Identification of an Arg-Gly-Asp (RGD) Cell Adhesion Site in Human Immunodeficiency Virus Type 1 Transactivation Protein, *tat*

David A. Brake, Christine Debouck, and Gregory Biesecker

Department of Molecular Genetics, SmithKline Beecham Pharmaceuticals, King of Prussia, Pennsylvania 19406-0939; Department of Pathology, Hahnemann University, Philadelphia, Pennsylvania 19102-1192

Abstract. *Tat*, the transactivation factor of human immunodeficiency virus type 1 (HIV-1), contains the highly conserved tripeptide sequence Arg-Gly-Asp (RGD) that characterizes sites for integrin-mediated cell adhesion. The *tat* protein was assayed for cell attachment activity by measuring the adhesion of monocytic, T lymphocytic, and skeletal muscle-derived cell lines to *tat*-coated substratum. All cell lines tested bound to *tat* in a dose-dependent manner and the *tat* cell adhesion required the RGD sequence because *tat* mutants constructed to contain an RGE or KGE tripeptide sequence did not mediate efficient cell adhesion. The *tat*-mediated cell attachment also required divalent cations and an intact cytoskeleton. In addition, cell adhesion to *tat* was inhibited in the presence of an RGD-containing peptide GRGDSPK or an anti-tat mAb that recognizes the RGD epitope. These results strongly suggest that cells are bound to *tat* through an integrin. Interestingly, myoblast cells bound to *tat* remained round, whereas the same cells attached through an integrin for a matrix protein typically flatten and spread. The role of this RGD-dependent cellular adhesion of *tat* in HIV-1 infection remains to be determined.

PROTEINS that interact with integrin cell adhesion receptors frequently contain the amino acid tripeptide RGD sequence (5, 20, 26, 30) within the integrin binding site. RGD sequences are found in fibronectin, vitronectin and collagen and constitute extracellular matrix attachment sites used for integrin-mediated cell adherence during development and differentiation (30). Integrin receptors on leukocytes bind to coagulation proteins (von Willebrand factor, fibrinogen, thrombospondin) and complement components (C3b), and participate in cell–cell adhesion (LFA-1 with I-CAM). These interactions are involved in homeostatic regulation, phagocytosis, cell migration, cell signaling, cellular trafficking, and lymphocyte recognition (11, 23, 30, 39). In addition, certain bacterial, parasitic, and viral proteins possess RGD sequences which recognize integrin receptors and may contribute to pathogenesis (1, 30, 31).

Human immunodeficiency virus type I (HIV-1),[1] the etiologic agent of AIDS (6, 17, 22, 28), encodes a gene for a transactivating protein, termed *tat*, which contains an RGD sequence. HIV-1 *tat* is an 86-amino acid-long protein, which greatly increases viral gene expression and replication (2, 4, 13, 14, 34, 35). The tripeptide RGD sequence in *tat* is located in the carboxy-terminal portion of the protein and is highly conserved among HIV-1 isolates (Fig. 1) (24). The presence of an RGD sequence within *tat* raised the intriguing possibility that this tripeptide could constitute a cell attachment site. In this study, purified *tat* protein was assayed for cell attachment to various cell types. The observed cell adhesion was further characterized using an RGD-containing peptide, anti-*tat* mAbs and mutant *tat* proteins which contained amino acid substitutions within the RGD sequence.

Dr. Brake's present address is Biological Research, SmithKline Beecham Animal Health Products, King of Prussia, PA, 19406-0939. Address all correspondence to Dr. C. Debouck.

1. *Abbreviations used in this paper*: HIV, human immunodeficiency virus; TBST, Tris-buffered saline, 0.5% Tween 20.

Materials and Methods

Construction of HIV-1 tat Bacterial Expression Vectors

Construction of the full-length *tat* (HTLV-IIIB isolate) bacterial expression plasmid, pOTS-TATIII, was previously described (2). The RGE *tat* (Fig. 1, mutant *1*) expression vector was constructed as follows. An Nde I-Xba I 582-bp fragment from pOTS-TATIII was gel purified and subcloned into the polylinker region of plasmid pUC19 using T4 DNA ligase. The resulting plasmid, pUC19TAT.WT, was digested with Ava I and Xba I and then ligated to a 35-bp Ava I-Xba I synthetic oligonucleotide to generate pUC19TAT.RGE. This synthetic oligonucleotide reconstitutes the 3′ end of the *tat* gene with a single base substitution changing Asp_{80} to Glu. A Bam HI–Xba I 253-bp fragment containing the full-length mutated *tat* gene was purified from pUC19TAT.RGE and then ligated into the Bam HI-Xba I site of pOTS-TATIII. The KGE *tat* (Fig. 1, mutant *2*) expression vector was similarly constructed except that a 35-bp Ava I–Xba I synthetic oligonucleotide containing a double base substitution (changing Arg_{78} to Lys and Asp_{80} to Glu) was used. These mutations were confirmed by dideoxy DNA sequencing (32) using an appropriate sequencing primer.

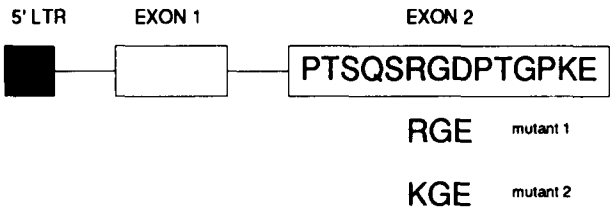

Figure 1. Coding exons and partial amino acid sequence of HIV-1 *tat*. *Tat* is composed of two coding exons, exon 1 (72 amino acids) and exon 2 (14 amino acids). The amino acid sequence of the second exon from the HTLV-IIIB isolate (3) is shown. The RGE (mutant *1*) and KGE (mutant *2*) *tat* mutants were constructed, expressed, and purified as described in Materials and Methods.

Purification of Wild-Type and Mutant tat Proteins

Escherichia coli (strain AR120) bacterial cells containing the respective pOTS expression vectors were grown in LB broth containing 50 μg/ml ampicillin at 37°C to OD 0.4 (650 nm) and induced by the addition of 60 μg/ml nalidixic acid as described (2). 5 h after induction sonicated cell lysates were centrifuged (15,000 *g*) and supernatants were acidified by slow addition of 1 M HCl to pH 3.0 to precipitate nucleic acids.

After centrifugation and neutralization to pH 7.5 using 1.5 M Tris base (pH 8.5), samples were applied to a Sephadex G-25F column (Pharmacia Fine Chemicals, Piscataway, NJ) (1 × 40 cm) equilibrated in 50 mM NaMES, pH 6.5. The protein peak was pooled and concentrated 10-fold using an Amicon YM-5 membrane (Amicon Corp., Danvers, MA).

Samples were then applied to individual anti-*tat* immunoaffinity columns (3 ml bed volume) equilibrated in PBS (pH 7.4). Columns were prepared by coupling purified anti-*tat* mAb (see below) to cyanogen bromide-activated Sepharose 4B (Pharmacia Fine Chemicals) using manufacturers' recommended conditions. The columns were washed with PBS and the flow-through material reapplied twice prior to elution. Bound samples were eluted (100 mM sodium citrate/0.5 M NaCl, pH 3.0), protein peaks pooled, and immediately neutralized to pH 7.5 using solid Tris base. Immunoaffinity purification was monitored by SDS-PAGE using Coomassie blue staining and Western blot analysis as previously described (2). Samples were concentrated, protein concentrations were determined by the method of Bradford (7), and samples were then stored at 4°C until use.

Cell Culture

The human T lymphocytic HUT-78 and MOLT-4 suspension cell lines, the human myelomonocytic THP-1 suspension cell line and the rat skeletal muscle-derived L_8 adherent myoblasts were obtained from American Type Culture Collection (Rockville, MD or Camden, NJ). All cell lines were grown and routinely subpassaged in DME supplemented with 10% FBS (Gibco Laboratories, Grand Island, NY), 2 mM L-glutamine, 10 U/ml penicillin G, and 10 μg/ml streptomycin (DME).

Cell Adhesion Assay

For the adhesion assay (29), a polystyrene plate (Linbro 76-203; non-tissue culture treated, Flow Laboratories, Maclean, VA) was coated overnight at 37°C with wild-type *tat*, mutant *tat* proteins, or the matrix protein vitronectin. Vitronectin was purified according to the method of Dahlback (12). The plate was washed with PBS, and with DME, and 0.1 ml of DME was added to each well. The adherent L_8 myoblasts were suspended by trypsinization and washed three times with trypsin inhibitor (0.5 mg/ml sterile PBS); HUT-78, MOLT-4, and THP-1 cells were washed three times in PBS; 100 μl of each cell suspension was added to wells in quadruplicate (1×10^6 cells/ml in DME). The cells were incubated in the coated wells for 1 h, fixed with 4% formalin and stained with 1% toluidine blue. Cell adherence was quantitated using a microtiter plate reader (Dynatech Laboratories, Chantilly, VA) set at 570 nM. In some experiments, the cells were incubated with 20 mM EDTA, or cytochalasin B and colchicine, separately or in combination, at 10 μg/ml each for 30 min at 4°C, and then added to the protein-coated wells. For the mAb inhibition experiments, the coated plate was incubated for 30 min with mAb and washed; then the cells were added.

ELISA

A polystyrene plate (Linbro) was coated with wild-type and mutant *tat* proteins (5 μg/ml in 100 mM $NaHCO_3/Na_2CO_3$, pH 9.4) overnight at 4°C. The plate was washed with Tris-buffered saline, 0.5% Tween-20, pH 8.0 (TBST) and unbound sites blocked with TBST/0.5% gelatin for 1 h. After washing, a 1/1,000 dilution (TBST) of a mouse anti-*tat* mAb specific for the amino terminal portion of *tat* (mAb 1, ref. 9) was added, incubated for 3 h, washed with TBST and a 1/2,000 dilution of biotin-conjugated affinity-purified goat anti-mouse IgG (Cappel Laboratories, West Chester, PA) added. After 1 h, wells were washed and a 1/2,000 dilution of alkaline phosphatase–conjugated avidin (Cappel Laboratories) was added for 1 h. Wells were washed thoroughly and bound enzyme was detected using the substrate *p*-nitrophenyl phosphate (Sigma 104; Sigma Chemical Co., St. Louis, MO) in 0.1 M glycine, pH 10.4, 1 mM $ZnCl_2$, 1 mM $MgCl_2$ buffer. The enzymatic reaction was stopped by the addition of 5 N NaOH, and the optical density measured at 405 nm.

Peptide Synthesis

The peptides GRGDSPK and GRADSPK were synthesized as carboxy-terminal amides using FMOC chemistry with the RaMPS system (Du Pont Corp., Wilmington, DE). Amino acids and other reagents were obtained from Du Pont Corp.; all solvents were of the highest grade available. The synthesis was performed according to manufacturer's instructions by deblocking the RapidAmide resin with piperidine/DMF; washing alternatively with DMF and methanol; adding the FMOC-amino acid and rocking for 2 h; washing with DMF and methanol, and then testing by ninhydrin reaction; repeating these steps for each amino acid. The peptide was cleaved from the resin and blocking groups removed with trifluoroacetic acid/1,2-ethanedithiol/phenol. The eluted peptide was precipitated with diethyl ether, and washed with ether and then with ether/ethyl acetate 1:1. The peptides were purified on a FPLC Mono S column (Pharmacia LKB Biotechnology, Piscataway, NJ). The sample was applied in 50 mM $NaPO_4$, pH 3.0, 30% acetonitrile, and the column developed with a linear NaCl gradient to 0.35 M. The column eluent was monitored at OD_{214}. The peptides eluted as single peaks, and each contained the appropriate molar ratios of amino acids by compositional analysis (Protein Structure Laboratory, Wistar Institute, Philadelphia, PA).

Anti-tat mAbs

The characterization of the murine anti-*tat* mAbs used has recently been reported (9). The mAbs were purified from ascitic fluid by passage through glass wool to remove cellular debris. The IgG fraction from each sample was purified by chromatography on 2-ml columns of Affi-Gel Protein A MAPSII (Bio-Rad Laboratories, Richmond, CA) according to the manufacturer specifications. Eluates were dialyzed overnight at 4°C against 50 mM Hepes pH 7.0, 150 mM NaCl, and concentrated using Centricon-10 (Amicon Corp., Danvers, MA) cartridges. Protein concentration was determined as above and mAbs were stored at 4°C until use.

Results

Cell Adhesion to tat and Mutant tat Proteins

To test the hypothesis that *tat* can mediate cell adhesion, purified recombinant *tat* protein was assayed for attachment of various cultured cell lines. Because of the known HIV-1 tropism for T lymphocytes and monocyte/macrophage cells, the HIV-1 replication-competent human T lymphocytic cell lines HUT-78 and MOLT-4 and the human myelomonocytic cell line THP-1 (21, 22, 36) were selected for initial studies. In addition to these cell lines, a rat skeletal muscle cell line, L_8, was also utilized because of its restricted adhesion properties (8). To assay for cell adhesion, microtiter wells were coated with immunoaffinity-purified *tat* protein, seeded with cells, and incubated for 1 h. *tat*-mediated cell adhesion was quantitated by optical density measurement after toluidine blue staining. As shown in Fig. 2, *tat* was highly efficient in

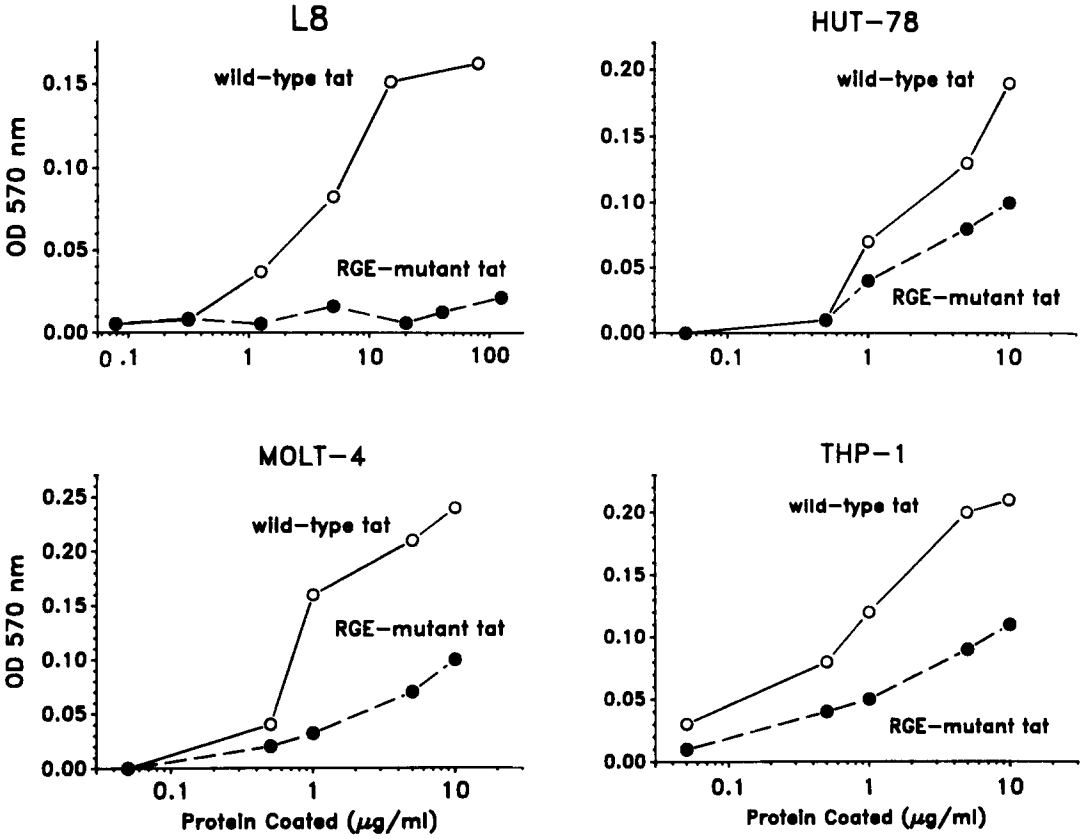

Figure 2. Dose response of the cell adhesion to wild-type and RGE mutant *tat*. The adherence of cells to protein-coated wells of a 96-well microtiter plate was measured by optical density reading (570 nm) after incubation and staining as described in Materials and Methods. Shown is the adherence of rat skeletal L_8 myoblasts, human T lymphocytic HUT-78 cells, human T lymphocytic MOLT-4 cells, and human monocytic THP-1 cells to wells coated with purified recombinant wild-type (○) or RGE mutant *tat* protein (●) at increasing concentrations. SE in all experiments were ±0.025 OD_{570}. Note the difference in the abscissa and ordinate scales since assays were performed at different times.

mediating cell adherence of all four cell lines tested. The *tat*-mediated adhesion was dose dependent and the *tat* coating concentration, which gave half-maximal cell adhesion, was between 1 and 5 μg/ml (0.1–0.5 μM). For each cell line the level of adherence to *tat*-coated wells was equivalent to the highest level of binding obtained with control matrix proteins (Fig. 4 and data not shown).

To determine the role of the *tat* RGD-tripeptide sequence in the cell adhesion, two *tat* mutants were constructed which contained the conservative replacement sequences RGE and KGE. The single amino acid D_{80}-E substitution was selected since the overall net charge of *tat* would not be affected and an RGD-RGE substitution has been shown to abrogate cell binding to fibronectin (25). This D-E change was sufficient to significantly reduce or completely eliminate *tat*-mediated cell binding (Fig. 2). The mutation completely eliminated the adherence of L_8 cells and reduced the binding (40–90%) of the HUT-78, MOLT-4 and THP-1 cells. The L_8 myoblasts did not adhere to the mutant RGE *tat* protein even when wells were coated at concentrations up to 120 μg/ml, whereas wild-type RGD *tat* protein coated at 1 μg/ml or more mediated efficient cell adhesion.

To further investigate the involvement of the tripeptide RGD sequence of *tat* in cell attachment, a second mutant *tat* protein was constructed containing conservative amino acid substitutions at both the arginine and aspartic residues (Fig. 1, mutant 2). This KGE mutant *tat* protein also completely lacked cell attachment activity for L_8 cells (Fig. 3). The adhesion of the other cell lines to the KGE double mutant was only slightly above background and was therefore more drastically reduced as compared with the adhesion to the single RGE mutant *tat* protein (Fig. 3). The adhesion was performed several times at a single protein concentration (5 μg/ml) since insufficient quantities of purified KGE mutant protein were available to perform a full dose response. The lack of cell adhesion to the RGE and KGE mutant *tat* proteins could not be attributed to binding of lower levels of mutant *tat* to the wells, since similar amounts of anti-*tat* mAb bound to wells coated with either wild-type *tat* (0.88 ± 0.04 OD_{405}), mutant RGE (0.93 ± 0.04), or mutant KGE (0.79 ± 0.07) *tat* protein as judged by ELISA.

tat-mediated Cell Adhesion Is Dependent on Divalent Cations and an Intact Cytoskeleton, and Is Inhibited by an RGD-containing Peptide

The negative effect of *tat* mutations at the RGD site on cell adhesion suggested that cell binding could involve the partic-

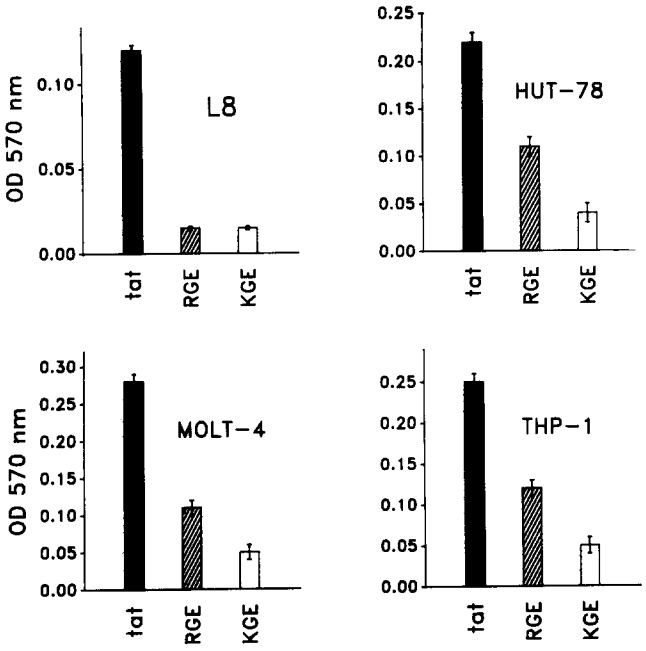

Figure 3. Comparison of cell adhesion of cultured cell lines to wild-type, RGE, and KGE mutant *tat*. The adherence of cells to protein-coated wells of a 96-well microtiter plate was measured by optical density reading (570 nm) after incubation and staining as described in Materials and Methods. Shown is the adherence of rat skeletal L_8 myoblasts, human T lymphocytic HUT-78 cells, human T lymphocytic MOLT-4 cells, and human monocytic THP-1 cells to wells coated with purified recombinant wild-type, RGE, or KGE mutant *tat* protein (5 μg/ml sterile PBS). Single point determinations were conducted due to insufficient quantities of purified KGE mutant *tat* protein. Note differences in the abscissa scales.

ipation of an integrin. To further investigate this possibility, the effect of EDTA, cytoskeletal blockers, and an RGD-containing peptide on cell adhesion was investigated. For these investigations the skeletal muscle–derived L_8 cell line was used because this line has been characterized for integrin expression and has been found to adhere primarily to vitronectin but not to fibronectin, laminin, or collagen (8). It was felt that the restricted integrin distribution of L_8 cells would allow for a more straightforward interpretation of the results.

Several integrins have been shown to require divalent cations such as Ca^{2+} or Mg^{2+} for binding to their ligand and there is strong evidence that integrins are connected to the cytoskeleton (20, 30). We observed that the interaction between *tat* and L_8 cells was dependent on divalent cations. Indeed, the addition of EDTA abrogated cell attachment

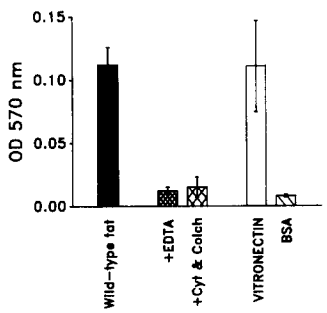

Figure 4. Effect of divalent cations and cytoskeletal blockers on *tat*-mediated cell adhesion. Shown is the adherence of rat skeletal muscle L_8 cells to wild-type *tat*, to wild-type *tat* plus EDTA (20 mM), to wild-type *tat* plus a combination of cytochalasin B and colchicine. Also shown is control binding of L_8 cells to 5 μg/ml vitronectin and 5 μg/ml BSA.

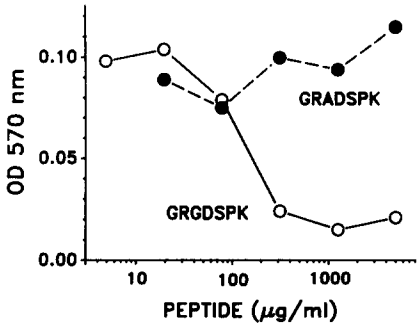

Figure 5. Peptide inhibition of *tat*-mediated cell adhesion. Shown is the adherence of rat skeletal muscle L_8 cells to wild-type *tat* in the presence of increasing concentrations of either an RGD-containing (○) or a control RAD-containing (●) peptide. SE for all data points were ±0.025 OD_{570}.

(Fig. 4) and adherence was restored by the addition of either Ca^{2+} or Mg^{2+} (data not shown). In addition, cell attachment required an intact cytoskeleton, as cell binding was eliminated in the presence of cytochalasin B plus colchicine (Fig. 4). These results are all consistent with integrin-mediated cell attachment (29, 30).

RGD-containing peptides are known to compete with several ligands for binding to their respective integrin (20, 30). Accordingly, we examined the effect of an RGD-containing peptide on the *tat*-mediated cell adhesion. As shown in Fig. 5, the L_8 cell attachment to *tat* was abrogated by addition of the synthetic peptide GRGDSPK. This peptide reduced the cell adherence to background levels when present at concentrations >100 μg/ml (0.1 mM). L_8 cell attachment to the control matrix protein vitronectin was inhibited at similar levels of the peptide (data not shown). In contrast, the control synthetic peptide, which contained an RAD sequence, did not block cell attachment at concentrations up to 5 mg/ml (Fig. 5). These results are also consistent with integrin-mediated cell attachment.

Specific Inhibition of tat-mediated Cell Attachment by Anti-tat mAb 9

A panel of murine mAbs reactive with HIV-1 *tat* have been recently mapped and epitopes defined using Pepscan analysis (9). One mAb, designated mAb 9, was found to react with nonapeptides spanning sequences at the carboxy-terminal end of *tat* and the critical residues for binding were found to be centered on the RGD site. Moreover, RGE and KGE mutant *tat* proteins were no longer recognized by mAb 9, but retained reactivity to other mAbs (Brake, D., unpublished observations). In the present study, we asked if mAb 9 could inhibit L_8 myoblast attachment to *tat*-coated wells (Fig. 6). A 50% reduction in cell attachment was found at 25 μg/ml and approached background levels (0.05 OD_{570}) at 250 μg/ml. Inhibition was specific since the control mAb 1, which recognizes an amino-terminal epitope, failed to efficiently block cell attachment. Neither mAb 1 nor mAb 9 inhibited cell attachment on vitronectin substrates (data not shown). These data further demonstrate that the RGD site on *tat* is involved in L_8 cell adhesion.

Cell Morphology of tat-mediated Cell Adhesion

An interesting observation of the *tat*-mediated cell adherence

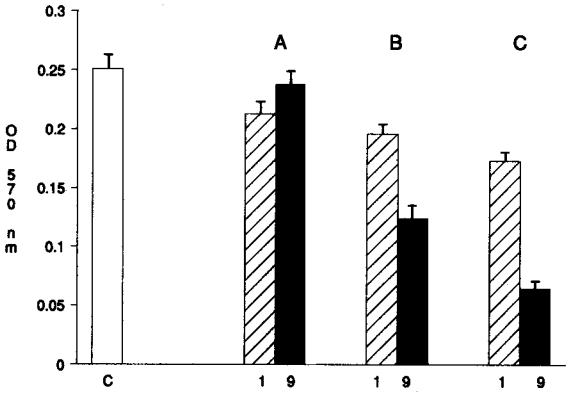

Figure 6 Anti-*tat* mAb inhibition of *tat*-mediated cell adhesion Shown is the adherence of rat skeletal muscle L_8 cells to wild-type *tat* in the presence of either no antibody (open bar) or mAb 1 (*hatched bar*) or mAb 9 (*solid bar*) In *A*, the concentration of mAb is 1 μg/ml In *B*, the concentration of mAb is 25 μg/ml In *C*, the concentration of mAb is 250 μg/ml Each bar is the average of values determined from triplicate wells (± SEM) Background adherence in the absence of *tat* was 0 05 OD_{570}

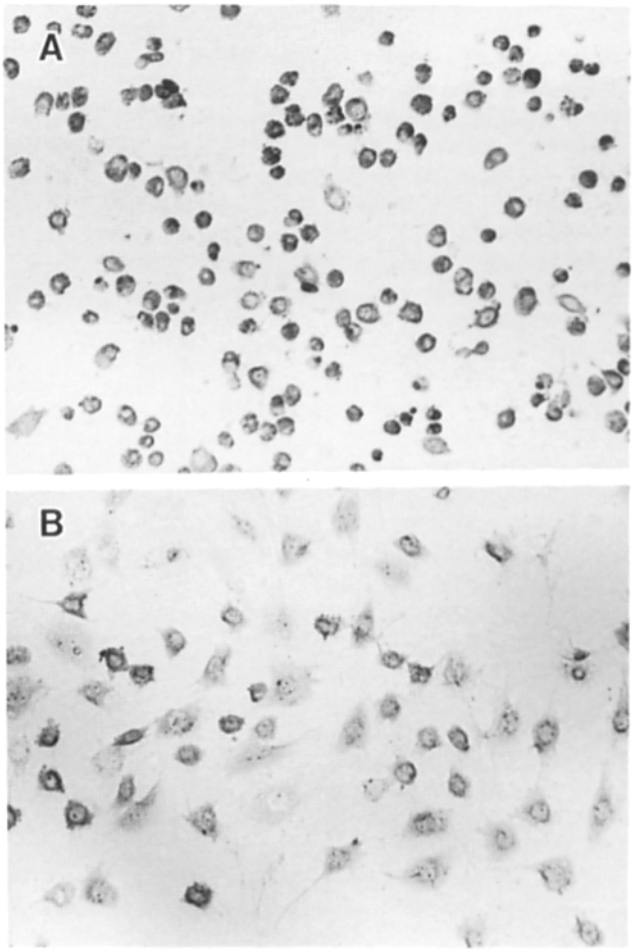

Figure 7 Morphology of cells attached to RGD-containing proteins L_8 cells seeded on *tat* remained round (*A*), in contrast to L_8 cells seeded on vitronectin-coated substratum (*B*), which were spread and flattened with numerous projections Similar results were obtained whether L_8 cells were incubated 1 h or overnight on the protein-coated substratum The cell adherence was analyzed after suspension of the mononucleated L_8 myoblasts with trypsin, washing with trypsin inhibitor (0 5 mg/ml PBS) and seeding at 10^5 cells/well in protein-coated wells of a microtiter plate

was that L_8 myoblasts remained round after attachment (Fig 7 *a*), whereas when bound to vitronectin the L_8 cells were spread, flattened, and had numerous projections (Fig 7 *b*) Cell spreading was also found for L_8 cells bound nonspecifically to tissue culture-treated plastic or poly-lysine (data not shown) Absence of cell spreading on *tat* was also noted for the T lymphocytic and myelomonocytic cell lines The distinct morphology of L_8 cells bound to *tat* suggests that events after binding leading to cytoskeletal reorganization differ for cells bound to *tat* compared with cells bound to extracellular matrix proteins such as vitronectin

Discussion

The HIV-1 *tat* gene is composed of two coding exons which direct the synthesis of an 86 amino acid-long protein The second exon codes for 14 amino acids, which include the tripeptide sequence RGD, which is highly conserved among HIV-1 isolates sequenced to date (24) Since the RGD sequence shared among many extracellular ligands is thought to play a crucial role in cell adhesion (30), purified recombinant *tat* protein was assayed for cell attachment using several different cell lines *tat* was shown to mediate attachment of T lymphocytic, myelomonocytic, and muscle-derived cell lines in a dose-dependent manner To test that adherence was specific, and not the result of ionic interactions with the highly basic *tat* protein (two lysines and six arginines over nine residues), the *tat*-mediated cell adhesion was further tested using *tat* mutations in the consensus RGD sequence

Two different mutants containing alterations at the consensus RGD sequence site were constructed, expressed, purified and tested for their cell attachment activity Initially, a conservative Asp_{80} to Glu amino acid substitution was chosen since this mutation could alter specific interactions without affecting any nonspecific ionic interactions and this type of mutation has been shown to abrogate the cell adhesion activity of fibronectin (25) The single RGD to RGE mutation completely abrogated *tat*-mediated cell adhesion to L_8 cells over a wide dose range, and produced a significant but less dramatic effect on the adhesion of the human T lymphocytic and monocytic cell lines Furthermore, a second *tat* mutant in which the RGD was changed to KGE also failed to efficiently bind L_8 myoblasts, and reduced the cell attachment of the HUT-78, MOLT-4 and THP-1 cell lines almost to background levels The observed differences between the various cell lines in their binding to the wild-type and *tat* mutants could result from different levels of expression of a specific cell receptor, possibly an integrin receptor To test the hypothesis that *tat* directly interacts with a functional integrin, additional experiments were conducted using reagents known to block ligand–integrin interactions

tat-mediated cell binding was completely eliminated in the presence of either the metal chelator EDTA or the cytoskeletal blockers cytochalasin B and colchicine, agents that have been shown to inhibit several other ligand–integrin interactions (20) In addition, the L_8 cell attachment to *tat* was also inhibited by an RGD-containing peptide but not by a control

RAD-containing peptide. The inhibition by GRGDSPK is somewhat surprising, since the RGD tripeptide in *tat* is followed by a highly conserved proline and a proline residue could markedly affect conformation-dependent interactions. However, initial attempts to inhibit *tat* cell attachment with a proline-containing peptide SRGDPT based on the *tat* sequence have not been successful, suggesting that protein regions outside of the *tat* tripeptide may stabilize a particular RGD conformation. The results obtained with anti-*tat* mAb 9 further support the notion that the RGD tripeptide in *tat* participates in cell adhesion. Immunoprecipitation experiments are currently in progress to further characterize the putative *tat* cell surface receptor.

Integrin alpha-subunits contain calcium-binding sequences involved in stabilizing the protein structure and in ligand binding (20, 30). The alpha and beta integrin subunits both contain a small cytoplasmic domain which can interact with the cytoskeleton matrix (10, 11, 37). Integrin-mediated binding to vitronectin and other matrix proteins is typically associated with integrin aggregation and cytoskeletal reorganization of microfilaments and microfilament-associated proteins, such as talin and vinculin (10, 11). L_8 myoblasts bound to vitronectin were spread and displayed numerous projections typical of integrin-mediated binding to matrix proteins. In contrast, although cell binding to *tat* involved the cytoskeleton, the L_8 cells on *tat* did not spread. The morphological appearance of cells bound to *tat* is similar to the morphology of hematopoietic cells bound to RGD-containing proteins, where reorganization of cytoskeletal structures occurs in the absence of cell spreading (10). These results indicate that while cells may bind to *tat* through an RGD-dependent integrin interaction, the cell binding may not involve the vitronectin receptor or related matrix protein integrin.

The identification of a highly conserved functional RGD site on HIV-1 *tat* is the first example, to our knowledge, of a specific cell adhesion site on a retrovirally encoded regulatory protein. The identification of a cell attachment site on *tat* indicates that *tat* binds to a specific cell surface protein, possibly an integrin. It will be interesting to examine the role of RGD-mediated adhesion in the exogenous transactivation of *tat* recently described (15, 16, 19). The RGD-mediated *tat* binding could promote *tat* cellular uptake *in vivo* and subsequent transactivation of virus in latently infected cells. Alternatively, *tat* uptake could lead to aberrant gene expression in uninfected cells, such as that observed in *tat* transgenic mice (38). *tat* binding could also directly stimulate viral and/or cell function without requiring cell uptake. The location of the highly conserved RGD sequence in a separate exon also raises the possibility that the cell attachment site could be incorporated into other viral proteins by alternate splicing. Characterization of this cell adhesion site and its importance for *tat* function and HIV-1 infection will require the precise elucidation of the mechanism of action of *tat* transactivation as well as the isolation of the *tat* cell receptor.

We thank G. Sathe and F. Watson for oligonucleotide synthesis and P. Kmetz for DNA sequencing. We also thank T. Smith and S. Franklin for help in the purification protocol and J. Klass and K. Little for their excellent assistance. We thank M. Rosenberg, J. Samanen, J. Stadel, and E. Wawrousek for critical reading of the manuscript.

This work was supported by National Institutes of Health grant AI24845 (D. Brake, C. Debouck) and a grant from the Muscular Dystrophy Association (G. Biesecker).

Received for publication 19 December 1989 and in revised form 23 May 1990.

References

1. Acharya, R., D. Fry, D. Stuart, G. Fox, D. Rowlands, and F. Brown. 1989. The three dimensional structure of foot-and-mouth disease virus at 2.9 Å resolution. *Nature (Lond.).* 337:709–716.
2. Aldovini, A., C. Debouck, M. B. Feinberg, M. Rosenberg, S. K. Arya, and F. Wong-Staal. 1986. Synthesis of the complete trans-activation gene product of human T-lymphotropic virus type III in *Escherichia coli*. *Proc. Natl. Acad. Sci. USA.* 83:6672–6676.
3. Alizon, M., S. Wain-Hobson, L. Montagnier, and P. Sonigo. 1986. Genetic variability of the AIDS virus: nucleotide sequence analysis of two isolates from African patients. *Cell.* 46:63–74.
4. Arya, S. K., C. Guo, S. F. Josephs, and F. Wong-Staal. 1985. Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). *Science (Wash. DC).* 229:69–73.
5. Barnes, D. W., J. Silnutzer, C. See, and M. Schaffer. 1983. Characterization of human serum spreading factor with monoclonal antibody. *Proc. Natl. Acad. Sci. USA.* 80:1362–1366.
6. Barre-Sinoussi, F., J. C. Chermann, R. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler-Blin, F. Brun-Vezinet, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1983. Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immunodeficiency syndrome (AIDS). *Science (Wash. DC).* 220:868–871.
7. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
8. Biesecker, G. 1990. The complement SC5b-9 complex mediates cell adhesion through a vitronectin receptor. *J. Immunol.* 145:209–214.
9. Brake, D. A., J. Goudsmit, W. J. A. Krone, P. Schammel, A. Appleby, R. H. Meloen, and C. Debouck. 1990. Characterization of murine monoclonal antibodies to the *tat* protein from human immunodeficiency virus type 1. *J. Virol.* 64:962–965.
10. Burn, P., A. Kupfer, and S. J. Singer. 1988. Dynamic membrane-cytoskeletal interactions: Specific association of integrin and talin arises *in vivo* after phorbol ester treatment of peripheral blood lymphocytes. *Proc. Natl. Acad. Sci. USA.* 85:497–501.
11. Burridge, K., K. Fath, T. Kelly, G. Nuckolls, and C. Turner. 1988. Focal adhesions: transmembrane functions between the extracellular matrix and the cytoskeleton. *Annu. Rev. Cell Biol.* 4:487–525.
12. Dahlback, B., and E. R. Podack. 1985. Characterization of human S-protein, an inhibitor of membrane attack complex of complement: demonstration of a free reactive thiol group. *Biochemistry.* 24:2368–2374.
13. Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine, 1986. The transactivator gene of the human T cell lymphotropic virus type III is required for replication. *Cell.* 44:941–947.
14. Fisher, A. G., M. B. Feinberg, S. F. Josephs, M. E. Harper, L. M. Marselle, G. Reyes, M. A. Gonda, A. Aldovini, C. Debouck, R. C. Gallo, and F. Wong-Staal. 1986. The trans-activator gene of HTLV-III is essential for virus replication. *Nature (Lond.).* 320:367–371.
15. Frankel, A. D., and C. O. Pabo. 1988. Cellular uptake of the *tat* protein from human immunodeficiency virus. *Cell.* 55:1189–1193.
16. Frankel, A. D., S. Biancalana, and D. Hudson. 1989. Activity of synthetic peptides from the tat protein of human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA.* 86:7397–7401.
17. Gallo, R. C., S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleska, B. Safai, G. White, P. Foster, and P. D. Markham. 1984. Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science (Wash. DC).* 224:500–503.
18. Gehlsen, K. R., L. Dillner, E. Engvall, and E. Ruoslahti, 1988. The human laminin receptor is a member of the integrin family of cell adhesion receptors. *Science (Wash. DC).* 241:1228–1229.
19. Green, M., and P. M. Lowenstein. 1988. Autonomous functional domains of chemically synthesized human immunodeficiency *tat* trans-activator protein. *Cell.* 55:1179–1188.
20. Hynes, R. O. 1987. Integrins: a family of cell surface receptors. *Cell.* 48: 549–554.
21. Kikukawa, R., Y. Koyanagi, S. Harada, N. Kobayashi, M. Hatanaka, and N. Yamamoto. 1986. Differential susceptibility to the acquired immunodeficiency syndrome retrovirus in cloned cells of human leukemic T-cell line Molt-4. *J. Virol.* 57:1159–1162.
22. Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuru, and L. S. Oshiro. 1984. Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science (Wash. DC).* 235:840–842.
23. Marlin, S. D., and T. A. Springer. 1987. Purified intercellular adhesion

molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 51:813–819.
24. Meyers, G. 1989. Human Retroviruses and AIDS 1989. Los Alamos National Laboratory, Los Alamos, NM. Section II, p. 37.
25. Obara, M., M. S. Kang, and K. M. Yamada. 1988. Site-directed mutagenesis of the cell-binding domain of human fibronectin: separable, synergistic sites mediate adhesive function. *Cell.* 53:649–657.
26. Pierschbacher, M. D., and E. Ruoslahti. 1984. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of molecule. *Nature (Lond.).* 309:30–33.
27. Pierschbacher, M. D., and E. J. Ruoslahti. 1987. Influence of stereochemistry of the sequence Arg-Gly-Asp-Xaa on binding specificity. *J. Biol. Chem.* 262:17294–17298.
28. Popovic, M., M. G. Sarngadharan, E. Read, and R. C. Gallo. 1984. Detection, isolation and continuous production of cytopathic retrovirus (HTLV-III) from patients with AIDS and pre-AIDS. *Science (Wash. DC).* 224:497–500.
29. Ruoslahti, E., E. G. Hayman, M. Pierschbacher, and E. Engvall. 1982. Fibronectin: purification, immunochemical properties, and biological activities. *Methods Enzymol.* 82:803–831.
30. Ruoslahti, E., and M. D. Pierschbacher. 1987. New perspectives in cell adhesion: RGD and integrins. *Science (Wash. DC).* 238:491–497.
31. Russell, D. G., and S. D. Wright. 1988. Complement receptor type 3 (CR3) binds to an Arg-Gly-Asp-containing region of the major surface glycoprotein, gp 63, of *Leishmania* promastigotes. *J. Exp. Med.* 168:279–292.
32. Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.
33. Seigel, L. J., L. Ratner, S. F. Josephs, D. Derse, M. B. Feinberg, G. R. Reyes, S. J. O'Brien, and F. Wong-Staal. 1986. Transactivation induced by human T-lymphotropic virus type III (HTLV III) maps to a viral sequence encoding 58 amino acids and lacks tissue specificity. *Virology.* 148:226–231.
34. Sodroski, J., R. Patarca, C. Rosen, F. Wong-Staal, and W. Haseltine. 1985. Location of the trans-activation region on the genome of human T-cell lymphotropic virus type III. *Science (Wash. DC).* 229:74–77.
35. Sodroski, J. G., C. R. Rosen, F. Wong-Staal, M. Popovic, S. Arya, R. C. Gallo, and W. A. Haseltine. 1985. Trans-acting transcriptional activation of human T-cell leukemia virus type III long terminal repeat. *Science (Wash. DC).* 227:171–173.
36. Tsuchiya, S., M. Yamabe, Y. Yamaguchi, Y. Kobayashi, T. Konno, and K. Tada. 1980. Establishment and characterization of a human acute monocytic leukemia cell line (THP-1). *Int. J. Cancer.* 26:171–176.
37. Turner, C. E., F. M. Pavalko, and K. Burridge. 1989. The role of phosphorylation and limited proteolytic cleavage of talin and vinculin in the disruption of focal adhesion integrity. *J. Biol. Chem.* 264:11938–11944.
38. Vogel, J., S. H. Hinrichs, R. K. Reynolds, P. A. Luciw, and G. Jay. 1988. The HIV *tat* gene induces dermal lesions resembling Kaposi's sarcoma in transgenic mice. *Nature (Lond.).* 335:606–611.
39. Wright, S. D., P. A. Reddy, M. T. Jong, and B. W. Erickson. 1987. C3bi receptor (complement receptor type 3) recognizes a region of complement protein C3 containing the sequence Arg-Gly-Asp. *Proc. Natl. Acad. Sci. USA.* 84:1965–1968.