Increased Expression of CD4 Molecules on Jurkat Cells Mediated by Human Immunodeficiency Virus *tat* Protein

PRASAD KOKA,^{1,2} JUAN YUNIS,^{2,3} A. LORENA PASSARELLI,^{3†} DEVENDRA P. DUBEY,^{2,3}
DOUGLAS V. FALLER,^{1,4*} AND EDMOND J. YUNIS^{2,3}

Divisions of Pediatric Oncology^{1} and Immunogenetics,³ Dana-Farber Cancer Institute, and Departments of Pathology² and Pediatrics,⁴ Harvard Medical School, Boston, Massachusetts 02115*

Received 1 April 1988/Accepted 12 July 1988

The *tat* gene of the human immunodeficiency virus, *tat-III*, when introduced into T-lymphoblastoid Jurkat cells by a Moloney retroviral recombinant DNA vector expressed high levels of the functional *tat* protein as measured by the chloramphenicol acetyltransferase assay. Immunofluorescence analysis with CD4-specific monoclonal antibodies demonstrated that the cell surface levels of the CD4 antigen were increased by 5- to 10-fold in the *tat-III*-infected Jurkat cells. Cellular cytoplasmic RNA analysis indicated that the enhanced CD4 expression was mediated at the mRNA level. Our findings suggest that the single expression of the human immunodeficiency virus *tat* protein in the absence of the other viral proteins causes an upregulation of CD4 gene expression on helper T cells, although infection of these cells by the virus, thus expressing all the viral gene products including *tat*, is known to downregulate CD4 antigen expression.

The human immunodeficiency virus (HIV), which is tissue-tropic for CD4⁺ cells, binds to the CD4A and some other epitopes of the CD4 antigen (10, 11, 20, 28, 37). CD4A-like epitopes expressed on different cells, including non-T cells and cells transfected with the CD4 gene, can also serve as receptors for HIV but with altered binding affinity (10, 20, 28). Following the binding of HIV to the CD4 molecules, the virus is internalized by receptor-mediated endocytosis (16). Infection of CD4⁺ cells with HIV produces a complex between the virus-encoded 110,000-molecular-weight *env* glycoprotein and the CD4 antigen (19). Surface expression of these two proteins on the HIV-infected cells is thought to promote the formation of syncytia or giant cells, leading to decreased numbers of CD4 molecules and CD4⁺ cells and the ultimate loss or destruction of the latter (14, 20, 29). It was found recently, however, that syncytium formation is not the primary cause for the cytopathic effects of HIV (31). Monoclonal antibodies directed against certain epitopes of the CD4 antigen prevent HIV binding, infection, and cell fusion (19, 28). Although such fusion events reduce the number of available CD4 molecules on the cell surface, other regulatory factors, such as the virus-encoded *tat* gene product, could potentially alter the expression of host genes including CD4. The *tat-III* gene product has been shown to regulate viral gene expression at the transcriptional or post-transcriptional level via the viral long terminal repeat (LTR), resulting in increased mRNA and protein synthesis, respectively (3, 4, 6, 23, 25, 26, 30).

The CD4 antigen, which is a surface marker of the T-lymphocyte helper cells, is involved in the recognition of major histocompatibility complex (MHC) class II antigens in conjunction with the Ti-CD3 complex and a foreign antigen such as a virus (5, 34). Although infection of CD4⁺ cells with HIV ultimately leads to their destruction, the viral genes responsible for all the changes which lead to the pathogenesis of HIV are not clear (13). One difficulty inherent in the study of virus-induced changes in host gene expression is the

loss of cells under investigation owing to the cytopathic effects of the virus (13, 37). Therefore, a study of such virus-controlled regulation of host gene expression leading to pathogenesis of HIV might best be accomplished by a study of the effect of the individual viral genes. We investigated the effect produced by the *tat* gene on the expression of CD4 antigen on T-lymphoblastoid Jurkat cells in the absence of expression of the other viral genes. Any significant modulation in the levels of the virus receptor CD4 molecule could potentially affect virus binding to the helper T cells. We found that the *tat-III* gene product increases the CD4 antigen levels by 5- to 10-fold and increases CD4 mRNA levels.

The cells were maintained in RPMI 1640 medium containing 10% fetal calf serum, 1% penicillin-streptomycin, and 1% L-glutamine (all from GIBCO Laboratories, Grand Island, N.Y.). The *tat-III*-infected, control antisense-*tat-II*-infected, and uninfected Jurkat cells were provided by C. Rosen, J. Sodroski, and W. Haseltine, Dana-Farber Cancer Institute (24). The medium for the *tat*- and control-infected Jurkat cells contained 350 to 700 µg of G418 (GIBCO) per ml. The Jurkat AW subclone was from D. Diamond and E. Reinherz, Dana-Farber Cancer Institute (36).

The T4 (CD4) cDNA clone was obtained from R. Axel, Columbia University (17). The plasmid containing the Jurkat (REX) T-cell-receptor cDNA was from O. Acuto and E. Reinherz, Dana-Farber Cancer Institute (1). MHC class II cDNA clones from E. Long of the National Institutes of Health (35) were transformed into *Escherichia coli* JS4 (from J. Sedivy and P. Sharp, Massachusetts Institute of Technology). HLA class I B7 cDNA clones were from J. Pan and S. Weissman, Yale University (32), and H. Orr, University of Minnesota (12). A mouse actin cDNA clone was obtained from M. Shipp and E. Reinherz, Dana-Farber Cancer Institute (33). The plasmid DNAs were prepared by the Triton lysis procedure (18).

The 1.8-kilobase T4 cDNA fragment was obtained by digestion of pT4B with *Bam*HI and *Eco*RI (17). The cDNA fragments from the purified HLA-DRβ and HLA-B7 cDNA-containing plasmids were obtained by digestion with *Bam*HI and *Pst*II, respectively. The β-chain constant-region frag-

* Corresponding author.

† Present address: Department of Genetics, University of Georgia, Athens, GA 30602.

TABLE 1. HIV LTR-directed CAT expression as a function of *tat-III* expression

Cell line	% Conversion ^{a,b}	pL3CATc/pSV2CAT ^{b,c}	nmol of [¹⁴ C]chloramphenicol converted/mg of protein ^b
Jurkat	13.1 ± 1.03	2.10 ± 0.19	3.47 ± 0.20
Jurkat, anti- <i>tat-II</i> , clone 11	10.2 ± 0.46	1.80 ± 0.05	3.00 ± 0.08
Jurkat, anti- <i>tat-II</i> , clone 12	17.8 ± 1.56	1.60 ± 0.04	2.64 ± 0.07
Jurkat, anti- <i>tat-II</i> , clone 13	6.9 ± 0.36	2.33 ± 0.15	3.84 ± 0.25
Jurkat, anti- <i>tat-II</i> , clone 14	5.9 ± 0.31	2.13 ± 0.13	3.51 ± 0.21
Jurkat, anti- <i>tat-II</i> , clone 17	12.5 ± 0.12	3.00 ± 0.06	4.95 ± 0.10
Jurkat, <i>tat-III</i> , clone A	84.6 ± 2.07	119.33 ± 3.30	196.89 ± 5.45
Jurkat, <i>tat-III</i> , clone B	63.5 ± 1.33	184.00 ± 29.3	303.60 ± 48.35
Jurkat, <i>tat-III</i> , clone C	50.9 ± 2.00	217.67 ± 41.0	359.16 ± 67.65

^a Percent [¹⁴C]chloramphenicol converted to acetylated forms.

^b The numbers represent the average of three different experiments, except clone C, which represents the average of only two experiments. Mean ± standard deviation.

^c Results were normalized to cells transfected with a control pSV2CAT plasmid DNA.

ment of the T-cell-receptor gene was isolated by digestion with *HincII* and *BglII* (1). The cDNA fragments were isolated after agarose gel electrophoresis by electroelution with an electroelutor (model UEA; International Biotechnologies, Inc., New Haven, Conn.) or by using low-melting-point agarose. The cDNA fragments were labeled with ³²P as described previously (7, 8). The ³²P-labeled cDNA samples (100 µl) were spin dialyzed on 1.5 ml of Sepharose CL-6B (Pharmacia, Inc., Piscataway, N.Y.) at 3,000 × *g* for 3 min for the removal of the unincorporated deoxynucleotide triphosphates.

Cellular DNA was isolated by standard procedures (18). The purified DNA samples (20 µg) were digested with the appropriate restriction enzyme and run on 0.7% agarose gels at 35 V for 18 h. The gels were UV irradiated for 10 min, denatured in 0.2 N NaOH, and neutralized in a Tris buffer and EDTA. Southern transfer and hybridization were done with a Gene Screen Plus filter (Dupont, NEN Research Products, Boston, Mass.) under conditions suggested by the manufacturer.

The Jurkat cell clones used in our study were all derived from the same parental cell lineage, as shown by DNA restriction fragment length polymorphism analysis. The genomic DNAs were digested with *PvuII* or *HindIII* and hybridized with ³²P-labeled HLA-B7 cDNA (12, 32) or the HLA-DRβ cDNA (35) probe. A positive-control DNA sample from a Jurkat AW subclone (36) was also used for comparison with the restriction fragment length polymorphisms of infected and uninfected Jurkat cells. The genomic blots of these cellular DNA samples showed identical restriction patterns within the MHC class I and class II genes between the uninfected and infected Jurkat clones (data not shown). As expected, however, the negative-control genomic DNAs from the human T-lymphoid line HUT78 and the B lymphoid line Raji showed restriction fragment length polymorphism patterns different from those of Jurkat cells. The same cellular DNA samples were also digested with *EcoRI* or *HindIII* and hybridized with the Cβ fragment of the T-cell-receptor cDNA clone (pβREX) (1). Again, no polymorphisms or clonal differences could be found to account for the significant increases in CD4 gene expression observed in the *tat-III*-expressing clones (data not shown).

The cloned neomycin-resistant Jurkat cells were assayed for expression of the *tat-III* gene by the chloramphenicol acetyltransferase (CAT) assay (15). The uninfected, *tat-III*-infected, and control antisense-*tat-II*-infected Jurkat cells were transfected by the DEAE-dextran procedure (15). The cells (5 × 10⁶) were incubated in 1.5 ml of RPMI 1640

medium containing DEAE-dextran (50 µg/ml) and the pL3CATc plasmid (5 µg of DNA), consisting of the HIV LTR directing expression of the bacterial CAT gene (38), at room temperature for 1 h. The transfected cells were harvested after growing for 48 h. The LTR-directed *trans*-activation of the CAT gene by the functional *tat-III* gene product in these Jurkat cells was measured by the acetylation of [¹⁴C]chloramphenicol (Table 1). All the enzyme assays were performed in the same manner by incubation at 37°C for 2 h with 0.6 mg of cellular protein contained in each CAT assay reaction. Thin-layer chromatography was performed on all the reaction products at the same time, and Kodak X-ray films were exposed to ¹⁴C from the thin-layer chromatography plates for the same duration. The results were normalized to a control parallel transfection with a plasmid, pSV2CAT, containing the simian virus 40 promoter-enhancer (27). The pL3CATc and pSV2CAT plasmids were from G. N. Pavlakis (Frederick Cancer Research Facility, National Cancer Institute) and N. Rosenthal (Harvard Medical School), respectively. The simian virus 40-driven CAT levels, which apparently are low for the *tat-III*-infected Jurkat clones (Table 1), are not due to differences in experimental conditions. We believe this is possibly due to a negative regulatory effect by the *tat* protein on the simian virus 40 transcriptional regulatory elements. This interesting and consistent effect is under investigation.

Monoclonal antibodies specific for the CD4 and CD4A epitopes of the CD4 antigen (OKT4 and OKT4A, respectively [Ortho Diagnostics, Inc., Raritan, N.J.]) were used for immunofluorescence analysis. For the two-color direct immunofluorescence analysis, the red fluorescence phycoerythrin-labeled OKT4 and the green fluorescence fluorescein isothiocyanate-labeled OKT4A monoclonal antibodies (both from Ortho Diagnostics) and the CD3 antigen-specific anti-Leu-4-fluorescein isothiocyanate (Becton Dickinson and Co., Paramus, N.J.) monoclonal antibody were used. A Jurkat cell clonotypic REX monoclonal antibody which was used for assay of the T-cell-receptor antigen levels was from O. Acuto and E. Reinherz, Dana-Farber Cancer Institute (2).

A total of 10⁶ cells per analysis were washed and suspended in 60 µl of RPMI 1640 medium containing the monoclonal antibody at the appropriate dilution (OKT4, 1/50; βREX, 1/400; and OKT3, 1/100) and incubated at 4°C for 45 min. The washed cells were stained with the second antibody, fluorescein isothiocyanate-goat anti-mouse immunoglobulin G (30 µl at 1/30 dilution [Bionetics Products]), for 45 min at 4°C. Cells not treated with the first antibody were used as a negative control. The RPMI-azide (0.02%)-washed

TABLE 2. Increased expression of the T-cell-specific antigens on the infected Jurkat cells estimated by two-color immunofluorescence analysis

Cell line	Monoclonal antibody						
	OKT4-Phycoerythrin				OKT4A-Fluorescein isothiocyanate		
	Expt no. ^a	% Positive cells ^b	MFI ^c	Fold increase ^d	% Positive cells	MFI	Fold increase
Jurkat	1	40.4	78	1.0	44.0	65	1.0
	2	38.0	78	1.0			
Jurkat, anti- <i>tat-II</i> , clone 11	1	46.7	84	1.4	60.9	73	1.7
	2	51.5	87	1.7			
Jurkat, anti- <i>tat-II</i> , clone 12	1	47.4	86	1.5	59.2	76	1.8
	2	53.6	93	2.1			
Jurkat, anti- <i>tat-II</i> , clone 13	1	28.0	75	0.6	27.1	61	0.6
	2	24.3	72	0.5			
Jurkat, anti- <i>tat-II</i> , clone 14	1	51.2	90	1.8	48.0	73	1.4
	2	47.5	87	1.6			
Jurkat, anti- <i>tat-II</i> , clone 17	1	38.7	82	2.3	35.4	66	0.8
	2	33.5	78	0.9			
Jurkat, <i>tat-III</i> , clone A	1	75.1	109	4.3	80.0	93	3.9
	2	76.2	112	4.9			
Jurkat, <i>tat-III</i> , clone C	1	81.8	120	9.3	84.3	101	5.1
	2	84.7	124	7.5			

^a Experiments 1 and 2 were performed on different days.^b Obtained by two-color immunofluorescence analysis.^c Mean fluorescence intensity, channel number.^d Fold increase is the ratio of the total intensity of all the percent positive cells of Jurkat clones relative to that of uninfected Jurkat cells.

cells were then subjected to forward-angle light scattering intensity measurements on the Coulter Epic V (Coulter Electronics, Inc., Hialeah, Fla.).

For the two-color immunofluorescence analysis, 10^6 cells were suspended in a total volume of 100 μ l of RPMI 1640 medium containing 20 μ l each of the green and red fluorescence-labeled monoclonal antibodies. The cells were incubated at 4°C for 30 min, washed, and suspended in 10% medium for analysis on the Coulter Epic V. The control antibodies used were fluorescein isothiocyanate-goat anti-mouse immunoglobulin G (1/30) (Bionetics) and phycoerythrin-anti-mouse immunoglobulin G (heavy plus light chains; Vector Laboratories).

Jurkat cells expressing a functional HIV *tat* (*tat-III*) protein as determined by the CAT assay (Table 1) and control cells infected with the Moloney retroviral pZIPNeoSVX vector carrying the human T-cell lymphotropic virus type II *tat* (*tat-II*) in an antisense orientation (24) were resistant to G418. All three *tat-III*-positive clones that were tested for binding of the CD4 antigen-specific monoclonal antibodies showed 5- to 10-fold increases in the levels of expression of the CD4 antigen when compared with the uninfected or control-infected Jurkat cells either by indirect immunofluorescence analysis (data not shown) or by a two-color fluorescence analysis by direct binding of the labeled monoclonal antibodies (Table 2). Comparable results were also obtained for clone B (data not shown). These cells did not suggest any epitope-specific intramolecular alterations in the binding of the CD4 and CD4A epitope-specific monoclonal antibodies (Table 2). Both antibodies showed concomitant increases in their binding levels to the *tat-III*-infected clones.

Total cellular RNA was extracted from the uninfected and infected cells by the guanidinium isothiocyanate-CsCl ultracentrifugation procedure (18). The RNA samples were then subjected to agarose (1%) gel electrophoresis at 35 V overnight. The RNA was then transferred to a nitrocellulose filter (Bethesda Research Laboratories, Inc., Gaithersburg, Md.) presoaked in 20 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M

sodium citrate), using the Bethesda Research Laboratories nucleic acid blot-transfer system. The RNA-bound filters were hybridized with the 32 P-labeled cDNA probes as follows.

The filters were prehybridized overnight at 42°C in a solution containing 5 $\times$ SSC, 1% sodium dodecyl sulfate, 5 $\times$ Denhardt solution, 100 μ g of single-stranded DNA per ml, and 50% formamide. Hybridization mixtures with the labeled cDNA contained 10% dextran T-500 in prehybridization solution and the 32 P-labeled probe at 5 $\times 10^6$ cpm per lane, and hybridization was performed at 42°C for 18 h. The filters were then washed sequentially in 2 $\times$ SSC-0.1% sodium dodecyl sulfate and in 0.2 $\times$ SSC-0.1% sodium dodecyl sulfate at room temperature and 42°C, respectively. The dot blots of CD4- and actin mRNA-containing filters were also performed with nitrocellulose filters, using a Bio-Rad apparatus. Filters were hybridized with 32 P-labeled probe at 42°C and treated as for Northern (RNA) blots. The dot blots were quantitated with an LKB scanning densitometer. All the RNA samples were analyzed at the same time, and the X-ray films were exposed for the same duration (48 h). The dot blot shown in Fig. 1B is of the same membrane.

The observed increases in the levels of the CD4 antigen in the *tat-III*-infected cells were secondary to increased levels of the 3.0-kilobase CD4 transcript (16) as seen in the total cellular RNA blot analysis (Fig. 1). Data on clone C was comparable but was not included (Fig. 1A). Densitometric scanning or scintillation counting quantitation showed that the CD4 mRNA levels in all three *tat-III*-infected clones were increased by factors of 8- to 10-fold relative to those in uninfected or control (antisense-*tat-II*) infected cells, while the actin mRNA levels remained unchanged. The five control-infected (anti-*tat-II*) Jurkat clones did not exhibit increases in the levels of CD4 antigen or CD4 mRNA, thus suggesting that the increased CD4 gene expression was mediated by the *tat-III* protein.

An incidental finding of this study was that the levels of expression of the clonotypic T-cell receptor (Ti)-CD3 anti-

FIG. 1. RNA blot analysis for CD4 and actin transcripts in uninfected, control anti-*tat-II*-infected, and *tat-III*-infected Jurkat cells. Filters shown in panels A and B were hybridized with a ^{32}P -labeled *Bam*HI-*Eco*RI fragment containing the CD4 cDNA (17). The dot blots were performed with nitrocellulose filters and a Bio-Rad blot apparatus. A parallel dot blot on the same RNA samples was obtained with a mouse actin cDNA probe (33) to normalize the levels of the mRNA in the *tat-III*-expressing cells to those in the control cells (panel C). The cellular sources of the mRNA used in panel A were: lane 1, Jurkat, uninfected; lane 2, Jurkat, anti-*tat-II*, pool of clones; lane 3, Jurkat, *tat-III*, clone A; and lane 4, Jurkat, *tat-III*, clone B. The dots in panels B and C contain 1.50 (a), 1.00 (b), 0.75 (c), 0.50 (d), 0.25 (e), and 0.10 (f) μg of RNA from the following cell lines: 1, Jurkat; 2, Jurkat, anti-*tat-II*, clone 11; 3, Jurkat, anti-*tat-II*, clone 14; 4, Jurkat, *tat-III*, clone A; 5, Jurkat, *tat-III*, clone B; and 6, Jurkat, *tat-III*, clone C.

gen complex were also increased in the *tat*-infected cells independent of *tat* expression. Control experiments indicate that this was due to the retrovirus vector that was used for the introduction of the *tat-III* DNA or the antisense-*tat-II* DNA into the Jurkat cells (data not shown).

HIV infection of CD4^+ T lymphocytes (via the CD4 antigen) leads to a substantial reduction in the numbers of these antigens on the cells (13). HIV infection of CD4^+ T cells, including Jurkat cells, caused a downregulation of the levels of CD4 antigen and its mRNA (9). We report here that the expression of the viral *tat* gene alone in the CD4^+ Jurkat cells and in the absence of expression of the other viral proteins caused increased levels of expression of the CD4 antigen and its mRNA. The mechanisms by which the HIV *tat* gene product increases the level of certain cellular proteins remain incompletely understood. The *tat* gene of HIV was previously found to increase the expression of certain HIV genes in these same Jurkat cells, via the viral LTR, by a disproportionately greater increase in viral protein synthesis relative to mRNA synthesis (25). Others have reported that the HIV *tat* gene acts predominantly at the level of transcriptional accumulation of mRNA from a CAT gene driven by the HIV LTR in vitro (21). It was also reported that an inducible cellular nuclear transcription factor in these same *tat-III*-infected Jurkat cells could act synergistically with the *tat* protein on the HIV LTR to promote additional transcription (22). Our results suggest that the increased CD4 antigen expression on these Jurkat cells which was mediated by the *tat* protein is due to a comparable increase in the level of the CD4 mRNA.

The interesting discrepancy between the reported suppression of CD4 gene expression by infection with the whole HIV and our observed enhancement of CD4 antigen and mRNA levels mediated by the HIV *tat* gene alone is not yet explained. This is the first example of human retroviruses affecting cellular gene expression in ways opposite from the action of the isolated *tat* gene that they encode.

It has been proposed that although both the human T-cell lymphotropic virus *tat* genes and the HIV *tat* genes activate transcription of the host genes in *trans*, activation by the human T-cell lymphotropic virus *tat* genes leads to T-cell

proliferation, whereas the HIV *tat* gene activates genes responsible for terminal differentiation leading to cell death (39). It is therefore necessary to determine the effects produced by the *tat-III* gene product on CD4 gene expression in a variety of CD4^+ T-cell lines to establish whether this increase in CD4 antigen levels induced by the *tat-III* protein is a universal or a cell lineage-specific event and to prove a correlation with the viral cytopathic effect.

We thank C. Rosen, J. Sodroski, and W. Haseltine for the *tat*-infected Jurkat cells; O. Acuto for helpful discussions; and U. Hansen for use of the LKB densitometer. We also thank B. Felber and G. N. Pavlakis for the pL3CATc DNA; N. Rosenthal for the pSV2CAT DNA; P. Maddon and R. Axel for the T4 cDNA clone; O. Acuto and E. Reinherz for the REX cDNA clone and the REX monoclonal antibody; E. Long for the class II cDNA clones; H. Orr, J. Pan, and S. Weissman for the class I cDNA clones; M. Shipp and E. Reinherz for the mouse actin cDNA clone; J. Sedivy and P. Sharp for *E. coli* JS4; and D. Diamond and E. Reinherz for the Jurkat AW subclone.

This work was supported by grants from the National Institutes of Health, Public Health Service grants CA37169 to D.V.F. and A117120 and CA20531 to E.J.Y., also from the National Institutes of Health, and American Cancer Society grants ACS-IM-427 to D.V.F. and ACS-NY-IM-437 to D.P.D. D.V.F. is the recipient of an American Cancer Society senior faculty research award, and P.K. was the recipient of a Leukemia Society of America special fellowship.

LITERATURE CITED

1. Acuto, O., T. J. Campen, H. D. Royer, R. E. Hussey, C. B. Poole, and E. L. Reinherz. 1985. Molecular analysis of T cell receptor (Ti) variable region (v) gene expression. *J. Exp. Med.* **161**:1326-1343.
2. Acuto, O., R. E. Hussey, K. A. Fitzgerald, J. P. Protentis, S. C. Meuer, S. F. Schlossman, and E. Reinherz. 1983. The human T cell receptor: appearance in ontogeny and biochemical relationship of γ and β subunits on IL-2 dependent clones and T cell tumors. *Cell* **34**:717-726.
3. 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* **229**:69-73.
4. Chen, I. S. Y. 1986. Regulation of AIDS virus expression. *Cell* **47**:1-2.
5. Cohn, M. 1983. The T-cell receptor mediating restrictive recog-

- nition of antigen. *Cell* 33:657-669.
6. Dayton, A. I., J. G. Sodroski, C. A. Rosen, W. C. Goh, and W. A. Haseltine. 1986. The trans-activator gene of the human T cell lymphotropic virus type III is required for replication. *Cell* 44:941-947.
 7. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6-13.
 8. Feinberg, A. P., and B. Vogelstein. 1984. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 137:266-267.
 9. Hoxie, J. A., J. D. Alpers, J. L. Rackowski, K. Huebner, B. S. Haggarty, A. J. Cedarbaum, and J. C. Reed. 1986. Alterations in T4 (CD4) protein and mRNA synthesis in cells infected with HIV. *Science* 234:1123-1127.
 10. Hoxie, J. A., L. E. Flaherty, B. S. Haggarty, and J. L. Rackowski. 1986. Infection of T4 lymphocytes by HTLV-III does not require expression of the OKT4 epitope. *J. Immunol.* 135:361-363.
 11. Klatzmann, D., F. Barre-Sinoussi, M. T. Nugeyre, C. Dauguet, E. Vilmer, C. Griscelli, F. Brun-Vezinet, C. Rouziou, J. C. Gluckman, J. C. Chermann, and L. Montagnier. 1984. Selective tropism of lymphadenopathy associated virus (LAV) for helper-inducer T lymphocytes. *Science* 225:59-63.
 12. Koller, B. H., B. Sidwell, R. DeMars, and H. T. Orr. 1984. Isolation of HLA locus-specific DNA probes from the 3'-untranslated region. *Proc. Natl. Acad. Sci. USA* 81:5175-5178.
 13. Lane, H. C., and A. S. Fauci. 1985. Immunologic abnormalities in the acquired immunodeficiency syndrome. *Annu. Rev. Immunol.* 3:477-500.
 14. Lipson, L. D., M. B. Feinberg, G. R. Reyes, L. Rabin, B. Banapour, S. Chakrabarti, B. Moss, F. Wong-Staal, K. S. Steimer, and E. G. Engleman. 1986. Induction of CD4-dependent cell fusion by the HTLV-III/LAV envelope glycoprotein. *Nature (London)* 323:725-728.
 15. Lopata, M. A., D. W. Cleveland, and B. Sollner-Webb. 1984. High level transient expression of a CAT gene by DEAE-dextran mediated DNA transfection. *Nucleic Acids Res.* 12:5707-5717.
 16. Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel. 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* 47:333-348.
 17. Maddon, P. J., D. R. Littman, M. Godfrey, D. E. Maddon, L. Chess, and R. Axel. 1985. The isolation and nucleotide sequence of a cDNA encoding the T cell surface protein T4: a new member of the immunoglobulin family. *Cell* 42:93-104.
 18. Maniatis, T., E. F. Fritsch, and J. Sambrook. 1982. Molecular cloning, a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
 19. McDougal, J. S., M. S. Kennedy, J. M. Sligh, S. P. Cort, A. Mawle, and J. K. A. Nicholson. 1986. Binding of HTLV-III/LAV to T4⁺ T cells by a complex of the 110K viral protein and the T4 molecule. *Science* 231:382-385.
 20. McDougal, J. S., A. Mawle, S. P. Cort, J. K. A. Nicholson, G. D. Cross, J. A. Scheppeler-Campbell, D. Hicks, and J. Sligh. 1985. Cellular tropism of the human retrovirus HTLV-III/LAV. *J. Immunol.* 135:361-363.
 21. Muesing, M. A., D. H. Smith, and J. D. Capon. 1987. Regulation of mRNA accumulation by a human immunodeficiency virus trans-activator protein. *Cell* 48:691-701.
 22. Nabel, G., and D. Baltimore. 1987. An inducible transcription factor activates expression of human immunodeficiency virus in T cells. *Nature (London)* 326:711-713.
 23. Okamoto, T., and F. Wong-Staal. 1986. Demonstration of virus-specific transcriptional activator(s) in cells infected with HTLV-III by an in vitro cell free system. *Cell* 47:29-35.
 24. Rosen, C. A., J. G. Sodroski, K. Campbell, and W. A. Haseltine. 1986. Construction of recombinant murine retroviruses that express the human T cell leukemia virus type II and human T cell lymphotropic virus type III trans-activator genes. *J. Virol.* 57:379-384.
 25. Rosen, C., J. G. Sodroski, W. C. Goh, A. I. Dayton, J. Lippke, and W. A. Haseltine. 1986. Post-transcriptional regulation accounts for the trans-activation of the human T lymphotropic virus type III. *Nature (London)* 319:555-559.
 26. Rosen, C., J. G. Sodroski, and W. A. Haseltine. 1985. The location of cis-acting regulatory sequences in the human T cell lymphotropic virus type III (HTLV-III/LAV) long terminal repeat. *Cell* 41:813-823.
 27. Rosenthal, N., M. Kren, P. Gruss, and G. Khoury. 1983. BK viral enhancer element and a human cellular homolog. *Science* 222:749-755.
 28. Sattentau, Q. J., A. G. Dalgleish, R. A. Weiss, and P. C. L. Beverly. 1986. Epitopes of the CD4 antigen and HIV infection. *Science* 234:1120-1127.
 29. Sodroski, J., W. C. Goh, C. Rosen, K. Campbell, and W. A. Haseltine. 1986. Role of the HTLV-III/LAV envelope in syncytium formation and cytopathicity. *Nature (London)* 322:470-474.
 30. Sodroski, J., R. Patarca, C. Rosen, F. Wong-Staal, and W. Haseltine. 1985. Location of the transactivating region on the genome of human T-cell lymphotropic virus type III. *Science* 229:74-77.
 31. Somasundaram, J., and H. L. Robinson. 1987. A major mechanism of human immunodeficiency virus-induced cell killing does not involve cell fusion. *J. Virol.* 61:3114-3119.
 32. Sood, A. K., D. Pereira, and S. Weissman. 1981. Isolation and partial nucleotide sequence of a cDNA clone for human histocompatibility antigen HLA-B by use of an oligodeoxynucleotide primer. *Proc. Natl. Acad. Sci. USA* 78:616-620.
 33. Spiegelman, B., M. Frank, and H. Green. 1983. Molecular cloning of mRNA from 3T3 adipocytes. *J. Biol. Chem.* 258:10083-10089.
 34. Takeuchi, T., S. F. Schlossman, and C. Morimoto. 1987. The T4 molecule differentially regulating the activation of subpopulations of the T4⁺ cells. *J. Immunol.* 139:665-671.
 35. Tonnel, C., R. DeMars, and E. O. Long. 1985. DO-beta: a new beta chain gene in HLA-D with a distinct regulation of expression. *EMBO J.* 4:2839-2847.
 36. Weiss, A., and J. D. Stobo. 1984. Requirements for the coexpression of T3 and the T cell antigen receptor on a malignant human T cell line. *J. Exp. Med.* 160:1284-1299.
 37. Wong-Staal, F., and R. C. Gallo. 1985. Human T-lymphotropic retroviruses. *Nature (London)* 317:395-403.
 38. Wright, C. M., B. K. Felber, H. Paskalis, and G. N. Pavlakis. 1986. Expression and characterization of the trans-activator of HTLV-III/LAV virus. *Science* 234:988-992.
 39. Zagury, D., J. Bernard, R. Leonard, R. Cheynier, M. Feldman, P. S. Sarin, and R. C. Gallo. 1986. Long-term cultures of HTLV-III-infected T cells: a model of cytopathology of T-cell depletion in AIDS. *Science* 231:850-853.