Upregulation of Human Immunodeficiency Virus-1 in Chronically Infected Monocytic Cell Line by Both Contact With Endothelial Cells and Cytokines

By Sao-Tah Fan, Karen Hsia, and Thomas S. Edgington

Cells of monocytic lineage (Mo) have been implicated in a variety of important roles in human immunodeficiency virus type 1 (HIV-1) infection. In addition to producing cytokines that directly influence virus replication, Mo may act as viral reservoirs for transmission of virus to susceptible target cells. Persistently infected Mo appear to have a broad tissue distribution and may be a major cellular reservoir in the central nervous system (CNS).2-4

At the clinically latent stage of HIV infection, it has been reported that there are many cells harboring virus, particularly in lymphoid tissues.5,6 Throughout the lymphoid system, a large number of T lymphocytes and Mo appear latently infected.6 In contrast to the cytopathic effect of HIV-1 on CD4+ T cells, the replication of virus in Mo is generally noncytopathic and persistent.7 Cytokines, exemplified by tumor necrosis factor α (TNFα), and cell-signaling activators such as phorbol esters, are reported to induce HIV-1 replication in both T lymphocytes and Mo.8-10 Some cellular agonists, such as granulocyte-macrophage colony-stimulating factor (GM-CSF),11,12 interleukin-6 (IL-6),13 and bacterial lipopolysaccharide (LPS),14 induce HIV-1 replication selectively in Mo. In common, these cellular activators possess the ability to induce nuclear factor κB activation and translocation to the nucleus.5,9,14

Cellular adhesion influences the patterns of gene expression in the participant cells. The integrins are important not only in cellular adhesion and cellular traffic but also mediate "outside-in" signaling leading to cellular events that change the phenotype, movement, gene expression, or activation state of the cell.15 We have described that engagement of Mac-1 (CR3, αM/β2, CD11b/CD18) with specific monoclonal antibodies (MoAbs) or natural ligand, independent of physical cellular adhesion, can significantly enhance expression of the immediate early inflammatory gene products tissue factor and TNFα in Mo.16,17

In response to foreign antigens, chemoattractants, and other stimuli, leukocytes localize and emigrate from the peripheral circulation by adherence to endothelium and transmigration to tissue. CD11/CD18 integrins play an important role in the tight binding of leukocytes to endothelium and transmigration steps during the process of cellular traffic.18,19

Interactions with endothelial monolayers via the engagement of CD11/CD18 integrins were also observed to greatly enhance the expression of Mo inflammatory mediators, ie, TNFα17 as well as early cytokines of activated T cells.20 We speculate that signaling through CD11/CD18 engagement might induce or enhance HIV-1 replication in chronically infected cells through similar mechanisms.

Using an established model system for persistent infection of Mo with very restricted viral expression, namely the U1 promonocytic cell line,10,11 we find that engagement of Mac-1 and interactions with endothelial cells (EC) have profound effects on activation of U1 endogenous HIV-1. This response appears to be mediated via both cell contact and EC-derived cytokine mechanisms.

MATERIALS AND METHODS

Cells and reagents. The U1 cell line, a promonocytic leukemia cell line persistently infected with HIV-1,11 was obtained from the AIDS Research and Reference Reagent Program (Ogün BioServices, Rockville, MD). Human umbilical vein endothelial cells (HUVEC) of less than 3 passages were provided by the Endothelial Cell Core (The Scripps Research Institute). The following MoAbs were used: anti-TNFα 104, anti-IL-6 CLB/8 (a gift from Dr D. Shealy, Centocor, Malvern, PA), anti-CD11a IgG1 TS1/22,21 anti-CD11b IgG2b (rat) M1/70,22 anti-CD11c IgG1 3.9,23 anti-CD18 IgG1 TS1/18,24 anti-HLA class I 6/32, and anti-ELAM-1 H18/7. Goat-anti-

From the Department of Immunology, The Scripps Research Institute, La Jolla, and the Department of Pediatrics, University of California, San Diego.

Submitted October 22, 1993; accepted April 22, 1994.

Supported in part by MH-47680 from the National Institutes of Health. This is manuscript 8031-IMM from the Department of Immunology.

Address reprint requests to Sao-Tah Fan, PhD, Department of Immunology, IMM 17, The Scripps Research Institute, 10666 N Torrey Pines Rd, La Jolla, CA 92037.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1994 by The American Society of Hematology.

0006-4971/94/8403-0020$3.00/0
human IL-1, goat-antihuman GM-CSF, and goat-antihuman platelet-derived growth factor (PDGF) were purchased from R&D systems (Minneapolis, MN). LPS Escherichia coli 011:B4 was purchased from Calbiochem (La Jolla, CA), and TNFα, IL-1β, and GM-CSF were purchased from R&D Systems. PGE2, fibrinogen, fibronectin, were purchased from Sigma (St Louis, MO). Platelet activating factor was provided by Dr R. Ye (The Scripps Research Institute). Monocyte chemotactic and activating factor was purchased from Genzyme (Cambridge, MA). Culture medium used was complete RPMI (CRPMI, RPMI 1640 supplemented with 2 mM L-glutamine, 10 mM HEPES, and 10% fetal calf serum). All media and solutions, except LPS solution, contained less than 0.03 ng/mL LPS.

Induction and assay for HIV-1 expression in U1. Serial dilutions of LPS, TNFα, or other agonists were added to 5 × 10⁴ U1 in each well of flat-bottom 96-well plates in a final volume of 200 μL. In some experiments, wells were coated as previously described with 70 μL of 100 μg/mL purified fibronectin, fibrinogen, CD18-specific MoAb TS1/18, or CD11b-specific MoAb M1/70 at 20 to 22°C for 2 to 4 hours or overnight at 4°C, and wells were washed twice with HEPES-saline. For studies of U1 adhered to HUVEC, confluent HUVEC were dissociated from T-75 flasks with HEPES-saline containing 5 mM EDTA and added to wells of gelatin-coated 96-well flat-bottom plates at 4 × 10⁴ cells per well in 100 μL of CRPMI. After 24 hours of incubation, monolayers were washed once and stimulated with serial concentration of TNFα (0.01 to 3 ng/mL) or 1 μg/mL LPS for 1 to 3 days. For MoAb-blocking experiments, various MoAbs were added at 10 μg/mL final concentration before adding U1 cells to HUVEC monolayers. Dilutions of supernatant from each well were assayed for p24 antigen content by enzyme-linked immunosorbent assay (ELISA) (Abbott, Abbott Park, IL; Coulter, Hialeah, FL) per manufacturers’ instructions.

Fractionation of HUVEC-derived factors. HUVEC monolayer in a T-75 flask (7 × 10⁶ cells) was stimulated with 1 μg/mL LPS or 1.3 ng/mL IL-1β in 10 mL CRPMI. Medium supernatant harvested 1 day after stimulation was concentrated eightfold by Centricon (Amicon, Beverly, MA). Two hundred microliters was applied to 0.9 × 52 cm S-200 Sephacryl column (Pharmacia, Piscataway, NJ) equilibrated with RPMI-1640. Each fraction of 0.5 mL was collected with an elution speed of 100 μL/min.

RESULTS

Effect of adhesion to ligand surfaces on HIV-1 expression. The effect of CD11b/CD18 engagement on the induction of HIV-1 biosynthesis by persistently infected U1 cells was analyzed for p24, which is found exclusively associated with virus particles in the supernatant medium of activated U1 cells. Cells were incubated for 1 to 3 days with or without 1 μg/mL LPS on plates coated with (1) a ligand for CD11b/CD18, ie, fibronectin; (2) MoAb specific for CD11b or CD18; or (3) fibronectin as a negative control. As shown in Fig 1, the p24 determined in the culture supernatant was markedly increased when cells adhered to fibronectin or MoAb specific for CD18 (TS1/18) but not when adherent to fibronectin. Because unstimulated cells do not adhere strongly to fibronectin, the HIV-1 expression by unstimulated U1 was not enhanced as much as in LPS-stimulated U1 adhered to fibronectin surface. On day 3, the degrees of enhancement are generally slightly less than that observed on day 2 (data not shown). Similar enhancement of p24 production was also observed with an MoAb specific for CD11b (M1/70, data not shown). U1 cells attached and spread extensively on fibronectin surfaces without exhibiting significant enhance-

ment of p24 production, indicating that the enhancement of p24 production is independent of physical contact, adhesion or extent of cell spreading per se on surfaces, but in contrast is specific for CD11b/CD18 integrin engagement. Additionally, cell numbers in all 3 days were found not to be influenced by these different culture conditions (data not shown).

Induction of HIV-1 expression by endothelial cell. We have shown previously that adherence of Mo to EC monolayers has a significant effect on the expression by Mo of certain inflammatory mediators. The effect of U1 adherence to EC monolayers was examined. As illustrated in Fig 2, production of HIV-1 p24 was considerably enhanced as a result of coculturing of U1 with endothelial monolayers. In the absence of stimulus, the enhancement was maximal at about eightfold after 2 days of coculturing with EC. Introduction of the cellular agonist LPS at 1 μg/mL directly increased HIV-1 expression about 6- and 10-fold after 1 and 2 days of stimulation, respectively (Fig 2A). In comparison, when U1 cells were also cultivated on HUVEC monolayers, HIV-1 expression was increased more than 10-fold over the levels of p24 generated with LPS alone and the maximal increase was found after 2 days of coculturing.

TNFα has been reported to be a potent inducer of HIV-1 expression in Mo. HIV-1 expression by U1 cells induced with TNFα was also markedly enhanced by coculture with HUVEC monolayers (Fig 2B). At the peak of enhancement (after 2 days) and with optimal TNFα stimulation, HIV-1 expression was more than 200-fold greater than the unstimulated cells.

CD11/CD18-specific MoAb inhibition of enhanced HIV-1 expression. Several MoAbs specific for the α- or β-chains of members of the CD11/CD18 integrins, and other cell surface receptors, ie, HLA, and ELAM-1, were used to assess specificity of the interaction between U1 and HUVEC that may be required for the enhancement of HIV-1 p24 synthesis. As shown in Fig 3 (top panel), enhanced HIV-1 expression resulting from interaction with HUVEC in the absence of LPS stimulation was inhibited 50% to 70% by
MoAbs specific for CD11b (M1/70), CD18 (TS1/18), a combination of M1/70, TS1/18, and CD11a-specific TS1/22, and only slightly by TS1/22 alone, but not by MoAbs specific for CD11c or other receptors tested. In addition, the absence of an effect of neutralizing TNFα with MoAb 104 suggests that the enhanced HIV-1 expression by U1 cells is not a result of TNFα production by U1 or HUVEC.

In the presence of LPS, the markedly enhanced HIV-1 expression resulted from interactions with HUVEC was not inhibited by any of these MoAbs (Fig 3, bottom panel). These results of MoAb-blocking experiments indicate that engagement of CD11/CD18 adhesive receptors was not required nor did it further enhance HIV-1 biosynthesis after LPS stimulation and coculture with HUVEC. The lack of inhibition by anti-TNFα MoAb 104 also suggests that TNFα does not participate in this response/pathway.

Enhancement of HIV-1 p24 expression by soluble agonists secreted by HUVEC. Results of MoAb-blocking experiments suggest that other factors are responsible for the enhancement effect in association with LPS stimulation and may play a role as well in the unstimulated cells. To determine if soluble secreted products of HUVEC participate, supernatant from unstimulated HUVEC or HUVEC cultures stimulated with agonists, such as LPS, TNFα, and IL-1β, were harvested daily for 3 days and then analyzed for induction of the p24 marker of HIV-1 expression. First, there is constitutive production of a small amount of soluble-enhancing factor by unstimulated HUVEC (Fig 4). This level of enhancing factor activity could account for the activity that was not inhibitable by CD11/CD18-specific MoAbs in experiments shown in Fig 3 (top panel). LPS, TNFα, and IL-1β stimulate HUVEC to produce high levels of soluble factors that enhanced HIV-1 expression (Fig 4).

Characterization of the HUVEC-derived enhancing factors. The enhancing factor activity from HUVEC was recovered only in the excluded fraction of a Sephadex G-25 column (Pharmacia) consistent with a macromolecule (data not shown). This ruled out the involvement of peptides or other small molecules produced by HUVEC. For further characterization, concentrated supernatant from HUVEC, stimulated either with LPS or IL-1β, were subjected to Sephacryl S-200 molecular exclusion gel chromatography (Fig
The effect of coculture of U1 cells with vascular endothelium results in a significant enhancement of HIV-1 expression in these chronically infected cells; and it appears to be mediated by complex events involving both cell contact and soluble factors derived from EC. Recently, it had been reported that HIV could be induced and recovered from latently infected Mo ex vivo by coculture with activated normal T lymphocytes.\(^{26}\) A plasma membrane fraction, but not secreted factors, of T lymphocytes was observed to mediate this induction of HIV.\(^{26}\) In light of our findings, the signal transduction by integrins may play a role. In addition, CD11a/CD18 (LFA-1) was found to be required for HIV-mediated cell fusion and syncytia formation of T lymphocytes,\(^{27,28}\) but not for cell-to-cell spreading of virus of these cells.\(^{29}\)

Mo are focused to the vessel wall by mediators released from stimulated HUVEC and HUVEC supernatant. HUVEC supernatant stimulated with LPS (top) or IL-1β (bottom) were concentrated eightfold and 0.2 mL was applied to a Sephadex S-200 column (0.9 x 52 cm). The p24 antigen produced by U1 cells was determined after incubation with individual fractions (0.5 mL) for 24 hours (top) or 36 hours (bottom). Polymixin B was added in all the fractions of LPS-stimulated HUVEC supernatant (top) to neutralize LPS.

The HIV-1-enhancing factor activity was completely recovered over a broad range from approximately 45 kD to 10 kD. It appears that both the IL-1β and LPS-induced HUVEC factors may be similar and heterogeneous in size.

Several cytokines, known to stimulate HIV-1 production in U1 cells, such as TNFα, IL-6, and GM-CSF, can be produced by stimulated endothelial cells. The involvement of TNFα in the HIV-1-enhancing factor activity was ruled out in previous experiments with neutralizing MoAb (Fig 3). Neutralizing antibodies specific for IL-1 and for GM-CSF were found to inhibit the enhancing factor activity by 20% to 30%, whereas neutralization of IL-6 blocked about 50% of the enhancing activity in HUVEC supernatant (Fig 6). A combination of all these three antibodies exhibited greater than 70% of inhibition (Fig 6). Inasmuch as these purified cytokines, i.e., IL-1, IL-6, and GM-CSF, induced low levels of HIV-1 expression individually (data not shown), it appears that these cytokines, notably IL-6, act synergistically with each other or with other cytokines in the enhancement of HIV-1 expression. In contrast, addition of neutralizing antibodies specific for PDGF did not block HIV-1 enhancement activity of HUVEC supernatant (Fig 6). Neither platelet-activating factor nor monocyte chemotactic and activating factor at concentrations ranging from 0.01 to 1 μmol/L or from 0.5 to 100 nmol/L, respectively, induced HIV-1 expression in U1 cells (data not shown).
during the initial stage of inflammation. Activation of endothelium and leukocytes induces loose association and rolling of leukocytes on endothelium followed by adhesion to endothelium, spreading, and then transmigration to perivascular location in response to appropriate chemotactic gradients. It appears that there are several mechanisms during this transmigration and subsequent encounter with extracellular matrix that may significantly induce or enhance HIV-1 expression by infected Mo. First, engagement of β2 integrins as part of the stage of tight binding to endothelium appears to upregulate HIV-1 expression as we have shown in this study. Second, there can be marked enhancement of monokine biosynthesis resulting from β2 engagement during adhesion, as well as from HIV infection. These monokines can induce HIV expression by Mo in an autocrine fashion. Third, one or more of these monokines stimulate synthesis of soluble macromolecular factors by EC, such as IL-6, GM-CSF, and others, that synergistically upregulate HIV-1 expression by Mo. Furthermore, the attached cells transmigrate into tissues through the basement membrane, where they are exposed to extracellular matrix. It has been reported that HIV-1 infection enhances the capacity of Mo to adhere and spread on extracellular matrix (ECM), and interactions between the infected monocyte and components of the matrix such as laminin, but not fibronectin, could upregulate HIV-1 replication in Mo. HIV-infected Mo were also found to have significantly higher capacity to bind to neural cells and endothelial cells as a result of upregulation of integrins.

The incidence of infected Mo in blood is exceedingly low, reported to be less than 0.001%. In contrast, 10% or more of cells of Mo lineage in brain, lung, or lymph nodes of HIV-infected individuals produce demonstrable HIV. The CNS contains an exquisitely small proportion of resident cells of monocytic lineage, yet in the brain these cells appear to be a principal reservoir for HIV. Do the interactions between Mo and brain EC encountered during the transmigration of Mo across the blood brain barrier account for overt expression of HIV by otherwise latently infected Mo?

The underlying mechanisms of HIV-1–associated dementia remain obscure. It has been observed that infected Mo, which have sharply increased ability to adhere to neural cells, might injure neural cells by direct contact and induce plaques of gliosis. Furthermore, it had been suggested that infected macrophages interact with astrocytes, and greatly amplifies the production of glial proliferatory and neurotoxic factors. This Mo-astroglia interaction was implicated to play major role in the progressive neurological impairment during HIV disease. Thus, these reports and our findings suggest that intervention with the adhesion of infected Mo to endothelium, or interruption of integrin mediated “outside-in” signaling, may reduce the CNS reservoir of HIV cells of Mo lineage.

ACKNOWLEDGMENT

We thank Dr Steven Spector (University of California, San Diego) for use of laboratory facilities during these studies, Dr Floyd E. Bloom (The Scripps Research Institute) for critical reading of the manuscript, and Barbara Parker for administrative assistance and preparation of the manuscript.

REFERENCES

8. Osborn L, Kunkel S, Nabel GJ: Tumor necrosis factor α and interleukin 1 stimulate the human immunodeficiency virus enhancer by the activation of the nuclear factor κB. Proc Natl Acad Sci USA 86:2336, 1989


Upregulation of human immunodeficiency virus-1 in chronically infected monocytic cell line by both contact with endothelial cells and cytokines

ST Fan, K Hsia and TS Edgington