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Expression of human T-cell leukaemia virus type I and associated antigens, and interleukin-2 and receptor in lymph nodes of adult T-cell leukaemia/lymphoma

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Abstract To examine the relationship between the expression of human T-cell leukaemia virus type (HTLV-I) mRNA and associated antigens and clinicopathological features, we studied 31 lymph nodes of patients with adult T-cell leukaemia/lymphoma (ATLL) and related diseases, using in situ hybridization and immunohistochemistry. We classified the patients into four types on the basis of their clinicopathological features (HTLV-I associated lymphadenitis, incipient ATLL, ATLL with complete HTLV-I provirus, and ATLL with defective HTLV-I provirus. The expression of HTLV-I mRNA was detected in all 3 patients with incipient ATLL, in 5 of 10 patients with defective-provirus ATLL, in 5 of 11 patients with complete-provirus ATLL, and 3 of 7 with HTLV-I associated lymphadenitis, but the amounts were very small; approximately 1 in 10000–200000 lymph node cells express the viral genomes. This suggests that expression of viral genomes may not be important for immortalization, but it is important that to note the capacity for HTLV-I infection is preserved in each group of non-neoplastic and neoplastic states. HTLV-I mRNA was detected only in lymphocytes and/or lymphoma cells, but the HTLV-I associated antigens (env, gag and pX) were found in histiocytes and endothelial cells, as well as in lymphocytes and/or lymphoma cells. Anti-interleukin 2 receptor (IL-2R) antibody reacted with the giant cells of incipient ATLL and with the transformed lymphocytes and immunoblast-like cells of the HTLV-I-associated lymphadenitis but not with the lymphocytes in the background . Of the typical ATLL, IL-2R was found in both lymphoma cells and giant cells. IL-2 was rarely detected.

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Introduction

Adult T-cell leukaemia/lymphoma (ATLL) is a human malignancy associated with T-cell leukaemia virus type I (HTLV-I) [23, 31, 32]. It can be diagnosed by characteristic clinicopathological findings and the presence of integrated proviral HTLV-I in tumour cells with a helper/inducer phenotype [29, 34]. HTLV-I is a human retrovirus and has a sequence, pX, that is not derived from cellular proto-oncogenes as are other mammalian retrovirus oncogenes [20]. This region is expressed as a doubly spliced mRNA, with two overlapping open reading frames, designated tax1 and rex1 [8]. tax1 encodes pX40tax, which has been shown to be a transactivator of the long terminal repeat of HTLV-I [4, 7, 21, 25, 26]. Moreover, pX40tax can transactivate many cellular genes, such as those of interleukin-2 (IL-2), the IL-2 receptor (IL-2R), IL-3, IL-4 and c-*fos* [1, 3, 6, 14, 15, 24]. Because many ATLL cells have the helper/inducer T-cell phenotype and express Tac antigen, a subunit of IL-2R [9], immortalization of virus-infected cells might occur by means of an autocrine circuit consisting of IL-2 and its own receptor. However, expression of HTLV-I cannot be detected by such conventional methods, as immunofluorescence analysis or RNA blot analysis [10, 11, 23, 28, 30], with rare exceptions [5]. However, mRNA for HTLV-I tax1/rex1 genes was detected in peripheral blood mononuclear cells of ATLL patients and asymptomatic HTLV-I carriers by using reverse transcription followed by the polymerase chain reaction (PCR). However, the amounts of mRNA detected corresponded to ~100000 to 1000000 times less than that in the HTLV-I infected MT-2 cell line [13]. These findings are consistent with the idea that expression of one or more HTLV-I gene may be involved in initiating transformation, but consistent expression is not needed for leukaemogenesis of immortalized cells. Expression of mRNA for HTLV-I in lymph

nodes was not reported. In order to examine the relationship between the expression of HTLV-I and the clinicopathological features, we performed in situ hybridization for RNA of HTLV-I and immunological staining for the HTLV-I associated antigens of gag, env, and pX.

Materials and methods

Tissue specimens filed in the Department of Pathology at Fukuoka University were used. We fixed the lymph nodes obtained in buffered formalin or B5 solution, embedded them in paraffin wax, and stained them with haematoxylin-eosin, Giemsa, periodic acid-Schiff, and Gomori's silver impregnation. Immunostainings were also used: L26 for B cells (Dakopatts; Glostrup, Denmark); UCHL-1 for T-cells (Dakopatts); Leu-M1 (Becton-Dickinson; Mountain View, Calif.); and Ber-H2 (Dakopatts). A portion of each lymph node was kept at −80° C in a deepfreeze, and the nodes were examined using monoclonal antibodies for T cells (CD2, CD3, CD4, CD8) and B cells (CD20, CD22) (Ortho, Becton-Dickinson, or Dakopatts), activated cells and others (CD30, Ki67, Ia) (Dakopatts), and IL-2 and IL-2R (Becton-Dickinson), using the PAP or ABC method.

For immunological staining of HTLV-I associated antigens, the paraffin-embedded sections were deparaffinized and dehydrated.We successfully incubated them at 37° C for 30 min with serial twofold dilutions of heat-inactivated sera antibodies from patients with guinea-pig complement and then with fluorescein isothiocyanate (FITC) conjugated anti-guinea-pig C3 rabbit serum [19]. Thereafter, the sections were processed for immunostaining by the avidin-biotin immunoperoxidase technique. The sera bodies were purified, corresponding to the peptide amino acids 100–130 gag p19, 175–199 env gp46, 73–93, 253–272, 324–343 pX40tax [35].

For Southern blot analysis, a part of the frozen material was used for DNA isolation and gene analysis. The details of the examination methods have been reported previously [16–18]. We used the T-cell receptor gene Cβ, Jγ, and immunoglobulin heavy chain (JH) gene, and proviral DNA of HTLV-1 (full length; gag, pol, env, pX, LTR) as probes. After the dehybridization, gag, env, and pX probes were used to detect the defective portion of proviral HTLV-I DNA. Because the HTLV-I DNA has a restriction enzyme site *Pst*I, but no *Eco*RI site, we used *Eco*RI for monoclonal integration and *Pst*I for polyclonal integration.

The isolation DNA was used for PCR. Specific primers were synthesized, based on the published DNA sequences [20] corresponding to the pX regions (primer pX1: ATGCTGTTTCGCCTTCTCAG; primer pX2: TAAGGACCTTGAGGGTCTTA), corresponding to the env region (primer env1: ATGGGTAAGTTTCTCGCCAC; primer env2: GTGCTTTGTAGGGTTAACTG), and corresponding to the gag region (primer gag1: ATGGGCCAAATCTTTTCCCG; primer gag2: GCTATCTAGCTGCTGGTGAT). The amplification was carried out at 30 cycles with the GeneAmp DNA amplification reagent kit and DNA Thermal Cycler (Perkin-Elmer Cetus, Norwalk, Conn.). After amplification, the samples were analysed by the Southern blot method with an HTLV-I probe.

Table 1 Clinical data

No.	Age	Sex	WBC (Aty)	LDH	Prognosis
HTLV-I associated lymphadenitis					
1	24	M	2300 (3%)	↑	A 12 months
2	55	M	7600 (0%)	→	A 42 months
3	59	F	10300 (2%)	→	A 113 months
4	53	M	5100 (3%)	→	A 6 months
5	55	M	7400 (2%)	→	A 8 months
6	52	M	4800 (1%)	→	A 9 months
7	67	M	6100 (0%)	↑	A 9 months
Incipient ATLL					
8	60	M	11400 (0%)	→	D 15 months
9	63	M	9990 (3%)	→	D 24 months
10	57	M	4480 (9%)	→	A 7 months
Typical ATLL (complete form)					
11	71	F	16900 (28%)	↑	D 1 months
12	69	F	20000 (70%)	→	D 5 months
13	40	F	62500 (70%)	→	D 4 months
14	58	M	11300 (0%)	↑	A 6 months
15	52	F	3300 (0%)	↑	D 5 months
16	81	F	32000 (70%)	↑	D 3 months
17	59	F	4240 (48%)	↑	D 2 months
18	56	F	3800 (0%)	↑	D 5 months
19	77	F	4600 (0%)	↑	D 6 months
20	76	M	17200 (0%)	↑	D 1 months
21	68	F	5400 (12%)	↑	D 3 months
Typical ATLL (defective form)					
22	54	M	8000 (50%)	↑	D 6 months
23	46	M	6400 (1%)	↑	D 2 months
24	58	F	2500 (0%)	↑	A 6 months
25	64	M	6500 (0%)	↑	D 1 months
26	56	M	12300 (0%)	↑	D 6 months
27	34	M	50400 (95%)	→	D 6 months
28	54	M	6400 (0%)	→	D 9 months
29	67	M	26500 (64%)	↑	A 21 months
30	63	M	3900 (0%)	↑	D 5 months
31	59	F	2700 (0%)	↑	D 24 months

F female, *M* male, *D* dead, *A* alive

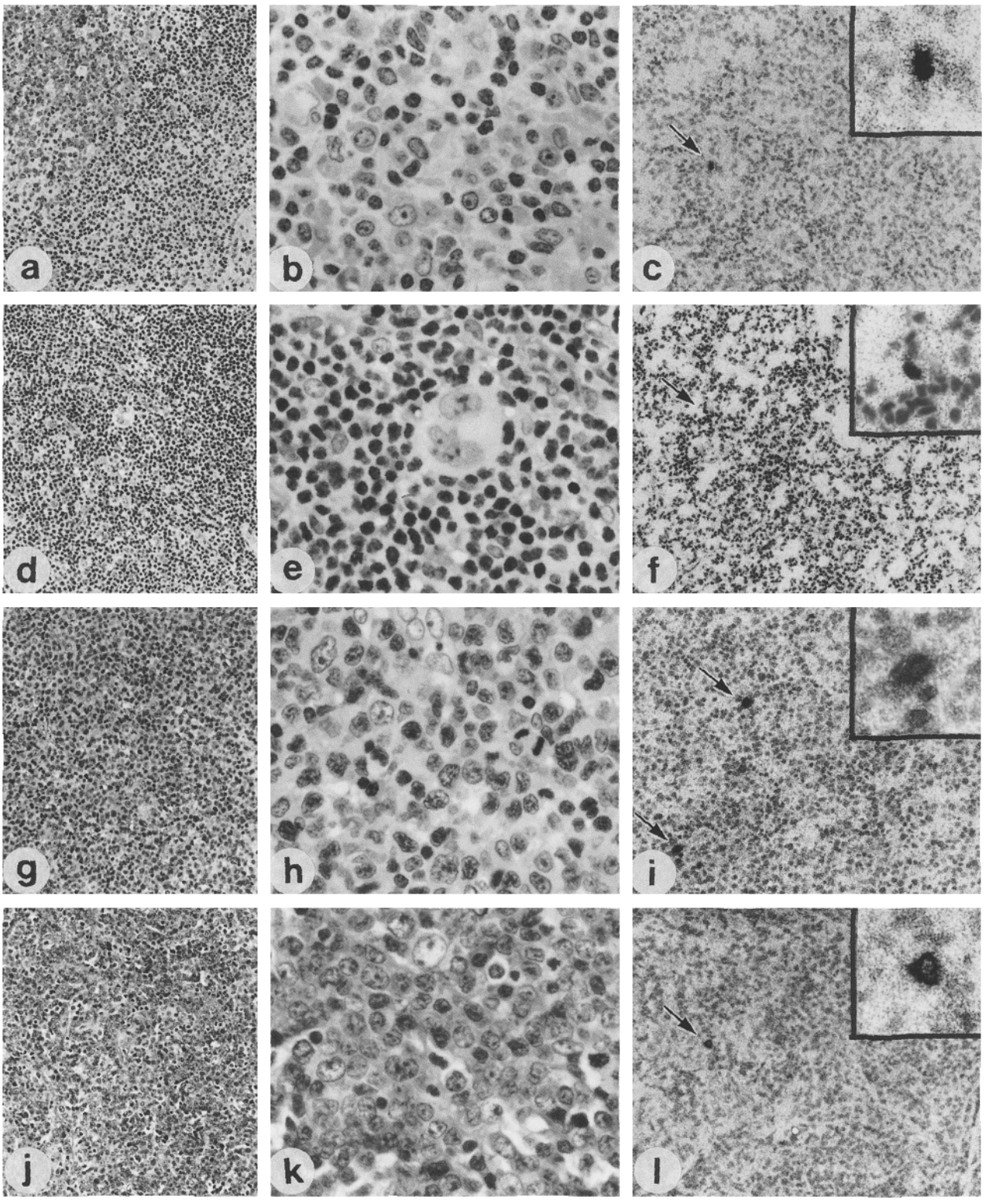

Fig. 1a–c Case 3, HTLV-I associated lymphadenitis. **a** Enlarged paracortex with diffuse infiltration of small or medium-sized lymphocytes. ×120. **b** Some scattered transformed lymphocytes and immunoblast-like cells. ×480. **c** In situ hybridization for retrovirus HTLV-I mRNA. A cell suspected to be a lymphocyte expresses silver grains. ×120. *Inset*, ×480. **d–f** Case 9, incipient ATLL. **d** Enlarged paracortex with diffuse infiltration of small or medium-sized lymphocytes and small aggregated foci or clusters of a few giant cells. **e** Giant cells with irregular lobulated or highly convoluted Reed-Sternberg or Hodgkin's cell-like nuclei. **f** In situ hybridization for retrovirus HTLV-I mRNA. A small lymphocyte expresses silver grains. **g–i** Case 17, ATLL with complete HTLV-I provirus. **g, h** A diffuse proliferation of atypical lymphoid cells, varying in size and form with the appearance of cerebriform giant cells. **i** A giant cell expresses silver grains. **j–l** Case 23, ATLL with defective HTLV-I provirus

We prepared frozen sections for in situ hybridization for retrovirus HTLV-I RNA using previously described methods [36]. We fixed air-dried sections in acetone and used immediately. The specimens of lymph nodes were hybridized overnight at 50° C with a ^{35}S DNA probe (HTLV-I, full length). After hybridization, the slides were washed twice in 50% formamide 2xSCC and 50% formamide 1xSCC for 30 min each at 42° C and twice in 1×SSC for 20 min each at 42° C.. We dehydrated the sections in graded ethanol solutions and air-dried them.

Autoradiographic exposure using an overlay of Konica NR-M2 emulsion (Tokyo, Japan) lasted 14–21 days. After the emulsion developed, we counterstained the sections with haematoxylin.

Results

Clinical features

We classified these 31 cases studied into four types on the basis of the clinicopathological findings (Table 1): HTLV-I associated lymphadenitis, incipient ATLL, ATLL with complete HTLV-I provirus, and ATLL with defective HTLV-I provirus. Malignant lymphomas were diagnosed according to the Lymphoma-Leukaemia Study Group of Japan classification [27]. All patients with HTLV-I associated lymphadenitis were healthy and free from disease after the nodal biopsy. The patients in the other three groups had received chemotherapy and/or radiation and many were dead. Most of the cases have been reported previously [16–18].

Histology

The lymph nodes of HTLV-I-associated lymphadenitis showed a well-preserved nodal architecture with small lymph follicles, and an enlarged paracortex with a diffuse infiltration of small or intermediate-sized lymphocytes with mild nuclear irregularity, indistinct nucleoli, and scanty cytoplasm (Fig. 1). Mitotic features were rare. Some transformed lymphocytes and immunoblast-like cells with round or indented nuclei were scattered in the expanded paracortex. The lymph nodes of incipient ATLL had a preserved nodal architecture and an enlarged paracortex, with a diffuse infiltration of small or intermediate-sized lymphocytes having mild nuclear irregularity. A few mitotic features are intermingled. Small aggregated foci or clusters consisting of a few giant cells with irregularly lobulated, highly convoluted, or Reed-Sternberg or Hodgkin's cell-like nuclei were scattered throughout the expanded paracortex. Some giant cells showed mitoses. The lymph nodes of ATLL show a diffuse proliferation of atypical lymphoid cells, varying in size and form with the appearance of cerebriform giant cells. Mitoses were frequently encountered.

Southern blot analyis

To investigate monoclonal HTLV-I integration, we used the restriction enzyme *Eco*RI; for polyclonal integration, we used *Pst*I [20] (Fig. 2, Table 2). Proviral DNA bands were found in two of seven cases of HTLV-I-associated lymphadenitis, in one of three patients with incipient ATLL, and in all cases of typical ATLL. However, the bands of lymphadenitis and incipient ATLL were fainter than those of typical ATLL, probably because of the minor population of HTLV-I integrated lymphocytes. Of the ten patients who had typical ATLL with defective provirus, six showed the defect to be of the gag region, three had defects of both of the gag and env, and one displayed a defect in the env region. However, all cases preserved the pX region. All cases of typical and incipient ATLL showed rearrangements or deletion of the T-cell-receptor gene Cβ and Jγ, but no cases of HTLV-I-associated lymphadenitis showed such rearrangement. No cases displayed rearrangement of immunoglobulin heavy chain gene (JH).

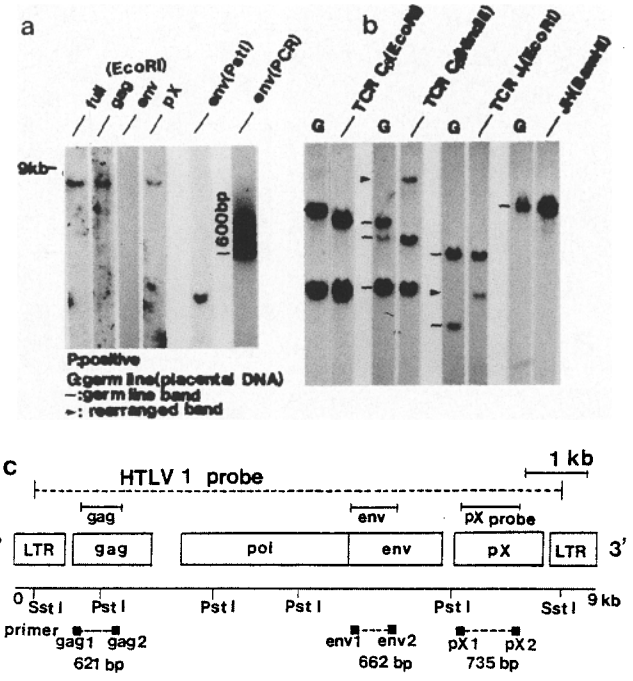

Fig. 2a–c Southern blot analysis and polymerase chain reaction. **a** The HTLV-I DNA has a *Pst*I restriction enzyme site but no *Eco*RI site. Thus, the monoclonal integration of HTLV-I DNA was examined by digestion of *Eco*RI and the polyclonal integration was digested by *Pst*I. **b** For T-cell receptor Cβ and Jγ, a rearranged band was found, but for immunoglobulin heavy chain gene JH, no such band was found. **c** Genomic structure of HTLV-I, restriction map, and probe regions (*kb* kilobases, *bp* base pairs)

Polymerase chain reaction

After 30 cycles of PCR amplification, we performed Southern blot analysis with an HTLV-I. Amplification of HTLV-I gag, env, and pX regions was detected in all patients, except cases 28 and 29, whose gag regions could not be amplified. The amplification band was not detected in the DNA from placenta and nonspecific lymphadenitis without anti-HTLV-I antibody in serum (Fig. 2, Table 2).

Table 2 Immunological and DNA analysis. *Eco EcoRI*, *Pst PstI*, *PCR* polymerase chain reaction, *TCR* T-cell-receptor gene, *Ig* immunoglobulin gene, ( ) defective region, *e* env, *g* gag, * gag region could not be detected, *G* germ line, *R* rearrangement, *D* deletion, *ND* not done

No.	Histology	HTLV-1 Southern		PCR	TCR		Ig JH	CD		
		Eco	*Pst*		Cβ	Jγ		3	4	8
HTLV-I associated lymphadenitis										
1		−	−	+	G	G	G	+	+	−
2		+	+−	+	G	G	G	+	+	−
3		+	+−	+	G	G	G	+	+	−
4		−	−	+	G	G	G	+	+	+−
5		−	−	+	G	G	G	+	+	+−
6		−	−	+	G	G	G	+	+	−
7		−	−	+	G	G	G	+	+	−
Incipient ATLL										
8		−	−	+	R	R	G	+	+	−
9		−	−	+	D	ND	G	+	+	−
10		+	+	+	R	R	G	+	+	−
Typical ATLL (complete form)										
11	intermediate	+	+	+	R	R	G	+	+	−
12	medium	+	+	+	R	R	G	+	+	+
13	medium	+	+	+	R	R	G	+	+	−
14	large	+	+	+	R	R	G	+	−	−
15	large	+	+	+	R	R	G	+	+	−
16	large	+	+	+	R	R	G	+	+	−
17	pleomorphic	+	+	+	R	R	G	+	+	−
18	pleomorphic	+	+	+	R	R	G	+	+	+
19	pleomorphic	+	+	+	R	R	G	+	+	−
20	pleomorphic	+	+	+	R	R	G	+	+	−
21	pleomorphic	+	+	+	R	R	G	+	+	+
Typical ATLL (defective form)										
22	medium	+	+(g)	+	D	R	G	+	+	−
23	large	+	+(e/g)	+	R	R	G	+	+	−
24	large	+	+(e)	+	R	R	G	+	+	−
25	pleomorphic	+	+(g)	+	R	R	G	+	+	+
26	pleomorphic	+	+(e/g)	+	R	R	G	+	+	−
27	pleomorphic	+	+(g)	+	R	R	G	+	+	−
28	pleomorphic	+	+(g)	+*	R	R	G	+	+	−
29	pleomorphic	+	+(g)	+*	R	R	G	+	+	−
30	pleomorphic	+	+(e/g)	+	R	R	G	+	+	−
31	pleomorphic	+	+(g)	+	R	R	G	+	+	+

Immunohistology

In the patients with HTLV-I-associated lymphadenitis and incipient ATLL, proliferation of small to intermediate-sized lymphocytes possessed a peripheral helper/inducer T-cell phenotype (CD1−, 2+, 3+, 4+, 8−) (Fig. 3; Tables 2, 3). Only a few B lymphocytes (L26+, CD19+, CD20+) were intermingled. Furthermore, the giant cells in cases of incipient ATLL, and the large cells of transformed lymphocytes and immunoblast-like cells in the HTLV-I-associated lymphadenitis, had a peripheral T-cell phenotype of CD4. Of the 21 patients with typical ATLL, 15 possessed the CD3+, 4+, 8− phenotypes, 5 had CD3+, 4+, and 8+, and 1 had CD3+, 4−, 8−. Regarding phenotype, there were no definite differences between the complete and defective forms. IL-2R reacted with the giant cells of the incipient ATLL and with the transformed lymphocytes and immunoblast-like cells of HTLV-I-associated lymphadenitis, but it did not react with the lymphocytes in the background. In the cases of typical ATLL, IL-2R was found in both lymphoma and giant cells, but three cases of tyical ATLL showed no IL-2R. IL-2 was rarely found in nodes with HTLV-I-associated lymphadenitis, incipient ATLL, and typical ATLL with complete provirus, but half of the cases of typical ATLL with defective provirus displayed IL-2. The difference in distribution between IL-2 and IL-2R probably arose from IL-2s having a shorter lifetime than IL-2R. There was no definite relationship between IL-2 and IL-2R and the expression of HTLV-I mRNA.

In situ hybridization and expression of HTLV-I associated antigens

We performed in situ hybridization with an HTLV-I DNA probe for retrovirus HTLV-I mRNA. Three of 7 patients with HTLV-I-associated lymphadenitis (43%), all of 3 incipient ATLL cases, and 5 of 11 having typical ATLL in the complete form (45%) and 5 of 10 having typical ATLL with defective provirus (50%) showed expression of HTLV-I mRNA. However, very few lympho-

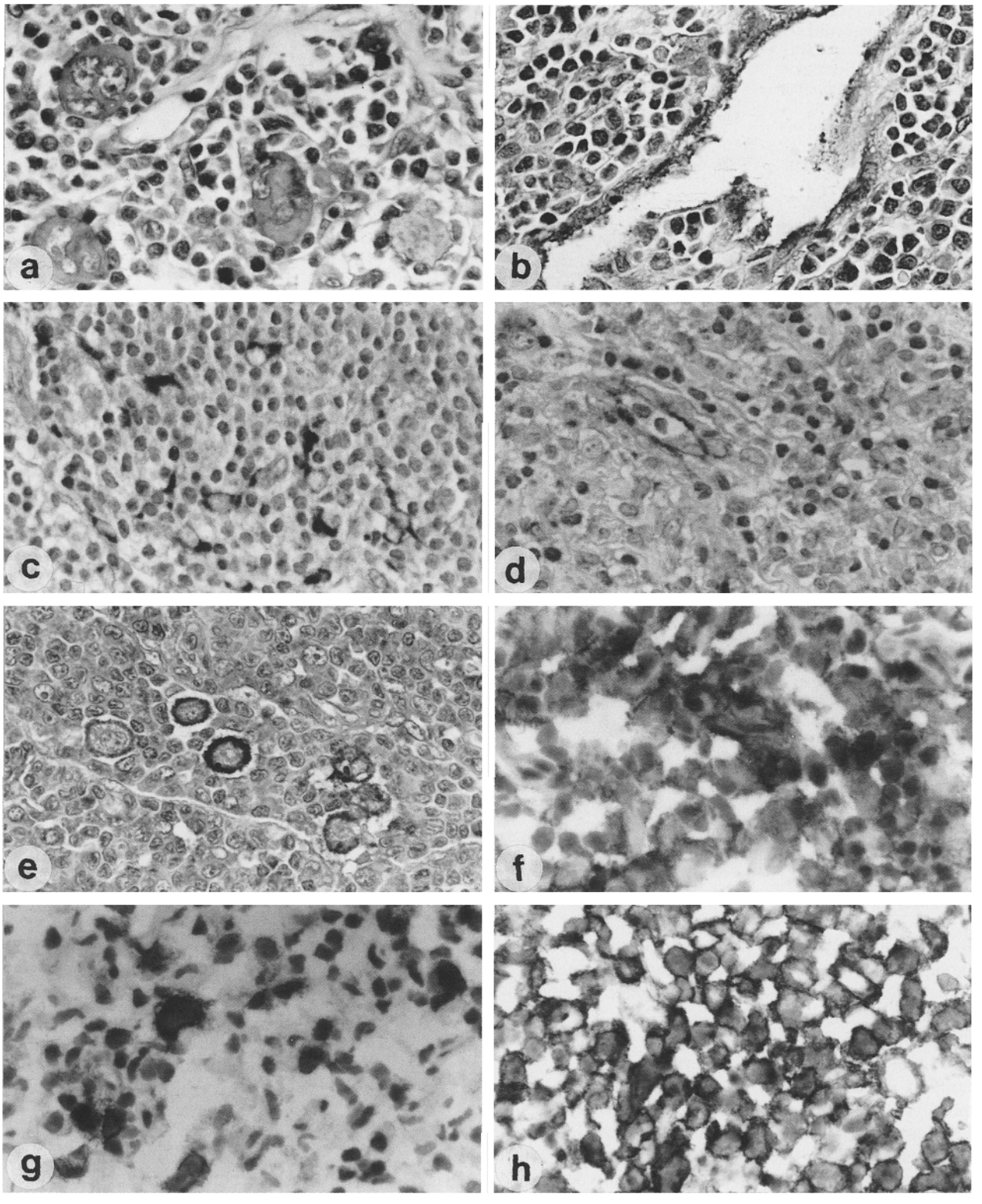

Fig. 3 **a** Case 8. Giant cells reacting with env. **b** Case 12. Endothelial cells reacting with gag. **c** Case 10. Histiocytes reacting with pX 73. **d** Case 10. Endothelial cells reacting with pX 253. **e** Case 11. Giant cells reacting with pX 324. **f** Case 12. Lymphoma cells diffusely reacting with interleukin-2. **g** Case 10. Giant cells reacting with interleukin-2 receptor. **h** Case 16. Lymphoma cells diffusely reacting with interleukin-2 receptor

cytes and/or lymphoma cells produced silver grains (Figs. 1, 3; Table 3). The reaction was not detected in the lymph nodes of the patients with nonspecific lymphadenitis without ATLL in serum. The number of expression cells was one to nine in the sections. The areas of the sections were 10–100 mm^2. Sections with an area of 1 mm^2 had about 1000–2000 cells, so approximately 1 in

Table 3 In situ and HTLV-I associated antigen. *H* histiocyte, *G* giant cells, *E* endothelial cells, *L* lymphoma cell or lymphocyte, *G* giant cell or immunoblast-like cells

No.	In situ[a]	gag	env		pX		IL-2		IL-2R	
		100 −130	175 −199	73 −93	253 −272	324 −343	L	G	L	G
HTLV-1 associated lymphadenitis										
1	–	H	–	H	H	–	–	+–	–	+
2	+1	–	H	–	–	–	–	–	–	+
3	+1	H	H	–	–	–	–	–	–	+
4	–	H	–	–	–	–	–	+–	–	+
5	–	–	–	–	–	–	–	–	–	+
6	–	–	–	–	–	–	–	–	–	+
7	+1	–	–	E	E	–	–	–	–	+
Incipient ATLL										
8	+1	G	G	G, E	G	–	–	–	–	+
9	+1	G	–	–	G	–	–	–	–	+
10	+3	–	G	G, H	E, G	–	–	–	–	+
Typical ATLL (complete form)										
11	–	–	H	H	H	G	–	–	+	+
12	+4	–	E	–	–	–	+	+	+–	+
13	–	–	–	G	–	–	–	–	+	+
14	–	–	–	G	–	–	–	–	–	–
15	–	–	–	G	–	–	–	–	+–	+
16	+1	–	–	–	–	–	–	–	+	+
17	+9	–	–	–	–	–	–	–	+	+
18	–	–	–	–	–	–	–	–	+–	+
19	+1	–	–	–	–	–	–	–	–	+
20	+1	G	G	–	–	–	–	–	–	+–
21	–	–	–	–	–	–	–	–	–	–
Typical ATLL (defective form)										
22	+1	–	–	–	–	–	+–	+	–	–
23	+4	H	–	–	–	–	+–	+–	+	+
24	+2	–	–	–	–	–	–	–	+–	+
25	–	–	–	–	–	–	–	–	+–	+
26	+1	–	–	–	–	–	–	–	+	+
27	+1	–	–	G	–	G	–	+–	–	–
28	–	–	–	–	–	–	+	+	+–	+
29	–	–	G	–	–	–	–	–	+–	+
30	–	–	G	–	–	–	+	+	+	+
31	–	–	–	–	–	–	–	–	+	+

[a] Number of cells with HTLV-I expression in the section

10000–200000 cells of the lymph nodes might express the viral genome. HTLV-I-associated antigens, corresponding to env gp46, gag p19, and pX40tax were detected not only in lymphocytes and/or lymphoma cells but also giant cells, histiocytes, and endothelial cells. All patients with incipient ATLL showed some HTLV-I-associated antigens, especially pX 253–272, but only a few with HTLV-I-associated lymphadenitis and typical ATLL showed a few reacted cells. No relationship existed between the HTLV-I-associated antigens and the expression of HTLV-I mRNA.

Discussion

ATLL is a mature T-cell malignancy closely related to HTLV-I [22, 31, 32]. HTLV-I is a human retrovirus that has been shown to be associated with human malignancy. However, expression of HTLV-I cannot be detected by conventional methods, such as immunofluorescence analysis or RNA blot analysis in fresh ATLL cells or fresh peripheral blood mononuclear cells of HTLV-I carriers [10, 11, 23, 28, 30], with rare exceptions [5]. It is therefore suggested that expression of the viral gene may not be important in leukaemogenesis of immortalized cells. However, it is not clear whether the provirus is not expressed in vivo or is expressed at too low a level to be detected by conventional methods. Nevertheless, mRNA for the HTLV-I tax1/rex1 genes was detected in fresh peripheral blood mononuclear cells of adult T-cell leukaemia patients and asymptomatic HTLV-I carriers by using reverse transcription followed by the polymerase chain reaction (RT-PCR). In fresh peripheral blood mononuclear cells, the expression of tax1/rex1 mRNA was detected in five of six adult T-cell leukemia patients and four of eight HTLV-I carriers examined. The amount of tax1/rex1 mRNA detected corresponded to approximately 100000–1000000 times less than that in the HTLV-I-infected MT-2 cell lines. These results indicate that, in some individuals infected with HTLV-I, the provirus in circulating blood cells is transcribed in vivo. Because approximately one in 100000–1000000 peripheral

blood mononuclear cells may express viral genomes, the expression of viral antigens in circulating blood cells in vivo is suggested [13].

We know of no published report on the expression of HTLV-1 mRNA in the lymph nodes. In our lymph node study using the in situ hybridization method, the expression of HTLV-I mRNA was detected in all 3 patients with incipient ATLL, 5 of 10 patients having ATLL with defect provirus (50%), 5 of 11 cases of ATLL with complete provirus (45%), and 3 of 7 with HTLV-I-associated lymphadenitis (43%). Some cases showed mRNA expression of HTLV-I, but in very small amounts. About 1 in 10000–200000 cells of lymph nodes may express the viral genomes. Our data on mRNA in lymph nodes were practically identical to those in circulating blood cells; the lymph nodes might express the viral genomes slightly more often than did the circulating blood cells. The data did not clarify which cells with lymph node expression might reach the circulating blood or which circulating blood cells might go into the lymph nodes. The ATLL with defective provirus form expressed the HTLV-1 mRNA, but the cases without gag region in the PCR methods did not. The expression probably originated in the latent non-ATLL lymphocytes with complete provirus. Therefore, expression of the viral gene may not be important for leukaemogenesis of immortalized cells. But it is important to note that the capacity for HTLV-I infection is preserved in each group of both the non-neoplastic and the neoplastic states.

Atypical lymphocytes are seen frequently in the peripheral blood of ATLL patients and in some healthy HTLV-I carriers. Tax1/rex1 mRNA was not detected in a patient with smouldering ATLL or in an HTLV-I carrier, both of whom had atypical lymphocytes. However, tax1/rex1 mRNA was detected in some HTLV-I carriers who were haematologically normal and who had no atypical lymphocytes. These findings suggest that the level of tax1/rex1 mRNA expression does not correlate with the number of atypical lymphocytes in the peripheral blood. The titres of antibodies against HTLV-I vary in the ATLL patients and HTLV-I carriers, as shown by the particle agglutination method, and this titre did not correlate with the expression of tax1/rex1 mRNA [13]. In our data, there was no relationship between the expression of mRNA and pX, env, or gag.

Previous reports have shown the presence of antigens crossreactive with monoclonal antibodies to HTLV-I p19 in cells not infected with HTLV-I [37]. In synovial tissues from patients with early proliferative rheumatoid arthritis, immunoreactivity could be demonstrated using monoclonal IgG antibodies reactive with the p19 and p24 protein of HTLV-I, which were encoded by the gag region of the viral genome. Predominantly large synovial lining cells that appeared transformed contained the immunoreactive antigen, and synovial vessel wall cells, including endothelial cells, were likewise reactive [37]. Clarke et al. [2] reported the detection of homology between the envelope gene region of HTLV-I and the region of an HLA-B locus gene that codes for an extracellular region of a class I histocompatibility antigen. Taken together, these data strongly suggest that an HTLV envelope region gene product with HLA-like determinants was being expressed on HTLV-I infected cells curing viral replication. Our study found that not only lymphocytes and/or lymphoma cells but also some endothelial cells and histiocytes showed a positive reaction for gag, env, and pX gene products.

Ten T-cell lines were established from patients with ATLL. During the establishment of these cell lines, it was found that when T-cell lines expressing the ATLL-associated retroviral antigen were cocultivated with 8C cat cells, multinucleated syncytia were formed. Retroviral antigen-negative T-cell lines did not induce syncytia. Peripheral blood lymphocytes obtained from ATLL patients did not express the retroviral antigen before cultivation in vitro but became positive for the retroviral antigen after a short period of cultivation; these retroviral antigen-positive lymphocytes (but not retroviral antigen-negative lymphocytes) induced syncytia upon cocultivation with 8C cells. Retroviral antigen was present in all established T-cell lines or freshly isolated peripheral blood lympocytes that were able to induce syncytia [11]. In our study, incipient (early stage) ATLL had more bizzare giant cells than ATLL and carrier. So the bizarre giant cells probably relate directly to HTLV-1 infection in leukaemogenesis.

ATLL cells contain the genome of the HTLV-I provirus and express large amounts of IL-2 subunits. Perhaps p40tax plays a key role in the early stage of leukaemogenesis of ATLL through its transactivation of the IL-2 system [3]. In other words, in the early stage of leukaemogenesis, tax1 gene expression induces multiclonal expansion of some subpopulations of the virus-infected T cells by an autocrine mechanism, that is by transactivation of IL-2 and IL-2R by p40tax [33]. In fact, normal peripheral blood lymphocytes can be immortalized by HTLV-I by cocultivating them in vitro with HTLV-I-producing cells. Our data showed no definite relationship between IL-2 and IL-2R and the expression of pX, but the giant cells of incipient ATLL, which was considered early-stage ATLL [17], expressed pX 253–272, corresponding to pX40tax, more frequently than did lymphadenitis (carrier state) and ATLL. The expression of pX253 of incipient ATLL may be related to the tumorigenicity of the giant cells.

Because IL-2 and IL-2R are required for T-cell proliferation, induction of IL-2 and its receptor by the pX gene product would induce selective proliferation of infected T cells without specific antigen stimulation if this activation occurs in primary T cells. Thus, HTLV-I infection resulted in an unregulatable growth of infected T cells, but not other cell types, and eventually may increase the number of putative target cells for malignant transformation. Primary leukaemic cells in ATLL patients express the IL-2R on their cell surface, but usually do not express any HTLV-I antigens, including p40x. Therefore, the viral function of p40x in activating the IL-2R gene may account for early ATLL development, but may not

be required for the constitutive expression of the IL-2R on ATLL cells [12]. In the lymph nodes we studied, all cases of early-stage ATLL and lymphadenitis expressed the IL-2R in the giant cells of early ATLL and in the immunoblast-like large cells of lymphadenitis. However 4 of 21 ATLL showed no expression (19%). The immunoblast-like large cells of the carrier state might develop into the giant cells of early ATLL. In the process of that development, IL-2R may play an important role.

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