HTLV-III in Saliva of People with AIDS-Related Complex and Healthy Homosexual Men at Risk for AIDS

Abstract. *Peripheral blood leukocytes and saliva from 20 individuals, including four with the acquired immune deficiency syndrome (AIDS), ten with AIDS-related complex (ARC), and six healthy homosexual males at risk for AIDS, were compared as sources of transmissible human T-cell leukemia (lymphotropic) virus type III (HTLV-III), the virus found to be the etiologic agent of AIDS. All of the AIDS and ARC patients and four of the six healthy homosexuals had evidence of prior exposure to HTLV-III as indicated by seropositivity for antibody to HTLV-III structural proteins. Infectious virus was isolated from the peripheral blood of one of the AIDS patients, four of the ARC patients, and two of the healthy homosexual males, consistent with previous reports. HTLV-III was also isolated from the saliva of four of the ARC patients and four of the healthy homosexuals. Virus was also observed by electron microscopy in material prepared by centrifugation of the saliva of one AIDS patient. Although AIDS does not appear to be transmitted by casual contact, the possibility that HTLV-III can be transmitted by saliva should be considered.*

The acquired immune deficiency syndrome (AIDS) is a transmissible disorder of the cellular immune system resulting in frequently fatal opportunistic infections or neoplasms (*1*). The groups at high risk for AIDS include homosexual or bisexual men and their partners, hemophiliacs, Haitians, Central Africans, and intravenous drug abusers (*2*). In addition, children of high-risk mothers and recipients of random blood products have developed AIDS (*3*). Epidemiological studies indicate that AIDS may be transmitted by an agent in blood and possibly other body fluids. Public health recommendations designed to limit the spread of the AIDS epidemic need to be based on data showing the presence or absence of the agent in each body fluid or excretion (*4*).

A primary pathogenic role for HTLV-III in AIDS and in the AIDS-related complex (ARC) has been demonstrated (*5*). HTLV-III is cytopathic for T4 helper-inducer lymphocytes (this population of cells decreases in function and in absolute number as the disease progresses), and has been isolated from peripheral blood lymphocytes of approximately 50 percent of AIDS patients and 80 percent of ARC patients (*5, 6*). In addition, 90 to 100 percent of AIDS and ARC patients have antibody to the 41,000-dalton HTLV-III antigen (*7*). HTLV-III appears to be serologically closely related to the virus named LAV that was independently isolated from AIDS and pre-AIDS patients in France (*8*). Although LAV was less frequently serologically associated with AIDS in earlier studies (*9*), recent data from Kalyanaraman *et al.* (*10*) indicate that 70 to 95 percent of AIDS and ARC patients are positive for LAV antibodies by an enzyme-linked immunosorbent assay (ELISA). Here we report a study of peripheral blood mononuclear cells and saliva from four patients with AIDS, ten patients with ARC, and six healthy asymptomatic homosexual males at risk for AIDS.

Samples of serum, heparinized peripheral blood, and saliva were obtained from the 20 individuals. The serum samples were tested for antibody to HTLV-III by means of ELISA with whole disrupted virus and by a more sensitive Western electroblot technique (*7, 11*). Mononuclear cells were obtained from heparinized peripheral blood banded in Ficoll-Hypaque, and cell cultures were initiated in complete medium supplemented with T-cell growth factor (interleukin-2). HTLV-III production was monitored as previously described (*5*). Saliva samples were diluted to a final volume of 2 ml in complete growth medium, incubated for 2 hours at 37°C, and centrifuged at 1000*g* for 10 minutes at 4°C. Pelleted materials were fixed for electron microscopy and supernatant fluids were filtered (0.45 μm) and used for transmitting virus to fresh peripheral blood lymphocytes (*5*). HTLV-III production by infected cells was monitored by means of assays for reverse transcriptase (RT), by electron microscopic observation, and by immunofluorescence and competition radioimmunoassays with the use of HTLV-III–specific antisera.

As shown in Table 1, all of the AIDS and ARC patients and four of the six healthy homosexuals were positive for antibody to HTLV-III structural proteins. HTLV-III was isolated from the peripheral blood cells of seven of the 18 subjects who were positive for HTLV-III antibody but from neither of the two seronegative healthy homosexuals. Virus was also isolated from the saliva of eight of the 18 individuals who were seropositive for HTLV-III–specific antibodies. As shown in Fig. 1, virus was observed in leukocytes pelleted directly from the saliva of one ARC patient. Cells

Table 1. Detection and isolation of HTLV-III from peripheral blood cells or saliva. Heparinized peripheral blood was obtained by venipuncture, and mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation and cultured with interleukin-2 as described (*13*). Saliva was obtained at the time of venipuncture, diluted to a final volume of 2 ml in complete growth medium, and incubated for 2 hours at 37°C. It was then filtered through a 0.45-μm filter (Millipore) and added to cultures of interleukin-2–stimulated peripheral blood mononuclear cells. Seropositivity for antibody to HTLV-III was determined by means of ELISA with whole disrupted virus and the Western electroblot technique as previously described (*7, 11*). HTLV-III was identified by assay for reverse transcriptase, electron microscopy, and specific antisera to viral proteins (*14*); PBL, peripheral blood lymphocytes.

Patient or donor	Diagnosis*	Antibody to HTLV-III†		Isolation of HTLV-III	
		ELISA	Western	PBL	Saliva
1	AIDS-KS	+/−	+	−	−
2	AIDS-KS	+/−	+	−	−
3	AIDS-KS	+/−	+	+	−
4	AIDS-OI	+	ND	−	−
5	ARC	+	ND	−	+
6	ARC	+/−	+	−	+
7	ARC	+	ND	−	−
8	ARC	+/−	+	+	+
9	ARC	+/−	+	+	−
10	ARC	+	ND	−	+
11	ARC	+/−	+	−	−
12	ARC	+/−	+	−	−
13	ARC	−	+	+	−
14	ARC	+	ND	+	−
15	HHM	+	ND	+	+
16	HHM	+	ND	−	+
17	HHM	+	ND	+	+
18	HHM	+	ND	−	+
19	HHM	−	−	−	−
20	HHM	−	−	−	−

*KS, Kaposi's sarcoma; OI, opportunistic infection; HHM, healthy homosexual male. †Symbols: +, present; −, absent; ND, not done; +/−, equivocal result.

infected by virus from saliva were tested for the presence of HTLV-III proteins by a homologous competition radioimmunoassay (Fig. 2). The competition patterns thus obtained demonstrated the identity of this virus with HTLV-III.

The inability to recover virus from some seropositive individuals, as noted here and elsewhere (*5*), may indicate prior exposure to viral antigens without infection or lack of current viremia due to restrictions on HTLV-III replication. Alternatively, HTLV-III may occur in a reservoir other than saliva or peripheral blood lymphocytes in certain persons or at a certain stage of the disease.

Studies of the biological activity of HTLV-III and its role in AIDS and associated clinical disorders have just begun. Transmission of two members of the HTLV family involved in the development of T-cell leukemias and lymphomas, that is, HTLV-I and HTLV-II, usually requires cocultivation procedures, indicating that these viruses are highly cell-associated. HTLV-III, in contrast, is efficiently transmitted by cell-free virus making it feasible to study its distribution in body fluids. Studies of feline leukemia viruses, which are associated with both a T-cell leukemia and a profound immunodeficiency, indicate that cell-free fluids, specifically saliva, contain infectious virus (*12*). The mechanism of virus survival in saliva has not been determined either in cats or in the HTLV-III–positive patients. However, cells producing virus (Fig. 1), as well as cell-free virus, were observed in the saliva of the patients described here.

These data should be taken into account in designing recommendations to limit the spread of AIDS. The recovery of HTLV-III from saliva suggests that direct contact with this body fluid should be avoided since saliva may form a protective matrix to support cell-free virus or virus-positive cells and could facilitate horizontal transmission.

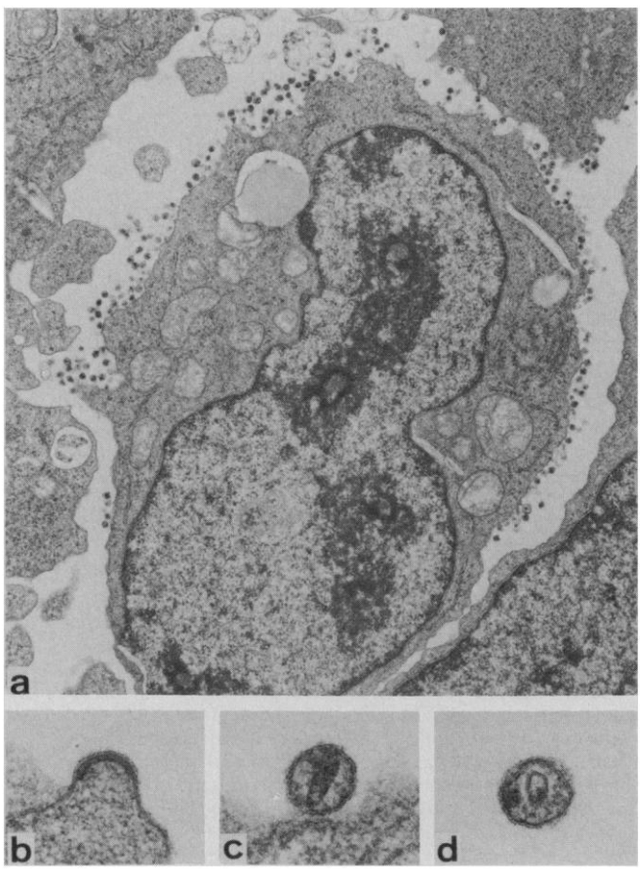

Fig. 1. Transmission electron micrograph of fixed cells obtained from the saliva of patient No. 8. Procedures for preparation of saliva are described in the legends to Table 1. (a) Leukocyte-expressing HTLV-III (×10,000). (b) Budding virus (×150,000). (c) Mature virus particles (×150,000).

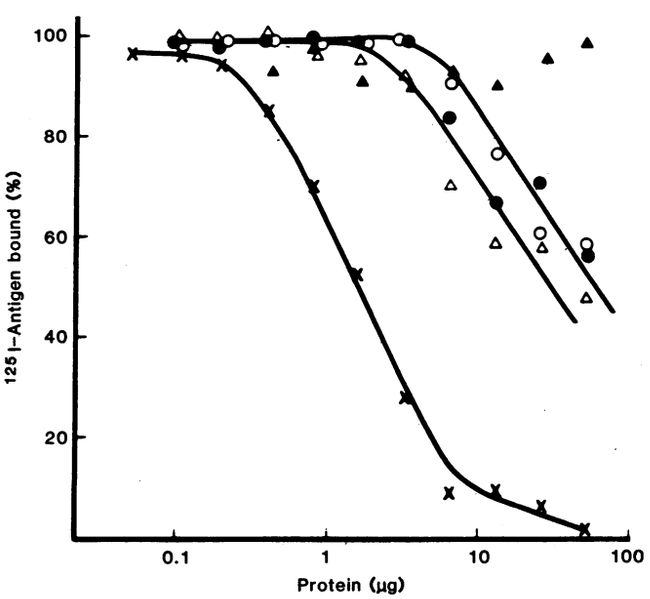

Fig. 2. Homologous competition radioimmunoassay of lysates of cells infected by virus from saliva. The indicated concentrations of disrupted cell proteins were used to compete with the precipitation of ^{125}I-labeled p24 of HTLV-III by antibody to this virus. Cell lysates from: x, H9 cells producing HTLV-III; ▲, H9 control cells; ●, ARC patient No. 8; ○, ARC patient No. 10; and △, HHM healthy homosexual male donor No. 16.

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HTLV-III in Cells Cultured from Semen of Two Patients with AIDS

Abstract. *Epidemiological results suggest that the etiological agent of the acquired immune deficiency syndrome (AIDS) is transmitted primarily through blood products, semen, and saliva. There is evidence that the human T-cell leukemia (lymphotropic) virus type III (HTLV-III) is this agent. HTLV-III has been isolated repeatedly from T cells obtained from peripheral blood or lymph node tissue of AIDS and pre-AIDS patients and of healthy people believed to have been exposed to the virus. In the present study, HTLV-III was detected in and isolated from T cells present in the seminal fluid of AIDS patients. Mononuclear cells from the semen of AIDS patients and normal individuals were cultured in the presence of T-cell growth factor (interleukin-2). After 6 to 8 days, HTLV-III antigens were transiently expressed by the cells from the AIDS patients but not by those from the normal individuals. When the mononuclear cells from the semen of AIDS patients were cocultured with a permissive human T-cell line, cell cultures were produced that expressed high levels of reverse transcriptase activity, showed retroviral particles by electron microscopy, and were positive for HTLV-III–specific antigens when tested by fixed-cell indirect immunofluorescence with the use of monoclonal antibodies to the p24 and p15 antigens of HTLV-III.*

Several findings indicate that the primary cause of the acquired immune deficiency syndrome (AIDS) is an infection with the human T-cell leukemia (lymphotropic) virus type III (HTLV-III) (*1–5*). Almost all patients with AIDS and related conditions have detectable specific serum antibodies to a member or members of this retrovirus family (*4–6*), and 48 isolates of HTLV-III were reported in one study (*3*). More than 95 isolates of HTLV-III have since been obtained (*7*). Epidemiological data indicate that transmission of the AIDS agent is through blood or blood products (*8*) or through intimate contact between homosexual males (*9*) or between heterosexual females and their male partners that have been exposed to the agent (*10*). Such data, obtained mostly from surveys of populations at risk within the United States, together with the observed lymphotropism of the causative virus, led us to suspect that lymphocytes infected with HTLV-III might be found in the semen of AIDS patients. In the study reported here we attempted to answer the following question: Can the lymphocytes from the seminal fluid of AIDS patients be grown in vitro and, if so, can HTLV-III be detected in and isolated from these cells?

Semen was obtained from two patients with AIDS and stored frozen in 10 percent dimethyl sulfoxide in liquid nitrogen. Both patients had disseminated Kaposi's sarcoma, low numbers of circulating T4 lymphocytes, and reversed T4/T8 ratios (0.5 and 0.2, respectively). Semen was also obtained from three healthy heterosexual males. The semen was collected under sterile conditions, thawed at 37°C, and subjected to low-speed centrifugation. Cell pellets were resuspended in RPMI 1640 medium containing 10 percent fetal calf serum. After thawing, a mononuclear cell–enriched fraction was isolated over Ficoll-Hypaque (Seromed, München, FRG). The number of cells obtained in this manner varied from 0.8×10^5 to 3×10^5 per milliliter in the semen of normal donors. The mononuclear cell–enriched fractions from AIDS patients 1 and 2 yielded 0.06×10^5 and 0.9×10^5 cells per milliliter, respectively. These fractions contained 70 percent of adherent macrophage-monocytes and 20 to 30 percent lymphocytes and residual spermatozoa (Fig. 1). The lymphocytes from the normal individuals consisted of 17 percent $T4^+$ cells (OKT4), 30

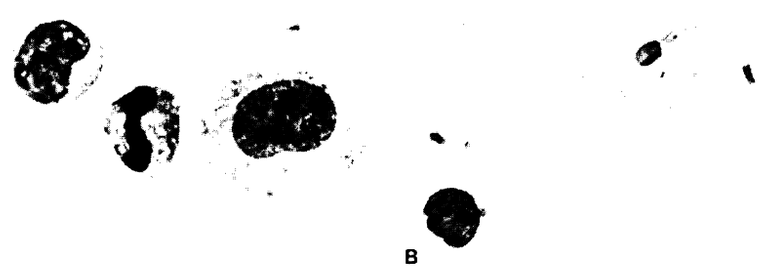

Fig. 1 (above). Mononuclear cells grown from the semen of AIDS patients. A mononuclear cell–enriched fraction was obtained after centrifugation over a Ficoll-Hypaque gradient. Portions were then smeared on a slide and stained (Giemsa-Wright). Note the lymphocytes in (A) and residual spermatozoa in (B). Fig. 2 (right). Reverse transcriptase activity in H9/AIDS semen cell cocultures. The RT was assayed in cell-free culture supernatants. Culture fluid (1 ml) was centrifuged for 10 minutes at 300*g*. Virus particles were precipitated from cell-free supernatants and the RT assay was performed as described (*3*). Samples were collected at different periods (days 2 to 14) after initiation of coculture. Culture fluids from H9 and H9/HTLV-III cells were collected according to the same procedure. Results are expressed in counts per minute per milliliter of culture medium. H9 is the cloned human T-cell leukemic line and H9/HTLV-III is the same cell infected with and producing HTLV-III. H9-AS1 and H9-AS2 are the cocultures of H9 cells with mononuclear cells derived from the semen of AIDS patients 1 and 2.