antiserum to α-IFN. The cultures were subcultured when the population reached a concentration of 10^6 cells per milliliter.

Ten days after the initiation of cocultivation the cell population was examined by light microscopy and the cells were tested for various immunological markers. The results were compared with those obtained with the reference H9 and H9/HTLV-III cell lines (*2, 3*). We observed a number of large cells (12 to 20 μm in diameter) with basophilic cytoplasm surrounding a large Golgi region and containing an indented nucleus; giant multinucleated syncytial cells; and numerous cells in mitosis. This morphological pattern is very similar to that seen with the reference H9/HTLV-III cell line, and all three types of cultured cells (the coculture, the H9/HTLV-III reference line, and the uninfected H9 reference line) showed a similar lymphocytic phenotype. No cells expressed T4 or T8 antigens in the cocultures. Thirty-five to 45 percent of the cells expressed HLA-DR antigen (OKI_2). The uninfected H9 cell cultures differed by showing less cellular degeneration and fewer multinucleated cells.

Cocultures were monitored for reverse transcriptase (RT) activity. Such activity was detectable as of day 8 of the coculture (Fig. 2). In contrast, RT activity was not detectable in long-term cultures (12 to 40 days) of semen T cells (not shown). Furthermore, 20 to 40 percent of the cells from 15-day-old cocultures expressed HTLV-III antigens (Table 1 and Fig. 3). Cell samples from the cocultures were also examined by electron microscopy. Retrovirus particles were observed at the surface of some cells (Fig. 4).

This study shows that semen from normal individuals and from patients with AIDS contains a number of mononuclear cells that proliferate at low cell densities in vitro in the presence of IL-2 and a feeder cell layer. We found that some cells from semen derived from the two AIDS patients contained HTLV-III that replicated in the culture system. However, expression was transient. Semen cell cultures older than 12 days contained neither HTLV-III antigens nor RT activity. In previous studies, long-term cultures derived from blood, bone marrow, or lymph node cells from AIDS patients were also frequently negative for HTLV-III (*3*). When the semen mononuclear cells from the primary short-term cultures were cocultured with H9, definitive results were obtained because of transmission and amplification of the virus. That the virus isolated belongs to the HTLV-III subgroup is indicated by its morphology (Fig. 4) and, more important, by the positive results with HTLV-III specific antisera (*12*). Thus, these results are consistent with the epidemiological data implicating semen as a source of the AIDS etiological agent (*9, 10*) and with results (*2–5, 8–10, 12–15*) indicating that HTLV-III is the agent.

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HTLV-III in the Semen and Blood of a Healthy Homosexual Man

Abstract. *Human T-lymphotropic virus type III (HTLV-III) is the probable etiologic agent for the acquired immune deficiency syndrome (AIDS). HTLV-III was isolated from semen and blood of a healthy homosexual man whose serum contains antibodies to HTLV-III. The finding of virus in semen supports epidemiologic data that suggest that AIDS can be transmitted sexually. In addition, the demonstration of HTLV-III in the blood and semen of a healthy individual establishes an asymptomatic, virus-positive carrier state which may be important in the dissemination of HTLV-III and, consequently, AIDS.*

The acquired immune deficiency syndrome (AIDS) was first recognized in 1981 as a generally fatal disorder of cell-mediated immunity manifested clinically by opportunistic infections or Kaposi's sarcoma (*1–3*). More than 6000 cases of AIDS have been reported to the Centers for Disease Control to date. Epidemiologic data in male homosexuals (*4, 5*) and female sexual partners of men with the syndrome (*6*) suggest that the disease can be transmitted sexually, possibly through contact with semen. Recently, a novel retrovirus, the human T-lymphotropic virus type III (HTLV-III), has been shown to be the likely etiologic agent for AIDS (*7, 8*). The lymphadenopathy-associated virus (LAV), initially isolated in France (*9*), appears to be closely related or identical to HTLV-III (*10*). Both viruses are now frequently isolated from blood of persons with AIDS or the AIDS-related complex (ARC) (*11*). Here we report the isolation of HTLV-III from semen of a healthy homosexual man who is seropositive for HTLV-III.

The subject, a 30-year-old homosexual male, has been evaluated at the Massachusetts General Hospital every 3 months since June 1983 as a participant in a prospective study of homosexual men with or without AIDS. His past medical history includes gonorrhea, hepatitis, and sexual contacts in 1982 with a man who subsequently developed Kaposi's sarcoma. However, he has shown no constitutional or localized signs or symptoms of AIDS. His peripheral blood leukocyte counts, lymphocyte proliferative responses to concanavalin A, and allogeneic cytotoxicity responses have been consistently normal. The subject's T helper/T suppressor (T4/T8) ratios have ranged from 1.0 to 2.4 (normal 1.7 ± 0.5) on five serial determinations. Four se-

men and four urine cultures for cytomegalovirus (CMV) have been negative. Serologically he is antibody positive for CMV (1:32 by complement fixation) and for Epstein-Barr viral capsid antigen (1:320) and nuclear antigen (1:80), and is negative for HTLV-I membrane antigen by an indirect immunofluorescence technique (*12*).

Tests for antibodies to HTLV-III were performed on five serum samples obtained from our study participant between June 1983 and August 1984. Approximately 1×10^4 H9 cells (*13*) infected with HTLV-III were placed in each 12-mm^2 well on glass slides. After fixation with acetone, 15 μl of a 1:10 dilution of serum were added to each well for 30 minutes. The samples were washed three times in phosphate-buffered saline (PBS), 15 μl of fluorescein-conjugated goat antibody to human immunoglobulin (Electronucleonics) was added for another 30 minutes, and the samples were again washed three times in PBS before being examined under ultraviolet light. Appropriate positive and negative control sera were included in each assay. All five serum samples were positive for antibodies to HTLV-III, with over 80 percent of the cells showing fluorescence.

In August 1984, semen and blood were obtained from the subject for virus isolation. The semen sample (3 ml) was immediately diluted 1:10 in RPMI 1640 medium with 20 percent fetal calf serum (FCS) in order to lessen the semen's toxic effect on cells. Ficoll-Hypaque (density of 1.078 g/ml) separation was then performed on the diluted semen preparation, which yielded 1.3×10^6 viable mononuclear cells. This low cell count prohibited adequate phenotypic analysis. However, the cells were heterogeneous with approximately 30 percent resembling lymphocytes. After treatment with phytohemagglutinin-P (PHA-P, 10 μg/ml; Sigma), the mononuclear cells were cultured in RPMI 1640 medium with 20 percent FCS and 10 percent interleukin-2 (Electronucleonics). No cellular proliferation was seen by day 2 of culture. Therefore, after treatment with Polybrene (Sigma; 2 μg/ml) the mononuclear cells were cocultivated with 3×10^6 H9 cells, a mature T-cell clone derived from an adult with lymphoid leukemia (*13*).

This cocultivation culture was examined every 2 to 3 days for cytopathic effects (CPE). On day 11 of culture, a few of the H9 cells were noted to be enlarged, "ballooned," and multinucleated. The changes were similar to those described by Popovic *et al.* for H9 cells infected with HTLV-III (*13*). Fixed-cell indirect immunofluorescence tests with a known HTLV-III–positive serum from another homosexual man demonstrated HTLV-III antigens in approximately 1 percent of the H9 cells (Fig. 1A). By day 15, the CPE and positive immunofluorescence were observed in 25 percent of the cells in culture. These progressed to 75 percent by day 19 (Table 1). We subsequently obtained murine monoclonal antibodies to the HTLV-III core proteins p15 and p24 (*14*). Tests with these antibodies in similar fixed-cell indirect immunofluorescence preparations were also positive (Table 1). The affected H9 cells were negative for antigens of Epstein-Barr virus (EBV) and cytomegalovirus and for p19 and p24 of HTLV-I by indirect immunofluorescence. Assays for reverse transcriptase (RT) activity were done as described (*13, 15*). Serial culture supernatant fluids were positive for RT activity beginning on day 11 (Table 1). In addition, on day 15, transmission electron microscopy showed many budding and mature viral particles consistent with HTLV-III (Fig. 1B).

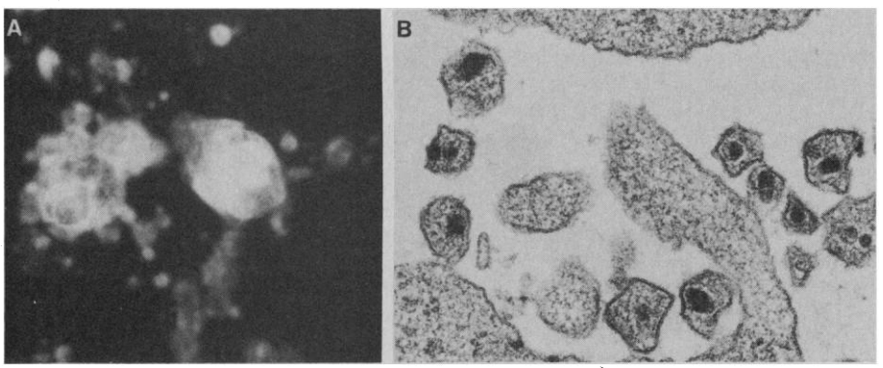

Fig. 1. (A) Multinucleated H9 cells showing positive immunofluorescence for HTLV-III antigens. H9 cells, cocultivated with mononuclear cells from the subject's semen, were placed on glass slides and fixed with acetone. The test serum was antibody positive for HTLV-III by an enzyme-linked immunosorbent assay (ELISA) and Western blot (*8*) but negative for HTLV-I by membrane immunofluorescence (*12*). Fifteen microliters of a 1:64 dilution of this serum were added to each well for 30 minutes. After three successive washes in PBS, 15 μl of a fluorescein conjugated goat antiserum to human immunoglobulin was added for an additional 30 minutes. The slides were then washed, dried, mounted, and examined with ultraviolet light (×500). (B) Transmission electron micrograph showing viral particles (×45,000).

Table 1. Detection of HTLV-III in semen and blood. Indirect immunofluorescence tests were performed with monoclonal antibodies against HTLV-III p15 and p24 antigens. Techniques were described in the legend to Fig. 1, except that the monoclonal antibodies were used at 1:200 dilutions and we used rabbit antiserum to mouse Fab_2-fluorescein isothiocyanate conjugate (1:40; Cappel). Before being assayed for RT, virus particles were precipitated from 5 ml of culture fluid by the addition of 2.5 ml of a solution containing 30 percent (w/v) polyethylene glycol (Fisher Carbowax 8000) in 0.4*M* NaCl. The suspension was placed at 0°C overnight and then centrifuged at 2100 rev/min at 4°C for 45 minutes. The precipitate was resuspended in 300 μl of 50 percent glycerol, 25 m*M* tris-HCl, *p*H 7.5, 5 m*M* dithiothreitol, 150 m*M* KCl, and 0.025 percent Triton-X 100. Virus particles were disrupted by the addition of 0.9 percent Triton-X 100 and 1.5*M* KCl (100 μl). The RT activity was assayed as described (*13, 15*) with the use of poly(rA)-oligo$(dT)_{12-18}$ (2.5 μg/ml, P-L Biochemicals). Results are expressed as counts per minute of methyl-[^{3}H]deoxythymidine triphosphate (16 to 18 Ci/mmol; New England Nuclear) incorporated per milliliter of culture fluid; NT, not tested.

Test	Days					
	5	11	15	19	25	29
H9-semen mononuclear cell cocultivation						
Cytopathic effects	−	+	+	+	+	+
Immunofluorescence (percent positive)						
Human serum positive for HTLV-III	0	1	25	75	90	90
Monoclonal antibodies to HTLV-III p15 and p24	0	0	N.T.	90	90	90
Reverse transcriptase activity (10^5 cpm/ml)	0	0.2	8	52	32	40
Peripheral blood mononuclear cell culture						
Immunofluorescence (percent positive)						
Monoclonal antibodies to HTLV-III p15 and p24	0	0	N.T.	0	5	5
Reverse transcriptase activity (10^4 cpm/ml)	N.T.	N.T.	0	0	1.0	0.8*

*Result from day 33.

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on a Ficoll-Hypaque gradient. PBMC (5×10^6) were then treated with PHA-P (10 μg/ml) and maintained on RPMI-1640 medium with 20 percent FCS and 10 percent interleukin-2. No abnormal cellular changes were observed by light microscopy throughout the culture period. However, after day 25, PBMC expressed HTLV-III antigens as detected by indirect immunofluorescence with a human serum positive for HTLV-III or with monoclonal antibodies to HTLV-III p15 and p24 (Table 1), but they were consistently negative for EBV, CMV, and HTLV-I antigens. The corresponding supernatant fluids showed significant RT activity on days 25 and 29 (Table 1). Furthermore, on day 12, addition of cell-free supernatant fluid (2 ml) from the PBMC culture to Polybrene-treated H9 cells (3×10^6) resulted in characteristic morphologic changes 14 days later. The CPE were similar to those induced by the mononuclear cells from the subject's semen described above. These H9 cells also become positive for HTLV-III antigens, and their supernatant fluids showed RT activity (0.4×10^5 to 4.8×10^5 cpm/ml).

That our isolates are HTLV-III is indicated by their (i) antigen expression, (ii) RT characteristics, (iii) morphology on electron microscopy, and (iv) ability to induce unique CPE in H9 cells (*7, 13*). That the isolates are not HTLV-I or HTLV-II is shown by the positive staining obtained with the HTLV-III–specific monoclonal antibodies (*14*) and the negative results with the HTLV-I–specific monoclonal antibodies.

HTLV-III was recovered from the mononuclear-cell fraction of semen. Attempts to find virus in the spermatozoa fraction of semen from 11 individuals have yielded negative results. Because of the toxic effects of semen on target cells used for these isolations, it is unclear whether there is also cell-free HTLV-III in seminal plasma. The coexistence of other viruses in semen, such as CMV (*16, 17*), may also interfere with the successful cultivation of HTLV-III.

The demonstration of HTLV-III in the semen of an asymptomatic individual who is at risk for AIDS supports epidemiologic data suggesting that AIDS can be sexually transmitted. It is unknown why one HTLV-III carrier remains well while another develops AIDS. Asymptomatic carriers should be closely followed for the possible development of AIDS. Recent surveys suggest that the prevalence of HTLV-III/LAV seropositivity in urban male homosexuals may be as high as 65 percent (*18*). Most of these men are healthy and without obvious immune deficits. The frequency of HTLV-III carriers in this population is unknown. These issues need to be addressed by careful prospective analyses of asymptomatic HTLV-III seropositive individuals.

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Prevalence of Antibodies to Lymphadenopathy-Associated Retrovirus in African Patients with AIDS

Abstract. *The presence of antibodies to lymphadenopathy-associated retrovirus (LAV) was determined by a radioimmunoprecipitation assay and by an enzyme-linked immunosorbent solid assay of sera from Zairian patients with the acquired immune deficiency syndrome (AIDS) in 1983. Thirty-five of 37 patients (94 percent) and 32 of 36 patients (88 percent), respectively, were seropositive by the two tests. In a control group of 26 patients, six (23 percent) showed positive results in these tests. Of these six control patients, five had clinically demonstrable infectious diseases and a low ratio of T4 to T8 lymphocytes. In addition, sera collected from a control group of Zairian mothers in 1980 were positive for LAV in 5 of 100 cases. Other serologic data suggest that LAV was present as early as 1977 in Zaire.*

The isolation of a new lymphotropic retrovirus from cultured lymphocytes of a patient with lymphadenopathy syndrome (LAS) was reported in May 1983 (*1*). This virus, named lymphadenopathy-associated virus (LAV), differed from the previous isolates of human T-cell leukemia virus (HTLV-I) by the lack of antigenic relatedness of its major core protein (p25) to HTLV-I p24 and by a peculiar morphology of mature virions, which was similar to those of D type particles and equine infectious anemia virus (*2*). In addition, antibodies produced in horses infected by the latter virus precipitated the p25 of the human virus (*2*).

Similar isolates have been made from AIDS or LAS patients belonging to the groups that are at risk for the disease: four from homosexuals, two from two hemophiliac siblings, two from Haitians, and three from Zairians (*3–6*). Such viruses display selective tropism for the $T4^+$ subset of lymphocytes, both in vitro and in vivo, in which they induce a depression of cell growth and a cytopathic effect (*3, 7*) upon activation. A high prevalence of antibodies to viral structural proteins was found in AIDS and LAS patients hospitalized in France, including patients of African origin (*3, 8*). By contrast, only one of 330 controls (French blood donors, laboratory workers, and prisoners) was serologically positive for these antibodies (*6, 8*). These data suggest that such a group of viruses could play a role in the etiology of AIDS,