HIV-1 Envelope Glycoprotein 120 Increases Intercellular Adhesion Molecule-1 Expression by Human Endothelial Cells

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SUMMARY: Human immunodeficiency virus type 1 (HIV-1) infection is often associated with central nervous system damage and vascular complications. However, the mechanisms of this association are largely unknown. We examined the effect of HIV-1 envelope glycoprotein 120 (gp120) on cell adhesion molecule expression by endothelial cells. We found, for the first time, that both soluble and membrane-bound gp120 could significantly increase the expression of human endothelial intercellular adhesion molecule-1 (ICAM-1) at both mRNA and protein levels, but not vascular cell adhesion molecule-1 and E-selectin. The specificity of gp120-mediated response was demonstrated by blocking experiments using a specific monoclonal antibody against gp120, which successfully abolished the gp120-mediated increase of ICAM-1 expression. Furthermore, there was a significant increase of human monocytic cell line THP-1 adherence onto the gp120-treated endothelial monolayers. This increased cell adhesion was effectively blocked by either anti-gp120 or anti-ICAM antibodies. These findings suggest that HIV-1 gp120-mediated endothelial ICAM-1 expression could be one of the important mechanisms of HIV-1 pathogenesis. (*Lab Invest* 2002, 82:245–255).

Both human immunodeficiency virus (HIV)-positive and acquired immune deficiency syndrome (AIDS) patients often have significantly increased levels of circulating cell adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular adhesion molecule-1 (VCAM-1) (Greenwood et al, 1998; Seigneur et al, 1997). Serum ICAM-1 levels correlate well with AIDS progression in HIV type 1 (HIV-1)-infected patients (Galea et al, 1997). Endothelial cells and leukocytes from these patients exhibit a significant increase of such adhesion molecules (Pillet et al, 1999; Zietz et al, 1996). HIV-1 particles also incorporate many cell adhesion molecules (Guo and Hildreth, 1995). HIV-associated ICAM-1 is biologically active and can enhance virus infectivity (Fortin et al, 1997) and virus-induced syncytium formation (Gruber et al, 1991). ICAM-1-bearing viruses are less susceptible to neutralization by anti-envelope glycoprotein 120 (gp120) monoclonal antibodies than the same virus produced in cells expressing no ICAM-1 (Sawyer et al, 1994). HIV-1 replication in monocytes/macrophages is significantly enhanced by interaction with endothelial cells through cell adhesion molecules (Gilles et al, 1995). In addition, alterations in expression of cell adhesion molecules by leukocytes and

endothelial cells can increase leukocyte migration into the tissues, which may result in certain organ damage. Indeed, HIV-1-infected leukocytes can transmigrate into the central nervous system and cause encephalopathy (Nottet, 1999). Endothelial cell dysfunction and leukocyte infiltration may also be attributable to the increased frequency of vascular complications such as atherosclerosis in HIV-1-infected patients (Constans et al, 1995; Tabib et al, 2000).

Several factors may contribute to the increased levels of cell adhesion molecules in HIV-1 infection. Elevated levels of proinflammatory cytokines such as TNF- α and IL-1 β have been detected in serum of AIDS patients and in macrophages isolated from HIV-1-infected individuals (Butl et al, 1993; Lahdevirta et al, 1988). Clearly, these cytokines are able to induce expression of endothelial adhesion molecules. HIV-1-infected monocytes/macrophages are able to induce the expression of E-selectin and VCAM-1 on brain microvascular endothelial cells (Lahdevirta et al, 1988). Furthermore, HIV-1 appears to be permissive for infection of brain endothelium (Moses et al 1993) and microvascular endothelium from liver sinusoids (Cenacchi et al, 1992), kidney glomerulus (Green et al, 1992), and adipose tissue (Steffan et al, 1992). Thus, direct HIV-1 infection might also activate these endothelial cells. On the other hand, HIV-1 viral proteins such as Tat and envelope glycoproteins may be involved in endothelial cell activation. Treatment of human umbilical vein endothelial cells (HUVECs) or human microvascular endothelial cells with Tat induces the cell surface expression of ICAM-1, VCAM-1, and E-selectin (Dhawan et al, 1997; Mrowiec

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et al, 1997). Circulating HIV-1 gp120 is found in HIV-1-infected individuals and it could be derived from shedding during viral turnover (Oh et al, 1992). HIV-1 gp120 is able to up-regulate ICAM-1 expression in glial cells through the activation of protein kinase C and tyrosine kinase (Shrikant et al, 1996). Peripheral blood mononuclear cell (PBMC) cultures exposed to gp120 have a dose-dependent increase of ICAM-1 expression (Capobianci, 1996). HIV-1 gp41 could also enhance ICAM-1 expression on H9 monocytoid cell line (Chen et al, 1994). In addition, gp120 could enhance monocyte adhesion to the exposed endothelial cells of both human saphenous vein and internal thoracic arteries in vitro (Stefano et al, 1998). However, little is known about the direct effect of gp120 on cell adhesion molecule expression by endothelial cells. It is believed that HIV-1-mediated endothelial dysfunction, including alteration of cell adhesion molecule expression, could have significant clinical consequences on HIV-1 infection, and it could affect virus infectivity, disease progression, neuropathogenesis, and atherosclerosis in the patients. In this study, we have investigated whether HIV-1 gp120, both in soluble and membrane-bound forms, could induce cell adhesion molecule expression by several types of endothelial cells. The observations made in this study suggest that HIV-1 gp120 increases ICAM-1 expression, but not VCAM-1 and E-selectin, by endothelial cells as well as enhances monocyte cell adhesion to the gp120-treated endothelial cells.

Results

HIV-1 gp120 Increases ICAM-1 mRNA Expression by Several Types of Endothelial Cells

To test whether recombinant HIV-1 gp120 could induce cell adhesion molecule expression by endothelial cells, we initially used immortalized human dermal microvascular endothelial cells (HMECs) (Ades et al, 1992) with treatment of soluble gp120, and analyzed ICAM-1, VCAM-1, and E-selectin expression using RT-PCR. Incubation of HMECs with gp120 (5 μ g/ml) enhanced ICAM-1 mRNA expression in a time-dependent manner (Fig. 1A). HMECs constitutively expressed low levels of ICAM-1 mRNA, which was maximally enhanced up to $140 \pm 10\%$ by 8- and 16-hour treatments with gp120 ($p < 0.05$; $n = 4$). Having established the optimal kinetics of expression, we performed dose-response experiments to determine the concentrations of HIV-1 gp120 that effectively enhanced ICAM-1 mRNA expression. We cultured HMECs with treatment of different doses of gp120 for 16 hours, and showed that ICAM-1/ β -actin density increased significantly by 65%, 89%, and 111%, corresponding to 0.5-, 5-, and 10- μ g/ml gp120 treatments, respectively, compared with controls without gp120 treatment (Fig. 1B; $p < 0.05$; $n = 4$).

To further confirm the effect of HIV-1 gp120 on ICAM-1 expression, we used four types of human primary endothelial cells, including dermal microvascular endothelial cells (MECs), HUVECs, lung micro-

Figure 1.

Soluble Human immunodeficiency virus-1 (HIV-1) envelope glycoprotein 120 (gp120) increases intercellular adhesion molecule-1 (ICAM-1) mRNA expression by human dermal microvascular endothelial cells (HMECs). A, Time-course study showing that, by semiquantitative RT-PCR analysis, the treatment of gp120 (5 μ g/ml) significantly enhanced endothelial ICAM-1 mRNA expression. The maximal ICAM-1 expression is at 8 and 16 hours after treatment. B, Dose-dependent study showing that the treatment with gp120 for 16 hours stimulates endothelial ICAM-1 expression in the dose ranging from 0.05 to 10 μ g/ml. The gp120 treatment at 0.5, 5, and 10 μ g/ml shows a significant stimulating effect. * $p < 0.05$ compared with the no-drug-treated group ($n = 4$). The no-drug treatment group acted as a negative control. TNF- α treatment group acted as a positive control.

vascular endothelial cells (HMECs-L), and coronary artery endothelial cells (HCAECs) and analyzed ICAM-1 expression after treatment of gp120 (5 μ g/ml) for 16 hours. Like HMECs, all primary cell types showed a significant increase of ICAM-1 expression, except for HUVECs, which had a limited increase (Fig. 2). In addition, to test whether membrane-associated gp120 could induce cell adhesion molecular expres-

Figure 2.

Soluble HIV-1 gp120 increases ICAM-1 mRNA expression by several types of endothelial cells. RT-PCR analysis demonstrating that HMECs, microvascular endothelial cells (MECs), human lung microvascular endothelial cells (HMVECs-L), and human coronary artery endothelial cells (HCAECs) show a significant ICAM-1 expression on the stimulation of gp120 (5 μ g/ml) for 16 hours, but human umbilical vein endothelial cells (HUVECs) exhibit only a limited increase of ICAM-1 expression. Duplicate experiments were performed.

sion by endothelial cells, we used HIV-1 virus-like particles (VLPs) (Yao et al, 2000) to treat HMEC cultures. RT-PCR results showed that HIV-1 VLPs also induced ICAM-1 expression (Fig. 3A), but not VCAM-1 (Fig. 3B) and E-selectin (data not shown). These data were confirmed by duplicated experiments.

HIV-1 gp120 Enhances ICAM-1 Protein Expression by HMECs

ICAM-1 expression can be modulated at the transcriptional, post-transcriptional, and/or translational level depending on the nature of the stimulus (Shrikant et al, 1994). We thus examined whether the increase in ICAM-1 steady state mRNA levels corresponded with an increase in ICAM-1 protein expression. We assessed ICAM-1 cellular protein expression using immunofluorescence staining. HIV-1 gp120 treatment

Figure 3.

HIV-1 virus-like particles (VLP) enhance the mRNA expression of ICAM-1, but not of vascular adhesion molecule-1 (VCAM-1), by HMECs. A, Time course study indicating that ICAM-1 mRNA is significantly expressed at 8 and 24 hours after the treatment of HIV-1 VLPs (50 μ g/ml). B, Time course study showing no VCAM-1 expression after the same treatment of HIV-1 VLPs. Duplicate experiments were performed.

increased ICAM-1 protein expression in HMECs as compared with the untreated cells (Fig. 4A). We also collected supernatants of cell cultures and determined soluble ICAM-1 levels using ELISA analysis. Consistent with ICAM-1 mRNA and cellular protein expression data, HIV-1 gp120 significantly increased soluble ICAM-1 production by 92% as compared with untreated controls (Fig. 4B; $p < 0.01$; $n = 3$).

Anti-gp120 Antibody Blocks HIV-1 gp120-Induced ICAM-1 Expression by HMECs

To test whether a neutralizing monoclonal antibody against HIV-1 gp120 could block gp120-induced ICAM-1 expression, we treated HMECs with a mixture of the antibody and soluble gp120, and analyzed ICAM-1 mRNA, cellular protein expression, and secreted ICAM-1 levels. HIV-1 gp120 (5 μ g/ml) combined with different doses of the antibody (0.1, 1, 10, 20 μ g/ml) significantly attenuated the effect of gp120-induced ICAM-1 mRNA expression in a dose-dependent manner (Fig. 5; $p < 0.05$; $n = 3$). Anti-gp120 antibody treatment also reduced gp120-enhanced cellular ICAM-1 protein expression as demonstrated by less immunofluorescence staining intensity (Fig. 4A). Furthermore, the anti-gp120 antibody significantly decreased gp120-induced ICAM-1 protein secretion by 54% as compared with gp120 treatment alone (Fig. 4B; $p < 0.05$; $n = 3$). Thus, these data demonstrate the specificity of gp120-mediated ICAM-1 expression by human endothelial cells.

HIV-1 gp120 Treatment Increases Monocyte Adhesion onto the HMEC Monolayer

To test the effect of HIV-1 gp120 on monocyte adherence, we analyzed the ability of THP-1 cells (monocytic cell line) to adhere to unstimulated or gp120-stimulated HMECs. We labeled THP-1 cells with a fluorescent compound (BCECF-AM) then allowed them to adhere to a monolayer of HMECs that had been previously exposed to medium or gp120 for 16 hours. After a proper wash procedure, we examined adherent THP-1 cells on the HMEC monolayer using a computer-assisted fluorescence microscope and image analysis system. There was a minimal adherence of THP-1 cells to untreated HMECs, whereas in cultures containing gp120-activated HMECs, adherence of THP-1 cells was significantly enhanced over that in control cultures by 3.26-fold (Fig. 6; $p < 0.01$; $n = 6$). This enhanced binding was inhibited approximately 64% on the inclusion of anti-gp120 antibody ($p < 0.01$; $n = 6$). Furthermore, the antibody against ICAM-1 also significantly reduced THP-1 adhesion onto gp120-treated HMECs by 53% as compared with those without anti-ICAM-1 antibody treatment (Fig. 6; $p < 0.01$; $n = 6$). These results indicate that monocytic cell line THP-1 adheres to gp120-stimulated HMECs via interactions involving endothelial ICAM-1 molecules.

A **B** **Figure 4.**

Soluble HIV-1 gp120 increases ICAM-1 protein levels in both cellular expression and secreted form in HMECs. A, Immunofluorescence microscopy (original magnification, $\times 200$) showing that gp120 treatment ($5 \mu\text{g/ml}$) for 16 hours enhances ICAM-1 cellular expression in HMECs (b) as compared with untreated cells (a). This effect of gp120 is blocked by anti-gp120 antibody (c). TNF- α -treated group acted as positive control (d). Scale bar, $100 \mu\text{m}$. B, ELISA analysis demonstrating that gp120 treatment ($5 \mu\text{g/ml}$) for 16 hours significantly increases the levels of secreted ICAM-1 protein from HMECs. This effect is also inhibited by the anti-gp120 antibody in a dose-dependent manner. * $p < 0.01$ compared with the no-drug treatment group ($n = 3$). ** $p < 0.05$ compared with the gp120 treatment group without antibody ($n = 3$).

Discussion

In this study, we examined the effect of HIV-1 gp120 on cell adhesion molecule expression by endothelial cells. We found, for the first time, that soluble HIV-1 gp120 significantly increased the expression of endothelial ICAM-1, but not VACM-1 and E-selectin, in a time- and dose-dependent manner. This effect was reflected by an enhancement of both ICAM-1 mRNA

and protein levels, including cellular expression and secreted form. The specificity of gp120-mediated response was demonstrated by blocking experiments using a specific monoclonal antibody against gp120, which successfully abolished a gp120-mediated increase of ICAM-1 expression in several types of endothelial cells. In addition, membrane-bound gp120 of HIV-1 VLPs was also able to increase ICAM-1 expression of human endothelial cells. These obser-

Figure 5.

Monoclonal antibody against HIV-1 gp120 effectively inhibits gp120-stimulated ICAM-1 mRNA expression by HMECs. RT-PCR analysis demonstrating that gp120 treatment (5 μg/ml) for 16 hours significantly increases the ICAM-1 mRNA expression by HMECs. This effect is significantly inhibited by the anti-gp120 antibody in a dose-dependent manner. * $p < 0.05$ compared with the no-drug treatment group ($n = 3$). ** $p < 0.05$ compared with the gp120 treatment group without antibody ($n = 3$).

vations demonstrate that HIV-1 gp120 alone can activate human vascular endothelium, indicating that this increased ICAM-1 may facilitate leukocyte adhesion onto the endothelium. Indeed, we found there was a significant increase of human monocytic cell line THP-1 adherence onto the gp120-treated endothelial monolayers as compared with the untreated endothelial monolayers. This increased cell adhesion was effectively blocked by either anti-gp120 or anti-ICAM-1 antibodies.

Although the direct effect of HIV-1 envelope proteins on endothelial ICAM-1 expression has not been reported previously, HIV-1 gp120 or gp41 is capable of inducing ICAM-1 expression in other cell types, such as glial cells and leukocytes (Capobianchi 1996; Chen et al, 1994; Shrikant et al, 1996). Shrikant et al (1996) reported that treatment of primary rat astrocytes and microglia with recombinant HIV-1 gp120 significantly enhanced ICAM-1 mRNA expression and cellular ICAM-1 protein levels. The optimal enhancement was at 2 hours after exposure of gp120. However, it is not clear whether gp120 affects the expression of other cell adhesion molecules such as VCAM-1 and E-selectin in these glial cells. In our study, several types of human endothelial cells were used, and they were derived from different sources, including human skin, lung, coronary, and umbilical cords. All cell types except for HUVECs strongly expressed ICAM-1 in response to the HIV-1 gp120. ICAM-1 expression reached maximal response after 8 hours of gp120 treatment. As well as ICAM-1 expression, we also demonstrated that soluble HIV-1 gp120 did not affect VCAM-1 and E-selectin expression in endothelial cells. In addition, membrane-bound gp120 of HIV-1 VLPs also showed a significant enhancement of ICAM-1 expression, but not VCAM-1 and E-selectin, in

human endothelial cells. Capobianchi (1996) observed a dose-dependent increase of the expression of ICAM-1 in PBMC cultures exposed to recombinant HIV-1 gp120. Interestingly, Chen et al (1994) reported that soluble HIV-1 gp41 enhanced ICAM-1 expression by 70% on monocytic cell line H9, but did not affect U937 cells, suggesting a selective biologic function of gp41 that is involved in the regulation of ICAM-1 expression. Moreover, a transgenic mouse model for HIV-1 gp120 has been established with secreted circulating levels of gp120 similar to those detected in AIDS patients (Finco et al, 1997), and significant up-regulation of ICAM-1 on brain endothelial cells has been found in this animal model (Toneatto et al, 1999). Thus, HIV-1 gp120 can directly increase ICAM-1 expression in several cell types, including endothelial cells, glial cells, and leukocytes.

Our results demonstrate a functional correlate for gp120-mediated ICAM-1 expression by endothelial cells, and increased adhesion of the monocytic cell line THP-1 to gp120-stimulated endothelial cells. This interaction was effectively blocked by 53% when endothelial cells were incubated with a monoclonal antibody to ICAM-1, indicating a specific interaction between monocytes and endothelial cells via ICAM-1. However, the complete inhibition of THP-1 cell adhesion by anti-ICAM-1 antibody was not achieved, suggesting other molecules, such as ICAM-2 and ICAM-3, may be involved in this adhesion event induced by gp120. There is evidence that ICAM-2 and ICAM-3, in addition to ICAM-1, can function as ligands in HIV-mediated syncytia formation (Butini et al, 1994). Thus, it will be important to determine whether gp120 affects the expression of ICAM-2 and/or ICAM-3 in endothelial cells. Stefano et al (1998) showed a direct effect of HIV-1 gp120 on enhancement of human monocyte adherence to the endothelial surface of human saphenous vein and internal thoracic artery segments *in vitro*. Although the expression of cell adhesion molecules was not studied, this enhanced monocyte adhesion appeared to result from endothelial nitric oxide synthase (eNOS) inhibition and decreased NO production by gp120-treated endothelial cells, because NO is an inhibitor of monocyte-endothelium adhesion (Adams et al, 1997). Another study demonstrated that treatment of CD4+ T cells with gp120 significantly altered their interaction with endothelium (Bragardo et al, 1997). HIV-1 gp120 treatment increased dynamic binding of lymphocytes to HUVECs in a dose-dependent manner *in vitro* as well as increased the early homing of lymphocytes into the spleen and the late homing into peripheral lymph nodes in a mouse model (Bragardo et al, 1997). Cocapping analysis indicated that the intermolecular aggregations induced by gp120 might alter lymphocyte adhesion to endothelium by inducing the formation of multimolecular adhesive patches (Bragardo et al, 1997). Therefore, HIV-1 gp120 can directly enhance adhesion between leukocytes and endothelial cells by an increase of cell adhesion molecule expression or by other mechanisms, such as eNOS activity and aggregations of cell surface molecules.

A

B

How HIV-1 gp120 induces the ICAM-1 expression by endothelial cells is not clear. It has been shown that the promoter for several adhesion molecules, including ICAM-1, contains a nuclear factor κ B (NF- κ B) element that is essential for transcription. TNF- α induces ICAM-1, VCAM-1, and E-selectin via activation of NF- κ B (Collins et al, 1995). Unlike TNF- α , HIV-1 gp120 only selectively increased ICAM-1 expression by endothelial cells in this study. Thus, we speculate that HIV-1 gp120 may have a different mechanism for the induction of ICAM-1 expression than TNF- α . Such differences have not been studied in endothelial cells. However, they have been observed in HIV-1 Tat-mediated expression of cell adhesion molecules. It has been reported that HIV-1 Tat by itself does not activate NF- κ B, but rather potentiates the TNF- α -mediated activation of NF- κ B (Westendorp et al, 1995). This potentiation was due to a change in the redox state of the cells caused by down-regulation of magnesium superoxide dismutase (Mn-SOD) expression (Flores et al, 1993; Westendorp et al, 1995). In contrast to Tat, gp120 is unable to inhibit activity and expression of Mn-SOD, but it can amplify TNF- α -induced NF- κ B-binding activity in CD4-positive Jurkat T cells (Shatrov et al, 1996). Although the direct effect of gp120 on TNF- α expression in endothelial cells has not been determined, our data imply that TNF- α may not be involved in gp120-mediated endothelial ICAM-1 expression because the expression of VCAM-1 and E-selectin are not affected by gp120 treatment. Unlike HIV-1 gp120, TNF- α is a potent inducer for expression of all ICAM-1, VCAM-1, and E-selectin in endothelial cells (Cominacini et al, 1999; Kalogeris et al, 1999). In glial cells, inclusion of a neutralizing antibody against TNF- α does not block gp120-induced ICAM-1 expression, suggesting a direct effect of gp120 on ICAM-1 gene expression (Shrikant et al, 1996). HIV-1 gp120 induces tyrosine phosphorylation of signal transducer and activator of transcription (STAT-1 α) as well as the Janus kinase (JAK2) in glial cells (Shrikant et al, 1996). Conversely, other studies suggest that the up-modulation of ICAM-1 in PBMC is not directly induced by HIV-1 gp120, but it may be mediated by the action of gp120-induced cytokines, such as TNF- α , IL-10, and interferon (Capobianci, 1996). Thus, HIV-1 gp120 seems to exhibit different mechanisms for modulation of ICAM-1 expression in different cell types.

HIV-1 gp120-mediated up-regulation of endothelial ICAM-1 may have clinical significance in HIV-1 pathogenesis. It could influence the intercellular communication events involved in immune cross-talking and in the leukocyte-endothelium multistep interactions

(Springer, 1994), and it could be involved in the cell-mediated transmission of HIV-1 infection (Scheglovitova et al, 1995). Increased ICAM-1 expression determines a stimulation of the transmission of HIV-1 from abortively infected endothelial cells to permissive CD4 lymphocytes (Scheglovitova et al, 1995). Furthermore, increased leukocyte-endothelium interaction enhances HIV-1 replication in in vitro infected macrophages and chronically infected monocytic cell lines (Borghi et al, 2000; Gilles et al, 1995). Anti-ICAM-1 antibody strongly inhibits adherence of monocytes to endothelial monolayers, and such treatments also result in the reduction of HIV-1 particle formation (Scheglovitova et al, 1995). Because endothelial cells lining blood vessels are important components of the blood-tissue barriers, it is possible that increased endothelial ICAM-1 expression could enable HIV-1 to cross anatomical barriers and to invade otherwise inaccessible body districts, such as the central nervous system (Scheglovitova et al, 1995). Evidence indicates that ICAM-1 is the predominant endothelial molecule governing lymphocyte or monocyte adhesion to and migration through central nervous system endothelium (Scheglovitova et al, 1995). Indeed, a large number of lymphocytes and monocytes are seen in the brain tissues of HIV-1-infected patients who suffer from AIDS dementia complex (Nottet, 1999; Nottet et al, 1996). HIV-1 infection is also associated with an increased frequency of vascular complications such as atherosclerosis (Constans et al, 1995; Tabib et al, 2000). Up-regulation of endothelial ICAM-1 could promote monocyte infiltration into the vascular wall, contributing to vascular lesion formation. Tabib et al (2000) examined coronary lesions in corpses of patients infected with HIV-1 who had died young. The lesions were constituted by hyperplastic endothelial cells lining a thickening of intima, which was composed of the proliferation of smooth muscle cells and monocytic macrophages. It is interesting to note that the vascular lesions associated with simian immunodeficiency virus in monkeys and with HIV-1 infection in children are morphologically identical (Chalifoux et al, 1992). By immunohistochemical examination, increased ICAM-1 expression is correlated with monocyte infiltration in the vascular wall of the HIV-infected patients (Pillet et al, 1999; Tabib et al, 2000; Zietz et al, 1996). In addition to HIV-1, ICAM-1 could also be subverted by viruses as receptors for cell infectivity, including rhinoviruses, Cocksackie viruses, and poliovirus (White and Littman, 1998). Additionally, ICAM-1 is one of two receptors characterized for *Plasmodium falciparum*-infected erythrocytes (Berendt et al, 1989). A key event in malaria is the sequestration of infected

Figure 6.

Monocytic cell line THP-1 increases its adhesion to the gp120-treated HMEC monolayer. THP-1 cells were labeled with a fluorescence dye. HMEC monolayers were treated with gp120 (5 μ g/ml) for 16 hours. A, Fluorescence microscopy (original magnification, $\times 200$) showing that there is a significant increase of THP-1 cell adhesion to the gp120-treated HMEC monolayer (b) as compared with the untreated monolayer (a). This enhancement of THP-1 cell adhesion is significantly inhibited by both anti-gp120 antibody (10 μ g/ml) (c) and anti-ICAM-1 antibody (10 μ g/ml) (d). TNF- α (1 ng/ml) treatment acted as a positive control (e). Phase contrast microscopy showing similar HMEC cell density for all adhesion experiments (f). Scale bar, 100 μ m. B, Quantitation of THP-1 cell adhesion by image analysis showing the corresponding results to Panel A. * $p < 0.01$ compared with the no-drug treatment group ($n = 6$). ** $p < 0.01$ compared with the gp120 treatment group without antibody ($n = 6$).

erythrocytes in the cerebral microvasculature, and ICAM-1 may play a role in this process. Therefore, HIV-1 gp120-induced endothelial ICAM-1 expression could contribute to organ damage and AIDS progression in HIV-1-infected patients.

Recent investigations have revealed several other effects of HIV-1 gp120 on endothelial functions in addition to its induction of endothelial ICAM-1 in this study. Annunziata et al (1998) has demonstrated that recombinant HIV-1 gp120 increases the permeability of rat brain endothelial cell cultures. This effect has also been confirmed by a model of transgenic mice for gp120 (Toneatto et al, 1999). Two recent studies have shown that HUVECs undergo apoptosis after their treatment with gp120 (Huang et al 1999; Ullrich et al, 2000). Chemokine receptors appear to mediate this process. The gp120 protein used in our experiments is derived from the HIV-1-IIIB isolate, which has been previously described as the prototype T-cell tropic isolate (Bazan et al, 1998). Differential patterns of chemokine receptor usage have been shown in studies, suggesting that the IIIB-derived clone HXB2 binds the CXCR4 receptor in preference to the CCR5 receptor (Bazan et al, 1998). In our study, we have also demonstrated that all types of endothelial cells used in the experiments are expressing CCR5 and CXCR4 by both flow cytometry analysis and immunofluorescence staining (data not shown), but it is not clear whether these chemokine receptors mediate the effects of gp120 on endothelial ICAM-1 expression. This issue is currently under active investigation in our laboratory. Furthermore, gp120 could bind to brain endothelial cells by a mechanism resembling adsorptive endocytosis (Banks et al, 1997). This vesicular-based internalization is stimulated by the plant lectin wheatgerm agglutinin and cytokines, and provides a pathway by which free viruses and infected immune cells could cross the blood-brain barrier (Banks et al, 1999). More evidence suggests that gp120 initially binds to N-acetylglucosamine on the brain endothelial cell surface with postinternalization membrane fusion involving a chemokine receptor (Banks et al, 1998). Thus, HIV-1 gp120 could exhibit several different mechanisms to interact with endothelial cells resulting in endothelial dysfunction.

Materials and Methods

Cells

The immortalized HMECs (Ades et al 1992) and primary culture of human dermal MECs were obtained from Dr. Wright S. Caughman, Department of Dermatology, Emory University (Atlanta, Georgia). HMVECs-L, HCAECs, and HUAECs were purchased from Clonetics Corporation (Walkersville, Maryland). HMECs and MECs were cultured as previously described (Ades et al, 1992). HUVECs, HMVECs-L, and HCAECs were cultured according to the instruction provided with the cells. When the cells grew to 80% to 90% confluence, the different drugs were added to the medium. At a proper time point, the medium and cells were collected for analysis of

ICAM-1 expression. THP-1, one leukemia monocytic cell line, was obtained from American Type Culture Collection (ATCC; Manassas, Virginia). THP-1 cells were cultured as instructed by ATCC.

Proteins and Antibodies

HIV-1 gp120 and anti-gp120 monoclonal antibody (mAb) were obtained from the National Institutes of Health AIDS Research and Reference Reagent Program (Rockville, Maryland). HIV VLPs were produced in a baculovirus expression system, as described previously (Yao et al, 2000). The sources of other antibodies used for the experiments were as follows: anti-Von Willebrand factor antibody (polyclonal) and anti-ICAM-1 mAb (DAKO, Carpinteria, California), and fluorescein-conjugated goat anti-mouse antibody and rhodamine-conjugated goat anti-rabbit antibody (ICN/Cappel, Aurora, Ohio). Serial concentrations of anti-gp120 and anti-ICAM-1 antibodies were used for blocking experiments. Recombinant human TNF- α (GIBCO BRL, Gaithersburg, Maryland) was used at the concentration of 1 ng/ml, which was tested to optimally induce ICAM-1 expression by HMECs.

Isolation of RNA and RT-PCR

The total RNA was isolated with TRIzol reagent (GIBCO). The RNA was resuspended in RNase-free water and the concentration was measured. The RT-PCR reagent kit (Promega, Madison, Wisconsin) and one-step RT-PCR method were used. The PCR mixture was prepared according to the kit instructions. The reaction was set up as follows: for all template RNA, 48° C for 45 minutes for reverse transcription and 94° C for 2 minutes for AMV reverse transcriptase inactivation and RNA/cDNA/primer denaturation. The conditions for PCR reactions were denaturation, 94° C, 30 seconds; annealing, 60° C for VCAM-1 and β -actin and 55° C for ICAM-1 for 1 minute; and extension, 72° C for 2 minutes. PCR primers for ICAM-1, VCAM-1, E-selectin, and β -actin were designed based on the published sequences (Grafe et al, 1997; Matsuzaki et al, 1996; Westergren et al, 1997). For quantification analysis, the PCR with primers for ICAM-1 and the internal standard β -actin were performed in separate tubes using the same conditions and amounts of template RNA. The PCR product was analyzed by electrophoresis on 2% agarose gel and stained with ethidium bromide. The band density was determined by using image analysis software (Scion, Frederick, Maryland). All of the PCR products were sequenced and found to match the target RNA sequences.

Immunofluorescence

The cells were cultured in 4-well chamber slides, which were fixed with 4% formaldehyde and incubated with 5% normal goat serum in PBS for 30 minutes. The slides were incubated with primary antibodies (0.1%) overnight at 4° C, then with secondary antibodies for 1 hour at room temperature in the dark.

The slides were cover slipped with Immuno Fluore Mounting Medium (ICN). The concentration for each antibody was as follows: anti-Von Willebrand factor antibody, 1:100; anti-ICAM-1 antibody, 1:100; FITC conjugated rabbit antibody, 1:50; and rhodamine conjugated goat anti-mouse immunoglobulins, 1:50.

Soluble ICAM-1 Immunoassay

The HMEC supernatants were collected and the ELISA kit (R and D Systems, Minneapolis, Minnesota) was used. This assay used the quantitative sandwich enzyme immunoassay technique, and the procedure was performed as described in the kit.

Monocyte Adhesion Assay

The adhesion of the THP-1 human monocytic cell line to HMECs was determined as described (Ouchi et al, 1999). Briefly, confluent HMECs in 12-well plates were exposed to gp120 (5 μ g/ml) for 16 hours, and then the monolayer was rinsed and overlaid with THP-1 monocytes labeled with BCECF-AM (Molecular Probes, Eugene, Oregon) at 1×10^6 cells/ml. After 1 hour of incubation with gentle rotational shaking, nonadherent cells were removed by gentle rinsing and the wells were washed extensively. Six random fields were captured using a CCD camera and fluorescence microscope. Adherent monocytes were counted as the number of adherent cells per field by image analysis (Alpha Innotech Corporation, San Leandro, California).

Statistical Analysis

The data were presented as mean $\pm$ standard error. Statistical differences were determined using Student's *t* test, where appropriate. *p* values less than 0.05 were considered statistically significant.

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