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Serologic Identification and Characterization of a Macaque T-Lymphotropic Retrovirus Closely Related to HTLV-III

Abstract. *Human T-lymphotropic virus type III (HTLV-III) is thought to play an etiologic role in the development of the acquired immune deficiency syndrome (AIDS). In this study the serologic characterization of a new simian retrovirus that is related to HTLV-III is described. This new virus, here referred to as STLV-III, was isolated from sick macaques at the New England Regional Primate Research Center. Radioimmunoprecipitation analysis revealed STLV-III–specific proteins of 160, 120, 55, and 24 kilodaltons, all similar in size to the major* gag *and* env *proteins of HTLV-III. These antigens were recognized by representative macaque serum samples and human reference serum samples positive for HTLV-III antibodies. Monoclonal antibodies directed to p24, the major core protein of HTLV-III, also immunoprecipitated a 24-kilodalton species in lysates of cells infected with the macaque virus. This HTLV-III–related virus, which naturally infects a nonhuman primate species, may provide a useful model for the study of HTLV-III and the pathogenesis of AIDS.*

The human T-lymphotropic retroviruses (HTLV) are a group of related exogenous agents that preferentially infect helper T lymphocytes. There are three known types. HTLV-I is characterized by its widespread yet geographically distinct distribution; in endemic regions it has been closely linked with the development of a unique lymphoma of mature T cells designated the adult T-cell leukemia/lymphoma (ATLL) (*1*). HTLV-II is closely related to the type I virus, but its distribution and relation to human disease has not been established (*2*). HTLV-III, also known as LAV and ARV, is the prototype virus isolated from patients with the acquired immune deficiency syndrome (AIDS) (*3*, *4*). Thus, it appears that at least two members of this virus family are highly associated with different types of human diseases that include a malignancy and an ablative disorder of the same $T4^+$ lymphocyte population.

The existence of a virus related to HTLV-I in nonhuman primates was first reported by Miyoshi and his colleagues (*5*). Studies with healthy Japanese macaques, *Macaca fuscata*, indicated that various proportions of healthy adults (of this species) had antibodies that reacted with HTLV-I–related antigens. Type C retroviruses that were presumably responsible for this activity were then detected in lymphoid cultures established from seropositive animals (*6*). Subsequent serologic studies have demonstrated the presence of antibodies to HTLV-I in Asian and African Old World primate species, whereas New World primates and prosimians have been uniformly seronegative (*7*, *8*). Proviral sequence analyses of viruses derived from seropositive baboons, African green monkeys, and *Macaca* species indicate that the nonhuman primate viruses are closely related to, yet distinct from, HTLV-I (*9*).

We recently reported an association between exposure to an HTLV-I–related virus and the development of spontaneous lymphoma and lymphoproliferative disorders in three species of macaques. Antibodies highly cross-reactive with HTLV-I–related antigens were detected by means of membrane immunofluorescence (MIF) and radioimmunoprecipitation (RIP) techniques (*8*). Macaque sera

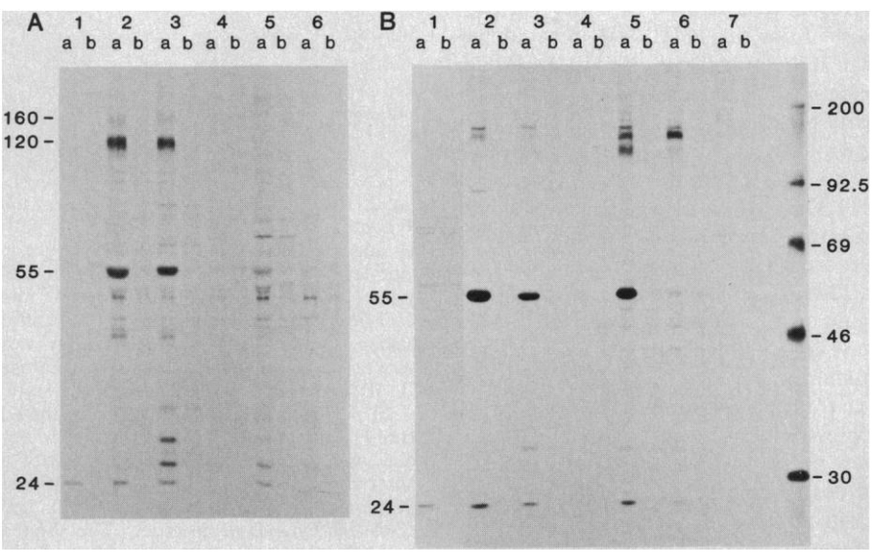

Fig. 1. (A) H9/HTLV-III and H9 cells were harvested at their peak of log-phase growth and were exposed to [^{35}S]cysteine [150 μCi/ml; specific activity, 1000 to 1050 Ci/mmole; New England Nuclear] for 8 to 10 hours. A soluble cell lysate was prepared by disruption of cells with RIPA buffer (0.15*M* NaCl, 0.05 tris-HCl, *p*H 7.2, 1 percent sodium deoxycholate, and 0.1 percent SDS) and centrifugation for 1 hour at 100,000*g*. The lysates [H9/HTLV-III (lanes a); H9 (lanes b)] were reacted with 10 μl of the following test sera bound to protein A–Sepharose CL-4B (protein A beads, Sigma): (Lane 1) Monoclonal antibody to HTLV-III p24; (lane 2) human reference positive serum to HTLV-III; (lane 3) human reference positive serum to HTLV-III; (lane 4) human reference negative serum to HTLV-III; (lane 5) representative macaque serum, positive for STLV-III; and (lane 6) representative macaque serum, negative for STLV-III. The immunoprecipitates were eluted in a sample buffer containing 0.1M Cleland's reagent, 2 percent SDS, 0.08*M* tris-HCl, *p*H 6.8, 10 percent glycerol, and 0.2 percent bromophenol blue by boiling at 100°C or 2 minutes. Samples were analyzed in a 10.0 percent acrylamide resolving gel with a 3.5 percent stacking gel according to the discontinuous buffer system of Laemmli (*19*). (B) HUT-78/STLV-III (lanes a) and uninfected HUT-78 (lanes b) cell lysates were prepared as described above. (Lane 1) Monoclonal anti-p24 HTLV-III; (lane 2) human reference positive serum to HTLV-III; (lane 3) human reference positive serum to HTLV-III; (lane 4) human reference negative serum to HTLV-III; (lane 5) representative macaque serum, positive for STLV-III; (lane 4) representative macaque serum, positive for STLV-III; and (lane 7) representative macaque serum, negative for STLV-III.

that were MIF-positive immunoprecipitated the same major HTLV-I proteins encoded by the *gag* and *env* genes that had previously been identified and characterized for the human virus (*8, 10*). The lymphoma could be transmitted in vivo to other macaques by inoculation of tumor cell suspensions and karyotypic analysis of the tumors so induced confirmed successful transmission by an infectious agent (*11*).

In other studies involving the inoculation of materials from a macaque with lymphoma, the induction of clinical and histopathologic features of macaque immune deficiency syndrome was observed (*11–13*). That this syndrome could be induced so easily prompted us to direct our efforts toward the identification of an HTLV-type virus from macaques with immune deficiency syndromes. We report here on the serologic identification and characterization of a new macaque retrovirus that has striking similarities to the human AIDS virus HTLV-III; we therefore refer to it as the simian T-lymphotropic virus of macaques related to HTLV-III (STLV-III) (*12*).

The viral-related proteins were identified by radioimmunoprecipitation and sodium dodecyl sulfate–polyacrylamide gel electrophoresis (RIP/SDS-PAGE) of [^{35}S]cysteine-labeled whole cell lysates of virus-infected cells. One reference cell line used was HUT-78/STLV-III (HUT-78 cells that were infected with STLV-III), a stable producer of the primate T-lymphotropic virus related to human HTLV-III (*12*). The uninfected HUT-78 cells represent a well-characterized mature human T-cell line (*12, 14*). All sera were similarly reacted with antigens prepared from the HTLV-III–infected human H9 reference cell line (*3, 4*). Human reference positive sera with known reactivity to all HTLV-III type–specific proteins, and monoclonal antibodies directed to p24, the major core protein of HTLV-III, were also subjected to RIP with the same cells (*4, 15*). Serum samples from representative macaques were included with serum from rhesus macaque Mm251-79, from which the virus was isolated. The four representative human and macaque sera that were positive for precipitation of either HTLV-III proteins or STLV-III proteins, or both, were prescreened for antibodies by MIF as described (*10, 15*) and found to be positive (four of four) on both types of infected cells but negative on the uninfected cells.

The major antigens recognized by antibody-positive human sera from patients with AIDS-related complex (ARC) have previously been described for the H9/HTLV-III cell line (*4, 15*). Monoclonal antibodies to the p24 of HTLV-III recognized a 24-kD species on H9/HTLV-III but not on the uninfected H9 line (Fig. 1A). Human reference positive sera from ARC patients precipitated 160-, 120-, 55-, and 24-kD species and these were not recognized by a human reference negative serum. A representative serum sample from the MIF-antibody–positive macaque Mm251-79, from which STLV-III was isolated, recognized a 55-kD and a 24-kD species upon reaction with H9/HTLV-III cell lysates, but not from lysates prepared from the control H9 cells.

HUT-78/STLV-III and uninfected HUT-78 cell lines were metabolically labeled with S[^{35}S]cysteine according to the same procedure (*10, 15*) and RIP of whole cell lysates was performed with the same sera as described above (Fig. 1B). Monoclonal antibody to p24 of HTLV-III recognized a 24-kD species in the STLV-III–infected antigen preparation. Human ARC reference serum positive for HTLV III proteins immunoprecipitated a 55-kD and 24-kD species on the STLV-III–infected HUT-78 cell line where weak reactivity to the 120- and 160-kD species was also observed. Similar species were not detected with uninfected HUT-78 cells. Proteins of the same size were immunoprecipitated by representative macaque sera including MIF antibody-positive sera taken from Mm251-79. The same serum samples reacted positively with the major *gag*-related proteins of HTLV-III. Human reference negative sera and representative negative macaque serum did not react with the STLV-III viral antigen preparation. Human sera with previously determined serologic reactivity to HTLV-I viral proteins failed to recognize STLV-III viral proteins in either MIF or RIP assays. Reference antisera to HTLV-I and macaque sera containing antibodies to the macaque virus that is related to HTLV-I (*8*) were also seronegative with STLV-III.

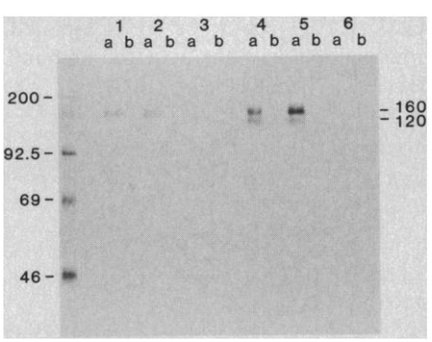

Fig. 2. Reactivity of serum samples with the glycoprotein preparation of HUT-78/STLV-III and uninfected HUT-78 (*10*). The glycoproteins were prepared from soluble cell lysates of HUT-78/STLV-III (lanes a) and HUT-78 (lanes b) made with RIPA buffer lacking sodium deoxycholate. The lysates were passed through a lentil-lectin–Sepharose CL-4B (Pharmacia, Sweden) column at a ratio of 20×10^6 cells to 1 to 2 ml of undiluted lentil-lectin–Sepharose Cl-4B. The glycoproteins were eluted from the column with a buffer consisting of 0.15*M* NaCl, 0.05*M* tris-HCl, *p*H 7.2, 1 percent Triton X-100, and 0.2*M* methyl-α-D-mannoside. The eluted bound fraction was reacted with 10 μl of test sera that had been bound to protein A beads. The same sera was used in lane 1 to 6, respectively, as described for lanes 2 to 7 in Fig. 1B. The immunoprecipitates were eluted from protein A beads and subjected to SDS-PAGE, as described above.

The same human and macaque serum samples were reacted with glycoproteins prepared from HUT-78/STLV-III cells by using lentil-lectin affinity chromatography (Fig. 2). Human positive reference sera and macaque positive sera similarly recognized two protein species from the eluate fraction that migrated at approximately 160 and 120 kD; these were not recognized by reference negative sera. The high molecular weight HTLV-III proteins gp160 and gp120 are glycosylated and encoded by the *env* region of the HTLV-III genome (*15, 16*). Seroepidemiologic studies indicate that these are the most immunogenic protein species recognized by antibody-positive humans exposed to HTLV-III (*15, 16*).

These data indicate that the viral proteins immunoprecipitated from the STLV-III–infected cell line are of the same molecular sizes as those seen for HTLV-III, and that the larger species (gp120 and gp160) are apparently glycosylated as they are for HTLV-III. Although the high molecular weight glycoproteins of HTLV-III are the most immunogenic antigens in exposed humans, sera from STLV-III antibody–positive macaques showed minimal reactivity to these proteins, indicating an apparent one-way cross-reactivity of antibodies directed to these glycoproteins. STLV-III may thus be distinct from HTLV-III, at least to the degree of the type-specific immunoreactivity to the *env*-encoded glycoproteins. This observation is consistent with the type-specific nature of *env*-encoded antigens of other previously described animal retroviruses.

From Mm251-79 we obtained tumor cells and cell-free preparations that were inoculated into seven rhesus macaques. All seven macaques, which were previously free of disease, succumbed to a variety of opportunistic infections with clinical signs and pathologic lesions similar to those observed in spontaneous immune deficiency disease of macaques (*11–13*). Other STLV-III isolates were

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