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18. We acknowledge the helpful discussions of S. J. Leibovich, N. Bouck, F. Schmid, and R. Pope. Supported in part by NIH grants AR30692 and AR41492 (A.E.K.), HL39926 (P.J.P.), HL02401 and 1P50HL46487 (R.M.S.), HL31693 and HL35276 (S.L.K.), EY07003I and EY09441 (V.M.E.); a Veterans Administration Merit Review (A.E.K.); an Arthritis Foundation Fellowship (A.E.K.); and an American Lung Association Research Grant. R.M.S. is an RJR Nabisco Research Scholar.

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Invariant Chain Peptides in Most HLA-DR Molecules of an Antigen-Processing Mutant

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Class II major histocompatibility complexes bind peptides in an endosome-like compartment. When the class II null cell line 721.174 was transfected with class II DR3 genes, DR molecules were produced in normal amounts. However, the DR molecules were abnormally conformed and unstable because deletion of an antigen-processing gene had impaired intracellular formation of most class II–peptide complexes. Yet, 70 percent of the DR molecules still bore peptides, 80 percent of which were 21– to 24–amino acid fragments of the class II–associated invariant chain. These peptides were rare on DR3 from control cells. Thus, a defect in the main antigen-processing pathway revealed a process in which DR molecules bind long peptides derived from proteins present in the same compartment.

The main biological function of class I and class II proteins encoded in the major histocompatibility complex (MHC) is to bind antigenic peptides. The peptides bind in a groove walled in by two parallel α-helical segments on top of a floor formed by a β-pleated sheet (*1–3*). Complexes between MHC proteins and antigenic peptides are normally produced in antigen-presenting cells as a result of antigen processing (*4*) and are then displayed on the cell surface. They can then be recognized by the antigen receptors of specific T lymphocytes and thus trigger T cell activation. Thus, any interference with the formation of MHC-antigen complexes will impair activation of peptide-specific T cells.

Studies of cell mutants (*5*) have shown that the binding of self or foreign peptides is necessary for the normal assembly, stability, intracellular trafficking, and cell surface display of class I molecules. These and other (*6, 7*) studies have demonstrated that TAP1 and TAP2 transporter genes in the class II region of the MHC are necessary for the formation in the endoplasmic reticulum of most class I–peptide complexes. At least one gene in the vicinity of the TAP genes is needed for the intracellular production of class II–peptide complexes. Human B lymphoblastoid cell lines (LCLs) that have homozygous deletions (*8, 9*) or presumably point mutations (*10*) in this region have the following characteristics: (i) They display normal amounts of class II heterodimers on their surfaces, but the molecules lack certain antigenic epitopes and dissociate more readily than class II molecules from normal cells in the presence of SDS (*9, 11*). (ii) They produce molecules that are not recognized by many DR3-specific T cells (*12*). (iii) They have reduced ability to process and present epitopes derived from exogenous whole protein antigens but efficiently present epitopes from exogenously added antigenic peptides (*8, 10, 11*). Proteolytic cleavage of the class II–associated invariant chain normally must precede binding of peptides to class II molecules and occurs in processing-defective mutants (*13*). The antigen-processing defects in these mutants may be functionally analogous to those causing the production of empty class I molecules in TAP1- or TAP2-deficient mutants (*5*). We show here that class II DR3 molecules produced in the cell line 721.174, which has the abnormalities described above (*8*), carried less self peptide than DR3 purified from control cells. Most of the mutant-derived DR molecules carried a limited variety of peptides, which were undetected or rare on DR3 isolated from nonmutant cells. The antigen-processing defect in these cells, therefore, has revealed an alternative processing pathway.

These observations (*8*) were made with cells created by the transfection of DR α and β genes into LCL mutants 721.82 (.82) (*14*) and 721.174 (.174) (*15*), which have homozygous deletions of the DR and DQ1 α and β genes. The MHC region bounded by and including the DP1 and DQ2 loci is present in .82 but absent in .174 (*16, 17*). This region includes the TAP1 and TAP2 transporter genes and LMP2 and LMP7 (*18*), which encode components of a multicatalytic proteinase (*5*). Because antigen processing and presentation with class II molecules apparently are normal in a mutant in which these four genes are not expressed, at least one undiscovered gene in this region is needed for antigen presentation and is deleted in .174 (*19*).

DR-expressing transfectants derived from .174 [.174(DR3)] have abnormalities associated with an impediment to intracellular antigen processing or presentation with class II molecules, but this impediment is absent in DR-expressing transfectants derived from .82 [.82(DR3)] (*8*). We thought that the impaired antigen processing in class II–expressing transfectants of .174 might result in empty DR3 molecules that would bind peptides in solution in larger quantities and at faster rates than DR3 derived from .82(DR3) and that fewer self peptides would be bound to DR3 from .174(DR3) than to DR3 produced by

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.82(DR3) when directly analyzed by reverse-phase high-performance liquid chromatography (HPLC). In a standard 48-hour binding assay (*20*), .174(DR3)-derived DR3 bound three to four times as much ^{125}I-labeled mycobacterium tuberculosis (MT) peptide as DR3 purified from .82(DR3) or from the DR3-homozygous LCL MAT, which is an Epstein-Barr virus–transformed B cell line (Fig. 1A). Binding was specific in that an excess of nonradiolabeled peptide completely blocked the binding of ^{125}I-labeled peptide. Similar results were obtained at room temperature and at 37°C, and little binding to DR3 from all three cell lines occurred at pH 7.0, as described for DR3 from nonmutant cells (*21*).

At equimolar concentrations of DR3, the binding of peptide to .174(DR3)-derived DR3 was two to three times more rapid than to DR3 isolated from .82(DR3) (Fig. 1B). The dissociation rates of preformed peptide–DR3 complexes indicated that, once formed, class II–peptide complexes were stable (Fig. 1C), as observed in other studies (*22*, *23*). Significant differences in the dissociation rates of DR-peptide complexes isolated from the .174(DR3) and .82(DR3) cell lines were not detected at pH 4.5 or 7.0 or at 37°C. Scatchard analysis revealed that the differences in amounts of peptide bound from the various cell lines (Fig. 1A) were not a result of differences in equilibrium constants between DR3-peptide complexes and the separate molecules (in the 6.4×10^{-7} to 9.0×10^{-7} M range); rather, the proportion of active peptide-binding sites was about 26% (that is, 0.55 μM/1.8 μM) for .174(DR3) but only about 7% (0.13 μM/1.8 μM) for the .82(DR3) and MAT cell lines (Fig. 1D).

Thus, as expected, a significantly larger proportion of DR3 molecules derived from .174(DR3) were empty, as compared with DR3 from the other two cell lines. But only one-third of the .174(DR3)-derived DR3 could bind exogenously added peptide, which suggests that most of the DR3 molecules might be occupied by self peptides. This indication was confirmed by HPLC analysis of peptides eluted from purified DR3 molecules (Fig. 2). Comparison of the HPLC profiles of peptides from .174(DR3) and .82(DR3) indicated that most of the prominent peaks in .82(DR3) were absent or diminished in .174(DR3) (regions B and D of the chromatogram), and at least three peaks (region C) that were prominent in .174(DR3) were absent or barely evident in .82(DR3). Peptides associated with DR3 from .174(DR3) and .82(DR3) were further analyzed by tandem mass spectrometry (*6*, *24*, *25*). About 80% of the peptides from DR3 of .174(DR3) were 21 to 24 residues in length and were derived from residues 80 to 103 of the invariant chain (Table 1). These

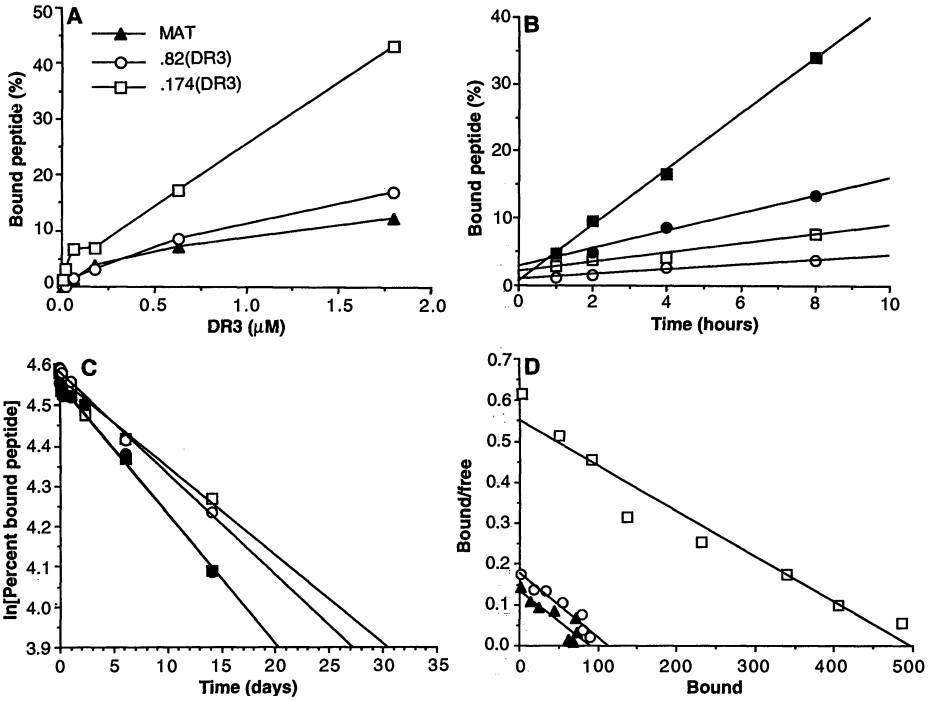

Fig. 1. Peptide-binding capacity of purified DR3 molecules isolated from .174(DR3) cells, .82(DR3) cells, and the MAT cell line. DR molecules were purified from 1×10^{10} cells by affinity chromatography with monoclonal antibody LB3.1 (*19*). DR3-specific peptide binding was assayed as described (*21*). Purified DR3 molecules were incubated with an ^{125}I-labeled analog of the MT(3–13) peptide (*34*) [M1 sequence YKTIAFDEEARR (*22*, *33*)] at pH 4.5 in the presence of a mixture of protease inhibitors. Bound and unbound peptides were separated by gel filtration over TSK 2000 columns, (Tolso Haas, Philadelphia, PA) and the percent bound was calculated as described (*35*). (**A**) Peptide-binding capacity of DR3 isolated from .174(DR3), .82(DR3), and MAT. Varying amounts of DR3 were incubated in the presence of 6.3 nM ^{125}I-labeled M1 for 2 days at room temperature. One of two experiments with similar results is shown. (**B**) Association rates. DR3 (0.95 nM) from various sources and 6.3 nM ^{125}I-labeled M1 were incubated at room temperature for the indicated periods of time. One of two independent experiments is shown. The early linear points of the binding isotherm shown express the association rates, *b*, of the binding reactions in $y = a + bx$ where *y* is percent bound radioactivity, *a* is the intercept, and *x* is the time. [Acid constant (K_a) values were not determined because of the unknown amount of active receptor present in each preparation.] □, .174(DR3) at room temperature, $b = 0.68$, $r^2 = 0.950$; ■, .174(DR3) at 37°C, $b = 4.16$, $r^2 = 0.998$; ○, .82(DR3) at room temperature, $b = 0.36$, $r^2 = 0.963$; ●, .82(DR3) at 37°C, $b = 1.32$, $r^2 = 0.985$, where *b* is expressed in s^{-1} and r^2 is the correlation coefficient of the linear regression. (**C**) Dissociation rates. Large amounts of preformed DR3–^{125}I-labeled M1 complexes were isolated by TSK 2000 filtration and then incubated at 37°C at either pH 7.0 or pH 5.0 in the presence of protease inhibitors and of at least a 1000-fold excess of unlabeled M1 to prevent reassociation (*23*). □, .174(DR3) at 37°C, pH 5.0; ○, .82(DR3) at 37°C, pH 5.0; ■, .174(DR3) at 37°C, pH 7.0; and ●, .82(DR3) at 37°C, pH 7.0. (**D**) Scatchard analysis of peptide binding to DR3 isolated from various cell lines. Binding assays in the presence of 1.9 μM DR3, 6.3 nM ^{125}I-labeled M1, and varied concentrations of unlabeled M1 were performed as described (*35*). ▲, MAT; □, 174(DR3); and ○, .82(DR3).

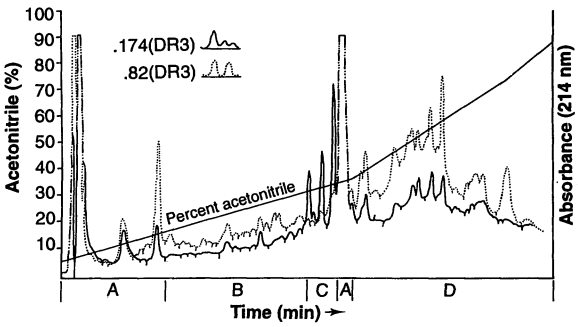

Fig. 2. HPLC profiles of acid-eluted peptides from DR3 isolated from .174(DR3) and .82(DR3) cells. We washed purified DR3 (150 μg) from each cell line by ultrafiltration in an Amicon filtration apparatus to remove detergent molecules and then treated the DR3 with 4 ml of 2.5 N acetic acid for 30 min at 37°C. Peptides were separated from class II molecules by filtration over a YM30 (30K) filtration apparatus (Amicon, Beverly, Massachusetts). Peptides were collected, lyophilized, redissolved in water, and then injected into a Vydac C_{18} column (particle size, 5 μm; 0.46 cm by 25 cm). The gradient used was as follows ($X = 0.06\%$ trifluoroacetic acid in water, $Y = 0.043\%$ trifluoracetic acid in 80% acetonitrile in water, and flow rate = 0.5 ml/min): 0 to 60 min, 2 to 37.5% *Y*; 60 to 90 min, 37.5 to 75% *Y*; and 90 to 105 min, 75 to 98% *Y*. The absorbance of eluate fractions at 214 nm is shown. Peaks labeled A are detergent or injection artifacts.

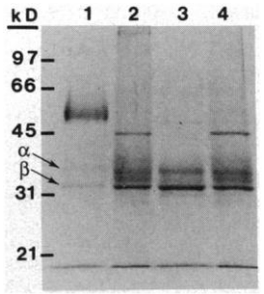

Fig. 3. DR3 isolated from .174(DR3) cells dissociated in the presence of SDS. DR3 molecules were purified from .82(DR3) (lanes 1 and 3) or .174(DR3) (lanes 2 and 4) cells as described in Fig. 1; purified proteins (3 μg) were then separated with SDS–polyacrylamide gel electrophoresis after incubation for 5 min in 4% SDS either at room temperature (lanes 1 and 2) or 100°C (lanes 3 and 4). Molecular size standards and the α and β chains are indicated at left.

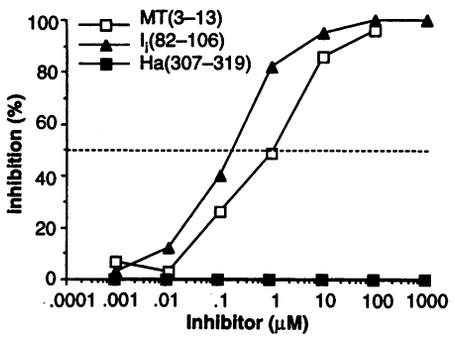

Fig. 4. Affinity of the I_i peptide for DR3. Affinity was measured by the inhibition of binding of ^{125}I-labeled M1 (5 nM) peptide to 1.6 μM DR3 purified from MAT cells in the presence of varied concentrations of nonradioactive peptides (*34*). Conditions were as described in Fig. 1. Ha(307–319) was derived from the influenza virus hemagglutin protein and served as a negative control. Peptides were synthesized as described (*34*). One of four experiments with similar results is shown.

peptides accounted for 71 pmol (~50%) of the DR3 molecules from .174(DR3) and were about 40 times more abundant than in .82(DR3), in which only I_i(80–103) and I_i(82–102) were detected.

Besides peptide binding, a parameter that is associated with the maturation of class II MHC molecules is the stability of the αβ heterodimer (*26*). Before peptide loading, the α and β chains are weakly associated and readily fall apart in the presence of SDS without heating. Later in the processing pathway and coincident with peptide binding, the association of the α and β chains is stabilized, and they resist SDS-mediated dissociation. After exposure to SDS at room temperature, DR3 molecules isolated from .82(DR3) migrated as a single band of ~60 kD, which suggests that all or nearly all of these molecules were dimers (*11*) (Fig. 3). In contrast, virtually all .174(DR3)-derived DR3 molecules dissociated into α and β monomers. Thus, in .174(DR3) cells, there is not a direct correlation between the amount (60 to 75%) of peptide-bound DR3 molecules and the amount (~0%) of class II molecules in a stable configuration.

Mature class II molecules react with certain conformation-specific antibodies (*11*). The DR3 molecules on .174(DR3) cells did not bind such an antibody [monoclonal (MAb 16.23)] (*8*). These data suggest that peptide binding per se is not responsible for the change in stability and conformation of MHC class II molecules as they move through the cell. This lack of correlation between peptide binding and mature conformation cannot be generally ascribed to low-affinity binding of the self peptides associated with DR3 in .174(DR3) cells; the most abundant DR3-bound peptide [I_i(82–106)] bound to DR3 with somewhat higher affinity [the inhibition constant (K_i) is ≈0.1 μM] than did the standard DR3 binding peptide MT(3–13) (Fig. 4). Almost all other described class II–associated peptides are 13 to 18 residues long (*24*, *27*, *28*). Perhaps the effect of greater length of the I_i peptides on .174(DR3)-derived DR3 is to prevent the conformational change usually associated with peptide binding.

Table 1. The amino acid sequences (*33*) of peptides bound to HLA-DR3 molecules (150 pmol) purified from .174(DR3) and .82(DR3) cells. DR3 molecules were purified (*20*), and peptides were isolated from them as described in Fig. 3. Peptides were analyzed by means of tandem mass spectrometry (*25*). We identified source proteins for the peptides by searching DNA and protein databases. The peptides shown for .174(DR3) account for ~80% of the amount of peptide bound to the DR3 isolated from .174 cells. Therefore, a total of ~87 pmol of peptide was associated with 150 pmol of DR3. Because of exon splicing differences, two main different forms of I_i exist: p33 and p41. I_i residues are numbered by counting the first methionine of the p33 form as number one. NA, not applicable; ND, not determined; Obs. *m/z*, observed mass-to-charge ratio; $(M+H)^+$ *m/z*, nominal *m/z* of the protonated molecule (molecular mass + 1 dalton).

Obs. *m/z*	$(M+H)^+$ *m/z*	Peptide sequence	Length	I_i residues	Yield (pmol)*		Oxidized residues†
					.174(DR3)	.82(DR3)	
677	2707	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	3	0.4	88(90), 101
621	2481	KPPKPVSKMRMATPLLMQALPM	22	82–103	1	–	101
673	2691	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	5	–	101
617	2465	KPPKPVSKMRMATPLLMQALPM	22	82–103	4	–	NA
637	2544	LPKPPKPVSKMRMATPLLMQALP	23	80–102	6	–	NA
673	2691	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	6	–	88(90)
669	2675	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	10	1.0	NA
649	2592	LPKPPKPVSKMRMATPLLMQALP	23	80–102	1	–	88(90), 96
592	2366	KPPKPVSKMRMATPLLMQALP	21	82–102	2	–	88(90), 96
621	2481	KPPKPVSKMRMATPLLMQALPM	22	82–103	2	–	101
645	2576	LPKPPKPVSKMRMATPLLMQALP	21	82–102	2	–	88(90), 96
681	2721	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	4	–	88(90), 96, 101
588	2350	KPPKPVSKMRMATPLLMQALP	21	82–102	6	0.4	88(90)
677	2707	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	4	–	88(90), 101
621	2481	KPPKPVSKMRMATPLLMQALPM	22	82–103	2	–	101
641	2560	LPKPPKPVSKMRMATPLLMQALP	23	80–102	2	–	88(90)
584	2334	KPPKPVSKMRMATPLLMQALP	21	82–102	8	–	NA
673	2691	LPKPPKPVSKMRMATPLLMQALPM	24	80–103	3	–	101
577	1729	Unknown	ND	NA	–	0.25	ND
447	2231	Unknown	ND	NA	–	1.0	ND

*A minus (–) sign indicates <0.1 pmol. †Oxidation at Met^{88} and Met^{90} cannot be distinguished from the collision-activated dissociation (CAD) mass spectrum.

Our results and others (*8–11*) are compatible with several possible defects in the main class II antigen-processing and presentation pathway. (i) Class II molecules and processed antigen may initially occupy separate compartments; conjunction of these compartments may be defective in .174. (ii) Processed antigenic peptides may have to be transported from the compartment in which they are produced to a compartment containing class II molecules; their transport may be impaired in .174. (iii) A chaperonin-like protein may facilitate the binding of peptides to class II molecules in the main antigen presentation pathway (*29*) and may be absent in .174 cells. (iv) Antigen-processing proteinases or conditions that affect their activities may be defective in .174. Proteolysis occurs in the class II pathway of .174, but it may not be the proteolysis predominantly used to generate antigenic peptides.

It is not yet possible to decide between these four hypotheses. Any explanation of the defect in .174 should also account for the relative scarcity of long, I_i-derived peptides on DR3 molecules produced in .82(DR3) and the presence of these same peptides on DR1 produced by another human cell line, in which antigen processing is apparently normal (*30*). The long, I_i-derived peptides may be produced in a compartment other than that in which cells generate the highly diverse shorter peptides that are associated with class II molecules in nondefective cells. This interpretation is supported by the absence on .174(DR3)-derived DR3 of the 14– to 15–residue long I_i peptides that were relatively abundant on DR1 of normal cells (*30*).

Peptides derived from homologous segments of the human and mouse invariant chains associate with DR3, DR1 (*30*), I-A^b (*27*), and I-A^d (*24*). However, such peptides do not bind to I-E^b (*27*), and the 17–amino acid peptide that binds to I-A^d binds poorly to I-E^d (*24*). The part of I_i that is bound to the DR3 and I-A molecules is unlikely to occlude the peptide-binding groove (*24*). This suggests that after proteolytic cleavage and dissociation of I_i from class II MHC molecules, the I_i peptides bind to certain class II molecules because those class II molecules have motifs that favor the association. However, the possibility that the I_i-derived peptides are not derived from the peptide-binding groove but are derived from some other binding site on the class II molecule cannot be excluded at present. Analysis of peptides that are bound to DR1, DR2, and DR52b that are produced in existing transfectants of .174 (*8*) should clarify this point.

Most class II–associated peptides derived from exogenous proteins are produced in the endocytic pathway, but some viral peptides presented on class II molecules are derived by the processing of cytosolic proteins (*31, 32*). Both pathways can contribute to the presentation of foreign antigens. Our data suggest that a third source of class II–peptide complexes is from a B cell compartment that contains abundant peptides derived from the invariant chain and perhaps other proteins. Possible contributions of this pathway of peptide loading to the presentation of foreign and autoantigens can now be investigated with class II–expressing derivatives of .174.

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33. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

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Expression Directed from HIV Long Terminal Repeats in the Central Nervous System of Transgenic Mice

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Infection with the human immunodeficiency virus (HIV) is frequently accompanied by the AIDS (acquired immunodeficiency syndrome) dementia complex. The role of specific HIV genetic elements in the pathogenesis of central nervous system (CNS) disease is not clear. Transgenic mice were constructed that contained the long terminal repeats (LTRs) of two CNS-derived strains and a T cell tropic strain of HIV-1. Only mice generated with CNS-derived LTRs directed expression in the CNS, particularly in neurons. Thus, some strains of HIV-1 have a selective advantage for gene expression in the brain, and neurons can supply the cellular factors necessary for their transcription.

During the course of AIDS an individual is host to many different strains of HIV-1 (*1*). The role of these different strains in the pathogenesis of AIDS and the frequently associated neurologic disease called AIDS dementia complex (ADC) is not clear. AIDS dementia complex is characterized by subacute encephalitis that results in progressive memory loss, cognitive impairment, and motor and behavioral abnormalities (*2*). Examination of the brains of patients with ADC has revealed significant neuronal cell loss (*3*). The cause of neuronal loss remains unclear because HIV-1 replication occurs primarily in the microglia and macrophages (*3*). A number of HIV-1 strains have been isolated from the CNS (*4*) of patients with ADC; however, it is difficult to determine