er retroviral subfamilies, antigenic variation involving the structure of the viral envelope is a characteristic feature of lentivirus isolates (*21–23*). Neutralization studies show that different antigenic strains of equine infectious anemia virus (EIAV), responsible for sequential febrile episodes, can be identified in individual diseased horses (*21*). These EIAV variants contain alterations confined to envelope glycoproteins as monitored by tryptic peptide mapping (*24*). Similar changes have been mapped to the *env* gene of visna virus antigenic variants (*25, 26*). Furthermore, molecular probes, respresentative of lentivirus genomes, do not hybridize to normal cellular DNA (*27*), indicating that these agents are exogenous, not endogenous retroviruses. The structural changes that appear in the viral genome are therefore not a consequence of recombination with related retroviral sequences that have been inserted into the germline of the infected cell.

These results clearly demonstrate the heterogeneity of AIDS RV genomes. Although the African isolates, as a group, were more diverse than those from North America, the U.S. samples were all different from one another and exhibited no geographical specificity. This conclusion differs, therefore, from that of Ratner *et al.* (*28*), who explain the genomic variation of HTLV-III and ARV on the basis of their New York and California origins, respectively. Since our analysis was based on restriction enzyme polymorphisms which, at best, measure single base changes, nucleotide sequencing of some of these new AIDS RV isolates will be required for a more complete assessment of the genomic relatedness. Such studies will be of importance in understanding how the AIDS RV exerts its immunopathogenic effects and in generating strategies for the development of new therapies and effective vaccines.

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16. Complementary DNA was synthesized by reverse transcription of viral RNA purified from 14 liters of A3.01 cells (*10*) infected with LAV (*9*) kindly provided by L. Montagnier. The cDNA was prepared in a reaction mixture (100 μl) containing 50 m*M* tris-HCl, *p*H 8.3, 60 m*M* KCl, 10 m*M* $MgCl_2$, 10 m*M* dithiothreitol, actinomycin D (20 μg/ml), RNasin (750 U/ml) (Promega Biotech), 80 U of RT (Seikagaku American, Inc.), 0.5 m*M* each deoxyadenosine triphosphate, deoxythymidine triphosphate, and deoxyguanosine triphosphate, 300 μCi of α^{32}P-labeled deoxycytosine triphosphate (400 Ci/mmol) (Amersham) and 5 μg of oligo dT (Pharmacia). LAV-infected A3.01 cellular DNA was digested to completion with Bam HI, an enzyme previously shown to cut the provirus at map position 8.4 kb (*18*). The restricted cellular DNA was then ligated to Bam HI cleaved λJ1 (*15*) arms, packaged in vitro (*30*), and propagated in *Escherichia coli* DP50 *sup*F.
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Isolation of T-Lymphotropic Retrovirus Related to HTLV-III/LAV from Wild-Caught African Green Monkeys

Abstract. *Present evidence suggests that the acquired immune deficiency syndrome (AIDS) emerged in Central Africa as a new disease in recent decades. This disease has recently approached epidemic proportions in many parts of the world. The etiologic agent of AIDS is believed to be the virus HTLV-III/LAV, which has been proposed as having originated from a recent simian-human transmission in Africa. This report describes the isolation of a designated STLV-III$_{AGM}$ retrovirus closely related to HTLV-III/LAV from seven healthy wild-caught African Green monkeys* (Cercopithecus aethiops) *that showed the presence of antibodies designated STLV-III$_{AGM}$. In vitro growth characteristics, ultrastructural morphology, and major proteins of 160,000 kilodaltons (kD), 120 kD, 55 kD, and 24 kD are similar to and cross-reactive with the analogous antigens of HTLV-III/LAV. The use of these serologic markers in the detection of STLV-III$_{AGM}$–infected monkeys may be important in assuring the continued safety of a variety of biologic reagents that are derived from these primate species. The existence of a retrovirus closely related to HTLV-III/LAV that naturally infects an African nonhuman primate in the apparent absence of disease may provide a unique model for the study of human AIDS and the development of an effective vaccine.*

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Studies on murine, feline, and bovine retroviruses have provided background and direction for much of the research on human retroviruses and the pathogenesis of leukemia and immunosuppression. The relevance of animal model systems is particularly exemplified by the recently described simian T-lymphotropic viruses (STLV), where present data on these exogenous type C retroviruses of nonhuman primates closely parallels observations of the biology of the human T-lymphotropic virus (HTLV) family. It is now widely recognized that a variety of Asian and African Old World primates are naturally infected with one or more retroviruses closely related to HTLV-I, designated STLV-I (1). In serologic studies, the association of STLV-I with spontaneous lymphoma in captive *Macaca* species has been noted paralleling the association of HTLV-I with adult T-cell leukemia/lymphoma (ATLL) in humans (*2*).

We recently described an exogenous retrovirus of captive ill macaques that is closely related to HTLV-III and designated it simian T-lymphotropic retrovirus type III (STLV-III$_{mac}$). The growth characteristics, T4 tropism, and ultrastructural morphology of STLV-III$_{mac}$

Table 1. Isolation of STLV-III_{AGM} from African Green monkeys. Seven isolates from eight seropositive African Green monkeys were identified as STLV-III_{AGM}.

Animal number	Geographic origin	Virus isolation	Antibodies to	
			STLV-III_{AGM}*	HTLV-III*
1	Kenya	+	gp160/120, p55, p24	+
2	Ethiopia	+	gp160/120, p55, p24	−
3	Kenya	+	gp160/120	+
4	Kenya	+	gp160/120	+
5	Kenya	+	gp160/120	−
6	Kenya	+	gp160/120, p55, p24	−
7	Kenya	+	gp160/120	+
8	Kenya	−	gp160/120	+

*Antibody status determined by RIP/SDS-PAGE analysis.

and HTLV-III are similar (*3*). STLV-III_{mac} viral proteins show size similarities and cross-reactivity with the *gag*-, *env*-, and 3′ *orf*-encoded proteins of HTLV-III (*4, 5*).

It is now widely believed that HTLV-III/LAV is the etiologic agent of AIDS in humans. Numerous serologic studies of HTLV-III suggest that the AIDS epidemic is expanding. A disproportionate number of AIDS cases has been reported in Central Africa; some of these cases were observed prior to recognition of the disease in the United States or Europe (*6*). It has therefore been speculated that HTLV-III and AIDS originated recently in Central Africa.

We therefore investigated the possibility that primates indigenous to Central Africa are carriers of an infectious virus serologically related to HTLV-III/LAV. In a survey of a variety of African primate species we found a high prevalence of antibodies to STLV-III in healthy African Green monkeys (*Cercopithecus aethiops*) (*7*). The study of STLV-III in African Green monkeys may not only lead to the development of a model system for the study and prevention of AIDS but may also be important in ensuring the safety of biologic reagents derived from these primates. Here we report on the isolation and characterization of an STLV-III from antibody-positive African Green monkeys. We have designated this virus STLV-III_{AGM}. We also describe the major antigens of this virus and their utilization for the detection of STLV-III–infected monkeys.

Serum samples from 67 healthy, wild-caught African Green monkeys were initially analyzed for the presence of antibodies to STLV-III of macaques by membrane immunofluorescence (MIF) and radioimmunoprecipitation with sodium dodecyl sulfate–polyacrylamide gel electrophoresis (RIP/SDS-PAGE) as previously described (*4, 7*). Twenty-seven of the 67 (40 percent) samples were positive for antibody to STLV-III_{mac}. Peripheral blood lymphocytes from eight different antibody-positive monkeys were cocultivated with Hut-78 cells, a well-defined human mature T-cell line (*8*). The procedure used was the same as that described for STLV-III_{mac} (*3*). Cell cultures were successfully established from seven of the eight animals, and all seven contained readily detectable STLV-III. Whether or not the failure to identify virus in the eighth animal was due to lack of infectivity of the virus or to the actual absence of the virus is not known. A cytopathic effect was observed after 7 to 28 days of culture, characterized by marked cytolysis of Hut-78 cells (Fig. 1A), cellular atypia, and the appearance of multinucleated giant cells. This characteristic cytolytic effect necessitated the addition of fresh Hut-78 cells at regular intervals for the first 14 to 21 days of culture. Similar results were observed in H9 cells infected with HTLV-III (*9*) and Hut-78 cells infected with STLV-III_{mac} (*3*). Cell cultures were monitored for viral protein expression by MIF and RIP/SDS-PAGE as described (*4*), with the use of a battery of reference sera with known antibody reactivity to viral proteins of STLV-III_{mac}, HTLV-III, HTLV-I, and type D Mason-Pfizer monkey virus (MPMV). After 30 days of culture, STLV-III–positive proteins were detected in each of the seven cultures by both MIF and RIP/SDS-PAGE assays, with no detection of HTLV-I or type D MPMV specific viral proteins.

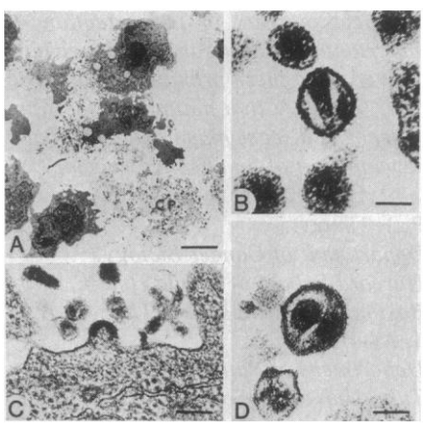

Fig. 1. (A to D) Electron micrographs of Hut-78 cells infected with STLV-III. (A) The human T lymphocytes have convoluted nuclei. The cells that release virus particles to the media contain numerous cytoplasmic processes. The cytopathic effect is exhibited in a dead cell. Original magnification ×2430 (bar, 4 μm). (B) Longitudinal section through an STLV-III_{AGM} mature virion showing a dense cylindrical nucleoid resembling HTLV-III. Original magnification ×150,000 (bar, 65 nm). (C) A C-type budding particle of STLV-III_{AGM}. Original magnification ×70,000 (bar, 143 nm). (D) A cross section through an STLV-III_{AGM} mature virion with a dense oval nucleoid resembling that of HTLV-III. Original magnification ×150,000 (bar, 65 nm).

All cell cultures expressing STLV-III viral proteins were examined by electron microscopy. The budding pattern observed at the cell membrane was characteristic for a type C retrovirus (Fig. 1C) and the mature extracellular virions were 100 to 120 nm in diameter with an electron-dense cylindrical core on longitudinal section (Fig. 1, B and D). This ultrastructural morphology is similar to that described for both STLV-III_{mac} and HTLV-III/LAV (*3, 9*).

The major viral antigens of STLV-III_{mac} have been identified (*4*) by RIP/SDS-PAGE as 160 kilodaltons (kD) (gpl60), 120 kD (gpl20), 55 kD (p55), and 24 kD (p24) species, the gpl60 and gpl20 species being glycosylated. The seven cell cultures derived by cocultivation of cells from seven different African Green monkeys as well as with Hut 78 uninfected cells were metabolically labeled with [^{35}S]cysteine, and RIP/SDS-PAGE of whole-cell lysates was performed with (i) an STLV-III antibody positive serum sample from an African Green monkey, (ii) an STLV-III antibody–negative serum sample from an African Green monkey, and (iii) an HTLV-III antibody–positive reference serum sample from a human patient with AIDS-related complex (ARC) (Fig. 2). Lysates from all seven cultures (lanes 1 to 7) showed bands of about 160, 120, 55, and 24 kD when reacted with serum from a reference antibody-positive African Green monkey. The same bands were not seen with lysate from uninfected Hut 78 cells (lane C). The proteins were also not detected when lysates from the seven infected cell cultures were reacted with a representative serum from an African Green monkey that lacked antibodies to STLV-III. Serum from a human ARC patient recognized the 55-kD and 24-kD proteins and showed faint reactivity with

proteins of about 120 kD and 160 kD in lysates prepared from isolates 1 to 7 but the same bands were lacking in uninfected Hut-78 cells. The relation of this virus to HTLV-III is further supported by the precipitation of the 24-kD protein of STLV-III$_{AGM}$ with a monoclonal antibody to the p24 of HTLV-III.

Serum samples from the seven different African Green monkeys from which virus was successfully isolated were reacted with cell lysates prepared from an STLV-III$_{AGM}$ cell line (isolate 1) and uninfected Hut-78 cells (Fig. 3, lanes 1 to 7). All seven sera from virus-positive monkeys specifically precipitated the gp160/120, whereas only three of these serum samples also showed reactivity with the p55 and p24. Analysis of sera from other African Green monkeys demonstrated a similar phenomenon, with only 11 of 42 (26 percent) of the serum samples that were positive for antibodies to STLV-III$_{AGM}$ gp160/120 were also positive for antibodies to p55 or p24, or both. Thus, it appears that the high molecular weight glycoproteins of this virus are the most immunogenic species in infected monkeys. A similar observation was made in the human system where the *env*-encoded gp160 and gp120 of HTLV-III/LAV are the most immunogenic proteins in people exposed to the human virus (*10*).

We previously showed that a proportion of macaque sera that possess antibodies to STLV-III$_{mac}$ viral proteins also have antibodies that cross-react with the major antigens of HTLV-III; however, this cross-reactivity was minimal with respect to the *env*-encoded glycoproteins gp160 and gp120. Of the seven African Green monkeys that were antibody-positive for STLV-III and also yielded infectious STLV-III$_{AGM}$, four had antibodies to HTLV-III proteins by RIP/SDS-PAGE analysis (Table 1). STLV-III$_{AGM}$ antibody positive sera from 20 other African Green monkeys were also analyzed for antibodies to HTLV-III/LAV proteins, by RIP/SDS-PAGE, and 50 percent of these sera demonstrated reactivity to HTLV-III proteins, including the gp160 and gp120. Thus, it appears that the possession of antibodies to STLV-III–specific proteins was the most closely associated with virus isolation in African Green monkeys; serologic screening to detect cross-reacting antibodies to the related virus HTLV-III/LAV was less sensitive in detecting STLV-III$_{AGM}$ infected monkeys.

These data indicate that healthy African Green monkeys are infected with a retrovirus closely related to HTLV-III, designated STLV-III$_{AGM}$. Seven isolates were identified by in vitro growth characteristics, specific viral protein expression, and ultrastructural morphology of retroviral particles. All seven isolates have since been used to productively infect the Hut-78 line and have been maintained in continuous cell culture for 6 months. The major STLV-III$_{AGM}$ viral proteins are similar in molecular weight to the major *gag*- and *env*-encoded proteins of HTLV-III/LAV. Like the STLV-III$_{mac}$ viral proteins they are recognized by reference HTLV-III–positive human sera and reference antisera as well as monoclonal antibodies to the HTLV-III major core protein, p24. The high molecular weight glycoproteins of STLV-III$_{AGM}$ were uniformly precipitated by all virus-positive monkeys whereas antibodies to the p55 and p24, the presumed *gag*-associated proteins, were less regularly detected. As in the case of HTLV-III–infected people, the gpl60/120 appear to be the best serologic markers for infection by these closely related viruses. These proteins therefore represent the most obvious antigen target for serologic screening purposes. In addition, serologic cross-reactivity directed to these presumed *env*-encoded antigens suggests that conserved regions of these proteins should be evaluated as potential immunogenic epitopes for development of a human AIDS vaccine. African Green monkeys are commonly used for biomedical research, diagnostic virology, and the production of biologic reagents; much of the oral poliovaccine (OPV) used throughout the world is produced on primary cultures of kidney cells from this species. The serologic screening of monkeys used for these purposes by means of STLV-III$_{AGM}$

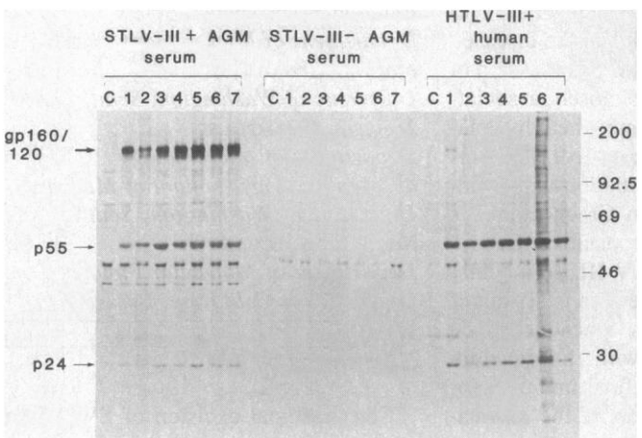

Fig. 2. Cells from cell cultures (lanes 1 to 7) and control uninfected Hut 78 cells (C) were harvested at their peak of log phase growth and were exposed to [^{35}S]cysteine [150 Ci/ml; specific activity 1000 to 1050 Ci/mmol; New England Nuclear (NEN)] for 4 to 6 hours. A soluble cell lysate was prepared by disrupting the cells with RIPA buffer (0.15*M* NaCl, 0.05*M* tris-HCl, *p*H 7.2, 1 percent sodium deoxycholate, and 0.1 percent SDS), and clearing by centrifugation for 1 hour at 100,000*g*. Each group of cell lysates was reacted with 10 μl of the following test sera bound to protein A–Sepharose CL-4B (protein A–beads; Sigma): STLV-III+ serum is from an antibody-positive African Green monkey; STLV-III− serum is from a reference antibody-negative African Green monkey; and HTLV-III+ reference antibody-positive serum is from a human ARC patient. The immunoprecipitates were eluted in a buffer containing 0.1*M* 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 for 2 minutes. Samples were analyzed in a 10.0 percent acrylamide resolving gel with 3.5 percent stacking gel according to the discontinuous buffer system of Laemmli (*14*).

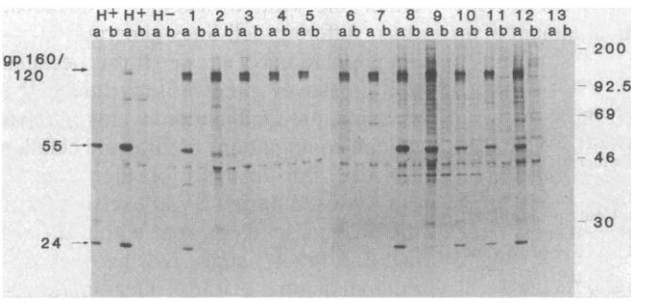

Fig. 3. Lysates of Hut-78 cells infected with STLV-III$_{AGM}$ (isolate 1) (lanes a) and of uninfected Hut-78 cells (lanes b) were prepared as described above. Lanes H+, contain serum samples from patients with AIDS or ARC who were positive for HTLV-III; lane H−, contains serum from a healthy control human who is antibody-negative; lanes 1 to 7 are serum samples from African Green monkeys from which virus isolates 1 to 7 were obtained; lanes 8 to 12 are serum samples from representative African Green monkeys that were positive for antibodies to STLV-III$_{mac}$; and lane 13 is a representative African Green monkey serum that was negative for antibodies to STLV-III$_{mac}$.

antigens would provide some assurance against potentially undesirable contamination (*11*).

Numerous subspecies of African Green monkeys (*C. aethiops*), also termed grivets, vervets, or guenons, are found throughout most of sub-Saharan Africa. In limited serologic surveys conducted to date, other African nonhuman primates such as the chimpanzee (*Pan troglodytes*), baboon (*Papio* sp.), patas monkey (*E. patas*), and colobus monkey (*C. polykomos*) were seronegative to STLV-III viral proteins. It is possible that other nonhuman primates from specific geographic locales in Africa are infected with an STLV-III. In Africa, the African Green monkeys are somewhat gregarious and are regarded as agricultural pests. They have been considered as reservoirs or vectors of certain other viruses that sometimes cause disease in humans including Ebola fever, Marburg disease, and African yellow fever.

$STLV\text{-}III_{mac}$ was isolated from macaques with an immunodeficiency syndrome. Serologic studies of the diseased macaques indicate that the possession of STLV-III antibodies is closely associated with this syndrome; healthy macaques have been seronegative (*12*). This is in contrast to the situation with wild-caught healthy African Green monkeys that are frequently seropositive. In preliminary studies $STLV\text{-}III_{mac}$ inoculation induced seroconversion in six out of six inoculated rhesus macaques (*12*). This was associated with the development of an acute and fatal immunosuppressive disease in four of the six macaques (*13*).

To date, there has been no evidence of disease in any African Green monkeys that have evidence of exposure to $STLV\text{-}III_{AGM}$. Understanding the biology of an HTLV-III–related virus in this primate species may help us understand the specific viral alterations or viral-host interactions that are involved in the pathogenicity of this family of T-lymphotropic retroviruses and perhaps provide a new approach in the development of an AIDS vaccine.

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Association of Crossover Points with Topoisomerase I Cleavage Sites: A Model for Nonhomologous Recombination

Abstract. *Nonhomologous DNA recombination is frequently observed in somatic cells upon the introduction of DNA into cells or in chromosomal events involving sequences already stably carried by the genome. In this report, the DNA sequences at the crossover points for excision of SV40 from chromosomes were shown to be associated with eukaryotic topoisomerase I cleavage sites in vitro. The precise location of the cleavage sites relative to the crossover points has suggested a general model for nonhomologous recombination mediated by topoisomerase I.*

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Chromosomal excision of SV40 from nontandem inserts proceeds via imprecise, illegitimate (nonhomologous) recombination events. As a result, single integrated copies of SV40 yield heterogeneous populations of excision products. (*1*). Nevertheless, excisional recombination is not random because a given cell line gives rise to a reproducible population of discrete excised forms (*2*). SV40 integration is not specific with respect to either the viral or cellular sequences; thus, different cell lines generate different populations of excised forms because the crossover points used during the excisional recombination events involve unique combinations of viral and cellular DNA's. Our analysis of the parental DNA's used during SV40 excision from the proviral locus present within the rat cell line 14B (*1*) suggested that the nonrandom nature of these illegitimate recombination events was dependent on features either at, or very close to, the crossover points (*2*). The crossover point features we identified included small homologies [2 to 3 base pairs (bp) long] at the point of strand exchange and sequences that were very similar to those reported to be eukaryotic topoisomerase I (topo I) cleavage sites (*3*, *4*). The observation that potential topo I cleavage sites were associated with the excision crossover points was interesting in light of reports that this enzyme can catalyze the ligation in vitro of heterologous DNA fragments lacking any sequence complementarity (*4–6*). That eukaryotic topo I is available to catalyze illegitimate recombination in vivo is suggested by studies that demonstrated that this abundant enzyme is constitutively expressed in somatic cells (*7*) and is associated with chromatin (*8–10*).

Eukaryotic topo I cleavage sites have sequence features in common; the great majority of these sites contain the sequence CTT or GTT immediately 5′ to the cleavage site (*3*, *4*, *11*). However, the specificity is not absolute, as (for example) is the case with a restriction endonuclease. Also, although single-stranded DNA is infrequently cleaved by topo I relative to duplex DNA, it is nonetheless a substrate for this enzyme (*11*). Therefore, the possible association of topo I cleavage sites with SV40 excisional recombination crossover points had to be tested in vitro and with both single-stranded and duplex DNA's. This report indicates that the excisional recombination crossover points in duplex DNA, but not in single-stranded DNA, are associated with rat liver topo I cleavage sites in a statistically significant manner.

Eukaryotic topo I preferentially interacts with certain sequences on DNA, and these sequences can be identified by stopping the nicking-closing reaction with sodium dodecyl sulfate (SDS) (*12*). Under these conditions, nicks are gener-