Infection of human pericytes by HIV-1 disrupts the integrity of the blood–brain barrier

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Abstract

Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system (CNS) affects cross-talk between the individual cell types of the neurovascular unit, which then contributes to disruption of the blood–brain barrier (BBB) and the development of neurological dysfunctions. Although the toxicity of HIV-1 on neurons, astrocytes and brain endothelial cells has been widely studied, there are no reports addressing the influence of HIV-1 on pericytes. Therefore, the purpose of this study was to evaluate whether or not pericytes can be infected with HIV-1 and how such an infection affects the barrier function of brain endothelial cells. Our results indicate that human brain pericytes express the major HIV-1 receptor CD4 and co-receptors CXCR4 and CCR5. We also determined that HIV-1 can replicate, although at a low level, in human brain pericytes as detected by HIV-1 p24 ELISA. Pericytes were susceptible to infection with both the X4-tropic NL4-3 and R5-tropic JR-CSF HIV-1 strains. Moreover, HIV-1 infection of pericytes resulted in compromised integrity of an *in vitro* model of the BBB. These findings indicate that human brain pericytes can be infected with HIV-1 and suggest that infected pericytes are involved in the progression of HIV-1-induced CNS damage.

Keywords: HIV-1 ● pericytes ● blood–brain barrier ● neurovascular unit

Introduction

Human immunodeficiency virus type 1 (HIV-1) infection of the central nervous system (CNS) causes disruption of the blood–brain barrier (BBB) and contributes to the development of neurological dysfunctions. However, the mechanisms underlying these effects are not fully understood. There are numerous reports related to the effects of HIV-1 and HIV-1-specific proteins on all components of the neurovascular unit [1–4] except for pericytes. Microglia and macrophages are the main cells that support productive HIV-1 replication in the CNS. Although HIV-1 has limited potential to infect astrocytes, they play an important role in HIV-1-mediated neurological dysfunctions by serving as a possible reservoir for the virus and an active producer of inflammatory mediators and neurotoxic factors [5–7]. Although HIV-1 does not productively infect brain capillary endothelial cells, it affects endothelial integrity, leading to the breakdown of the BBB and the development of vascular inflammatory reactions [2, 4, 5]. Therefore, it has been hypothesized that specific HIV-1 proteins and/or interaction with infected cells may be responsible for the disruption of the BBB in HIV-1-infected brains. Perturbation of the cross-talk between cells of the neurovascular unit may also contribute to altered functions of the BBB and to the development of CNS disorders [2, 8, 9].

Pericytes encircle endothelial cells and contribute to the maturation and stabilization of the capillaries. Recent studies have revealed that brain pericytes play pivotal roles in a variety of brain functions such as regulation of capillary flow, angiogenesis and the formation and maintenance of the BBB during brain development [10–14]. In addition, pericytes have been shown to promote endothelial cell survival through induction of autocrine VEGF-A signalling and Bcl-w expression [15]. Pericyte dysfunction plays an important role in CNS disorders as a reduced ratio of pericytes to brain endothelial cells has been detected in a number of brain disorders, including traumatic brain injury, stroke, multiple sclerosis, brain tumours, ageing and angiopathies [11, 16, 17].

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Because there are no reports on the effects of HIV-1 infection on pericytes, the present study focused on brain pericytes as targets of HIV-1. This study is the first report that HIV-1 can infect, although at a low level, brain pericytes *in vitro*. In addition, infected pericytes negatively influence the barrier function of brain endothelial monolayers.

Materials and methods

Cell cultures and co-culture models

Primary human brain vascular pericytes and human astrocytes were obtained from ScienCell Research Laboratories (Carlsbad, CA, USA) and cultured for additional three to five passages. Pericytes were positive for α-smooth muscle actin, NG2 chondroitin sulphate proteoglycan and desmin, but negative for glial (GFAP) and endothelial (von Willebrand factor) markers. Both cell types were cultured in medium provided by the supplier, containing 2% foetal bovine serum (FBS; HyClone, Logan, UT, USA), pericyte or astrocyte growth supplement and antibiotics (penicillin and streptomycin). Immortalized human brain microvascular endothelial cells (hCMEC/D3 cells) [18] were cultured in endothelial cell growth medium (EBM-2) supplemented with EGM-2 SingleQuots (Lonza, Walkersville, MD, USA) and 5% FBS.

Generation of HIV-1 stock and infection of BBB cells

HIV-1 stock was generated in human embryonic kidney 293T cells as described earlier [19]. The cells were grown in DMEM containing 10% FBS and an antibiotic mixture (100 U/ml penicillin and 100 μg/ml streptomycin). When cultures reached ~70% confluence, the cells were transfected with NL4-3 plasmid containing full-length proviral DNA or pYK-JRCSF plasmid carrying 0.5 kb of 3′-flanking sequences and 2.2 kb of 5′-flanking DNA. The transfection procedure was performed with calcium phosphate. After transfection, the cells were incubated for 36 hrs in growth medium. Culture supernatants were then collected, filtered through 0.45-μm filters (Millipore, Bedford, MA, USA) and frozen at −80°C. HIV-1 p24 levels in the supernatants were determined by ELISA (ZeptoMetrix, Buffalo, NY, USA) according to the manufacturer's instructions.

HIV-1 stock was used to infect the BBB cells. Briefly, the cells were incubated with culture medium containing viral stock (10 ng p24/ml) for 12 hrs at 37°C. Then, the medium was removed, the cells washed extensively to remove unbound virus, and fresh growth medium added. HIV-1 p24 levels were determined periodically in the cell culture medium. No notable changes in cell morphology or growth were observed in HIV-1-infected pericytes compared with the non-infected group for up to 14 days post injection.

Effects of anti-retroviral drugs and the reverse transcriptase assay

Pericytes were cultured in 24-well tissue culture plates and infected with the X4-tropic HIV-1 Lai (2×10^6 cpm) or the R5-tropic HIV-1 ADA (1×10^6 cpm) by incubation for 16 hrs at 37°C under 5% CO_2. Medium was then replaced with fresh growth medium containing either 0.3 μM Plerixafor (AMD3100, dissolved in water) (Sigma Aldrich, St. Louis, MO, USA), 2 nM Maraviroc (UK-427857, dissolved in DMSO) (Sigma Aldrich), or 50 μl vehicle. Cell density was monitored throughout the experiment and confluency was consistently maintained above 85% during the experiments.

HIV-1 replication was assayed by HIV reverse transcriptase (RT) assay [20] 4 days after the initial infection. Briefly, 5 μl medium was incubated with 10 μl disruption buffer (100 mM Tris-HCl pH 7.9, 300 mM KCl, 10 mM DTT, and 0.1% NP-40) for 15 min. Then, 25 μl reaction mixture (50 mM Tris-HCl pH 7.9, 150 mM KCl, 5 mM DTT, 15 mM $MgCl_2$, and 0.05% NP-40), containing 10 μg/ml Poly A (GE Healthcare, Waukesha, WI, USA), 0.25 U/ml pd(T)12-18 (GE Healthcare) and 4 μl/ml ^{3}H-TTP (NEN, 10 μCi/ml) were added to the samples and incubated for 18 hrs at 37°C. The samples were placed onto glass fibre filters and measured in a scintillation counter.

Immunostaining

For immunofluorescence, cells were fixed in 4% paraformaldehyde for 20 min. and permeabilized with 0.1% Triton-X 100 for 10 min. After washing with phosphate-buffered saline (PBS) and blocking with 3% bovine serum albumin (BSA) in PBS for 30 min., samples were incubated overnight at 4°C with anti-CD4 (Millipore), anti-CXCR4, anti-CCR5 (BD Pharmingen, San Diego, CA, USA), or anti-p24 (Dako, Carpinteria, CA, USA) antibodies. All antibodies were diluted 1:100 in PBS containing 0.1% BSA. Excess primary antibody was removed, slides were washed with PBS, and samples were then incubated with Alexa Fluor 488- or 548-conjugated secondary antibodies (Invitrogen, Carlsbad, CA, USA) for 30 min. at 37°C. After washing with PBS, slides were mounted using ProLong Gold Antifade reagent containing 4′,6-diamidino-2-phenylindole (Invitrogen) to visualize the nuclei. Specimens were covered with cover slips and evaluated under an epifluorescence Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan). Images were captured using a Spot charge-coupled device camera system.

For immunocytochemistry, cells were fixed and permeabilized as above. Endogenous peroxidases were inhibited with 0.3% H_2O_2 for 30 min. and non-specific binding was blocked with 3% BSA in 2% goat serum in PBS for 30 min. Cultures were incubated with anti-p24 primary antibody overnight at 4°C. Incubation with biotinylated antimouse secondary antibody followed by horseradish peroxidase (HRP)-labelled streptavidin and diaminobenzidine (DAB) chromogen was performed as directed by the manufacturer (Vector, Burlingame, CA, USA).

Immunoblotting

Cells were lysed using the radioimmunoprecipitation assay buffer (RIPA; Santa Cruz Biotechnology, Santa Cruz, CA, USA) and centrifuged at $15,000 \times g$ for 15 min. The supernatants were collected and protein levels were determined using a BCA protein assay kit (Pierce, Rockford, IL, USA). Samples were separated on 4–15% Tris-HCL Ready SDS-polyacrylamide gels (Bio-Rad Laboratories, Hercules, CA, USA), transferred onto polyvinylidene difluoride membranes (Bio-Rad Laboratories), and incubated with the aforementioned antibodies. Anti-CCR5 and anti-CXCR4 antibodies were purchased from BD Pharmingen, anti-CD4 antibody was purchased from Millipore, anti-occludin and anti-ZO-1

antibodies were obtained from Invitrogen, and all secondary antibodies were from Santa Cruz Biotechnology. Antibodies were diluted 1:500 (CCR5 and CXCR4), 1:1000 (CD4), 1:2500 (occludin) and 1:5000 (ZO-1) in 3% BSA in PBS. For visualization of detected proteins, immunoblots were analysed using the enhanced chemiluminescence (ECL) Western blot detection kit (Amersham Biosciences, Piscataway, NJ, USA).

IL-6 detection

Total RNA was isolated and purified using the RNeasy Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. First-strand cDNA was synthesized from 1 μg of total RNA with the Reverse Transcription System (Promega, Madison, WI, USA). Polymerase chain reaction (PCR) amplifications were performed using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using TaqMan Universal PCR Master Mix and a pre-developed human IL-6 primer pair and probe (Applied Biosystems). Expression of mRNA was calculated and analysed using the comparative CT method. PCR amplification of β-actin was performed for each sample to normalize IL-6 mRNA levels. IL-6 levels in the cell culture media were determined by ELISA according to the manufacturer's protocol (Thermo Fisher Scientific, Rockford, IL, USA). Results were normalized to total cellular protein.

Permeability studies

Endothelial barrier function was evaluated by measuring the permeability of fluorescein isothiocyanate dextran (FD-4, MW 4000) flux across hCMEC/D3 monolayers in a monoculture system or in a co-culture model with pericytes. In the monoculture system, hCMEC/D3 cells were cultured on the upper side of the Transwell insert (Costar, Corning, NY, USA). To generate a co-culture model, pericytes (2×10^4 cells/cm^2) were seeded on the bottom side of the collagen-coated polyester membrane of the Transwell inserts [21]. The cells were left overnight to adhere firmly, followed by incubation with HIV-1 NL4-3 or JR-CSF stock for 12 hrs. Then, hCMEC/D3 cells (1×10^5 cells/cm^2) were seeded on the upper side of the Transwell inserts and the co-culture model was maintained in hCMEC/D3 medium. In specific experiments, hCMEC/D3 cells were exposed for 48 hrs to conditioned medium collected from pericyte cultures 3 days after a 12-hr infection with HIV-1 NL4-3 or JR-CSF, both at 10 ng p24/ml.

To initiate the permeability assay, the medium was removed and Hank's balanced salt solution (HBSS; 0.5 ml) containing 100 μM FD-4 was placed in the luminal chamber of the Transwell insert. Aliquots from the abluminal chamber were removed 15 and 45 min. after adding FD-4, and the concentration of FD-4 was determined using a fluorescence multi-well plate reader (Ex. 485 nm; Em. 530 nm). The permeability coefficient and clearance were calculated as previously described [21].

Statistical analysis

All data presented are means ± the standard error of the mean (SEM). The values were compared using the analysis of variance followed by the Tukey-Kramer method. Changes were considered statistically significant at $P < 0.05$.

Results

Human brain pericytes express CD4 and chemokine co-receptors

The primary receptor for HIV-1 entry into the majority of target cells is the CD4 receptor. In addition, expression of the chemokine co-receptors CXCR4 and CCR5 is essential for infection with the NL4-3 and JR-CSF HIV-1 strains respectively. Therefore, we examined the expression of these receptors on cultured human pericytes using immunofluorescence and immunoblotting. Pericytes expressed strong immunoreactivity for CD4, CXCR4 and CCR5 (Fig. 1A). Although CXCR4 and CCR5 were distributed evenly throughout the cells, CD4 exhibited relatively strong nuclear staining. Because CD4 is a cell surface receptor, this localization may be an artefact related to sample preparation or the antibody used. Control experiments in which no primary antibodies were added revealed no staining. Figure 1B shows the Western blot analysis, confirming expression of CD4, CXCR4 and CCR5 on human brain pericytes. Human monocytic U937 cells and Jurkat cells were used as controls in immunoblotting analyses.

HIV-1 replicates at low levels in human pericytes

To examine whether or not HIV-1 can invade human pericytes, these cells (6×10^4 cells/well) were plated onto collagen-coated 4-well culture slides. Pericytes were exposed to the NL4-3 or the JR-CSF HIV-1 strains (both at 10 ng p24/ml) for 12 hrs, washed extensively and stained with anti-p24 antibody. HIV-1 entry into pericytes was compared with entry into two other cell types of the neurovascular unit, astrocytes and brain endothelial cells. As illustrated in Figure 2, numerous HIV particles were detected in all three types of cells.

In parallel experiments, cultured pericytes were incubated with HIV-1 NL4-3 or JR-CSF (10 ng p24/ml for 12 hrs), followed by washing the cells and maintaining the cultures in growth medium for 14 days. Supernatants were collected and periodically assayed for p24 content. Both NL4-3 and JR-CSF strains replicated in pericytes, as indicated by a constant rise in p24 levels (Fig. 3A and B). The replication rate was low, with the maximum levels of ~75 pg p24/ml occurring 14 days post pericyte infection. HIV-1 replication in pericytes was compared to that in astrocytes and brain endothelial cells. p24 levels in the supernatants of cultured astrocytes were higher than in pericytes, especially for the NL4-3 strain. The release of p24 into the culture supernatant of brain endothelial cells did not change throughout the observation period.

p24 expression was also analysed in pericytes, astrocytes and brain endothelial cells by immunohistochemistry 7 days after a 12-hr incubation with HIV-1. As shown in Figure 3C, small numbers of p24-positive cells were detected in astrocyte and pericyte cultures, whereas p24 was undetectable in brain endothelial cells.
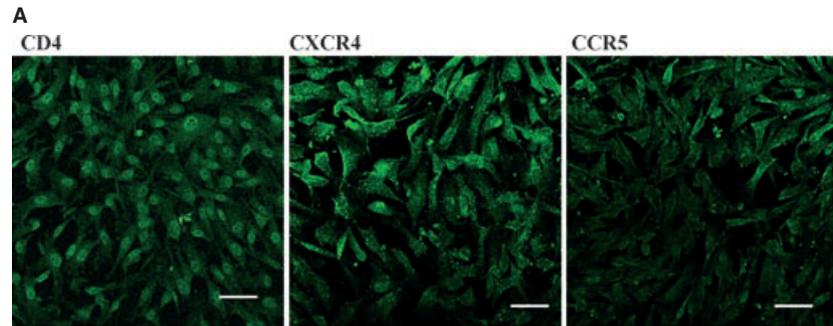
Fig. 1 Human brain pericytes express the CD4 receptor and chemokine co-receptors. (**A**) Cultured pericytes were fixed with 4% paraformaldehyde and stained with mouse anti-CD4, anti-CXCR4, or anti-CCR5 antibodies, followed by incubation with Alexa 488-conjugated secondary antibodies. The results were visualized by confocal microscopy. Scale bar, 20 μm. (**B**) Expression of CD4, CXCR4, and CCR5 was determined in cell extracts by Western blotting. Human monocytic cell line (U937 cells) and T cell line (Jurkat cells) were used as positive controls for individual receptors.
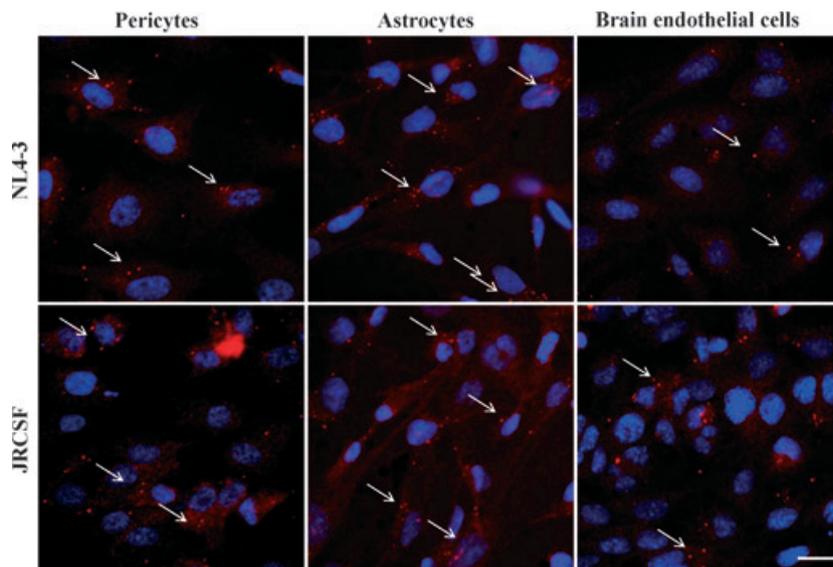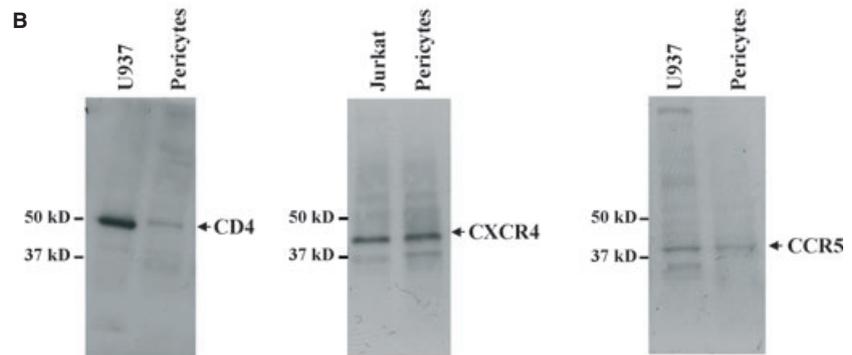
Fig. 2 HIV-1 enters human brain pericytes. Pericytes were plated on collagen-coated culture slides and incubated for 12 hrs with HIV-1 NL4-3 or HIV-1 JR-CSF at 10 ng p24/ml. Control experiments were performed using human astrocytes and brain endothelial cells. Following incubation with HIV-1, cells were vigorously washed to remove unabsorbed virus, fixed with 4% paraformaldehyde, and stained for p24 expression (red staining). DAPI staining was performed to visualize the nuclei (blue staining). Scale bar, 20 μm. Arrows indicate p24 immunoreactivity associated with pericytes, astrocytes, and brain endothelial cells.

To further confirm that HIV-1 replicates in pericytes and to demonstrate the role of chemokine coreceptors, pericytes were 2infected with HIV-1 Lai in the presence of Plerixafor (a specific inhibitor of CXCR4) or with HIV-1 ADA in the presence of Maraviroc (a specific inhibitor of CCR5). HIV-specific RT activity was determined by incorporation of radiolabelled dTTP. As shown in Figure 3D, both drugs effectively decreased replication of the corresponding HIV strains.

HIV-1-infected pericytes produce IL-6

IL-6 mRNA and protein levels were determined as markers of pericyte activation. As indicated in Figure 4A, a 12-hr incubation with the NL4-3 or JR-CSF HIV-1 strains (both at 10 ng p24/ml) markedly induced IL-6 gene expression. Importantly, these results were associated with a significant increase in IL-6 protein levels as determined in cell cul-
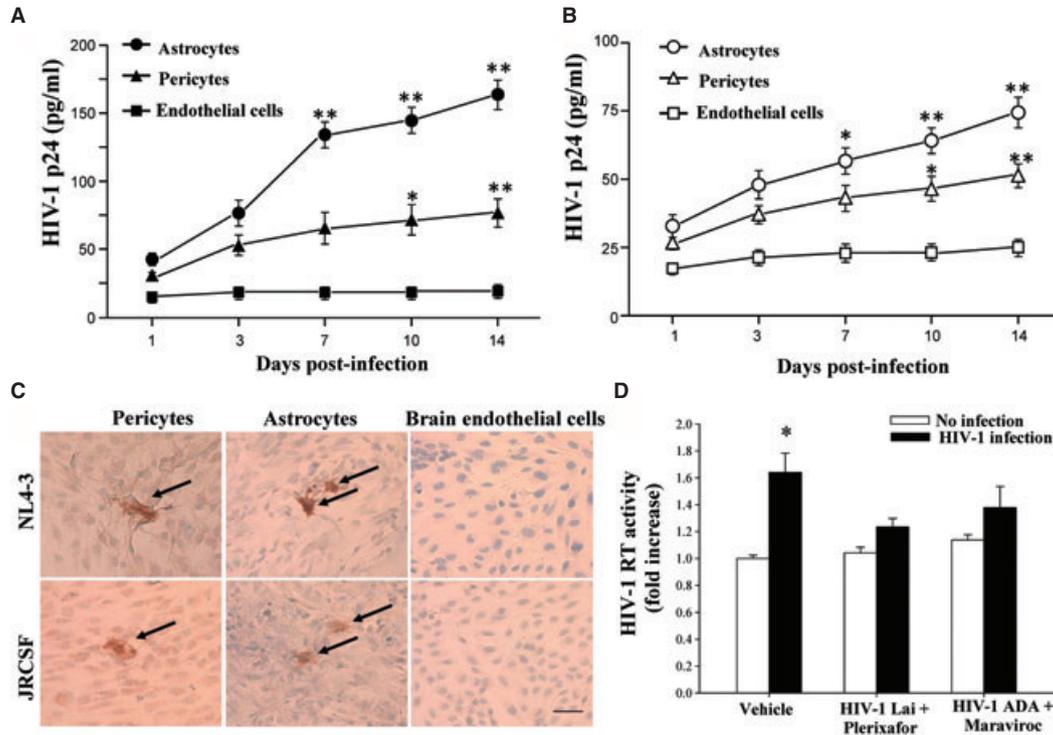
Fig. 3 HIV-1 replicates in human pericytes. Pericytes were incubated for 12 hrs with NL4-3 (**A**) or JR-CSF (**B**) HIV-1 strains at 10 ng p24/ml. Control experiments were performed with human astrocytes and brain endothelial cells. Supernatants were collected at the indicated days post HIV-1 exposure and p24 levels were measured by ELISA. Values are the means $\pm$ SEM from three separate experiments performed in triplicate. *$P < 0.05$ or **$P < 0.01$ relative to day 1 post infection. (**C**) Pericytes, astrocytes and brain endothelial cells were plated on collagen-coated culture slides and incubated with HIV-1 NL4-3 or JR-CSF as in A and B. Cultures were stained for p24 expression at day 7 post infection. p24-immunoreactive peri-cytes and astrocytes (arrows) were visualized using diaminobenzidine (DAB, brown staining) and counterstained with hematoxylin. No p24-positive cells were detected in cultures of brain endothelial cells. Scale bar, 50 μm. (**D**) Pericytes were infected with HIV-1-Lai (1×10^6 cpm) or HIV-1 ADA (2×10^6) in the presence of 0.3 μM of Plerixafor or 2 nM of Maraviroc, respectively. The activity of HIV-specific reverse transcriptase (RT) was determined 4 days post infection. Values are normalized CPM counts $\pm$ SEM. The vehicle for Plerixafor was water and for Maraviroc was DMSO. Because there were no differences in HIV-1 RT activity when water or DMSO was added, the results were combined into one vehicle group. *Significantly different from non-infected cells at $P < 0.05$.

ture media collected 48 hrs after the initial 12-hr incubation with the virus (Fig. 4B).

HIV-1 infection of pericytes disrupts the integrity of endothelial monolayers

To investigate whether or not HIV-1 infection of pericytes affects the integrity of brain endothelial cells, pericytes were co-cultured with hCMEC/D3 cells. The barrier properties created by brain endothelial cells increased in co-cultures with normal (*i.e.* non-infected) peri-cytes, supporting the hypothesis that pericytes strengthen the integ-rity of the BBB. Indeed, the FD-4 permeability coefficient decreased from 0.69 ± 0.02 in a monoculture of hCMEC/D3 cells to $0.49 \pm 0.01 \times 10^{-3}$ cm/min ($P < 0.01$) in co-cultures with pericytes (Fig. 5A). By contrast, co-cultures of hCMEC/D3 cells with pericytes infected with HIV-1 NL4-3, or JR-CSF increased the permeability of FD-4 by 126.9% or 134.6% respectively. Disruption of barrier integ-

rity was also observed in hCMEC/D3 cells incubated with conditioned medium collected from pericyte cultures 3 days post infection with HIV-1 (Fig. 5B). In addition, incubation with conditioned medium from HIV-1-infected pericytes resulted in a decrease in the tight junc-tion proteins occludin and ZO-1 (Fig. 5C and D respectively).

Discussion

Pericytes are pluripotent cellular components of the capillaries and post capillary venules [17]. Although they are ubiquitously present in different tissues and organs, pericytes are most abundant in the brain and retina. In fact, the density of pericytes in the brain is estimated to be several-fold higher than in other organs, illustrating their impor-tance in the CNS [12]. They communicate with other cell types of the neurovascular unit, namely, brain endothelial cells, astrocytes and neurons, by direct contact *via* gap junctions and through autocrine and paracrine mechanisms. In addition to regulation of BBB integrity,

Fig. 4 HIV-1-infected pericytes overexpress IL-6. (**A**) Pericytes were infected with HIV-1 strains NL4-3 or JR-CSF (10 ng p24/ml) for 12 hrs, followed by determination of IL-6 mRNA by real-time RT-PCR. (**B**) Pericytes were infected with HIV-1 as in (A), and medium IL-6 levels were determined by ELISA 48 hr post infection. Values are the means ± SEM from three separate experiments, $n = 6$. *Significantly different from controls at $P < 0.05$.
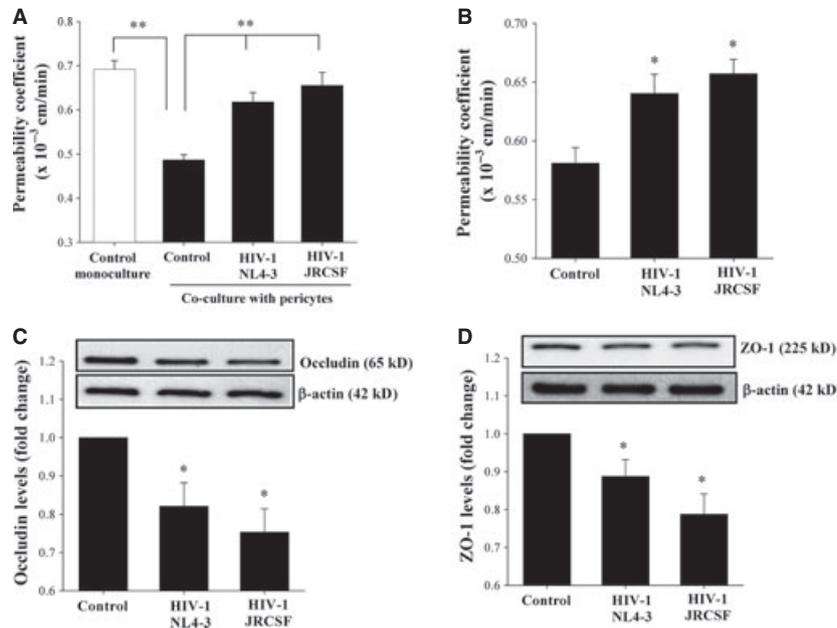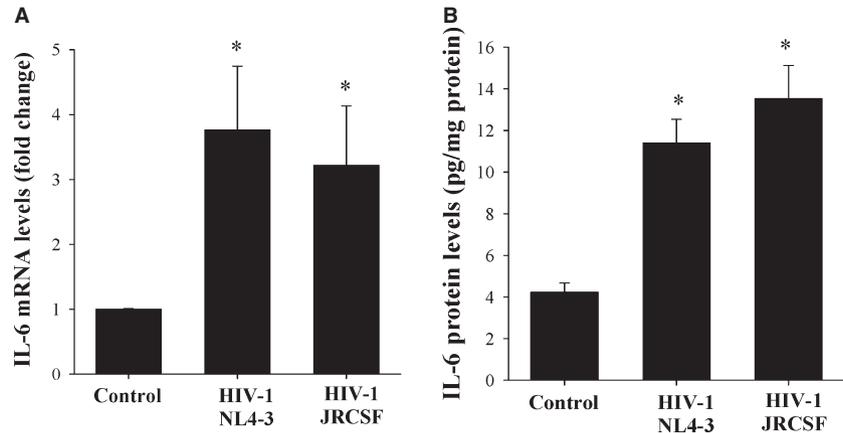

Fig. 5 HIV-1 infection of pericytes disrupts the integrity of endothelial monolayers. Pericytes were infected with HIV-1 as in Figure 2. (**A**) Brain endothelial cells were maintained in monoculture (open bar) or were co-cultured with HIV-infected or non-infected pericytes (black bars) for 4 days on a Transwell system. (**B**) Brain endothelial cells were maintained in monoculture and exposed to conditioned medium collected 3 days postinfection of the pericytes. Dextran (MW 4000) labelled with fluorescein isothiocyanate (FD-4) was used as an indicator of endothelial integrity. An increase in permeability to FD-4 reflects disruption of the barrier function. (**C** and **D**). Brain endothelial cells maintained in monoculture were exposed to conditioned medium collected from HIV-1-infected pericytes as in (B). Following a 48-hr incubation period, the expression of occludin (C) and ZO-1 (D) was determined by Western blotting of total cell extracts. The blots are representative images from three separate experiments performed in triplicate, and the graphs represent densitometric measurements from these experiments. Values are the means ± SEM. *$P < 0.05$ or **$P < 0.01$ relative to controls.

pericytes have been demonstrated to be involved in the maintenance of brain homeostasis, angiogenesis, neovascularization and the regulation of cerebral blood flow [10–14]. Although it has been postulated that communication between pericytes and other cells of the neurovascular unit is fundamental to the maintenance of brain homeostasis, there is no information currently available on the role of pericytes in HIV-1 brain infection. This lack of information is partially caused by the fact that there is no selective marker for pericytes [17], making it difficult to distinguish these cells from other components of the neurovascular unit on brain slides.

Although a precursor cell for pericytes remains elusive, brain pericytes are believed to be of mesodermal origin and derived from the bone marrow [22]. They express markers of the monocyte/macrophage lineage, such as ED1 or CD11b, and are involved in immunoresponses in the brain [23]. Therefore, we hypothesize that pericytes are conducive to HIV-1 infection. In the first series of experiments, we found that human pericytes express CD4, the main receptor utilized by HIV-1 for cellular entry [24]. Although expression of CD4 in pericytes is relatively low compared with U937 cells, the presence of this receptor differentiates pericytes from several other cell types in the CNS. In fact, CD4 is highly expressed on microglia and macrophages, which are the main target cells for HIV-1 infection in the CNS, whereas astrocytes, endothelial cells and neurons do not have detectable levels of CD4 [25, 26].

Although HIV-1 viral turnover in the circulation is primarily due to infection of CD4 + cells (*i.e.* T-lymphocytes and monocytes/macrophages), expression of the CD4 receptor is not the sole factor determining viral entry [24]. Important results of the present study demonstrate that human pericytes express CXCR4 and CCR5, which are the main co-receptors used by specific HIV-1 strains for cell infection [27, 28]. As indicated by immunoblotting, the expression of CXCR4 and CCR5 in pericytes is similar to that in Jurkat and U937 cells respectively. The presence of both co-receptors makes pericytes susceptible to both the X4-tropic NL4-3 and R5-tropic JR-CSF strains. Although several other receptors can be utilized by HIV-1 to infect transfected cells *in vitro* [24], only these two co-receptors appear to play a role *in vivo*. CXCR4 was the first HIV-1 co-receptor identified and is involved in HIV infection by most of the common strains of HIV-1 [29]. The importance of CCR5 was demonstrated in population studies that found that absence or deficiency of this receptor protects against HIV-1 infection [30]. Nevertheless, despite expression of CD4, CXCR4, and CCR5, HIV-1 infection of human pericytes appears to be restricted and does not support productive viral replication. Analysis of HIV-1 p24 core protein in the supernatant of infected pericytes revealed values below the levels detectable in cultures of infected astrocytes. These results corresponded to a limited number of p24-immunoreactive pericytes.

The relatively low infection rate of pericytes by HIV-1 does not preclude the importance of this process in the neuropathology of the virus. For example, few infected astrocytes can induce bystander effects and propagate inflammatory effects to neighbouring cells *via* cell–cell communication [5, 6]. Although astrocyte infection is typically restricted and non-productive due to replication defects at several stages of the virus life cycle, HIV-1 production in infected astrocytes can be transiently activated by treatment with inflammatory cytokines. Thus, infected astrocytes can be induced to transmit HIV-1 to other cells and function as viral reservoirs [7]. Although the rate of HIV-1 infection in pericytes appears to be lower than in astrocytes, it is possible that pericytes play a similar role to astrocytes as viral reservoirs.

Owing to the importance of pericytes as an element of the neurovascular unit, we also determined the effects of normal and HIV-1-infected pericytes on the barrier function of cultured brain endothelial cells. A co-culture of brain endothelial cells with normal (*i.e.* uninfected) pericytes resulted in a strengthening of the barrier properties compared with endothelial monocultures. These effects are consistent with the hypothesis that one of the main functions of brain pericytes is the development and maintenance of the BBB. Pericytes were shown to contribute to the integrity of the BBB by producing elements of the basal lamina and enhancing the barrier properties of the brain endothelium. In addition, they can stimulate expression of BBB-specific genes in brain endothelial cells and induce polarization of astrocyte end-feet surrounding brain capillaries [10–15, 17, 23]. In further support of the role of pericytes in BBB functions, it has been observed that disruption of BBB integrity is proportional to the degree of pericyte loss in experimental animals [31, 32].

Blood–brain barrier integrity is disrupted in HIV-1 brain infection, allowing the entry of virotoxins and HIV-1 virions into the CNS [4, 33]. Clinical studies have reported the absence or disruption of tight junction proteins ZO-1 and occludin in HIV-1-associated encephalitis or dementia [2, 34, 35]. Importantly, it has been shown that HIV-1-related disruption of tight junction proteins correlates with the intensity of monocyte infiltration in human brain tissue [34, 36]. In agreement with these reports, we observed that co-cultures of brain endothelial cells with HIV-1-infected pericytes resulted in a significant disruption of barrier function. Such changes were not detected in cultures exposed to 10 ng p24/ml cell-free HIV-1, suggesting that soluble factors released from HIV-1-infected pericytes induce BBB disruption. Indeed, increased permeability across endothelial monolayers and a decrease in expression of occludin and ZO-1 were observed in monocultures of brain endothelial cells exposed to conditioned medium from infected pericytes. These observations are consistent with reports that inflammatory mediators secreted by HIV-1-infected cells in the brain play a critical role in HIV-1-induced brain dysfunction [37–39]. In fact, HIV-1-infected pericytes were stimulated to produce IL-6, a proinflammatory cytokine that was shown to be involved in increased endothelial permeability [40]. Although other cytokines can also be released in response to HIV-1 brain infection, this is the first report suggesting that IL-6 released from HIV-infected pericytes is associated with a loss of integrity of the cerebral endothelium.

In conclusion, the present study indicates that human brain pericytes express cellular receptors used by HIV-1 for cell entry and can be infected with both X4-tropic and R5-tropic HIV-1 strains. Although HIV-1 replication in pericytes is restricted, it leads to increased production of IL-6 and disrupted barrier properties of endothelial monolayers. Overall, these results suggest that HIV-1 infection of brain pericytes contributes to the formation of viral reservoirs in the brain and to BBB dysfunction in the course of HIV-1 infection.

Acknowledgements

Conflict of interest

The authors have no conflicting interests to disclose.

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