CONCISE REPORT

Antibody Seronegative Human T-Lymphotropic Virus Type III (HTLV-III)–Infected Patients With Acquired Immunodeficiency Syndrome or Related Disorders

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The human T-lymphotropic virus type III (HTLV-III) is the primary cause of the acquired immunodeficiency syndrome (AIDS) and related disorders (ARC). Prior studies have reported that nearly all symptomatic patients with AIDS or ARC manifest antibody to HTLV-III. This observation has engendered efforts to screen for HTLV-III, especially prior to blood donation, with assays for antibody to HTLV-III. We report the first two cases, one with AIDS and one with ARC, that are HTLV-III virus positive but antibody negative. Accurate diagnosis of HTLV-III infection in some cases may require direct virus culture or tests for antigen. In addition, lack of HTLV-III antibody may indicate an atypical clinical course of AIDS.

THE HUMAN T-lymphotropic virus type III (HTLV-III) is the primary etiologic agent in the pathogenesis of the acquired immunodeficiency syndrome (AIDS) and related disorders.[1-4] Individuals with AIDS as well as those at risk for the syndrome who manifest prolonged generalized unexplained lymphadenopathy have been studied and have virtual uniformity of antibody to HTLV-III–related antigens.[5-9] Indeed, screening both asymptomatic blood donors and high-risk persons for HTLV-III infection has recently been instituted using assays that detect antibodies to this virus. We have previously reported on asymptomatic healthy individuals exposed to HTLV-III who had no detectable antibody, yet had this virus recovered from peripheral blood and/or saliva.[10] We now report on two symptomatic high-risk individuals, one with AIDS and the other with lymphadenopathy who are antibody seronegative but infected with HTLV-III.

CASE REPORTS

Case 1. A 55-year-old homosexual man presented in July 1982 with a violaceous nontender skin lesion on the lateral aspect of his right knee, which he had noted six months previously. Biopsy revealed Kaposi's sarcoma. He had a history of both receptive and insertive oral-genital and anal-genital contact. The results of his physical examination were otherwise entirely normal. The patient did well without any treatment until one year later when another skin lesion appeared on the right arm. That one was also biopsy-proven Kaposi's sarcoma. His physical exam at this time was otherwise unremarkable. Again, there was no treatment of the patient, and the findings of extensive evaluation for other Kaposi's sarcoma lesions, including panendoscopy, were negative. There have been no new Kaposi's sarcoma lesions noted for nearly two years.

Case 2. A 24-year-old bisexual man was evaluated in October 1984 for generalized lymphadenopathy. This had been present for greater than four months and was not associated with fever, malaise, weight loss, oral candidiasis, herpes zoster, or diarrhea. Physical exam revealed 1-cm to 2-cm nontender moveable lymph nodes in cervical, inguinal, and axillary regions. He was a sexually active bisexual man who had experienced oral-genital but not anal intercourse with several men as well as vaginal intercourse with women. These women were not prostitutes and denied contact with other bisexual men. The patient was taking Mysoline and intermittent Dexedrine for temporary lobe epilepsy. He had a prior history of mononucleosis in 1980.

MATERIALS AND METHODS

Sera and heparinized peripheral blood were obtained from both patients at the time of diagnosis and periodically thereafter. Total lymphocyte count was determined by the percentage of lymphocytes on a peripheral blood smear. Peripheral T cells were measured using the monoclonal antibodies OKT4 (helper-inducer), OKT8 (suppressor-cytotoxic), and OKT3 (pan-T cells) (Ortho Pharmaceutical Co, Piscataway, NJ).[8] Serum immunoglobulins were measured as previously described.[10] Titers to Epstein-Barr virus capsid antigen, hepatitis virus B, and cytomegalovirus were measured as described.[8] Skin tests were performed using *Candida*, mumps, and PPD antigens.[8]

Four assays were used to measure serum antibody to HTLV-III: an enzyme-linked immunosorbent assay (ELISA) using semipurified whole virus;[3,7] indirect membrane immunofluorescence using HTLV-III–infected H9 cells as targets;[11] radioimmunoprecipitation using ^{35}S-cysteine–labeled whole cell lysates from HTLV-III–infected H9 cells;[11] and Western immunoblot.[3,6] Virus cultures for HTLV-III were established by separating peripheral blood mononuclear cells on a Ficoll-Hypaque density gradient and stimulating these cells with T cell growth factor and anti-alpha interferon antibody as previously described.[1,8,10] Similarly, HTLV-III was cultured in patient saliva specimens using established T cell cultures from healthy individuals.[10] Controls consisted of peripheral blood mononuclear cells similarly cultured from healthy asymptomatic HTLV-III–negative donors at no epidemiologic risk for AIDS. Identification of HTLV-III in cultures of patient peripheral blood

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mononuclear cells or saliva was done by measuring reverse transcriptase activity and using a monoclonal antibody against the HTLV-III core-related antigen p24.

RESULTS

Standard laboratory testing revealed normal complete blood count in patient 1 but leukopenia and thrombocytopenia in patient 2 (Table 1). The bone marrow aspirate and biopsy results were normal in patient 2, and his abnormal blood counts were thought consistent with the diagnosis of ARC. T cell studies revealed mild abnormalities in absolute T4 number in patient 2 and in T4/T8 ratio in both patients. Serum immunoglobulins were normal, and antibodies were detected to previously encountered viruses in both cases.

Antibody to HTLV-III antigens was not detected in either patient using the enzyme-linked immunosorbant assay, indirect membrane immunofluorescence assay, Western blot, or radioimmunoprecipitation techniques. Both IgG and IgM antibodies were sought using these techniques and were not found. HTLV-III was recovered from the peripheral blood of the patient with Kaposi's sarcoma and from both peripheral blood and saliva from the patient with leukopenia, thrombocytopenia, and generalized lymphadenopathy. Both patients are currently asymptomatic without any therapeutic intervention.

DISCUSSION

HTLV-III is the primary etiologic agent of the acquired immunodeficiency syndrome and related disorders.[1-4] It appears that virtually all symptomatic patients with the syndrome manifest antibody to HTLV-III, detected by either ELISA or Western blot assays.[3,5-9] This has led to efforts to screen for HTLV-III, particularly among blood donors, using assays that detect serum antibody to this virus. We now report the first two cases, one with AIDS and one with a related disorder of prolonged lymphadenopathy, who are HTLV-III antibody negative, but virus positive. The clinical course of the patient with Kaposi's sarcoma is particularly interesting in that he has been asymptomatic and untreated without appearance of any new lesions for almost two years. The patient with generalized lymphadenopathy, mild thrombocytopenia, and leukopenia is similarly asymptomatic. We have previously reported on asymptomatic individuals at risk for the acquired immunodeficiency syndrome who carry HTLV-III in peripheral blood and/or saliva without detectable antibody.[10] Although these persons were entirely normal by immunologic testing, these cases of AIDS and ARC show some abnormality in peripheral T cell subsets and skin test reactivity to recall antigens.

It is possible that development of antibody is correlated with a poor clinical outcome, perhaps due to sufficient in vivo

Table 1. Laboratory Evaluation

	Case 1		Case 2	
	(AIDS/KS)		(ARC)	
White blood cells per mm^3	6,200		3,900	
Total lymphocyte count per mm^3	1,425		900	
Percentage				
OKT4 (helper-inducer)	36		30	
OKT8 (cytoxic-suppressor)	41		48	
OKT4/OKT8 ratio	0.88		0.72	
Platelets	168,000		125,000	
Serum Immunoglobulins (mg/dL)				
IgG	1,600		1,520	
IgA	355		380	
IgM	285		255	
Skin Testing				
Mumps	+		−	
Candida	−		−	
PPD	−		−	
Serum Viral Titers				
Cytomegalovirus	1:64		1:8	
Epstein-Barr virus capsid	1:80		1:40	
Hepatitis B core	+		−	
Hepatitis B surface	+		−	
HTLV-III Antibody				
ELISA	−		−	
IFA	−		−	
RIP	−		−	
Western blot	−		−	
HTLV-III Virus Culture*	day 9	day 14	day 9	day 14
PBL	32,000	130,000	26,000	175,000
Control	173	802	168	712

*Cultures expressed as reverse transcriptase activity (counts per minute per mL supernatant) on days 9 and 14.

ELISA, enzyme-linked immunosorbent assay; IFA, indirect immunofluorescence assay; and RIP, radioimmunoprecipitation.

proliferation of HTLV-III to expose the host to viral antigens that elicit an antibody response. Lane and co-workers have reported an acquired B lymphocyte abnormality in response to neoantigen challenge in persons with the AIDS and ARC.[12] Although the two cases reported here had normal total serum immunoglobulins, it is possible that an appropriate antibody response to newly encountered HTLV-III–related antigens is unable to develop. This B cell dysfunction could explain the antibody seronegativity of these two cases but certainly does not explain the response to HTLV-III in the vast majority of patients with AIDS or ARC since such patients are nearly uniformly antibody positive.[3,6]

We are currently following these two patients closely, and over a six-month period since recovery of virus, antibody has not developed in either of them. Preliminary data indicate that antibody serum conversion to HTLV-III occurs within four to 16 weeks after exposure in both man and chimpanzees (M. Sarngadharan, Litton Bionetics, Kensington, Md, personal communication, March 1985). Nonetheless, some individuals may require long periods to seroconvert or, indeed, may never seroconvert. Observations such as ours on patients with AIDS or ARC suggest that diagnosis of HTLV-III infection may require direct assay for virus or viral antigens rather than antibody in certain hosts. This might be of importance for both symptomatic individuals as well as possibly in screening of asymptomatic persons such as blood donors. Furthermore, antibody seronegativity may have prognostic significance in HTLV-III–infected persons.

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