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Presence of Antibodies to the Human T-Cell Leukemia Virus HTLV I in German Patients with Symptoms of AIDS *

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A. Introduction

The incidence of the acquired immune deficiency syndrome (AIDS) in North America has increased steadily since 1979. This syndrome has been recognized mainly in male homosexuals in the Western hemisphere, in intravenous drug users, and occasionally in hemophiliacs and inhabitants of some tropical areas (Caribbean, Central Africa).

The human T-cell leukemia virus HTLV I is a type-C retrovirus isolated in North America initially from a patient with a malignant variant form of mycosis fungoides [1] and independently in Japan from a patient with T-cell leukemia [2]. Many additional isolates have since been identified, primarily from patients with clinical manifestations of adult T-cell leukemia-lymphoma (ATLL).

HTLV II, which is also associated with T-cell malignancies, has so far only been isolated once from a patient with hairy-cell leukemia [3]. A newly discovered subgroup of the human T-cell leukemia virus family, designated HTLV III, has been described recently [4, 5]. The virus has been isolated in North America and France from more than one-third of patients with AIDS or the lymphadenopathy syndrome.

Antibodies to HTLV III have been found in 90%–100% of American AIDS patients; antibodies against HTLV I had been found in about 30% of North and Middle American AIDS patients [6].

During a study of the presence of HTLV I in the Federal Republic of Germany we examined serum samples of patients from Munich with symptoms of AIDS (11 sera) or the lymphadenopathy syndrome (LAS) (20 sera) for the presence of antibodies to viral antigens of HTLV I.

B. Material and Methods

I. Cells and Virus Purification

HTLV-I-producing MT-2 cells, which have a OKT^{4+} phenotype [7], were donated by Dr. Hinuma. The cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum. Culture fluids were harvested daily and concentrated at 40 000 *g* for 12 h. Virus pellets were resuspended in 0.02 *M* Tris hydrochloride (pH 7.5), 0.1 *M* NaCl, and 1 m*M* ethylenediaminetetraacetate (EDTA), and were further purified by sucrose gradient centrifugation. Fractions with a density of 1.14–1.17 g/cm³ were pooled and subjected to a second run of density gradient centrifugation, from which fractions were pooled in a range of density corresponding to 1.15–1.16 g/cm³.

II. Enzyme-Linked Immunoadsorbent Assay

Sera were screened by enzyme-linked immunoadsorbent assay (ELISA) for antibodies to antigens derived from virus prep-

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arations of MT-2 cells. Briefly, microtiter plates were coated overnight at room temperature with aliquots of sonified, highly purified virus in 0.1 *M* carbonate buffer, pH 9.5. The plates were rinsed with phosphate-buffered saline (PBS) and incubated with 200 µl/well swine skin gelatin (Sigma) at a concentration of 5 mg/ml for 1 h at room temperature. Aliquots of test sera, diluted 1:100 with PBS containing 3% Tween 20 and 2% sheep serum, were added in duplicates and incubated for 2 h at room temperature. The plates were washed once with swine skin gelatin, twice with PBS + Tween 20, and three times with PBS. Peroxidase-conjugated rabbit immunoglobulin against human IgG (gamma-chains) in PBS + Tween 20, containing 2% sheep serum, were then added and incubated for 2–4 h at room temperature. After thorough washing, 100 µl substrate solution (0.001% H_2O_2 and 0.1% orthophenylenediamine in 0.1 *M* phosphate buffer, pH 6.0) was added to each well. The reaction was allowed to proceed for 10 min at 37 °C in the dark and then stopped by addition of 100 µl 1 *N* HCl/well. Absorbance of each well was determined at 486 nm. Specimens with a threefold higher absorbance than negative control sera were further analyzed in the radioimmunoassay.

III. Immunoprecipitation

MT-2 cells were harvested and exposed to [^{35}S]cysteine (100 µCi/ml; specific activity 1000 Ci/mmol, Amersham, Buckinghamshire, England) for 3–5 h. A soluble cell lysate was obtained after disruption with lysis buffer (0.01 *M* Tris hydrochloride, pH 7.5, 0.05 *M* NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate) and centrifuged for 15 min at 18 000 *g*. Aliquots of the lysate supernatant were reacted with 8–12 µl patient's sera preabsorbed with protein A beads. Immunoprecipitates were eluted in electrophoresis sample buffer by boiling for 3 min and analyzed in a 12.5% acrylamide resolving gel with 3.5% stacking gel according to Laemmli [8]. Dried gels were exposed to Kodak x-Omat film with an intensifying screen.

C. Results

Two different procedures have been used to survey serum samples for evidence of exposure to HTLV I. These include [1] an enzyme immunoassay (ELISA), with highly purified virus as antigen, and (2) radioimmunoassays with the use of solubilized [^{35}S]cysteine-labeled MT-2 cells.

1. Five of the 31 patient's sera tested by ELISA were positive for antibodies against viral antigens of HTLV I with a factor of more than 3 over the negative control (Table 1). None of the 34 sera from healthy donors contained detectable antibodies against HTLV I. The five positive sera were further examined in the radioimmunoassay.

2. Three of the five sera which showed a positive reaction in the ELISA precipitated the viral envelope antigen gp68 (Fig. 1, lanes 2–4); one serum of the three also precipitated p28 (lane 3). Also samples from ELISA-negative patients were reacted in the radioimmunoassay and none showed a positive reaction for HTLV I (data not shown).

Table 1. Results of ELISA and immunoprecipitation examinations

	AIDS	LAS	Healthy homosexuals	Others
ELISA				
Total sera tested	11	20	7	27
Sera positive	5	0	0	0
Immunoprecipitation				
Total sera tested	5	0	0	3
Sera positive	3			0

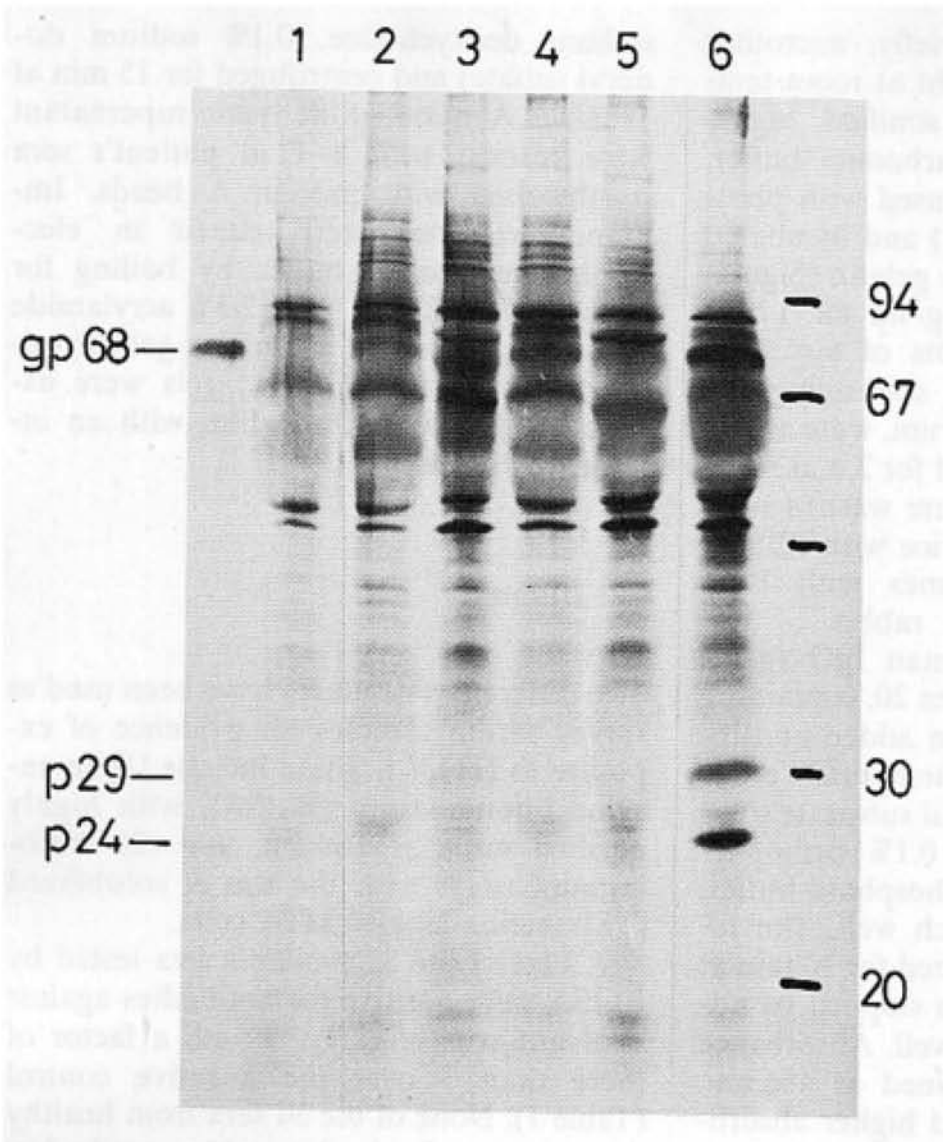

Fig. 1. Immunoprecipitation of radiolabeled HTLV-III-producing MT-2 cells. *Lanes 1–5,* ELISA-positive patient sera; *lane 6,* positive reference serum

D. Discussion

We describe here the presence of antibodies to HTLV I in five of 31 sera from patients with AIDS or LAS. Two of the five sera showing a positive reaction in the ELISA possibly represent false-positive results, probably due to nonspecific fractions with protein contaminations of the antigen preparation. Our results are compatible with the finding that 20%–30% of AIDS patients from North America are positive for antibodies to HTLV I. Not all of the 31 patients tested here were suffering from a clearly diagnosed AIDS, but all belonged to high-risk groups. Eleven patients had symptoms specific for AIDS, including opportunistic infections, Kaposi sarcoma, and altered immunological status; 20 patients suffered from lymphadenopathy syndrome (LAS). The three sera which were able to precipitate HTLV I gp68, as well as the two sera found positive only in the ELISA, came from typical AIDS patients.

Recently the detection of HTLV III as the probable cause of AIDS has been reported [4, 5]. HTLV III is a member of the HTLV family with antigens related to but distinguishable from HTLV I and II. Our findings that some sera from AIDS patients react with antigens from HTLV I are compatible with the reports of Schüpbach et al. and Sarngadharan et al. [9, 10] that AIDS sera with high titers against envelope antigens of HTLV III frequently also recognize the HTLV I envelope antigen.

The gp68 of HTLV I recognized by our three positive sera is known to be a precursor to the viral gp46 envelope protein, which is antigenically related to the gp41 of HTLV III. We thus assume that at least our three positive sera might have high titer antibodies against envelope antigens of HTLV III which cross-react with the enve-

lope antigens of HTLV I. Our results suggest that at least some of the AIDS patients in the Federal Republic of Germany became infected with a member of the HTLV I group and produced antibodies against it.

Postscript. During the preparation of this manuscript, we tested sera from AIDS patients for antibodies against HTLV III antigens. Seventy percent of these sera were positive for Anti-HTLV III antibodies, two sera also showing a reactivity against HTLV I antigens.

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