral DNA, we fractionated NDK- or NLHXADA-GP–infected MDM into nuclei and cytoplasm, and then performed PCR upon the isolated compartments (Figure 3). We used amplification of the chromosomal gene μ globin as a control for fractionation. Nuclear DNA was standardized in reference to the μ globin signal, and cytoplasmic fractions were diluted by the same factor, as indicated in Figure 3. The localization of viral DNA was similar in NLHXADA-GP– and NDK-infected MDM. In both infections, viral DNA was associated with the nuclear fraction by 3 days after infection, and almost absent from the cytoplasm at that time. The very low signals of μ globin DNA in cytoplasmic fractions indicated that they contained little nuclear DNA. Because viral DNA synthesis continued until 14 to 18 days after infection in other experiments (Figure 2), it is possible that some reverse transcription takes place in the nucleus. We conclude that nuclear entry of viral DNA is efficient in X4 HIV-1–infected MDM.

MDM permit transcription of X4 HIV-1

To determine whether the nuclear DNA of X4 HIV-1 is transcribed in MDM, we extracted total cellular RNA from infected MDM and subjected it to reverse transcriptase (RT)-PCR using primers to amplify doubly spliced or unspliced viral RNA (Klotman *et al*, 1991) (Figure 4). In this experiment we also included HTLV-III_B infected MDM, because they produced detectable p24 (Table 1) (Collman *et al*, 1989). Cell lysates were diluted prior to amplification to obtain signal within the standard curve to facilitate comparison of RNA levels among infected cells. MDM infected by NLHXADA-GP, NDK, or HTLV-III_B produced comparable levels of doubly spliced messenger RNA (Figure 4, panel **A**). That the amplified product arose from viral RNA and not DNA is indicated in the absence of product when reverse transcriptase was omitted. The experiment was repeated and PCR was conducted using primers for the *gag* region, which amplify unspliced or genomic RNA, as well as primers for doubly spliced RNA (Figure 4, panels **B** and **C**). All RNAs were detected in each infected MDM population. In this experiment, the levels of *tat* and *rev* mRNAs differed among the infected cells; however, this may be a result of MDM donor variation, because there were no pronounced quantitative differences in RNA levels in the experiment shown in panel **A**. Thus viral RNA can be synthesized in MDM infected by X4 HIV-1, confirming that X4 viral DNA can enter the nucleus and integrate in MDM.

MDM restrict the production of cell-free but not cell-associated progeny X4 HIV-1

To evaluate completion of the HIV-1 life cycle, we chose to measure the production of infectious progeny virus, which requires genomic RNA, the replicative enzymes, and processed envelope but may not require mature viral particles (McCune *et al*, 1988; Sato *et al*, 1992; Vogt, 1996). To be able to detect changes in titer of progeny virus with time, we cocultured MDM at different times after infection with autologous peripheral blood lymphocytes (PBL). After 6 h of culture, nonadherent PBL were collected and cultured separately from MDM. As shown in Figure 5, the levels of progeny virus, determined by transmission to PBL, rose over the course of MDM infection by NDK. Day 7 after infection, no progeny

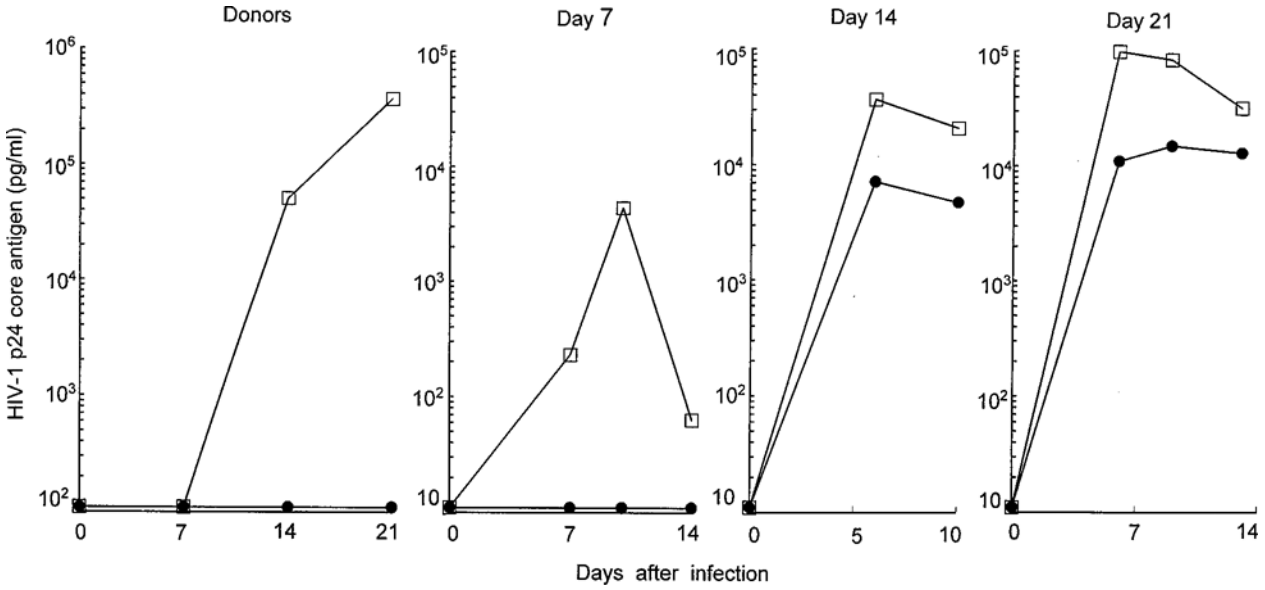

Figure 5 Kinetics of production of infectious progeny virus in MDM infected with R5 or X4 HIV-1. MDM were infected with 0.1 pg p24 ADA per cell or 2.0 pg p24 NDK per cell, and at 7, 14, and 21 days after infection, an equal number of autologous PBL were exposed to MDM for 6 h and then cultured separately. The levels of extracellular p24 produced by MDM (Donors) or by PBL from each infection was measured at the indicated time points. Open squares indicate ADA-infected cells, closed circles indicate NDK-infected cells.

virus was detectable, indicating that the NDK used to infect donor MDM was not carried over into the target PBL. However, on day 14 or 21 after their infection, NDK-infected cells produced infectious progeny within fivefold the level produced by ADA-infected MDM. Thus, HIV-1 produced by NDK-infected MDM was efficiently transmitted to T cells by cell-cell contact. In contrast, the amount of p24 in HIV-1 secreted by MDM infected with R5 or X4 virus differed significantly. Fourteen days after infection, ADA-infected MDM produced 51,000 pg p24 per ml and NDK-infected MDM produced 17 pg p24 per ml culture medium. These findings suggest that the amount of p24 secreted does not reflect the extent of transmissible HIV-1 produced by infected MDM. Knowing that virus transmissible by cell contact was synthesized by X4 HIV-1–infected MDM, we compared the production of cell-associated and cell-free progeny virus. PBL were infected for 6 h with supernatants of autologous macrophages standardized for p24 content or by coculture with equal numbers of ADA- or NDK-infected macrophages. Extracellular p24 was assayed in infected PBL as a measure of the virus transmitted (Figure 6). Both ADA- and NDK-infected MDM efficiently transmitted HIV-1 to PBL by transient coculture (Figure 6**B**). However, only ADA-infected MDM produced infectious virions; NDK secreted by infected MDM was not infectious (Figure 6**A**). Our results support the view that there are qualitative differences in the requirements for cell-free and cell-cell transmission of X4 HIV-1 from macrophages and the conclusion of other investigators that mature viral particles are dispensable for cell-cell transmission (Sato *et al*, 1992). These findings indicate that certain X4 HIV-1–infected MDM can produce infectious progeny virus capable of spreading to susceptible lymphocytes. Our findings also introduce a new cell-specific control of retroviral life cycle through which MDM restrict the production of infectious viral particles, although permitting synthesis of other viral products.

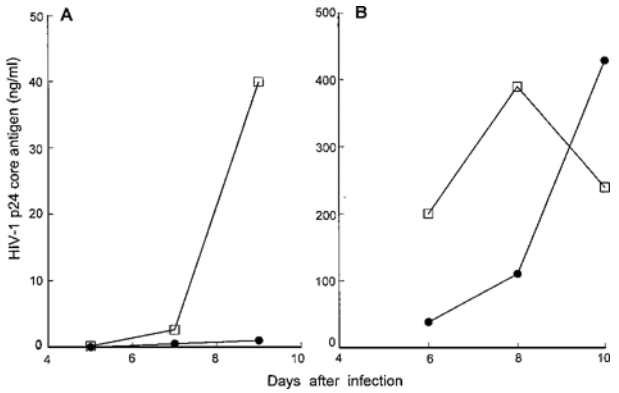

Figure 6 Comparison of infectivity of progeny of MDM infected with R5 or X4 HIV-1 by cell-free and cell-to-cell transmission. MDM were infected with 0.1 pg p24 ADA per cell or 2.0 pg p24 NDK per cell and 14 days after infection, extracellular virions were harvested, standardized by p24 content, and used to infect autologous PBL (panel **A**) or PBL were infected by 6-h coculture with infected MDM (panel **B**). HIV-1 expression by PBL was measured by the levels of extracellular p24 at the indicated time points. Open squares indicated ADA-infected cells, closed circles indicate NDK-infected cells.

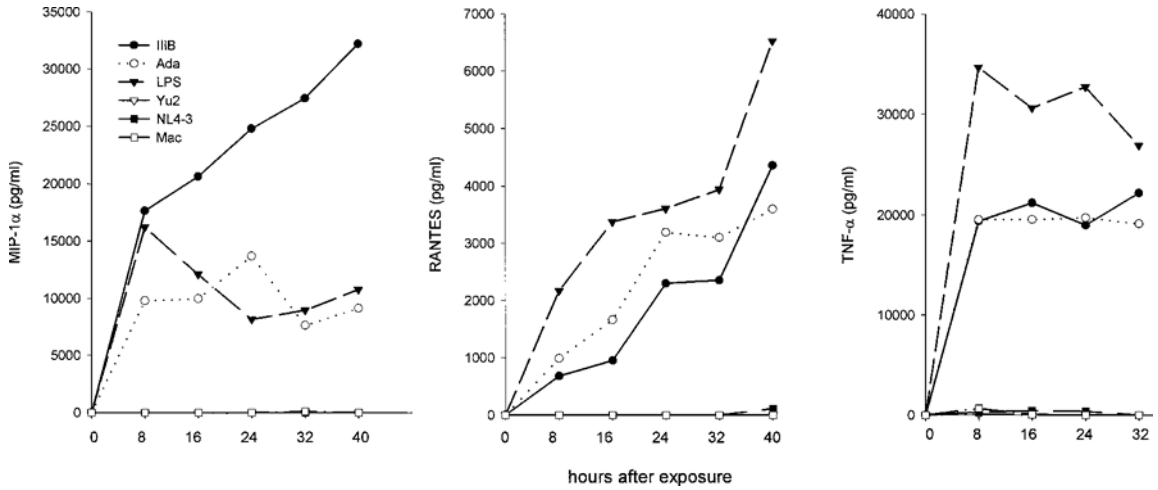

Figure 7 Time course of secretion of μ-chemokines and TNF-μ by MDM infected with R5 and X4 HIV-1. MDM were infected with the indicated HIV-1 species or were cultured with 1 μg per ml LPS and supernatants were collected at the indicated times for measurement of the levels of MIP-1μ, RANTES, and TNF-μ by ELISA.

X4 and R5 HIV-1 elicit the rapid production of μ-chemokines and TNF-μ

We have recently found that certain strains of HIV-1, independent of coreceptor phenoytpe or replication, induce MDM to secrete μ-chemokines and TNF-μ (Choe *et al*, 2001). Here, we tested four virus strains for the time course of induction of these factors as a first step to constructing a pathway of MDM activation (Figure 7). As we previously reported, ADA and HTLV-III$_B$ induced synthesis of macrophage inflammatory protein (MIP)-1μ, RANTES, and TNF-μ, but YU-2 and NL4-3 did not. We can exclude that differences in cells used to produce virus contribute to the effect in our previous observation that NLHXAD4-GP activates cells whereas YU-2 does not, although both are produced by transfection (Choe *et al*, 2001). Although each of these factors was induced by 8 h after exposure, the kinetics of their production differs. TNF-μ reached a very high peak at 8 h after ADA or HTLV-III$_B$ infection that was maintained over 24 h. By contrast, RANTES production increased over 40 h after infection. We were able to distinguish exposure to HTLV-III$_B$ from exposure to ADA in the kinetics of MIP-1μ synthesis. HTLV-III$_B$ induced continued synthesis over 40 h but ADA- or lipopolysaccharide (LPS)-induced synthesis reached a peak at 8 h after exposure. Similar results were obtained with different macrophage donors (data not shown). This difference may relate to affinity or occupancy of a receptor for HIV-1 that is unknown, but that is different from CD4, CCR5, or CXCR4 (Choe *et al*, 2001). Similarly, the specificity for sequences within the envelope required for this activation is not straightforward, appearing to consist of determinants both within and outside the V3 region, as we previously discussed (Choe *et al*, 2001).

Discussion

The results presented here demonstrate that MDM actively control HIV-1 replication at several levels. MDM arrested X4 HIV-1 infection prior to the production of mature p24 and transmissible virions. Certain X4 and R5 HIV-1 also induce innate immune responses by macrophages in synthesis of R5 virus, inhibiting μ-chemokines and the inflammatory cytokine TNF-μ (Choe *et al*, 2001). Such studies support the view that a global appreciation for HIV-1 replication must include awareness of the set of cellular functions modulated by virus exposure. Considering that a "smoldering" infection may persist despite apparently efficacious antiviral therapy (Fraser *et al*, 2001; Zhu *et al*, 2002), cellular responses to HIV-1 unlinked to high-level virus replication may have significant impacts upon health.

One important conclusion of our results is that the host cells may impose restriction on virus replication after entry into cells at stages involving maturation of structural proteins and virions. The recognition that the host cell can impose both early (at entry) and late (after proviral formation) restrictions on viral replication is consistent with more than 30 years of retroviral research (Weiss, 1982). In quantitative terms, R5 NLHXADA-GP–infected MDM synthesized sixfold more viral DNA than NDK-infected cells (Figure 2), but NLHXADA-GP–infected MDM synthesized up to 200-fold more p24 than NDK-infected cells (Table 1). This places a defect in the replication of these X4 HIV-1 strains in MDM after viral entry and viral DNA synthesis. Very similar results on NDK infection of MDM have been reported (Schmidtmayerova *et al*, 1998). A final subset of X4 HIV-1 that productively infects macrophages has also been identified (Simmons *et al*, 1998). This unique group broadens

the definition of tropism; but the efficiency of their infection obviates their use for investigation of the macrophage responses to nonproductive X4 HIV-1 infection.

We continued our comparison of R5 and X4 HIV-1 infection of MDM in evaluation of nuclear import of viral DNA, transcription of viral RNA, and the synthesis of progeny virus. Using cell fractionation, we demonstrated that X4 HIV-1 DNA can enter the macrophage nucleus. Schmidtmayerova *et al*. (1998), in similar experiments, found inefficient nuclear entry of viral DNA in NDK-infected MDM and attributed the absence of p24 to this block in replication. We interpret their data somewhat differently. They reported a 1000-fold difference in p24 production between MDM infected with R5 and with X4 HIV-1, consistent with our findings, and only a 10-fold difference between these cells in HIV-1 DNA localization in the nucleus (Schmidtmayerova *et al*, 1998). We suggest that their data support ours in identifying a step following nuclear entry of viral DNA that restricts X4 HIV-1 infection of MDM prior to p24 synthesis. From our studies, this block follows DNA integration and RNA synthesis. We found comparable levels of both doubly spliced and unspliced viral RNA in NDK-, HTLV-III_B–, and NLHXADA-GP–infected MDM (Figure 4). Because X4 HIV-1–infected macrophages produce viral RNA and some viral proteins, we speculated that they might also produce a p24-poor form of virus transmissible by cocultivation.

Previous studies have shown that cell-to-cell transmission of HIV-1 is extremely efficient and may dispense with the requirement for mature virions characteristic of cell-free virus transmission (Sato *et al*, 1992). Studies reported here confirmed this proposal: X4-infected MDM transmitted virus to lymphocytes by coculture (Figures 5 and 6). The increase in titer of transmissible progeny HIV-1 over the course of NDK infection of MDM is a clear indication of active synthesis of an infectious form of virus. However, it was necessary to compare the titer of cell-free and cell-associated forms of progeny virus to determine whether the minor component of p24 produced by X4 HIV-1–infected MDM accounted for viral transmission. If so, standardization of cell-free X4 and R5 virus by p24 content should also standardize virion infectivity. Because NDK-infected MDM produced very little p24, their supernatants were concentrated relative to those of ADA-infected MDM, but cocultures were carried out with equal numbers of NDK- and ADA-infected MDM. We found that cell-free NDK produced by MDM was not infectious, but that progeny virus from the same cells was infectious through coculture. We conclude that there are circumstances in which mature Gag protein is dispensable for HIV-1 transmission, such as in the instances shown here, during cell-cell transmission from infected MDM to T cells.

At this time, we can only speculate about the reason that X4 HIV-1 fails to produce p24 in MDM, despite the completion of preceeding steps in viral replication and its replication competence in T lymphocytes. It has been suggested that signals induced by HIV-1 envelope binding to a coreceptor determine the outcome of viral infection after entry (Littman, 1998). However, dual-tropic HIV-1 can employ CXCR4 on CCR5-negative MDM for entry and productive infection (Yi *et al*, 1998), indicating both that signals through CXCR4 do not obligatorily abort HIV-1 production and that signals through CCR5 are not necessary for productive MDM infection. Ligation of CCR5- and G_i protein–mediated signal transduction have been shown to enhance the replication of X4 HIV-1 in CD4-bearing T cells (Kinter *et al*, 1998), a clear demonstration that ligand binding of chemokine receptors can influence the efficiency of HIV-1 replication, independent of virus entry. Our observations and those of other investigators (Schmidtmayerova *et al*, 1992, 1998; Huang *et al*, 1993; Mori *et al*, 1993; Bazan *et al*, 1998; Yi *et al*, 1998) indicate that the function of the viral envelope glycoprotein in controlling host cell range of HIV-1 extends beyond interactions with receptors on cell surfaces to influence intracellular processes during viral replication. There are also precedents for induction of antiviral activity during nonproductive HIV-1 infection, such as X4 infection of MDM studied here. We have recently shown that infection of primary or transformed T lymphocytes by attenuated virus induced the synthesis of still unknown antiviral factors, which block HIV-1 transcription within the cell of origin as well as in by stander cells exposed to conditioned medium (Simm *et al*, 2000, 2002).

The majority of X4 viruses characterized do not productively infect macrophages, as determined by viral protein production. As shown here, such X4 HIV-1 can persist in MDM and can be transmitted to T cells during cell-cell contact. Our findings raise the possibility that macrophages in the human host similarly carry and transmit such X4 HIV-1. The finding of a key residue determining X4 utilization in viral envelope sequences from alveolar macrophages of HIV-1–infected persons supports this proposition (Itescu *et al*, 1994). More recently, an end-stage simian immunodeficiency virus (SIV) was described with properties of transmission very similar to those described for X4 HIV-1 here. SIV_{F359} can infect primary macrophages, although infected cells do not produce detectable viral core antigen (Holterman *et al*, 2001). Nonetheless SIV_{F359}-infected macrophages produce virus transmissible by cocultivation to primary lymphocytes as we observe for X4 HIV-1. It is tempting to suggest that X4 virus characteristic of disease can reside within a macrophage reservoir, producing transmissible virus. Macrophages thereby create a barrier to disease progression by blocking the production of infectious virions, but the barrier is imperfect because cell-to-cell transmission of virus is maintained. Cell-to-cell transmission of X4 HIV-1 from infected macrophages or microglial cells may

have particular significance in the context of HIV-1–associated dementia. Multinucleated giant cells, generally referred to as the hallmark of HIV-1 infection of the nervous system, consist of synctia of macrophages and/or microglial cells, presumably as a result of the display of fusogenic viral envelope on the cell surface permitting fusion with cells displaying viral receptors (Sharer, 1992). Because cell-to-cell transmission of HIV-1 requires surface deposition of viral envelope (Sato *et al*, 1992), our study indicates that X4 HIV-1–infected macrophages have the potential to contribute to the generation of such pathological cellular structures. Moreover, reports from many investigators indicate that X4 HIV-1 or gp120 is toxic to neurons (reviewed in Kaul *et al*, 2001). Our findings support the possibility that macrophages display X4 HIV-1 envelope, available to bind receptors on other cells, initiating this apoptotic cascade.

Many functional abnormalities have been attributed to HIV-1 infection of macrophages (Meltzer and Gendelman, 1992). It may now be appropriate to consider protective pathways initiated in macrophages by HIV-1 exposure or infection. Unlike infected T lymphocytes, macrophages infected by X4 HIV-1 fail to secrete infectious progeny virus and constitute a dead-end for virus propagation through cell-free HIV-1. However, such interaction is not neutral. Findings reported here and previously by ourselves and others indicate that certain X4 and R5 virus strains induce innate immune responses by macrophages (Merrill *et al*, 1989; Choe *et al*, 2001) and may block local productive infection of both lymphocytes and macrophages by R5 virus through secretion of μ-chemokines (Schmidtmayerova *et al*, 1996; Swingler *et al*, 1999; Choe *et al*, 2001; Fantuzzi *et al*, 2001). It is important to note that the same virus-inhibiting factors, MIP-1μ and MIP-1μ, were found in brain macrophages from HIV-1–infected patients with dementia, but not without dementia (Schmidtmayerova *et al*, 1996), suggesting that such responses accelerate, rather than diminish, disease progression. Another report indicates that patients with higher concentrations of MIP-1μ/μ and RANTES in cerebrospinal fluid experience less neurological impairment than those with lower levels of μ-chemokines (Letendre *et al*, 1999). Only further studies will determine the balance of protective versus destructive consequences of the interaction of HIV-1 with macrophages, an essential step for shifting this balance in favor of the HIV-1–infected human host.

Materials and methods

Cells

Human monocytes were isolated by elutriation from blood collected from healthy, HIV-1–negative donors and were cultured at 2–3 ∘ 10^6 cells per well in Dulbecco's modified Eagle's medium (DMEM) with 10% antibody-positive human serum and 10% giant cell tumor conditioned medium (Sigma, St. Louis, MO), antibiotics, and glutamine. Greater than 98% of cells were nonspecific esterase positive. Monocytes were allowed to adhere and differentiate to macrophages (MDM) for 5 to 7 days prior to infection or transfection. PBL were harvested from the same elutriations and were cultured in phytohemagglutinin and IL-2 (Biotest, Denville, NJ).

HIV-1 propagation and infection

The X4 molecular clones, HIV-1 pN1T-A (Sakai *et al*, 1988), pNDK (Ellrodt *et al*, 1984), pNL4-3 (Adachi *et al*, 1986), pNLHXADA-PG (Westervelt *et al*, 1991), pZ6 (Srinivasan *et al*, 1987), and strain HTLV-III_B (Popovic *et al*, 1984); R5 clones pNLHXADA-GP (Westervelt *et al*, 1991), and Yu-2 (Li *et al*, 1991), and R5 isolate ADA (Gendelman *et al*, 1988; Li *et al*, 1991) were used in this study. Virus stocks were prepared by calcium phosphate–mediated transfection of proviral DNA into RD cells, by chronic infection of H9 cells, or by passage in macrophages (Gendelman *et al*, 1988). Virus was harvested by low-speed centrifugation of cell supernatants. Viral particles were concentrated by centrifugation at 12,000 ∘ *g* and were quantified by the p24 enzyme-linked immunosorbent assay (ELISA) kit for p24, HIV Ag kit (Coulter, Hialeah, FL), and frozen in aliquots at ∘ 80°C until use. MDM were infected with 1 to 2 pg p24 per cell by X4 viruses and 0.1 to 0.5 pg p24 per cell by R5. Infection was monitored by measurement of p24 in cell supernatants, by analysis of viral DNA or RNA synthesis by PCR, or by virus transmission studies as described below. For experiments involving PCR, viral particles were collected by ultracentrifugation, filtered through 0.45-μm filters, and treated with type II bovine pancreatic DNase (Sigma) at 4000 U per ml in 40 mM Tris-HCl, 6 mM $MgCl_2$, and 2 mM $CaCl_2$ for 1 h at 37°C, prior to infection. Infections were performed by incubating virus with target cells for 2 h at 37°C; unfused virions were then washed off and cells were cultured under standard condition as described above.

PCR amplification of HIV-1 DNA and RNA

At the time points indicated below, MDM were harvested for PCR by gentle scraping and centrifugation at 200 ∘ *g*. After two washes in phosphate-buffered saline (PBS), dry cell pellets were frozen at ∘ 80°C until the completion of the experiment, at which time all samples were processed in parallel. Lysates were prepared by resuspending cell pellets in equal volumes of solution A (10 mM Tris, pH 8.3, 100 mM KCl, 2.5 mM $MgCl_2$) and solution B (10 mM Tris, pH 8.3, 2.5 mM $MgCl_2$, 1% Tween, 1% NP40, 60 μg proteinase K per ml) to a final concentration of 5000 cell equivalent per μl, followed by incubation at 55°C for 1 h and at 95°C for 15 min. PCR was performed under standard conditions (Inis *et al*, 1990) as we described previously (Sova and Volsky, 1993;

Chowdhury *et al*, 1996). In brief, 2 μl of the cell lysate (equivalent to 10,000 cells) were mixed in 25 μl buffer consisting of 10 mM Tris (pH 9.0), 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.2 to 0.6 mM each primers, and 2.5 U Taq polymerase (Perkin-Elmer-Cetus, Norwalk, CT). Amplification was performed for 30 to 40 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1 min, followed by extension at 72°C for 15 min. Cell equivalents in lysates were determined by amplification of the cellular μ globin gene as described (Bauer *et al*, 1991) and densitometry of the PCR products; cell lysates were accordingly normalized for amplification of HIV-1 DNA. Two sets of primer pairs were used for amplification of different regions of the HIV-1 genome, corresponding to incomplete (strong stop) and *gag* regions, respectively. Map positions are reported in reference to the NL4-3 genome. Primers M667 and AA55 (Zack *et al*, 1990) were used to amplify a region of 140 bp of strong stop DNA. HIV-1 DNA containing the *gag* region was detected using the primer pair RR1 (1312–1337) and RR2 (1695–1720), which produce a fragment of 408 bp. The radiolabeled probe for strong stop DNA was M669 (Zack *et al*, 1990) and for *gag* DNA was SK19 (Ou *et al*, 1988). Blotting, transfer, hybridization, and autoradiography were performed according to standard techniques (Ausubel *et al*, 1989). HIV-1 DNA was quantified by densitometry in reference to PCR products of a standard curve of graded numbers of 8E5 cells, which contain 1 copy of viral DNA per cell (Folks *et al*, 1986). To permit reliable quantitation of viral DNA in infection kinetics experiments, after initial amplification and gel analysis as described above, samples with signals above the linear range of the standard curve were diluted and reamplified along with other samples. The dilution factor is noted in figures. For analysis, HIV-1 RNA was isolated from HIV-1–infected MDM 14 days after infection using Trizol (GIBCO-BRL, Gaithersburg, MD) according to the manufacturers' instructions. cDNA synthesis was conducted using the Superscript RT kit (GIBCO-BRL) and the downstream primer SK39 (Ou *et al*, 1988), for the detection of unspliced RNA. *gag* region cDNA was amplified and detected as described above. The doubly spliced viral mRNAs were reverse transcribed using the downstream primer ART7, and cDNA was amplified using the sense primer US and the antisense primer ART-7 as described (Klotman *et al*, 1991). *tat, rev*, and *nef* products were detected by using ^{32}P-labeled probe S3, and *tat* and *rev* messages were detected using the probe S2 as described (Klotman *et al*, 1991). The RT-PCR product from 1 ○ 10^5 cells was loaded per lane.

Cell fractionation

MDM were harvested 3 to 6 days after infection for isolation of nuclear and cytoplasmic fractions by standard methods (Ausubel *et al*, 1989) for the analysis of compartmentalization of viral DNA. Briefly, MDM were washed three times with ice-cold PBS, resuspended in PBS containing 2 mM EDTA for 5 min, and were washed in PBS. The pellet was resuspended at 10^7 cells per ml in lysis buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl, 5 mM MgCl, and 0.5% NP40), mixed, and incubated on ice for 5 min. The cell lysate was centrifuged at 400 ○ *g* for 2 min at 4°C, the supernatant constituting the cytoplasmic fraction was collected, and the pellet constituting the nuclear fraction was washed twice in ice-cold PBS. The fractions were subjected to PCR for μ globin amplification and quantified by densitometry of the autoradiograms, nuclear fractions were standardized accordingly, and the cytoplasmic fractions were diluted similarly to the nuclear fractions. HIV-1 *gag* region DNA was amplified in these standardized fractions as described in the previous section.

Assays of virus transmission from infected MDM

The production of infectious progeny virus was tested by infection of primary autologous lymphocytes with supernatants of infected MDM or by cocultivation of MDM. To limit the exposure of target cells to infected MDM, day 7, 14, or 21 after infection, MDM supernatants were collected and mitogen-stimulated autologous PBL were added to wells containing MDM for 6 h; nonadherent PBL were collected and cultured separately. In parallel, virions in supernatants of infected MDM were collected by sedimentation, standardized by p24 content, and used for infection of PBL.

ELISA for MIP-1μ, RANTES, and TNF-μ

μ-chemokines MIP-1μ and RANTES and the cytokine TNF-μ were measured by using the Quantikine ELISA kit (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. Macrophage cultures in triplicate in 24-well plates were exposed to infectious species of HIV-1 and culture supernatants were sampled for ELISA assay. At each time point, 50% of the medium was harvested and replaced by fresh medium. Macrophages were stimulated with 1 μg/ml LPS (Sigma) to mimic the maximum immune stimulation.

References

Adachi A, Gendelman HE, Koenig S, Folks T, Willey R, Rabson A, Martin MA (1986). Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *J Virol* **59:** 284–291.

Alkhatib G, Combadiere C, Broder CC, Feng Y, Kennedy PE, Murphy PM, Berger EA (1996). CC CKR5: a RANTES, MIP-1μ, MIP-1μ receptor as a fusion cofactor for macrophage-tropic HIV-1. *Science* **272:** 1955–1958.

Ausubel F, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (1989). *Current protocols in molecular biology*. John Wiley & Sons: New York.

Bauer H, Ting Y, Grier C, Chambers J, Tashiro C, Chimera J, Reingold A, Manos M (1991). Genital human papilloma virus infection in female university students as determined by a PCR-based method. *JAMA* **265:** 472–477.

Bazan HA, Alkhatib G, Broder CC, Berger EA (1998). Patterns of CCR5, CXCR4, and CCR3 usuage by envelope glycoproteins from HIV-1 primary isolates. *J Virol* **72:** 4485–4491.

Bjorndal A, Deng H, Jansson M, Fiore JR, Colognesi C, Karlsson A, Albert J, Scarlatti G, Littman DR, Fenyo EM (1997). Coreceptor usage of primary HIV type 1 isolates varies according to biological phenotype. *J Virol* **71:** 7478–7487.

Bleul CC, Wu L, Hoxie JA, Springer TA, Mackay CR (1997). The HIV coreceptors CXCR4 and CCR5 are differentially expressed and regulated on human T lymphocytes. *Proc Natl Acad Sci USA* **94:** 1925–1930.

Capon DJ, Ward RHR (1991). The CD4-gp120 interaction and AIDS pathogenesis. *Annu Rev Immunol* **9:** 649–678.

Cheng-Mayer C, Liu R, Landau NR, Stamatatos L (1997). Macrophage tropism of HIV-1 and utilization of the CC-CKR5 coreceptor. *J Virol* **71:** 1657–1661.

Choe H, Farzan M, Sun Y, Sullivan N, Rollins B, Ponath PD, Wu L, Mackay CR, Larosa G, Newman W, Gerard N, Gerard C, Sodroski J (1996). The μ-chemokine receptors CCR3 and CCR5 facilitate infection by primary HIV-1 isolates. *Cell* **85:** 1135–1148.

Choe W, Volsky DJ, Potash MJ (2001). Induction of rapid and extensive μ-chemokine synthesis in macrophages by human immunodeficiency virus type 1 and gp120, independently of their coreceptor phenotype. *J Virol* **75:** 10738–10745.

Chowdhury IH, Chao W, Potash MJ, Sova P, Gendelman HE, Volsky DJ (1996). *vif* negative human immunodeficiency virus type 1 persistently replicates in primary macrophages, producing attenuated progeny virus. *J Virol* **70:** 5336–5345.

Collman RG, Hassan N, Walker R, Godfrey B, Cutilli J, Hastings J, Friedman H, Douglas S, Nathanson N (1989). Infection of monocyte-derived macrophages with human immunodeficiency virus type 1. *J Exp Med* **170:** 1149–1163.

Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR (1996). Identification of a major co-receptor for primary isolates of HIV-1. *Nature* **381:** 661–666.

Dragic T, Litwin V, Allaway GP, Martin SR, Huang Y, Nagashima KA, Cayabab C, Maddon PJ, Koup RA, Moore JP, Paxton WA (1996). HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature* **381:** 667–673.

Ellrodt A, Barré-Sinoussi F, Le Bras P, Nugeyre MT, Palazzo L, Rey F, Brun-Vezinet F, Rouzioux C, Segond P, Caquet R, Montagnier L, Chermann J-C (1984). Isolation of human T-lymphotropic retrovirus (LAV) from Zairian married couple, one with AIDS, one with prodromes. *Lancet* **1:** 1383–1385.

Fantuzzi L, Canini I, Belardelli F, Gessani S (2001). HIV-1 gp120 stimulates the production of b-chemokines in human peripheral blood monocytes through a CD4-independent mechanism. *J Immunol* **166:** 5381–5387.

Feng Y, Broder CC, Kennedy PE, Berger EA (1996). HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science* **272:** 872–877.

Folks TM, Powell D, Lightfoote M, Koenig S, Fauci AS, Benn S, Rabson A, Daugherty D, Gendelman HE, Hoggan MD, Venkatesan S, Martin MA (1986). Biological and biochemical characterization of a cloned Leu-3-cell surviving infection with the acquired immune deficiency syndrome retrovirus. *J Exp Med* **164:** 280–290.

Fouchier RAM, Brouwer M, Kootstra NA, Huisman HG, Schuitemaker H (1994). HIV-1 macrophage tropism is determined at multiple levels of the viral replication cycle. *J Clin Invest* **94:** 1806–1814.

Fraser C, Ferguson NM, Anderson RM (2001). Quantification of intrinsic residual viral replication in treated HIV-infected patients. *Proc Natl Acad Sci USA* **98:** 15167–15172.

Gendelman HE, Orenstein JM, Martin MA, Ferrua C, Mitra R, Phipps T, Wahl LA, Lane HC, Fauci AS, Burke DS, Skillman D, Meltzer MS (1988). Efficient isolation and propagation of human immunodeficiency virus on recombinant colony-stimulating factor-1 treated monocytes. *J Exp Med* **167:** 1428–1441.

Giulian D, Wendt E, Vaca K, Noonan CA (1993). The envelope glycoprotein of human immunodeficiency virus type 1 stimulates release of neurotoxins from monocytes. *Proc Natl Acad Sci USA* **90:** 2769–2773.

Herbein G, Mahlknecht U, Batliwalla F, Gregersen P, Pappas T, Butler J, O'Brien WA, Verdin E (1998). Apoptosis of CD8+ T cells is mediated by macrophages through interaction of HIV gp120 with chemokine receptor CXCR4. *Nature* **395:** 189–194.

Hirsch I, de Mareuil J, Salaun D, Chermann J-C (1996). Genetic control of infection of primary macrophages with T-cell tropic strains of HIV-1. *Virology* **219:** 257–261.

Ho W-Z, Lioy J, Song L, Cutilli J, Polin R, Douglas S (1992). Infection of cord blood monocyte-derived macrophages with human immunodeficiency virus type 1. *J Virol* **66:** 573–579.

Holterman L, Dubbes R, Mullins J, Learn G, Niphuis H, Koornstra W, Koopman G, Kuhn E-M, Wade-Evans A, Rosenwirth B, Haaijman J, Heeney J (2001). Characteristics of a pathogenic molecular clone of an end-stage serum-derived variant of simian immunodeficiency virus (SIV_{F359}). *J Virol* **75:** 9328–9338.

Huang Z-B, Potash MJ, Simm M, Shahabuddin M, Chao W, Gendelman HE, Eden E, Volsky DJ (1993). Infection of macrophages with lymphotropic human immunodeficiency virus type 1 can be arrested after viral DNA synthesis. *J Virol* **67:** 6893–6896.

Inis MA, Gelfand DH, Sninsky JJ, White TJ (1990) *PCR protocols*. Academic Press: San Diego.

Itescu S, Simonelli F, Winchester RJ, Ginsberg HS (1994). HIV-1 strain in the lungs of infected individuals evolve independently from those in peripheral blood and are highly conserved in the C-terminal region of the envelope V3 loop. *Proc Natl Acad Sci USA* **91:** 11378–11382.

Kaul M, Garden GA, Lipton SA (2001). Pathways to neuronal injury and apoptosis in HIV-associated dementia. *Nature* **410:** 988–994.

Kinter A, Catanzaro A, Monaco J, Ruiz M, Justement J, Moir S, Arthos J, Oliva A, Ehler L, Mizell S, Jackson R, Ostrowski M, Hoxie J, Offord R, Fauci AS (1998). CC-chemokines enhance the replication of T-tropic strains

of HIV-1 in CD4(+) T cells: role of signal transduction. *Proc Natl Acad Sci USA* **95:** 11880–11885.

Klotman EM, Kim S, Buchbinder A, DeRossi A, Baltimore D, Wong-Staal F (1991). Kinetics of expression of multiply spliced RNA in early human immunodeficiency virus type 1 infection of lymphocytes and monocytes. *Proc Natl Acad Sci USA* **88:** 5011–5015.

Letendre SL, Lanier ER, McCutchan JA (1999). Cerebrospinal fluid μ-chemokine concentrations in neurocognitively impaired individuals infected with human immunodeficiency virus type 1. *J Infect Dis* **1801:** 310–319.

Li Y, Kappes JC, Conway JA, Price RW, Shaw GM, Hahn BH (1991). Molecular characterization of human immunodeficiency virus type 1 cloned directly from uncultured brain tissue, identification of replication competent and defective viral genomes. *J Virol* **65:** 3973–3985.

Littman DR (1998). Chemokine receptors: keys to AIDS pathogenesis? *Cell* **93:** 677–680.

Liu Q-H, Williams DA, McManus C, Baribaud F, Doms RW, Schols D, De Clercq E, Kotlikoff MI, Collman RG, Freedman BD (2000). HIV-1 gp120 and chemokines activate ion channels in primary macrophages through CCR5 and CXCR4 stimulation. *Proc Natl Acad Sci USA* **97:** 4832–4837.

McCune JM, Rabin LB, Feinberg MB, Lieberman M, Kosek JC, Reyes GR, Weissman IL (1988). Endoproteolytic cleavage of gp160 is required for activation of HIV. *Cell* **53:** 55–67.

McKnight A, Wilkinson D, Simmons G, Talbot S, Picard L, Ahura M, Marsh M, Hoxie JA, Clapham PR (1997). Inhibition of HIV fusion by a monoclonal antibody to a coreceptor (CXCR4) is both cell type and virus strain dependent. *J Virol* **71:** 1692–1696.

Meltzer MS, Gendelman HE (1992). Mononuclear phagocytes as targets, tissue reservoirs, and immmunoregulatory cells in human immunodeficiency virus disease. *Curr Top Microbiol Immunol* **181:** 239–263.

Merrill JE, Koyanagi Y, Chen ISY (1989). Interleukin-1 and tumor necrosis factor μ can be induced from mononuclear phagocytes by human immunodeficiency virus type 1 binding to the CD4 receptor. *J Virol* **63:** 4404–4408.

Mori K, Ringler DJ, Desrosiers RC (1993). Restricted replication of simian immunodeficiency virus strain 239 in macrophages is determined by *env* but is not due to restricted entry. *J Virol* **67:** 2807–2814.

Naif HM, Li S, Alali M, Sloane A, Wu L, Kelly M, Lynch G, Lloyd A, Cunningham AL (1998). CCR5 expression correlates with susceptibility of maturing monocytes to human immunodeficiency virus type 1 infection. *J Virol* **72:** 830–836.

O'Brien WA, Koyanagi Y, Namazie A, Zhao J-Q, Diagne A, Idler K, Zack JA, Chen ISY (1990). HIV-1 tropism for mononuclear phagocytes can be determined by regions of gp120 outside the CD4-binding domain. *Nature* **348:** 69–73.

Ou C-Y, Kwok S, Mitchell S, Mack D, Sninsky J, Krebs J, Feorino P, Warfield D, Schocketman G (1988). DNA amplification for direct detection of HIV-1 in DNA of peripheral blood mononuclear cells. *Science* **239:** 295–297.

Popovic M, Sarangadharan MG, Read E, Gallo RC (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224:** 497–500.

Potash MJ, Zeira M, Huang Z-B, Pearce TE, Eden E, Gendelman HE, Volsky DJ (1992). Virus-cell membrane fusion does not predict efficient infection of alveolar macrophages by human immunodeficiency virus type 1 (HIV-1). *Virology* **188:** 864–868.

Sakai K, Dewhurst S, Ma XY, Volsky DJ (1988). Differences in cytopathogenicity and host cell range among infectious molecular clones of human immunodeficiency virus type 1 simultaneously isolated from an individual. *J Virol* **62:** 4078–4085.

Sato H, Orenstein J, Dimitrov D, Martin MA (1992). Cell-to-cell spread of HIV-1 occurs within minutes and may not involve participation of virus particles. *Virology* **186:** 712–724.

Schmidtmayerova H, Alfano M, Nuovo G, Bukrinsky M (1998). HIV-1 T-lymphotropic strains enter macrophages via a CD4- and CXCR4-mediated pathway: replication is restricted at a postentry level. *J Virol* **72:** 4633–4642.

Schmidtmayerova H, Bolmont C, Baghdiguian S, Hirsch I, Chermann J-C (1992). Distinctive pattern of infection and replication of HIV-1 strains in blood-derived macrophages. *Virology* **190:** 124–133.

Schmidtmayerova H, Nottet HSLM, Nuovo G, Raabe T, Flanagan CR, Dubrovsky L, Gendelman HE, Cerami A, Bukrinsky M, Sherry B (1996). Human immunodeficiency virus type 1 infection alters chemokine μ peptide expression in human monocytes: implications for recruitment of leukocytes into brain and lymph nodes. *Proc Natl Acad Sci USA* **93:** 700–704.

Sharer LR (1992). Pathology of HIV-1 infection of the central nervous system. A review. *J Neuropathol Exp Neurol* **51:** 3–11.

Shioda T, Levy JA, Cheng-Mayer C (1991). Macrophage and T cell-line tropisms of HIV-1 are determined by specific regions of the envelope gp120 gene. *Nature* **349:** 167–169.

Simm M, Miller LS, Durkin HG, Allen M, Chao W, Lesner A, Potash MJ, Volsky DJ (2002). Induction of secreted human immunodeficiency virus type 1 (HIV-1) resistance factors in CD4-positive T lymphocytes by attenuated HIV-1 infection. *Virology* **294:** 1–12.

Simm M, Pekarskaya O, Potash MJ, Volsky DJ (2000). Prolonged infection of peripheral blood lymphocytes by Vif-negative HIV type 1 induces resistance to productive HIV type 1 infection through soluble factors. *AIDS Res Hum Retrovir* **16:** 943–952.

Simmons G, Reeves JD, McKnight A, Dejucq N, Hibbitts S, Power CA, Aarons E, Schols D, De Clercq E, Proudfoot AEI, Clapham PR (1998). CXCR4 as a functional coreceptor for human immunodeficiency virus type 1 infection of primary macrophages. *J Virol* **72:** 8453–8457.

Sova P, Volsky DJ (1993). Efficiency of viral DNA synthesis during infection of permissive and nonpermissive cells with *vif*-negative human immunodeficiency virus type 1. *J Virol* **67:** 6322–6326.

Srinivasan A, Anand R, York D, Ranganathan P, Feorino P, Schochetman G, Curran J, Kalyanaraman VS, Luciw PA, Sanchez-Pescador R (1987). Molecular characterization of human immunodeficiency virus from Zaire: nucleotide sequence analysis identifies conserved and variable domains in the envelope gene. *Gene* **52:** 71–82.

Swingler S, Mann A, Jacque J, Brichacek B, Sasseville VG, Williams K, Lackner AA, Janoff EN, Wang R, Fisher D, Stevenson M (1999). HIV-1 Nef mediates lymphocyte chemotaxis and activation by infected macrophages. *Nat Med* **9:** 997–103.

Tokunaga K, Greenberg ML, Morse MA, Cumming RI, Lyerly HK, Cullen BR (2001). Molecular basis for cell tropism of CXCR4-dependent human immunodeficiency virus type 1 isolates. *J Virol* **75:** 6776–6785.

Vogt VM (1996). Proteolytic processing and particle maturation. In: *Current topics in microbiology and immunology.* Krausslich H-G (ed). Springer-Verlag: New York, pp 95–131.

Weiss R (1982). Experimental biology and assay of RNA tumor viruses. In: *RNA tumor viruses*. Weiss R, Teich N, Varmus H, Coffin J (eds). Cold Spring Harbor Press: Cold Spring Harbor, NY, pp 226–260.

Westervelt P, Gendelman HE, Ratner L (1991). Identification of a determinant within the human immunodeficiency virus 1 surface envelope glycoprotein critical for productive infection of primary monocytes. *Proc Natl Acad Sci USA* **88:** 3097–3101.

Yi Y, Rana S, Turner JD, Gaddis N, Collman RG (1998). CXCR-4 is expressed by primary macrophages and supports CCR5-independent infection by dual-tropic but not T-tropic isolates of human immunodeficiency virus type 1. *J Virol* **72:** 772–777.

York-Higgins D, Cheng-Mayer C, Bauer D, Levy J, Dina D (1990). HIV-1 cellular host range, replication, and cytopathicity are linked to the envelope region of the viral genome. *J Virol* **64:** 4016–4020.

Zack JA, Arrigo SJ, Weitsman SR, Go AS, Haislip A, Chen ISY (1990). HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61:** 213–222.

Zaitseva M, Blauvelt A, Lee S, Lapham CK, Klaus-Kovtun V, Mostowski H, Manischewitz J, Golding H (1997). Expression and function of CCR5 and CXCR4 on human Langerhans cells and macrophages: implications for HIV primary infection. *Nat Med* **3:** 1369–1375.

Zhang L, He T, Huang Y, Chen Z, Guo Y, Wu S, Kuntsman KJ, Brown CR, Phair JP, Neumann AU, Ho DD, Wolinsky SM (1998). Chemokine coreceptor usage by diverse primary isolates of human immunodeficiency virus type 1. *J Virol* **72:** 9307–9312.

Zheng J, Ghoparde A, Niemann D, Cotter RL, Thylin MR, Epstein L, Swartz JM, Shepard RB, Liu X, Nukuna A, Gendelman HE (1999). Lymphotropic virions affect chemokine receptor-mediated neural signaling and apoptosis: implications for human immunodeficiency virus type 1-associated dementia. *J Virol* **73:** 8256–8267.

Zhu T, Muthui D, Holte S, Nickle D, Feng F, Brodie S, Hwangbo Y, Mullins JI, Corey L (2002). Evidence for human immunodeficiency virus type 1 replication in vivo in $CD14^+$ monocytes and its potential role as a source of virus in patients on highly active antiretroviral therapy. *J Virol* **76:** 707–716.