Journal of NeuroVirology, 7: 155–162, 2001
 ISSN 1355–0284/01 $12.00+.00

Short Communication

CD4/CXCR4-independent infection of human astrocytes by a T-tropic strain of HIV-1

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HIV-1 establishes a low-level persistent infection in astrocytes. In this study, we studied the susceptibility of a human astrocyte cell line (SVG-A) to infection with luciferase expressing reporter viruses pseudotyped with envelopes derived from five isolates of HIV-1. SVG-A cells were susceptible to infection by a T-cell tropic isolate and the infection was both CD4 and CXCR4 independent. These data confirm the susceptibility of astrocytes to infection with T-tropic strains of HIV-1 and suggest a novel mechanism by which T-tropic strains of HIV can infect cells. *Journal of NeuroVirology* (2001) **7,** 155–162.

Introduction

Human immunodeficiency virus (HIV)-associated dementia affects approximately 20% of HIV-infected individuals (Lipton, 1994; McArthur *et al*, 1997). It is characterized by progressive cognitive and motor dysfunction (Atwood *et al*, 1993; Kolson *et al*, 1998). The neuropathology of HIV dementia includes diffuse damage to white matter, loss of synaptic density, vacuolization of dendritic processes, the appearance of multinucleated giant cells, and reactive astrogliosis (Epstein and Gendelman, 1993; Everall *et al*, 1991; Kolson *et al*, 1998).

In the CNS, microglial cells and perivascular macrophages are the predominant cell types productively infected by HIV-1 (Gabuzda *et al*, 1986; Wiley *et al*, 1986; Jordan *et al*, 1991). HIV proviral DNA as well as early and late viral gene products are readily detected within these cell types *in vivo* (Koenig *et al*, 1986; Wiley *et al*, 1986; An *et al*, 1999a). Infection of macrophages by HIV involves the cellular receptors CD4 and CCR5 (Alkhatib *et al*, 1996; Deng *et al*, 1996; Doranz *et al*, 1996). Microglia are also CD4-positive and have been shown to be highly susceptible to infection by macrophage tropic strains of HIV (Watkins *et al*, 1990; Jordan *et al*, 1991). Microglia express the β-chemokine receptors, CCR5 and CCR3. HIV infection of microglial cells can be inhibited by pretreatment with the ligands of these two coreceptors, RANTES and eotaxin respectively, implying that both CCR3 and CCR5 play a role in HIV infection of microglia *in vivo* (He *et al*, 1997; Shieh *et al*, 1998; Albright *et al*, 1999).

HIV infected astrocytes have also been detected in the CNS of HIV positive individuals (Conant *et al*, 1994; Nuovo *et al*, 1994; Tornatore *et al*, 1994a; Tornatore *et al*, 1994b; Ranki *et al*, 1995; Bagasra *et al*, 1996; An *et al*, 1999a; An *et al*, 1999b). Several groups have reported that primary astrocytes and astrocyte cell lines are susceptible to T-tropic strains of HIV (Tornatore *et al*, 1993; Tornatore *et al*, 1994a; Tornatore *et al*, 1994b; Nath *et al*, 1995; Keir *et al*, 1997; DiRienzo *et al*, 1998; Bencheikh *et al*, 1999; Hao and Lyman, 1999; Sabri *et al*, 1999). The majority of these studies documented infection based on the detection of low levels (picomoles) of viral p24 antigen in infected cultures.

In this report, we evaluated the susceptibility of a human fetal astrocyte cell line (SVG-A) to infection with luciferase expressing reporter viruses pseudotyped with envelopes from T-tropic, macrophage tropic, and primary brain derived isolates of HIV-1. This is a highly sensitive and accurate assay to study HIV infection that does not rely on detection of low levels of the viral capsid antigen p24. SVG-A cells were reproducibly infected with the NL4-3 T-tropic pseudotype in a CD4- and CXCR4-independent manner. These data suggest that T-tropic strains of HIV-1 infect astrocytes by a novel pathway. The elucidation

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Received 5 September 2000; revised 1 November 2000; accepted 27 November 2000

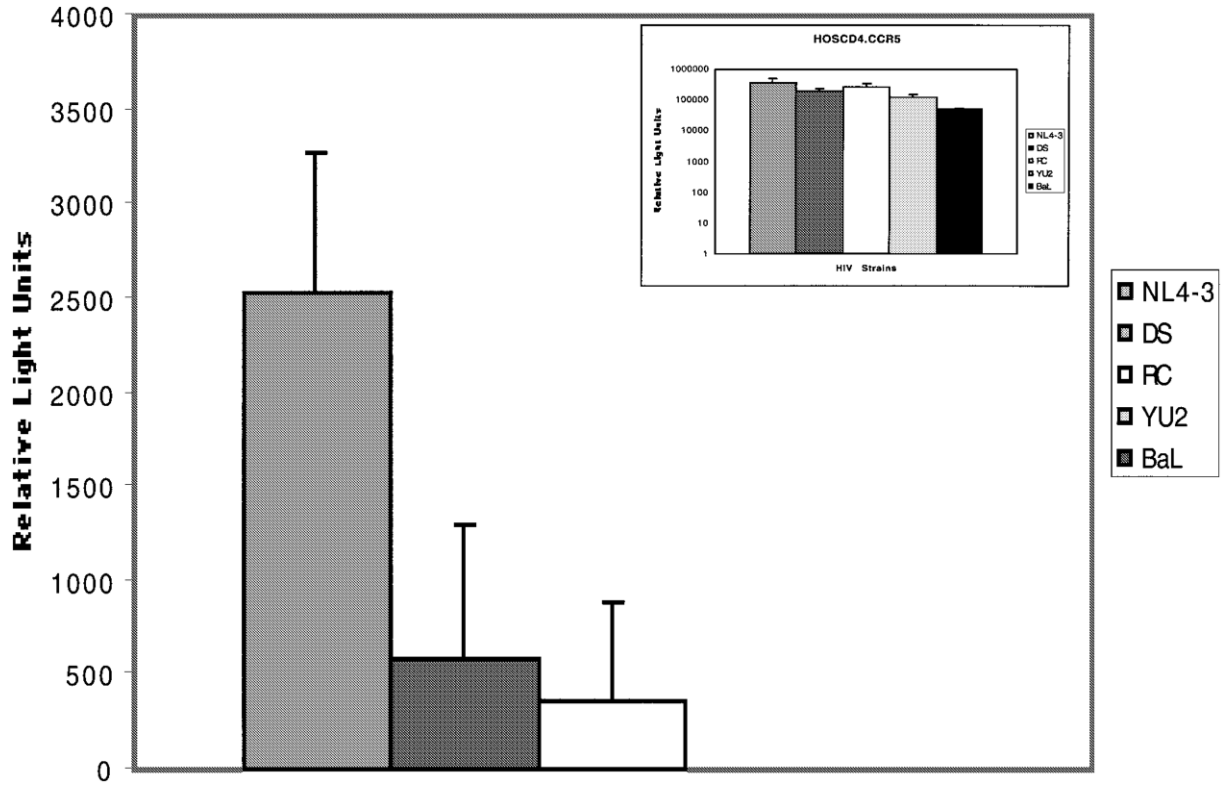


Figure 1 SVG-A cells are susceptible to infection by the T-tropic strain of HIV-1, NL4-3. SVG-A cells were cultured in a 96-well plate (2.5×10^4 cells per well) and incubated with 300 ng of each pseudotyped virus strain in the presence of 8 μg/ml polybrene. After a 16-h incubation at 37°C, the virus inoculum was removed and fresh media added. After an additional incubation of 72 h, cells were lysed and luciferase activity assayed in a luminometer. Luciferase activity is reported as relative light units per 10 s. Inset: HOSCD4.CCR5 cells were infected with 300 ng of each pseudotyped virus strain as a positive control. Error bars represent the standard deviation of triplicate samples.

of this pathway will broaden our understanding of the mechanisms by which T-tropic strains of HIV-1 can infect host cells.

SVG-A cells are susceptible to infection by NL4-3, a T-tropic strain of HIV-1 The SVG-A cell line was established by limited dilution cloning of the original SV40 transformed human fetal glial cell line, SVG. SVG-A cells maintain a glial cell phenotype as they are highly susceptible to the glial tropic virus, JCV (not shown). To investigate the susceptibility of these cells to infection with HIV-1, we used luciferase expressing reporter viruses pseudotyped with envelopes from T-tropic (NL4-3), macrophage tropic (BaL), and primary brain-derived isolates (YU2, DS-br, RC-br) of HIV-1. The pseudoptyped viruses were constructed by cotransfection of 293T cells with an HIV luciferase backbone plasmid (pNL4-3-LucR^+E^-) together with one of the following five envelope-expressing plasmids: NL4-3, DS-br, RC-br, YU2, or BaL. The virus-containing supernatants were harvested and virion content quantitated by p24 ELISA. SVG-A cells were then infected, in triplicate, with identical concentrations of each pseudotyped virus strain (300 ng of p24 units). Infection was detected by assaying for luciferase activity in cell lysates at 72 h postinfection. The cells were susceptible to infection by the NL4-3 T-tropic strain of HIV-1, yet resistant to significant levels of infection by all other virus strains tested (Figure 1). All of the pseudotyped viruses were capable of infecting HOSCD4.CCR5 positive control cells (Figure 1, inset). These cells were derived by transfection of a human osteosarcoma cell line with CD4 and CCR5. The cells also express endogenous levels of CXCR4 and are therefore susceptible to both macrophage tropic and T-cell tropic strains of HIV-1. Negative controls included cells infected with a no-envelope control virus, constructed by transfecting 293T cells with the backbone plasmid alone. The susceptibility of SVG-A cells to infection with the NL4-3 pseudotyped virus was both reproducible and dose dependent (Figure 2).

Functional expression of CXCR4 on SVG-A cells As SVG-A cells were susceptible to infection by a T-tropic strain of HIV-1, we hypothesized that the T-tropic coreceptor, CXCR4, may be involved in CD4-independent mechanisms of viral entry into these cells. To investigate this hypothesis, we first analyzed CXCR4 expression on the SVG-A cells by flow cytometry. The cells were incubated with 12G5, a mouse monoclonal antibody directed against the human CXCR4 receptor. Antibody binding was then detected using a FITC-conjugated goat anti-mouse

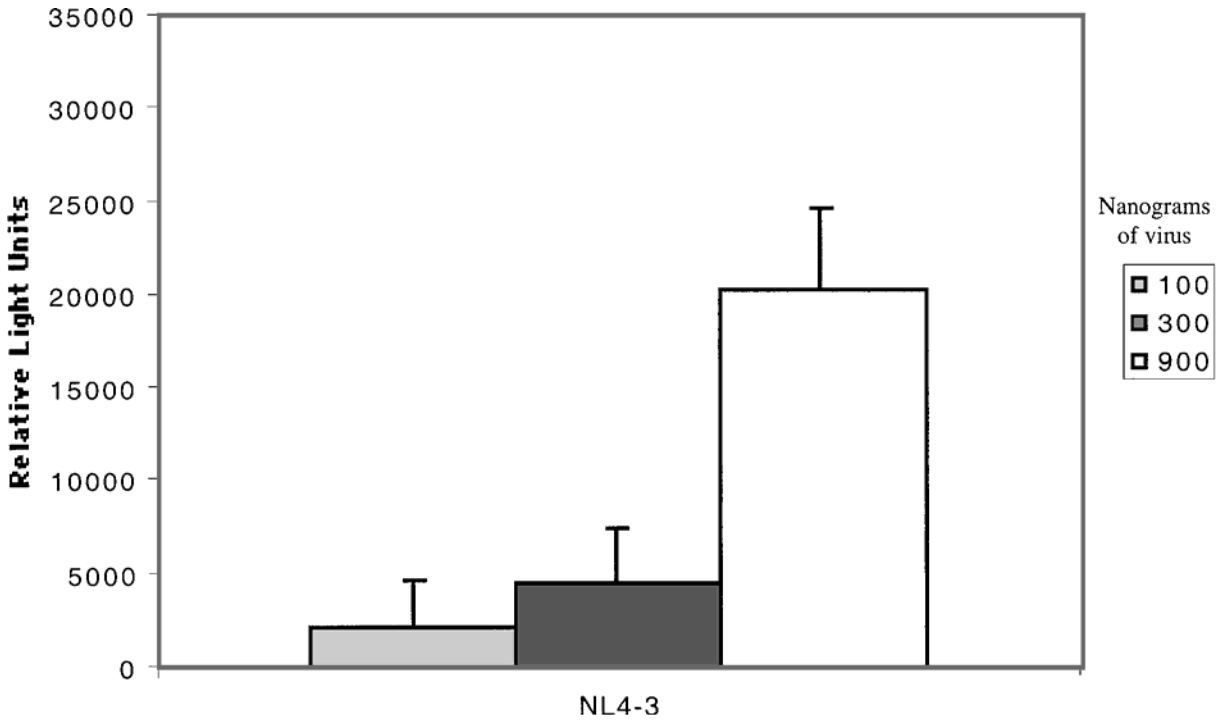


Figure 2 Infection of SVG-A cells with increasing concentrations of NL4-3. SVG-A cells were cultured in a 96-well plate and incubated with 100 ng, 300 ng, or 900 ng of NL4-3. At 72 h postinfection, cells were lysed and luciferase activity assayed. Luciferase activity is reported as relative light units per 10 s. Error bars represent the standard deviation of triplicate samples.

secondary antibody. SVG-A cells were found to express a low level of CXCR4 (Figure 3A, thick solid line). As a negative control, cells were incubated with an equivalent concentration of an irrelevant isotype-matched control antibody (Figure 3A, thin solid line).

We next examined CXCR4 mRNA expression in SVG-A cells by RT-PCR. Total RNA from SVG-A and Jurkat T cells was extracted and reverse transcribed. The resulting cDNA was amplified using oligonucleotide primers specific for CXCR4. After a single round of PCR, we detected a 619-bp CXCR4 specific band in the positive control cell type, Jurkat, however, no band was detected in SVG-A cells (Figure 3B). We next amplified the products from the first round of PCR with a set of nested oligonucleotide primers specific for CXCR4. Using nested PCR (N-PCR), we detected a 395-bp CXCR4 specific band in Jurkat and SVG-A cells (Figure 3B). As a negative control RT-PCR was performed in the absence of reverse transcriptase. These results are consistent with the low levels of CXCR4 expression detected on SVG-A cells by flow cytometry.

To determine whether the low level of CXCR4 on SVG-A cells was functional, we measured the ability of the natural ligand of CXCR4, SDF-1, to mobilize intracellular calcium. SVG-A cells were grown on coverslips, and then incubated with the intracellular calcium indicator, Fluo-3AM, which fluoresces if hydrolyzed and bound by calcium within the cell. The coverslips were adhered to the bottom of a perfusion chamber, which was then mounted onto a confocal microscope. SDF-1α or buffer alone was loaded onto the SVG-A cells, and a time series of fluorescent images of individual cells was acquired by confocal microscopy. A peak in fluorescence was observed in the SVG-A cells following treatment with SDF-1α, but not in response to buffer alone, signifying a signaling event in response to the ligand of CXCR4 (Figure 3C).

Does CXCR4 mediate CD4 independent infection of SVG-A cells by the NL4-3 pseudotyped reporter virus?

We took two approaches to evaluate the role of CXCR4 in mediating CD4 independent infection of SVG-A cells by NL4-3. In the first approach, we overexpressed CXCR4 in the SVG-A cell line (SVG-X4) and asked whether this would increase their susceptibility to infection by the NL4-3 pseudotyped virus. We included CD4 negative HeLa cells as a negative control. We also infected SVG cells expressing CD4 and basal levels of CXCR4 (SVG-CD4) and SVG-X4 cells expressing CD4 (SVGCD4-X4) (Figure 4A). SVG-X4 and SVGCD4-X4 cells expressed significantly higher levels of CXCR4 than the parental cell lines (Figure 4A). Note that HeLa cells also express higher levels of CXCR4 than either the SVG-A cells or the SVG-CD4 cells (Figure 4A). RT-PCR analysis confirmed that SVG-X4 and SVGCD4-X4 cells expressed increased amounts of CXCR4 (Figure 4B).

The susceptibility of each of these cell lines to infection by the NL4-3 pseudotyped virus was then investigated. Each cell type was infected with an

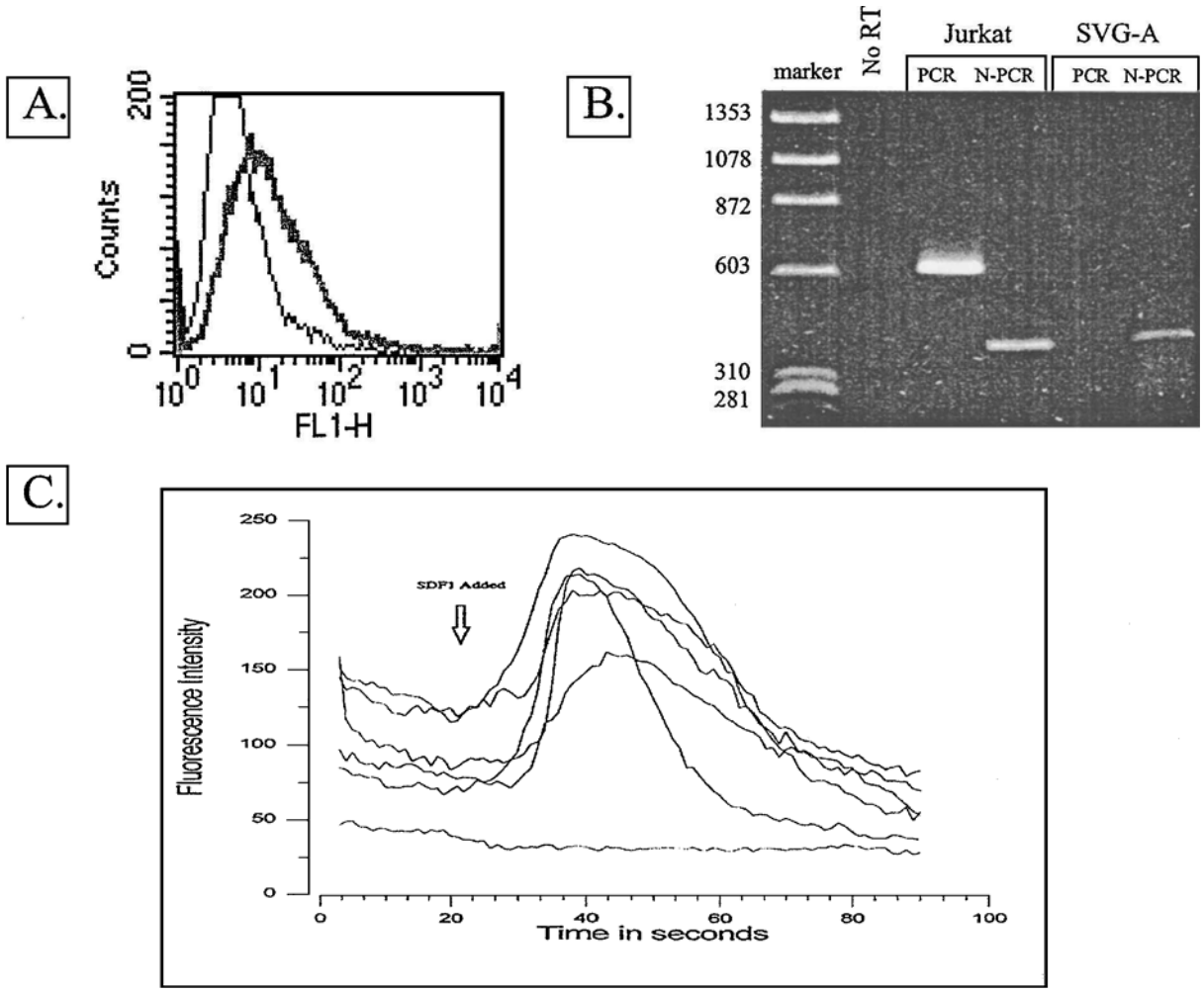
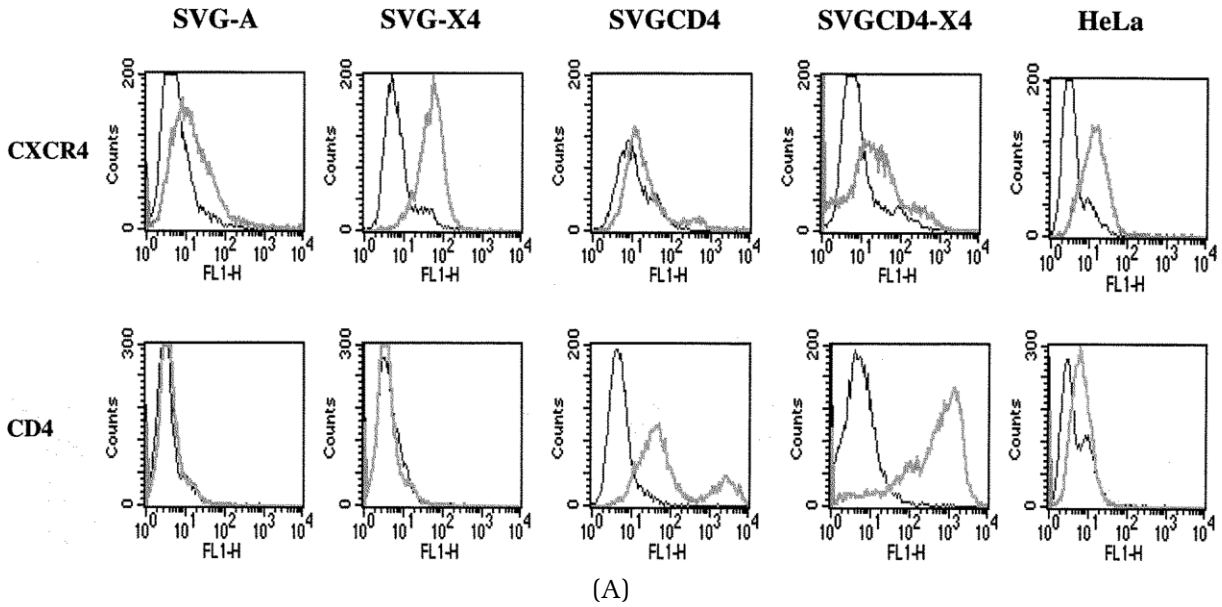


Figure 3 CXCR4 is a functional receptor on SVG-A cells. (**A**) SVG-A cells were incubated with 12G5, a monoclonal antibody directed against CXCR4. Specific antibody binding was detected with a FITC-labeled secondary antibody followed by flow cytometric analysis (thick solid line). Negative control: cells incubated with isotyped matched control antibody (thin solid line). (**B**) CXCR4 transcripts detected in SVG-A cells by nested RT-PCR. RT-PCR products were resolved on a 1% agarose gel and visualized by ethidium bromide staining. Molecular weight marker = ϕX174 DNA-Hae III digest. Positive control: CXCR4 transcript detected in Jurkat T cells. Negative control: reaction run in the absence of reverse transcriptase. (**C**) SVG-A cells were incubated with the intracellular calcium indicator, Fluo-3AM, which fluoresces if hydrolyzed and bound by calcium within a cell. The graph shows a peak in fluorescence in individual SVG-A cells over time following treatment with SDF-1. This result was not seen when buffer alone was applied to the samples or when SDF-1 was added a second time immediately following the initial stimulation.

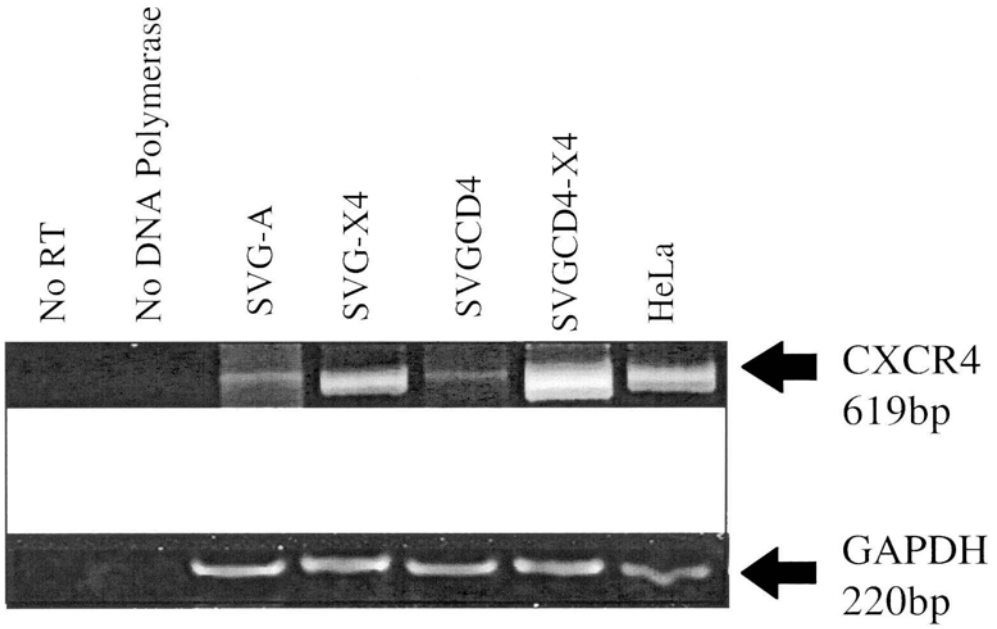
equivalent amount (300 ng/2.5×10^4 cells) of the NL4-3 pseudotyped virus. As expected, SVG-A cells were susceptible to infection with the NL4-3 pseudotyped virus (Figure 5). By comparison, HeLa cells, which express abundant levels of CXCR4 were refractory to infection with NL4-3 (Figure 5). Surprisingly, over-expression of CXCR4 in the SVG-A cells did not lead to increased susceptibility to infection by the NL4-3 pseudotyped virus (Figure 5). Also, SVG-CD4 cells, which express basal levels of CXCR4, were no more susceptible to infection with NL4-3 than were the parental SVG-A cells (Figure 5). In contrast, overexpression of the prototypical T-tropic receptors, CXCR4 and CD4 in SVG cells (SVGCD4-X4), led to a marked increase in the susceptibility of these cells to infection by NL4-3 (Figure 5).

We next asked whether the natural ligand of CXCR4, SDF-1, could inhibit infection of SVG-A cells by NL4-3. Treatment of SVG-A cells with SDF-1 had little to no effect on the ability of the NL4-3 pseudotype to infect these cells when compared to the control chemokine, eotaxin (Figure 6). SDF-1, however, did block infection of the SVG-CD4-X4 cells, but only to baseline levels (Figure 6). A control chemokine, eotaxin, had no effect on the ability of NL4-3 to infect either the SVG-A or the SVGCD4-X4 cells (Figure 6).

Several groups have presented data indicating that T-tropic strains of HIV-1 establish a low level, noncytopathic infection in primary cultures of human fetal glial cells (Tornatore *et al*, 1991; Tornatore *et al*, 1993; Tornatore *et al*, 1994b; Nath *et al*, 1995). In addition, the human glial cell line, SVG, has been



(A)



(B)

Figure 4 SVG-X4 and SVGCD4-X4 cells express significantly higher levels of CXCR4 than the parental cell lines. (**A**) Flow cytometric analysis of cell surface expression levels of CXCR4 and CD4. Cells were incubated with a monoclonal antibody directed against CXCR4 or CD4, followed by detection with a FITC-conjugated secondary antibody (thick line in each panel). Negative controls included cells incubated with isotype matched control antibody (thin line in each panel). HeLa cells served as a positive control for CXCR4 expression. (**B**) RT-PCR analysis of CXCR4 expression. CXCR4 transcripts were amplified from total RNA using CXCR4 specific primers. Equivalent amounts of GAPDH expression were detected in all cell types, serving as an internal gel loading control. Negative controls included a reaction run in the absence of reverse transcriptase and a reaction run in the absence of DNA polymerase.

reported to be susceptible to infection by a T-tropic strain of HIV-1 (Keir *et al*, 1997). The majority of these data have been based on detection of very low levels of the viral capsid protein, p24, in tissue culture supernatants. It is possible that the low levels of p24 detected in these experiments represent release of p24 from degraded input viral particles or from the death of cells that had phagocytosed virion particles into a noninfectious pathway. Also, many of these experiments have relied upon transfection rather than infection to study HIV gene expression in astrocytes. In this study, we evaluated the susceptibility of a human fetal astrocyte cell line, SVG-A, to infection with luciferase expressing reporter viruses

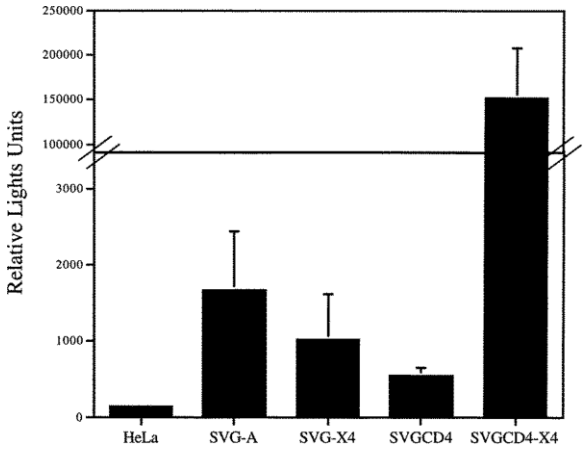


Figure 5 Increased CXCR4 expression on SVG-A cells did not result in increased susceptibility to NL4-3 infection in the absence of CD4. HeLa cells, which express abundant levels of CXCR4, were not susceptible to infection by the NL4-3 pseudotyped virus. Each cell type was incubated with 300 ng of NL4-3. At 72 h postinfection, cells were lysed and luciferase activity assayed. Luciferase activity is reported as relative light units per 10 s. Each experiment was performed in triplicate. The mean and standard deviation of three independent experiments are plotted.

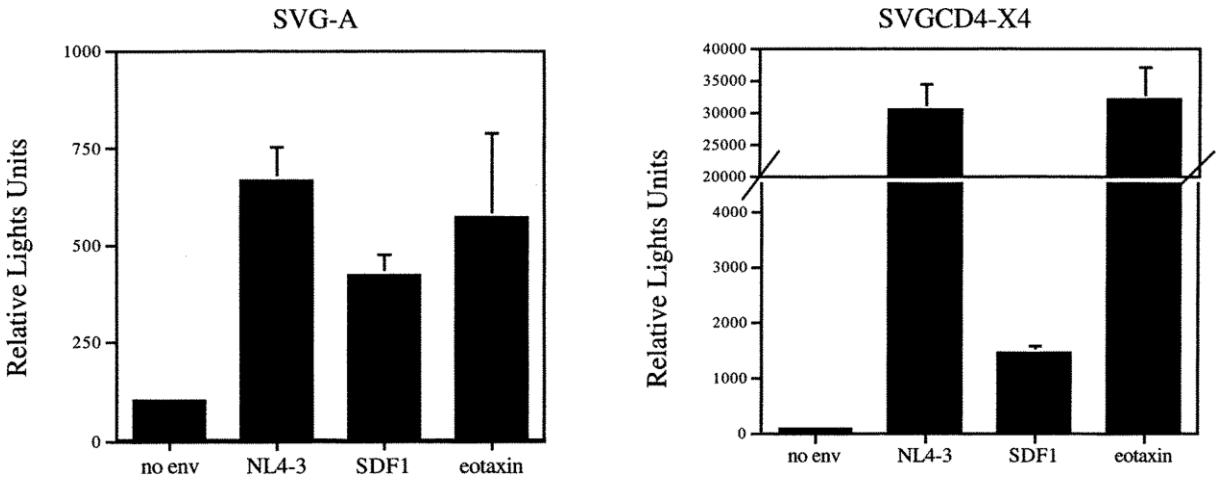


Figure 6 SDF-1 does not block infection of SVG-A cells by NL4-3. SVGCD4-X4 cells and SVG-A cells were treated with 5 μg/ml SDF-1, 5 μg/ml eotaxin, or with media alone for 1 h at 37°C. The cells were then infected with the NL4-3 pseudotyped virus and luciferase activity assayed at 72 h postinfection. Luciferase activity is reported as relative light units per 10 s. Error bars represent the standard deviation of triplicate samples.

pseudotyped with envelopes from several strains of HIV-1. The luciferase assay system is highly sensitive and accurate as it depends upon viral entry into a pathway that leads to proviral integration and early HIV gene expression from multiply spliced transcripts. The luciferase gene is expressed from the nef open reading and is therefore independent of rev function which has been shown to be compromised in astrocytes (Brack-Werner, 1999; Ludwig *et al*, 1999).

Our results show that the SVG-A cell line was reproducibly infected by the NL4-3 T-tropic isolate of HIV-1 and were resistant to infection with Bal, YU2, DS-br, and RC-br isolates. This confirms earlier reports that astrocytes are susceptible to low-level infection by T-tropic isolates of HIV-1. We then asked whether infection of the SVG-A cell line by NL4-3 was mediated by the T-tropic chemokine coreceptor, CXCR4. Our data clearly indicate that infection of SVG-A cells occurs independently of CXCR4, as overexpression of CXCR4 in these cells did not increase susceptibility to infection. Also, blocking of CXCR4 with SDF-1 did not inhibit infection of the SVG-A cells. SVG-A cells expressing the prototypical T-tropic receptors, CXCR4 and CD4, were highly susceptible to infection. The high level of activity in SVG-CD4-X4 cells was blocked by SDF-1, but only to the baseline levels of activity seen in SVG-A cells.

This indicates that the low efficiency of astrocyte infection is due to inefficient viral entry in the absence of the prototypical receptors. Identification of the mechanism of infection in this cell line may lead to the development of reagents useful in identifying astrocytes *in vivo* that may harbor latent HIV genomes.

Acknowledgements

This work was supported by an NIH-funded Lifespan/Brown/Tufts Center for AIDS Research (CFAR) AI 42853 to WA, a Brown University Salomon Foundation grant #6-32320 to WA, and by Grants-in-aid of Research from Sigma Xi, the Scientific Research Society, to BS. BS was also supported by a Department of Education training grant in Areas of National Need (GANN). We wish to thank EO Major for providing glial cell lines, the laboratories of Drs Gonzalez-Scarano and Landau for generously providing the plasmids necessary to generate the HIV pseudotypes, Dr Daniel Yip for help with the calcium mobilization assays, and Dr Paul Skolnich and the CFAR Immunovirology Core for p24 assays.

References

Albright AV, Shieh JTC, Itoh T, Lee B, Pleasure D, O'Connor MJ, Doms RW, Gonzalez-Scarano F (1999). Microglia express CCR5, CXCR4, and CCR3, but, of these, CCR5 is the principal coreceptor for HIV-1 dementia isolates. *J Virol* **73:** 205–213.

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

An SF, Groves M, Giometto B, Beckett AAJ, Scaravelli F (1999a). Detection and localisation of HIV-1 DNA and RNA in fixed adult AIDS brain by polymerase chain reaction/insitu hybridization technique. *Acta Neuropathol* **98:** 481–487.

An SF, Groves M, Gray F, Scaravilli F (1999b). Early entry and widespread cellular involvement of HIV-1 DNA in brains of HIV-1 positive asymptomatic individuals. *J Neuropathol Exp Neurol* **58**(11)**:** 1156–1162.

Atwood WJ, Berger JR, Kaderman R, Tornatore CS, Major EO (1993). Human immunodeficiency virus type 1 infection of the brain. *Clin Microbiol Rev* **6:** 339–366.

Bagasra O, Lavi E, Bobroski L, Khalili K, Pestaner JP, Pomerantz RJ (1996). Cellular reservoirs of HIV-1 in the central nervous system of infected individuals: identification by the combination of in situ PCR and immunohistochemistry. *AIDS* **10:** 573–585.

Bencheikh M, Bentsman G, Sarkissian N, Canki M, Volsky DJ (1999). Replication of different clones of human immunodeficiency virus type 1 in primary fetal human astrocytes: enhancement of viral gene expression by Nef. *J NeuroVirol* **5:** 115–124.

Brack-Werner R (1999). Astrocytes: HIV cellular reservoirs and important participants in neuropathogenesis. *AIDS* **13:** 1–22.

Conant K, Tornatore CS, Atwood WJ, Meyers K, Traub R, Major EO (1994). In vivo and in vitro infection of the astrocyte by HIV-1. *Adv Neuroimmunol* **4:** 1–3.

Deng H, Liu R, Ellmeier W, Choe S, Unatmaz D, Burkhart M, DiMarzio 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.

DiRienzo AM, Aloisi F, Santarcangelo AC, Palladino C, Olivetta E, Genovese D, Verani P, Levi G (1998). Virological and molecular parameters of HIV-1 infection of human embryonic astrocytes. *Arch Virol* **143:** 1599–1615.

Doranz BJ, Rucker J, Yi Y, Smith RJ, Samson M, Peiper SC, Parmentier M, Collman RG, Doms RW (1996). A dual tropic primary HIV-1 isolate that used fusin and the beta-chemokine receptors CCR5, CCR3 and CKR-2b as fusion cofactors. *Cell* **85:** 1149–1158.

Epstein L, Gendelman H (1993). Human immunodeficiency virus type 1 infection of the nervous system: pathogenetic mechanisms. *Ann Neurol* **33:** 429–436.

Everall I, Luthert P, Lantos P (1991). Neuronal loss in the frontal cortex in HIV infection. *Lancet* **337:** 1119–1121.

Gabuzda D, Ho D, de la Monte S, Hirsch M, Rota T, Sobel R (1986). Immunohistochemical identification of HTLV-III antigen in brains of patients with AIDS. *Ann Neurol* **20:** 289–295.

Hao H, Lyman WD (1999). HIV infection of fetal human astrocytes: the potential role of a receptor-mediated endocytic pathway. *Brain Res* **823:** 24–32.

He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, Busciglio J, Yang X, Hofmann W, Newman W, Mackay CR, Sodroski J, Gabuzda D (1997). CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* **385:** 645–649.

Jordan CA, Watkins BA, Kufta C, Dubois-Dalcq M (1991). Infection of brain microglial cells by human immunodeficiency virus type 1. *J Virol* **65:** 736–742.

Keir SD, Miller J, Yu G, Hamilton R, Samulski RJ, Xiao X, Tornatore C (1997). Efficient gene transfer into primary and immortalized human fetal glial cells using adeno-associated virus vectors: establishment of a glial cell line with a functional CD4 receptor. *J Neurovirol* **3:** 322–330.

Koenig S, Gendelman H, Orenstein J, Dal Canto M, Pezeshkpour G, Yungbluth M, Janotta F, Aksamit A, Martin M, Fauci A (1986). Detection of AIDS virus in macrophages in brain tissue from AIDS patients. *Science* **233:** 1089–1093.

Kolson DL, Lavi E, Gonzalez-Scarano F (1998). The effects of human immunodeficiency virus in the central nervous system. *Adv Virus Res* **50:** 1–700.

Lipton SA (1994). HIV-related neuronal injury. *Mol Neurobiol* **8:** 181–196.

Ludwig E, Ceccherini-Silberstein F, van Empel J, Erfle V, Neumann M, Brack-Werner R (1999). Diminished rev-mediated stimulation of human immunodeficiency virus type 1 protein synthesis is a hallmark of human astrocytes. *J Virol* **73:** 8279–8289.

McArthur JC, McClernon DR, Cronin MF, Nance-Sproson TE, Saah AJ, St Clair M, Lanier ER (1997). Relationship between human immunodeficiency virus-associated dementia and viral load in cerebrospinal fluid and brain. *Ann Neurol* **42:** 689–698.

Nath A, Hartloper V, Furer M, Fowke KR (1995). Infection of human fetal astrocytes with HIV-1: viral tropism

and the role of cell to cell contact in viral transmission. *J Neuropathol Exp Neurol* **54:** 320–330.

Nuovo GJ, Gallery F, MacConnell P, Braun A (1994). In situ detection of polymerase chain reaction-amplified HIV-1 nucleic acids and tumor necrosis factor-alpha RNA in the central nervous system. *Am J Pathol* **144:** 659–666.

Ranki A, Nyberg M, Ovod V (1995). Abundant expression of HIV Nef and Rev proteins in brain astrocytes in vivo is associated with dementia. *AIDS* **9:** 1001–1008.

Sabri F, Tresoldi E, Di Stefano M, Polo S, Monaco MC, Verani A, Fiore JR, Lusso P, Major E, Chiodi F, Scarlatti G (1999). Nonproductive human immunodeficiency virus type 1 infection of human fetal astrocytes: independence from CD4 and major chemokine receptors. *Virology* **264:** 370–384.

Shieh JTC, Albright AV, Sharron M, Gartner S, Strizki J, Doms RW, Gonzalez-Scarano F (1998). Chemokine receptor utilization by human immunodeficiency virus type 1 isolates that replicate in microglia. *J Virol* **72:** 4243–4249.

Tornatore C, Chandra R, Berger JR, Major EO (1994a). HIV-1 infection of subcortical astrocytes in the pediatric central nervous. *Neurology* **44:** 481–487.

Tornatore C, Meyers K, Atwood W, Conant K, Major E (1994b). Temporal patterns of human immunodeficiency virus type 1 transcripts in human fetal astrocytes. *J Virol* **68:** 93–102.

Tornatore C, Nath A, Amemiya K, Major EO (1993). Persistent human immunodeficiency virus type 1 infection in human fetal glial cells reactivated by T-cell factor(s) or by the cytokines tumor necrosis factor alpha and interleukin-1 beta. *J Virol* **65:** 6094–6100.

Tornatore CS, Nath A, Amemiya K, Major EO (1991). Persistent human immunodeficiency virus type 1 infection in human glial cells reactivated by T cell factors or by the cytokines tumor necrosis factor alpha and interleukin 1 beta. *J Virol* **65:** 6094–6100.

Watkins BA, Dorn HH, Kelly WB, Armstrong RC, Potts BJ, Michaels F, Kufta CV, Dubois-Dalcq M (1990). Specific tropism of HIV-1 for microglial cells in primary human brain cultures. *Science* **249:** 549–552.

Wiley C, Schrier R, Nelson J, Lampert P, Oldstone M (1986). Cellular localization of human immunodeficiency virus infection within the brains of aquired immune deficiency patients. *Proc Natl Acad Sci USA* **83:** 7089–7093.