HIV envelope gp120 activates human arterial smooth muscle cells

Alison D. Schecter*†‡, Adriane B. Berman*‡, Lin Yi*‡, Arevik Mosoian‡, Carrie M. McManus§, Joan W. Berman¶‖, Mary E. Klotman‡, and Mark B. Taubman*‡††**

*Zena and Michael A. Wiener Cardiovascular Institute and Departments of ‡Medicine, **Microbiology, and ††Physiology and Biophysics, Division of Infectious Diseases, Mount Sinai School of Medicine, New York, NY 10029; §DuPont Pharmaceuticals, Experimental Station, Wilmington, DE 19803; and Departments of ¶Pathology and ‖Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, NY 10461

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There have been increasing reports of acute coronary thrombotic events in patients with HIV. Although these clinical events have been attributed primarily to dyslipidemia associated with protease inhibitor therapy, autopsy studies in children with HIV suggest the presence of an underlying arteriopathy. This study demonstrates that the HIV envelope protein, gp120, activates human arterial smooth muscle cells to express tissue factor, the initiator of the coagulation cascade. The induction of tissue factor by gp120 is mediated by two biologically relevant coreceptors for HIV infection, CXCR4 and CCR5, and is also dependent on the presence of functional CD4. Induction of tissue factor by gp120 requires activation of mitogen-activating protein kinases, activation of protein kinase C, and generation of reactive oxygen species, signaling pathways that have protean effects on smooth muscle cell physiology. The activation of smooth muscle cells by gp120 may play an important role in the vascular, thrombotic, and inflammatory responses to HIV infection.

Recent reports of acute coronary thrombotic events have suggested that young patients with HIV may be prone to accelerated coronary artery disease (reviewed in ref. 1). This has been attributed to the use of protease inhibitors, which have adverse side effects such as hypercholesterolemia, hypertriglyceridemia, and insulin resistance. However, evidence from autopsy studies (2, 3) performed in the ''preprotease era'' also demonstrated eccentric atherosclerotic lesions in the proximal coronary arteries and intimal fibrosis, in the absence of traditional cardiac risk factors.

HIV envelope glycoprotein gp120 (gp120) uses chemokine receptors CCR5 or CXCR4, usually with or rarely without the coreceptor CD4, to gain entry into target cells (4). In addition to facilitating viral entry, gp120 initiates signaling events that affect postentry stages of infection and modulate cellular functions, even in the absence of infection.

Smooth muscle cells (SMC) are the major cellular components of the arterial wall, and their proliferation and migration is key to the development of atherosclerotic plaques. Tissue factor (TF), the initiator of coagulation (5), is abundant in the plaque and in the injured arterial wall. Exposure of TF by plaque rupture may cause thrombosis and lead to acute myocardial infarction and unstable angina. Similarly, acute arterial injury, such as that produced during percutaneous coronary interventions, may also expose TF and promote thrombosis (reviewed in ref. 6).

We have recently shown that SMC possess functional CCR5 (7). We now report that treatment of human SMC with HIV gp120, at concentrations similar to those found in the blood of patients with HIV, induces TF activity. We also report that in addition to CCR5, human SMC possess functional CXCR4. The induction of TF activity by agonists of CXCR4 involves different signaling pathways from those activating CCR5. Importantly, the induction of TF activity by gp120 via either chemokine receptor requires functional CD4. These results provide evidence for direct viral activation of human SMC and may provide insight into the mechanism underlying the increased incidence of acute coronary syndromes and prothrombotic states in patients with HIV.

Methods

Reagents. Recombinant human macrophage inflammatory protein-1β (MIP-1), platelet-derived growth factor, recombinant human IL-16, recombinant human stromal cell-derived growth factor 1-α (SDF-1), and monoclonal antibodies to human CXCR4 and CCR5 were from R & D Systems. FBS, 8-bromoadenosine 3′:5′-cyclic monophosphate sodium (Tiron), actinomycin D, phorbol 12,13-dibutyrate, a monoclonal anti-human CD4 antibody, clone Q4120, and its isotype-matched IgG control were from Sigma. PD98059, SB203580, U0126, and *N*-acetylcysteine were from Calbiochem.

A monoclonal antibody to CD4 (Leu3A) and its corresponding nonimmune IgG were from Becton Dickinson Immunocytometry Systems. Monoclonal antibody to CD4 (clone SK3) for Western blot was purchased from BD Biosciences (San Jose, CA). Rabbit antiserum to HIV_{SF2} gp120 was provided by Dr. Kathelyn Steimer (Chiron, Emeryville, CA) through the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health) (8, 9). Rabbit 1169 antisera to HIVgp120 was a generous gift from Dr. R. W. Doms (University of Pennsylvania). Recombinant gp120 from HIV-SF2 strain (10) was a generous gift from Dr. Avindra Nath (University of Kentucky Medical Center) and Chiron. CCR5-specific gp120 (r5gp120), derived from the macrophage tropic HIV-1 strain JRFL; CXCR4-specific gp120 (x4gp120), a lab adapted HIV strain (HXB) (11); and HXBD368R, a mutant x4gp120 lacking a functional CD4 binding site generated by mutagenesis at site 368 (12) were generous gifts from Dr. R. W. Doms. The oligomeric gp120 (HIV-1 IIIB), recombinant protein 4683 was from the AIDS Research and Reference Reagent Program (Division of AIDS, National Institute of Allergy and Infectious Diseases, National Institutes of Health).

Cell Culture. Human aortic SMC were prepared and cultured in DMEM supplemented with 10% FBS as described (13). To achieve quiescence, cells were incubated in 0.3% serum for 24 h. Under these conditions, 24-h incorporation of [^{3}H]thymidine into DNA is $<$10% of that seen with 10% serum (data not shown). THP-1 cells were obtained from the American Type Culture Collection and were maintained in 25 mM Hepes-buffered RPMI-1640 containing 10% FBS/1 mM glutamine/100

Abbreviations: r5gp120, CCR5-specific gp120; x4gp120, CXCR4-specific gp120; gp120, glycoprotein gp120; MAPK, mitogen-activated protein kinase; MIP-1, macrophage inflammatory protein-1β; PKC, protein kinase C; ROS, reactive oxygen species; SMC, smooth muscle cells; SDF-1, stromal cell-derived growth factor-α; TF, tissue factor.

†To whom reprint requests should be addressed. E-mail: alison.schecter@.mssm.edu.

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units/ml penicillin/100 μg/ml streptomycin. Primary human endothelial cells were harvested from umbilical veins treated with 1% collagenase as described (14).

Western Blot Analysis was performed as described (15). Proteins were separated by 7.5% SDS/PAGE and transferred onto nitrocellulose. Membranes were blocked with 5% milk and treated with clone SK3, a monoclonal antibody (0.5 μg/ml) against human CD4 followed by a horseradish peroxidase-labeled goat polyclonal antibody against mouse IgGκ. Signals were visualized by chemiluminescence according to manufacturer's protocol (Amersham Pharmacia).

Reverse Transcription–PCR. Total RNA was isolated as described (16). Primer pairs for the human CD4 cDNA were from Maxim Biotech (San Francisco). The Titan One Tube reverse transcription–PCR system (Roche Molecular Biochemicals) was used as per the manufacturer's instructions. Initial incubation was at 50°C for 30 min. This was followed by 42 cycles of 30 s at 94°C, 60 s at 55°C, 60 s at 72°C, and a final incubation for 10 min at 72°C.

Determination of TF Activity. For most experiments, SMC in 35-mm culture plates were incubated in 0.3% FBS 24 h before treatment. Agonists were added directly to the plates, and TF activity was measured 4 h after treatment. In antibody-blocking experiments, SMC were incubated with antibody at 37°C for 1 h before addition of agonists. TF activity was measured as described (7). Cells were washed two times with 10 mM Hepes (pH 7.6)/140 mM NaCl/4 mM $CaCl_2$, trypsinized, and then resuspended in 0.5 ml of the same solution. An 80-μl aliquot of the suspension was diluted 1:1 with 15 mM octyl-β-D-glycopyranoside. Factors VIIa and X were added to final concentrations of 1 and 150 nM, respectively. Aliquots (40 μl) were then taken at 2, 4, and 6 min and placed in a 96-well plate containing 100 μl of Bicine buffer (pH 8.5, 1 g/liter BSA/25 mM EDTA). Factor Xa was assayed by adding 25 μl of Spectrozyme (2 mM) and measuring the absorption at 405 nm at 35°C. The concentration of Xa was calculated from the slope of the absorption over time. Femtomoles of TF/plate were obtained assuming a k_{cat} of the TF–VIIa complex of 300 min^{-1} at 20°C.

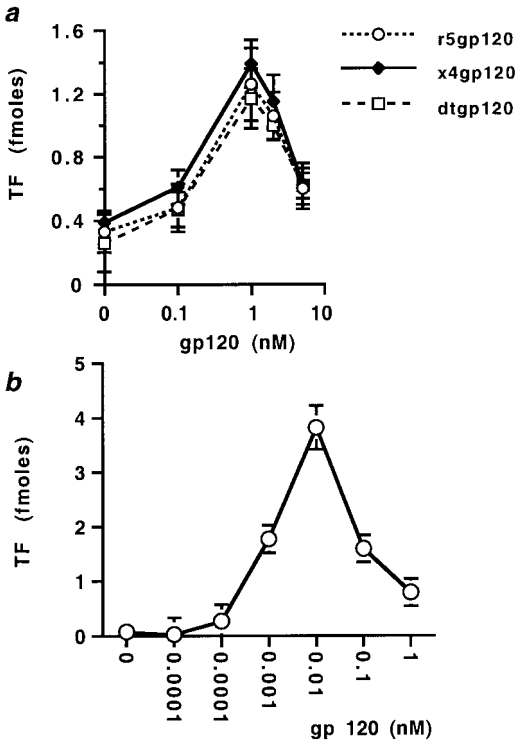

Fig. 1. Induction of TF activity by gp120 in human arterial SMC. SMC, at 80% confluence in 35-mm plates, were incubated in 0.3% FBS for 24 h to induce quiescence and were subsequently treated for 4 h with monomeric x4gp120, r5gp120, or dtgp120 (*a*) or oligomeric CXCR4-specific gp120 (*b*) at the concentrations indicated (nM). TF activity (calculated from factor Xa generation) was measured from cells lysed in detergent and is expressed as fmol/plate. Values represent the average ± SD of three experiments performed on duplicate plates.

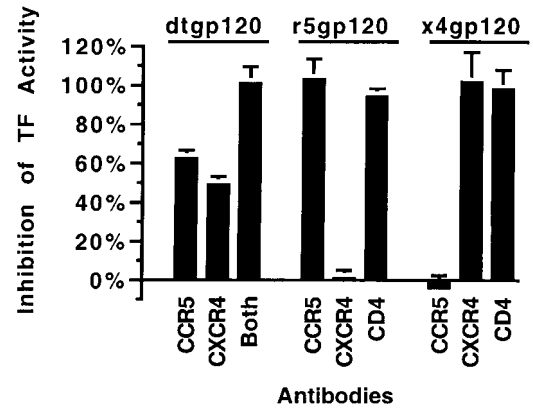

Fig. 2. Role of CD4 and chemokine receptors in the induction of TF by gp120. Quiescent SMC were treated for 4 h with monomeric dtgp120 (1 nM), r5gp120 (1 nM), or x4gp120 (1 nM) (indicated at top) alone or in the presence of monoclonal blocking antibodies (indicated at the bottom) to CCR5, CXCR4, or CD4. Some SMC were also treated with a combination of CCR5 and CXCR4 antibodies (both). TF activity was measured from cells lysed in detergent. Values are expressed as percent inhibition of TF activity. All experiments were performed in duplicate on duplicate plates and are expressed as the average ± SD.

Results

Induction of TF Activity by gp120. Quiescent SMC were treated with monomeric CCR5-specific (r5gp120), CXCR4-specific (x4gp120), and dual tropic (dtgp120) gp120 for 4 h. These gp120 induced a concentration-dependent increase in TF activity (Fig. 1*a*), which peaked at 1 nM. An oligomeric CXCR4-specific gp120 induced peak TF activity at 0.01 nM (Fig. 1*b*).

Antibodies to CXCR4 (10 μg/ml) and CCR5 (10 μg/ml) completely blocked the induction of TF activity by x4gp120 or r5gp120. There was an ≈50% reduction in TF activity induced by the dtgp120 in the presence of either CXCR4 or CCR5 antibody; treatment with both antibodies completely abrogated the induction of TF by dtgp120 (Fig. 2). These data suggest that CXCR4 and CCR5 contribute equally to the effect of dtgp120 and that other HIV coreceptors are not involved in gp120 activation of human SMC.

Treatment with 10 μM actinomycin D inhibited induction of TF activity by 100% ± 3.5% for x4gp120 and 97% ± 4.2% for r5gp120, suggesting that the increase in TF activity was secondary to new transcription. Two different antisera, HIV-1_{SF2} gp120 (1:500) and HIV1169 (1:500) against recombinant gp120, completely inhibited the induction of TF activity by dtgp120 or r5gp120 (data not shown).

SDF-1 Is a Ligand for Human Arterial SMC. CXCR4 has not previously been found on SMC (17). Because the x4gp120 induced TF activity, SMC were treated with SDF-1, the native CXCR4 ligand. SDF-1 induced TF activity at a concentration (0.1 μg/ml) reported to result in calcium mobilization in other cell types (17)

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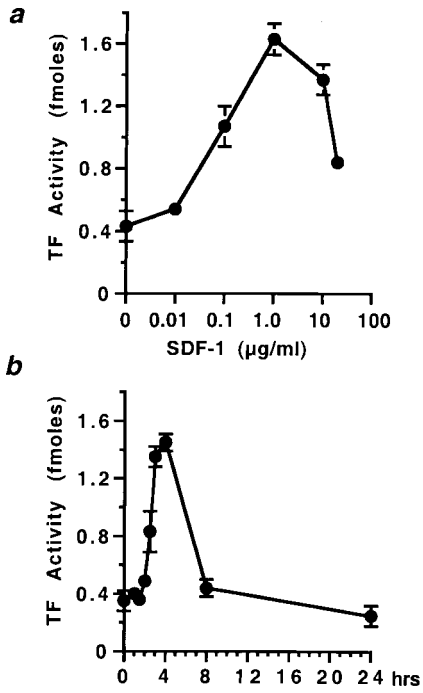

Fig. 3. Induction of TF by SDF-1. Quiescent SMC in 35-mm plates were treated for 4 h with human recombinant SDF-1α at the concentrations indicated (*a*) and with 1.0 μg/ml SDF-1 for the times indicated (*b*). TF activity was measured from cell lysates and is expressed as fmol/plate. Values represent the average ± SD from three experiments performed on duplicate plates.

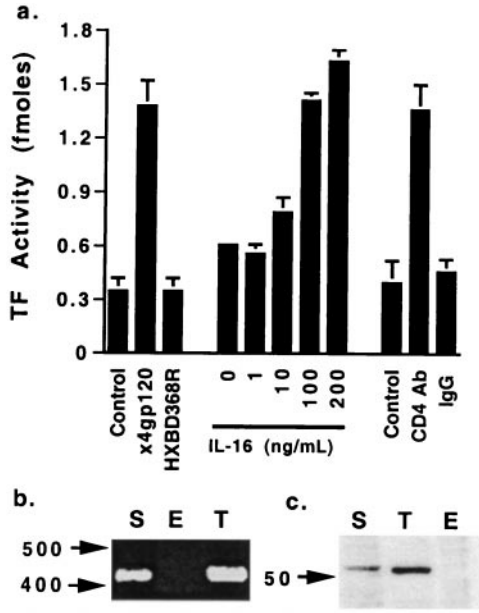

Fig. 4. Human SMC possess functional CD4. (*a*) Quiescent SMC were treated for 4 h with DMEM (control), 1 nM x4gp120, a mutant x4gp120 (HXBD368R; 1 nM) lacking a functional CD4 binding site, or IL-16 at the concentrations indicated. In separate experiments, SMC were also treated for 4 h with an activating CD4 antibody (CD4Ab) or its isotype-matched IgG. TF activity was measured from cell lysates and is expressed as fmol/plate. Values represent the average ± SD from three experiments performed on duplicate plates. (*b*) Reverse transcription–PCR was performed on total RNA from SMC (S), THP-1 cells (T), or human umbilical vein endothelial cells (E) by using primers spanning a 438-bp region of the human CD4 mRNA. The experiment was performed twice. The positions of 400- and 500-bp size markers are indicated. (*c*) Western blot analysis with a monoclonal antibody to CD4 was performed on cell lysates from human SMC (S), THP-1 cells (T), and human umbilical vein endothelial cells (E). The experiment was performed three times. The position of a 50-kDa size marker is shown.

(Fig. 3*a*). TF activity peaked 4 h after treatment (Fig. 3*b*), similar to the time course seen with other TF agonists in SMC (18).

CD4 Is Required for Envelope-Mediated Induction of TF Activity in SMC. Gp120 signals via CD4-dependent and -independent mechanisms (19). To determine whether CD4 was required for the induction of TF activity, SMC were treated with r5gp120 or x4gp120 in the presence of a monoclonal antibody to human CD4. The CD4 antibody (leu 3A, 10 μg/ml) blocked the induction of TF activity by x4gp120 and r5gp120 (Fig. 2).

To establish further that gp120 binding to CD4 was required for gp120 signal transduction, SMC were treated with 1 nM HXBD368R, a mutant x4gp120 that is unable to bind CD4. HXBD368R did not induce TF activity (Fig. 4*a*). To determine whether binding to CD4 alone activates SMC, cells were treated with either a native ligand of CD4, interleukin-16 (20), or with a monoclonal anti-human CD4 antibody, Q4120 (5 μg/ml), that activates CD4 (21). Both induced TF activity, demonstrating that CD4 can function as an independent receptor in SMC (Fig. 4*a*).

To our knowledge, CD4 has not been found on SMC. By reverse transcription–PCR, a product of the expected size (≈438 bp) was present when using SMC RNA. A band of identical size was seen with THP-1 (T), a myelomonocytic cell line known to possess high levels of CD4 mRNA (Fig. 4*b*). Human umbilical vein endothelial cells, which are not known to possess CD4, yielded no detectable product(s). Western blot analysis using the CD4 antibody SK3 revealed a major species at the expected molecular mass of ≈55 kDa from extracts of SMC, identical in size to that from THP-1 cell extracts (Fig. 4*c*). Extracts from human umbilical vein endothelial cells produced no detectable band at this molecular weight.

TF Induction Requires Activation of Protein Kinase C (PKC) and Generation of Reactive Oxygen Species (ROS). We have previously reported that the induction of TF in human SMC by the CC chemokine monocyte chemoattractant protein-1 is PKC-dependent (13). To determine whether PKC is involved in the induction of TF activity by agonists of CCR5 and CXCR4, SMC were treated with agonists in the presence of staurosporine, an inhibitor of PKC. Staurosporine blocked the increase in TF activity in response to x4gp120, r5gp120, MIP-1, and SDF-1 (Fig. 5*a*). SMC were also treated for 24 h with phorbol 12,13-dibutyrate to down-regulate cytoplasmic PKC activity (22). Subsequent treatment with MIP-1, SDF-1, or coreceptor-specific gp120 failed to induce TF activity (Fig. 5*a*).

ROS are associated with inflammation and act as mediators of SMC activation by numerous agonists (23). Tiron, an ROS chelator, and *N*-acetyl cysteine, an antioxidant, inhibited the induction of TF activity by x4gp120, r5gp120, MIP-1, and SDF-1. This suggests that in SMC, CXCR4- and CCR5-mediated induction of TF activity requires the generation of ROS (Fig. 5*b*).

Induction of TF Activity via CXCR4 and CCR5 Is Mediated by Distinct Mitogen-Activated Protein Kinase (MAPK) Pathways. Many chemokines signal in part via MAPKs (15). In some cases, different MAPK cascades are used depending on whether the chemokine receptor, CD4, or both are activated (21). SMC were treated with agonists in the presence of p42/44 MAPK inhibitors, PD98059 or U0126, or a p38 MAPK inhibitor, SB203580. The p42/44 inhibitors blocked the induction of TF activity by MIP-1 and r5gp120 but had no effect on the induction of TF activity by SDF-1 and x4gp120 (Fig. 5*c*). The p38 inhibitor had no effect on the induction of TF activity by MIP-1 or r5gp120 but completely

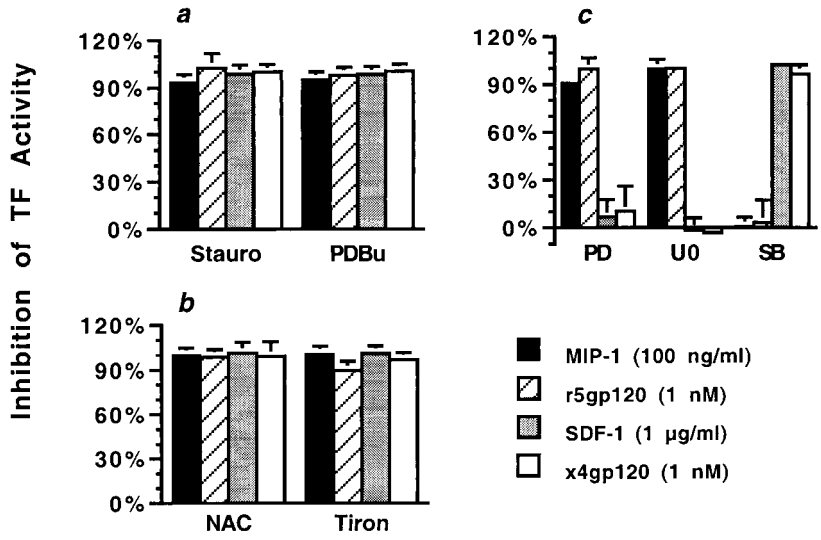

Fig. 5. The role of MAPK pathways, ROS generation, and PKC activation in the induction of TF by gp120. (*a*) SMC, at 80% confluence in 35-mm plates, were incubated in 0.3% serum for 24 h to induce quiescence and were then treated with agonists for 4 h alone or in the presence of 1.0 nM staurosporine (Stauro). In parallel experiments, cells were treated with 1 μM phorbol 12,13-dibutyrate during the 24-h incubation in 0.3% serum to down-regulate PKC and were then treated for 4 h with agonists. (*b*) Quiescent SMC were treated for 4 h with agonists alone or in the presence of 10 mM *N*-acetyl cysteine (NAC) and 10 mM Tiron, inhibitors of ROS. (*c*) SMC were treated for 4 h with agonists alone or in the presence of inhibitors of p42/44 MAPK [PD98059 (PD) and U0126 (UO)] or p38 MAPK [SB203580 (SB)], all at concentrations of 20 μM. Cells were then lysed in detergent, and TF activity was measured. Values are expressed as percent inhibition of TF activity compared with SMC treated with agonists alone. Values represent the average ± SD from three experiments performed on duplicate plates.

blocked its induction by SDF-1 and x4gp120. These data demonstrate that in SMC, CCR5 and CXCR4 are coupled to TF induction via different MAPK pathways and that the engagement of CD4 by gp120 does not alter MAPK signaling.

Concomitant activation of both chemokine receptors by maximal effective concentrations of either their natural ligands (i.e., SDF-1 + MIP-1) or by a combination of ligand and gp120 (i.e., r5gp120 + SDF-1, or x4gp120 + MIP-1) resulted in a doubling of TF activity compared with each agonist alone (Fig. 6). In contrast, activation of a single receptor with both the ligand and gp120 (i.e., r5gp120 + MIP-1, or x4gp120 + SDF-1) did not increase TF activity. These data suggest that activation of the two MAPK pathways acts additively on TF transcription and further suggests that each receptor is maximally stimulated. Simultaneous treatment with x4gp120 and r5gp120 failed to produce an additive effect on TF activity, suggesting that the amount or the localization of CD4 on the cell surface may be limiting.

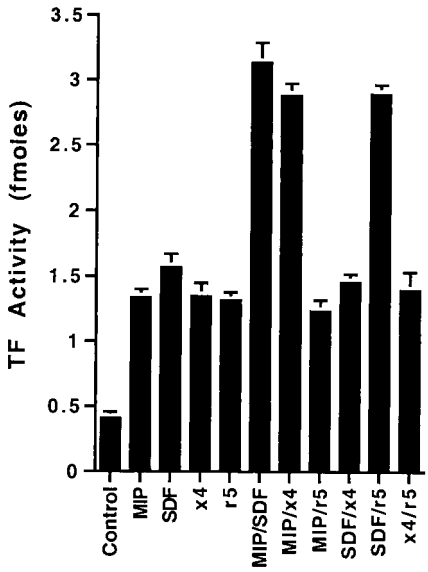

Fig. 6. CCR5- and CXCR4-mediated induction of TF is additive. Quiescent SMC were treated with MIP-1, SDF-1, CCR5-specific gp120 (r5), CXCR4-specific gp120 (x4) alone, or in the combinations indicated for 4 h (concentrations as in Fig. 5). TF activity was measured from cell lysates and is expressed as fmol/plate. Values represent the average ± SD from three experiments performed on duplicate plates.

Discussion

SMC are the major cellular elements of the vessel wall. Abnormal SMC activation is associated with hypertension (24), atherosclerosis (25), and arteriogenesis (26). The major findings of this study are that (*i*) human arterial SMC are activated by the HIV envelope protein, gp120, (*ii*) CD4 is present on SMC and is necessary for SMC activation by gp120, (*iii*) functional CXCR4 is present on SMC, and (*iv*) CCR5 and CXCR4 transduce signals in SMC via distinct MAPK pathways.

The effects of HIV infection, such as immune dysfunction and cell death, occur either as a result of direct viral infection or from the activation of bystander cells, in part by its viral proteins. Initially, the role of gp120 was thought to be primarily that of viral fusion. More recently, gp120 has been shown to activate cells in the absence of viral infection, leading to apoptosis of endothelial cells (27), T lymphocytes (28), and neurons (29) and to cell migration in T cells (30). To our knowledge, human arterial SMC have not been reported to be infected by HIV; in addition, our own preliminary studies have not detected HIV infection in SMC. However, we do demonstrate that SMC are targets for gp120 and represent novel bystander cells for HIV activation.

The concentration of monomeric gp120 that induced maximal TF activity is similar to that reported in other studies examining the effects of gp120 on migration (31) and apoptosis (27, 29) in T cells, endothelial cells, and neurons. The relationship between the tissue concentrations of HIV virions and levels measured in serum is unknown. There is evidence that in certain tissues, such as lymph nodes, there may be very high, localized concentrations of virus. For example, Gray *et al.* (32) found $52{,}795 \times 10^6$ HIV RNA copies/g of lymph tissue in the interstitial space. This is in contrast to the usual concentration of viral particles detected in infected patients' blood (10^5–10^6/ml). The concentration of viral particles in the arterial wall or atherosclerotic plaque has yet to be determined. Because leukocyte accumulation is a cardinal feature of atherosclerotic plaque progression, it is certainly possible that arterial SMC could be exposed to unusually high levels of virions from infected leukocytes.

Virtually all of the studies examining the effects of HIV envelope in cell culture have used monomeric gp120. Because gp120 *in vivo* oligomerizes (33), we performed experiments using gp120 oligomers obtained from the AIDS Research and Refer-

ence Reagent Program (National Institutes of Health). Oligomeric gp120 induced TF activity at a concentration 100- to 1,000-fold less than that observed with the monomeric form. This concentration may be more likely to be attainable under physiologic conditions. In addition, a recent study demonstrated that noninfectious HIV virions, which maintain structural integrity, induced T cell apoptosis at concentrations 10,000 to 100,000 times less than those required for recombinant monomeric gp120 (34). Thus, even using oligomeric gp120 may overestimate the amount of virus required to transduce intracellular signals *in vivo*. Ultimately, *in vivo* experiments will be required to fully assess the impact of HIV on SMC biology.

Gp120 activates a variety of signaling pathways in lymphocytes, including phosphatidylinositol-3 kinase, the protein tyrosine kinases Pyk2 and Lck, focal adhesion kinase, the serine/threonine kinase Raf-1, and MAPK p42/44 (reviewed in ref. 19). We demonstrate that in SMC, gp120 induces two different MAPK pathways. MAPK is important in HIV replication and virulence (21). In SMC, many proteins including monocyte chemoattractant protein-1 (15) initiate MAPK signal transduction. MAPK signal transduction in SMC is critical to such processes as migration and proliferation. The activation of p42/44 or p38 MAPKs also results in the induction of numerous transcription factors including c-fos, c-jun, and ATF-2 (reviewed in ref. 35). It is therefore likely that the effects of gp120 on SMC will not be limited to TF induction and will include other MAPK-dependent cellular functions. Likewise, the induction of TF by gp120 is not likely to be SMC-specific, and other cells possessing CXCR4 and CCR5, such as macrophages, may also respond to gp120 by producing TF.

We found no differences in signaling between native ligand and gp120 in SMC. In studies using human T cells, SDF-1 induced phosphorylation of p42/44 MAPK, whereas x4gp120 did not (19). Similarly, in primary macrophages, differences in ionic channel activation were found in response to treatment with gp120 as compared with MIP-1 and SDF-1 (36). The coupling of CXCR4 to p38 MAPK in SMC differs from that reported in other cell types, where SDF-1 activates p42/44 MAPK (21). One explanation for the differences in MAPK signaling is that gp120 from different HIV strains, which the other studies used (21), may bind to different domains of the CXCR4 receptor and activate different signaling pathways. In addition, glycosaminoglycans on the SMC surface may affect signaling pathways used by gp120 or native chemokines in a cell-specific manner (37). Whatever the cause, in SMC, the chemokine coreceptor seems to be a major determinant of the MAPK pathway used.

In addition to MAPKs, activation of PKC and generation of ROS are involved in mediating TF regulation in SMC by gp120, MIP-1, and SDF-1. ROS participate in chronic inflammation, HIV replication, and apoptosis of immune system cells seen in HIV-infected subjects (38). In SMC, ROS are mediators of growth factor- and cytokine-induced cell migration, mitosis, and apoptosis (15). Dysregulation of signal transduction pathways involving PKC and ROS have been postulated as potential mechanisms of nervous system alterations in HIV-1-related dementia complex (39). As with MAPK, the importance of PKC and ROS in regulating numerous cell functions suggests that gp120, MIP-1, and SDF-1 may have protean effects on SMC biology.

This study also demonstrates the presence of functional CD4 on SMC. CD4 has been found on thymocytes, T cells, macrophage/monocytes, and Langerhans cells but not, to our knowledge, on arterial SMC. CD4 is required for gp120 signaling in SMC, in that a mutant x4gp120 defective for CD4 binding failed to activate SMC. In addition, pretreatment with an antibody against CD4 blocked the response to r5gp120 and x4gp120. It is not clear whether the requirement is only to ensure proper conformational changes in gp120 or if CD4 activation plays a role in the signaling in SMC. Although CD4 generally functions by interacting with the T cell receptor, it also has been identified as the receptor for IL-16 (20). We used IL-16 and a CD4-activating antibody (21) to establish that CD4 can act independently of its coreceptors to transduce a procoagulant signal.

We have also established that SMC possess functional CXCR4. CXCR4 has been previously detected on neurons (40), astrocytes (40), microglia (40), endothelial cells (17), and T cells. Mice lacking CXCR4 or its ligand SDF-1 die *in utero* and are defective in vascular development, hematopoiesis, and cardiogenesis (41). SDF-1 is highly expressed in atherosclerotic but not in normal vessels (42). In the atherosclerotic plaque, TF is present in the same cellular elements (SMC, endothelial cells, and macrophages) as SDF-1 (6). SDF-1 may act as an agonist for TF induction in these cells and thus contribute to the procoagulant milieu of the atherosclerotic plaque.

Concomitant activation of CXCR4 and CCR5 results in levels of TF activity that are double that seen by activating either receptor alone or by treating with platelet-derived growth factor, the most potent activator of TF in SMC (18). TF-mediated coagulation is a threshold phenomenon that depends on exposure of active TF on cell surfaces (5). We have recently demonstrated (43) that a single agonist was not sufficient to generate significant TF activity on the surface of cultured endothelial cells, whereas treatment with two agonists was. The coactivation of CCR5 and CXCR4 by a combination of their natural ligands and/or circulating viral proteins may potentiate TF activity and thus enhance plaque thrombogenicity.

TF is a primary determinant of the thrombogenicity of human atherosclerotic plaques and has been implicated in SMC proliferation and neointimal thickening following vascular injury (44) and in the development of cardiac vasculopathy (45). Factor Xa, the product of proteolytic cleavage of factor X by the TF–FVIIa complex, can cleave and thus activate protease-activated receptor 2 (PAR2) (46). TF–FVIIa may also directly activate PAR2 (46). Thrombin, generated during activation of coagulation, regulates cell proliferation, migration, the synthesis of matrix, and the secretion of inflammatory cytokines (47). Additionally, TF may be important in tumor metastasis by activating extracellular protease-dependent signaling pathways and through intracellular links to the actin cytoskeleton (48). Thus, the generation of TF by HIV-related proteins may have protean pathologic effects on the arterial wall beyond initiating thrombosis.

Until recently, the prognosis for patients with HIV was so poor that concerns regarding chronic diseases such as atherosclerosis were not considered germane. In the preprotease era, two studies (2, 49) that examined coronary arteries at autopsy from AIDS patients (23–32 years old) found evidence of accelerated atherosclerosis and arteriopathy. The arteriopathy involved thickening of the SMC-rich intima, similar to that seen with chronic rejection in cardiac transplant patients. Joshi *et al.* (3) reported a distinct arteriopathy in pediatric AIDS patients involving small and medium-sized arteries, which resulted in coronary luminal narrowing. There have also been increasing reports of acute coronary and arterial thrombotic events in patients with HIV on protease inhibitors (1). The cause of these events has yet to be determined. Hypotheses include dyslipidemia secondary to protease inhibitors, a primary effect of HIV infection on the arterial wall, or acceleration of atherosclerosis by AIDS-related arterial wall inflammation in patients already at risk for coronary artery disease. Other HIV-related thrombotic sequelae, without an established mechanism, include thrombotic microangiopathy, acute large artery thromboses, and deep vein thromboses (reviewed in ref. 50). The induction of TF in SMC and other cells in HIV may contribute to these thrombotic states.

In addition to its role as a procoagulant, TF is also a marker of SMC activation. Our studies suggest that MAPK, PKC activation, and ROS generation are involved in the induction of TF by gp120. We therefore postulate that gp120 will affect myriad cellular phenomena in SMC that may facilitate the progression of HIV infection and the inflammatory response to HIV.

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