Vascular Biology, Atherosclerosis and Endothelium Biology

Human Immunodeficiency Virus (HIV) Infects Human Arterial Smooth Muscle Cells in Vivo and in Vitro

Implications for the Pathogenesis of HIV-Mediated Vascular Disease

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Human immunodeficiency virus (HIV) infection is associated with accelerated atherosclerosis and vasculopathy, although the mechanisms underlying these findings have not been determined. Hypotheses for these observations include: 1) an increase in the prevalence of established cardiac risk factors observed in HIV-infected individuals who are currently experiencing longer life expectancies; 2) the dyslipidemia reported with certain HIV anti-retroviral therapies; and/or 3) the proinflammatory effects of infiltrating HIV-infected monocytes/macrophages. An unexplored possibility is whether HIV itself can infect vascular smooth muscle cells (SMCs) and, by doing so, whether SMCs can accelerate vascular disease. Our studies demonstrate that human SMCs can be infected with HIV both in vivo and in vitro. The HIV protein p24 was detected by fluorescence confocal microscopy in SMCs from tissue sections of human atherosclerotic plaques obtained from HIV-infected individuals. Human SMCs could also be infected in vitro with HIV by a mechanism dependent on CD4, the chemokine receptors CXCR4 or CCR5, and endocytosis, resulting in a marked increase in SMC secretion of the chemokine CCL2/MCP-1, which has been previously shown to be a critical mediator of atherosclerosis. In addition, SMC proliferation appeared concentric to the vessel lumen, and minimal inflammation was detected, unlike typical atherosclerosis. Our data suggest that direct infection of human arterial SMCs by HIV represents a potential mechanism in a multifactorial paradigm to explain the exacerbated atherosclerosis and vasculopathy reported in individuals infected with HIV. (Am J Pathol 2008, 172:1100–1111; DOI: 10.2353/ajpath.2008.070457)

In 2006, an estimated 33 million people worldwide are living with human immunodeficiency virus (HIV) infection (World Health Organization and United Nations estimates). The advent of more successful antiviral therapies has increased dramatically the life expectancy of HIV-infected individuals. As the HIV-infected population lives longer, an understanding of the impact of the virus on chronic disease processes such as atherosclerosis becomes increasingly relevant. The rates of atherosclerotic lesion development, myocardial infarction, and restenosis after coronary angioplasty are significantly higher in HIV-infected people as compared to uninfected individuals.2–5 HIV-infected persons show particularly high risk for atherosclerosis for several reasons. Inflammation induced by HIV infection or its associated proteins may promote atherosclerosis and formation of high-risk plaque, thus increasing the risk of myocardial infarction and stroke.6–11 HIV infection itself has been associated with an abnormal lipid profile.12 Highly active antiretroviral therapy also may cause lipid abnormalities and insulin resistance, both risk factors for atherosclerosis.13 However, the role of HIV infection itself within the vessel wall in the pathogenesis of atherosclerosis is not well understood. Other studies investigating the vascular effects of HIV infection have suggested a potential role for direct infection of arterial smooth muscle cells (SMCs) by HIV.

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HIV have focused on endothelial dysfunction and the infiltration of HIV-infected monocyte/macrophages but have not examined the effects of HIV on the smooth muscle cells (SMCs) of the vessel wall.

We demonstrated previously that human arterial SMCs express the three biologically relevant HIV receptors, CD4, CCR5, and CXCR4, that participate in HIV entry into leukocytes. SMCs are the predominant cells of the arterial media. The present study underscores the potential role of SMCs in the pathogenesis of HIV-associated vasculopathy by demonstrating that HIV can infect SMCs, both in vivo and in vitro, by using CD4, chemokine coreceptors and endocytosis as a mechanism of entry. HIV infection of SMCs also resulted in an enhanced secretion of the chemokine CCL2.

**Materials and Methods**

**Materials**

Dulbecco’s modified Eagle’s medium, fetal bovine serum, penicillin/streptomycin, and trypsin-EDTA were purchased from GibcoBRL/Invitrogen (Carlsbad, CA). Purified mouse IgG2a and IgG, myeloma protein were purchased from Cappel Pharmaceuticals Inc. (Westchester, PA). CD4 blocking antibody was purchased from BD Biosciences (San Jose, CA). The MCP-1/CCL2 enzyme-linked immunosorbent assay (ELISA) was purchased from R&D systems (Minneapolis, MN). The in situ cell death detection kit, TMR red terminal deoxynucleotidyl transferase dUTP nick-end labeling, was purchased from Roche (Mannheim, Germany). The HIV isolates, CXCR4 and CCR5 blockers, and blocking antibodies were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, National Institutes of Health (Germantown, MD). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO), unless otherwise designated.

**Human Tissue Sections**

Coronary artery sections from four individuals without histological evidence of atherosclerosis, designated as HIV-negative/no plaque (HIVneg/no plaque), were obtained from the University of Kentucky Department of Pathology (courtesy of Dr. P. Moreno and Dr. K. Purushothaman). Coronary artery sections from eight HIV-infected persons, 10 uninfected individuals with documented atherosclerosis, and four uninfected individuals without demonstrable atherosclerosis were examined. Established risk factors are listed in Table 1. The sections from atherosclerotic coronaries had plaque classified as either type IV (5 cases) or V (3 cases) (classification based on) and were obtained from either the Manhattan HIV Brain Bank (R24MH59724) or the University of Kentucky Department of Pathology (courtesy of Dr. P. Moreno and Dr. K. Purushothaman). All specimens were examined by confocal microscopy for p24 antigen and/or smooth muscle actin (SMA), a marker for smooth muscle cells. Clinical information was obtained in accordance with HIPAA regulations (Table 1). The investigators were blinded to any patient identifiers.

**Cell Culture**

Human SMCs were prepared as described. Briefly, SMCs were isolated from donor human thoracic aortas harvested from explanted hearts at the time of cardiac transplantation. SMCs were grown in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 μg/ml streptomycin.
and were serially passaged before reaching confluence. Cells were identified as smooth muscle by their typical appearance on light microscopy and by the presence of immunostaining with an antibody to human SMA. In several experiments, primary SMC cultures purchased from Cambrex (Baltimore, MD) were used to compare with the results obtained from primary cultures prepared by our laboratory. We did not detect any differences between the primary cells and those that were purchased commercially.

**HIV-1 Infection of Human SMCs**

Cell-free viral inocula were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program. Three isolates were used: the HIV\_\textsubscript{ADA}, an R5 isolate that infects human monocytes/macrophages; the HIV\_\textsubscript{JR-CSF}, an R5 isolate that infects primary human peripheral blood lymphocytes as well as certain mononuclear phagocyte populations, obtained from the cerebrospinal fluid of a patient with Kaposi’s sarcoma and severe acquired immunodeficiency syndrome encephalopathy; and the HIV\_\textsubscript{92UG021}, an X4 isolate that infects peripheral blood lymphocytes, that was obtained from an asymptomatic Ugandan patient. Subconfluent SMCs were incubated in Dulbecco’s modified Eagle’s medium with 50 to 90 ng of p24/ml of HIV-1 (ADA, JR-CSF, or 92UG021) for 1 to 2 hours, washed extensively to eliminate unbound virus, resuspended in fresh media, and then maintained in culture for an additional 2, 4, 7, 9, or 12 days. HIV-1 p24 protein levels were determined by an ELISA according to manufacturer’s instructions (PerkinElmer, 12 days. HIV-1 p24 protein levels were determined by an ELISA according to manufacturer’s instructions (PerkinElmer, 12 days).

Ten fields per coverslip were counted. The data were expressed as the percentage of total SMCs that were terminal deoxynucleotidyl transferase dUTP nick-end labeling-positive were counted.

**Assessment of CCL2 Secretion**

Uninfected or HIV-infected SMCs were grown to confluence in 96-well tissue culture plates and the supernatants were collected, centrifuged, and analyzed. CCL2/MCP-1 levels were analyzed by quantitative sandwich ELISA, according to the manufacturer’s instructions (R&D Systems Inc, Minneapolis, MN). This ELISA has a 15 pg/ml detection limit. Three independent experiments were performed.

**Apoptosis Assay**

The percentage of apoptotic SMCs was determined by terminal deoxynucleotidyl transferase dUTP nick-end labeling staining (Roche, Mannheim, Germany) using confocal microscopy. On each coverslip, the total number of SMCs, identified by positive staining with \( \alpha \)-actin, and the number of SMCs that were terminal deoxynucleotidyl transferase dUTP nick-end labeling-positive were counted. Ten fields per coverslip were counted. The data were expressed as the percentage of total SMCs that were apoptotic (\( n = 3 \)).

**Statistical Analysis**

Student’s one-tailed, paired t-test was used to compare MCP-1/CCL2 and p24 production and to quantify the numbers of p24-positive cells as compared to designated control conditions. A \( P \) value <0.05 was considered significant.

**Immunofluorescence and Immunohistochemistry**

Tissue sections or SMCs in culture were analyzed by triple or four-color immunostaining with antibodies to p24 antigen, SMA, for nuclear chromatin (4,6-diamidino-2-phenylindole, DAPI) or CD68, as previously described.\(^{23,24}\) Briefly, tissue sections or cultured cells were incubated in a blocking solution for 1 hour and then in diluted primary antibody (anti-p24, 1:20; National Institutes of Health repository, catalog no. 4121, anti-human SMA 1:500; Abcam, catalog no. ab21027 or anti-CD68, 1:200; Santa Cruz, catalog no. sc-7083) overnight at 4°C. Sections or cells were washed and secondary antibody conjugated to fluorescein isothiocyanate, Cy3 (Sigma anti-mouse and anti-rabbit, 1:300), and/or Alexa Fluor 647 (Molecular Probes, anti-goat) were added for 2 hours. Cells and sections were washed and mounted in anti-fade reagent with DAPI (Molecular Probes, Carlsbad, CA) and examined by confocal microscopy (Leica Microsystems GmbH, Germany).
Results

SMCs in Arterial Tissue Sections Obtained from Individuals with HIV and Atherosclerosis Are Infected by HIV

To examine whether SMCs are infected in vivo, sections from normal and atherosclerotic arteries were obtained from uninfected and HIV-infected individuals and were analyzed by confocal microscopy for SMA (a marker for SMCs), HIV-p24 (a protein produced by HIV-infected cells), and DAPI (nuclei) staining. As described below, our data demonstrated that, in vivo, a population of SMCs is infected with HIV within the atherosclerotic vessel wall of infected individuals.

Arterial sections obtained from uninfected individuals, either without (Figure 1, designated as HIVneg/no plaque; A–D) or with detectable atherosclerotic plaque (Figure 1, designated HIVneg/plaque; E–H), had a typical SMA staining pattern with respect to the lumen (Lu) of the blood vessel and no detectable p24 staining (C). In sections obtained from uninfected individuals with plaque (E–H), SMC-rich areas (F) were present without detectable p24 antigen (G). Similar studies were done on atherosclerotic arterial sections from HIV-infected individuals (denoted HIVpos/plaque in I–P). In these samples, HIV p24 staining (K and O) was detected. The distribution of SMA and p24 appeared concentric with respect to the lumen (J) in the arterial sections with plaque from the HIV-infected individuals. At higher magnification, these sections (M–P) demonstrated the colocalization of the SMA-positive cells and p24 (P), as indicated by the arrows. A–L, scale bar = 25 μm; M–P, scale bar = 170 μm.

Figure 1. HIV infects SMCs in vivo. Immunohistochemical analyses of arteries from uninfected individuals without demonstrable plaque (denoted HIVneg/no plaque in A–D) or with atherosclerotic plaque (denoted HIVneg/plaque in E–H) were performed using antibodies to either human smooth muscle α-actin (SMA, red staining), HIV-p24 (green staining, p24), and DAPI (blue staining, nuclei), and were analyzed by confocal microscopy. DAPI staining was used to identify individual cells, blood vessel morphology, and cell accumulation (A, E, I, M). The arterial sections obtained from uninfected individuals without plaque (A–D) had a typical SMA staining (B) pattern with respect to the lumen (Lu) of the blood vessel and no detectable p24 staining (C). In sections obtained from uninfected individuals with plaque (E–H), SMC-rich areas (F) were present without detectable p24 antigen (G). Similar studies were done on atherosclerotic arterial sections from HIV-infected individuals (denoted HIVpos/plaque in I–P). In these samples, HIV p24 staining (K and O) was detected. The distribution of SMA and p24 appeared concentric with respect to the lumen (J) in the arterial sections with plaque from the HIV-infected individuals. At higher magnification, these sections (M–P) demonstrated the colocalization of the SMA-positive cells and p24 (P), as indicated by the arrows. A–L, scale bar = 25 μm; M–P, scale bar = 170 μm.
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protein produced by HIV-infected cells), CD68 (a mono-

cyte/macrophage marker), and DAPI staining (nuclei).

Arterial sections obtained from uninfected individuals, 
either without (Figure 2, designated as HIVneg/no plaque; A–E) or with detectable atherosclerotic plaque (Figure 2, designated HIVneg/plaque; F–J), had no detectable p24 staining in the vessel wall (Figure 2, C and H). SMC distribution in HIVneg/no plaque were concentric to the lumen (Figure 2, B–E). In sections obtained from uninfected individuals with atherosclerotic plaque, both SMC (Figure 2, G and J) and CD68 (Figure 2, I and J) staining were intense in the fibrous plaque. These two parameters, SMC proliferation and significant monocyte/macrophage infiltration, are characteristic features of atherosclerosis.

In all arterial specimens obtained from HIV-infected individuals with clearly detectable atherosclerotic plaque there was a population of p24-positive SMCs (Figure 2, L, M, O, Q, R, T). In contrast to the inflammatory character-

istic of atherosclerotic plaques in uninfected sections, fewer monocytes/macrophages, ie, CD68-positive cells, were detected (Figure 2, N, O, S, T). There was a distinct population of CD68-positive cells that were HIV-infected (Figure 2, N, O, S, T, arrowheads). The HIV-positive SMCs did not colocalize with CD68-positive cells, suggesting that the infected SMCs are a distinct population from the CD68-positive cells (Figure 2, M–O, S, T). These results indicate that, in vivo, SMCs and the few infiltrated monocyte/macrophages are infected by HIV.

Tissue sections from HIV-Infected Individuals with Atherosclerosis Have Fewer Monocyte/ Macrophages in the Plaque Than Those Specimens Examined from Uninfected Individuals with Atherosclerosis

To demonstrate that HIV-infected SMCs are a distinct cell population from HIV-infected monocyte/macrophages, we performed double staining for SMCs and monocyte/macrophages on sections from normal and atheroscle-

rotic arteries obtained from uninfected and HIV-infected 

individuals. These sections were analyzed by confocal 

microscopy for SMA (a marker for SMCs), HIV p24 (a 

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istic of atherosclerotic plaques in uninfected sections, fewer monocytes/macrophages, ie, CD68-positive cells, were detected (Figure 2, N, O, S, T). There was a distinct population of CD68-positive cells that were HIV-infected (Figure 2, N, O, S, T, arrowheads). The HIV-positive SMCs did not colocalize with CD68-positive cells, suggesting that the infected SMCs are a distinct population from the CD68-positive cells (Figure 2, M–O, S, T). These results indicate that, in vivo, SMCs and the few infiltrated monocyte/macrophages are infected by HIV.
SMC supernatants at 1, 2, 4, 5, 7, 9, and 12 days postinfection. Preincubation with blocking antibody for CD4 (bCD4) reduced viral replication of SMCs infected with HIV92UG021 (Figure 4A), HIV JR-CSF (Figure 4B), and HIVADA (Figure 4C) at least for the first 48 to 72 hours postinfection (Figure 4, asterisk). No nonspecific effects of IgG1, the isotype-matched control antibody (control for bCD4), were detected (Figure 4, IgG). These results suggest that HIV entry into SMCs is mediated, in part, by CD4. To determine whether chemokine receptors are involved in SMC infection, blocking antibodies to CCR5 (dilution, 1:100, 1:200, 1:500, 1:1000, or 1:3000) or to CXCR4 (1, 10, or 30 μg/ml) were used. These antibodies significantly decreased SMC infection by R5-specific or X4-specific virus, respectively (Figure 5, A and B, *P ≤ 0.005, n = 4), without altering entry and subsequent replication of the virus with the unrelated tropism (Figure 5, A and B). Data after 7 days postinfection are shown in Figure 5. Similar results were found at days 9 and 12 after HIV infection (data not shown). CCR5 (TAK-779) and CXCR4 (bicyclam) antagonists also blocked HIV entry and subsequent viral replication, resulting in amounts of p24 similar to those observed with the neutralizing antibodies and did so without altering the replication of virus with the alternative tropism (Figure 5, C and D, *P ≤ 0.005).

As controls for HIV infection and for the efficiency of CD4 neutralizing antibody and the chemokine receptor blockers, human PBMCs were used (data not shown). The CD4 neutralizing antibody reduced HIV92UG021 replication in PBMCs at days 2 and 3 postinfection by 42 ± 3.7 and 32 ± 6.8% as compared to PBMCs just exposed to the virus. CCR5 and CXCR4 blocking agents were

**Figure 2.** HIV-infected tissue sections of atherosclerotic lesions have fewer monocyte/macrophages that are distinct from SMCs. Immunohistochemical analyses of arterial sections from uninfected individuals without demonstrable plaque (denoted HIVneg/no plaque in A–E) or with atherosclerotic plaque (denoted HIVneg/plaque in F–J) were performed using antibodies to human smooth muscle α-actin (SMA, red staining), HIV-p24 (green staining, p24), CD68 (Cyan staining, monocyte/macrophages), and DAPI (blue staining, nuclei), and then were analyzed by confocal microscopy. DAPI staining was used to identify individual cells, blood vessel morphology, and cell accumulation (A, F, K, P). The arterial sections obtained from uninfected individuals without plaque (A–E) had a typical SMA staining (B) pattern with respect to the lumen (Lu) of the blood vessel and no detectable p24 staining (C) and few macrophages, CD68 staining (D). In sections obtained from uninfected individuals with atherosclerotic plaque (F–J), SMC-rich areas (G) were present without detectable p24 antigen (H) and many monocyte/macrophages (I) were detectable, indicating inflammation. In atherosclerotic arterial sections from HIV-infected individuals (denoted HIVpos/plaque in K–O and P–T), HIV p24 staining (N, S) was detected. The distribution of SMA and p24 appeared concentric with respect to the lumen (L, Q) in the arterial sections with plaque from the HIV-infected individuals. CD68 staining in HIVpos/plaque individuals was minimal (N, S) compared to sections obtained from uninfected individuals (D, I) with atherosclerotic plaque. At higher magnification, these sections (P–T) demonstrated the colocalization of the SMA-positive cells and p24 (P), as indicated by the arrows. Some CD68 staining also colocalized with p24 staining (R, S, T, arrowheads), but the SMA-positive population of cells was distinct from the CD68-positive cells, indicating that HIV-infected SMCs in vivo are distinct from the monocyte/macrophages. A–O, scale bar = 25 μm; P–T, scale bar = 170 μm.
HIV infects SMCs in vitro. Human SMCs were stained with antibodies to either human smooth muscle α-actin (αSMA) (B, D) or p24 antigen (p24) (A, C) following infection for 7 days with either HIV-ADA (C, D) or uninfected (A, B). Control uninfected SMCs did not show detectable p24 staining (A). SMCs exposed to HIV (C) had intracellular vesicular p24 staining, notably with the X4 virus; scale bar = 50 μm. E: HIV-infected SMCs produce increasing amounts of p24. To determine the p24 production following HIV exposure, human SMCs were exposed to either an R5 (HIV-ADA or HIV-JR-CSF) or an X4 (HIV-R5G021) virus for 2, 4, 7, 9, and 12 days. Supernatants were analyzed for p24 viral antigen by ELISA. No p24 was detected in uninfected cultures (data not shown). No significant differences were detected between the two R5 viruses and the X4 virus was significantly different from R5 viruses after 7 days postinfection (*P < 0.003, n = 7 independent experiments, mean ± SD).

**HIV Infection Increases SMC Production of the Chemokine CCL2/MCP-1**

CCL2 is a potent chemoattractant for monocytes and plays an important role in both the pathogenesis of HIV infection and of atherosclerosis. We previously demonstrated that CCL2 induces SMCs to express tissue factor, a procoagulant protein that may participate in plaque progression and rupture. It was unknown whether infection by HIV alters SMC production of CCL2. Our hypothesis was that HIV infection would serve as a proinflammatory and/or atherogenic stimulus, leading to augmentation of CCL2 secretion by SMCs. Supernatants from SMC cultures infected with different viral strains were collected at the designated time points and assayed using an ELISA for CCL2. SMC infection by all three viral isolates significantly increased SMC production of CCL2 (*P < 0.005) at 14 days postinfection compared to uninfected SMCs (Figure 7). CCL2 levels remained significantly elevated through day 28, the last time point assayed, as compared to control, uninfected SMCs (Figure 7). The time course of CCL2 production was similar regardless of which strain of viral isolate was used to infect the SMCs.

**Discussion**

The results indicate that SMCs can be infected by HIV, in vivo and in vitro, by a CD4, chemokine receptor-, and assessed in the presence of agents that are known to disrupt endocytosis, including NH₄Cl, chloroquine, bafilomycin-A1, or chlorpromazine. Concentrations of these reagents were selected as those that had been optimized to inhibit maximally the entry of avian leukosis virus (ALV-B) into cells, a virus that requires endocytosis for infection. HIV infection of PBMCs was used as a negative control because HIV entry into leukocytes is not mediated by endocytosis. NH₄Cl (50 mmol/L) and chloroquine (100 μg/ml) are lysosomotropic agents that selectively accumulate in endocytic compartments and increase the endosomal pH. Bafilomycin-A1 (50 μmol/L) is a specific blocker of v-type H⁺-ATPase, and chlorpromazine (50 μmol/L) disrupts clathrin mediated uptake. Blocking endocytosis reduced the infectivity of the SMCs to both R5 and X4 HIV strains and the subsequent viral production, as assessed by p24 production in the SMC supernatant, at 2, 4, 7, and 12 days after viral exposure (Figure 6).

To determine whether endocytosis contributes to viral entry into SMCs, in vitro HIV infection of SMCs was effective in reducing viral entry and subsequent replication after 2 days following viral exposure. The percentage of reduction in viral replication in PBMCs using the higher concentrations of chemokine receptor blockers that were used in SMC cultures were HIV-ADA, 78 ± 9.1%; HIV-JR-CSF, 75 ± 7.5%; or HIV-R5G021, 81 ± 10.7%, as compared to HIV-infected PBMCs without blockers. Thus, our data indicate that HIV infection of human SMCs is CD4- and chemokine receptor-dependent.

**HIV Entry into SMCs Is Mediated by Endocytosis**

To determine whether endocytosis contributes to viral infection of SMCs, in vitro HIV infection of SMCs was
endocytosis-dependent mechanism. This infection also results in increased CCL2 production by SMCs. Based on these data, we propose that HIV infection of SMCs may contribute to the pathogenesis of atherosclerosis observed in HIV-infected individuals.

The advent of highly active antiretroviral therapy coupled with effective prophylaxis for opportunistic infections has resulted in a dramatic increase in survival in individuals with HIV infection. This population of individuals represents one of the most rapidly growing groups with cardiovascular disease globally. The importance of cardiovascular disease in HIV-infected people receiving highly active antiretroviral therapy has been underscored by the reported descending artery. In agreement with the human studies, a single case of HIV-associated coronary arteritis in an infected individual who had died of myocardial infarction and was found to have HIV sequences, as detected by in situ hybridization, in the intima and media of the left anterior descending artery. In agreement with the human studies, a transgenic mouse model carrying a replication-defective HIV-1 provirus develops an extensive vasculopathy characterized by intimal thickening and luminal narrowing.
Our finding of p24 antigen in SMCs in arterial sections from individuals infected with HIV with vascular disease indicates that HIV directly can infect SMCs and therefore may contribute to the pathogenesis of atherosclerosis and the vasculopathy that has been reported in individuals infected with HIV. In all of the tissue sections from different HIV-infected individuals with atherosclerosis, a population of HIV-infected SMCs was detected by p24 staining, suggesting active HIV replication. A striking difference between the sections obtained from atherosclerotic vessels from uninfected people compared to those from HIV-infected individuals was the symmetry of the SMC accumulation in the intima of tissues from HIV-infected people. In the latter sections, the SMC proliferation was concentric to the vessel lumen, and minimal inflammation was detected. These histological findings are reminiscent of allograft vasculopathy as opposed to the more eccentric, focal, lipid-rich plaques that are characteristic of atherosclerosis. In addition, in uninfected individuals with atherosclerosis, more inflammation was detected as evidenced by the infiltration and accumulation of macrophages and SMCs forming a more classic asymmetric atherosclerotic plaque.

SMCs, as a target for HIV infection, had not yet been examined in depth. We previously demonstrated that human SMCs express the CD4 receptor at low levels, as well as the two biologically relevant chemokine receptors for HIV entry, CCR5 and CXCR4. The presence of these receptors on SMCs supports the possibility that SMCs may be a target for HIV infection.

Our data demonstrate that anti-CD4 blocking antibodies, anti-CCR5 antibodies, and TAK-779, as well as anti-CXCR4 antibodies and bicyclam JM-2987, block infection and subsequent viral replication of SMCs as determined by p24 ELISA, indicating that these receptors are essential for viral entry into these cells. HIV-p24 immunostaining of these infected cultures pretreated with chemokine receptor inhibitors were also negative, underscoring that entry is mediated by these receptors. The tropism of the virus also was important, because blockers of CCR5 did not alter activity of CXCR4-dependent viruses, suggesting an entry effect dependent on the gp120 expressed on the surface of the virus. To determine whether endocytosis was required for HIV infection of SMCs, various blockers of endocytosis were used.
of the atherosclerotic process, a more significant infiltration was not observed, it is possible that at earlier stages in inflammation in the HIV-infected atherosclerotic sections filtration in areas with SMC infection. Although significant surprisingly, we observed minimal mononuclear cell infiltration in areas with SMC infection. These viral proteins have been shown to activate the vasculature in other organ systems. 

In HIV infection of T cells, the delivery of its genome into the cytoplasm is by fusion of the envelope with the plasma membrane, mediated by viral interaction with CD4 and CCR5 and/or CXCR4. Viral fusion is a pH-independent mechanism and only requires binding of the virus to CD4 and its coreceptors, CCR5 and/or CXCR4. In contrast, in some other cell types, HIV enters into the cell by endocytosis. For example, in macrophages and dendritic cells, HIV particles bind to surface receptors, including CD4, CCR5, and/or CXCR4, and are taken up by macroinocytosis/endocytosis, suggesting that active infection and subsequent viral release into the cytoplasm can be effectively mediated by vesicular trafficking. 

Our current studies of HIV entry into SMCs demonstrate the participation of the chemokine receptors CCR5 and CXCR4 in binding of the virus to the cell, but also show that endocytosis is essential to trigger infection and subsequent viral replication in SMCs. When the gp120 of the virus binds to these chemokine receptors on SMCs, we propose that HIV is retained on the surface of SMCs and then uses the normal recycling/trafficking of these receptors mediated by endocytosis, to enter into SMCs. Whether small populations of HIV-infected cells with low viral replication contribute to the pathogenesis of HIV is still in debate. Nevertheless, it is clear that even low numbers of HIV-infected dendritic cells and astrocytes can play an important role as potential viral reservoirs. These reservoirs may be silent and persistent for long periods and, under specific inflammatory conditions, can be reactivated and transmit the virus to cells that support high viral replication, such as T cells and monocyte/macrophages. This mechanism of cross-infection is termed dissemination in trans. We propose that HIV-infected SMCs may participate in this trans process. One of the potential signals that recruit these inflammatory cells that support high HIV replication is CCL2. CCL2 is, to date, the most potent monocyte chemoattractant and is also chemotactic for activated T cells. Thus, the high levels of CCL2 expressed by HIV-infected SMCs may recruit monocytes and T cells into the vessel wall, facilitating their subsequent infection. Surprisingly, we observed minimal mononuclear cell infiltration in areas with SMC infection. Although significant inflammation in the HIV-infected atherosclerotic sections was not observed, it is possible that at earlier stages in the atherosclerotic process, a more significant infiltration of HIV-infected monocyte/macrophages and T cells could exist and potentially play a role in HIV infection of the arterial wall in vivo. In addition, CCL2 has other functions in that it promotes a thrombotic potential in the vessel wall by inducing tissue factor, the initiator of the coagulation cascade. CCL2 also has also been shown to increase SMC proliferation in vivo.

Our findings demonstrate HIV infection of SMCs in vivo and in vitro. HIV infection of SMCs in the atherosclerotic plaque and in vitro suggests that direct infection of human arterial SMCs by the virus may be a potential mechanism in a multifactorial paradigm to explain the atherosclerosis and vasculopathy reported in individuals infected with HIV.

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