obtained from three rhesus macaques with clinical or histopathologic features of an immunosuppressive disorder (*12*). We therefore hypothesize that this virus may be immunosuppressive and etiologically linked to the macaque immune deficiency syndrome. Type D retroviruses have been isolated at the New England Regional Primate Research Center (NERPRC) and at two other primate colonies where spontaneous immune deficiency syndromes in macaques have been described (*17*). Three of the four macaques from which MTLV-III isolates were obtained were apparently free of the type D retrovirus on the basis of repeated unsuccessful isolation attempts (type D retrovirus was recovered from Mm142-83) (*12*). There have been no studies at the NERPRC involving inoculation of HTLV-III or AIDS materials into any primates, nor have HTLV-III–infected cell lines been maintained at this facility. The inadvertent inoculation of multiple macaques or contamination of cell cultures therefore seems highly unlikely.

Serologic studies of macaques at the NERPRC indicate that at least some animals possess antibodies reactive to both HTLV-I and HTLV-III proteins. Three of four macaques that yielded STLV-III isolates were also seropositive for the HTLV-I–related virus of macaques (Mm251-79, Mm239-82, and Mm220-82). There are at least two possible explanations for this observation. The existence of three distinct members of the HTLV family has been established in humans; it is therefore possible that analogous macaque T-lymphotropic virus types could also exist and multiply infect a given animal. Second, cross-reactivity between antibodies directed to different HTLV types has been demonstrated and is presumably due in part to *env* gene conservation between HTLV types (*18*). Therefore, it is conceivable that some of the macaques in our study that had antibodies to an HTLV-I–related agent also had antibodies to STLV-III that were cross-reactive to the same conserved epitopes of the two viruses. Macaques with both HTLV-III– and HTLV-I–related viruses should provide useful models for studies of this family of viruses. The availability of a nonhuman primate naturally infected with a virus related to HTLV-III may facilitate studies of the pathogenesis and treatment or prevention of AIDS.

Noted added in proof: In seroepidemiologic studies of a variety of African primate species we have noted that a significant number of healthy African green monkeys (*Ceropithecus aethiops*) possess antibodies reactive to the STLV-III viral proteins described herein, and that these show significant cross-reactivity with viral proteins of HTLV-III. This observation may be significant with regard to our understanding of the origin of HTLV-III and the pathobiology of AIDS in Africa (*20*).

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Isolation of T-Cell Tropic HTLV-III–Like Retrovirus from Macaques

Abstract. *The isolation of a T-cell tropic retrovirus from three immunodeficient macaques and one macaque with lymphoma is described. The morphology, growth characteristics, and antigenic properties of this virus indicate that it is related to the causative agent of acquired immune deficiency syndrome in humans (HTLV-III or LAV). This virus is referred to as simian T-lymphotropic virus type III (STLV-III) of macaques. The existence of a cytopathic, T-cell tropic virus resembling HTLV-III in monkeys may facilitate study of disease induction and vaccine development in an animal model.*

Converging lines of research strongly suggest that a T-cell tropic retrovirus called HTLV-III or LAV is the cause of the acquired immune deficiency syndrome (AIDS) in humans (*1–6*). Recent data on the increasing prevalence of infection with HTLV-III indicate that this is a public health problem of major proportions (*7*). An immune deficiency syndrome of macaque monkeys with many similarities to human AIDS has been described (*8–10*); affected animals at the New England Regional Primate Research Center (NERPRC) die with opportunistic infections, impaired T-cell function, and lymphoproliferative disorders (*8*). Because of the need to develop animal systems for the study of HTLV infection and to define the etiological agent (or agents) of the spontaneous immune deficiency syndrome at the NERPRC, we have been studying T-cell tropic retroviruses of macaques.

Serological surveys have indicated

Table 1. Isolation of HTLV-III–like virus from macaques.

Animal*	Blood sample used for isolation	Clinical-pathological features
Mm251-79	Frozen splenocytes†	Malignant lymphoma
Mm239-82	Frozen serum	Macrophage infiltrates in brain, oroesophageal candidiasis, cryptosporidiosis, intestinal trichomoniasis
Mm220-82	Frozen serum	Macrophage infiltrates in brain, oroesophageal candidiasis, cryptosporidiosis, intestinal trichomoniasis
Mm142-83‡	Lymphocytes	Diarrhea, facial rash, generalized lymphadenopathy, splenomegaly

*Mm, *Macaca mulatta* (rhesus monkey). †Splenocytes were stored viably frozen in 15 percent dimethyl sulfoxide in liquid nitrogen. ‡The only live animal from which virus was isolated.

that several Old World primate species are extensively infected with an HTLV-I–related virus (*11*), and HTLV-I seropositivity has been correlated with lymphomas and lymphoproliferative disorders in the NERPRC macaque colony (*12*). T-cell tropic retroviruses have been isolated from Old World primates, and the biologic, antigenic, and genetic characteristics of these viruses suggest that they are closely related to HTLV-I (*13*). Evidence for natural infection of nonhuman primates with a retrovirus that has properties similar to those of HTLV-III has not been presented. Here we describe the isolation of such a virus from four sick macaques of the NERPRC colony.

For virus isolation, peripheral blood lymphocytes, splenic lymphocytes, or cell-free serum samples were cocultivated with the following cell types: (i) HUT-78 cells, a human tumor T-cell line (*14*), and (ii) human T cells that had been cultured in the presence of T-cell growth factor [interleukin-2 (IL-2)]. Cell cultures were monitored for the appearance of reverse transcriptase activity in cell-free supernatants (Fig. 1). In cultures established with lymphocyte or serum samples from four of the macaques, reverse transcriptase activity was detected 12 to 18 days after initiation of cocultivation and reached maximum levels approximately 10 days later. High levels of reverse transcriptase activity have been maintained for more than 3 months of continuous cultivation of infected HUT-78 cells.

We have found no difference in the sensitivity of the two types of cultured cells for isolation or growth of these macaque retrovirus isolates. However, the use of T-cell tumor lines (*15*) has several advantages over the use of normal T cells. HUT-78 cells grow continuously in culture and are independent of exogenously added IL-2. Also, infection of HUT-78 cells with these macaque retroviruses resulted in the appearance of a characteristic cytopathic effect (Fig. 2, a and b). Infected HUT-78 cells became pleomorphic, and multinucleated giant cells were formed. The multinucleated giant cells appeared quite similar to those previously described for HT cells that had been infected with HTLV-III (*1*). Furthermore, like HTLV-III, but unlike HTLV-I and -II, filtered cell-free supernatants from cells infected with the new macaque virus isolates could infect T-cell cultures with high efficiency. Unlike type D retroviruses (*16*), these new retrovirus isolates did not replicate detectably in Raji cells, a B-cell line. Conversely, type D retroviruses did not replicate significantly in HUT-78 cells.

Peripheral blood lymphocytes prepared from heparinized human blood, were treated with monoclonal antibody plus complement and cell populations enriched in helper (T4) and suppressor (T8) cells were isolated. The replication of the macaque virus in fractionated as well as unfractionated cells growing in the presence of IL-2 was determined. The new macaque retrovirus replicated efficiently in $T4^+$ cells but much less efficiently in $T8^+$ cells (Table 2). These results are similar to previous findings with HTLV-III and LAV (*17*).

Table 2. STLV-III replication occurs preferentially in $T4^+$ lymphocytes.

Cell population*	Infection†	Reverse transcriptase activity (cpm)‡	
		Day 9	Day 15
Unseparated	STLV-III	34,631	12,141
	None	537	281
T4-enriched	STLV-III	33,905	6,853
	None	574	344
T8-enriched	STLV-III	4,537	237
	None	526	297

*Human peripheral blood lymphocytes (PBL) were treated twice with monoclonal antibody to T4 (19Thy5D7) + complement (C) or monoclonal antibody to T8(7PT3F9) + C, stimulated in vitro with phytohemagglutinin for 3 days, and then similarly treated a third time. Cell staining and fluorescence-activated cell-sorter analysis of these populations after the last treatment demonstrated that the unseparated PBL were comprised of 80 percent $T11^+$, 48 percent $T4^+$, and 29 percent $T8^+$ lymphocytes; the T4-enriched PBL were 72 percent $T11^+$, 72 percent $T4^+$, and 0 percent $T8^+$ lymphocytes; the T8-enriched PBL were 68 percent $T11^+$, 1 percent $T4^+$, and 63 percent $T8^+$ lymphocytes. †Cell populations were incubated for 3 days with aliquots of culture medium containing cell-free virus or control medium, washed, and then maintained in culture with IL-2, the cell number being adjusted to 1×10^6/ml every 3 to 4 days. Growth of these cell populations was comparable during the course of this experiment. ‡Assay performed as described (legend to Fig. 1) on supernatants sampled immediately prior to addition of fresh IL-2 and adjustment of cell concentration; cpm, count/min.

Cell cultures containing high reverse transcriptase activity (Fig. 1) were processed for electron microscopy as described (*16*). The four macaque T-cell tropic retrovirus isolates displayed a similar morphology. Budding particles with the morphology of type C retroviruses were observed in infected cells (Fig. 2c). Nucleoids were seen only in those viral particles that were in the process of budding from the cell membrane; preformed nucleoids were not observed. Mature, extracellular enveloped viral particles 110 to 130 nm in diameter typically had a cylindrical nucleoid (Fig. 2d). This type of nucleoid is a characteristic of HTLV-III that is not found with HTLV-I or -II. Thus, the formation of budding particles and the morphology of extracellular particles appeared identical to that previously observed for HTLV-III (*3*). Intracytoplasmic type A particles, typically seen in type D retrovirus-infected cells, were not observed.

The histories of the four macaques from which virus was isolated may be relevant to the potential pathogenicity of this virus and are summarized in Table 1. A rhesus monkey (*Macaca mulatta*), designated Mm251-79, died with a lymphoma 26 months after inoculation with minced tissue from a spontaneous *M. mulatta* lymphoma (*18*). Inoculation of seven macaques with minced Mm251-79 lymphoma tissue resulted in the death (range, 47 to 511 days; median, 63 days) of all seven animals with one or more of the following opportunistic infections: candidiasis, generalized cytomegalovirus (CMV), and cryptosporidiosis. Two of these animals were described in more detail previously (*19*). These findings were consistent with an immune deficiency disorder. Pooled blood samples from three of these seven animals were inoculated into an additional six animals with similar results; all six animals died 50 to 65 days after inoculation. Filtered, cell-free plasma from one of these six animals (Mm61-82) was inoculated into Mm220-82 and Mm239-82, both of which died 71 and 85 days later, respectively, with oroesophageal candidiasis, cryptosporidiosis, intestinal trichomoniasis, and multifocal perivascular macrophage infiltrates in the brain. Retrovirus was isolated, not only from viably frozen

splenocytes of the original Mm251-79 but also from serum frozen for longer than 1 year from the time of death of Mm220-82 and Mm239-82.

HTLV-III has recently been detected in the brains of AIDS encephalopathy patients (*20*). Histopathologic features of the brain lesions of Mm220-82 and Mm239-82 appeared similar to brain lesions sometimes seen in AIDS encephalopathy. Extensive electron microscopic examination of thin sections of lymph nodes taken from Mm220-82 and Mm239-82 just prior to death revealed budding and extracellular retrovirus particles indistinguishable in morphology from HTLV-III (Fig. 2, e and f).

Virus was also isolated from a living rhesus monkey Mm142-83. This monkey had lymphadenopathy, splenomegaly, hemoglobin of 6.8 g/dL, and a peripheral blood smear demonstrating atypical lymphocytosis and monocytosis. Peripheral blood lymphocytes from this animal exhibited depressed blastogenic responses after in vitro stimulation with pokeweed mitogen, xenogeneic cells, and *Candida* antigen. The ratio of helper to suppressor T lymphocytes (T4/T8) of this animal was 1.2. Mm142-83 had no connection with the transmission study described above. The retrovirus was isolated from Mm142-83 on two separate occasions, 4 months apart. Mm142-83 died 3 weeks after the second virus isolation.

In a separate study, the antigens of this newly isolated macaque retrovirus have been shown to be closely related to the proteins previously defined for HTLV-III (*21*). Since the morphology, growth characteristics, and antigenic properties of the new macaque retrovirus clearly indicate that it is similar to HTLV-III, we refer to this virus as simian T-lymphotropic virus type III (STLV-III) of macaques.

The STLV-III isolates described in this report cannot be due to HTLV-III introduced artificially through cell culture contamination or infection by HTLV-III–inoculated animals; HTLV-III has never been present in the laboratories where STLV-III was isolated nor have animals been inoculated with HTLV-III or AIDS material at NERPRC. Thus, these macaques were naturally infected with STLV-III.

We have readily isolated type D retrovirus from NERPRC macaques with an immune deficiency syndrome (*16, 22*), and type D retroviruses have also been isolated from macaques at other centers where immune deficiency syndromes are endemic (*10, 23*). However, experimental infection of naive macaques with D/New England isolates did not induce any features suggestive of the immune deficiency syndrome (*24*). Three of the four samples which yielded STLV-III (Mm251-79, Mm220-82, and Mm239-82) did not yield type D retrovirus upon cocultivation with Raji cells and with canine thymus cells (cell lines which are the most sensitive for type D retrovirus recovery) (*10, 16*). Cell-free supernatants from the four T-cell lines producing STLV-III did not yield a type D retrovirus when incubated with Raji and canine thymus cells. We were also not able to isolate type D retrovirus from the Mm61-82 filtered plasma used for inoculation of Mm220-82 and Mm239-82 nor from blood samples of Mm220-82 or Mm239-82 themselves on five separate occasions following inoculation, including those taken at the time of death. Continued investigation will be needed to determine the roles of STLV-III and type D retrovirus in spontaneous disease in our colony.

Studies of the pathogenesis of AIDS as well as the development of an effective

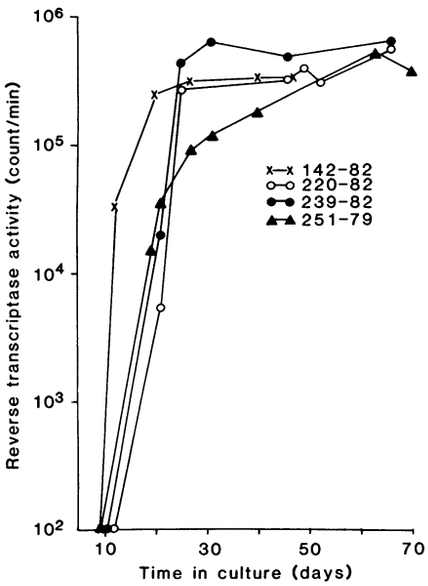

Fig. 1. Appearance of reverse transcriptase activity after cocultivation of macaque samples with HUT-78 cells. The samples indicated in Table 1 were cocultivated with HUT-78 cells (American Type Culture Collection). Lymphocytes and splenocytes were prepared by banding cells over Ficoll-Hypaque solutions. The four positive samples are a composite of cocultivations started on different dates. At each time point, 2 to 3 ml of cells were centrifuged at 1300*g* for 10 minutes. The supernatant was removed and centrifuged again at 1300*g* for 10 minutes to ensure that all cells were removed. Supernatant (1.4 ml) was centrifuged at 12,000*g* for 90 minutes to pellet virus. The virus pellet was resuspended on ice in 20 μl of dissociation buffer [0.01*M* tris-HCl, *p*H 7.3, 0.2 percent Triton X-100, 0.001*M* EDTA, 0.005*M* dithiothreitol (DTT), and 0.06*M* KCl]. To this was added 60 μl of a solution containing 0.05*M* tris-HCl, *p*H 8.3, 0.007*M* $MgCl_2$, 0.06*M* KCl, 0.08 mg/ml poly(ribocytidylate)oligo(deoxyguanylate) [(rC)-oligo(dG)]primer, 0.007*M* DTT, and 3.3 μCi of α-^{32}P-labeled dGTP (3000 Ci/mmol). Samples were incubated at 37°C for 60 minutes and 60 μl of each sample was spotted onto a Whatman No. 3 disk. Radioactivity incorporated in trichloroacetic acid–precipitable material was measured by Cerenkov counting. Backgrounds from uninfected cells varied from 500 to 1000 count/min. Samples from 68 other macaques gave negative results. These 68 samples were of two types: peripheral blood lymphocytes of live, healthy macaques (54 samples) and frozen sera from macaques that had died previously with immune deficiency (14 samples).

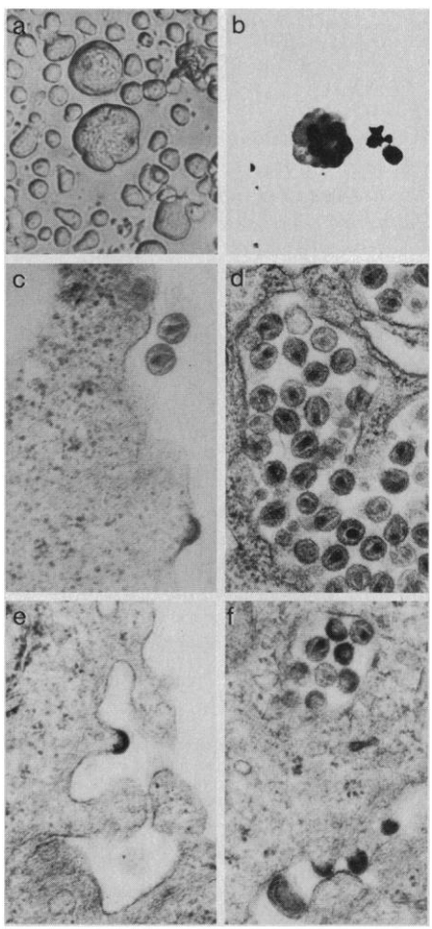

Fig. 2. Photomicrographs of HUT-78 cells infected with STLV-III. (a) Cytopathic effect of STLV-III infection of HUT-78 cells. (b) Large multinucleated cell after hematoxylin and eosin staining. (c) Electron micrograph of a portion of an STLV-III infected HUT-78 cell in which there is a single, budding retrovirus particle and two mature, extracellular particles with dense cylindrical nucleoids. Preformed, cytoplasmic, A-type particles were not observed. (d) Numerous mature, retrovirus particles with dense cylindrical nucleoids resembling HTLV-III. (e) Electron micrograph of a portion of a lymphocyte from a lymph node of Mm 239-82 in which there is a single retrovirus particle budding from the cell membrane. (f) Electron micrograph of a portion of the same cell as in (e) in which there are several tangentially sectioned, budding retrovirus particles and an aggregate of mature extracellular particles with dense, cylindrical nucleoids.

vaccine would be aided if HTLV-III had the ability to infect and cause disease in a laboratory animal. However, attempts to infect nonhuman primates other than chimpanzees with HTLV-III have generally been unsuccessful (*25*). Because of the endangered status of chimpanzees, their use for this purpose will probably be limited. If STLV-III is indeed pathogenic in macaques, useful approaches to the development and testing of a vaccine for AIDS may emerge.

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A Novel Mechanism of Somatic Rearrangement Predicted by a Human T-Cell Antigen Receptor β-Chain Complementary DNA

Abstract. *The T-cell antigen receptor is a cell surface molecule vital in mediating the cellular immune response. The arrangement and rearrangement of the gene segments encoding the β-chain polypeptide of the receptor are similar to those of immunoglobulin gene segments. The two constant region genes of the human T-cell antigen receptor are 8 kilobases apart with a cluster of joining segments located 5′ of each constant region gene. Although most β-chain gene rearrangements involve the variable, diversity, and joining segments, analysis of a β-chain complementary DNA clone suggests the occasional occurrence of another type of rearrangement.*

The antigen receptor expressed on the surface of T cells allows the specific recognition of a wide variety of antigens. This receptor contains two disulfide-linked polypeptide chains designated α and β (*1–4*). The β-chain genes of both murine and human T-cell receptors are encoded in variable (V), diversity (D), joining (J), and constant (C) region segments in germline DNA (*5–14*). These segments rearrange during somatic development to produce an active gene in a fashion precisely analogous to immunoglobulin heavy-chain gene segments.

Murine D, J, and C gene segments are arranged D-J-C_1-D-J-C_2 in a 15-kilobase

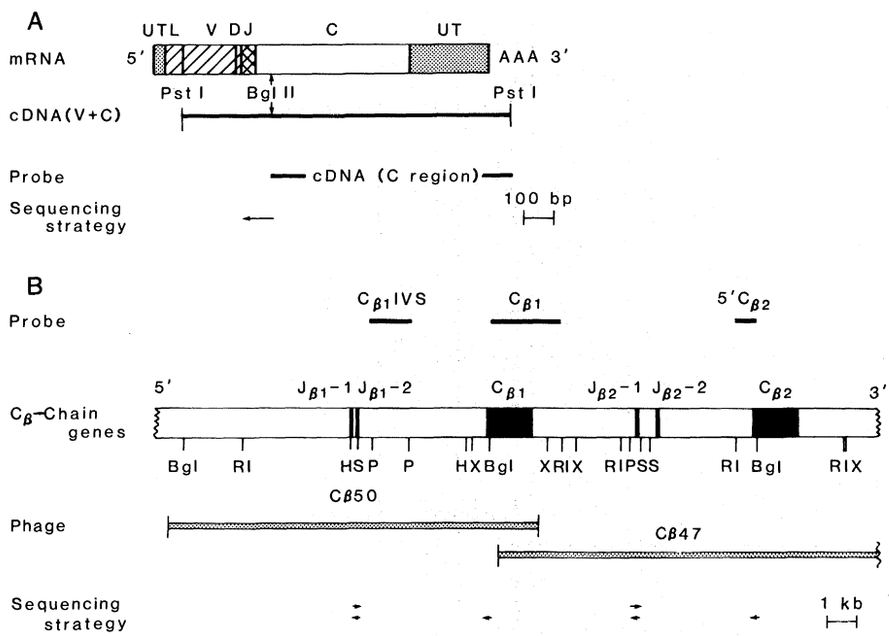

Fig. 1. Organization of the β chain mRNA (A) and constant region genes (B) of the human T-cell antigen receptor. A cDNA library was screened with two 20-base oligonucleotide probes (*21*) constructed to hybridize to the C region of a human β chain cDNA (*14*). The nucleotide sequence pTβ1 [cDNA (V + C)], containing a 1.1-kb nearly full-length β-chain cDNA insert, was isolated. Four J segments have been mapped near the C-region locus; other J segments probably exist close to the $J_{\beta1}$ and $J_{\beta2}$ clusters. The location of Eco RI (RI), Bgl II (Bgl), Hind III (H), Sma I (S), Pvu II (P), and Xba I (X) restriction enzyme sites are indicated. Probes used for analysis of C-region genes are restriction enzyme fragments. Other restriction enzyme fragments were cloned into the single-stranded bacteriophage M13 and their nucleotide sequence determined with the dideoxy chain termination method as described (*22*). The direction and extent of sequencing are indicated by individual arrows.