

High incidence of human type-C retrovirus (HTLV) in family members of a HTLV-positive Japanese T-cell leukemia patient

(adult T-cell leukemia and lymphoma/T-cell growth factor)

P. S. SARIN*, T. AOKI†, A. SHIBATA‡, Y. OHNISHI§, Y. AOYAGI‡, H. MIYAKOSHI†, I. EMURA§, V. S. KALYANARAMAN¶, M. ROBERT-GUROFF*, M. POPOVIC*, M. SARNGADHARAN¶, P. C. NOWELL||, AND R. C. GALLO*

*Laboratory of Tumor Cell Biology, National Cancer Institute, Bethesda, Maryland 20205; †Research Division, Shinrakuen Hospital, Nishiariakecho 1-27, Niigata 950-21, Japan; ‡First Department of Internal Medicine and §Second Department of Pathology, Niigata University School of Medicine, Asahimachi-1, Niigata 951, Japan; ¶Littion Bionetics Laboratories, Rockville, Maryland 20850; and ||Department of Pathology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

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ABSTRACT Sera and peripheral blood cells of an adult T-cell leukemia patient and several clinically normal members of his family from the northwest coast of Japan were examined for evidence of infection with human T-cell leukemia (lymphoma) virus (HTLV). The sera of the patient and his parents had antibodies to HTLV, whereas these antibodies were absent in the sera of the patient's brother and sister. T-cell lines were established from the peripheral blood lymphocytes of all of the family members, and all except the patient's sister expressed HTLV antigens (p19, p24, and reverse transcriptase) and type-C virus particles. Not only the fresh peripheral blood lymphocytes from the patient but also those from his clinically normal mother showed abnormal morphology of the kind characteristic of some patients with T-cell leukemia. These studies are consistent with previous evidence indicative of a high rate of HTLV infection within families, and they show that people whose sera are negative for antibodies may still be infected by HTLV. In addition, the results indicate that infection of T cells by HTLV can be associated with morphological transformation of the cells without other signs of leukemia.

A type-C retrovirus known as human T-cell leukemia (lymphoma) virus (HTLV) was first isolated in the United States in some cases of adult T-cell lymphoma and leukemia (1-3). This virus has been shown subsequently to be associated with T-cell leukemia patients from Japan (4, 5) and the West Indies (6, 7) by the detection of HTLV-specific antibodies in the serum of these patients. The presence of HTLV-specific antibodies in the serum and the expression of HTLV in cultured T cells have also been observed in T-cell leukemia patients originating from other regions of the United States, Israel, and Ecuador (6-10).

A form of adult T-cell leukemia (ATL) involving mature T lymphocytes has been described in Japan (11-13) and has been distinguished from other adult T-cell malignancies, chiefly by its tendency to cluster in Japanese originating from the islands of Kyushu and Shikoku. Recently, apparent clusters of patients with ATL have also been identified in the northwest coast of Japan (unpublished data), and leukemias and lymphomas with features similar to Japanese ATL have been observed in other geographic areas (6-10, 14). Recently, other investigators have also reported the isolation of a type-C retrovirus from Japanese ATL patients (15, 16). The Japanese virus isolate is a member of the HTLV group because (i) antibodies in sera of Japanese ATL patients and of some normal people in Kyushu are highly

and specifically reactive with purified HTLV proteins (5) and (ii) proteins and nucleic acids of virus in the Japanese ATL cell line MT-1 are highly related or even indistinguishable from HTLV proteins and nucleic acids (17).

Recently, our seroepidemiologic studies identified a unique Japanese family infected with HTLV to an extent not previously seen. One member has ATL, and another member has morphologically abnormal lymphocytes with convoluted nuclei, typically found in T-cell leukemia or lymphoma patients. Other family members, with the exception of the patient's sister, either have HTLV-related serum antibodies or express HTLV-related antigens (or both) in cultured T cells and express type-C virus particles. The distribution of HTLV in this family is described in this report.

MATERIALS AND METHODS

Establishment of T-Cell Lines. T-cell lines were established according to an earlier procedure (18). Briefly, the mononuclear cells were separated from peripheral blood and were cultured in tissue culture flasks in a final volume of 5 ml containing 10^6 viable cells per ml in RPMI 1640 with 20% fetal calf serum and 10% crude or partially purified T-cell growth factor. The p24 (19), p19 (20), and reverse transcriptase (RT) activities (21) of HTLV were determined in the third or fifth passage in cells and in culture fluids.

Antibodies in Sera to HTLV. Sera were screened for HTLV-specific natural antibodies by a solid-phase radioimmunoassay (RIA) (22), a competition binding assay using a monoclonal antibody to HTLV p19 (23), or a radioimmunoprecipitation assay using homogeneous HTLV p24 (5, 19).

Assays for Expression of HTLV Proteins. Expression of HTLV p19 was monitored by an indirect immunofluorescence assay on cells fixed with methanol/acetone, 1:1 (vol/vol), by using a monoclonal antibody to HTLV p19 (20, 24). Expression of HTLV p24 was monitored by a competition radioimmunoprecipitation assay as described (19).

RT. HTLV RT activity in cell culture supernatants was assayed at 37°C in 0.05-ml reaction mixtures containing 50 mM Tris-HCl (pH 7.5), 100 mM KCl, 7 mM MgCl₂, 1.4 mM dithiothreitol, 0.1% Triton X-100, 5 µg of (dT)₁₅-(A)_n as the template primer, and [³H]dTTP (5,000 cpm/pmol) (21, 25). Cell culture supernatants were concentrated 30-fold prior to assays.

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Abbreviations: HTLV, human T-cell leukemia (lymphoma) virus; ATL, adult T-cell leukemia; RT, reverse transcriptase; RIA, radioimmunoassay.

Chromosome Analysis. Air-dried chromosome preparations were made from the T-cell lines of three family members and were trypsin-Giemsa banded by standard methods (26). At least 30 metaphases were counted and four karyotype analyses were done on each cell line.

RESULTS

Family and Patient History. Paternal and maternal ancestors of this family were from Akita prefecture in the northwest part of Honshu, Japan. There were no known family ties to the islands of Kyushu or Shikoku, areas known to be endemic for

ATL (11–13) and for HTLV (4–7, 16, 27). The patient (S.K.), a 21-yr-old male college student, had generalized skin tumors and subsequently developed enlarged inguinal lymph nodes, fever, and a pruritic skin rash on his feet and legs. His leukocyte count was 71,000 cells per mm^3 containing 65% lymphocytes. The mature T cells had convoluted, lobulated, or cleaved nuclei (Fig. 1A), and the diagnosis of ATL was made. After a short remission with vincristine, vinblastine, and prednisone, the patient developed numerous subcutaneous tumor nodules, severe edema of the eyelids, and a diffuse, erythematoid rash over much of his body. He had extensive peripheral lymphadenopathy, slight

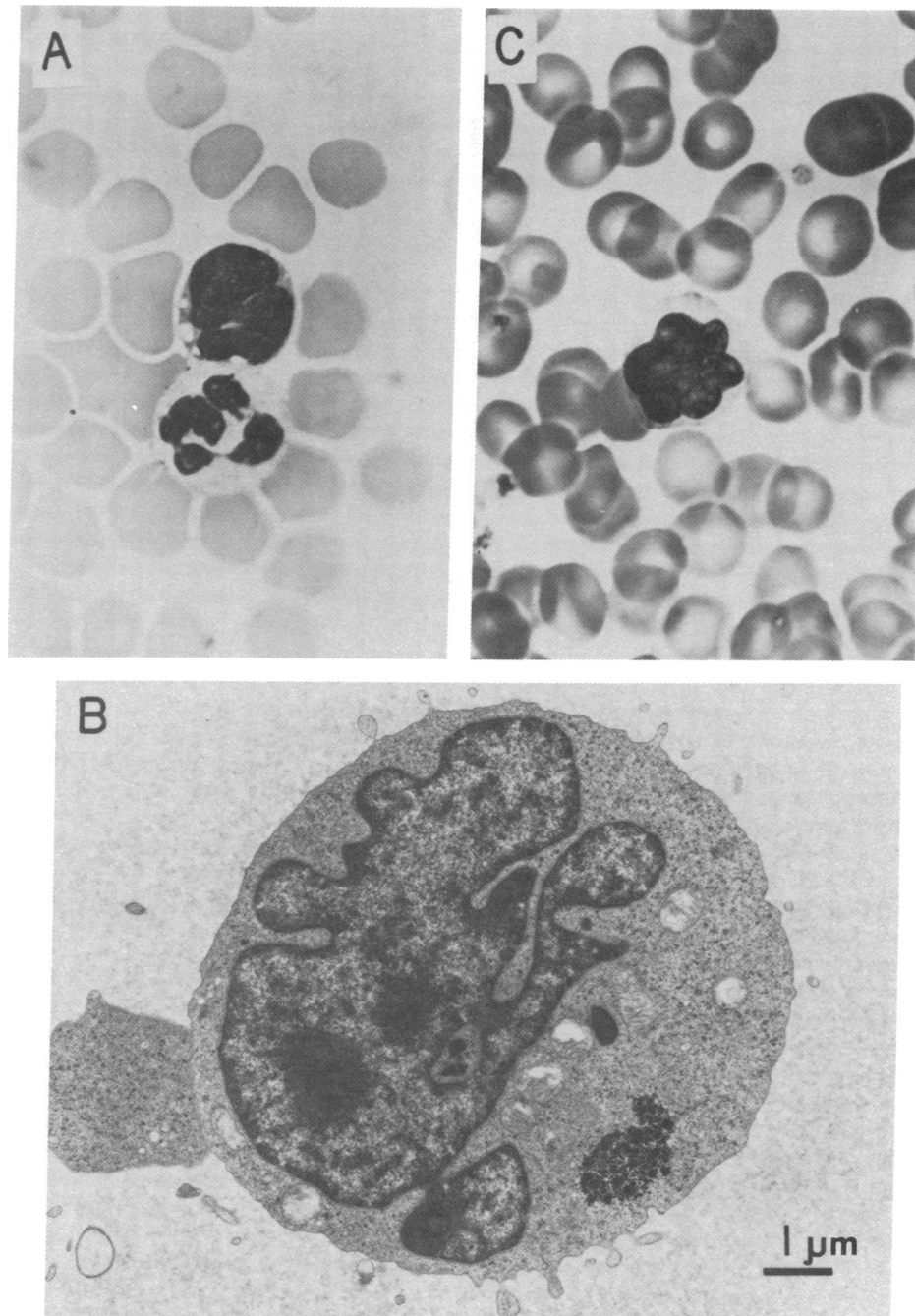


FIG. 1. (A) Light microscopy of patient's cells. An atypical lymphocyte with convoluted nucleus in the peripheral blood of the patient with T-cell leukemia (cell on top). Lower cell is a normal granulocyte. ($\times 1,300$.) (B) Electron micrograph of a representative atypical lymphocyte from the peripheral blood of the patient with T-cell leukemia exhibiting convoluted nucleus. (Bar = $1 \mu\text{m}$.) (C) Light microscopy of the T cells of the patient's mother. An atypical lymphocyte with convoluted nucleus. This atypical lymphocyte seems quite like that found in the peripheral blood of the patient. ($\times 1,300$.)

Table 1. Summary of clinical and experimental results on an ATL patient and his family

Characteristics	Family members				
	Patient (son) (S.K.)	Father (H.K.)	Mother (T.K.)	Brother* (B.K.)	Sister* (M.K.)
Age, yr	21	49	45	18	18
Abnormal morphology of peripheral blood lymphocytes, % of total cells	80	None	7	None	None
Antibody to HTLV in serum	+	+	+	-	-
HTLV antigens in T-cell lines	+	+	+	+	-
RT in cell culture fluids	+	+	+	+	-
Type-C virus particles (EM)	+	+	+	+	-

EM, electron microscopy.

* B.K. and M.K. are twins.

liver and spleen enlargement, became unresponsive to therapy, and died 3 yr after the onset of disease. Examination of his bone marrow (in relapse) showed infiltration with atypical lymphocytes to the same degree as found in the peripheral blood (about 30%). These cells were strongly periodic acid-Schiff positive and acid phosphatase negative. Electron microscopy examination revealed markedly atypical nuclei, some with nuclear convolutions (Fig. 1B), similar to those of Sezary T-cell leukemia. The majority of tumor cells from biopsies of skin tumors and peripheral blood were erythrocyte-rosette positive, confirming their T-cell nature.

Evidence for HTLV Infection in the Family of Patient S.K. This is a unique Japanese family from northwest Japan showing a high degree of HTLV infection. Table 1 summarizes the experimental findings on the blood specimens from the patient and his family members. Three members of this family, including the patient (S.K.), were positive for serum antibodies to HTLV (Table 2). The cultured T cells from all of the family members, with the exception of the patient's sister, expressed HTLV antigens (p19, p24, and RT) (Table 2) and type-C virus particles (Table 1). Representative examples of extracellular type-C virus particles released by cultured T cells of both the patient (S.K.) and his parents are shown in Fig. 2. The peripheral blood of the patient's clinically normal mother had abnormal lymphocytes (7%) containing convoluted nuclei (Fig. 1C), similar to those seen in other ATL patients. Her peripheral blood lymphocytes also showed high spontaneous DNA synthesis (data not shown).

Cytogenetic Studies. Chromosome studies on T-cell lines from the patient (S.K.), his mother (T.K.), and his brother (B.K.) showed a normal karyotype in each instance. Occasional meta-

phases from the patient's cell line had dicentric and trivalent chromosomes, but there was no evidence of a consistent cytogenetic abnormality.

DISCUSSION

The incidence of ATL is relatively high among native residents of Kyushu and Shikoku in southwestern Japan and in people who were born in these areas but moved to other parts of Japan later in life (11-13). Our seroepidemiologic studies with 19 families identified a unique Japanese family with a high degree of HTLV infection (24). We have examined this family with a 21-yr-old male T-cell leukemia patient from the northwest coast of Japan who have had no family ties to the islands of Kyushu or Shikoku. Most members of this family are infected with this virus. High-titer HTLV antibodies were observed in the serum of the patient and his parents. T-cell lines were established from the peripheral blood of all of the family members and all of the T-cell lines, with the exception of the one from the patient's sister, expressed HTLV proteins and extracellular type-C virus particles. The mother (T.K.) of this ATL patient (S.K.) has a small number of abnormal (7%) lymphocytes in her peripheral blood, suggesting a "preleukemic" state. HTLV integration sites are unique in a population of tumor cells, indicating that these diseases are monoclonal (28). It will be of considerable interest to determine if this is also the case in the apparent preleukemic state.

Recent seroepidemiologic studies on family members of patients with HTLV-associated T-cell malignancies in Japan, the United States, and the Caribbean have shown that the family members of ATL patients are more likely to possess serum antibodies to HTLV than random healthy donors from the same

Table 2. Detection of HTLV in family members of a patient with ATL

Family member	Natural antibodies to HTLV proteins			Expression of HTLV proteins in cultured T cells		
	Solid-phase RIA, titer*	RIP of p24, titer*	Competitive binding with α -p19, titer*	RIP of p24, ng/ml	IFA of p19, % positive cells	RT, pmol of [³ H]dTMP incorporated per ml of extract
S.K. (patient)	80	1,260	4	174	39	6
H.K. (father)	35	—	—	400	38	14
T.K. (mother)	640	600	20	2,700	54	33
B.K. (brother)	—	—	—	20	8	3
M.K. (sister)	—	—	—	0	0	0

IFA, immunofluorescence; RIP, radioimmunoprecipitation.

* Titers are expressed as follows: for the solid-phase RIA, as the reciprocal of the serum dilution at which binding is 50% of maximal; for the RIA of p24, as the reciprocal of the serum dilution giving 20% precipitation of the labeled protein; for the competitive binding with p19 antibody (α -p19), as the reciprocal of the serum dilution giving 50% competition of binding with p19 antibody.

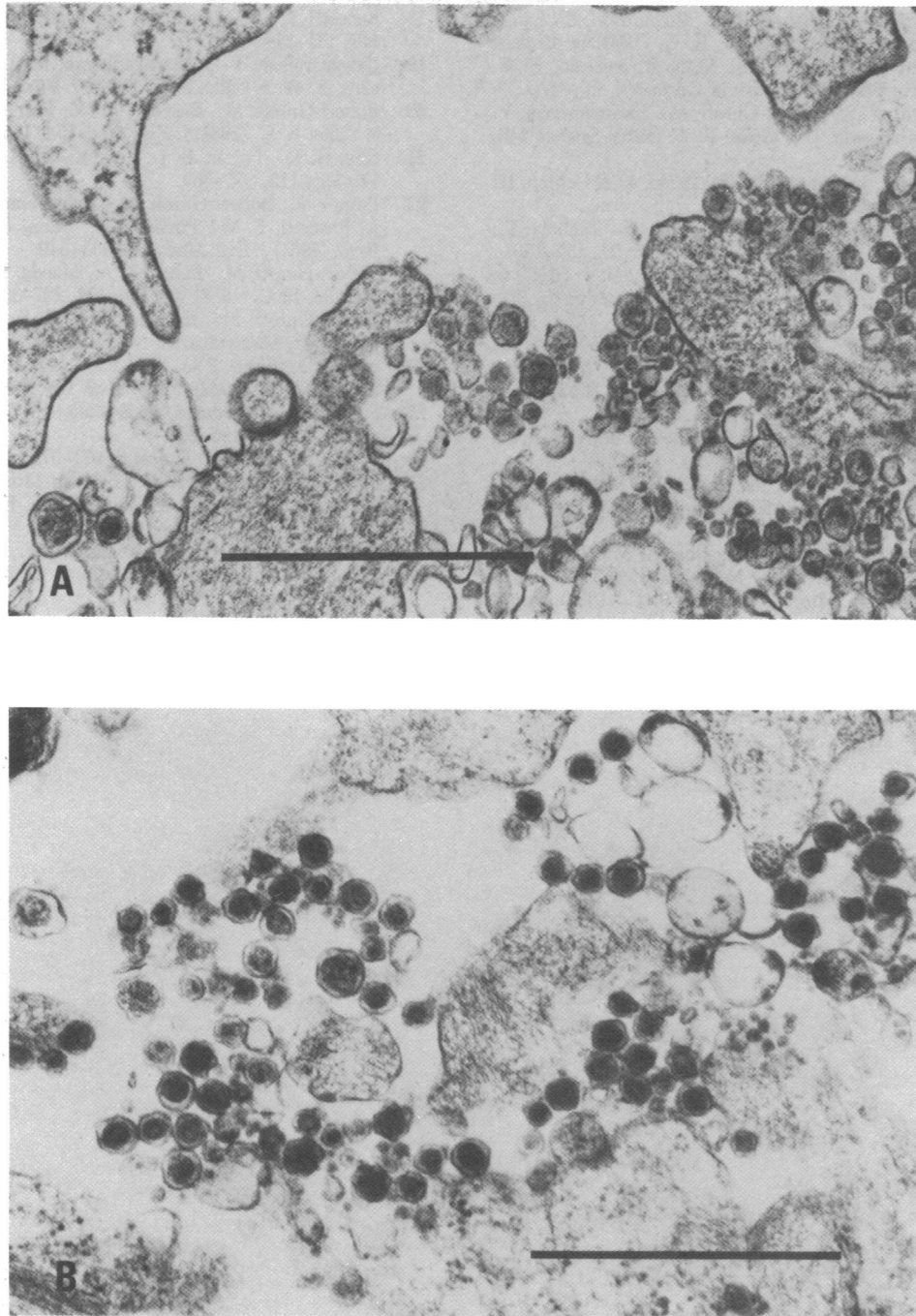


FIG. 2. Electron micrograph of the type-C virus particles released by cultured T cells of (A) the ATL patient and (B) his mother. (Bar = 1 μ m.)

region (24). The HTLV infection rate of this unique family from northwest Japan far exceeds the level of HTLV infection observed in other families in this geographic region. Further studies on the distribution of HTLV in various parts of the world may prove to be useful in discerning the involvement of this virus in other patients with T-cell malignancies.

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