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Advances in Brief

Regulation of TCL1 Expression in B- and T-Cell Lymphomas and Reactive Lymphoid Tissues[1]

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Abstract

Chromosomal rearrangements observed in T-cell prolymphocytic leukemia involve the translocation of one T-cell receptor gene to either chromosome 14q32 or Xq28, deregulating the expression of cellular proto-oncogenes of unknown function, such as *TCL1* or its homologue, *MTCP1*. In the human hematopoietic system, *TCL1* expression is predominantly observed in developing B lymphocytes, whereas its overexpression in T cells causes mature T-cell proliferation in transgenic mice. In this study, using a newly generated monoclonal antibody against recombinant TCL1 protein, we extended our analysis mainly by immunohistochemistry and also by fluorescence-activated cell sorting and Western blot to a large tumor lymphoma data bank including 194 cases of lymphoproliferative disorders of B- and T-cell origin as well as reactive lymphoid tissues. The results obtained show that in reactive lymphoid tissues, TCL1 is strongly expressed by a subset of mantle zone B lymphocytes and is expressed to a lesser extent by follicle center cells and by scattered interfollicular small lymphocytes. In B-cell neoplasia, TCL1 was expressed in the majority of the cases, including lymphoblastic lymphoma, chronic lymphocytic leukemia, mantle cell lymphoma, follicular lymphoma, Burkitt lymphoma, diffuse large B-cell lymphoma (60%), and primary cutaneous B cell lymphoma (55%). TCL1 expression was observed in both the cytoplasmic and nuclear compartments, as confirmed by Western blot analysis. Conversely, TCL1 was not expressed in Hodgkin/Reed-Sternberg cells, multiple myelomas, marginal zone B-cell lymphomas, $CD30^+$ anaplastic large cell lymphoma, lymphoblastic T-cell lymphoma, peripheral T-cell lymphoma, and mycosis fungoides. These data indicate that TCL1 is expressed in more differentiated B cells, under both reactive and neoplastic conditions, from antigen committed B cells and in germinal center B cells and is down-regulated in the latest stage of B-cell differentiation.

Introduction

Chromosome rearrangements involving either *TCR*[3] or *Ig* genes are often observed in human hematological malignancies. These chromosome breakpoints juxtapose enhancer elements of *Ig* and *TCR* loci to proto-oncogenes, leading to oncogene deregulation and tumor initiation (1, 2).

The *TCL1* gene (3) and the newly cloned *TCL1/MTCP1*-like 1 (*TCL1b*) gene at human chromosome 14q32.1 (4, 5) and their homologue, the *MTCP1* gene at Xq28 (6), are involved in the leukemogenesis of mature T cells. Indeed, overexpression of *TCL1/MTCP1* genes is observed in more than 90% of T-PLLs (3, 7–9). This T leukemia often occurs in patients with the genetic syndrome ataxia telangiectasia and is frequently associated with mutations in the *ATM* gene (10).

Transgenic animals overexpressing either activated *TCL1* or *MTCP1* gene develop mature T-cell leukemias, indicating that these genes are directly involved in leukemogenesis (11, 12). However, few data have been obtained regarding their physiological function. Recently, the crystal structures of human recombinant TCL1 and MTCP1 proteins have been determined, showing a novel β barrel topology that resembles the structure of lipocalin and calycin proteins (13, 14). This structural similarity suggests that TCL1 and MTCP-1 form a unique family of proteins that is predicted to bind small hydrophobic ligands (14). Analysis of TCL1 expression in organs, tissues, subpopulations of B and T cells of bone marrow and thymus, and a large panel of cell lines indicates that TCL1 is mainly expressed in cells of lymphoid lineage (3, 15, 16). Constitutive expression observed in normal fetal B cells sorted into subpopulations from bone marrow showed that TCL1 expression begins as early as at the $CD34^+CD19^+$ pro-B-cell stage. Its expression peaks in pro-B cell $CD19^{hi}$ IgM^-, persists in immature $CD19^+\mu^{lo}$, and decreases to a minimal level in more mature B cells ($CD19^+\mu^{hi}$; Ref. 3). Conversely, TCL1 expression is detectable in fetal thymocytes sorted into subpopulations in very immature $CD4^-CD8^-CD3^-$ thymocytes, but not in more mature $CD4^+CD8^+$ and $CD4^+CD8^-$ or $CD4^-CD8^+$ cells. Moreover, the study of a variety of T-ALL cell lines for TCL1 expression indicated that it is not present in these cells (8).

In this report, we used an immunohistochemical approach by using a recently generated MoAb against TCL1 to study its expression in reactive lymphoid tissues of lymph nodes and spleen and in 194 cases of B- and T-cell lymphomas/leukemias, spanning the whole spectrum of these neoplasms. In addition, we used this new antibody in FACS to detect TCL1 protein in normal and pathological peripheral blood, and we refined the analysis of subcellular extracts by Western blot to localize the TCL1 protein to the cytoplasm and nucleus compartment.

Materials and Methods

Patient Cases. A series of biopsies was selected from the files of the Department of Pathology of the Università degli Studi "La Sapienza," Istituto Dermopatico dell'Immacolata and the Division of Pathology of Azienda Ospedaliera S. Giovanni-Addolorata (Rome, Italy) to include: (*a*) 8 cases of reactive lymphoid hyperplasia; (*b*) 5 spleen specimens from patients who

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[3] The abbreviations used are: TCR, T-cell receptor; Ig, immunoglobulin; GC, germinal center; T-PLL, T-prolymphocytic leukemia; FACS, fluorescence-activated cell sorting; HD, Hodgkin's disease; MaZ, marginal zone; MALT-L, mucosa-associated lymphoid tissue lymphoma; PCBCL, primary cutaneous B-cell lymphoma, BL, Burkitt lymphoma; MoAb, monoclonal antibody; T-ALL, T-acute lymphatic leukemia; TBS-T, Tris-buffered saline; GST, glutathione *S*-transferase; CLL, chronic lymphocytic leukemia; PBMC, peripheral blood mononuclear cell; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; FL, follicular lymphoma.

Table 1 *Summary of TCL1 immunostaining results*

Diseases	No. of cases	% of TCL1-positive cases
Lymphoblastic B-cell lymphoma	4	75%
CLL	11	100%
Mantle cell lymphoma	8	87.5%
BL	5	100%
Follicular lymphoma	12	75%
Diffuse large B-cell lymphoma	15	60%
Cutaneous B-cell lymphoma	9	55%
MaZ B-cell lymphoma (nodal and extranodal type)	12	0%
Myeloma/plasmacytoma	12	0%
Classical HD	40	0%
Lymphocyte predominance HD	5	0%
$CD30^+$ anaplastic large cell lymphoma	5	0%
Lymphoblastic T-cell lymphoma	5	0%
Nodal peripheral T-cell lymphoma	10	0%
Mycosis fungoides	40	0%

underwent splenectomy because of traumatic rupture of the spleen; (*c*) 55 cases of nodal B-cell non-Hodgkin's lymphoma; (*d*) 45 cases of HD; (*e*) 5 cases of $CD30^+$ anaplastic large cell lymphoma; (*f*) 5 cases of lymphoblastic T-cell lymphoma; (*g*) 10 cases of nodal peripheral T cell lymphoma; (*h*) 1 case of T-PLL; (*i*) 7 cases of myeloma/plasmacytoma; (*j*) 5 cases of extramedullary plasmacytoma; (*k*) 12 cases of MaZ B-cell lymphomas [8 cases of low-grade gastrointestinal MALT-L, 1 case of low-grade salivary gland MALT-L, and 3 cases of MaZ cell lymphomas (2 from spleen and 1 from lymph node)]; (*l*) 9 cases of PCBCL; and (*m*) 40 cases of primary cutaneous T-cell lymphomas, *i.e.*, mycosis fungoides (see Table 1 for details). Pathological specimens were classified according to the Revised European and American Lymphoma classification. Primary cutaneous lymphomas were diagnosed according to the European Organization for Research and Treatment of Cancer classification.

Antibodies. The MoAb raised against purified recombinant human TCL1 protein (17, 18) was generated by Dr. Maria Luisa Nolli (Biosearch Laboratories, Gerenzano, Italy) and is named 27D6/20 MoAb (isotype IgG1). Anti-β-tubulin ascites antibody, TUB2.1, was obtained from Sigma.

Immunohistochemistry. For immunohistochemical studies, paraffin-embedded sections were heated for 1 h at 55°C. After dewaxing, the slides were rehydrated through a graded ethanol series and distilled water, immersed in PBS (pH 7.4), and then treated with 0.1% trypsin solution in Tris buffer for 30 min at 37°C. Endogenous peroxidase was blocked with 3% hydrogen peroxide, and nonspecific binding was blocked with 10% normal serum. Immunostaining was performed by incubating the 27D6/20 MoAb specific for recombinant human TCL1 protein at a concentration of 0.01 μg/μl.

Immunohistochemical staining was performed by using alkaline phosphatase anti-alkaline phosphatase and streptoavidin-biotin peroxidase labeling methods (19, 20).

Cell Culture. SUPT11 is a T-ALL cell line established from a patient with mature T-lymphocytic leukemia carrying a t(14;14)(q11;q32.1) and overexpressing TCL1. MOLT4 is a T-ALL cell that does not express TCL1, and it has been used as a negative control. These cells were grown in suspension in RPMI 1640 (Life Technologies, Inc., Gaithersburg, MD) supplemented with 10% bovine calf serum (Life Technologies, Inc.). All cells were maintained in a humid incubator at 5% CO_2 and 37°C.

Flow Cytometry Analysis. For detection of intracellular TCL1 expression, FACS was performed as described previously (21). PBMCs from normal donors and from a T-PLL patient were purified by Ficoll-Hypaque centrifugation. Because TCL1 is an intracellular antigen, mononuclear cells were previously fixed in 4% paraformaldehyde in PBS for 10 min at 4°C and permeabilized in 0.1% saponin in PBS for 10 min at room temperature. The treated cells were incubated with unconjugated mouse antihuman TCL1 antibody (27D6/20) diluted in saponin buffer at a final concentration of 1 ng/μl for 30 min at room temperature. Cells stained with an irrelevant mouse IgG1 MoAb (Becton Dickinson, San Jose, CA) were used as a negative control to rule out nonspecific staining. After this step, phycoerythrin-conjugated goat antimouse IgG (Dakopatts, Glostrup, Denmark) diluted in saponin buffer was used to detect TCL1 protein. Flow cytometry was performed by FACScan (Becton Dickinson). The data were analyzed using WinMDI (Joseph Trotter, Scripps Institute, La Jolla, CA) or Cell Quest (Becton Dickinson) software.

Preparation of Cellular Fractions and Immunoblotting. To determine the presence of TCL1 in both nuclear and cytoplasmic fractions, SUPT11 and MOLT4 cells were fractionated by a modification of the procedure described previously (22). Suspension cells were harvested at 1200 rpm and washed once with PBS at 4°C (all of the subsequent steps were performed at 4°C). Cell pellets were lysed in a 1.5 packed cell pellet volume of 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM DTT, and 0.5 mM phenylmethylsulfonyl fluoride on ice. The cells were collected at 1300 $\times$ *g* for 10 min, and the supernatant (cytoplasmic fraction) was carefully decanted. The resultant pellet was resuspended again as described previously in 1 pellet volume with the same buffer and centrifuged again. This step was repeated twice more, and the supernatants were pulled together. Contaminating organelles and whole cells were removed by adding 0.l volume of detergent solution (one part 10% sodium deoxycholate and two parts 10% NP40) to the last wash. Nuclear and cytoplasmic fractions were adjusted to equivalent volumes with Laemmli Sample Buffer. The extracts were resolved on 12% polyacrylamide gels and electrotransferred to nitrocellulose membranes using standard procedures (23). Membranes were then washed three times (5 min each) with TBS-T containing 3% milk, incubated with primary antibody (27D6/20 MoAb), diluted in the same buffer (1 μg/ml) for a minimum of 2 h at room temperature, washed again as outlined above (three times, 5 min each), and then incubated with antimouse horseradish peroxidase-conjugated secondary antibody for 1 h. Membranes were subsequently washed (three times, 5 min each) with TBS-T/milk and then washed once with 0.5% TBS-T and washed twice with 50 mM Tris (pH 6.8). The optimal primary antibody dilution was determined by titrating 27D6/20 MoAb against GST-TCL1 fusion protein. Antigen-antibody interaction was detected by enhanced chemiluminescence (Amersham, Arlington Heights, IL).

Results

Immunohistology of Reactive Lymphoid Tissues. In reactive lymphoid hyperplasia, TCL1 was expressed by the cells of the GCs (centrocytes and centroblasts) of the secondary lymphoid follicles and by a subset of small lymphocytes of the lymphoid mantles. A few scattered, TCL1-positive, small lymphoid cells were also observed within the paracortical areas. The GC cells showed a different degree of nuclear and cytoplasmic immunoreactivity, sometimes with evidence of polarization [the "dark" zone was more positive (data not shown)]. The TCL1-positive, small lymphocytes of the lymphoid mantles showed a strong nuclear immunoreactivity, as did the TCL1-positive cells of the paracortical areas. In the latter cells, a cytoplasmic positivity was also observed in some cases (Fig. 1*A*). Expression analysis performed in spleen tissues showed that cells of the MaZ were consistently TCL1 negative (Fig. 1*B*).

B-cell Lymphoproliferative Diseases. TCL1 was expressed in 75% of B-cell lymphoblastic lymphomas (Fig. 1*L*) and follicular lymphomas (Fig. 1*C*), in all cases of lymphocytic lymphoma/CLL (Fig. 1*F*), in all but one case of mantle cell lymphoma (Fig. 1*G*), in 60% of nodal diffuse large B-cell lymphomas (Fig. 1*H*), in 100% of BLs (Fig. 1*I*), in 60% of primary cutaneous follicle center cell lymphomas, and in 50% of PCBCLs other than follicular lymphoma. TCL1 was not expressed in nodal and extranodal MaZ cell lymphomas (including MALT-L) or in neoplastic plasma cells in all seven cases of multiple myeloma and all five cases of extramedullary plasmacytoma (Table 1). The percentage of positive cells and the degree and pattern (nuclear and/or cytoplasmic) of immunoreactivity were somewhat variable in the different subtypes of lymphoproliferative diseases, with well-differentiated lymphocytic lymphoma/CLL, mantle cell lymphoma, and BL usually showing a higher percentage of positive cells, which is often associated with a stronger immunoreactivity. On the contrary, follicular lymphoma and diffuse large B-cell lymphoma cases showed a more variable distribution in terms of the number of positive cells and the degree of immunoreactivity. As far as the immunolocalization of TCL1 is concerned, in most cases, we observed both nuclear and cytoplas-

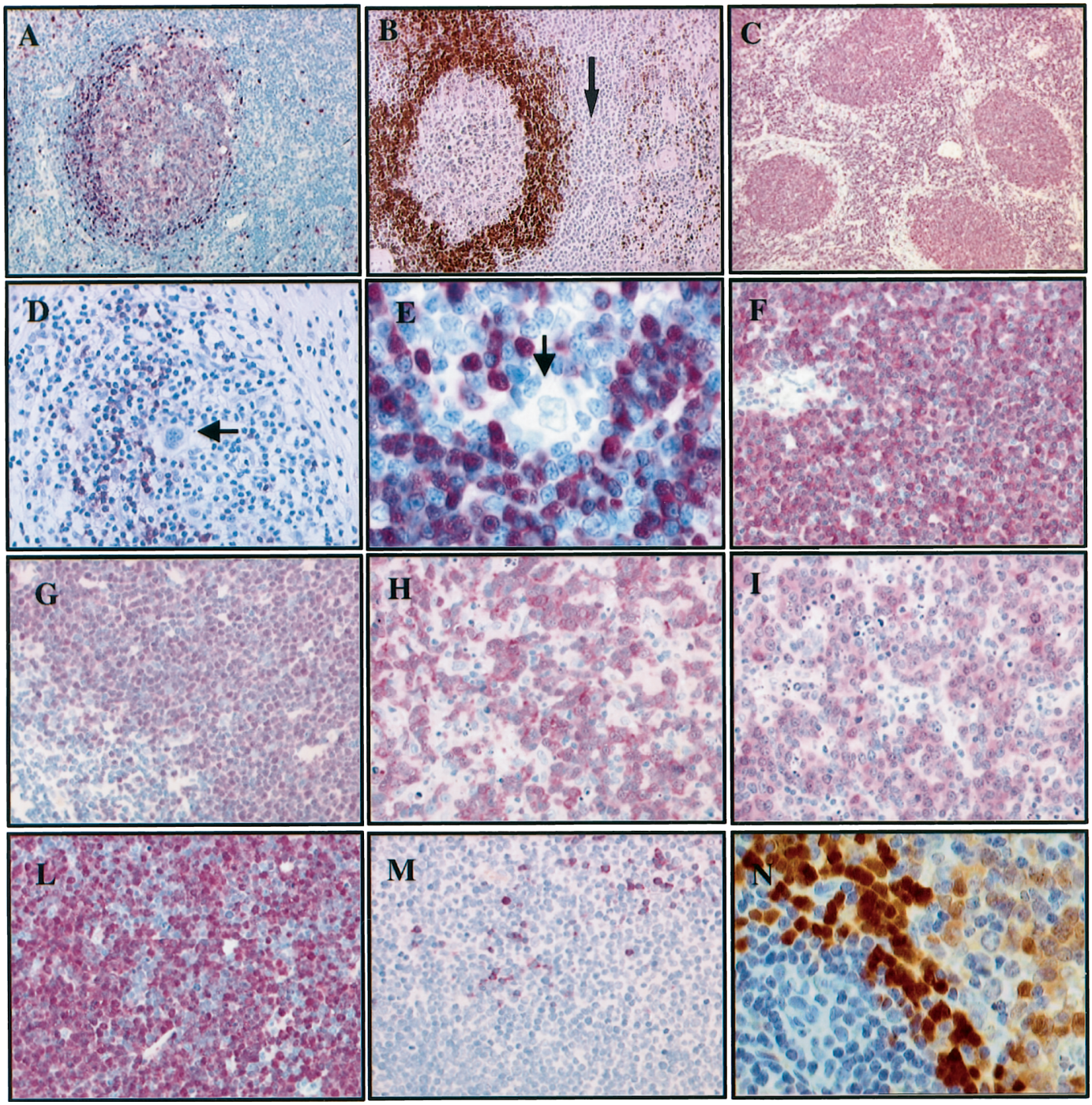

Fig. 1. TCL1 immunostaining of reactive lymph node, spleen tissue, and B- and T-cell lymphomas (*A–N*). *A*, reactive lymph node (×100) showing an enlarged follicle with moderately TCL1-immunopositive GC cells. A subset of mantle zone lymphocytes and a few scattered small lymphocytes within paracortical areas showed a strong TCL1 immunoreactivity. *B*, spleen tissue (×100) showing MaZ cells (*arrow*) to be consistently negative for TCL1. Follicular lymphoma (*C*; ×40), nodular sclerosis HD (*D*; ×200), and lymphocyte predominance variant HD (*E*; ×400) showing Hodgkin/Reed-Sternberg and "pop corn" Reed-Sternberg cells are consistently TCL1 negative. *F*, CLL (×200) showing a large majority of cells with cytoplasmic and nuclear TCL1 immunoreactivity. *G*, mantle cell lymphoma (×200) showing neoplastic cells with TCL1 immunoreactivity. Immunoreactivity of TCL1 in diffuse large B-cell lymphoma (*H*; ×200), BL (*I*; ×200), and B-cell lymphoblastic lymphoma (*L*; ×200). *M*, TCL1 is consistently negative in the neoplastic cells of T-cell lymphoblastic lymphoma (×200). *N*, reactive lymph node (×250) showing mantle cells with a strong nuclear and cytoplasmic TCL1 immunoreactivity.

mic immunoreactivity, with only a minority of cases of diffuse large B-cell lymphoma and BL showing either nuclear or cytoplasmic immunoreactivity. These findings are shown in Fig. 1, *A–N*.

HD and CD30$^+$ Anaplastic Large Cell Lymphoma. Twenty cases of classical HD [14 cases of the nodular sclerosis variant (Fig. 1*D*) and 6 cases of the mixed cellularity variant] have been investigated. In all of these cases, the Hodgkin/Reed-Sternberg cells were consistently TCL1 negative. Areas/nodules and scattered, TCL1-positive, small lymphoid cells were observed in all cases. Furthermore, in those cases in which residual lymphoid follicles with GCs were present, the pattern of TCL1 immunoreactivity was similar to that observed in the follicles of reactive lymphoid hyperplasia. Five cases of nodular lymphocyte predominance HD have been studied. In all cases, the "pop corn" Reed-Sternberg cells typical of nodular lymphocyte predominance HD were consistently TCL1 negative (Fig. 1*E*). In all cases, the nodules developed from progressively trans-

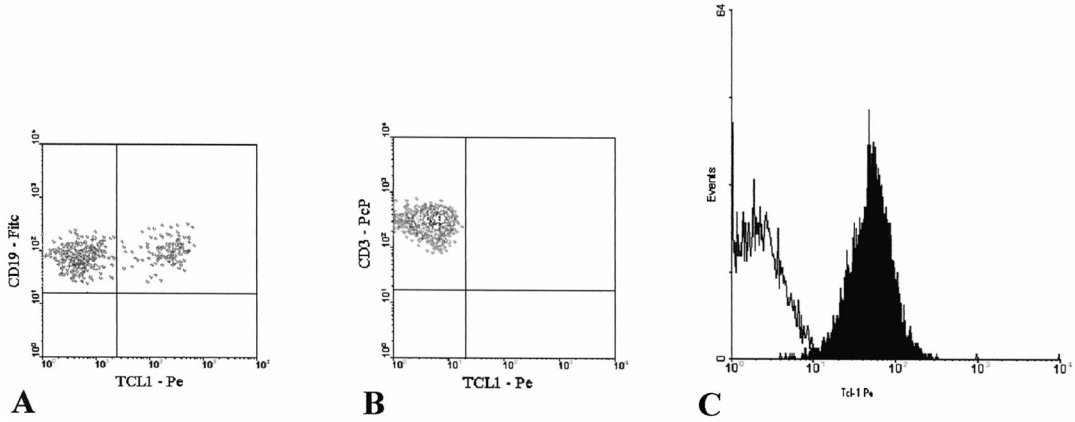

Fig. 2. FACS analysis on PBMCs from normal donors and from a T-PLL patient showing the three-color flow cytometry of CD19/TCL1/CD3 combination on normal donors and T-PLL patient PBMCs. Live lymphocytes were gated on forward and side light scatter. *A* and *B* represent cytoplasmic TCL1 expression in a normal control within electronically gated $CD19^+$ (*A*) and $CD3^+$ (*B*) subsets; *C* indicates TCL1 expression in electronically gated $CD3^+$ lymphocytes in a case of T-PLL. The *white curve* in this histogram represents an isotype control with an irrelevant murine IgG1.

formed GCs contained a significant number of TCL1-positive, small lymphoid cells.

Five cases of $CD30^+$ anaplastic large cell lymphoma have been studied, and all of them were consistently TCL1 negative (Table 1).

T-cell Lymphomas. Five cases of T-cell lymphoblastic lymphoma, 10 cases of nodal peripheral T-cell lymphoma, and 40 cases of primary cutaneous T-cell lymphoma have also been investigated, and all of them were consistently TCL1 negative. In every case, scattered, TCL1-positive, small lymphoid cells were present. In those cases in which residual lymphoid follicles were observed, TCL1 immunoreactivity was similar to that observed in the follicles of reactive lymphoid hyperplasia (Fig. 1*A*).

FACS Analysis on PBMCs from Normal Donors and from a T-PLL Patient. To assess whether the MoAb 27D6/20 was also a valuable tool in FACS analysis, we analyzed permeabilized PBMCs derived from normal donors and from one case of T-PLL. FACS analysis revealed that TCL1 is expressed in $CD19^+$ cells, but no TCL1 expression is detectable in $CD3^+$ cells of normal donors (Fig. 2, *A* and *B*). Conversely, FACS analysis performed on cells from a 75-year-old T-PLL patient (high WBC count, splenomegaly, >90% of circulating T lymphocytes with a T mature phenotype, $CD3^+$, $TCR\alpha\beta^+$, $TCR\gamma\delta^-$, $CD4^+$, $CD8^-$, $CD2^+$, $CD5^+$, $CD7^+$, $CD25^{+/-}$, $CD16^-$, $CD56^-$, and $CD19^-$) revealed a strong TCL1 expression in these lymphocytes (Fig. 2*C*), confirming that TCL1 is directly activated as described previously by other methods (9, 24).

MoAb Specificity and TCL1 Cellular Localization. The specificity of the MoAb 27D6/20 is shown in Fig. 3*A*, in which the MoAb recognizes only an approximately M_r 14,000 protein in cell extracts derived from the SUPT11 cell line on Western blot analysis. Furthermore, the 27D6/20 antibody is specific to recombinant TCL1 protein and does not cross-react with the recombinant TCL1b gene product and MTCP1 (Fig. 3*A*). The localization of TCL1 to both the cytoplasm and nuclear fractions was confirmed by immunoblots of subcellular fractions from SUPT11 cells. As shown in Fig. 3*B*, TCL1 is present in both the cytoplasm and isolated nuclei. The distribution of TCL1 between the two compartments is not equivalent, and a densitometer quantification shows a distribution ratio of 1:7 between the nucleus and cytoplasm. Effective separation of nuclei from cytoplasm has been confirmed by immunoblots for β-tubulin, which show that isolated nuclei are devoid of cytoskeletal and cytoplasmic contamination (Fig. 3*B*). TCL1 localization to both the cytoplasm and nucleus is also shown in high magnification of an immunohistochemistry performed on reactive lymph node tissue (Fig. 1*N*).

Discussion

In this study, we report the analysis of TCL1 expression with a newly generated and specific MoAb in 194 cases of B- and T-cell lymphomas, HD, and reactive hyperplastic secondary lymphoid tissues. This study allowed us to clarify the expression of this lymphoid

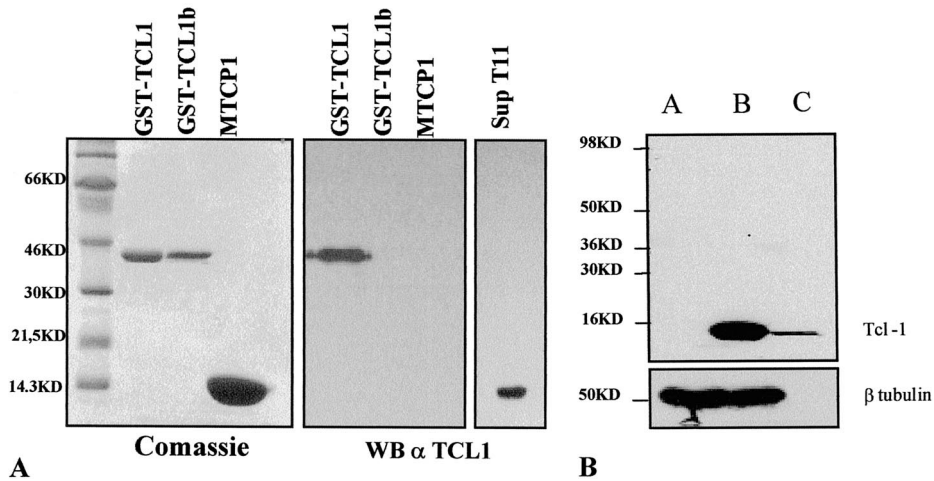

Fig. 3. *A*, SDS-PAGE analysis of GST-hTCL1 (10 μg), GST-hTCL1b (8 μg), and purified hMTCP1 (18 μg) using Coomassie BBR-250 staining (*left*) and α-hTCL1 27D6 (1 μg/ml) antibody Western blot showing hTCL1 specificity (*center*). Western blotting analysis with α-hTCL1 27D6 (1 μg/ml) of whole SUPT11 cell extracts (50 μg) shows a hTCL1-specific band (*right*). *B*, TCL1 detection on cytoplasmic cell extract and isolated nuclei is shown. Lanes are loaded on the basis of cellular equivalence. *Lane 1*, MOLT3 cytoplasmic extract; *Lane 2*, SUPT11 cytoplasmic extract; *Lane 3*, SUPT11 nuclei. Samples were also exposed to anti-β-tubulin antibodies to show the purity of the nuclear fraction.

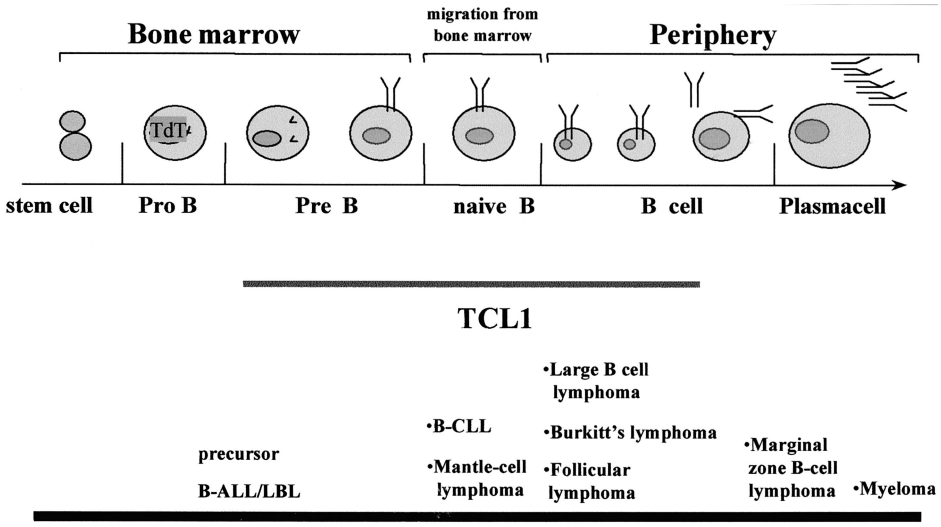

Fig. 4. TCL1 expression in B-cell development. The diagram shows the stages of B-cell development and the timing of TCL1 gene expression. The *black bar* shows the leukemias and/or lymphomas corresponding to B-cell developmental progression.

gene in more mature normal cells and in B- and T-cell tumors, indicating that this gene might be important in the differentiation of B cells (Fig. 4). TCL1 expression studies have been conducted predominantly in bone marrow and thymic fetal cells, normal and malignant B- and T-cell lines, T-cell leukemias, and peripheral blood primarily by reverse transcription-PCR and Northern blotting (3, 8, 9, 15, 16), and, using molecular analysis and immunocytochemistry, Teitell *et al.* (25) recently reported TCL1 involvement in reactive tonsils and lymph nodes and TCL1 up-regulation in the majority of AIDS immunoblastic lymphoma plasmacytoid tumors examined.

We observed TCL1 expression in B-cell type lymphoid tumors such as lymphoblastic lymphoma, CLL, MCL, FL, BL, and DLBCL cases distinctive of pre-GC, GC and, at least partially, of post-GC differentiation stages. The positivity of TCL1 in most cases of lymphoblastic lymphoma, which is characterized mainly by pre B-cell markers, is consistent with our previous observation that TCL1 is expressed in pre B-cells (3). As far as DLBCLs are concerned, the finding that only 60% of cases in the present series do express TCL1 likely reflects their heterogeneity in terms of morphology, immunophenotype, and pathogenesis because some DLBCLs may be of GC cell origin, whereas others may originate from B-cells at a post-GC differentiation stage (26, 27). When GC-associated lymphomas were analyzed, a strong positivity was observed in BL and FL, but TCL1 was entirely absent in all our cases of HD (classical and lymphocyte predominance), for which GC B-cell origin has been proposed because of a somatic mutation within the rearranged immunoglobulin gene (28). This negativity was also maintained in more mature (closer to plasma cells than GC-B cells) derived B-cell lymphomas such as MaZ cell lymphoma, including all MALT-Ls, neoplasms for which a MaZ origin has been proposed (29). Similarly, in all multiple myeloma/extramedullary plasmacytoma, no TCL1 expression was detected, indicating that this gene is not required in terminal differentiated B cells.

TCL1 was observed in only 55% of PCBCLs, but it was not observed in the remaining 45% of PCBCLs, suggesting that this subset of lymphomas could originate from B cells in an advanced stage of differentiation (post-GC) and supporting the recent proposal that histological subtypes of PCBCLs could be derived from MaZ B cells (30, 31).

The expression pattern depicted above was also reflected in the staining of normal reactive lymph nodes and spleen. TCL1 expression was highly positive in a subset of mantle zone small lymphocytes, mainly within the nucleus. Centroblasts and centrocytes of the GC showed a lesser and more variable TCL1 expression ranging from slight to moderate, with both nuclear and cytoplasmic localization. TCL1 expression was consistently negative in the MaZ cells of the normal spleen follicles. Our results are consistent with the recent findings of Teitell *et al.* (25), who reported that hyperplastic lymph node and tonsil also exhibit strong TCL1 protein expression in mantle zone B cells and in rare interfollicular zone cells, whereas follicle-center B-cells show weaker expression.

Analysis of T-cell neoplasms, including nodal, cutaneous, and T-cell lymphomas and $CD30^+$ anaplastic large cell lymphoma, revealed no TCL1 expression except in a T-PLL, in which TCL1 plays a causative role.

The pattern of immunoreactivity of TCL1 in B cells showed that TCL1 is detectable within both the nuclei and cytoplasm. Moreover, the nuclear immunoreactivity appears moderately strong in some B cells, such as in the subset of mantle B lymphocytes. These results, together with new data obtained concerning the protein structure (13, 14), suggest a possible active role for TCL1 as a "shuttle protein" between the cytoplasm and the nucleus.

Whereas its function has yet to be unraveled, TCL1 and its cognate, MTCP1, and probably TCL1b as well are clearly involved in lymphoid tumorigenesis and differentiation; antiapoptotic activity might be envisaged, based on the long latency of the tumors associated with TCL1/MTCP1 expression in ataxia telangiectasia patients (9, 24) and transgenic mice (11, 12) and on TCL1 expression, which seems to parallel that of BCL-2 (25). However, although it is evident that TCL1 is predominately expressed in quiescent mantle zone B cells, no reactivity is observed in most normal T cells, plasma cells, and MaZ cell lymphoma as seen with BCL-2 (32); therefore, it is possible that TCL1 might also act on a pathway other than BCL-2-associated apoptosis. At the moment, its window of expression in normal and pathological B lymphocytes, excluding the possibility that specific chromosomal translocations could activate it, suggests its involvement in B-cell differentiation and highlights the observation that this gene is highly expressed in antigen-committed B cells, persists in antigen-activated GC B cells, and is down-regulated in the latest stage of B-cell differentiation (Fig. 4).

Acknowledgments

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