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, myoblasts 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, fibronogen, 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 1 (HIV-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 that 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.

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 Asp62 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 Arg37 to Lys and Asp62 to Glu) was used. These mutations were confirmed by dideoxy DNA sequencing (32) using an appropriate sequencing primer.
and washed three times with trypsin inhibitor (0.5 mg/ml sterile PBS); tin. Vitronectin was purified according to the method of Dahlback (12). The culture treated, Flow Laboratories, Mclean, VA) was coated overnight at 20 mM EDTA, or cytochalasin B and colchicine, separately or in combination; quantitated using a microtiter plate reader (Dynatech Laboratories, Chantilly, VA) 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.}

**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 pP76 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 naldixid 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 x 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 cell line THP-1 (21, 22, 36) were selected for initial studies. In addition to these cell lines, a rat skeletal muscle cell line, rat 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, Ls, 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

**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 were selected for initial studies. In addition to these cell lines, a rat skeletal muscle cell line, Ls, 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
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-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 L₈ cells and reduced the binding (40-90%) of the HUT-78, MOLT-4 and THP-1 cells. The L₈ 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 L₈ 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 mAb tat to the wells, since similar amounts of anti-tat mAb bound to wells coated with either wild-type tat (0.88 ± 0.04 OD₄₀₅), 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-
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 muscle L8 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.

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 muscle L8 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.

Figure 4. Effect of divalent cations and cytoskeletal blockers on tat-mediated cell adhesion. Shown is the adherence of rat skeletal muscle L8 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 L8 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 L8 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 ±0.0025 OD₅₇₀.

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 L8 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 OD₅₇₀) 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 L8 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 Ls 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

was that Ls myoblasts remained round after attachment (Fig 7 a), whereas when bound to vitronectin the Ls cells were spread, flattened, and had numerous projections (Fig 7 b). Cell spreading was also found for Ls 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 Ls 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. Since the RGD sequence shared among many extracellular ligands is thought to play a crucial role in cell adhesion, 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 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. The single RGD to RGE mutation completely abrogated tat-mediated cell adhesion to Ls cells over a wide dose range, and produced a significant but less dramatic effect on the adhesion of the human T lymphocytic and monocylic cell lines. Furthermore, a second tat mutant in which the RGD was changed to KGE also failed to efficiently bind Ls 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. In addition, the Ls cell attachment to tat was also inhibited by an RGD-containing peptide but not by a control
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). L4 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 L4 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 retroviral 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 A resolution. Nature (Lond.). 337:709-716.
2. Allevi, A., C. Debouck, M. B. Feinberg, M. Rosenberg, S. K. Arja, and W. Wong-Staal. 1986. Synthesis of the complete trans-activation gene product of human T-lymphotropic virus type III from cDNA library (Excherichia 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. Arja, S. K., C. Guo, S. F. Josephs, and W. Wong-Staal. 1985. Trans-activator gene of human T-lymphotropic virus type III (HTLV-III). Science (Wash. DC). 229:59-73.
5. Barnes, D. W., J. Silnaufter, 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. Cernmann, R. Rey, M. T. Nugeyre, S. Chamaret, J. Gruess, C. Dauguet, C. Axler-Blin, F. Brun-Vezinet, C. Rouzioux, W. Rozenbaum, and L. Montagnier. 1985. 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 complete sequence of tat-5 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 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. Fathi, 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 third 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. Fishner, S. G., M. B. Feinberg, J. W. Schuppe, M. E. Harper, L. M. Marcelle, G. Reyes, M. A. Goeda, A. Allevi, C. Debouck, R. C. Gallo, and W. 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. Bicanalana, and D. Hudson. 1989. Activity of synthetic peptides from the tat protein of human immunodeficiency virus type 1. Proc. Natl. Acad. Sci. USA. 86:1229-1232.
17. Gallo, R. C., S. Z. Salahuddin, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palmer, R. Redfield, J. Oteska, B. Safai, G. White, F. Foster, and P. D. Markham. 1984. Frequent detection and isolation of cytopathetic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. Science (Wash. DC). 224:500-503.
18. Geilsen, 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). 241:1229-1232.
19. Green, M., and P. M. Lownesten. 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. Kikikawa, 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 Mol-4. J. Virol. 57:1159-1162.
22. Levy, J. A., A. D. Hoffman, S. M. Kramer, J. A. Landis, J. M. Shimabukuro, and L. S. Osbior. 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 cyopathic 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. Seigle, 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. 25:171–176.

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. Hinterichs, 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.