# HIV-1 Infection of Human T Lymphocytes Results in Enhanced $\alpha_5\beta_1$ Integrin Expression

**Benjamin S. Weeks,* Mary E. Klotman,‡ Subhash Dhawan,§ Maura Kibbey,* Jay Rappaport,‡ Hynda K. Kleinman,* Kenneth M. Yamada,* and Paul E. Klotman***

*Laboratory of Developmental Biology, National Institute of Dental Research; ‡Laboratory of Oral Medicine, National Cancer Institute; and §Laboratory of Oral Medicine, National Institute of Dental Research National Institutes of Health, Bethesda, Maryland 20892

*Abstract.* Altered T cell adherence after human immunodeficiency virus 1 (HIV-1) infection may contribute to viral pathogenesis in the acquired immune deficiency syndrome. To address this hypothesis, we assessed mechanisms of T cell adherence to extracellular matrix proteins in vitro. We found that after HIV-1 infection, both chronically infected H9 CD4+ T cells and acutely infected primary peripheral blood lymphocytes acquired the ability to adhere to the extracellular matrix glycoprotein fibronectin, to a lesser extent to type IV collagen and laminin, but not to type I collagen. H9 cells chronically infected with two of the three HIV-1 strains studied showed approximately a sevenfold increase in attachment to fibronectin, while the same cells infected with the human retrovirus HIV-2 did not. Adhesion was accompanied by changes in morphology, including marked spreading and increased filopodia. These alterations were not blocked by the protein kinase C inhibitor H-7, which did inhibit TPA-induced T cell attachment to fibronectin. Monoclonal antibodies against both the $\alpha_5$ and the $\beta_1$ subunits of the classical fibronectin receptor as well as an Arg-Gly-Asp (RGD) peptide inhibited attachment, whereas anti-$\alpha_4$ monoclonal antibodies and the CS1 peptide did not. Binding to collagen IV was also inhibited by the anti-$\beta_1$ monoclonal antibody, but not the other antibodies. Cells metabolically labeled with [$^{35}$S]methionine and analyzed by immunoprecipitation with polyclonal anti-$\beta_1$ integrin antibody showed a 2.5-fold increase in integrin synthesis in infected cells compared to uninfected controls. This increase in synthesis was associated with an increase in cell surface expression of both $\alpha_5$ and $\beta_1$ integrins by FACS® (registered trademark of Becton Dickinson for a fluorescence-activated cell sorter) analysis. Enhanced expression of integrins such as $\alpha_5\beta_1$ may cause T cell adherence to a variety of tissues, where released viral gene products may induce some of the tissue-specific manifestations of HIV-1 infection.

INFECTION with HIV-1 is associated with a progressive reduction in the number of circulating CD4+ T cells (8, 41). The resultant clinical syndrome is dominated by the sequelae of immunodeficiency, AIDS. Recent evidence, however, suggests that a number of the protean manifestations of HIV-1 infection including Kaposi's sarcoma (6), AIDS-associated dementia (23), and HIV-associated nephropathy (Adler, S. A., J. B. Kopp, P. Dickie, N. J. Marinos, J. L. Bryant, J. M Felser, A. L. Notkins, and P. E. Klotman. 1990. *J. Am. Soc. Nephrol.* 1:513*a*) may result from complex interactions between infected T cells, viral gene products, and host tissues.

One potential mechanism for T cell targeting is through an interaction with extracellular matrix proteins including fibronectin, collagen IV, laminin, entactin, and heparan sulfate proteoglycan. (2, 12, 18, 26, 33, 34, 46). Lymphocytes must attach to and then traverse basement membranes and connective tissues to arrive at target tissues. Lymphocytes possess cell surface receptors for the extracellular matrix proteins known as integrins which recognize fibronectin, fibrinogen, vitronectin, and laminin. (1, 14–16, 20, 27, 28, 51, 53, 54, 60, 61). This family of receptors appears to play an important role in tissue targeting, activation, and cell recognition by lymphocytes. In the present study, we have addressed potential mechanisms of T cell targeting by examining whether HIV-1 infection alters the interaction of T cells with extracellular matrix molecules (for review see reference 53).

Dr. Rappaport's present address is Department of Medicine, University of California, San Diego.

## Materials and Methods

### Cell Lines

H9 cells (39) and H9 cells chronically infected with the HIV-1 strains $III_B$

(HIV-$1_{IIIB}$) (39), MN (HIV-$1_{MN}$) (48), and RF (HIV-$1_{RF}$) (48) as well as the HIV-2 strain NIH Z (HIV-$2_{NIH\ Z}$) (62) were used in the initial studies.

### *Infection of Human Peripheral Blood Lymphocytes (PBL)*

PBL were obtained from three single donor packs from normal blood donors. T cells were isolated by Ficoll gradient centrifugation, stimulated with 5 μg/ml phytohemagglutinin (PHA) for 48 h in RPMI supplemented with 10% FCS, followed by removal of adherent cells. Stimulated cells were subsequently cultured in RPMI supplemented with 10% FCS and 10% human IL2 (Advanced Biotechnologies, Inc., Columbia, MD). After stimulation for 48 h, $2 \times 10^7$ cells in 5 ml were treated with polybrene (5 μg/ml), and HIV-$1_{IIIB}$ was added to the medium at 0.1 and 1.0 infectious virus particles/cell. After 2 h of virus adsorption, the volume was expanded to 20 ml with medium. After 24 h, cells were washed free of virus, and cell attachment to fibronectin was determined at 1, 2, and 5 d after infection. Infection of cells was confirmed by reverse transcriptase activity and the presence of the core protein, P24, by immunofluorescence microscopy.

### *Cell Attachment Assays*

Cell attachment was determined by a modification of a previously described method (21). Tissue culture plates (Costar, Cambridge, MA) were coated with one of the following substrates: bovine serum albumin (Boehringer Mannheim Biochemicals, Indianapolis, IN), collagen I (Sigma Chemical Co., St. Louis, MO), laminin (58), collagen IV (22), or fibronectin (31). Plates were coated by adding 10 μg of each substrate dissolved in 200 μl water to 16-mm-diam wells in 24-well cell culture plates and air dried overnight at 4°C.

Cells were harvested and washed three times in Dulbecco's phosphate buffered saline, pH 7.4 (PBS) and resuspended in RPMI containing 100 μg/ml of transferrin, 100 μM putrescine, 20 nM progesterone, 30 nM $NaSeO_3$, and 5 μg/ml of insulin. Cells were seeded in the coated plates at a density of 0.4–0.5 $\times$ $10^6$ cells/0.5 ml serum-free medium/well. Cells were incubated for 5 h at 37°C. Unattached cells were then removed by washing the wells twice with 0.5 ml PBS. The attached cells were fixed with methanol and stained with Diff Quick (Baxter Scientific Products, McGraw Park, IL). Three 1.5-$mm^2$ fields per well were counted by observers blinded to the experimental groups and the numbers of attached cells were expressed as the mean ± SEM of three fields in each of six wells.

The effect of nonspecific T cell activation on adherence was assessed by preincubation of H9 cells with 10 μg/ml Con A (Sigma Chemical Co.) or 10 μg/ml PHA (Sigma Chemical Co.) for 6 h before plating. Cells were washed and plated in coated wells in serum-free medium. Previous studies suggest that activation with 12-*O*-tetradecanoylphorbol-13-acetate (TPA; Sigma Chemical Co.) induces adhesion by protein kinase C activation. To evaluate the role of protein kinase C in virus-induced adhesion, H9 cells chronically infected with HIV-$1_{IIIB}$ were plated in coated wells containing serum-free medium alone or medium supplemented with 90 μM H-7, a protein kinase C inhibitor (Seikagaku America Inc., Rockville, MD). As an additional control, the role of protein kinase C in T cell adhesion induced by nonspecific mitogen was also explored. Uninfected H9 cells were plated in medium alone, medium supplemented with 100 nM TPA or medium supplemented with both TPA and 90 μM H7.

To determine the specific cell-surface receptors for fibronectin, inhibition assays were performed using peptides or receptor subunit-specific antibodies. HIV-1-infected cells ($5 \times 10^5$) were cultured on increasing amounts of fibronectin with the addition of 150 μg of peptide at the time of plating. These peptides contained the amino acid sequences RGD (GRGDS), RGE (GRGES), or DELPQLVTLPHPNLHGPEILDVPST, the CS1 (connecting segment) peptide from an alternatively spliced region of fibronectin (9). After 5 h, the numbers of attached cells were determined as above.

In additional experiments, HIV-1-infected H-9 cells were plated on fibronectin in the presence of 0.5 mg/ml control IgG (rat) or 0.5 mg/ml of the monoclonal antibodies to the $\beta_1$ (mAb 13), $\alpha_4$ (8F2), or $\alpha_5$ (mAb 16) integrin subunits. The $\alpha_4$ antibody was generated in ascites fluid (36) and the control for this antibody was an equal volume of nonimmune ascites fluid (Bethesda Research Laboratories, Bethesda, MD). After 5 h, the number of attached cells was determined.

### *Immunoprecipitation of Integrin Subunits*

Chronically infected and uninfected H9 cells ($30 \times 10^6$ were cultured in 5 ml methionine-free RPMI containing 10% FCS and 2.0 mCi [$^{35}$S]methionine for 24 h. After labeling, cells were washed four times in a total of 200 ml RPMI. The sedimented pellet was resuspended in 1.5 ml 2% Triton-X 100 containing 0.2 mM PMSF. The samples were briefly sonicated and pre-absorbed with protein A–Sepharose (Sigma Chemical Co.). The protein content of the cell lysate was determined by the Bradford assay (Bio-Rad Laboratories, Richmond, CA). An equal quantity of protein (5 mg) from both H9 and HIV-1-infected cell homogenates was diluted in a volume of 1.0 ml of PBS. The H9 lysate contained 286,624 TCA-precipitable cpm/μl and the HIV-1-infected lysate contained 231,856 TCA-precipitable cpm/μl. Lysates were precipitated at 4°C with a 1:200 dilution of either nonimmune or anti-$\beta_1$ polyclonal antiserum (Rb 3847) (43). Samples were analyzed by electrophoresis on a 5% polyacrylamide gel, which was then dried and exposed to x-ray film. Bands on the autoradiogram were quantitated by laser densitometry (Shimadzu scanner; Shimadzu, Tokyo, Japan), lane background was subtracted, and the density was expressed as a ratio of infected to control cells.

### *Fluorescence Activated Cell Analysis (FACS)*

Cell surface expression of $\beta_1$ integrin on HIV-1-infected and uninfected H-9 cells was determined by FACS analysis. 100,000 cells were incubated in 100 μl RPMI 1640 containing 5 μl of nonimmune serum (control) or 5 μl of anti-$\beta_1$ polyclonal antiserum for 1 h at 37°C. Cells were washed seven times with 1 ml 0.05% Tween-20 in PBS, resuspended, and incubated with a 1:50 dilution of the appropriate FITC-conjugated antibody at 37°C for 1 h. Cells were then washed seven times, fixed in 2% paraformaldehyde overnight, and analyzed by FACS. To determine the cell surface expression of the $\alpha_5$ integrin subunit, cells were analyzed essentially as above but 100 μg of anti-$\alpha_5$ monoclonal antibody were used for cell surface labeling and 100 μg rat IgG were used as control.

## *Results*

### *Morphology and Adhesive Properties of Infected Cells Cultured On Fibronectin*

H9 cells, grown in suspension and chronically infected with HIV-$1_{IIIB}$, exhibited striking morphological changes characterized by marked spreading and by the production of numerous filopodial projections in the presence of fibronectin substrate (Fig. 1). These changes were associated with increased attachment to fibronectin, collagen IV, and laminin (Fig. 2 *A*); attachment was greatest to fibronectin and least to laminin. No enhanced attachment was observed to interstitial collagen type I. The specificity of the enhanced attachment of HIV-1-infected lymphocytes to extracellular matrix proteins was tested using a number of viral strains. H9 lymphocytes chronically infected with HIV-$1_{MN}$ also showed a sevenfold increase in attachment to fibronectin. H9 cells chronically infected with HIV-$1_{RF}$ or HIV-$2_{NIH\ Z}$ failed to show enhanced attachment (data not shown). Attachment of HIV-1-infected cells occurred within 1 h of plating onto the substrate and was maximal after 3–5 h.

Qualitatively identical results were observed when human PBL were acutely infected with HIV-$1_{IIIB}$ (Fig. 2 *B*), although the magnitude of the response was blunted by the nature of the mixed T cell population. Similar to the results observed for H9 cells, acute HIV-$1_{IIIB}$ infection stimulated a significant increase in attachment of PBL to fibronectin which was maximally observed 2 d after infection.

Since T cell activation alone can induce attachment to several extracellular matrix components, we compared the attachment of H9 cells to fibronectin when activated either by nonspecific mitogens or by HIV-$1_{IIIB}$ infection. Neither Con A nor PHA stimulated increased attachment of uninfected cells to fibronectin (Table I). Treatment with TPA, however, significantly increased uninfected H9 cell attachment to

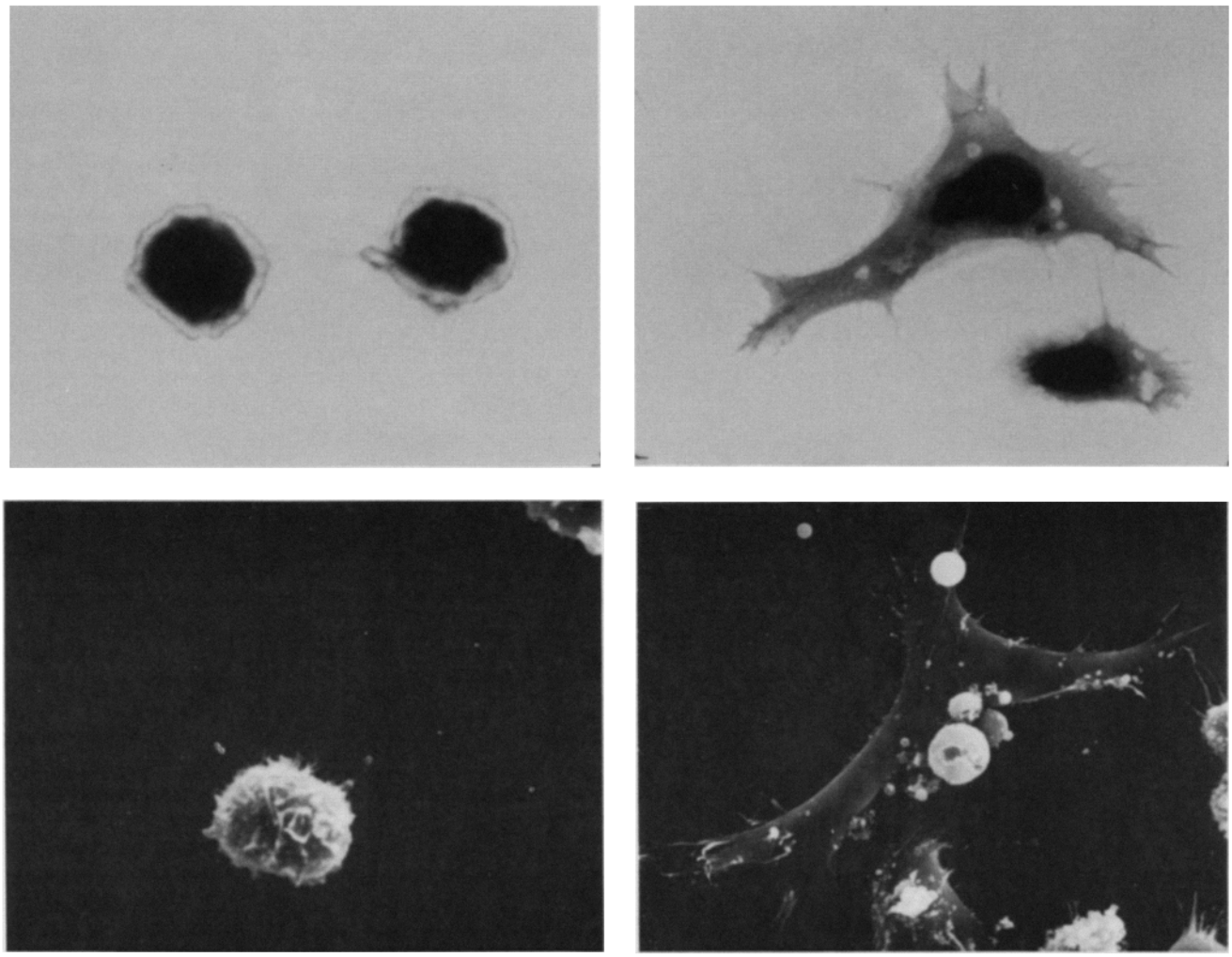


*Figure 1.* Morphology of CD4+ T lymphocytes plated on fibronectin. Uninfected and HIV-$1_{IIIB}$-infected H9 cells were plated ($5 \times 10^5$) in glass 1 $cm^2$ multi-chamber slides which had been coated with 10 $\mu$g of human plasma fibronectin. After 5 h, cells were washed with RPMI and fixed in methanol, stained with hematoxylin and eosin, and viewed by light microscopy (*top*), or fixed in 2.5% glutaraldehyde and viewed by scanning electron microscopy (*bottom*).

fibronectin by fivefold within 1 h of plating. TPA treatment of infected cells further enhanced attachment to fibronectin (~twofold). The increase in attachment of uninfected H9 cells in response to TPA could be blocked almost completely by the protein kinase C inhibitor H-7. In contrast, addition of H-7 to HIV-1-infected cells had no significant effect (Table I). These data suggest that TPA-induced attachment occurs by a mechanism different from that of HIV-1-induced attachment.

### *Identification of Cell Surface Receptors Responsible for Enhanced Attachment of Infected Cells*

To identify the fibronectin receptor used by HIV-$1_{IIIB}$ infected cells, monoclonal antibodies specific for integrin subunits were tested for their ability to block attachment to fibronectin. Monoclonal antibodies to both the $\beta_1$ and the $\alpha_5$ subunits of the classical fibronectin receptor significantly inhibited attachment (Fig. 3 *A*) while an antibody to another fibronectin receptor subunit $\alpha_4$ did not. The combination of anti-$\alpha_4$ and anti-$\alpha_5$ antibodies was not additive and, thus, did not reveal a subtle effect of the $\alpha_4$ subunit. Because HIV-1-infected cells also attached to collagen IV, although to a much lesser extent, antibodies to the collagen-binding $\beta_1$ integrins were tested for their ability to inhibit attachment to collagen IV. The anti-$\beta_1$ antibody inhibited cell attachment to collagen IV but, as expected, anti-$\alpha_5$ and anti-$\alpha_4$ antibodies (which are not collagen IV receptors) had no effect (data not shown). Because the $\alpha_5$ integrin of T lymphocytes recognizes a domain containing the RGD amino acid sequence in fibronectin and $\alpha_4$ recognizes the CS1 alternatively spliced region of fibronectin (2, 12, 14, 17, 20, 27, 28, 34, 36, 46, 51, 54, 61) peptides containing these sequences were tested for their ability to inhibit the attachment of HIV-1-infected H9 cells to fibronectin. Consistent with the

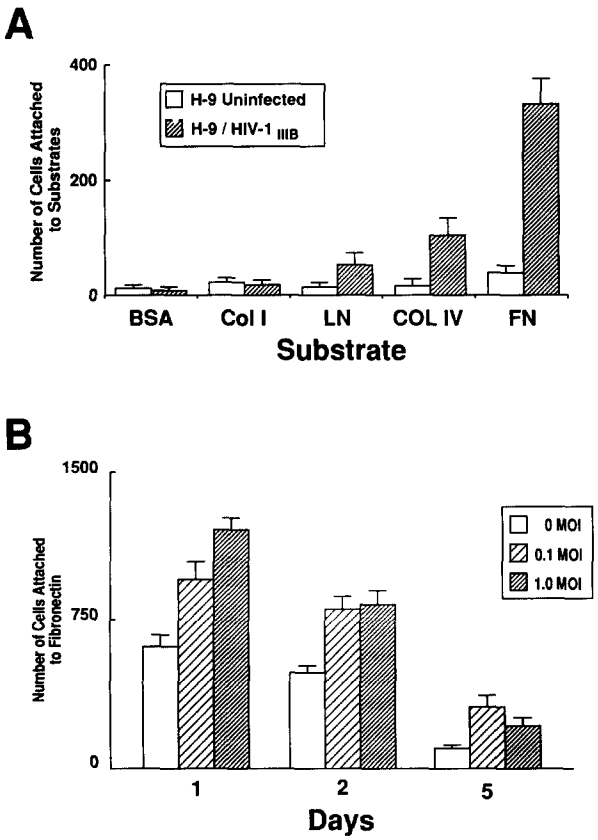


*Figure 2.* HIV-1-induced T lymphocyte attachment to substrates. (*A*) Chronically infected CD4+ T lymphocytes (*H9*) cultured on various substrates in 48-well culture dishes (11.3 $mm^2$ wells) coated with 10 $\mu$g of: bovine serum albumin (*BSA*); collagen I (*Col I*); laminin (*LN*); collagen IV (*Col IV*); or fibronectin (*FN*). (*B*) Acutely infected peripheral blood lymphocytes cultured on fibronectin. HIV-$1_{IIIB}$ was added to the medium at a multiplicity of infection (*MOI*) of 0.1 or 1.0 and cell attachment to fibronectin was determined 1, 2, and 5 d after infection. At all times, HIV-1-infected PBL attachment was significantly greater than that observed in uninfected cells. Each bar represents the mean ± SEM for results from PBL using three individual donors.

*Table 1. Effect of T Cell Activation on Attachment to Fibronectin by HIV-1-infected and Uninfected Cells*

| | Substrate | |
|---|---|---|
| Treatment | BSA | FN |
| Control | 7 ± 4 | 31 ± 13 |
| Con A | 15 ± 8 | 17 ± 7 |
| PHA | 13 ± 7 | 27 ± 10 |
| TPA | 18 ± 10 | 157 ± 32* |
| TPA + H-7 | 12 ± 7 | 54 ± 17‡ |
| HIV-$1_{IIIB}$ | 32 ± 15 | 289 ± 46§ |
| HIV-$1_{IIIB}$ + TPA | ND | 672 ± 69‖ |
| HIV-$1_{IIIB}$ + H-7 | ND | 209 ± 42 |

Before plating, cells were preincubated for 6 h with 10 $\mu$g/ml Con A or 10 $\mu$g/ml phytohemagglutinin (*PHA*). These cells and unstimulated cells were washed and plated in control medium or medium containing 100 nM TPA, or 90 $\mu$M H-7, a protein kinase C inhibitor. Cells were cultured on either bovine serum albumin or fibronectin as described in Materials and Methods.
* <0.05 vs. control.
§ <0.005 vs. control.
‡ <0.025 vs. TPA.
‖ <0.005 vs. HIV-$1_{IIIB}$.

antibody studies, the RGD-containing peptide but not the CS1 peptide inhibited attachment to fibronectin (Fig. 3 *B*). HIV-1-induced attachment of PBL to fibronectin was inhibited by the anti-$\beta_1$ antibody and RGD peptide as well (data not shown).

Since increased attachment to fibronectin by HIV-1-infected cells did not appear to involve phosphorylation (TPA data, Table I), we examined whether HIV-1 induced an increase in $\beta_1$ integrin synthesis. As demonstrated by immunoprecipitation (Fig. 4), HIV-1 infection stimulated a 2.5-fold increase in $\beta_1$ integrin synthesis by H9 cells when compared to uninfected controls. To determine whether this increase in synthesis was associated with an increase in cell surface expression, infected and uninfected H9 cells were analyzed by FACS. HIV-1 infection induced an increase in both $\beta_1$ (Fig. 5 *A*) and $\alpha_5$ integrin (Fig. 5 *B*) subunits on the cell surface of chronically infected H9 (HIV-$1_{IIIB}$) cells when compared to uninfected controls.

## *Discussion*

Several tissue-specific manifestations of HIV-1 infection do not appear to be related to immunodeficiency directly. For example, the development of Kaposi's sarcoma (47) and HIV-associated nephropathy can occur before the development of overt immunodeficiency (37). It is possible that these entities involve a more direct role of the virus and/or viral gene products released from localized T cells. The present study addresses mechanisms involving the initial step of the localization process, the attachment of HIV-1-infected T cells to extracellular matrix molecules. We have found that T cells that normally grow in suspension become markedly adherent to fibronectin but not collagen I when chronically infected with the HIV-$1_{IIIB}$ or the HIV-$1_{MN}$ strains of HIV-1. Acute infection of either the H9 cell line or human PBL with HIV-$1_{IIIB}$ produced a qualitatively identical result suggesting that this was not simply a property of clonal selection of a chronically infected cell line. The response was strain specific and HIV-2, which may be less pathogenic (25), did not stimulate a similar degree of adherence. T cell adhesion induced a marked change in morphology with cell spreading and an increase in the number and length of filopodia. T cell attachment to fibronectin did not appear to share a common mechanism with the protein kinase C–mediated attachment induced by the co-mitogen TPA; T cell attachment induced by HIV-1 was not inhibited by a protein kinase C inhibitor H7 whereas TPA-induced attachment was almost completely blocked by H7. HIV-1-infected T cell attachment to fibronectin was mediated by the $\alpha_5\beta_1$ member of the integrin family of cell surface receptors which recognizes the Arg-Gly-Asp (RGD) amino acid sequence in fibronectin. HIV-1 infection stimulated a 2.5-fold increase in $\beta_1$ integrin synthesis as determined by radioimmunoprecipitation and was associated with an increase in cell surface expression of both $\alpha_5$ and $\beta_1$ integrins by FACS analysis.

Lymphocytes are known to adhere to other cells as well as to extracellular matrix proteins using cell-surface adhesion molecules. For example, the lymphocyte function-related antigens (LFAs) and the very late antigens (VLA-1 to VLA-6) have been found to be members of the integrin gene family of adhesion receptors (for reviews see references 50, 53, 55). Regulation of the receptors has been shown to in-

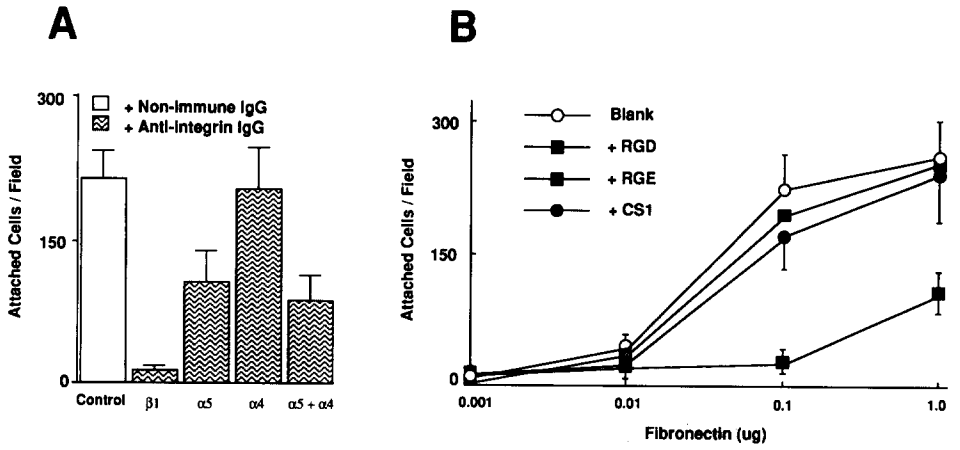


*Figure 3.* Inhibition of HIV-1-infected H9 cell attachment to fibronectin. (*A*) Inhibition of attachment to fibronectin by antibodies generated to the integrin subunits. HIV-1-infected H-9 cells were cultured on fibronectin as described previously in the presence of 0.5 mg/ml control IgG (rat), or 0.5 mg/ml of the monoclonal antibodies to the $\beta_1$ (mAb 13), $\alpha_4$ (8F2), or $\alpha_5$ (mAb 16) integrin subunits. (*B*) Inhibition of HIV-1-infected H9 cell attachment to fibronectin using an RGD-containing peptide. HIV-1-infected cells ($5 \times 10^5$) were cultured on increasing amounts of fibronectin. At the time of plating, 150 $\mu$g of peptides were added; these peptides contained the amino acid sequences RGD (GRGDS), RGE (GRGES), or DELPQLVTLPHPNLHGPEILDVPST, the CS1 peptide from the type III connecting segment of fibronectin.

volve at least two possible mechanisms. First, phorbol ester activation of protein kinase C leads to neutrophil and macrophage adhesion to laminin (4, 30). Macrophage treatment with phorbol ester stimulates $\alpha_6\beta_1$ integrin phosphorylation, which suggests that posttranslational events can influence integrin activity (49). Second, antigen stimulation of lymphocytes or lymphocyte activation by cytokines can result in increased synthesis and cell surface expression of adhesive molecules (5, 13, 57). Our results indicate that HIV-1-induced lymphocyte adhesion results from increased synthesis of integrin and is probably not associated with a protein kinase C-dependent pathway involving integrin phosphorylation.

Integrins facilitate a variety of lymphocyte functions including infiltration, recirculation, and tissue-specific targeting and homing (for reviews see references 50, 53, 55). To achieve these tasks, circulating lymphocytes must attach to the vascular endothelium, then attach to and traverse the extracellular matrix, and ultimately bind to cells in lymph nodes and tissue. The lymphocyte LFA-1 and VLA-4 integrins bind to the endothelial cell adhesion molecules ICAM-1 and VCAM-1, respectively (for review see reference 53) and are induced on lymphocytes and endothelium at sites of inflammation in vivo (11, 35). Furthermore, lymphocytes can be targeted to specific tissues through specialized interactions of lymphocyte integrins and endothelial adhesion molecules. For example, the process by which circulating T cells migrate to target tissues, as in the localization of lymphocytes to Peyer's patch in the gut, is mediated, in part, by the interaction of $\alpha_4\beta_p$ on lymphocytes with the high endothelial venules (15, 16). The $\alpha_4\beta_p$ integrin is distinct from Mel/LAM1, which targets lymphocytes to peripheral lymph

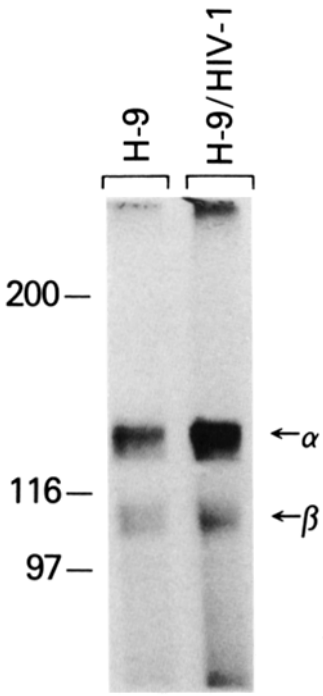


*Figure 4.* Effects of HIV-1 infection on integrin synthesis. Immunoprecipitation of integrin subunits in uninfected and HIV-1-infected H9 cells. Polyclonal rabbit antiserum to $\beta_1$ integrin was used to evaluate changes in [$^{35}$S]methionine incorporation. Molecular mass standards, expressed in kilodaltons, are also indicated.

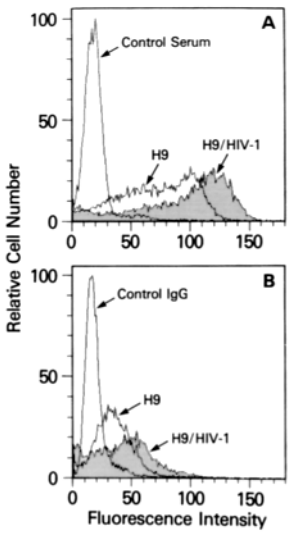


*Figure 5.* (*A*) Cell surface expression of $\beta_1$ integrin on HIV-1-infected and uninfected H-9 cells by FACS analysis. Uninfected H-9 cell fluorescence intensity is indicated in the unshaded area and infected cell fluorescence is shown by the shaded area. The $\beta_1$ subunit fluorescence intensity of HIV-1-infected cells was 132% greater than that of uninfected cells. (*B*) Cell surface expression of $\alpha_5$ integrin subunit on HIV-1-infected and uninfected H-9 cells. These studies were performed identically to those in *B*, but 100 $\mu$g rat IgG was used as a control and 100 $\mu$g of anti-$\alpha_5$ monoclonal antibody was used as the primary specific antibody. The $\alpha_5$ subunit fluorescence intensity of HIV-1-infected cells was 79% greater than that of uninfected cells. Increased intensity of fluorescence was calculated by the following formula: % increase in intensity = $\log^{-1}$ (number of channels/64) $\times$ (100) where the number of channels is equal to the number of channels shifted in the mean fluorescence intensity by HIV-1-infection. The shift in mean $\beta_1$ = 25 channels and the shift in mean $\alpha_5$ = 12 channels. These experiments were performed in duplicate.

nodes (24, 55, 56). Recently an endothelial leukocyte cell adhesion molecule (ELAM) has been shown to serve as a specific target for skin-homing memory T cells (38, 52). Finally, the ability of lymphocytes to interact with and traverse the extracellular matrix is facilitated by the $\beta_1$ integrin group, also known as the lymphocyte VLA molecules (for review see references 2, 3, 14, 29, 45). These molecules are induced in activated and memory T cells and have binding specificities for the extracellular matrix proteins laminin, collagens, and fibronectin.

The mechanism of cellular attachment to fibronectin and other adhesion proteins appears to be highly conserved and one that may serve as a relatively common pathway in pathogenesis. For example, several microbial pathogens are internalized by phagocytic and nonphagocytic tissues by interactions of integrins with their ligands, commonly but not exclusively associated with the Arg-Gly-Asp (RGD) tripeptide sequence (for review see references 19, 32, 40, 42). These previous observations in combination with those in the present study suggest the possibility that the pathogenesis of HIV-1-related syndromes such as Kaposi's sarcoma, AIDS-associated dementia, and AIDS-associated nephropathy may also result from the exploitation of normal T cell adherence properties. Our results suggest that HIV-1-infected T cells may take advantage of an initial step in this process, T cell attachment to extracellular matrix components, via integrin adhesive systems. Thus, increased adhesion to fibronectin and collagen IV which are found in endothelial cell basement membranes or connective tissues could facilitate infected T cell entry and localization. Once localized in tissues, the infected lymphocytes could infect local cells and/or release viral gene products. In this regard, the viral gene product, Tat, has recently been shown to undergo uptake by cells and to affect cell behavior and gene expression (6, 7, 10, 44). Since VLA integrins play a critical role in lymphocyte localization in tissues, our finding that HIV-1 induces the RGD-dependent integrin $\alpha_5\beta_1$ (VLA-5) on infected T cells provides a mechanism whereby HIV-1 and/or secreted HIV-1 gene products could be localized to organ tissues. Such localization of infected T cells to target tissues such as the skin, brain, or kidney, as well as the host response to virus or viral gene products, could help explain the tissue specificity of AIDS-related syndromes.

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