

## TCL1 oncogene expression in AIDS-related lymphomas and lymphoid tissues

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**ABSTRACT** AIDS-related non-Hodgkin's lymphoma (AIDS NHL) comprises a diverse and heterogeneous group of high-grade B cell tumors. Certain classes of AIDS NHL are associated with alterations in oncogenes or tumor-suppressor genes or infections by oncogenic herpesviruses. However, the clinically significant class of AIDS NHL designated immunoblastic lymphoma plasmacytoid (AIDS IBLP) lacks any consistent genetic alterations. We identified the *TCL1* oncogene from a set of AIDS IBLP-associated cDNA fragments generated by subtractive hybridization with non-AIDS IBLP. Aberrant *TCL1* expression has been implicated in T cell leukemia/lymphoma development, and its expression also has been seen in many established B cell tumor lines. However, *TCL1* expression has not been reported in AIDS NHL. We find that *TCL1* is expressed in the majority of AIDS IBLP tumors examined. *TCL1* protein expression is restricted to tumor cells in AIDS IBLP tissue samples analyzed with immunohistochemical staining. 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 (centroblasts and centrocytes) show weaker expression. These results establish *TCL1* as the most prevalent of all of the surveyed oncogenes associated with AIDS IBLP. They also indicate that abundant *TCL1* expression in quiescent mantle zone B cells is down-regulated in activated germinal center follicular B cells in parallel to the known expression pattern of *BCL-2*. High-level expression in nonproliferating B cells suggests that *TCL1* may function in protecting naïve preactivated B cells from apoptosis.

AIDS-related non-Hodgkin's lymphoma (AIDS NHL) occurs in up to 10% of HIV-infected individuals who have moderate to severe immunodeficiency (1–3). These lymphomas are biologically and genetically heterogeneous, are derived from germinal center or postgerminal center B cells, and are classified according to body location and histologic criteria (reviewed in refs. 4–7). Certain AIDS NHL classes are associated with specific oncogenic lesions or viral involvement. For example, AIDS-related Burkitt's lymphoma usually contain activating *c-MYC* translocations, whereas AIDS-related primary-effusion lymphoma consistently contain human herpesvirus-8 (8–24). AIDS diffuse large B cell lymphoma accounts for 70% of systemic lymphomas and is the second most common type of cancer after Kaposi's sarcoma in AIDS patients (25, 26). Systemic AIDS diffuse large B cell lymphoma are further classified into two subclasses (6, 7, 27–29). AIDS large noncleaved-cell lymphoma is postulated to originate from germinal center B cells and often exhibits dysregulated

expression of the *BCL-6* protooncogene because of chromosomal translocations or promoter mutations. AIDS immunoblastic lymphoma plasmacytoid (AIDS IBLP) is thought to derive from postgerminal center B cells and is not associated with any predominant genetic alteration (30, 31). AIDS IBLP are monoclonal tumors that usually contain Epstein–Barr virus, indicating that they are not simply Epstein–Barr virus-driven polyclonal proliferations. The lack of any consistently associated oncogene involvement in AIDS IBLP strongly suggests that these tumors arise through novel patterns of dysregulated gene expression (7, 15, 32).

We sought to identify differentially expressed genes in AIDS IBLP patient samples versus non-AIDS lymphoma samples by using suppression subtractive hybridizations (SSH) (33, 34). Large cell lymphomas with immunoblastic/plasmacytoid features consistent with postgerminal center derivations (IBLP) were selected for these subtractions from HIV-infected or uninfected patient samples. The *TCL1* oncogene was identified among multiple differentially expressed genes isolated from AIDS IBLP in these studies.

*TCL1* is developmentally regulated and is normally expressed in fetal thymocytes, in bone marrow pre-B and immature B cells, and weakly in CD19<sup>+</sup> peripheral blood lymphocytes (40, 41). Abnormal *TCL1* expression caused by chromosomal translocations or inversions near T cell receptor-enhancer elements have been previously demonstrated in T cell leukemia/lymphoma (35–39). Continuous tissue-specific expression of *TCL1* also caused transgenic mice to develop a polyclonal T cell expansion with subsequent progression to T cell leukemia (40). Widely variable levels of *TCL1* expression have been reported in many established B lymphoblastoid and B cell tumor lines. The combined results of several studies showed that most immortalized B lymphoblastoid cell lines (10/12) as well as B cell tumor lines established from acute lymphoblastic leukemia (15/18), Burkitt's lymphoma (9/11), and non-Hodgkin's lymphoma (8/9) expressed *TCL1* (40–42). *TCL1* expression was not detected in myeloma cell lines (0/9). In contrast to these findings on cell lines, *TCL1* expression has not been reported in primary B lymphoid tumors, including AIDS-related B cell malignancies. Comparison of the levels of *TCL1* protein expression in AIDS IBLP samples with the normal pattern of expression in hyperplastic lymph node (HYP) suggests that *TCL1* is aberrantly regulated and overexpressed in many AIDS IBLP tumors. Up-regulation of *TCL1* in AIDS IBLP cannot occur by the same chromosomal alterations involved in T cell leukemia/lymphoma because the T cell receptor loci are transcriptionally silent in normal and malignant B cells.

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Abbreviations: NHL, non-Hodgkin's lymphoma; IBLP, immunoblastic lymphoma plasmacytoid; HYP, hyperplastic lymph node; SSH, suppression subtractive hybridization; DRC, dendritic reticulum cell.

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## MATERIALS AND METHODS

### Patient Samples, cDNA Manufacture, and Subtractions.

Fresh-frozen patient samples were derived from HIV-infected and uninfected individuals (Table 1). These tissues are part of a catalogued bank of 750 AIDS-associated malignancies and related non-AIDS tissue samples maintained at UCLA. Eighty of the AIDS NHL samples in this bank have been extensively characterized for known oncogenes, tumor-suppressor genes, and viral involvement. Subtraction sample AIDS IBLP 1 was chosen from these characterized tumor samples based on the demonstrated absence of Epstein-Barr virus and herpesvirus-8 and the lack of oncogenic mutations or alterations in *p53*, *RAS*, *c-MYC*, *BCL-2*, or *BCL-6* (19, 43–46). AIDS IBLP, non-AIDS IBLP, and HYP samples were examined histologically, and tissue blocks were trimmed to exclude areas of necrosis or surrounding non-lymphoid tissues. Microtome sections (5–10  $\mu\text{m}$ ) were placed directly into 5 ml of RNA STAT-60 for total RNA extraction (Tel-Test, Friendswood, TX). cDNA was synthesized from 0.4  $\mu\text{g}$  of total RNA (Superscript II, GIBCO/BRL) by using a Smart PCR cDNA Synthesis kit and then PCR-amplified 17–19 cycles by using conditions recommended by the manufacturer (CLONTECH) (34).

SSH were performed between AIDS IBLP 1 versus HYP 1 or non-AIDS IBLP 1 by using the PCR-Select cDNA Subtraction kit (CLONTECH) (33). Subtracted gene fragments were cloned into the TA cloning vector (Invitrogen) and transformed into DH5 $\alpha$  *Escherichia coli*. White colonies containing gene inserts were selected by isopropyl- $\beta$ -D-thiogalactoside/5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside screening and seeded into 96-well microtiter plates for growth with antibiotic selection.

### Miniarray Filter Preparation, Hybridization, and Analyses.

Samples of bacterial culture lysates in 96-well plates were stamped with a replicating tool (V&P Scientific, San Diego, CA) into fresh 96-well Thermowell plates (Costar) for PCR amplification of cDNA inserts. PCR was performed for 30 cycles (94°C for 30 s, 68°C for 3 min), and the average size of insert fragments was  $\approx$ 1 kilobase. These PCR products were identically stamped onto quadruplicate NitroPlus nitrocellulose filters (Micron Separations). The first position on each filter was stamped with an 850-bp *Pst*I fragment of the plant *Lemna gibba* RuBPCase gene. The filters were denatured and neutralized for 5 min, followed by baking at 80°C for 1 h. Random-primed, radiolabeled probes were made by using a

Prime-it II kit (Stratagene) from 50 ng of unsubtracted or subtracted cDNA. Probes were spiked with 0.3 ng of the 850-bp RuBPCase gene fragment before radiolabeling to allow semi-quantitative comparisons of hybridization intensities between filters. Hybridizations were performed at 42°C for 18 h, and filters were washed with 0.1 $\times$  SSC/0.1% SDS for 45 min at 52°C. Hybridization signals were determined visually by autoradiography and quantitatively with a PhosphorImager (Molecular Dynamics) by using the program IMAGEQUANT.

**“Virtual” Northern Analyses of Tissues.** PCR-amplified cDNA (0.5  $\mu\text{g}$ ), obtained from equivalent amounts of reverse-transcribed total RNA, was fractionated in a 1% agarose gel and alkaline-transferred for 1 hr to a MagnaCharge nylon membrane by using a TurboBlotter (Schleicher and Schuell). The blots were hybridized with random-primed, radiolabeled *TCL1* or *BCA-1* cDNA fragment probes. Equal lane loading was determined by ethidium bromide staining (data not shown).

**Immunohistochemical Staining of *TCL1* and Fascin in Tissues.** Rabbit antisera were generated against a purified glutathione *S*-transferase–*TCL1* fusion protein (data not shown). Formalin-fixed tissues on glass slides were incubated with blocking buffer and subsequently with *TCL1* antisera. Slides were rinsed, and biotinylated goat anti-rabbit Ig (DAKO) was added. Slides were washed, and streptavidin was added, followed by diaminobenzidine HCl incubation and counterstaining in Mayer’s hematoxylin (DAKO). Formalin-fixed tissue sections were also stained with a fascin mAb to detect dendritic reticulum cells (DRC) as described (47, 48).

## RESULTS

### Identification of *TCL1* and *BCA-1* in Fresh-Frozen Patient Samples.

Two independent bidirectional SSH were performed to identify AIDS lymphoma-expressed genes. In the first subtraction, AIDS IBLP 1 cDNA was paired with HYP 1 cDNA and in the second, this AIDS IBLP sample was paired with non-AIDS IBLP 1 cDNA (Table 1). The first subtraction reduced genes that were transiently up- or down-regulated because of immune system activation as often occurs in premalignant persistent generalized lymphadenopathy in early HIV infection (4, 49). The second subtraction removed cDNAs expressed in common between two lymphomas of the same histologic type and enriched for up- or down-regulated sequences in severely immunosuppressed individuals. Over 2,000 cloned cDNA fragments from these subtractions were arrayed on nitrocellulose filters and independently hybridized with tester, driver, and subtracted cDNA probes (data not shown). Approximately 120 of the clones that differentially hybridized were sequenced. *TCL1*, an oncogene implicated in T cell leukemia/lymphoma development, and *BCA-1*, a recently identified B cell CXC-family chemoattractant, were identified in these characterized isolates and were differentially expressed in opposite directions (40, 50, 51). *TCL1* was identified in the AIDS IBLP 1 minus non-AIDS IBLP 1 subtraction, whereas *BCA-1* was found in both HYP 1 and non-AIDS IBLP 1 minus AIDS IBLP 1 subtractions (data not shown).

**Differential Expression Profiles of *TCL1* and *BCA-1*.** Northern blots to confirm the differential expression of *TCL1* and *BCA-1* were not possible because of the exceptionally small sizes of the tissue samples used in this study. Therefore, virtual Northern analyses were performed on PCR-amplified cDNA from tissue samples (Fig. 1). *TCL1* was abundantly expressed in AIDS IBLP samples 1 and 2 (which both lack known genetic lesions or viral involvement) but was absent in non-AIDS IBLP samples 1 and 2. Low levels of *TCL1* were detected in HYP samples from HIV-infected and uninfected individuals. In contrast to *TCL1*, *BCA-1* was highly expressed in HYP samples 1 and 2 as well as in non-AIDS IBLP 1 but was absent in non-lymph node-derived tumors as well as lymph node-derived

Table 1. Features of patient samples

Tissue type	Site	Lesion(s)
HYP 1	LN	HIV(–)
HYP 2	LN	HIV(+)
AIDS IBLP 1	LN	None
AIDS IBLP 2	Muscle	None
AIDS IBLP 3	Parotid	None
AIDS IBLP 4	LN	EBV(+), p53 E6, LOH
AIDS IBLP 5	Bowel	c-MYC, p53 E7, LOH
AIDS IBLP 6	Lung	EBV(+), p53 E7, LOH
AIDS IBLP 7	LN	EBV(+), p53 E6, LOH
AIDS IBLP 8	LN	k-RAS, p53 E5,6,7, LOH
AIDS BL	Salivary	Unknown
Non-AIDS IBLP 1	LN	HIV(–)
Non-AIDS IBLP 2	Bowel	HIV(–)
Hyperplastic Tonsil	Oropharynx	

Tissue types, biopsy sites, and characteristic genetic lesions and/or viral involvement in patient samples used in this study. AIDS IBLP samples were analyzed for Epstein-Barr virus, human herpesvirus-8, *c-MYC*, *RAS*, *p53*, *BCL-2*, and *BCL-6* genetic alterations (19, 43–46). Lymphomas with *p53* mutations in exons 5, 6, and/or 7 terminate translation early or contain frameshift mutations. These five tumors also contain loss-of-heterozygosity (LOH) alterations in the non-mutated alleles.

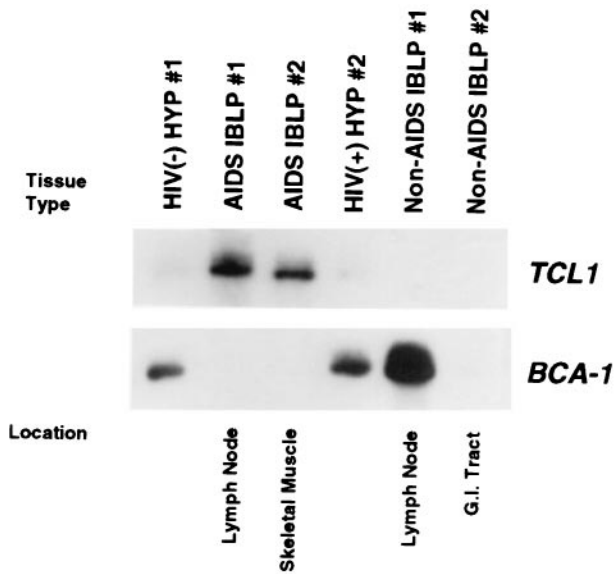


FIG. 1. Virtual Northern analysis of *TCL1* and *BCA-1* gene expression in tissue samples. (Upper) Hybridization with a 350-bp random-primed *TCL1* cDNA fragment. AIDS IBLP samples from lymph node or muscle expressed abundant *TCL1*, whereas low levels were expressed in both HYP samples. (Lower) The filter from Upper was stripped and rehybridized with a 200-bp random-primed *BCA-1* cDNA fragment. *BCA-1* was expressed in both HYP and non-AIDS IBLP 1 derived from a lymph node but was not detected in either AIDS IBLP 1 or 2 or in non-AIDS IBLP 2 derived from the gastrointestinal tract. Ethidium bromide gel staining demonstrated equal lane loading (data not shown).

AIDS IBLP 1. *BCA-1* is expressed in secondary human lymphoid tissues (50). Mice also express a *BCA-1* homologue,

termed *BLC*, and antisense *in situ* hybridization has demonstrated a reticular staining pattern for *BLC* in lymphoid follicles (51). This staining pattern suggested that mouse DRC may secrete *BLC* and, by analogy, that human DRC also secrete *BCA-1*, although neither association has been proven.

**TCL1 Protein Is Expressed in Distinct Locations in AIDS IBLP, HYP, and Tonsil.** Tissue-localization studies were performed to definitively identify the cell types expressing *TCL1* and *BCA-1* in AIDS IBLP and non-AIDS IBLP tumor samples. *TCL1* rabbit antisera staining of formalin-fixed lymphoma tissues demonstrated intense, widespread expression of *TCL1* protein restricted to tumor cells in AIDS IBLP samples (Fig. 2 *A* and *B*, data not shown). *TCL1* protein staining was mainly cytoplasmic, although some nuclear staining was also seen. *TCL1* protein was not detected within heterogeneous nonlymphoma cell elements of these same tissue sections. In contrast, no *TCL1* protein expression was detected in non-AIDS IBLP samples (Fig. 2 *C* and *D*, data not shown). In agreement with SSH isolation results and virtual Northern analyses (Fig. 1), *TCL1* protein was also detected in distinctive locations in HYP from HIV-infected and uninfected individuals. Staining was intense and widespread within mantle zone B cells. Intense *TCL1* staining was also detected in rare interfollicular zone cells, none of which morphologically resembled transformed lymphoid cells or immunoblasts (Fig. 2*E*, data not shown). Lower levels of *TCL1* staining was seen in follicle center cells, including both centroblasts and centrocytes. Hyperplastic tonsil showed a pattern of intense *TCL1* staining in germinal centers preferentially located in mantle-zone B cells similar to that seen in HYP (Fig. 2*F*).

Although the cells are not distinguishable by routine histologic examination with light microscopy, non-AIDS IBLP 1 tissue contained residual fascin antibody-stained DRC that were not detected in AIDS IBLP 1 (results not shown). AIDS IBLP 1 may lack DRC or, alternatively, may contain DRC that

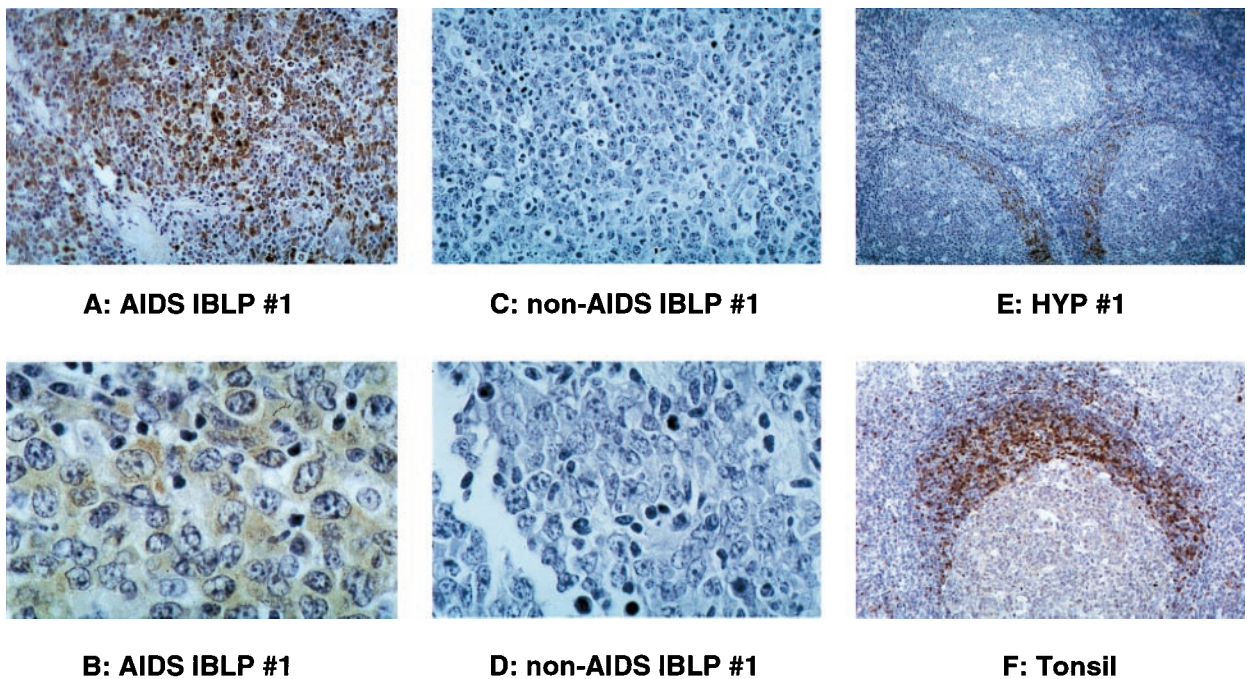


FIG. 2. *TCL1* antisera staining of formalin-fixed tissues. An intense positive signal was confined to the large immunoblastic/plasmacytic tumor cells in AIDS IBLP 1 (*A*,  $\times 400$ X; *B*,  $\times 1,000$  magnification), whereas non-AIDS IBLP 1 (*C*,  $\times 400$ ; *D*,  $\times 1,000$  magnification) was negative. Non-tumor cell elements were negative for *TCL1* protein expression in both samples. *TCL1* staining appeared mainly within the cytoplasm. Germinal center B cells also stained with *TCL1* antisera (*E*,  $\times 100$ ; *F*,  $\times 200$  magnification). Both uninfected and HIV-infected (data not shown) HYP contained activated germinal centers that reacted with *TCL1* antisera. In these samples, *TCL1* staining was most intense in naive, nonproliferating mantle-zone B cells and in rare interfollicular zone cells of unknown phenotype. A reduced staining intensity was seen in follicle-center cells (centroblasts and centrocytes). Overall, the level of staining in the different tissues analyzed correlated with the *TCL1* transcript levels detected by virtual Northern analysis.

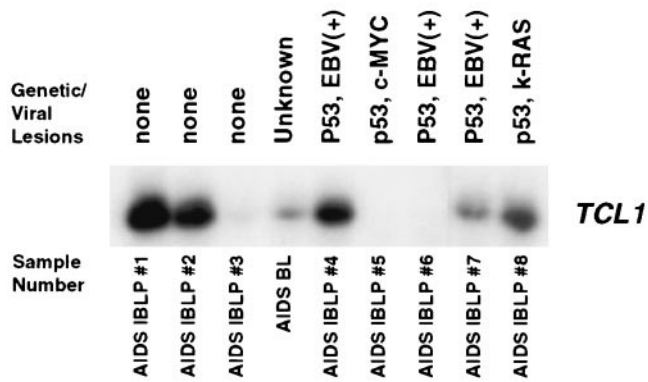


Fig. 3. Virtual Northern analysis of *TCL1* gene expression in AIDS IBLP and AIDS-related Burkitt's lymphoma samples. Blots were probed with a 350-bp random-primed *TCL1* cDNA fragment. All of the IBLP samples tested were originally classified as B cell immunoblastic lymphomas by Working Formulation criteria, and as AIDS IBLP by the Revised European-American Lymphoma (REAL) AIDS-related classification, except lane 4, which is an AIDS-related Burkitt's lymphoma. AIDS IBLP and an AIDS-related Burkitt's lymphoma with and without known genetic or viral lesions express *TCL1*. Ethidium bromide gel staining demonstrated equal lane loading, and detection of glyceraldehyde-3-phosphate dehydrogenase in each lane showed that the original RNA templates of each tissue sample were of similar quality (data not shown).

do not express fascin or *BCA-1*, in agreement with the spectrum of genes isolated by SSH and with virtual Northern results (47, 48). Detection of *BCA-1* in non-AIDS IBLP 1, coupled with residual fascin-positive antibody staining in this tumor, compared with a complete lack of both *BCA-1* and fascin-stained cells in AIDS IBLP 1, also suggest that DRC express *BCA-1*. The concordance of *BCA-1* expression and fascin antibody staining in these two tumor samples, along with concordant expression in HYP 1 and 2, also further confirms that both subtractions efficiently isolated differentially expressed genes.

***TCL1* Expression in AIDS NHL with or Without Genetic/Viral Alterations.** AIDS IBLP samples 1 and 2 expressed abundant *TCL1* protein in lymphoma cells without additional genetic alterations or viral involvement. This observation, coupled with detection of high levels of *TCL1* protein in nonproliferating mantle-zone B cells and in scattered, rare interfollicular-zone cells of HYP and tonsil, suggests that *TCL1* expression occurs early in AIDS lymphomagenesis. We therefore considered whether AIDS NHL tumors with identified oncogenic alterations, (perhaps representing later stage lymphomas) also expressed *TCL1*. Virtual Northern analyses demonstrated that the large majority (i.e., six of eight) AIDS IBLP patient samples tested expressed *TCL1*, including three of five lymphoma samples of this class with additional identified genetic aberrations (Fig. 3). These findings indicate that *TCL1* is more consistently expressed in AIDS IBLP than any other known oncogene previously analyzed. This prevalent expression strongly supports a possible role for *TCL1* in the development of this class of AIDS NHL. Interestingly, an AIDS-related Burkitt's lymphoma derived from a salivary gland tumor also expressed moderate *TCL1* mRNA. This latter observation suggests that additional classes of AIDS NHL should also be examined for *TCL1* expression.

## DISCUSSION

Two independent rounds of SSH were performed to isolate genes that are differentially expressed in AIDS IBLP versus non-AIDS IBLP tumors or activated lymph node (33, 34). The differential expression of SSH isolated genes was then confirmed by virtual Northern blots and assigned to specific cell

populations by immunohistochemical staining (and antisense *in situ* hybridizations; results not shown) of tumor and normal tissue samples. These combined steps define a powerful strategy for the isolation of differentially expressed genes from specific cells in heterogeneous surgical or biopsy tissue samples. Among the differentially expressed genes identified with this approach, we focused on *TCL1* because of its compelling biology in B cell development and its reported oncogenic activity in T cell leukemia/lymphoma. *TCL1* encodes a predominantly cytoplasmic protein of unresolved function that belongs to a novel family of recently identified oncogenes that now includes the *MTCPI* and *TCL1b* genes (42, 52–55). Although its expression was originally reported to be limited to developing lymphocytes (i.e., fetal thymocytes, pre-B cells, and immature surface IgM-expressing B cells in bone marrow) and to endemic (primarily Epstein-Barr virus<sup>+</sup>) Burkitt's lymphoma tumor cell lines, *TCL1* expression recently has been documented in many immortalized B lymphoblastoid and B cell tumor lines (40–42). *TCL1* transcripts have also been reported in CD19<sup>+</sup> sorted peripheral-blood lymphocytes and in spleen and lymph nodes, although the cell(s) of origin was not defined in these latter tissues (41). Using immunohistochemical staining, we show that HYP and tonsil express *TCL1* protein, with the highest levels of expression in mantle-zone B cells and in rare interfollicular-zone cells (none of which resembled immunoblasts). The mantle zone contains naive, unactivated B cells that may become activated within follicles and differentiate into either mature memory B cells or Ig-secreting plasma cells (ref. 56, reviewed in refs. 57–59). The finding of widespread *TCL1* expression in quiescent mantle-zone B cells suggests that *TCL1* expression may be involved in B cell survival rather than in proliferation or Ig/T cell receptor gene rearrangements as previously speculated (40). Mantle-zone B cells express abundant BCL-2 protein that serves to protect these resting B cells from apoptosis (58, 60–62). Elevated *TCL1* expression within this same quiescent B cell population also may reflect a role in conferring resistance to apoptosis, although this possibility remains to be tested. Finally, the mantle zone is physically juxtaposed to the follicle center, which contains areas of extensive apoptotic activity (along with reduced *BCL-2* expression) in which we also find decreased levels of *TCL1* protein expression. This concurrence lends further support for an antiapoptotic role for *TCL1*.

Aberrant *TCL1* expression has been previously reported in T cell leukemia/lymphoma with chromosomal rearrangements that juxtapose the *TCL1* gene in proximity to the T cell-specific enhancers of either the T cell receptor  $\alpha/\delta$  or  $\beta$  loci (35–39). Transgenic mice expressing either *TCL1* or *MTCPI* from promoters that restrict expression to T cells developed early T cell proliferative expansions that progressed to T cell leukemia by 15 months of age (63, 64). Interestingly, *BCL-2* transgenic mice also developed B cell lymphomas slowly and in moderate numbers, in a manner paralleling the time course and occurrence of T cell tumors in *TCL1* transgenic mice (reviewed in refs. 65 and 66). Humans may also sustain preleukemic *TCL1* translocations for many years before overt leukemia/lymphoma formation (67). All of these observations provide strong support for the oncogenic potential of *TCL1* and suggest that *TCL1* may function to promote cell survival rather than affecting cell proliferation.

AIDS IBLP are derived from postgerminal center cells and not from resting mantle-zone B cells (reviewed in refs. 6 and 7). Elevated *TCL1* expression in these cells may result from an abnormal up-regulation of the gene rather than the down-regulation that normally occurs in B cell maturation in germinal centers. Lower protein expression in follicle-center B cells and weak mRNA expression in circulating peripheral-blood lymphocytes is consistent with a physiologic down-regulation of *TCL1* from the high levels seen in quiescent mantle-zone B cells. Alternatively, AIDS IBLP may originate

from the rare intensely stained interfollicular-zone cells that express high levels of *TCL1* protein.

AIDS lymphomas develop under unique and extreme pathological conditions. These include moderate to severe immunodeficiency, chronic antigenic stimulation, profound alterations of cytokine expression profiles, and the loss of normal lymph-node architecture because of progressive immune system destruction (reviewed in refs. 1–3, 6, 7). It is conceivable that abundant *TCL1* expression in AIDS lymphoma results from the radically altered cytokine profiles in AIDS or from the absence of critical cell-mediated regulatory influences (e.g., such as those provided by DRC or T cells) that occur in normal lymph nodes. The lack of these cell-mediated regulatory influences may also be responsible for the widely varying levels of *TCL1* expression seen in multiple B cell tumor lines. Resolution of the mechanism(s) controlling *TCL1* expression in AIDS lymphoma versus normal developing B cells will provide important insights into the contribution of *TCL1* expression in AIDS lymphomagenesis.

Unchecked, high-level *TCL1* expression is reported to cause early polyclonal, proliferative T cell expansions in transgenic mice. The findings presented here strongly suggest that elevated *TCL1* expression also may be involved in an abnormal cellular regulatory program that creates an equivalent, early, or premalignant lymphoproliferative state in B cells. Subsequent transforming events, such as *p53* mutations, *c-MYC* rearrangements, or other genetic alterations could then promote frank B cell tumor formation, in analogy to the effect of introducing *c-MYC* mutations into *BCL-2* transgenic mice (reviewed in refs. 65 and 66).

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- Hamilton-Dutoit, S. J., Pallesen, G., Franzmann, M. B., Karkov, J., Black, F., Skinhoj, P. & Pedersen, C. (1991) *Am. J. Pathol.* **138**, 149–163.
- Raphael, M., Gentilhomme, O., Tulliez, M., Byron, P. A. & Diebold, J. (1991) *Arch. Pathol. Lab. Med.* **115**, 15–20.
- Raphael, M. M., Audouin, J., Lamine, M., Delecluse, H. J., Vuillaume, M., Lenoir, G. M., Gisselbrecht, C., Lennert, K. & Diebold, J. (1994) *Am. J. Clin. Pathol.* **101**, 773–782.
- Herndier, B. G., Kaplan, L. D. & McGrath, M. S. (1994) *AIDS* **8**, 1025–1049.
- Diebold, J., Raphael, M., Prevot, S. & Audouin, J. (1997) *Cancer Surv.* **30**, 263–293.
- Knowles, D. M. (1997) *Semin. Diagn. Pathol.* **14**, 67–82.
- Gaidano, G., Carbone, A. & Dalla-Favera, R. (1998) *Am. J. Pathol.* **152**, 623–630.
- Jaffe, E. S. (1996) *Am. J. Clin. Pathol.* **105**, 141–143.
- Carbone, A. & Gaidano, G. (1997) *Br. J. Haematol.* **97**, 515–522.
- Cesarman, E. & Knowles, D. M. (1997) *Semin. Diagn. Pathol.* **14**, 54–66.
- Chaganti, R. S., Jhanwar, S. C., Koziner, B., Arlin, Z., Mertelsmann, R. & Clarkson, B. D. (1983) *Blood* **61**, 1265–1268.
- Groopman, J. E., Sullivan, J. L., Mulder, C., Ginsburg, D., Orkin, S. H., O'Hara, C. J., Falchuk, K., Wong-Staal, F. & Gallo, R. C. (1986) *Blood* **67**, 612–615.
- Subar, M., Neri, A., Inghirami, G., Knowles, D. M. & Dalla-Favera, R. (1988) *Blood* **72**, 667–671.
- Ballerini, P., Gaidano, G., Gong, J. Z., Tassi, V., Saglio, G., Knowles, D. M. & Dalla-Favera, R. (1993) *Blood* **81**, 166–176.
- Gaidano, G., Parsa, N. Z., Tassi, V., Della-Latta, P., Chaganti, R. S., Knowles, D. M. & Dalla-Favera, R. (1993) *Leukemia* **7**, 1621–1629.
- Cesarman, E., Chang, Y., Moore, P. S., Said, J. W. & Knowles, D. M. (1995) *N. Engl. J. Med.* **332**, 1186–1191.
- Carbone, A., Ghoghini, A., Vaccher, E., Zagonel, V., Pastore, C., Dalla Palma, P., Branz, F., Saglio, G., Volpe, R., Tirelli, U. & Gaidano, G. (1996) *Br. J. Haematol.* **94**, 533–543.
- Gaidano, G., Pastore, C., Ghoghini, A., Cusini, M., Nomdedeu, J., Volpe, G., Capello, D., Vaccher, E., Bordes, R., Tirelli, U., *et al.* (1996) *AIDS* **10**, 941–949.
- Nador, R. G., Cesarman, E., Chadburn, A., Dawson, D. B., Ansari, M. Q., Sald, J. & Knowles, D. M. (1996) *Blood* **88**, 645–656.
- Lyons, S. F. & Liebowitz, D. N. (1998) *Semin. Oncol.* **25**, 461–475.
- Hsi, E. D., Foreman, K. E., Duggan, J., Alkan, S., Kauffman, C. A., Aronow, H. D. & Nickoloff, B. J. (1998) *Am. J. Surg. Pathol.* **22**, 493–499.
- Delecluse, H. J., Raphael, M., Magaud, J. P., Felman, P., Alsamad, I. A., Bornkamm, G. W. & Lenoir, G. M. (1993) *Blood* **82**, 552–563.
- Shiramizu, B., Herndier, B., Meeker, T., Kaplan, L. & McGrath, M. (1992) *J. Clin. Oncol.* **10**, 383–389.
- Shibata, D., Weiss, L. M., Hernandez, A. M., Nathwani, B. N., Bernstein, L. & Levine, A. M. (1993) *Blood* **81**, 2102–2109.
- Beral, V., Peterman, T., Berkelman, R. & Jaffe, H. (1991) *Lancet* **337**, 805–809.
- Schulz, T. F., Boshoff, C. H. & Weiss, R. A. (1996) *Lancet* **348**, 587–591.
- Knowles, D. M., Chamulak, G. A., Subar, M., Burke, J. S., Dugan, M., Wernz, J., Slywotzky, C., Pelicci, G., Dalla-Favera, R. & Raphael, B. (1988) *Ann. Intern. Med.* **108**, 744–753.
- Gaidano, G. & Carbone, A. (1995) *Br. J. Haematol.* **90**, 235–243.
- Carbone, A., Gaidano, G., Ghoghini, A., Larocca, L. M., Capello, D., Canzonieri, V., Antinori, A., Tirelli, U., Falini, B. & Dalla-Favera, R. (1998) *Blood* **91**, 747–755.
- Gaidano, G., Carbone, A., Pastore, C., Capello, D., Migliazza, A., Ghoghini, A., Roncella, S., Ferrarini, M., Saglio, G. & Dalla-Favera, R. (1997) *Blood* **89**, 3755–3762.
- Gaidano, G., Lo Coco, F., Ye, B. H., Shibata, D., Levine, A. M., Knowles, D. M. & Dalla-Favera, R. (1994) *Blood* **84**, 397–402.
- Ganser, A., Carlo-Stella, C., Bartram, C. R., Boehm, T., Heil, G., Henglein, B., Muller, H., Raghavachar, A., von Briesen, H., Griesinger, F., *et al.* (1988) *Blood* **72**, 1255–1260.
- Diatchenko, L., Lau, Y. F., Campbell, A. P., Chenchik, A., Moqadam, F., Huang, B., Lukyanov, S., Lukyanov, K., Gurskaya, N., Sverdlov, E. D. & Siebert, P. D. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 6025–6030.
- Peterson, L. A., Brown, M. R., Carlisle, A. J., Kohn, E. C., Liotta, L. A., Emmert-Buck, M. R. & Krizman, D. B. (1998) *Cancer Res.* **58**, 5326–5328.
- Russo, G., Isobe, M., Pegoraro, L., Finan, J., Nowell, P. C. & Croce, C. M. (1988) *Cell* **53**, 137–144.
- Mengle-Gaw, L., Albertson, D. G., Sherrington, P. D. & Rabbitts, T. H. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9171–9175.
- Davey, M. P., Bertness, V., Nakahara, K., Johnson, J. P., McBride, O. W., Waldmann, T. A. & Kirsch, I. R. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 9287–9291.
- Virgilio, L., Isobe, M., Narducci, M. G., Carotenuto, P., Camerini, B., Kurosawa, N., Abbas, R., Croce, C. M. & Russo, G. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 9275–9279.
- Narducci, M. G., Virgilio, L., Isobe, M., Stoppacciaro, A., Elli, R., Fiorilli, M., Carbonari, M., Antonelli, A., Chessa, L., Croce, C. M., *et al.* (1995) *Blood* **86**, 2358–2364.
- Virgilio, L., Narducci, M. G., Isobe, M., Billips, L. G., Cooper, M. D., Croce, C. M. & Russo, G. (1994) *Proc. Natl. Acad. Sci. USA* **91**, 12530–4.
- Takizawa, J., Suzuki, R., Kuroda, H., Utsunomiya, A., Kagami, Y., Joh, T., Aizawa, Y., Ueda, R. & Seto, M. (1998) *Jpn. J. Cancer Res.* **89**, 712–718.
- Pekarsky, Y., Hallas, C., Isobe, M., Russo, G. & Croce, C. M. (1999) *Proc. Natl. Acad. Sci. USA* **96**, 2949–2951.
- Said, J. W., Sasso, A. F., Shintaku, I. P., Corcoran, P. & Nichols, S. W. (1990) *Mod. Pathol.* **3**, 659–663.
- Nakamura, H., Said, J. W., Miller, C. W. & Koeffler, H. P. (1993) *Blood* **82**, 920–926.
- Lones, M. A., Mishalani, S., Shintaku, I. P., Weiss, L. M., Nichols, W. S. & Said, J. W. (1995) *Hum. Pathol.* **26**, 525–530.
- Said, J. W., Rettig, M. R., Heppner, K., Vescio, R. A., Schiller, G., Ma, H. J., Belson, D., Savage, A., Shintaku, I. P., Koeffler, H. P., *et al.* (1997) *Blood* **90**, 4278–4282.

47. Said, J. W., Pinkus, J. L., Yamashita, J., Mishalani, S., Matsumura, F., Yamashiro, S. & Pinkus, G. S. (1997) *Mod. Pathol.* **10**, 421–427.
48. Said, J. W., Pinkus, J. L., Shintaku, I. P., deVos, S., Matsumura, F., Yamashiro, S. & Pinkus, G. S. (1998) *Mod. Pathol.* **11**, 1–5.
49. Pelicci, P. G., Knowles, D. M. d., Arlin, Z. A., Wieczorek, R., Luciw, P., Dina, D., Basilico, C. & Dalla-Favera, R. (1986) *J. Exp. Med.* **164**, 2049–2060.
50. Legler, D. F., Loetscher, M., Roos, R. S., Clark-Lewis, I., Baggiolini, M. & Moser, B. (1998) *J. Exp. Med.* **187**, 655–660.
51. Gunn, M. D., Ngo, V. N., Ansel, K. M., Ekland, E. H., Cyster, J. G. & Williams, L. T. (1998) *Nature (London)* **391**, 799–803.
52. Fu, T. B., Virgilio, L., Narducci, M. G., Facchiano, A., Russo, G. & Croce, C. M. (1994) *Cancer Res.* **54**, 6297–6301.
53. Madani, A., Choukroun, V., Soulier, J., Cacheux, V., Claisse, J. F., Valensi, F., Daliphard, S., Cazin, B., Levy, V., Leblond, V., *et al.* (1996) *Blood* **87**, 1923–1927.
54. Stern, M. H., Soulier, J., Rosenzweig, M., Nakahara, K., Canki-Klain, N., Aurias, A., Sigaux, F. & Kirsch, I. R. (1993) *Oncogene* **8**, 2475–2483.
55. Hoh, F., Yang, Y. S., Guignard, L., Padilla, A., Stern, M. H., Lhoste, J. M. & van Tilbeurgh, H. (1998) *Structure (London)* **6**, 147–155.
56. Koppers, R., Zhao, M., Hansmann, M. L. & Rajewsky, K. (1993) *EMBO J.* **12**, 4955–4967.
57. Hummel, M., Tamaru, J., Kalvelage, B. & Stein, H. (1994) *Blood* **84**, 403–407.
58. MacLennan, I. C. (1994) *Annu. Rev. Immunol.* **12**, 117–139.
59. Liu, Y. J. & Arpin, C. (1997) *Immunol. Rev.* **156**, 111–126.
60. Pezzella, F., Tse, A. G., Cordell, J. L., Pulford, K. A., Gatter, K. C. & Mason, D. Y. (1990) *Am. J. Pathol.* **137**, 225–232.
61. Chleq-Deschamps, C. M., LeBrun, D. P., Huie, P., Besnier, D. P., Warnke, R. A., Sibley, R. K. & Cleary, M. L. (1993) *Blood* **81**, 293–298.
62. Liu, Y. J., Mason, D. Y., Johnson, G. D., Abbot, S., Gregory, C. D., Hardie, D. L., Gordon, J. & MacLennan, I. C. (1991) *Eur. J. Immunol.* **21**, 1905–1910.
63. Virgilio, L., Lazzeri, C., Bichi, R., Nibu, K., Narducci, M. G., Russo, G., Rothstein, J. L. & Croce, C. M. (1998) *Proc. Natl. Acad. Sci. USA* **95**, 3885–3889.
64. Gritti, C., Dastot, H., Soulier, J., Janin, A., Daniel, M. T., Madani, A., Grimber, G., Briand, P., Sigaux, F. & Stern, M. H. (1998) *Blood* **92**, 368–373.
65. Cory, S., Vaux, D. L., Strasser, A., Harris, A. W. & Adams, J. M. (1999) *Cancer Res.* **59**, 1685s–1692s.
66. Korsmeyer, S. J. (1999) *Cancer Res.* **59**, 1693s–1700s.
67. Levitt, R., Pierre, R. V., White, W. L. & Siekert, R. G. (1978) *Blood* **52**, 1003–1011.