Release of Human Immunodeficiency Virus by THP-1 Cells and Human Macrophages Is Regulated by Cellular Adherence and Activation

ROBIN J. SHATTOCK, JON S. FRIEDLAND, AND GEORGE E. GRIFFIN*

*Division of Communicable Diseases, St. George's Hospital Medical School,
Tooting, London SW17 0RE, United Kingdom*

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Macrophage adherence, an important regulatory signal, has the potential to affect human immunodeficiency virus (HIV) production either directly or by priming monocytes to respond to other activating signals. We have investigated the role of adherence as an activator of HIV-1 transcription and release. The effects of adherence on HIV-1 transcription were examined by using THP-1 cells, a human monocytic cell line, transfected with HIV long terminal repeat (LTR)-chloramphenicol acetyltransferase (CAT) constructs. The effects of adherence on release of HIV-1 were investigated in both HIV-1-infected THP-1 cells and human peripheral blood monocyte-derived macrophages (MDM). Adherence of lipopolysaccharide (LPS)-stimulated THP-1 cells to either tissue culture plastic or endothelial cells was crucial for enhanced HIV-1 transcription as measured by LTR-CAT expression. Such increased LTR-CAT expression did not occur with an HIV LTR construct containing mutated NF- κ B binding sites. In contrast, release of whole HIV, measured by reverse transcriptase (RT) activity in tissue culture medium, was reduced upon adherence of stimulated HIV-1-infected THP-1 cells without suppression of HIV LTR-CAT transcription or p24 release. This finding suggested that activation of adherent monocytic cells interfered with HIV assembly and release. Although the reduction of RT activity following activation of HIV-1-infected MDM was independent of adhesion, adherence alone of nonstimulated HIV-infected MDM to endothelial cells was sufficient to induce a reduction in RT release. This study demonstrates that LPS stimulation of monocytic cells enhances HIV LTR transcription under adherent conditions. In contrast, activation of adherent monocytic cells infected with HIV reduced viral release.

Monocytes and macrophages are an important reservoir of human immunodeficiency virus (HIV) and may represent the largest reservoir of this virus in tissue (11). HIV replication in monocytes has previously been shown to be induced by binding of the transcriptional activator NF- κ B to the enhancer region of the viral long terminal repeat (LTR) sequence and is dependent upon the state of cellular differentiation (13). Monocyte differentiation may be modified by many exogenous or endogenous stimuli. However, the effects of cellular adhesion on NF- κ B-mediated HIV replication are not known.

NF- κ B in the cytoplasm of monocytes and macrophages is complexed to its inhibitor, I κ B. Dissociation of this complex, which arises from phosphorylation of I κ B (18), allows translocation of NF- κ B to the nucleus, where it may bind to the promoter region of many cellular genes. Induction of NF- κ B binding activity occurs in a number of promonocytic cell lines by a variety of stimuli, including bacterial lipopolysaccharide (LPS) and phorbol esters (20). NF- κ B consists of a family of heterodimeric protein subunits, different subunit combinations have distinct binding kinetics for the HIV-1 provirus, and their release into the nucleus is inducer specific (27). However, in mature monocyte cell lines, including the THP-1 cells used in this study, blood monocytes, and adherent macrophages, NF- κ B binding activity is constitutively present (13).

The phenomenon of cellular adherence is known to induce mRNA production for several proto-oncogenes and monokines, including tumor necrosis factor alpha and interleu-

kin-8, which contain NF- κ B binding sites in their promoter regions (14, 16, 23). In addition, the phenomenon of adherence is important for monocyte migration and function (6, 19). Monocyte adhesion, therefore, has the potential to directly influence HIV production in infected cells through NF- κ B-dependent mechanisms or modulate the effects of other stimuli known to affect HIV production. Entry of peripheral blood monocytes into tissue follows adherence to and then translocation across vascular endothelium (6). This process of adhesion and translocation is mediated through the interaction of a variety of specific cellular proteins (5) and extracellular matrix proteins (8, 19).

The effects of monocyte activation on HIV transcription and release reported in previous studies are often conflicting. Such disparate results may have arisen from differences in experimental conditions, for example, use of different cell lines, divergence of subclones, differences in culture conditions (adherent and nonadherent), and differences in activating stimuli. Previous studies have shown that LPS stimulation enhanced HIV transcription in 24-h-old human monocytes and the promonocytic cell line U937 while reducing HIV transcription in 12-day-old monocyte-derived macrophages (MDM) (2). In addition, activation of MDM with LPS or gamma interferon (IFN- γ) prior to HIV infection has been shown to protect MDM from productive HIV replication (17). Indeed, monocyte activation can downregulate viral production, as treatment of HIV-infected macrophages with IFN- γ reduces viral release (26). Whether cell culture in adherent versus nonadherent conditions may have influenced these previous studies is unknown. We have therefore investigated the effects of macrophage adherence to tissue culture plastic or endothelial cells on the control of HIV

* Corresponding author.

transcription and release from monocytes stimulated by a variety of soluble mediators.

MATERIALS AND METHODS

Cell culture. The monocyte cell line THP-1 (25) was maintained in RPMI 1640 supplemented with 5% fetal calf serum (FCS), 2 mM glutamine, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml. Cells were cultured at 37°C in 5% CO₂ and passaged every 4 to 5 days. The endothelial cell line SGHEC 7 (gift of G. Whitley, St. Georges Hospital Medical School, London, United Kingdom) represents simian virus 40-transfected human umbilical vein endothelial cells. Such endothelial cells were seeded in six-well tissue culture plates (Marathon, United Kingdom) at 2×10^5 to 3×10^5 cells per well. Cells were cultured for 72 h at 37°C in 5% CO₂ in medium 199 (Sigma, Poole, United Kingdom) supplemented with Earle's modified salts-RPMI 1640 (1:1). This culture medium was supplemented with 5% FCS, 5% newborn calf serum, 2.5 µg of endothelial cell growth supplement (Sigma) per ml, and 0.9 mg of heparin per ml. Plates were seeded with cells between passages 12 and 20 and were used in adhesion experiments on reaching >90% confluency.

Human peripheral blood monocyte isolation. Human mononuclear cells were prepared from buffy coat preparations (The National Blood Transfusion Service, Tooting, London, United Kingdom) by Ficoll density gradient separation (Histopaque; Sigma). Contaminating platelets were depleted by four washes in phosphate-buffered saline (PBS) containing 3 mM EDTA. Cells were resuspended in RPMI 1640 with 5% human serum and incubated for 60 min in gelatin-coated tissue culture flasks (15). Nonadherent cells were removed by four washes with warm (37°C) RPMI 1640 plus 5% FCS. Adherent monocytes were eluted by 10-min incubation at 4°C in chilled elution buffer (a 1:1 ratio of PBS–10 mM EDTA and RPMI 1640 with 10% human serum). Monocytes were washed and resuspended in RPMI 1640 supplemented with 10% human serum, 5% FCS, 2 mM glutamine, 100 U of penicillin per ml, and 0.1 mg of streptomycin per ml. The final concentration of monocytes was 10^6 cells per ml of culture medium. Cells matured into MDM after 5 days of incubation in Teflon vials (Pierce and Warriner, Chester, United Kingdom) at 37°C in 5% CO₂.

Plasmid constructs. Two plasmid constructs (generous gifts of G. Nabel, University of Michigan, Ann Arbor) were used in experiments: HIV-1 LTR-CAT, containing the HIV-1 LTR upstream of the reporter chloramphenicol acetyltransferase (CAT) gene, and a corresponding construct with mutated κB sites which could no longer bind NF-κB (ΔHIV-1 LTR-CAT).

Transfections. The control of HIV LTR transcription was investigated by using cells transfected with HIV LTR-CAT constructs. Cells were transfected as previously described (13). In brief, 10^7 washed cells were incubated in 1 ml of transfection cocktail containing an HIV LTR-CAT plasmid (10 µg) and DEAE-dextran for 30 min at 37°C in 5% CO₂. Cells were washed in PBS and incubated in growth medium containing 1 mM chloroquine for 30 min at 37°C in 5% CO₂. Cells were then washed and resuspended in RPMI with 5% FCS and cultured either under nonadherent conditions in Teflon vials or under adherent conditions in six-well tissue culture plates (10^7 cells per 5 ml per well or vial).

At 24 h posttransfection, cells were exposed to the following stimuli: 1 µg of *Escherichia coli* O127:B8 LPS per ml, 500 U of IFN-γ (Boehringer Ingelheim, Bracknell, United King-

dom) per ml, or 20 nM phorbol 12-myristate 13-acetate (PMA; Sigma). In addition, transfected THP-1 cells were added to cultures of confluent SGHEC 7 endothelial cells that were pretreated as follows: nonstimulated SGHEC 7 cells washed three times with PBS, SGHEC 7 cells stimulated with 1 µg of LPS per ml for 4 h and then washed four times with PBS, and LPS-stimulated SGHEC 7 cells fixed with 0.2% glutaraldehyde and then washed four times with PBS. THP-1 cells were also exposed to endothelial cells in the continual presence of LPS. As a control for the endothelial cell experiments, THP-1 cells were cultured in Teflon vials with conditioned medium produced by collecting tissue culture supernatant from endothelial cells 24 h after the cells had been stimulated with LPS for 4 h and washed four times in PBS.

CAT assay. CAT activity was assayed by standard methods 48 h after transfection of the cells (12). Samples were assayed in a 150-µl volume containing [¹⁴C]chloramphenicol (0.5 µCi; 18.5 kBq; Amersham International Ltd., Aylesbury, United Kingdom), 4 mM acetyl coenzyme A (Sigma), 0.25 M Tris-HCl (pH 7.4), and 300 µg of cell lysate. Protein concentrations of cell extracts were standardized by the Bradford method (4). Following separation by thin-layer chromatography, percentage conversion of chloramphenicol to its acetylated forms was determined by liquid scintillation counting.

HIV infection. THP-1 cells in log-phase growth were infected with HIV-1_{RF}. Then 4×10^6 cells were incubated in 1 ml of cell-free viral supernatant (6×10^4 50% tissue infective doses derived from Jurkat TAT cells [22]) for 2 h at 37°C in 5% CO₂. Peak infection was established after 10 days of subculture. MDM were infected with the monocyctotropic virus HIV-1_{Ba-L} (9) 5 days after isolation of monocytes. Then 8×10^6 cells were incubated for 2 h in 1 ml of cell-free viral supernatant (10^5 50% tissue culture infective doses derived from MDM cultures) in Teflon vials at 37°C in 5% CO₂. Peak infection was established after 7 days of subculture in Teflon vials.

HIV detection. The control of HIV release was investigated by using HIV-infected THP-1 cells (10 days postinfection) and MDM (7 days postinfection). Immediately before experimentation, THP-1 and MDM cells were washed three times in PBS to remove all cell-free virus. Cells were resuspended in fresh medium (2×10^6 cells per 2 ml) and cultured either under nonadherent conditions in Teflon vials or under adherent conditions in six-well tissue culture plates in the presence of soluble stimuli. In addition, such cells were cocultured with confluent endothelial cells as previously described. Cell-free supernatants were assayed for reverse transcriptase (RT) activity or p24 levels at 24, 48, and 96 h poststimulation.

RT activity was determined on 10-µl samples of cell-free tissue culture supernatant, using [³²P]dTTP incorporation (21) determined with a direct β plate reader (Canberra Packard, Pangbourne, United Kingdom). To control for degradation of RT activity resulting from the action of soluble factors released by stimulated THP-1 cells, HIV of known RT activity was diluted 1/10 with supernatants from PMA- or LPS-stimulated adherent cells taken at 4, 8, 24, 48, and 72 h poststimulation. Virus diluted in such conditioned supernatant was incubated for 24 h at 37°C in 5% CO₂, and subsequently RT activity was analyzed.

Viral p24 levels were determined on cell-free tissue culture supernatant by using a p24 antigen capture enzyme-linked immunosorbent assay (ELISA). Viral supernatants treated with 1% Empigen (Calibrochem, Nottingham, United King-

FIG. 1. CAT expression in THP-1 cells transfected with HIV-1 LTR-CAT (▨) or Δ HIV-1 LTR-CAT with mutated κ B sites (■). Transfected THP-1 cells were cultured either under nonadherent conditions in Teflon vials (A) or under conditions permissible for adherence in tissue culture plastic (B). At 24 h after transfection, cells were treated with different agents (LPS [1 μ g/ml], PMA [20 nM], or IFN- γ [500 U/ml]). PMA or LPS stimulation induced adherence to tissue culture plastic but not to Teflon vials; IFN- γ did not induce adherence in either condition. Cell extracts were harvested for CAT assay 48 h after transfection. Bar graphs show fold increase in conversion of [14 C]chloramphenicol compared with untreated controls transfected with HIV-1 CAT, assigned a value of 1. Graphs illustrate the arithmetic mean of a minimum of three independent transfections.

dom) were added to enzyme immunoassay microtiter plates (Costar, Cambridge, Mass.) coated with an affinity-purified sheep anti-HIV p24^{gag} antibody (Aalto Bioreagents, Dublin, Eire). After washing, bound p24 was detected with a biotinylated anti-p24 antibody, EH12-EI (JGV 68 1543; MRC Aids Reagent Project, Potters Bar, United Kingdom). The plates were washed and incubated with streptavidin-alkaline phosphatase (Boehringer Mannheim, Lewes, United Kingdom). After repeat washing, p24 was detected by the addition of the substrate *p*-nitrophenyl phosphate (Sigma). p24 release was not measured for MDM infected with HIV-1_{Ba-L}, as this virus reacted poorly in this p24 ELISA.

RESULTS

Adherence of THP-1 cells and HIV LTR transcription. Unstimulated THP-1 cells do not adhere to tissue culture plastic. Treatment of THP-1 cells with LPS and PMA resulted in adherence to tissue culture plastic, while IFN- γ had no effect on cellular adherence. The effects of such

FIG. 2. CAT expression in THP-1 cells transfected with HIV-1 LTR-CAT upon adhesion to endothelial monolayers. At 24 h after transfection, cells were cultured in nonadherent conditions in tissue culture plastic or in tissue culture plates with confluent SGHEC 7 endothelial cells. The endothelial cells were either unstimulated, prestimulated with LPS (1 μ g/ml) for 4 h and washed four times in PBS before use, or stimulated in the continual presence of LPS (1 μ g/ml). In addition, transfected THP-1 cells were cultured in Teflon vials with conditioned medium collected from endothelial cells 24 h after they had been stimulated with LPS for 4 h and washed four times in PBS. THP-1 cell extracts were harvested for CAT assay 48 h after transfection. Bar graphs show fold increase in conversion of [14 C]chloramphenicol compared with untreated controls transfected with HIV-1 CAT, assigned a value of 1. Graphs illustrate the arithmetic mean of a minimum of three independent transfections.

cellular adhesion on HIV transcription were investigated by using THP-1 cells transfected with HIV-1 LTR-CAT. LPS stimulation of transfected THP-1 cells cultured in nonadherent conditions in Teflon vials had no effect on basal CAT expression (Fig. 1A). However, LPS stimulation of identical cells in adherent conditions enhanced CAT expression threefold upon cellular adherence to plastic (Fig. 1B). PMA treatment had no effect on HIV-1 LTR-CAT transcription in nonadherent or adherent conditions. IFN- γ treatment resulted in a small increase in CAT expression in both adherent and nonadherent conditions. Stimulation of THP-1 cells transfected with Δ HIV-1 LTR-CAT cultured in nonadherent conditions resulted in reduced levels of CAT expression; in contrast, transfection with HIV-1 LTR-CAT resulted in no increase in CAT levels in adherent conditions.

Adherence of THP-1 cells to endothelial cells and HIV LTR transcription. THP-1 cells adhered to unstimulated or stimulated SGHEC 7 endothelial cells regardless of their state of activation. THP-1 cells transfected with HIV-1 LTR-CAT showed minimal enhancement of CAT expression upon adherence to unstimulated or LPS-prestimulated SGHEC 7 endothelial cells. THP-1 cells incubated in Teflon vials with conditioned medium from LPS-stimulated endothelial cells produced similar minimal enhancement. However, when both THP-1 cells and SGHEC 7 cells were treated with LPS, adherence of THP-1 cells to SGHEC 7 cells enhanced HIV LTR-CAT expression threefold (Fig. 2). In control experiments, adherence of THP-1 cells transfected with Δ HIV-1 LTR-CAT to endothelial cells under all conditions produced negligible levels of transcription (data not shown).

Effects of cellular adherence on RT activity released from THP-1 cells. Washed, unstimulated HIV-infected THP-1 cells demonstrated increasing supernatant RT activity over 4 days of culture. LPS stimulation of HIV-infected THP-1 cells cultured nonadherent in Teflon vials resulted in reduced supernatant RT activity. In addition, LPS stimulation of identical cells under adherent conditions further reduced

FIG. 3. Production of HIV from stimulated THP-1 cells in nonadherent and adherent conditions. Ten days after infection with HIV-1_{RF}, THP-1 cells were washed three times in PBS to remove all cell-free virus, resuspended in fresh medium, cultured in either Teflon vials or tissue culture plastic, and stimulated with LPS (1 μ g/ml; A), PMA (20 nM; B), or IFN- γ (500 U/ml; C). Supernatants were analyzed for RT activity on days 1, 2, and 4 poststimulation. Symbols: $\square$, no treatment, nonadherent conditions; $\bullet$, treatment, nonadherent conditions; $\blacktriangle$, treatment, adherent conditions. Graphs illustrate the arithmetic mean of a minimum of three independent experiments.

supernatant RT activity upon adhesion to tissue culture plastic (Fig. 3A). PMA stimulation of HIV-infected THP-1 cells cultured under nonadherent conditions produced little reduction in RT activity in comparison with unstimulated controls, in which activity was detected only at 4 days poststimulation. PMA treatment of identical cells in adherent conditions removed all supernatant RT activity upon cellular adherence to tissue culture plastic (Fig. 3B). In control experiments, incubation of viral supernatant of known RT activity with conditioned supernatant from non-infected THP-1 cells treated with any of the stimuli in adherent conditions (data not shown) did not reduce RT activity. This finding indicated that the detected reduced RT activities were not due to degradation of the enzyme in culture supernatants. Stimulation of HIV-1_{RF}-infected THP-1 cells with IFN- γ produced a reduction in accumulation of supernatant RT activity compared with unstimulated controls (Fig. 3C). Such reduction in RT activity was similar in supernatant from cells cultured in either tissue culture plastic or Teflon vials, as IFN- γ did not induce adherence.

Effects of cellular adhesion on p24 release from THP-1 cells. The increase in supernatant p24 antigen levels from washed, unstimulated HIV-infected THP-1 cells over 4 days of culture paralleled the increase in RT activity. LPS stimulation of HIV-infected THP-1 cells cultured in nonadherent conditions in Teflon vials resulted in a similar pattern of p24 antigen release compared with released RT activity. In contrast to the RT data, cellular adherence to tissue culture plastic upon treatment with LPS resulted in little or no reduction of p24 levels (Table 1). PMA stimulation of cells

cultured in nonadherent conditions in Teflon vials produced a small reduction in p24 levels. Similarly, in contrast to the RT data, tissue culture plastic-adherent cells stimulated with PMA exhibited no reduction in p24 levels compared with controls. IFN- γ stimulation of HIV-infected THP-1 cells cultured in tissue culture plastic or Teflon vials reduced p24 levels.

Effects of adhesion to endothelial cells on viral release from HIV-infected THP-1 cells. Adhesion of washed, unstimulated HIV-infected THP-1 cells to unstimulated confluent SGHEC 7 endothelial cells resulted in a reduction in released RT activity compared with identical cells cultured in nonadherent conditions in Teflon vials. A significant reduction in RT activity was also seen in nonadherent cells cultured in Teflon vials with conditioned medium from LPS-stimulated SGHEC 7 cells. Such reduction in released RT activity was increased upon adherence of unstimulated THP-1 cells to LPS-prestimulated endothelial cells or adherence to endothelial cells in the presence of LPS (Fig. 4).

Effects of cellular activation on LTR-directed transcription in HIV-infected THP-1 cells. To determine whether control of HIV LTR transcription was different in HIV-infected cells, THP-1 cells were transfected with HIV LTR-CAT constructs 10 days after HIV infection. Cells were stimulated and harvested as in previous experiments. CAT expression in unstimulated HIV-1-infected THP-1 cells transfected with HIV-1 LTR-CAT produced very high levels of chloramphenicol acetylation. Fifteen micrograms of cytoplasmic extract protein from unstimulated HIV-infected cells produced 89% acetylation of [¹⁴C]chloramphenicol after 2 h of incubation at

TABLE 1. Comparison of HIV LTR-CAT expression, supernatant RT activity, and supernatant p24 levels

Type of analysis	Sample time point (h)	Value obtained in:					
		Nonadherent conditions			Adherent conditions		
		LPS	PMA	IFN- γ	LPS	PMA	IFN- γ
CAT expression (fold increase)	24	1.0	1.2	2.0	3.0	1.6	1.5
RT activity (relative to control)	24	0.61	0.93	0.67	0.63	All	0.45
	48	0.57	0.93	0.54	0.43	<0.01	0.82
	96	0.96	0.48	0.59	0.49		0.59
	96	0.96	0.48	0.59	0.49		0.59
p24 release (relative to control)	24	0.36	0.73	0.92	1.1	1.1	0.92
	48	0.77	0.48	1.12	1.1	1.2	1.12
	96	0.77	0.77	0.61	0.73	0.96	0.61

FIG. 4. Effects on viral release of adherence to endothelial cells. Ten days after infection with HIV-1_{RF}, THP-1 cells were washed three times in PBS to remove all cell-free virus, resuspended in fresh medium, and cultured in nonadherent conditions in tissue culture plastic (■), in six-well tissue culture plates with confluent untreated SGHEC 7 endothelial cells (○), with SGHEC 7 cells treated with LPS at 1 μ g/ml for 4 h and washed four times in PBS (▽), with SGHEC 7 cells in the presence of LPS at 1 μ g/ml (□), or in Teflon vials with conditioned medium (collected from endothelial cells 24 h after they had been stimulated with LPS for 4 h and washed four times in PBS) (●). Supernatants were analyzed for RT activity on days 1, 2, and 4 poststimulation. Graphs illustrate the arithmetic mean ($\pm$ standard error of the mean) of three independent experiments.

37°C. In comparison, 300 μ g of protein from uninfected cells transfected with HIV-1 LTR-CAT produced only a 1% acetylation of [¹⁴C]chloramphenicol after 6 h of incubation at 37°C. This high level of CAT expression was not reduced upon cellular stimulation; LPS, PMA, and IFN- γ produced 98, 94, and 91% acetylation, respectively. HIV-infected THP-1 cells transfected with Δ HIV-1 LTR-CAT showed low-level CAT expression, with unstimulated and stimulated cells producing acetylation values of below 3%.

Effects of cellular activation on RT activity from MDM. MDM spontaneously adhere to tissue culture plastic without stimulation. There was a negligible difference in RT activity between adherent and nonadherent controls. Stimulation of washed MDM infected with HIV-1_{Ba-L} with LPS, PMA, or IFN- γ resulted in a similar reduction in released RT activity from cells cultured under nonadherent and adherent conditions (Fig. 5A to C).

Effects of adhesion to endothelial cells on viral release from HIV-infected MDM cells. Adhesion of washed MDM infected with HIV-1_{Ba-L} to unstimulated or LPS-stimulated endothelial cells resulted in a large reduction in RT activity. A similar reduction in supernatant RT activity was seen upon adherence of MDM either to endothelial cells cultured in the presence of LPS or to glutaraldehyde-fixed LPS prestimulated SGHEC 7 cells. Culture of HIV-infected MDM in conditioned medium from LPS-treated endothelial cells resulted in a minimal reduction in RT activity (Fig. 5D).

DISCUSSION

The data presented in this study have demonstrated that cellular adherence may enhance HIV-1 LTR-CAT transcription in stimulated THP-1 cells. A significant enhancement of basal HIV LTR-CAT expression was observed following LPS stimulation of THP-1 cells in adherent conditions, in agreement with previous studies (20). We have shown that such enhancement was dependent upon cellular adhesion, as LPS stimulation of identical cells in nonadherent conditions failed to induce enhanced HIV LTR-CAT transcription. Basal HIV LTR-CAT expression in unstimulated THP-1

FIG. 5. Production of HIV from nonadherent and adherent MDM. Seven days postinfection, MDM infected with HIV-1_{Ba-L} were washed three times in PBS to remove all cell-free virus, resuspended in fresh medium, stimulated with LPS (1 μ g/ml; A), PMA (20 nM; B), or IFN- γ (500 U/ml; C), and incubated under either nonadherent conditions in Teflon vials or adherent conditions in tissue culture plastic. Symbols: ■, no treatment, nonadherent conditions; ○, treatment, nonadherent conditions; △, treatment, adherent conditions. (D) Effects on viral release of adherence of HIV-infected MDM to endothelial cells. Ten days postinfection, MDM were cultured in nonadherent conditions in tissue culture plastic (■), in six-well tissue culture plates with confluent untreated SGHEC 7 endothelial cells (○), with SGHEC 7 cells treated with LPS at 1 μ g/ml for 4 h and washed four times in PBS (△), with SGHEC 7 cells in the presence of LPS at 1 μ g/ml (□), or with glutaraldehyde-fixed SGHEC 7 cells which had been prestimulated with LPS at 1 μ g/ml for 4 h and washed four times in PBS (◇) or cultured in Teflon vials with conditioned medium (collected from endothelial cells 24 h after they had been stimulated with LPS for 4 h and washed four times in PBS) (●). In all experiments, supernatants were analyzed for RT activity on days 1, 2, and 4 poststimulation. Graphs illustrate the arithmetic mean ($\pm$ standard error of the mean) of three independent experiments.

cells was in part mediated by NF- κ B, constitutively expressed in such cells (13), as reduced LTR-CAT expression was observed in cells transfected with Δ HIV-1 LTR-CAT. Enhancement of HIV LTR-CAT expression following LPS stimulation of THP-1 cells in adherent conditions was dependent upon functional NF- κ B binding sites in the LTR, since no enhancement was seen following adherence of LPS-stimulated THP-1 cells transfected with Δ HIV LTR-CAT. However, a requirement for intact NF- κ B sites does not preclude the involvement of additional transcription factors in induction of LPS-enhanced CAT expression observed in adherent conditions.

In contrast, adherence of THP-1 cells to tissue culture plastic following PMA stimulation did not enhance basal HIV-1 LTR-CAT expression. Such lack of enhancement of LTR-CAT expression following PMA stimulation is in agree-

ment with our previous observations (13) but at variance with another report (20). Such differences may be due to variable basal expression of NF- κ B in different subclones of THP-1 cells. Furthermore, LPS and PMA have been shown to have very different time courses for induction of NF- κ B and are thought to act via different mechanisms (20). The fact that the combination of LPS stimulation and adherence enhanced HIV LTR-CAT expression whereas PMA stimulation and adherence did not adds support to this hypothesis. Monocyte adhesion may enhance HIV transcription only upon costimulation of the specific synergistic intracellular pathways. The nature of such pathways and their effects on HIV transcription remain to be determined.

As adhesion to tissue culture plastic may have artificial activating properties (19), we examined the effects of adherence of monocytes to nonstimulated and stimulated human umbilical endothelial cells (SGHEC 7 cells). Such studies demonstrated that enhancement of HIV LTR-CAT expression was similar following adhesion of LPS-stimulated THP-1 cells to either endothelial cells or tissue culture plastic. Enhanced CAT expression was also observed upon adherence of unstimulated THP-1 cells to endothelial monolayers. This effect was in part mediated by soluble mediators released from the endothelial cells, as culture of THP-1 cells in conditioned medium produced similar results.

In contrast to HIV LTR-CAT transcription, stimulation of HIV-infected THP-1 cells with LPS or PMA resulted in reduced RT activity in nonadherent conditions. More importantly, such reduction was greatly potentiated under adherent conditions, indicating an adherence-dependent reduction in viral release (Table 1). The measured reduction in RT activity was not due to degradation of virus particles in supernatant, since RT activity was unchanged in control experiments in which tissue culture supernatant from LPS- or PMA-stimulated adherent THP-1 cells was incubated for 24 h with supernatant containing high-level RT activity.

Exposure of HIV-infected THP-1 cells to IFN- γ reduced release of RT activity in both nonadherent and adherent conditions by equivalent amounts, demonstrating an adherence-independent mechanism of reduction. The reduction in RT activity following IFN- γ treatment was consistent with previous reports (26). Stimulation of HIV-infected MDM also reduced RT supernatant activity. A similar pattern of reduction in supernatant RT activity was observed in adherent and nonadherent conditions. The observed reduction in RT activity released from HIV-infected MDM in nonadherent conditions may have been facilitated by signals generated through autoadherence, as a high level of MDM clumping was observed in Teflon vials which was never observed with THP-1 cells.

A parallel reduction in supernatant RT activity was observed upon adherence of LPS-stimulated HIV-infected THP-1 cells to tissue culture plastic or endothelial cell monolayers. In addition, adhesion of unstimulated HIV-infected THP-1 cells to LPS-stimulated endothelial cells induced a significant reduction in release of RT activity. This finding suggested that adhesion per se to stimulated endothelial cells was sufficient to induce a reduction in viral release. The reduction in RT activity upon adhesion of HIV-infected THP-1 cells to unstimulated SGHEC 7 endothelial cells was, at least in part, mediated by soluble mediators released from endothelial cells, as similar data were obtained with use of culture conditioned medium. Furthermore, adhesion of MDM to unstimulated or prestimulated confluent endothelial cells greatly reduced RT activity independent of soluble mediators released from endothelial

cells. This finding suggested that adhesion of MDM may be sufficient to reduce RT activity. Endothelial cells appear to have a passive role in such reduced RT release from MDM, as similar results were obtained upon adherence of MDM to glutaraldehyde-fixed LPS-stimulated endothelial cells. Thus, these data suggest that in vivo adherence may downregulate viral release from infected macrophages.

The described reduction in RT activity occurred even in the presence of high levels of HIV transcription, as HIV LTR-CAT expression was active at a rate far higher than that seen in non-HIV-infected cells. Such high levels of LTR-CAT expression were not surprising and may reflect the action of *tat* expressed in HIV-infected cells (24). Hence, reduction in released RT activity may be mediated by posttranscriptional events. Such posttranscriptional downregulation is different from previously described transcriptional downregulation of HIV replication following LPS stimulation of MDM (2). However, in agreement with previous studies (1), use of HIV constructs with mutated κ B sites demonstrated that the efficiency of transactivation of HIV LTR-CAT in infected cells was dependent on the presence of intact κ B binding sites.

In contrast to data described above on RT activity, there was little difference in p24 release upon stimulation with LPS, PMA, or IFN- γ of HIV-infected THP-1 cells in adherent conditions. RT activity detected in tissue culture supernatants of cells infected with HIV has been shown to be associated almost exclusively with virus particles, whereas capsid and envelope proteins may be shed as free (soluble) antigen (7). IFN- γ has been shown to reduce released supernatant RT activity from the persistently HIV-infected promonocytic cell line U1 (3). Such reduction in released RT activity occurred without suppression of HIV mRNAs or proteins and reflected a redistribution of virions to intracytoplasmic vacuoles. In addition, IFN- α treatment of HIV-infected T cells and human mononuclear cells has previously been shown to reduce supernatant RT activity while little affecting p24 antigen levels (7, 10). Differences in RT and p24 release may reflect disruption of viral assembly and release but not transcription and translation (7, 10). Our observation that a reduction in RT activity occurred even in the presence of high levels of HIV transcription and without suppression of p24 antigen release suggests that activation of adherent monocytic cells interferes with HIV assembly and release. It remains to be determined whether such a reduction in release of intact HIV reflects an accumulation of intracytoplasmic virions or disrupted viral assembly.

The relative importance in vivo of the differential effects of adhesion and activation on HIV transcription and release remain to be determined. In latently infected immature cells, adherence may prime cells to respond to other stimuli which initiate transcription (14), while in mature cells, adhesion alone and in conjunction with other cellular activators may, as was observed, downregulate viral release. Thus, macrophage activation during an immune response may reduce rather than increase the amount of HIV released from infected macrophages.

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