Persistent Infection of Chimpanzees with Human T-Lymphotropic Virus Type III/Lymphadenopathy-Associated Virus: A Potential Model for Acquired Immunodeficiency Syndrome

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The lymphadenopathy-associated virus (LAV) prototype strain of human T-lymphotropic virus type III/LAV was transmitted to juvenile chimpanzees with no prior immunostimulation by (i) intravenous injection of autologous cells infected in vitro, (ii) intravenous injection of cell-free virus, and (iii) transfusion from a previously infected chimpanzee. All five animals that received more than one 50% tissue culture infective dose were persistently infected with LAV or chimpanzee-passaged LAV for up to 18 months. During this time they developed no illnesses, but they exhibited various degrees of inguinal and axillary lymphadenopathy and significant reductions in rates of weight gain. Detailed blood chemistry and hematologic evaluations revealed no consistent abnormalities, with the exception of immunoglobulin G (IgG) hypergammaglobulinemia, which became apparent in one animal 6 months postinfection and continued at more than 1 year postinfection. Transient depressions followed by increases in the numbers of T4 cells to levels greater than normal were observed in all animals after virus inoculation. However, the number of LAV-infected peripheral blood cells decreased with time after infection. Results of enzyme immunoassays showed that all infected animals seroconverted to IgG anti-LAV within 1 month postinfection and that antibody titers remained high throughout the period of observation. In contrast, only three of the five LAV-infected chimpanzees had detectable IgM antibody responses, and these preceded IgG-specific serum antibodies by 1 to 2 weeks. Virus morphologically and serologically identical to LAV was isolated from peripheral blood mononuclear cells of all infected animals at all times tested and from bone marrow cells taken from one animal 8 months after infection. One chimpanzee that was exposed to LAV only by sharing a cage with an infected chimpanzee developed lymphadenopathy and an IgM response to LAV, both of which were transient; however, no persistent IgG antibody response to LAV developed, and no virus was recovered from peripheral blood cells during a year of follow-up. Thus, LAV readily infected chimpanzees following intravenous inoculation and persisted for extended periods despite the presence of high titers of antiviral antibodies. However, the virus was not easily transmitted from infected to uninfected chimpanzees during daily cage contact.

The etiologic agent of the acquired immunodeficiency syndrome (AIDS) is a retrovirus presently designated human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) (10, 13, 32) after two prototype strains (HTLV-III and LAV) (2, 13, 32). A third prototype strain, AIDS-associated retrovirus, also has been described (22). HTLV-III/LAV replicates in and is cytopathic for helper T lymphocytes bearing OKT4 (Leu 3), but not OKT8 (Leu 2), cell surface antigens (20). Probably because of this cellular tropism, a large number of people infected with the virus have a deficiency in OKT4 cell numbers that impairs their immune responses to pathogens. In its most virulent form, HTLV-III/LAV infection results in death due to opportunistic infections or malignancies.

At present there is no apparent abatement of the AIDS epidemic in high-risk groups: homosexual men, intravenous (i.v.) drug abusers, and recipients of blood or blood products (hemophiliacs and transfused patients) (5, 16). In addition, there is evidence now that HTLV-III/LAV is spreading by heterosexual transmission (21, 31, 33). An animal model system would be valuable in understanding the pathogenesis

of HTLV-III/LAV, especially early in the course of infection, and also for evaluating potential methods for preventing and treating AIDS.

We and others (1, 11, 12) have reported that chimpanzees (*Pan troglodytes*) can be infected with HTLV-III/LAV with concomitant seroconversion. In our previous report (11) we showed that transmission of LAV to chimpanzees could be accomplished by immunostimulation followed by intravenous and subcutaneous injection of a mixture of concentrated cell-free virus and peripheral blood mononuclear cells (PBMC) infected in vitro. It was of interest to determine whether infection could be established (i) without prior immunostimulation, (ii) with cell-free virus alone, and (iii) by transfusion.

The results show that productive infections were established in naive chimpanzees irrespective of the inoculum and that immunoglobulin G (IgG) seroconversion occurred within 1 month postinfection. Detailed blood chemistry and hematologic measurements revealed that most parameters tested remained essentially normal in all animals. Determinations of T4 and T8 cell numbers, however, revealed decreases in the T4 population early after infection, followed by increases to numbers equal to or greater than those

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TABLE 1. Infection of chimpanzees with LAV by various inoculation protocols

Chimpanzee	Inoculation date	Age at inoculation (yr, mo)	Inoculum	Immunostimulation[a]	Route
C-477[b]	3-05-84	3, 3	LAV-autologous cells, LAV-human PBMC, LAV	DT, Pneumovax, human PBMC	i.v., s.c.[c]
C-459[b]	3-05-84	4, 4	LAV-autologous cells, LAV	DT, Pneumovax	i.v., s.c.
C-487	7-30-84	2, 11	LAV-autologous cells	None	i.v.
C-497		2, 4	Cage exposure (C-487)	None	
C-463	8-02-84	4, 8	Transfusion from C-459	None	i.v.
C-469		4, 5	Cage exposure (C-463)	None	
C-560	10-17-84	4, 2	1,100 TCID$_{50}$s of LAV cell-free supernatant	None	i.v.
C-499	10-17-84	2, 8	0.1 TCID$_{50}$ of LAV cell-free supernatant	None	i.v.

[a] Vaccines and human PBMC were administered 5 to 11 days and 5 days preinfection, respectively. DT, Diptheria-tetanus vaccine.
[b] Infection of these animals was reported previously (11).
[c] s.c., Subcutaneous.

present before infection. Even though the total number of lymphocytes in blood remained relatively constant, the number of LAV-infected PBMC decreased with time after inoculation. To date no LAV-infected chimpanzee has developed clinical disease, although some animals have exhibited intermittent lymphadenopathy and have failed to maintain their preinfection rate of weight gain.

MATERIALS AND METHODS

Animals. Eight chimpanzees, 2 to 5 years old, were used in this study (Table 1). They were colony raised at the Yerkes Regional Primate Research Center, Emory University, Atlanta, Ga., and were housed in isolation facilities. All were negative by enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay for antibodies to HTLV-III/LAV and HTLV-I prior to infection. However, all had IgG antibody titers between 40 and 70 (by solid-phase fluorescence assay) to simian cytomegalovirus at the time of inoculation with LAV. The chimpanzees listed in Table 1 include C-477 and C-459, which were infected as reported previously (11). Also included are two animals, C-497 and C-469, which were not infected experimentally but were housed with infected animals and, thus, were experimental subjects for horizontal transmission of the virus.

Virus. The virus strain used to inoculate chimpanzees was LAV and was the generous gift of L. Montagnier, J.-C. Chermann, and F. Barre-Sinoussi.

Isolation of virus. Virus in PBMC and selected tissues was detected as described previously (9). Briefly, this involved (i) cocultivation of chimpanzee material with leukocytes (from normal human female donors) that had been stimulated in vitro with phytohemagglutinin P for 3 days (PHA-AWBC); (ii) monitoring culture fluids for Mg^{2+}-dependent, particulate reverse transcriptase (RT) activity (22); and (iii) direct immunofluorescence assay for HTLV-III/LAV-specific antigen expression. Throat swabs were placed in tissue culture medium (RPMI-1640) and stored at −70°C until used as inocula for cultures of PHA-AWBC. Bone marrow was collected from the iliac crest, and the cells were separated from fluid by Ficoll-Hypaque (lymphocyte separation medium; Litton Bionetics) gradient centrifugation and washed with phosphate-buffered saline (PBS) before being placed in culture with PHA-AWBC. Spinal fluid was collected by lumbar puncture and filtered through 0.45-μm (pore size) filters before being used as an inoculum for PHA-AWBC.

Detection of antibodies. (i) IgG. The virus from which antigen was prepared for use in the ELISA for IgG was isolated at the Centers for Disease Control, Atlanta, Ga.,

from PBMC of a hemophiliac AIDS patient and is termed HTLV-III/LAV-CDC451. HTLV-III/LAV-CDC451 was concentrated by ultracentrifugation and banding in a sucrose gradient. Virus was disrupted in 0.5% Triton X-100–800 mM NaCl–20 mM Tris hydrochloride–0.5 mM phenylmethylsulfonyl fluoride (pH 7.5) and clarified by centrifugation for 1 h at 100,000 × g. Viral antigen was solubilized and diluted in carbonate buffer (pH 9.6), and 200 ng in 100 μl was used to coat each well of 96-well Dynatech II microtiter plates. After overnight incubation at 4°C, the wells were washed with 0.05% Tween 20 in PBS (PBS-Tween 20). Unbound sites in the wells were assumed to be blocked after 1 h of incubation with PBS-Tween 20 containing 5% bovine serum albumin (BSA-PBS). BSA-PBS was removed, and 100 μl of a 1:50 dilution (in BSA-PBS) of test serum was added to appropriate wells. After overnight incubation at 4°C and three washes with PBS-Tween 20, 100 μl of 1:2,000 diluted peroxidase-conjugated, affinity-purified goat anti-human IgG (Cappel Laboratories) was added to each well, and the plates were incubated for 1 h at 25°C. Each well was washed three times with PBS-Tween 20, and to each was added 100 μl of peroxidase substrate solution (0.01% phenylenediamine and 0.01% H_2O_2 in 50 mM citrate buffer [pH 3]). After 20 min at room temperature the reaction was stopped with 100 μl of 2 N H_2SO_4 and the A_{490} was read with a Dynatech ELISA reader.

(ii) IgM. Virus for the solid-phase ELISA for IgM was purified from supernatants of H9/HTLV-III cells provided by R. C. Gallo and M. Popovic. Cell-free supernatant was filtered through a 0.45-μm (pore size) Millipore Corp. filter, concentrated 20- to 40-fold by ultrafiltration in a Pellicon apparatus (Millipore), then pelleted on a cushion of 50% Renografin (E. R. Squibb & Sons), and further purified in a 15%/50% Renografin gradient. The virus band was suspended in PBS, pelleted at 28,000 rpm for 1 h and then inactivated with Triton X-100–deoxycholate. Chimpanzee sera were diluted in PBS-Tween 20 and tested for IgM antibodies specific for HTLV-III/LAV with a glucose-oxidase-conjugated, affinity-purified F(ab′)$_2$ fragment of a goat anti-human IgM (μ chain specific) serum that was obtained from Jackson ImmunoResearch Laboratories, Avondale, Pa. The chimpanzee test serum with antigen was incubated overnight at 4°C, followed by incubation at room temperature for 60 min with the goat anti-human IgM antiserum. Wells were washed three times with PBS-Tween 20, and then 200 μl of substrate solution containing 25 ml of 0.1 M phosphate buffer (pH 6), 5 μg of azino-bis-benzthiazoline-sulfonic acid, 0.25 μg of peroxidase type VI, and 3 ml of 18% β-D-glucose was added to each well. Following 30 min of

incubation at room temperature, the A_{405} was read with a Flow Laboratories, Inc., Multiscan reader. It should be mentioned that monoclonal and polyclonal antibody reagents to human antigens show a high degree of cross-reactivity to analogous chimpanzee antigenic determinants.

Determination of virus titer. Cell-free virus titers (50% tissue culture infective dose [$TCID_{50}$]) were determined by LAV-capture ELISA and 50% infective dose assay as described by McDougal et al. (26). The number of LAV-infected cells in peripheral blood was estimated by coculture of PHA-AWBC and lymphocyte separation medium-separated chimpanzee PBMC that were serially diluted 1:5. Culture supernatants were monitored for RT activity for 35 days. The least number of chimpanzee cells that produced a coculture with RT activity was assumed to contain at least one infected cell; thus, the numbers of infected cells probably are minimum estimates. Similar dilution experiments with an established cell line persistently infected with LAV showed that one infected cell could produce an RT-positive culture (data not shown).

RESULTS

Infection of chimpanzees with HTLV-III/LAV without prior immunostimulation. Our previous study showed (11) that chimpanzees could be infected with the LAV prototype strain of HTLV-III/LAV after immunostimulation with multiple antigens followed by injection of virus and autologous cells that had been infected with the virus in vitro. To determine whether a productive infection with LAV could occur without prior immunostimulation, we attempted to infect chimpanzees by injecting virus in various ways.

(i) **Autologous cells.** PBMC obtained from a chimpanzee (C-487; Table 1) were stimulated for 3 days with phytohemagglutinin P and then infected with the same pool of LAV that was used to infect chimpanzees C-459 and C-477 in our original study (11). After 3 additional days of incubation in vitro, a total of 61×10^6 cells were returned to C-487 by combined i.v. injection and injection into the left inguinal lymph node. Serum, plasma, and PBMC were obtained from the animal after 15 days, 1 month, and once a month thereafter for 12 months. Virus was detected in PBMC at all sampling times (by cocultivation with PHA-AWBC), and IgG antibodies were detected initially at 1 month postinfection (see below).

(ii) **Transfusion.** Chimpanzee C-463 (Table 1) was transfused with 100 ml of blood from C-459 5 months after the latter animal was infected with LAV (chimpanzee 2; reference 11). At the time of transfusion, virus was isolated from C-459's PBMC, but not from his plasma, and his serum anti-LAV IgG titer was 1:400 by ELISA. The number of infected cells in 1 ml of blood from C-459 at the time of transfusion was estimated to be approximately 250 by serial dilution and coculture experiments (see below). Following the transfusion, virus isolations from PBMC and detection of antibodies to LAV in sera obtained from C-463 followed a pattern similar to that seen with C-487.

(iii) **Cell-free virus.** To determine whether injection of cell-free virus also would result in infection and seroconversion, we injected two animals (C-560 and C-499) with a virus stock that was generated from a coculture of PHA-AWBC and PBMC taken from C-463 12 days after his transfusion of blood from C-459. This, therefore, was the third chimpanzee-to-chimpanzee passage for this LAV-derived pool. C-560 received $10^{3.1}$ $TCID_{50}$s, and C-499 received 0.1 $TCID_{50}$ of the virus stock. The latter dosage was given to determine whether a small inoculum would result in infection and, if so,
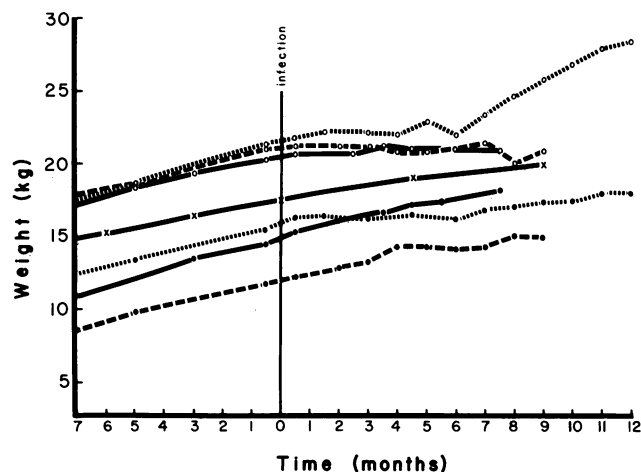

FIG. 1. Weight gain of chimpanzees before and after infection with LAV. C-459, O · · · · O, C-560, O———O; C-463, O- - - -O; C-477, ● · · · · ●; C-499, ●———●; C-487, ●- - - -●; uninfected chimpanzee, ×———×.

whether it occurred at the same time or at a later time than in the animal given the larger dose. C-560 was negative for LAV-specific antibodies at 12 days but had seroconverted for IgM by 19 days postinjection (see below). Infectious virus was isolated from C-560's PBMC during the first attempt at 12 days postinoculation and was isolated at every subsequent attempt. Compared with those from C-560, sera and PBMC taken from C-499 consistently were negative for anti-LAV antibodies and for virus isolation, respectively. These observations indicate that large doses of LAV readily establish infections in chimpanzees following inoculation of cell-associated or cell-free virus without prior immuno-stimulation.

Clinical status. Physical examinations of the chimpanzees were performed monthly. There were no signs of acute disease; however, the following were noted. (i) At 4.5 months postinfection, C-477 had a small ulcer on the lower gums, which resolved within 2 weeks; and (ii) at 2 months postinfection, C-459 had erythema of axillae and pectoral regions, which resolved. Although no animal exhibited prolonged, persistent lymphadenopathy, all five animals that seroconverted had various degrees of lymphadenopathy of the inguinal or axillary lymph nodes or both. The most prominent lymphadenopathy occurred in an inguinal node that measured approximately 1.7 by 3 cm (C-463 at 5 months postinfection); 1 month later this node had decreased in size to approximately 1 by 2.5 cm. Transient enlargement of inguinal lymph nodes was detected in some uninfected chimpanzees and in LAV-seronegative, exposed cagemates.

Because the chimpanzees were juveniles, their weight increased steadily before virus was injected; however, four of the five chimpanzees that were virus positive and antibody positive showed significantly less ($P < 0.01$; determined by a matched-pair partial correlation procedure that controlled for preinfection rates of weight gain) weight gain during the 6 months following infection than seven control animals (Fig. 1). Two uninfected, exposed cagemates, four uninfected control chimpanzees, and C-499 (inoculated with the low dose of LAV) showed consistent increases in weight during the last 12 months of observation (average of 0.33 kg/month). C-487 was the only animal that continued to gain weight immediately after being infected with LAV; however, between December 1984 (4 months postinfection) and March
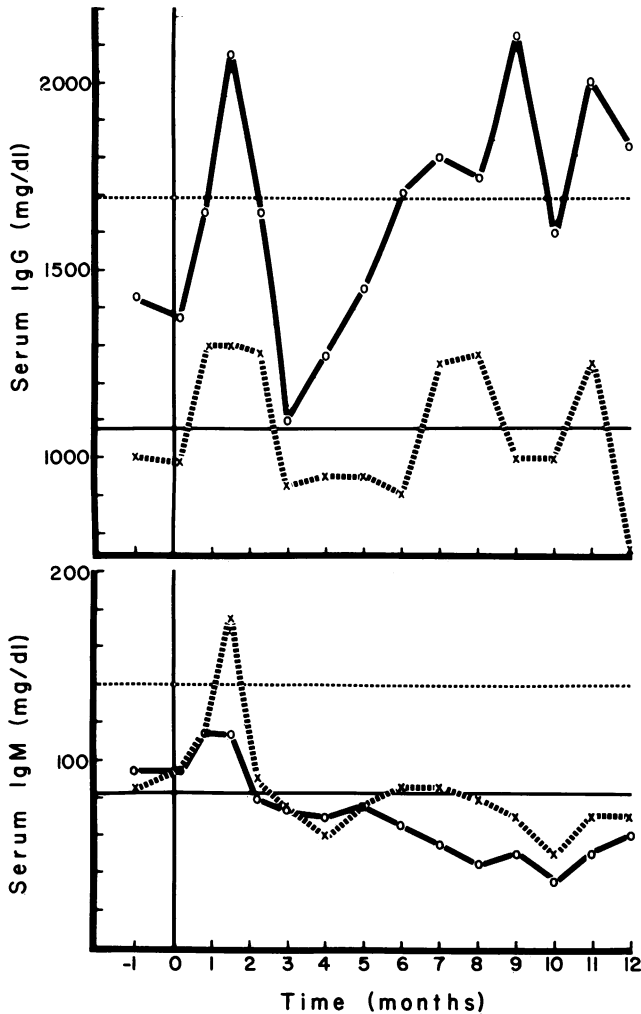
FIG. 2. Total serum IgG and IgM following infection of chimpanzees with LAV. Data for two animals are shown: C-459, O————O; C-477, ×· · · ·×. The solid and dashed horizontal lines represent mean values and 2 standard deviations above the means, respectively; they were calculated from preinfection values for the eight chimpanzees listed in Table 1 and for four control chimpanzees.

enzymes, complement levels, and serum proteins, with the exception of total serum IgG in one animal.

Antibody responses. (i) Total immunoglobulins. Increases and then declines in total serum IgM and IgG occurred following infection of chimpanzees with LAV (data for C-477 and C-459 are shown in Fig. 2). C-459 became hypergammaglobulinemic for IgG (>2 standard deviations above the mean) 6 months after LAV infection, and IgG levels remained elevated after that time. In contrast, C-477's IgG levels remained within the normal range. There were no fluctuations in total serum IgA in any animal.

(ii) LAV-specific antibodies. Sera obtained from all chimpanzees (including uninfected, exposed cagemates) were tested by ELISA for IgM and IgG anti-LAV antibodies (Table 2). Anti-LAV IgM antibodies were detected in sera from only three of the five infected animals: C-477, C-463, and C-560. Of the two animals (C-459 and C-477) that were immunostimulated prior to infection, only C-477 had detectable LAV-specific IgM response (Fig. 3A). IgM antibodies to LAV were detected in serum from C-463, the transfusion recipient, at 12 days postinfection (the earliest time tested) and persisted for 7 additional weeks (Fig. 3B). Serum specimens from C-560 were negative for IgM antibodies at 12 days but were positive for IgM at 19 days postinfection and remained positive for 3 additional weeks. In all animals that seroconverted for both IgM and IgG LAV-specific antibodies, IgM LAV-specific antibodies were present 1 to 2 weeks before the detection of IgG antibodies.

All animals injected with virus or virus-infected cells, with the exception of C-499 (the low-titer virus recipient), seroconverted for IgG within 1 month postinfection. The fact that C-499 had not seroconverted up to 5.5 months postinoculation suggests that the dose given (0.1 $TCID_{50}$) was subimmunogenic and was not sufficient to establish a productive infection. Low levels of IgG anti-LAV were detected in C-463's serum at 12 days postinfection, and it is assumed that these early IgG-specific antibodies were passively transferred from C-459 during transfusion. Both uninoculated, exposed cagemates (C-469 and C-497; Table 1) remained virus negative and IgG antibody negative during 12 months of observation. However, C-497, the cagemate of C-487, seroconverted to IgM, but not IgG, LAV-specific antibodies 3 months after his cagemate was infected (see below).

Results of titration (by ELISA) of antiviral antibodies in sera from C-477, C-459, C-487, and C-463 are shown in Fig. 3. They indicate a transient IgM response of low titer in sera

1985, he gained only 0.05 kg (0.017 kg/month). The uninfected control animals that continued to gain weight were from 4 years 7 months to 5 years 5 months old on 4 March 1984, the date C-459 and C-477 were infected. Since the ages of the uninfected controls were approximately the same as those of C-459 and C-463, the two oldest infected chimpanzees, the reductions in rate of weight gain apparently were not due to age.

Hematology and blood chemistry. Monthly examinations of all infected or exposed chimpanzees included complete hematologic and blood chemistry analyses. Total numbers of leukocytes fluctuated within normal ranges. The only hematologic abnormality was transient lymphocytosis (greater than 9,600 leukocytes per μl with more than 60% lymphocytes) at 3 to 5 months postinfection in four of five infected animals and at 10 to 11 months in two of the animals (C-477 and C-459). No significant changes occurred in any of the chemical parameters that were measured, including liver

TABLE 2. Humoral immune response to HTLV-III/LAV

Chimpanzee	Inoculum	Antibody[a]	
		IgM+	IgG+
C-477	LAV + LAV-infected autologous cells + immunostimulation	+ +	+ + + +
C-459	LAV + LAV-infected autologous cells + immunostimulation	−	+ + + +
C-487	LAV-infected autologous cells	−	+ + + +
C-497	Cage exposure (C-487)	+ +	−[b]
C-463	Transfusion (C-459)	+	+ + + +
C-469	Cage exposure (C-463)	−	−
C-560	1,100 $TCID_{50}$s of LAV	+	+ + + +
C-499	0.1 $TCID_{50}$ of LAV	−	−

[a] IgM (Cytotech): + +, ≥1:100; +, 1:50. IgG (Centers for Disease Control): + + + +, ≥1:800; −, <1:50.

[b] The response was considered negative because it was transient and at the ELISA cutoff value (see the text).
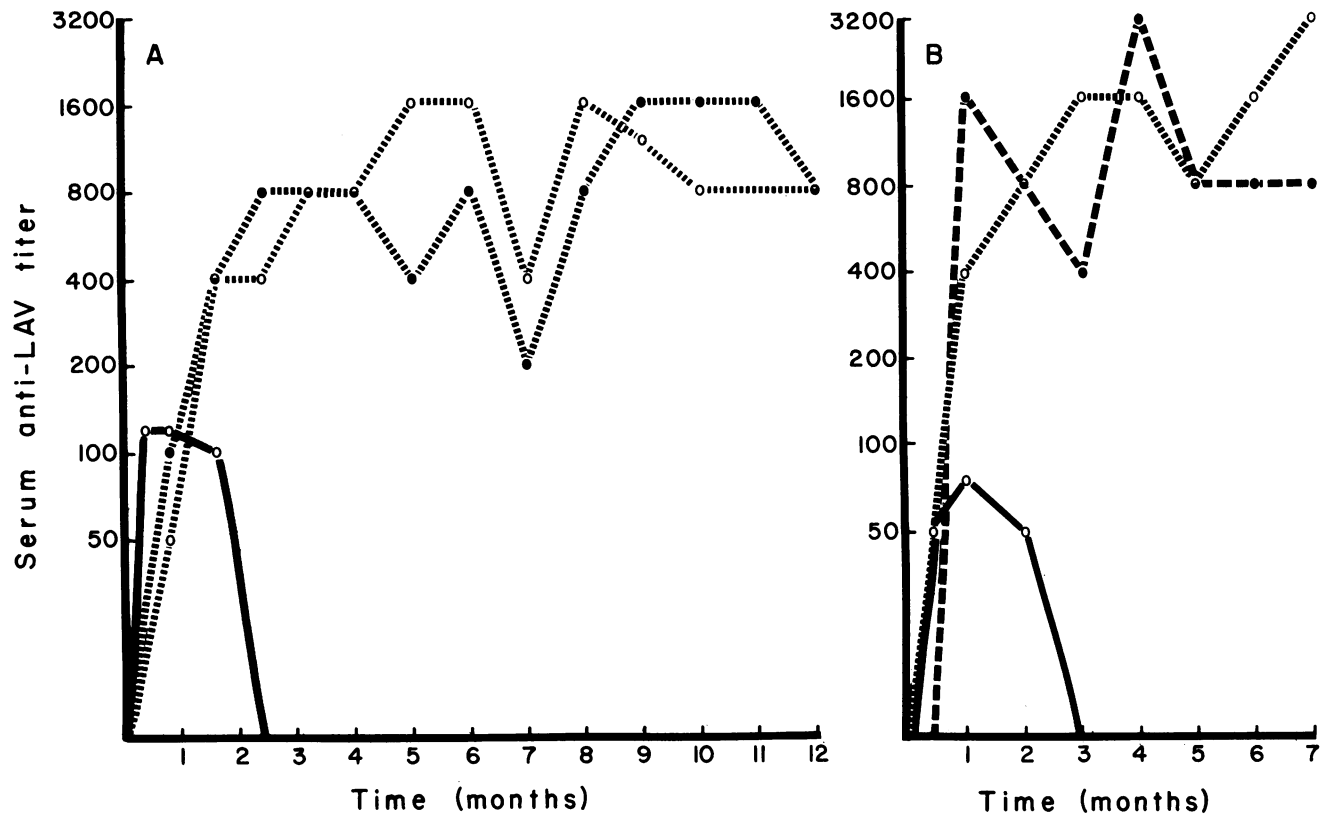
FIG. 3. LAV-specific serum IgM (━━━) and IgG (ııııı) titers following infection of chimpanzees. Data shown represent serum specimens from (A) C-459 (●) and C-477 (O) and (B) C-487 (●) and C-463 (O). Antibody titers are expressed as the highest dilution of serum that gave a positive reading by ELISA.

from C-477 and C-463, followed by an IgG response that, once established, remained at high titers in all animals.

T4:T8 lymphocyte ratios. The percentages and absolute numbers of OKT4 and OKT8 cells in infected chimpanzees were determined (Fig. 4). T4:T8 ratios showed some fluctuations, but overall, they remained relatively constant.

Isolation of virus from tissues. We attempted to isolate virus at various times postinfection from different tissues and fluids of LAV-infected chimpanzees (Table 3). Virus was isolated from PBMC of all IgG-antibody-positive animals each time attempted, whether before or after the appearance of anti-LAV antibodies. Since virus was isolated from serum taken from C-560 at 6 weeks postinfection, when her anti-LAV IgG titer was high, we know that cell-free virus was present in blood, but apparently not all, or very little, virus was neutralized. In contrast, all attempts to isolate virus from multiple plasma samples obtained from C-477 and C-459 at intervals from 2 to 12 months after inoculation failed.

Quantitation of infected cells. An estimate of the number of LAV-infected PBMC in blood from five infected chimpanzees was determined by serial dilution of cells obtained at various times after infection. When data from individual chimpanzees were pooled, an inverse correlation between the number of infectious cells and time after infection was observed (Fig. 5). During the first 2 months after infection, 10^3 to 10^4 cells were infected per 10^7 total PBMC. The number of infected cells decreased to 10 or fewer infected cells per 10^7 PBMC over a 6-month period. The number of infected cells in PBMC of C-477 and C-459 appeared to plateau, since at 16 months after infection (data not shown)

the numbers of infected cells were similar to the numbers found for both animals at 12 and 14 months after infection.

Transient LAV infection. C-497 had slight inguinal and axillary lymphadenopathy 3 months after his cagemate, C-487, was infected with LAV. The left inguinal node reached a maximum size of 2 by 2.5 cm by 4 to 5 months after infection of C-487, then it diminished in size. Also, during this same period there was a reversal in C-497's T4:T8 ratio, which was due to an increased number of OKT8 cells, and a transient IgM LAV-specific response that was followed by an equivocal and transient IgG response (Table 4). These data suggest that, as a result of being housed with an infected chimpanzee, C-497 was exposed to sufficient LAV antigen to elicit a transient immune response. No virus was isolated from PBMC of C-497 at any time or from bone marrow obtained 4 months after the IgM response.

DISCUSSION

We showed that naive chimpanzees could be infected with HTLV-III/LAV by various methods without prior immunostimulation and that persistent infection was established and maintained in the presence of high titers of anti-LAV IgG antibodies. Low titers of IgM antibodies to the virus were detected in sera from three of five infected animals. HTLV-III/LAV was isolated readily from PBMC of all animals that seroconverted for IgG. Because virus was isolated only rarely from serum or plasma, we concluded that infectious virus persisted primarily in chimpanzee PBMC (with little or no free virus in blood). This is in contrast to results obtained with human serum or plasma, which indicate that approximately 50% of infected individu-
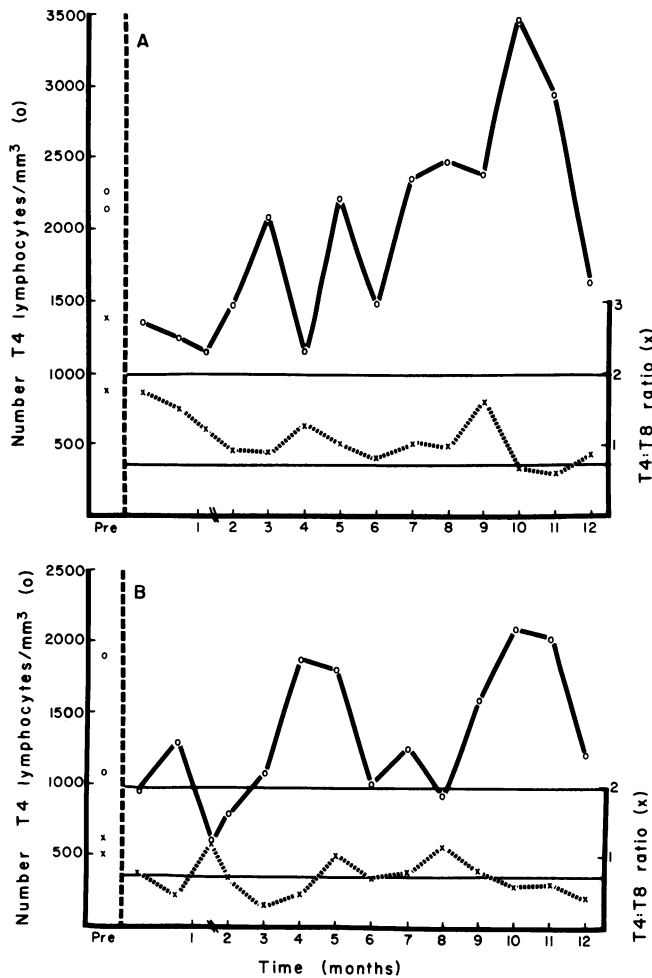
FIG. 4. Absolute numbers of peripheral blood OKT4+ lymphocytes and T4:T8 ratios following infection of C-477 (A) and C-459 (B) with LAV. The horizontal lines represent 1 standard deviation on either side of the mean T4:T8 ratio calculated from preinfection values for the same 12 animals as in Fig. 2.

als have cell-free virus in blood (24, 36). The fact that chimpanzee C-463 became infected following transfusion from C-459 confirms that LAV can establish infection in the presence of passively transferred heterologous antibodies (anti-LAV IgG titer was 400 at the time of transfusion). Whether the antibodies transfused along with virus and virus-infected cells were able to participate in neutralization, complement fixation, or antibody-dependent cell-mediated cytotoxic reactions is not yet known. Neutralizing antibodies against HTLV-III/LAV have been demonstrated infrequently in serum from infected persons and, if present, neutralizing titers are low compared with total antiviral antibodies (6, 17, 34, 39).

The fact that no prior immunostimulation was required for infection of chimpanzees indicates that HTLV-III/LAV can infect and replicate in susceptible target cells that may be in a resting state. Whether low-level replication in vivo occurs and perturbs the immune system to such an extent that infected lymphocytes are activated and produce amounts of virus capable of establishing a persistent infection is not known. While the chimpanzees that received virus without immunostimulation appeared well at the time of LAV inoculation, it is impossible to know the exact status of their

immune systems. We can conclude, however, that exogenous stimulation by multiple antigens is not required for HTLV-III/LAV to establish infection.

Chimpanzee C-560, which became infected after i.v. inoculation of virus only, received 1,100 TCID$_{50}$s. We do not know the minimum dose of virus that can successfully establish infection in chimpanzees (or in humans). The fact that the low-titer recipient showed no signs of infection during 7 months of follow-up suggests that extremely low doses are not sufficient for infection (at least in a healthy animal). Furthermore, the fact that C-497, the uninfected cagemate, seroconverted to IgM, but not to IgG, may indicate that HTLV-III/LAV infection can elicit an immune response capable of aborting a low-dose infection but not infection with a larger amount of virus. Alternatively, C-497 may have been transiently exposed to antigen only and not to infectious virus. The IgM response was confirmed independently in a second laboratory by indirect immunofluorescence assay on HTLV-III/LAV-infected cells. Repeated attempts to isolate virus from C-497's PBMC failed (including induction with iododeoxyuridine).

The establishment of persistent and inapparent LAV infections in chimpanzees without prior immunostimulation suggests that HTLV-III/LAV probably can establish an infection in healthy persons. The authors of a recent report (4) of a hemophiliac AIDS patient whose wife had antibodies to HTLV-III/LAV that disappeared after cessation of exposure to semen also suggested that the immune systems of some persons can effectively contain the virus. This same woman had increased numbers of OKT8 cells during and following the period of transient HTLV-III/LAV-specific antibody, as did chimpanzee C-497. Our data also show that

TABLE 3. Retrovirus isolation from tissues and fluids of LAV-infected chimpanzees

Chimpanzee	Tissue or fluid	Virus	Time post-infection
C-477	PBMC	+	1 wk–18 mo
	Bone marrow cells	–	8 mo
	Spinal fluid	–	8 mo
	Saliva	–	8 mo
	Plasma	–	2, 7, 12 mo
C-459	PBMC	+	1 wk–18 mo
	Bone marrow cells	+	8 mo
	Spinal fluid	–	8 mo
	Saliva	–	8 mo
	Plasma	–	5 mo
C-487	PBMC	+	2 wk–12 mo
	Saliva	–	3 mo
C-497	PBMC	–	2 wk–12 mo
	Saliva	–	3 mo
C-463	PBMC	+	12 days–12 mo
	Saliva	–	3 mo
C-469	PBMC	–	12 days–12 mo
	Saliva	–	3 mo
C-560	PBMC	+	12 days–9 mo
	Saliva	–	3, 4 wk
	Serum	+	6 wk
C-499	PBMC	–	12 days–9 mo
	Saliva	–	3, 4 wk

close physical contact between infected and uninfected chimpanzees for extended periods of time (more than 1 year) did not lead to active infections (Tables 2 and 3; C-497 and C-469).

The apparent transient depression in absolute OKT4$^+$ cell numbers (Fig. 4), followed by a return to normal numbers of this cell population, suggests that T4 cells are not eliminated by HTLV-III/LAV, but rather a comparative steady state exists between cell generation and cell destruction. LAV is cytopathic for chimpanzee T4 cells (unpublished data). The observed decreases in the number of infected PBMC in chimpanzees with time after infection may be important in establishing a healthy carrier state. No analogous data on numbers of infected PBMC in relation to time after infection are available for humans. It is known that some lymphadenopathy and AIDS-related complex patients have either decreased numbers of T4 cells or increased numbers of T8 cells with normal numbers of T4 cells, while AIDS patients generally have very low numbers of helper T4 lymphocytes (8, 29). It will be interesting to study LAV-infected chimpanzees to see whether development of disease is accompanied by changes in numbers of infected PBMC or in numbers of the different T-cell subsets or both.

That virus was isolated from bone marrow cells of one chimpanzee suggests that some cell type other than, or in addition to, T4 cells may be harboring virus. We have preliminary evidence that virus is present in both the adherent and nonadherent fractions of chimpanzee PBMC and that human macrophages can be infected by HTLV-III/LAV. This is not unexpected, because other retroviruses, e.g., visna, equine infectious anemia, and feline leukemia viruses can replicate in macrophages (7, 18, 28).

HTLV-III/LAV has been isolated from saliva from healthy individuals and patients with AIDS-related complex (15) and also from brain tissue and cerebrospinal fluid of AIDS-related complex and AIDS patients (23). Approximately one-third of AIDS patients develop central nervous

TABLE 4. Immunologic status of C-497 during exposure to LAV by housing with an LAV-infected cagemate (C-487)

Time (mo) after cagemate was infected	IgM[a]	IgG[a]	T4:T8 ratio	Lymph nodes enlarged
0.5	−	−	1.58	−
1	−	−	1.55	−
2	−	−	0.99	−
3	+ +	−	0.54[b]	+
4	+	−	0.63[b]	+
4.5	−	+/−	NT[c]	+ +
5	−	+/−	0.95	+ +
6	−	−	1.26	+
7	−	−	1.28	+

[a] LAV-specific antibodies. IgM titers (by ELISA): + +, 1:200; +, 1:100. IgG: +/−, a 1:50 dilution of serum gave an ELISA absorbance reading equal to the control cutoff value.

[b] Due to increased T8$^+$ cell numbers.

[c] NT, Not tested.

system disorders (38). Gajdusek et al. (12) have transmitted HTLV-III/LAV from human brain material to chimpanzees, and Shaw et al. (37) identified the viral genome in human brain tissue by in situ hybridization. However, we failed to isolate infectious virus from two samples of spinal fluid and from saliva of all of the animals studied. It is possible that our failure to do so using saliva from infected chimpanzees may be due to the very small volumes of the samples we tested. However, the frequency of virus isolation from saliva of persons infected with HTLV-III/LAV is, in general, low (24).

The chimpanzee-HTLV-III/LAV model system offers (i) a unique opportunity to study the natural history of HTLV-III/LAV infection, especially early after infection—a time that rarely can be identified with certainty in human HTLV-III/LAV infections—and (ii) a means of testing potential vaccines. The first few weeks following infection may be critical in determining the final outcome of infection, as has been shown for feline leukemia virus infection in cats, i.e., early events in virus-host interactions determine whether feline leukemia virus infection will be self-limiting or progress to persistent infection and permanent immunodeficiency (35). Because the time from seroconversion or from transfusion to diagnosis of AIDS in humans ranges from 6 to at least 84 months (11a, 25, 30), the fact that no chimpanzee developed opportunistic infections or other symptoms of AIDS could indicate a similarly long incubation time in these primates. In addition, to date, approximately 25 to 30% of persons infected with HTLV-III/LAV have developed lymphadenopathy or AIDS-related complex, and only about 5 to 10% have developed AIDS (14, 19); therefore, not all infected chimpanzees would be expected to develop AIDS. It has been suggested (3, 27) that one or more cofactors or host factors may play a role in the development of AIDS. Experiments are in progress to assess the role(s) of possible cofactors (e.g., other viruses, immunosuppression) in the development of AIDS with the chimpanzee model system.
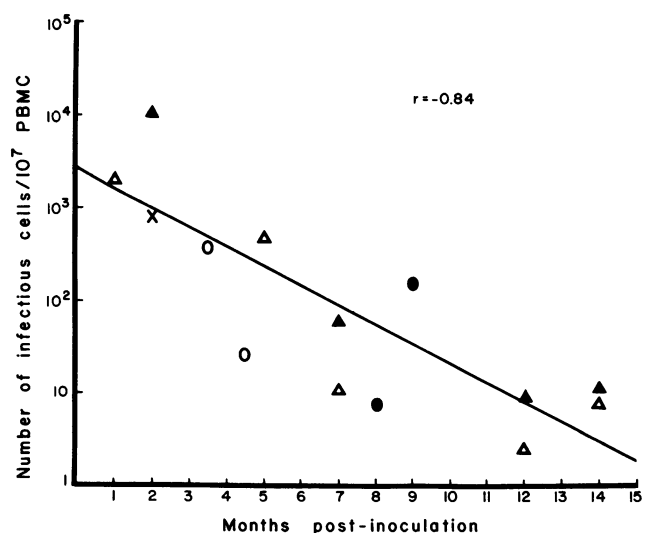

FIG. 5. Correlation between number of infectious cells in PBMC from LAV-infected chimpanzees with time after infection. PBMC were obtained from chimpanzees at various times after LAV infection and were serial diluted prior to coculture with PHA-AWBC. The coefficient of correlation, r, is shown. Symbols: △, C-459; ▲, C-477; ○, C-560; ●, C-487; ×, infected chimpanzee not described in this communication.

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