$V_0 - Z_0I$, where V_0 is the value that would occur in the absence of the Bay of Fundy and Gulf of Maine, I is the tidal volume flux across the shelf edge, and Z_0 is the ocean impedance. If nodal modulation increases V_0 by a factor of $1 + n$, with $n = 0.0373$, and I by $1 + m$, then $V_0 - Z_0I$ increases by a factor $[1 + n + C(n - m)]$ with $C = Z_0I(V_0 - Z_0I)^{-1}$. C. Garrett and D. A. Greenberg [*J. Phys. Oceanogr.* **7**, 171 (1977)] estimated C to have a magnitude of 0.2 with a small phase that we ignore here. Hence, with $m \approx 0.025$, the shelf-edge tide increases by 1.0398, which we round off to 1.040. The model 3.73 and model 4.0 predictions in Table 1 more or less straddle the results of data analysis, suggesting that C may even be a little less than 0.2. This would reduce Garrett and Greenberg's small corrections to Greenberg's (*2*) estimates, with $Z_0 = 0$, of the impact of tidal power.

7. The root-mean-square lag at Saint John, Bar Harbor, and Boston from our analysis is about 8° or 0.14 radians. This is small, but suggests that the value of R' may be wrong by about 14 percent, due to random and systematic errors. Thus R' is uncertain to ±0.3, although agreement between the data and the models actually seems better than this. A better estimate of the error limits could be obtained from the power spectral density of the M_2 amplitude in the neighborhood of the 18.6-year spectral line, but this cannot be estimated without longer records than are available.
8. This value, $Q_0 \approx 5$, is probably accurate to about 10 percent (*1*, *2*).
9. A period of less than the 12.42-hour period of M_2 could also be a solution; we adopt a longer period to be consistent with the results of Garrett (*1*) and Greenberg (*2*).
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11. Proper attention must, of course, be paid to the difficult problem of open-boundary conditions, as discussed by Garrett and Greenberg [see (*6*)].
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Induction of AIDS-Like Disease in Macaque Monkeys with T-Cell Tropic Retrovirus STLV-III

Abstract. *The T-cell tropic retrovirus of macaque monkeys STLV-III has morphologic, growth, and antigenic properties indicating that it is related to HTLV-III/LAV, the etiologic agent of the acquired immune deficiency syndrome (AIDS) in humans. Four of six rhesus monkeys died within 160 days of STLV-III inoculation with a wasting syndrome, opportunistic infections, a primary retroviral encephalitis, and immunologic abnormalities including a decrease in T4+ peripheral blood lymphocytes. These data show that an immunodeficiency syndrome can be produced experimentally in a nonhuman primate by an agent from the HTLV-III/LAV group of retroviruses. The STLV-III-macaque system will thus provide a useful model for the study of antiviral agents and vaccine development for human AIDS.*

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A T-cell tropic retrovirus with striking similarities to HTLV-III/LAV, the etiological agent of the acquired immune deficiency syndrome (AIDS) in humans, was recently isolated from macaques (*Macaca mulatta*) (*1*). We have called this virus simian T-lymphotropic virus type III (STLV-III) of macaques. Like HTLV-III/LAV, STLV-III can be isolated from T lymphocytes and grows preferentially in $T4^+$ rather than $T8^+$ lymphocytes. By electron microscopy, it has a cylindrical nucleoid and buds in a fashion typical of type C retroviruses (*1*). Furthermore, radioimmunoprecipitation of proteins from STLV-III–infected cells, with the use of a monoclonal antibody as well as sera from humans seropositive for HTLV-III/LAV, has revealed virus-specific proteins of 160, 120, 55, and 24 kilodaltons, all similar in size to the major *gag* and *env* proteins encoded by HTLV-III/LAV (*2*).

All the isolates of STLV-III to date have been obtained from animals with evidence of an immune deficiency syndrome or lymphoma. Macaques with this syndrome develop profound T-lymphocyte dysfunction and eventually die of lymphomas or opportunistic infections by a spectrum of agents similar to those seen in humans with AIDS (*3*). It is therefore important to determine the pathogenicity of this macaque retrovirus and to explore the possibility of developing an animal model for human AIDS. Such a model would facilitate both the testing of new treatment modalities for this disease and the development of a vaccine to protect against this syndrome. We now report the results of experimental infection of macaques with STLV-III.

The STLV-III used in these studies was isolated from a rhesus monkey designated Mm251-79, which had been inoculated with tissue from another rhesus monkey with a naturally occurring retroorbital lymphoma (*4*). Mm251-79 died 26 months after inoculation and at necropsy was found to have a poorly differentiated lymphocytic lymphoma. Viably frozen splenocytes from this animal were cocultivated with phytohemagglutinin (PHA)-stimulated human peripheral blood lymphocytes (PBL) that were maintained in cultures to which crude supernatants containing interleukin-2 (IL-2) were added. The growth of STLV-III was detected by the measurement of reverse transcriptase (RT) in the culture supernatant and was confirmed by electron microscopy (*1*). The virus stock was expanded by seeding the day-14 supernatant from this culture onto normal human T cells growing in the presence of IL-2. Inoculation of this supernatant onto Raji cells and canine thymus cells yielded no virus growth as determined by RT assay of culture supernatants, indicating that no simian type D retrovirus was present. Inoculation of this material onto Vero cells and human embryonic lung cells yielded no virus growth as determined by the appearance of cytopathic effect. Thus there was no evidence that other viral agents were present in the inoculum.

Six monkeys were selected for inoculation with this agent. Four of them were 6 months old and had not been used in previous studies; the other two were 18 months old and had been inoculated 15 months previously with the macaque retrovirus D/New England. While it has been suggested that type D retroviruses may play some part in immune deficiency syndromes in macaques (*5*), the two previously inoculated animals used in the present study appeared to be free of disease when the study began (*6*). They did, however, have a few enlarged axillary and inguinal lymph nodes. Except for lymphadenopathy in the D/New England infected animals, the six monkeys were found to be normal when they underwent physical examinations at the beginning of the study. Cocultivation of PBL from the four 6-month-old animals with Raji cells and canine thymus cells yielded no type D retrovirus, and cocultivation of PBL from all six animals with HUT-78 cells yielded no type C retroviruses. That the animals were normal was also indicated by the results of routine hematologic and serum chemistry studies, by their PBL absolute T4 and T8 numbers (Table 1), and by the blastogenic responses of their PBL to concanavalin A (Con A), pokeweed mitogen (PWM), xenogeneic stimulating cells, and *Candida* antigen.

The virus used in the inoculations was grown on human T cells maintained in culture with IL-2. Each animal received an intravenous inoculation of 1.7 ml of pooled supernatants from day 14 and 18 cultures of the second passage of this STLV-III isolate. The RT activity of the

supernatants was 199,000 and 92,000 cpm/ml [assayed as described (*1*)]. This isolate had only been passaged in normal human T lymphocytes that were shown to generate no RT activity unless they had been seeded with the virus-containing supernatant. Two weeks after the animals were inoculated, plasma was withdrawn and added to HUT-78 cells. Within 2 weeks these cells showed cytopathic effects, RT activity was detected, and STLV-III particles were observed by electron microscopy. STLV-III was also isolated from PBL of all the animals 19 weeks after they were inoculated and from brain, lymph node, spleen, and salivary gland as well as PBL at the time of necropsy. Other tissues were not tested. These data show that the monkeys became infected with STLV-III after inoculation.

Three of the monkeys that were 6 months old at the time of inoculation, Mm91-84, Mm99-84, and Mm101-84, became moribund and were killed with pentobarbital at 127, 160, and 142 days following inoculation, respectively. One of the monkeys that had previously been inoculated with D/New England virus (Mm74-84) became moribund and was killed at 148 days after the inoculation of STLV-III. These four monkeys had diarrhea by 1 month after inoculation. The diarrhea persisted and *Trichomonas* species were demonstrable in their stools at 2 months after inoculation. Three of the animals, Mm91-84, Mm101-84, and Mm106-83, developed a wasting syndrome, losing 13, 34, and 60 percent of their body weight during the study. No remarkable changes were seen in their blood hemoglobin levels, white cell counts, differential white cell counts, or the morphologic appearance of their white cells during the course of their illness.

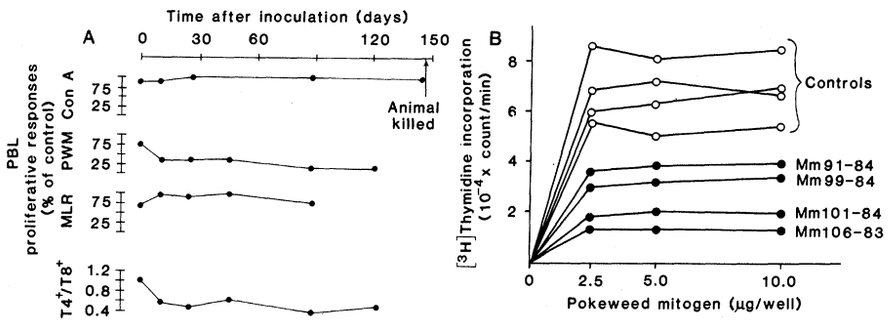

Fig. 1. (A) Immunologic status of Mm106-83 after inoculation with STLV-III. This animal had previously been inoculated with the retrovirus D/New England. The proliferative responses of PBL from this animal to lectins and xenogeneic antigenic stimulation (B cell clones derived from *Saguinus oedipus* PBL, transformed with Epstein-Barr virus and treated with mitomycin C) are expressed as a percentage of the response shown by PBL from two age-matched control rhesus monkeys assayed simultaneously. Staining and analysis of PBL for the cell surface antigens T4 and T8 were carried out as previously described (*10*). The immunologic changes observed in Mm91-84, Mm99-84, and Mm101-84 were very similar to those in Mm106-83. (B) Proliferative responses to PWM of PBL of the four rhesus monkeys 3 months after STLV-III inoculation. Triplicate 0.2-ml wells containing 10^5 responder PBL and the noted concentration of PWM were incubated for 3 days, exposed to [^{3}H]thymidine (0.15 mCi per well) overnight, and harvested onto glass fiber filter strips for measurement of [^{3}H]thymidine incorporation.

Table 1. Changes in numbers of $T4^+$ and $T8^+$ lymphocytes in monkeys inoculated with STLV-III. Mononuclear cells were isolated from peripheral blood by Ficoll/Diatrizoate gradient centrifugation, reacted with the indicated monoclonal antibodies, and then with FITC conjugated goat antiserum to mouse immunoglobulin G and analyzed by flow cytometry. The absolute $T4^+$ or $T8^+$ count was determined by the following calculation: peripheral blood white cell count times the percentage of lymphocytes times the percentage of cells reactive with that monoclonal antibody. Results show total numbers of reactive cells per cubic millimeter.

Animal	Before inoculation		After inoculation			
			At 6 weeks		At 12 weeks	
	T4	T8	T4	T8	T4	T8
Animals that became moribund						
Mm91-84*	1462	1174	598	933	1089	1053
Mm99-84*	1245	903	1231	1204	516	860
Mm101-84*	1718	1938	732	1164	824	1957
Mm106-83†	1308	1290	466	928	840	2058
Animals inoculated but still alive						
Mm74-84*	1420	1521	1091	1785	909	1515
Mm127-83†	1873	887	1803	1803	1439	1738
Age-matched control animals						
Mm100-84	1282	797	1562	634	1309	972
Mm130-83	1069	907	972	1004	1055	1021
Mm164-83	1564	1138	1232	1138	1422	1943

*Six months old at the time of inoculation. †Eighteen months old at the time of inoculation and previously inoculated with retrovirus D/New England.

The four animals showed similar immunologic abnormalities. The number of PBL bearing T4 and T8 determinants was quantitated and the blastogenic responses of these PBL were assessed 2, 3, 6, and 12 weeks and then on a monthly basis after inoculation. The number of $T4^+$ PBL decreased in all four animals until their last few weeks of life (Table 1) at which time a small increase in $T4^+$ PBL was observed.

Changes in the PBL of a representative animal (Mm106-83) are shown graphically in Fig. 1A. The blastogenic response of the PBL of each monkey to Con A and xenogeneic stimulator cells did not show a consistent decrement when compared with the response of PBL from two normal age-matched control animals. When stimulated with PWM, however, the proliferative response (a T-cell dependent B-cell response) was dramatically diminished. By 2 weeks after inoculation with STLV-III, the blastogenic responses of their PBL to PWM ranged from 35 to 75 percent of those of PBL from control animals. This abnormality persisted throughout the remainder of the study (Fig. 1B).

The gross and histopathologic findings at necropsy were similar for each of these four animals. The lymph nodes were not enlarged, and there was a paucity of follicles with hyalinized germinal centers in the nodes. The mesenteric nodes of one animal (Mm101-84) were surrounded and infiltrated by proliferating fibroblastic tissue. The thymus glands of all four of these monkeys were atrophic with marked cortical lymphocyte depletion.

Extensive areas of the pancreas in three of the animals (Mm91-84, Mm99-84, Mm101-84) were necrotic and infiltrated with mononuclear cells and neutrophils. Basophilic intranuclear inclusion bodies were present in acinar cells (Fig. 2A) which, on electron microscopic examination, were shown to contain adenovirus particles (Fig. 2B). Similar infiltrates and inclusion bodies were also present in the biliary system of one ani-

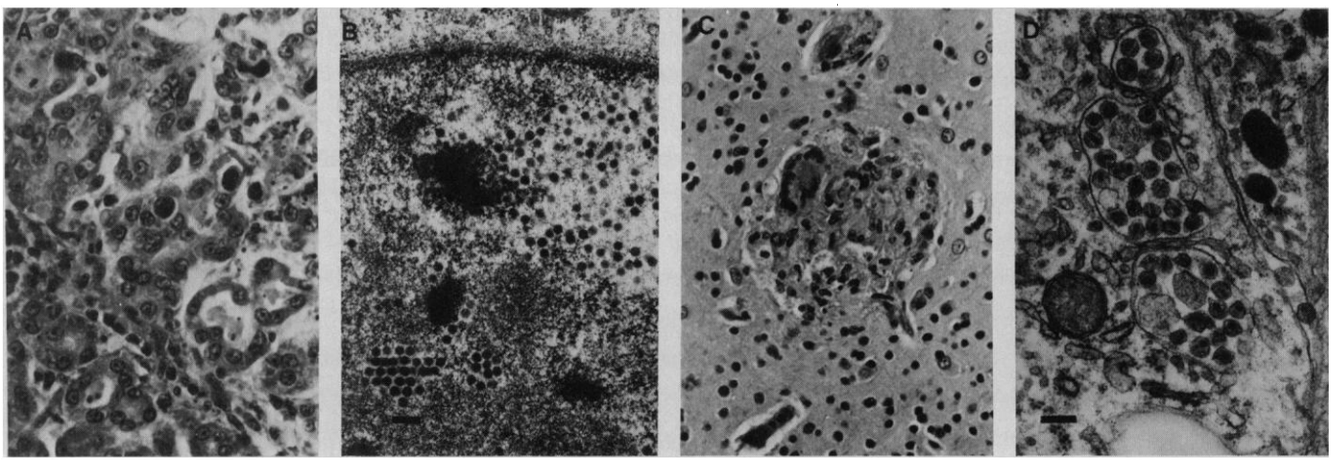

Fig. 2 (A) Photomicrograph of a portion of a necrotic pancreas. The residual acini are dilated and scattered epithelial cells contain densely basophilic, intranuclear inclusion bodies. Inflammatory cells are present in the interstitium (hematoxylin and eosin, ×560). (B) Electron micrograph of a portion of a nucleus of a pancreatic acinar cell illustrating scattered and crystalline arrays of adenovirus particles in the inclusion body (scale bar, 200 nm). (C) Photomicrograph of a portion of the cerebral cortex in which there are two (one large and one small) perivascular infiltrates of macrophages. The larger infiltrate also contains a multinucleated, syncytial giant cell. The vessels in each lesion are sectioned tangentially (hematoxylin and eosin, ×350). (D) Electron micrograph of a portion of the cytoplasm of a macrophage from a perivascular infiltrate in the brain. There are many STLV-III retrovirus particles contained within membrane-bound vacuoles, presumably phagosomes. Note the cylindrical-shaped nucleoids in several of the particles. No budding particles were found in these cells (scale bar, 200 nm).

mal (Mm101-84). In this animal, adenovirus inclusion bodies in type II pneumocytes were also seen in areas of pneumonic lung, and colonies of *Candida albicans* were present on the skin of its chin and eyelids.

The cerebrospinal fluid in all four monkeys was tinged with blood. The leptomeningeal vessels were markedly congested. Perivascular infiltrates of large, foamy macrophages were present throughout the white and gray matter of the brain (Fig. 2C). An occasional infiltrate also contained one or more multinucleated giant cells. The giant cells contained no viral inclusion bodies. Staining with periodic acid-Schiff reagent revealed no cryptococcal organisms, and acid-fast staining revealed no evidence of tubercle bacilli or encephalitozoon. No trophozoites suggesting lesions due to toxoplasmosis were seen.

Examination of the brain lesions by electron microscopy revealed cytoplasmic retroviral particles with nucleoids identical in appearance to those of STLV-III in the foamy macrophages (Fig. 2D). These retroviral particles were contained in large phagosomes, and no budding particles were seen. Viral particles were not observed in other types of cells in these brains. Virus was isolated from the brain tissue of two animals by cocultivation of tissue samples with HUT-78 cells. Shaw *et al.* (*7*) noted the presence of HTLV-III/LAV in the brain of humans with AIDS encephalopathy. The type of cell harboring the virus, however, was not determined.

Two monkeys inoculated with STLV-III remain alive at the time of this report. One (Mm74-84) has developed a clinical syndrome characterized by diarrhea and wasting with immunologic abnormalities. This monkey developed axillary and inguinal adenopathy approximately 200 days after inoculation. Microscopic examination of biopsy specimens of these nodes revealed marked follicular hyperplasia with confluent germinal centers and a reduction in the amount of normal paracortical tissue. The other monkey (Mm127-83) remains clinically well without immunologic abnormalities.

The disease that developed in the four monkeys that became moribund had certain elements of an immune deficiency syndrome, but the disease differed in its course from both the naturally occurring macaque immune deficiency syndrome and AIDS in humans. These two naturally occurring syndromes are characterized by a prolonged incubation period, a prodrome of lymphadenopathy, and a slowly progressive disease course punctuated by a multitude of opportunistic infections and tumors (*8*). The disease in the macaques experimentally infected with STLV-III had an incubation period of only a few weeks. STLV-III infection caused no demonstrable lymphadenopathy, but did cause rapid wasting and remarkably consistent pathologic changes. All four animals that became moribund developed a primary retroviral encephalitis, and three of them developed overwhelming adenovirus infections. The young age of the inoculated monkeys and the large dose of virus administered intravenously might explain the fulminant nature of the disease. These animals had lived together as a group prior to their experimental infection and had not been exposed to adult monkeys since birth. They were therefore exposed to the same limited number of infectious agents. This may account for the fact that the same opportunistic pathogen, an adenovirus, was found in all of them.

Although chimpanzees can be infected by HTLV-III/LAV, they do not appear to develop a disease as a result of such infection (*9*). The ability to produce an AIDS-like disease in macaques by means of STLV-III may prove useful for testing antiviral agents for AIDS patients and for developing a vaccine for the prevention of HTLV-III/LAV infection.

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