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Research Article

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The Cutaneous T Cell Lymphoma, Mycosis Fungoides, Is a Human T Cell Lymphotropic Virus-associated Disease

A Study of 50 Patients

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Abstract

For nearly two decades it has been suspected that the cutaneous T cell lymphoma, mycosis fungoides (MF), and its leukemic variant, the Sézary syndrome, are caused by the human T lymphotropic virus (HTLV-I/II). Arguments against this concept included the finding that only a small number of MF patients have antibodies to HTLV-I/II and that attempts to detect proviral sequences by mere Southern hybridization of extracted DNA usually met with failure. However, we have reported repeatedly that HTLV-like particles emerge in blood mononuclear cell (PBMC) cultures of practically all patients with this disease. In several instances, the particles were identified as HTLV by immunoelectron microscopy as well as biomolecular analysis. With the assumptions that the virus in MF patients may have become defective and that the remaining signal may be too weak for detection by Southern hybridization alone, the extracts of freshly isolated PBMC of 50 consecutive patients were subjected to combined PCR/Southern analysis. Here we report the presence of HTLV pol and/or tax proviral sequences in 46 out of 50 (92%) of the patients tested. In addition, five of the patients, who lacked antibodies to HTLV-I/II structural proteins, were found to be seropositive for tax. It thus seems reasonable to conclude that MF/Sézary syndrome is an HTLV-associated disease and that lack of an immune response does not preclude infection with this type of virus. (*J. Clin. Invest.* 1995. 95:547–554.) Key words: tax • leukemic lymphocytes • human T cell lymphotropic virus type I • Sézary syndrome • retroviruses

Introduction

The human T cell lymphotropic virus type I (HTLV-I)[1] is known to be the etiologic agent of adult T cell leukemia/lymphoma (ATLL) (1–4) and the neurological conditions tropical spastic paraparesis (5) and HTLV-associated myelopathy (6). These relationships were demonstrated by the consistent detection of antibodies to HTLV virion proteins (gag, env) in patients' sera and sequences representing intact HTLV-I proviral genomes in peripheral blood mononuclear cells (PBMC) (2–4, 7). Although an association between HTLVs and the cutaneous T cell lymphoma, mycosis fungoides (MF), and its leukemic variant, the Sézary syndrome (SS), has long been suspected, definitive evidence for such a relationship has been difficult to establish. Only 1–10% of MF patients, who live in HTLV nonendemic areas, have detectable antibodies to HTLV-I/II (8–12), and HTLV-related proviral sequences are usually not detected in their PBMC by Southern analysis alone. Other observations, which have put the relatedness of ATLL and MF in question, concern the nature of the neoplastic cell itself. Although both the ATLL and MF/SS cells are $CD4^+$ helper T lymphocytes (13, 14), MF or Sézary cells, terms used interchangeably here, usually lack receptors for IL-2 (15) and fail to proliferate spontaneously, in vitro (13, 15, 16). Their cerebriform nucleus is distinct from the "flower nucleus" of ATLL cells (14, 17–20). Rearrangements of the T cell receptor β chain are not consistently demonstrable (21). Although karyotypic abnormalities have been observed in some cases (22–24), no disease-specific chromosomal alterations have been identified. It was not until hematopoietic growth factors became available that attempts to culture Sézary cells became feasible (16). This led to the discovery that PBMC from almost all patients with MF become immortalized after a few weeks in culture when growth factors can be withdrawn and that they release particles which, ultrastructurally, are indistinguishable from HTLV-I (16). In several instances, the identity of these particles as HTLV could be confirmed by immunoelectron microscopy and/or immunohistochemistry (16) and the polymerase chain reaction (PCR), combined with Southern analysis, detected relevant proviral DNA sequences (16, 25). One patient's PBMC were shown to harbor DNA sequences related to HTLV-II (25). On the basis of these tantalizing observations, a more systematic effort was undertaken to prove that MF/SS is also an HTLV-associated disease. Firstly, biomolecular analyses were carried out on freshly isolated blood specimens rather than on cultured cells, and, secondly, a major effort was focused on the detection of tax, the putative HTLV-transforming gene (4, 26–30) which, so far, has not been found among human endogenous retroviral or other human genomic sequences (4, 31–33). We are now able to report that most, if not all, MF patients possess proviral DNA sequences related to HTLV tax and/or pol-I in their circulating mononuclear cells.

Methods

Patients. 50 patients with biopsy-proven cutaneous T cell lymphoma, MF, were the subject of this study (Table I). The diagnoses had been

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1. *Abbreviations used in this paper:* ATLL, adult T cell leukemia/lymphoma; CLL, chronic lymphocytic leukemia; HTLV-I/II, human T cell lymphotropic virus types I and II; MF, mycosis fungoides; SS, Sézary syndrome.

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Table I. Clinical and Laboratory Features of 50 MF Patients Included in This Study

Patient	Sex/Age	Race risks*	SCC‡	Serology§		PCR/ Southern	
				HTLV	tax	pol	tax
			%				
R. A.	F/71	C/N	70	+	+	+	+
A. B.	M/57	C/N	59	−	−	+	+
J. B.	M/73	A/Caribbean	20	−	−	+	+
M. B.	F/74	AA/±	46	−	−	+	+
R. B.	F/80	C/N	60	−	−	+	+
S. Be.	M/63	C/N	31	−	−	−	+
S. Bo.	M/63	C/N	45	−	−	+	+
H. Cl.	M/34	AA/±	30	−	−	−	−
H. Co.	M/59	AA/±	43	−	−	+	−
F. D.	M/31	H/Colombian	53	−	−	+	−
O. D.	M/76	C/N	38	−	−	−	−
F. F.	M/46	C/HS	18	−	nd	+	+
G. Fo.	F/77	C/N	42	−	−	+	+
G. Fx.	M/40	C/HS	nd	−	nd	+	+
H. F.	F/69	C/N	26	−	−	−	+
G. Ge.	M/73	C/N	65	−	−	+	+
G. Gl.	M/54	AA/±	19	−	−	+	+
J. G.	M/73	C/N	34	−	−	−	+
L. G.	M/66	AA/IVDA	27	+	−	+	+
M. Gg.	M/63	C/N	72	−	−	+	+
M. Gt.	F/67	AA/±	78	−	−	+	+
N. G.	M/43	H/Caribbean	16	−	−	+	+
W. G.	M/63	C/N	33	−	−	+	+
B. H.	F/51	AA/±	40	−	−	+	+
H. H.	M/64	AA/±	61	−	−	−	−
R. H.	F/76	C/N	94	−	nd	−	+
D. J.	F/32	AA/±	21	−	−	+	−
B. L.	M/44	C/N	71	−	−	+	+
G. L.	M/62	H/N	25	−	−	+	+
M. L.	F/51	AA/±	30	−	−	+	−
P. L.	F/48	C/N	22	−	−	+	+
T. L.	M/43	C/N	56	−	−	−	+
A. M.	M/66	C/N	25	−	−	−	+
C. M.	F/60	C/N	18	−	−	+	+
F. M.	M/76	C/N	52	−	+	+	+
L. M.	M/67	C/N	20	−	+	+	+
M. M.	F/62	A/Caribbean	47	−	−	−	+
A. P.	M/7	A/Caribbean	27	−	+	+	+
M. P.	F/55	C/Sicillian	29	−	+	+	+
J. P.	M/72	AA/±	27	−	−	+	+
J. R.	M/62	C/N	27	−	−	+	−
S. R.	F/69	C/transfusion	26	−	−	+	+
F. S.	M/35	C/N	29	−	−	+	+
M. S.	F/64	AA/±	43	nd	nd	−	+
A. W.	M/60	C/N	29	−	−	+	+
C. W.	M/70	AA/±	58	+	−	+	+
D. W.	M/75	C/N	47	−	−	+	+
J. W.	M/68	C/N	60	−	−	+	+
S. W.	M/43	AA/±	25	−	−	−	−
I. Y.	M/86	C/Iranian	41	−	−	+	−

* AA, African American; A, African/Caribbean; H, Hispanic; and C, Caucasian. § HTLV, ELISA or Western blot against HTLV structural proteins, and tax, ELISA against tax synthetic peptide. ‡ Sézary cell count (%); nd, not done. ±, Endemic origin questionable; N, No apparent risk factors elicited by history; HS, Homosexual; and IVDA, Intravenous drug abuse.

made on clinical grounds and by skin biopsy and/or peripheral blood Sézary cell counts carried out on the ultrastructural level (14, 20). Only three of the patients (R. A., L. G., and C. W., see Table I) proved to be seropositive for antibodies to HTLV-I/II when this was tested by routine ELISA and Western blot as defined by established guidelines (34). Plasma samples from all of our MF patients are routinely tested for HTLV-I/II antibodies at the NY Blood Center, using ELISA and Western blot assays obtained from Abbott Laboratories (North Chicago, IL), Cambridge Biotech (Worcester, MA), and Cellular Products (Buffalo, NY). Recently we have used the HTLV-I (r p21e enhanced), HTLV-II Western blot kit from Cambridge Biotech to confirm that patients tested previously and found to be negative for HTLV-I/II antibodies do not have detectable antibodies to HTLV structural proteins even using a more sensitive test than was earlier available. Two patients were known also to be infected with HIV (patients F. F. and G. Fx.). 35 of the patients were male and 15 were female; 17 were African American, 30 were Caucasian, and 3 were Hispanic. Their ages ranged from 7 to 86 yr. Their Sézary cell percentages among nonadherent mononuclear cells, determined ultrastructurally as described (14, 20), ranged from 16 to 94% with a mean of 40.3±18.3. Evaluable risk factors are listed in Table I. The 7-yr-old patient, A. P., is noteworthy because his skin lesions appeared at the age of 4. He has no detectable antibodies to HTLV-I/II structural proteins by routine tests.

Isolation of cells and cell culture. PBMC were isolated from heparinized blood by Ficoll/Hypaque gradient centrifugation using procedures routinely carried out in this laboratory (14). Some samples were further depleted of monocytes using the lymphocyte separating reagent as described before (35). Cells for use in the PCR were washed in Ca^{2+}- and Mg^{2+}-free PBS and stored either in suspension at 4°C or pelleted and frozen at −70°C. PBMC were also placed in culture in the presence, initially, of: PHA (15 μg/ml), GM-CSF (100 U/ml), and IL-2 (10 U/ml), and supplemented with 10% fetal calf serum, as described elsewhere (16). The prototypic HTLV-I and -II cell lines C91PL (36) and MoT (37), were obtained from Dr. Wade Parks (Department of Pediatrics, New York University), and were used throughout these studies as positive controls for PCR/Southern analyses. PBMC from 26 randomly chosen healthy adult volunteers and from 10 patients with chronic lymphocytic leukemia of B cell phenotype were collected and processed by the same procedures and in parallel with specimens of MF patients.

Electron microscopy. Freshly isolated and cultured cells fixed in 3% phosphate-buffered glutaraldehyde were postfixed with osmium, dehydrated, and embedded in Polybed 812 (Polysciences Inc., Warrington, PA). Thin sections stained with uranyl acetate and lead hydroxide were viewed with a Siemens Elmiskop I electron microscope.

PCR. Whole cell lysates of PBMC from patients with MF, 10 patients with B cell chronic lymphocytic leukemia (B CLL), 26 healthy volunteers, and cell lines C91PL and MoT were processed for PCR essentially as described by Kim and Smithies (38), as modified by Joyner et al. (39), and used by us as described previously (40). In brief, ~ 1×10^5 cells washed in PBS and resuspended in distilled water were lysed by boiling 8 min, freezing at −70°C, thawing, and mild sonication in a bath sonicator (Heat Systems Incorporated, Farmingdale, NY). Cell lysates were then incubated for 90 min at 50°C in the presence of proteinase K (2 μg/reaction), followed by boiling for 6 min to inactivate the protease. Samples were then subjected to 30 or 60 cycles of PCR consisting of 2 min at 94°C, 1 min at 55°C, and 30 s at 72°C per cycle, using reagents from the Perkin Elmer (Norwalk, CT) Gene Amp core reagents kit and the Perkin Elmer Thermal Cycler. The reaction conditions were as described previously (16, 25) except that the $MgCl_2$ concentration was 2.5 mM and the *Taq* polymerase concentration was increased to 0.8 U/80 μl reaction. The primers used for amplification of HTLV-I/II pol and tax proviral sequences, SK110/111 and SK43/44, respectively (41) (see Fig. 1), and β globin, PCO4 and GH20 (42), were synthesized locally by Dr. Bernard Goldschmidt (Department of Environmental Medicine at this institution). Specimens from 4 MF patients found to be negative for tax and pol sequences after 30 cycles of PCR (H. Cl., O. D., H. H., and S. K.) were subjected to 30

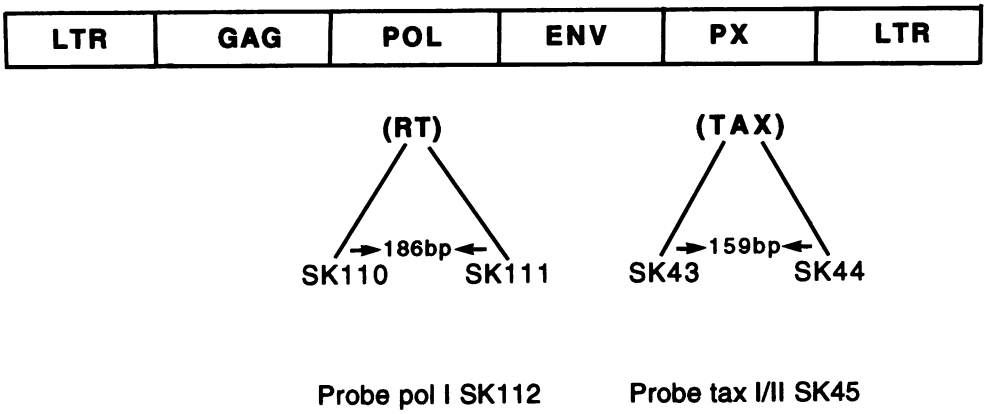

Figure 1. Genomic structure of HTLV indicating primers and probes used in PCR/Southern analyses.

additional cycles, as were the 26 normal blood donors and the 10 patients with B CLL. Primers to β globin were included as amplification controls to validate HTLV-negative results.

Southern blot hybridization. PCR products were resolved through 4% agarose gels in the presence of ethidium bromide in TAE (0.04 M Tris-acetate, 0.001 M EDTA) buffer as described previously (16, 25). DNAs in gels were then denatured in 1.5 M NaCl, 0.5 M NaOH, neutralized in 3 M Na acetate, pH 5.5, followed by overnight transfer to Biotrans nylon membranes (ICN Biomedicals Inc., Irvine, CA) by the method of Southern (43). Prehybridization, hybridization, and posthybridization washes were conducted essentially as described previously (16, 25), with the following exceptions. Prehybridization and hybridization were performed for 2 h and overnight, respectively, at 43°C. Probes SK112 (HTLV-I pol) and SK45 (for HTLV I/II tax) (Fig. 1) were 3′-tailed with digoxigenin-11-dUTP, using reagents and procedures from the Genius 6 nonradioactive probe labeling kit (Boehringer Mannheim Biochemicals, Indianapolis, IN) (44). Hybridization to detect HTLV tax was performed in the presence of 50% formamide. After hybridization, filters were subjected to 2–15-min washes in 2 × SSC, 0.1% SDS and, 2–15-min washes in 0.2 × SSC, 0.1% SDS, all at room temperature, followed by a wash at 55°C in 0.2 × SSC, 0.1% SDS. Bound probe was detected using reagents and procedures included in the Genius 3 kit (Boehringer Mannheim Biochemicals). Filters were blocked and further incubated in the presence of Fab′ fragments of antibodies to digoxigenin that were conjugated with alkaline phosphatase and then in the substrates 4-nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (44). HTLV-positive and -negative cell samples were included in all experiments.

Cloning and sequencing of HTLV tax PCR products. The full length 159-bp tax PCR products amplified from PBMC of patient F. M. were eluted from 1% agarose gels, phosphorylated by T4 polynucleotide kinase, and blunt-end ligated to SmaI-cleaved pUC19 (45). Recombinant plasmid DNA was introduced into *Escherichia coli* strain DHFα by electroporation. After amplification in the presence of carbenicillin, colonies harboring plasmids containing HTLV tax–related sequences were identified by colony hybridization, using digoxigenin-tailed SK45. (See the previous section on Southern analysis for details of probe labeling, hybridization, posthybridization filter washing, and detection of bound probe.) Nitrocellulose rather than nylon filters were used for colony hybridization. After alkaline denaturation in 1.5 M NaCl, 0.5 M NaOH, filters were neutralized in 1.5 M NaCl, 0.5 M Tris, pH 7.5, and baked in a vacuum oven at 80°C for 1 h. Plasmid DNAs were selectively isolated using the Promega Corp. (Madison, WI) Magic MiniPreps system. DNAs shown to undergo release of ~ 200-bp fragments (159-bp tax insert + ~ 43-bp pUC19 vector sequences) that hybridized to HTLV tax, after double digestion with EcoRI and PstI and electrophoresis through 1% agarose gels containing ethidium bromide, were sequenced by the dideoxy chain termination single strand DNA sequencing method of Sanger (46), using the Sequenase kit reagents and procedures obtained from the U. S. Biochemical Corp. (Cleveland, OH). Reactions were labeled by incorporation of ^{35}S-dATP, and chain-terminated reaction products were electrophoresed through 6% polyacrylamide sequencing gels. Sequences were determined after autoradiography of dried gels and were compared with those published for prototypic HTLV-I and -II tax sequences (31, 37, 47, 48). PCR products from PBMC of patients R. A. and R. B., in addition to those from the HTLV-I–infected cell line C91PL, were cloned directly, using the pGEM-T vector system (Promega Corp.) and sequenced by Dr. Bernard Goldschmidt using the Applied Biosystems (Foster City, CA) automated sequencer.

tax ELISA. Because of the unavailability of commercial tests to detect HTLV tax antibodies, a 20–amino acid peptide corresponding to the carboxy terminus of HTLV-I tax (QISPGGLEPPSEKHFRETEV) (47), a region known to be immunodominant in humans infected with HTLV-I (49), was synthesized by Dr. Bernard Goldschmidt, to serve as antigen. An ELISA test was performed essentially as described by Ehrlich et al. (50), using reagents obtained from Pierce Chemical Co. (Rockford, IL). Microtiter wells (Immulon 4; Dynatech, Chantilly, VA) were coated with 2 μg/well of peptide, and reactions were developed using goat anti–human IgA + IgG + IgM antibodies conjugated with horseradish peroxidase, in the presence of the substrate ABTS. Tests were read at 410 nm using the Dynatech MR600 microplate reader. Samples with OD > 3 SDs above the mean obtained after measuring responses in plasmas from 15 PCR tax sequence–negative control individuals were considered positive (51).

Results

Detection of HTLV-like virus particles in cultures of PBMC from MF patients. Particles indistinguishable from HTLV-I were detected by electron microscopy in cultures of PBMC of all patients presented in this report (Fig. 2). Such particles have never been observed in lymphocyte cultures from healthy individuals.

Detection of HTLV proviral sequences by PCR/Southern analysis. Fig. 3 shows the results of representative Southern blots of pol-I and tax sequences amplified from PBMC of 10 MF patients of the 50 tested. As seen in Fig. 3 *A*, when PCR-amplified DNAs were subjected to Southern analysis, the pol-I probe hybridized to DNA amplified from PBMC from nine of these patients, as it did to DNA from the prototypic HTLV-I cell line, C91PL. No pol-related bands were detected in samples from the HTLV-II cell line, MoT, the healthy control, or in the sample obtained from patient M. M.

The tax probe bound to DNA amplified from both the prototypic HTLV-I and HTLV-II cell lines (I and II in Fig. 3 *B*) and to PBMC-derived DNA amplified from all 10 MF patients.

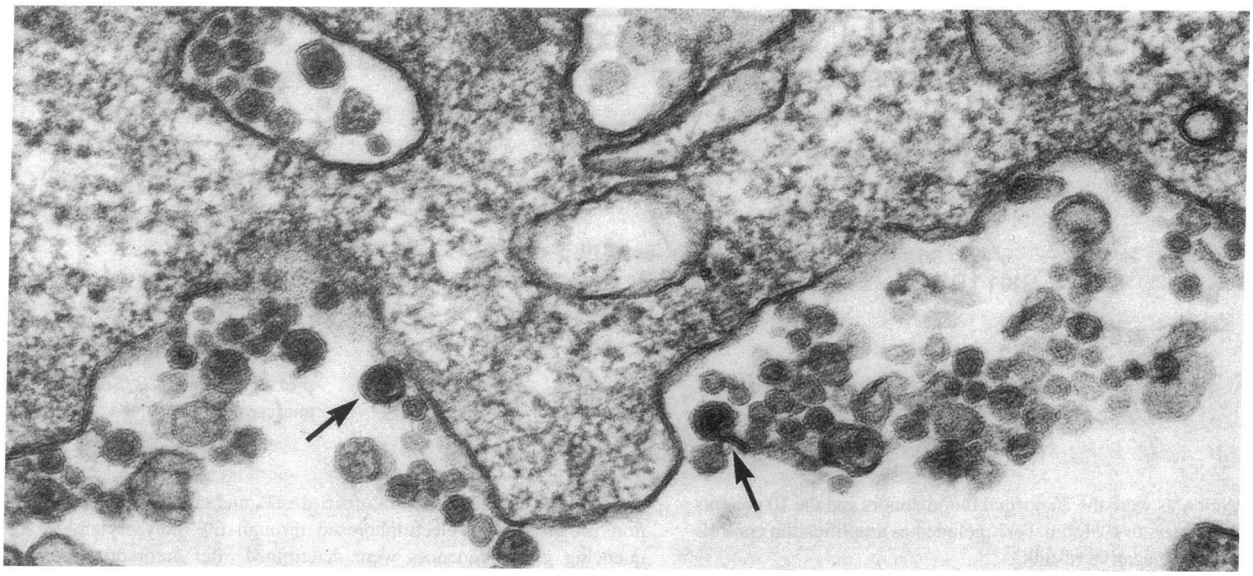

Figure 2. Detail of a mononuclear cell in a 64-d-old culture generated from PBMC of patient G. G. Arrows indicate virus particles. ×90,000.

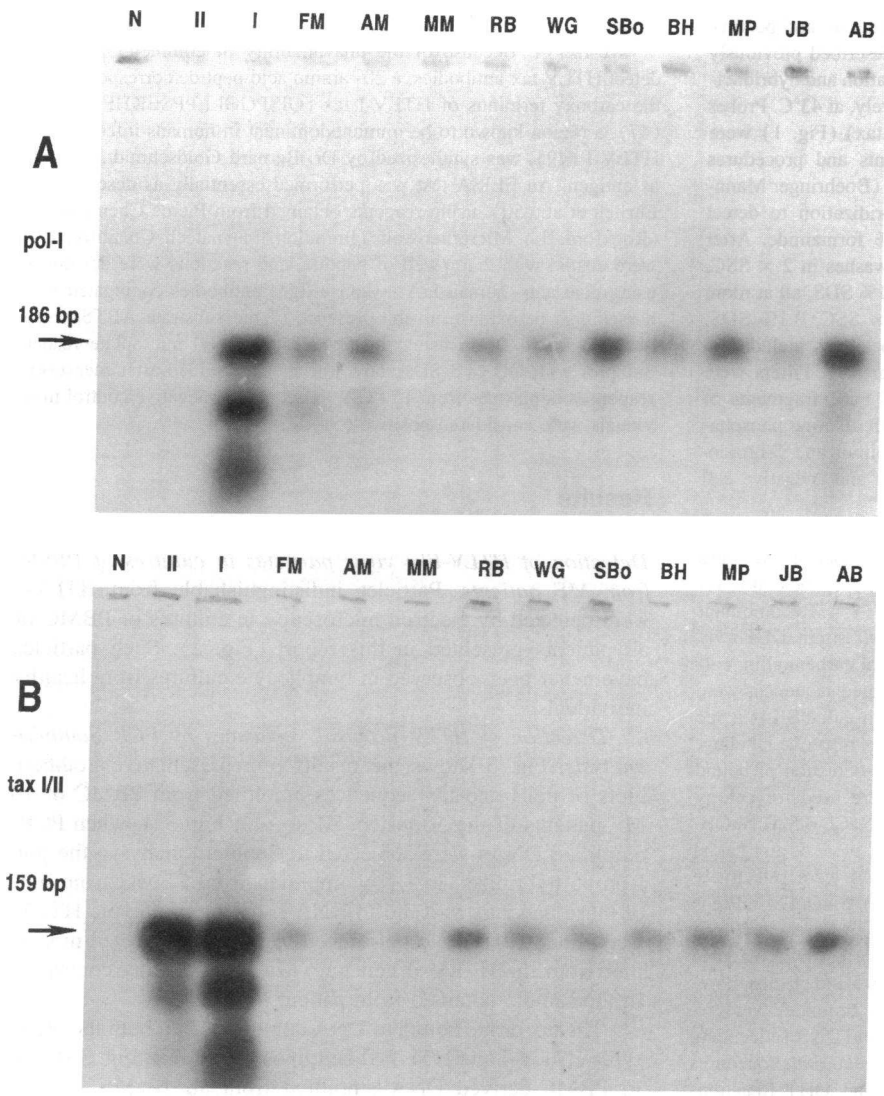

Figure 3. Southern blot detection of HTLV proviral DNA sequences amplified by PCR from PBMC of MF patients. Samples in *A* were amplified with HTLV I/II pol primers SK 110/111 and detected with pol-I–specific, digoxigenin-labeled probe SK112. tax sequences visualized in *B* were amplified using SK43/44 and probed with SK45. Arrows indicate expected 186-bp pol-I and 159-bp tax I/II amplification products observed in positive samples. Sources of samples: lane *N,* PBMC from a healthy volunteer, lane *I,* HTLV-I–infected cell line, C91PL; lane *II,* HTLV-II–infected cell line, MoT; lanes *1–10,* PBMC from 10 different MF patients.

Table II. Summary of PCR/Southern Blot Hybridization Data

pol$^+$	
MF patients	38/50 (76%)
Healthy controls	0/26
B CLL patients	0/10
tax$^+$	
MF patients	40/50 (80%)
Healthy controls	0/26
B CLL patients	0/10
pol$^+$ and/or tax$^+$	
MF patients	46/50 (92%)
Healthy controls	0/26
B CLL patients	0/10

$P < 0.0001$.

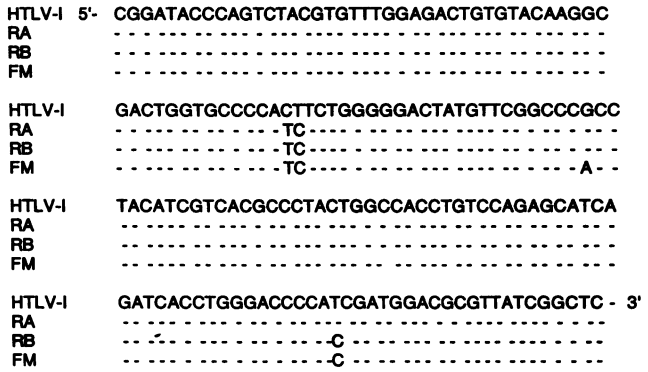

Figure 4. Comparative nucleotide sequence of amplified HTLV-I tax regions. Sequence alignment of the 159-bp tax proviral DNA sequences detected in PBMC from MF patients R. A., R. B., and F. M. with those of the published sequence for the prototypic HTLV-I–infected cell line, MT-2 (31, 48).

Overall, 92% of the patients with biopsy-proven MF had HTLV pol and/or tax proviral DNA sequences (Table II). In specimens from 33 of the 38 patients found to be positive for pol sequences, positive results were obtained after 30 cycles of PCR, whereas samples from the other 5 required 30 additional PCR cycles to detect these sequences. tax sequences were detected after 30 cycles of PCR in specimens from 34 of the 46 tax$^+$ patients. Samples from 6 patients (M. Gt., L. M., F. F., G. Fx., P. L., and M. Gg.) proved positive after a total of 60 cycles of PCR. These sequences were not detected in samples from 26 healthy controls which were processed and run simultaneously with those from the MF patients. In addition, and perhaps more importantly, such sequences also failed to be detected in samples obtained from 10 patients with B cell CLL. Amplification of β globin sequences was evident, as expected, in all samples prepared from healthy controls, the patients with CLL, as well as in the 4 MF specimens which were negative for HTLV sequences, even after 60 cycles of PCR.

With the exception of patient G. Gl., the PCR/Southern results for 50 MF patients summarized in Table II are based on analysis of tax and pol sequences in PBMC isolated from Ficoll-Hypaque gradients. Specimen G. Gl. was monocyte depleted. Specimens J. B., A. B., M. B., M. Gg., W. G., G. L., B. L., and J. P. have been subjected to PCR/Southern analyses for tax proviral sequences before and after depletion of monocytes. Paired total PBMC and monocyte-depleted specimens from all eight patients proved positive. Although the results support the idea that viral sequences may be harbored in lymphocytes of MF patients, these data do not rule out the possibility that monocytes might also be infected.

Nucleotide sequence determination of the amplified tax region. The cloned nucleotide sequences of the HTLV tax region amplified and detected in the PBMC from three of these patients, R. A., R. B., and F. M., are compared with those published for the prototypic HTLV-I–infected cell line MT-2 (31, 48), as shown in Fig. 4. Only four nucleotide base pair differences were detected in this region between HTLV-I and the patients' amplified sequences. Of these, one G to A transition observed for patient F. M. only would be predicted to result in an arginine (CGC) to histidine (CAC) exchange and the other (T to C) (patients R. B. and F. M.) in replacement of isoleucine (ATC) with threonine (ACC). The double base pair change (CT to TC), observed in all three MF patients, would not be expected to alter the amino acid sequence. Sequencing analysis of the tax region of the C91PL cell line used as a positive HTLV-I control in PCR/Southern analysis has not revealed any sequence differences from that in MT-2, in the region of tax under consideration.

Detection of HTLV-I tax antibodies in plasmas of patients with MF by ELISA. 5 of the 46 MF patients tested by ELISA using a synthetic peptide prepared to the carboxy-terminal 20 amino acids of HTLV-I tax showed tax antibodies in plasma dilutions of 1:10 or higher. All but one of these patients had been considered serologically negative when their sera were tested for antibodies against viral structural proteins by routinely used methods that would not have measured antibodies to tax.

Discussion

Previous studies in this laboratory have shown retrovirus-like particles indistinguishable from HTLV-I (16) or HTLV-II (25) in PBMC cultures of practically all patients with the cutaneous T cell lymphoma, MF, and its leukemic variant, the Sézary syndrome. Such particles have never been seen in cultures derived from the PBMC of healthy individuals. We have now demonstrated that > 90%, if not all, of these patients, harbor HTLV proviral DNA sequences in their freshly isolated PBMC, when this is tested by PCR/Southern analysis. The HTLV-I–specific pol probe SK112 hybridized to DNA sequences amplified from extracts of PBMC of 37 of the 50 patients tested, implicating a virus related to HTLV-I in the majority of these patients.[2, 3]

The question as to why it was so difficult to prove that most MF patients are infected with HTLV seems pertinent. Several

2. In independent studies, Dr. Mary Mancewicz in the Department of Dermatology of New York University has detected HTLV-related proviral sequences in archival skin biopsies of 61 out of 89 (68%) patients with MF (unpublished observations, personal communication).

3. Since submission of this manuscript, the authors have become aware of the study of Ghosh et al., published as an abstract (1994. *AIDS Res. Hum. Retroviruses.* 10:468*a*), wherein they reported the detection of tax sequences in a high percentage of patients with MF/SS who had no antibodies to structural proteins of the virus.

reasons can be offered. First of all, patients with MF are usually seronegative for antibodies to the virus, discouraging further analysis in a clinical setting, especially since antibody positivity can usually be relied on in the diagnosis of HTLV-associated ATLL. Furthermore, patients with the endemic form of ATLL usually harbor proviral sequences spanning the entire HTLV-I genome. As a rule, their PBMC possess sufficient levels of proviral DNA to be detected by standard Southern blot hybridization techniques. This proved to be futile in the case of mycosis fungoides. It has taken the development of the PCR and the use of a variety of primers and probes to uncover evidence of HTLV infection in the majority of MF patients. It is not clear why our efforts were more successful than those of others (11, 52–54) in showing a high incidence of HTLV in MF patients, many of whom are Caucasian and do not originate from HTLV-I–endemic areas (see Table I). Part of the reason for the inconsistent detection of HTLV sequences is likely to be due to the low levels of virus or to the presence of incomplete or variant proviral sequences in the samples studied. It is too early to tell whether patients who have been shown to harbor tax but not pol-I sequences, or those who have pol but no tax sequences, actually have defective viruses, or whether variant HTLV sequences not amplified or detected by the primers/probes used, so far, are responsible for these results. On the other hand, only one of the patients, reported in more detail elsewhere (25) has been shown to be infected with HTLV-II. Use of pol-II–specific primers/probes has not resulted in detection of homologous sequences in the cells of any of the other patients studied (data not shown). Although the tax primers and probe used did not distinguish HTLV-I from HTLV-II, the sequence analysis of DNA amplified from three of the patients (Fig. 4) resembles HTLV-I in the region of tax examined (31, 37, 47, 48). HTLV-I and -II differ in this region by 25 bp (48). The significance of finding the same TC-CT base pair change in all three patients has yet to be established in that no predicted amino acid change would be expected. However, the observations that the patients do each differ from each other and from the prototypic HTLV-I sequence in the region of tax studied, albeit by only a few base pairs, essentially rule out the possibility that the viral sequences detected are contaminants. Multiple clones derived from DNA from PBMC of patients F. M. and R. B. were used to exclude artifactual PCR sequences. Therefore, the data suggest that MF is more likely to be associated with HTLV-I.

Despite the finding that peripheral blood cells of > 90% of MF patients possess HTLV-related proviral sequences, only three patients had antibodies to HTLV-I/II structural proteins (see Table I). The antibody-negative status of most of the MF patients warrants comment. It is possible that MF patients carry incomplete or variant proviral genomes as has been suggested by others (10, 11, 55). However, it is not generally appreciated that even patients with ATLL, living in endemic areas, may lack antibodies to the virus (56), and their PBMC may reveal only defective HTLVs (32, 57, 58). Where characterized in detail, gene sequences encoding viral structural proteins, such as *env* and *gag*, have been found to be missing (32, 57–59). Therefore, it is conceivable that MF patients fail to mount an immune response to HTLV structural proteins simply because the relevant viral gene products are no longer made. Alternatively, these patients may be infected with a virus sufficiently different in *gag* and *env* from those of the HTLV-I or -II prototypes, such that their antibodies would escape detection in standard serological tests using prototypic HTLV antigens. It is also possible that, in some cases, such particles may result from activation of endogenous retroviral gag sequences known to exist in the human genome (60). Lastly, the patients may have a selective type of immunosuppression which remains to be defined.

In any event, the salient observation communicated here is that proviral HTLV sequences have been detected in the vast majority of patients with MF. Since tax has never been reported among endogenous human retroviruses, this observation is probably significant. At the very least, the presence of pX region sequences in such patients is of interest because of the demonstrated role of tax, one of the gene products from this region, in neoplastic transformation (26, 28, 29, 61, 62). HTLV-I and -II are capable of transforming $CD4^+$ T lymphocytes, in vitro, (28, 61) and numerous studies have implicated tax in this process. tax has also been shown capable of transactivating a number of cellular genes known to be essential for stimulating the proliferation of $CD4^+$ T lymphocytes, as reviewed by Sherman et al. (4). An animal model that mimics many of the clinical features of ATLL has been established (30). PBMC from HTLV-I–infected ATLL patients, but not cells infected in vitro by HTLV, produce lymphoid tumors in SCID mice that maintain tumor cell characteristics indistinguishable from those of the cells derived from ATLL patients. Defective HTLV proviral genomes have also been demonstrated in these animals. The tumor cells, like the cells freshly isolated from patients, were found to have preferentially retained HTLV sequences encoded by the pX region. These sequences were detected by the same primers and probes used in the present study. In one MF patient, retention of the pX region, but loss of the regulatory sequence elements required for expression of functional tax, has been reported (10). In the present study, only a 159-bp region of tax was used for the purpose of revealing infection. Because of the high incidence of tax sequences, frequently in the absence of proviral sequences coding for HTLV structural proteins, antibodies to this gene product were also looked for. So far, only an antigen consisting of the 20 carboxy-terminal amino acids of the tax-I protein has been available for testing. This region is distinct from the sequences we detected by PCR/Southern analysis. Nevertheless, 5 of the 46 patients tested had detectable antibodies to this sequence, even though 4 of the 5 were serologically negative for antibodies to HTLV structural proteins, when tested by standard ELISA and Western blot. Therefore, it would not be surprising if these patients also had antibodies to other epitopes including those generated by the 159-bp tax region, which was demonstrable in their cells. The demonstration of such antibodies must await synthesis of additional tax peptides for use as antigens in these tests. Nonetheless, preliminary studies show that several of the patients with detectable tax proviral sequences also appear to express tax mRNA as detected by reverse transcription PCR.

Regardless, the finding that most, if not all, MF patients possess HTLV-I–related proviral sequences in their PBMC, even in the absence of antibodies to the virus, greatly strengthens the long-held hypothesis that MF is an HTLV-associated neoplasm.

These observations also suggest a need to include biomolecular analyses, in addition to serological assays, to establish whether or not a patient with a T cell lymphoma is infected with this virus.

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