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Expression of the *TCL1* Gene at 14q32 in B-Cell Malignancies but Not in Adult T-Cell Leukemia

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The *TCL1* gene was recently cloned as a candidate target within the 14q32.1 breakpoint cluster region observed in T-cell malignancies. We examined the *TCL1* gene expression in 21 patients with adult T-cell leukemia (ATL) and 5 cell lines, because ATL is reported to have frequent chromosome 14 band q32 aberrations. However, 20 of the ATL patients and all 5 cell lines lacked any TCL1 expression on northern blot analysis, and TCL1 transcripts were only very faintly detected in the remaining one patient. Expansion of our analysis to include other types of hematopoietic malignancies revealed strong expression of the *TCL1* gene in almost all tumor cells of B-cell lineage except myelomas. However, no TCL1 signals were encountered in cells of T-cell or myeloid lineages. In normal human tissues *TCL1* was found to be expressed in the spleen, lymph nodes and B-lymphocytes of peripheral blood. These results indicate that *TCL1* is not a major target gene for ATL, but that it may play a role in B-cell differentiation and proliferation.

Key words: *TCL1* gene — Adult T-cell leukemia — B-cell malignancies

In hematopoietic malignancies particular types of translocations are associated with histologically distinct neoplasms.[1)] Several breakpoints at 14q32.1, designated as the *TCL1* (T-cell lymphoma/leukemia-1) locus, have been analyzed and found to be scattered over about 400 kb of DNA.[2, 3)] A large proportion of patients with T-cell prolymphocytic leukemia (T-PLL) and T-cell leukemia developing from ataxia telangiectasia carry t(14;14)(q11;q32), inv(14)(q11;q32) or t(7;14)(q35;q32), which juxtapose the *TCL1* locus to the α/δ or β loci of the T cell receptor (TCR).[4–6)] This points to the presence of a gene which is activated by the juxtaposition of TCR, as found for *MYC* or *BCL2* translocations with activation by the immunoglobulin gene (Ig) in B-cell malignancies. Virgilio *et al.*[7)] recently identified a transcript from the *TCL1* locus and designated it as the *TCL1* gene, although direct evidence for activation by juxtaposition to *TCR* genes remains elusive. The open reading frame of this gene encodes a 14-kDa protein that is predominantly localized in the microsomal fraction.[8)] The product has a sequence similarity to the protein encoded by the *MTCP-1* (mature T cell proliferation-1) gene on chromosome Xq28, which is known to be involved in T-cell lymphoproliferative diseases.[9, 10)]

Although adult T-cell leukemia (ATL) is associated with human T-cell leukemia virus type-I (HTLV-I), the virus alone can not explain the development of ATL because of the presence of a long latent period between HTLV-I infection and manifestation of the disease, as well as the very low occurrence rate of ATL among carriers of the virus. Therefore it has been considered that ATL leukemogenesis might be the result of accumulation of a number of genetic events.[11)] A recent cytogenetic study of 107 ATL cases revealed that chromosomal translocations occurred most frequently in band 14q32,[12)] suggesting that a gene involved in leukemogenesis of ATL exists at this locus. To determine whether *TCL1* might play a role, its expression in ATL as well as various hematopoietic malignancies was investigated in the present study.

MATERIALS AND METHODS

Patients Clinical features of the 21 ATL patients are listed in Table I. Diagnosis was made using the criteria described previously by Shimoyama *et al.*[13)] All patients were positive for serum antibody to HTLV-I and CD4 surface markers. HTLV-I proviral genome integration was examined by Southern blot analysis for 18 cases. Karyotypic analysis has been performed in 8 patients. None of the patients showed chromosomal abnormalities involving 14q32. In UPN001, who showed very faint TCL1 expression, the karyotype was normal; 46, XY. Patients with other types of T-cell malignancies, including four with acute lymphocytic leukemia (T-ALL), two with lymphoblastic lymphoma (T-LBL), three with T-PLL and one

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Table I. Clinical Features and TCL1 Expression in ATL Patients

Case	Age/Sex	Subtype[a)]	Sample	HTLV-I integration	Expression[b)] of TCL1
UPN001	58/M	A	PB	+	0.1
UPN002	70/M	A	PB	+	–
UPN003	43/F	A	Acites	+	–
UPN004	34/M	A	PB	+	–
UPN005	66/M	A	PB	ND	–
UPN006	64/F	A	PB	+	–
UPN007	78/F	A	PB	+	–
UPN008	71/F	A	PB	+	–
UPN009	73/F	A	PB	+	–
UPN010	57/F	A	PB	+	–
UPN011	47/F	A	PB	+	–
UPN012	51/M	A	PB	+	–
UPN013	47/M	A	LN	+	–
UPN014	53/F	A	PB	+	–
UPN015	52/M	A	PB	+	–
UPN016	49/F	C	PB	+	–
UPN017	40/F	C	PB	+	–
UPN018	54/M	C	PB	+	–
UPN019	72/M	UD	PB	ND	–
UPN020	52/F	UD	PB	ND	–
UPN021	71/M	UD	PB	+	–

Abbreviations: UPN, unique patient number; ND, not done.
a) Subtype: A, acute type; C, chronic type; UD, undefined.
b) The level of expression was normalized by arbitrarily defining the SP-49 signal as 10.

with a diffuse lymphoma of T-cell type (T-DL), were also studied. The karyotype of the UPN201 patient who showed faint TCL1 expression is 44, XY, der(2) t(2;3)(p21;p13), del(3p13), del(7q22), –7, –8, der(7)t(7;14)(q32;q24), t(8;11)(p21;q13), t(7;12;14)(q22;q32;q24).

Cell lines Sixty-five hematopoietic cell lines (13 from T-cell tumors, 31 from B-cell tumors, 13 from myeloid tumors and 8 others) were examined (Table II). Some of these cell lines were kindly provided by Drs. A. Karpas, A. Epstein, N. Kamada, T. Nakagawa, K. Yanagisawa, M. Ogura, M. Abe, I. Kubonishi, S. Nakazawa, M. Nitta, T. Ohtsuki and S. Shimizu.

Phytohemagglutinin or IL-2 stimulation of peripheral blood mononuclear cells Peripheral blood mononuclear cells (PBMNCs) from normal individuals were separated by Ficoll-Hypaque gradient centrifugation and cultured in RPMI 1640 with 5% fetal calf serum (FCS)(RPMI-FCS) in the presence of 0.1% phytohemagglutinin-P (PHA-P; Difco Laboratories, Detroit, MI) in a 5% CO_2 incubator at 37°C for 72 h. Anti-CD19 beads (MBL, Nagoya) were used for cell fractionation analysis, conducted according to the manufacturer's protocol.

Northern blot analysis Total RNA was isolated by ultracentrifugation on a guanidine isothiocyanate/$CsCl_2$ gradient and northern blot analysis was performed as described previously[14]. The TCL1 probe was synthesized by means of reverse transcription-polymerase chain reaction using Daudi cell line mRNA with 5′-CGGGGATCCGACGC-CATGGCCGAGTGCCCGA-3′ and 5′-GCTGAATTCT-CACAAGTTCACGGAGCT-3′ and cloned into the pBluescript II vector at the *Bam*HI/*Eco*RI site. The TCL1 sequence was confirmed by dideoxy sequencing (Sequencing kit, Amersham-Japan, Tokyo). mRNA signals were normalized to the corresponding 28S rRNA levels visualized by ethidium bromide staining. Hybridization signals of mRNA were quantitated by scanning densitometry (Image Analyzer V10, TOYOBO, Osaka). mRNA of the SP-49 cell line was applied to every gel for northern analysis as a standard, and the SP-49 signal was arbitrarily defined as 10. The human TCRβ chain gene probe, CTβ, and the human IgM gene probe, Cμ, were also used to evaluate cell types in blots of PBMNCs.

RESULTS

TCL1 expression in normal human tissues Northern blot analyses of TCL1 expression in various human tissues (Fig. 1) revealed signals in the spleen and lymph

Table II. Expression of TCL1 mRNA in Hematopoietic Cell Lines

Cell line	Type/origin	Northern[a)]
AML cell lines		
HL-60	AML M2	—
KASUMI-1	AML M2	—
SKNO-1	AML M2	—
NKM-1	AML M4	—
KOCL-48	PreB(ALL) →M4	—
HE-1R	AML M4E	—
NOMO-1	AML M5	—
THP-1	AML M5	—
HEL	AML M6	—
CMK	AML M7	—
CML cell lines		
K562	CML-BC (E)	—
NCO-2	CML-BC (My)	—
MEG-01	CML-BC (Meg)	—
T-Lymphoid cell lines		
CCRF-CEM	T(ALL)	—
HPB-ALL	T(ALL)	—
Jurkat	T(ALL)	—
MOLT-3	T(ALL)	—
MOLT-4F	T(ALL)	—
Karpas-299	T(NHL)	—
Hut-78	T(CTCL)	—
HT-1	T(LBL)	—
Hut-102	T(ATL)	—
ATN-1	T(ATL)	—
IPAT-1	T(ATL HTLV-I(–))	—
WHN-2	T(ATL HTLV-I(–))	—
ATL-5T	T(ATL)	—
AST-1	T(HTLV-I(–))	—
KPNT-1	T(HTLV-I(+))	—
Other cell lines		
U937	Histiocytic	—
RC-K8	Histiocytic(DL)	—
SU-DHL-1	Histiocytic	—
B-Lymphoid cell lines		
NALM-6	Pre-B(ALL)	23.1
NALM-18	Pre-B(ALL)	7.5
NALL-1	Pre-pre-B(ALL)	4.0
RS4;11	Pre-pre-B(ALL)	0.7
KOPN-1	Pre-B(ALL)	63.2
KOCL-45	Pre-B(ALL)	5.8
KOCL-58	Pre-B(ALL)	36.9
KOCL-33	Pre-B(ALL)	62.3
KOCL-44	Pre-B(ALL)	49.3
KOCL-50	Pre-B(ALL)	3.4
KOCL-51	Pre-pre-B(ALL)	5.7
KOPB-26	Pre-B(ALL)	10.4
SU-DHL-4	B(NHL)	3.6
SU-DHL-6	B(NHL)	4.7
SU-DHL-9	null cell(NHL)	15.5
SU-DHL-10	B(NHL)	—
Karpas-422	B(NHL)	0.5
ACOL-1	B(NHL)	0.8
SP-49	B(NHL)	10.0
SP-53	B(NHL)	9.0
NOL-3	B(NHL)	0.9
Daudi	B(Burkitt)	9.0
Raji	B(Burkitt)	10.9
Monca	B(Burkitt)	9.0
NCU-L3	B(Burkitt)	11.0
SP-52-EB	B(EB(+))	14.0
ISHI	B(EB(+))	21.9
Myeloma cell lines		
NOP-1	Myeloma	—
NOP-2	Myeloma	—
Oda	Myeloma	—
KMS-12-PE	Myeloma	—
FLAM-76	Myeloma	—
AMo1	Myeloma	—
KM-1	Myeloma	—

a) The level of expression was normalized by arbitrarily defining the SP-49 signal as 10.0.

nodes, with intensities of one-third and two-thirds, respectively, of those of the SP-49 signal. A very weak signal was also found in PBMNCs at one-fiftieth of the SP-49 level. No signals were detected in the thymus or other tissues. These results indicate that the TCL1 expression is restricted to lymphoid organs.

TCL1 expression in tumor samples of T-cell malignancies We examined the TCL1 expression in 12 hematopoietic T-cell lines including five ATL cell lines, four T-ALL cell lines, one T-LBL cell line (HT-1), one CTCL cell line and one Ki-1 lymphoma cell line (Karpas-299), but no signals were detected in any case (Fig. 2, Table II). HT-1 cells established from a patient with lymphoblastic lymphoma carrying inv(14)(q11;q32)[15] did not exhibit any TCL1 signals. We next examined 21 cases of ATL patients and 20 were found to be negative (Fig. 3, Table I), the remaining patient UPN001 showing a very faint TCL1 signal at one-hundredth of SP-49. Ten patients with other types of T-cell malignancies (4 with T-ALL, 3 with T-PLL, 2 with T-LBL, and one with T-DL) were also studied (Fig. 3). Only one T-PLL case demonstrated a signal (two twenty-fifths of the SP-49

level), although no 14q32 abnormality was evident on karyotypic analysis.

TCL1 expression in samples of B-cell and myeloid-cell malignancies We expanded our analysis to include various types of B-cell malignancies (Fig. 4, Table II) and found the *TCL1* gene to be strongly expressed in all of 12 cell lines derived from pre-B ALL and 12 cell lines derived from B-cell lymphomas. No signal was found in the SU-DHL-10 cell line derived from a B-NHL or in seven myeloma cell lines. Eleven cell lines derived from either AML or CML were also studied (Fig. 2, Table II) but no TCL1 signals were observed in any case.

TCL1 expression in normal peripheral blood mononuclear cells stimulated by phytohemagglutinin (PHA) Since the cell line study suggested TCL1 expression to be associated with the B-cell lineage, we investigated fractionated normal PBMNCs with anti-CD19 beads. The TCL1 signal was found in CD19-positive cells, but not in CD19-negative cells (Fig. 5). This result indicated that the TCL1 expression is restricted to B-lymphocytes in peripheral blood. In an attempt to study TCL1 expression in response to a growth signal, we investigated the mRNA expression of normal PBMNCs stimulated with PHA-P. TCL1 mRNA levels decreased to one-tenth of the unstimulated case after 6 h, but the signal was then increased to 31% at 24 h, 81% at 48 h and 102% at 72 h relative to unstimulated PBMNCs. Since the kinetics of both Cμ and CTβ mRNAs were similar to that of TCL1 when the membrane was reprobed with CTβ and Cμ (Fig. 6), it is not possible to distinguish which lineage is more involved in the TCL1 expression on PHA-stimulation. However, this experiment demonstrated that the TCL1 expression is regulated upon growth stimulation.

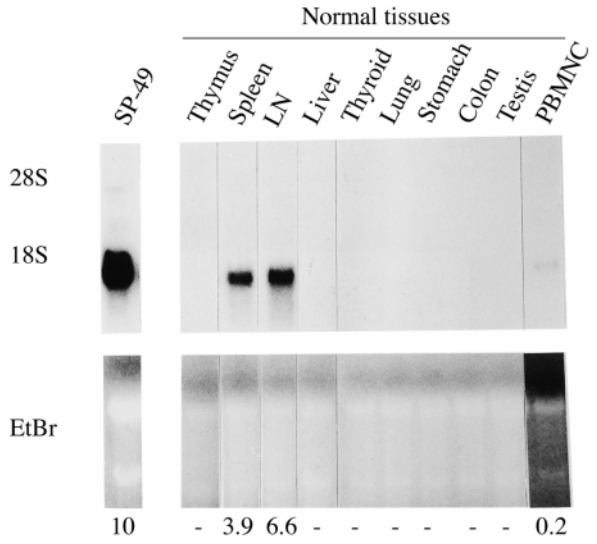

Fig. 1. Northern analysis of TCL1 expression in normal human tissues. Five micrograms aliquots of total RNAs were applied to the lanes. SP-49, a B-non Hodgkin's lymphoma cell line was used as the standard. EtBr, ethidium bromide-stained gel. The arbitrary units based on comparison with SP-49 expression are given below.

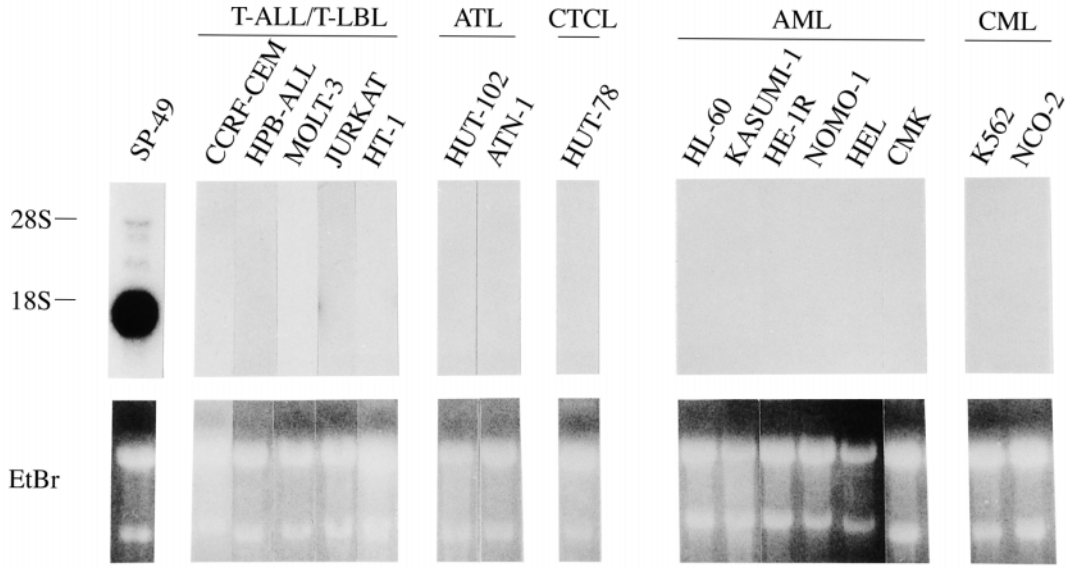

Fig. 2. Representative northern analysis of TCL1 expression in T-cell and myeloid cell lines. Ten micrograms aliquots of total RNAs were applied to the lanes. Arbitrary units based on comparison with SP-49 expression are summarized in Table II.

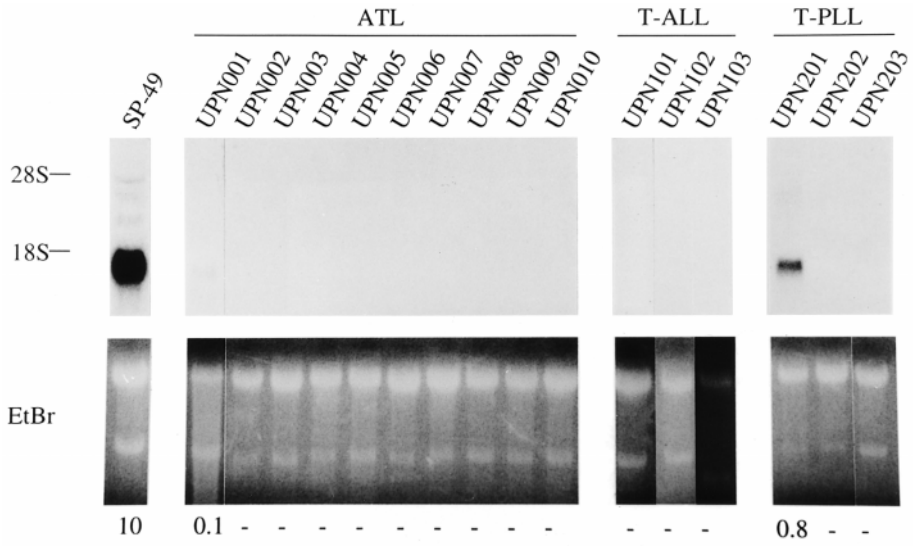

Fig. 3. Northern analysis of TCL1 expression in patients with T-cell malignancies. Five micrograms aliquots of total RNAs were applied to the lanes. See the legend to Fig. 1.

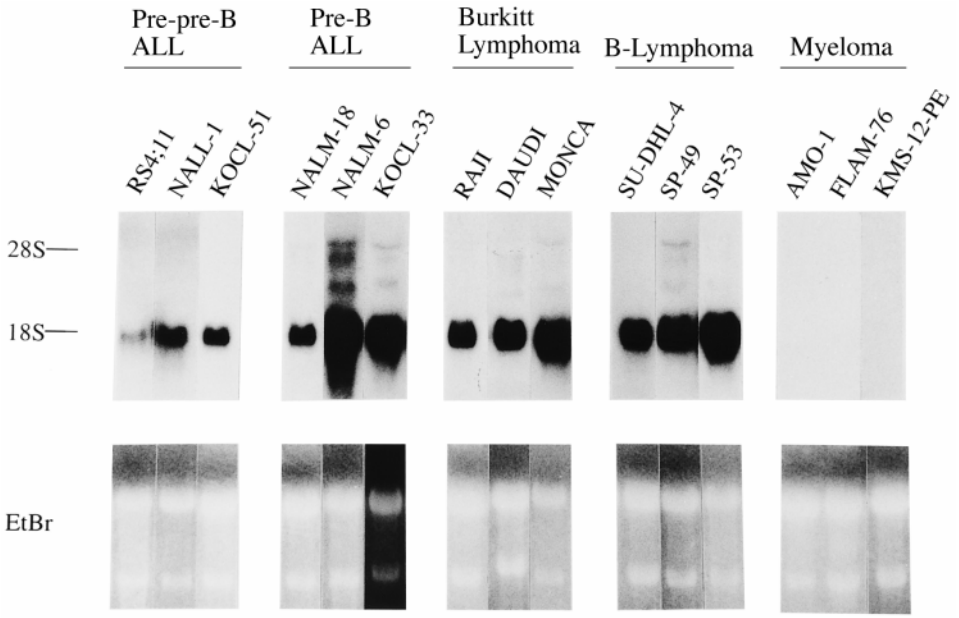

Fig. 4. Northern analysis of TCL1 expression in B-cell lines. Ten micrograms aliquots of total RNAs were applied to the lanes. Arbitrary units based on comparison with SP-49 expression are summarized in Table II.

DISCUSSION

Chromosomal translocations involving 14q32 have been reported to be the most frequent in ATL patients. The report showed that 25 out of 78 cases (32%) with acute and lymphoma type ATL had 14q32 abnormality.[12] Recently, a candidate gene in the *TCL1* locus activated by a juxtaposed *TCR* gene was isolated.[7] This prompted us to examine the expression of this gene in ATL cell lines and patients to assess its involvement. Northern blot anal-

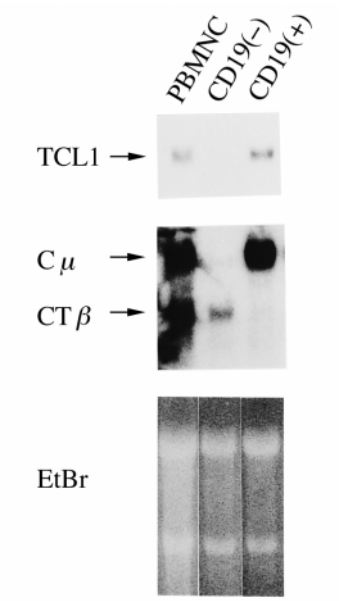

Fig. 5. Northern analysis of anti-CD19 antibody bead-fractionated PBMNC. CD19(–) represents a sample prepared from PBMNC cells depleted with anti-CD19 antibody beads, while CD19(+) indicates cells separated by the beads. TCL1, Cμ and CTβ signals are indicated by arrows.

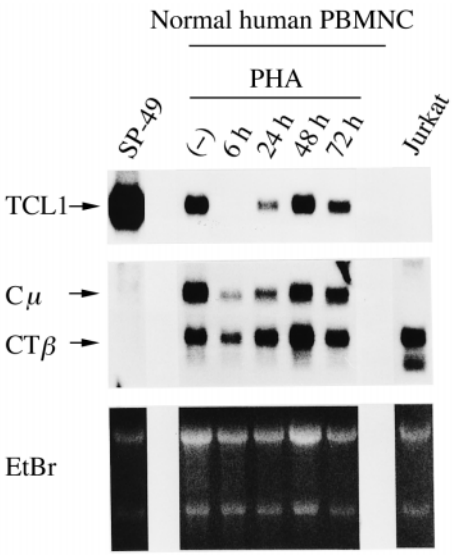

Fig. 6. Northern analysis of PHA-stimulated PBMNCs. See the legend to Fig. 5.

ysis, however, produced no signals in any of the five ATL cell lines or in 20 of the 21 ATL patients, and only a very weak signal in the remaining case, UPN001. This suggests that the *TCL1* gene is not activated in the majority of ATL patients' cells, and is in direct contradiction to the data recently presented by Narducci *et al.*,[16] who found overexpression of TCL1 products in all ATL cases examined by immunostaining. Our analysis, on the other hand, revealed that the majority of B-cell malignancies other than myelomas show very intense TCL1 signals. Furthermore, the fact that TCL1 expression was seen in normal spleen, lymph nodes and PBMNCs, but not in other tissues examined, including the thymus, suggests that TCL1 is transcribed predominantly in lymphoid cells of B-cell lineage. Indeed, cell fractionation analysis using anti-CD19 beads demonstrated that CD19-positive cells are responsible for the TCL1 in PBMNCs. Thus, it should be emphasized that the expression of TCL1 in ATL leukemic cells needs to be more carefully examined, because contamination by B cells might generate strong positive signals. It is therefore significant that Narducci *et al.*'s data showing positive reaction on ATL cells do not correlate to the percentage of leukemic cells in the samples.[16] Furthermore, the expression of various cell lineages examined in their reports[7, 8, 16] has not been examined by means of northern blot analysis, but only reverse transcriptase-polymerase chain reaction. Therefore, the positivity on ATL cells needs to be re-examined in conjunction with mRNA levels, which should clarify the relevance of *TCL1* gene involvement in ATL.

In an attempt to study their possible role in TCL1 expression, PBMNCs were stimulated with PHA. In response, the TCL1 signal was reduced to about one-tenth after 6 h, and gradually increased thereafter. Although the precise function of the *TCL1* gene remains unknown, this decrease caused by PHA might indicate some role in cell proliferation. The down-regulation of the signal during the first 6 h after the stimulation also suggests that TCL1 may be expressed during a limited period of the cell cycle.

The present study revealed one PLL patient with significant TCL1 expression. Because the number of blastic cells in this patient was 76,300/μl and 95% of the cells were CD3-positive, it is unlikely that contaminating B-cells were responsible for this level of mRNA expression. This suggests that either a distinct type of T-cell malignancy might aberrantly express TCL1, or a T-cell subset, presenting itself as such a T-cell malignancy, might express TCL1 as a normally regulated gene expression in the course of development. Although the TCL1 expression in one PLL patient raises the possibility that TCL1 can indeed be aberrantly expressed in ATL, the fact that the 21 cases we examined did not show any strong mRNA expression suggests that TCL1 does not represent a major gene alteration in ATL. Thus, it is important to study TCL1 in many T-cell and B-cell malignancies of various types, as was done in the present study to clarify the role of the *TCL1* gene in cell differentiation and proliferation.

In conclusion, our data presented here demonstrate that *TCL1* gene involvement is not a major cause of ATL development, but rather indicate that this gene expression may play some role in B-cell development.

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