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Short Communication

TIA-1 Expression in Lymphoid Neoplasms

Identification of Subsets with Cytotoxic T Lymphocyte or Natural Killer Cell Differentiation

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TIA-1 is a 15-kd cytotoxic granule-associated protein expressed in natural killer (NK) cells and cytotoxic T lymphocytes. TIA-1 expression was evaluated by paraffin immunohistochemistry in 115 T- or NK-cell neoplasms, 45 B-cell neoplasms, and 45 Hodgkin's lymphomas. TIA-1-positive granules were identified within the cytoplasm of neoplastic cells in 6/6 large granular lymphocytic leukemias, 11/11 hepatosplenic T-cell lymphomas, 15/15 intestinal T-cell lymphomas, 6/6 NK-like T-cell lymphomas of no special type, 2/2 NK-cell lymphomas, 8/9 nasal T/NK-cell lymphomas, 7/8 subcutaneous T-cell lymphomas, 4/5 pulmonary T- or NK-cell angiocentric lymphomas (lymphomatoid granulomatosis), 12/19 T-cell anaplastic large-cell lymphomas, 2/12 nodal peripheral T-cell lymphomas, 1/3 CD8$^+$ cutaneous T-cell lymphomas, and 5/38 classical Hodgkin's disease. All B-cell neoplasms, nodular lymphocyte-predominant Hodgkin's disease (7 cases), CD4$^+$ cutaneous T-cell lymphomas (6 cases), adult T-cell leukemia/lymphomas (3 cases), T-cell chronic or prolymphocytic leukemias (3 cases), and T-cell lymphoblastic leukemia/lymphomas (7 cases) were TIA-1 negative. These findings indicate that most large granular lymphocytic leukemias, hepatosplenic T-cell lymphomas, intestinal T-cell lymphomas, NK-like T-cell lymphomas, NK-cell lymphomas, nasal T/NK-cell lymphomas, subcutaneous T-cell lymphomas, pulmonary angiocentric lymphomas of T or NK phenotype, and anaplastic large-cell lymphomas are cytotoxic T- or NK-cell neoplasms. (Am J Pathol 1997, 150:1893–1900)

Both natural killer (NK) cells and NK-like cytotoxic T lymphocytes (CTLs) are phenotypically mature lymphocytes with cytoplasmic granules, NK-associated antigens (eg, CD11b, CD11c, CD16, CD56, and CD57), and the ability to recognize and kill certain target cells, especially tumor and virally infected cells with altered expression of class I major histocompatibility complex (MHC) antigens.[1–7] Proteins within cytoplasmic granules of activated cytolytic lymphocytes (of both NK and CD8$^+$ CTL type) are capable of mediating cellular lysis *in vitro*.[8,9] Among the cytolytic proteins isolated from these granules are perforin (cytolysin), a series of serine esterases (granzymes), and a recently characterized, granule membrane-associated cytotoxic protein known as TIA-1.[10,11] TIA-1 is a 15-kd protein structurally related to the tumor necrosis factor receptor family, which induces apoptotic cell death when introduced

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into permeabilized target cells.[12] Antibodies to TIA-1 have been shown to be highly specific for the cytolytic granules of NK cells and CTLs.[10,12,13] Thus, antibodies to TIA-1 should be useful in identification of specific subsets of lymphoid neoplasms derived from CTLs or NK cells.

Recently, we and others have characterized a group of aggressive hepatosplenic T-cell lymphomas that express γδ T-cell receptors (TCRs) and have features consistent with cytolytic lymphocyte origin, including expression of NK-cell-associated antigens, the presence of cytotoxic granules, and expression of cytolytic effector proteins, including Fas ligand (CD95L), perforin, granzyme B, and TIA-1.[14,15] Other studies have also suggested CTL or NK-cell origin for large granular lymphocytic leukemia (LGL),[16] intestinal or enteropathy-associated T-cell lymphoma,[17,18] nasal T/NK-cell lymphomas,[17,19,20] cutaneous angiocentric T/NK-cell lymphomas,[21] subcutaneous panniculitic T-cell lymphoma,[22] anaplastic large-cell lymphoma (ALCL),[17,23,24] and approximately 10 to 20% of classical Hodgkin's disease.[23–25] In addition, Emile et al[26] and Macon et al[18] have identified subsets of aggressive NK-cell and NK-like T-cell lymphomas that may be derived from cytotoxic lymphocytes. These neoplasms represent many of the categories of T-cell and NK-cell lymphomas and leukemias recently recognized as distinct entities by the International Lymphoma Study Group in the Revised European-American Lymphoma (REAL) classification.[27]

Identification of cytotoxic lymphoid neoplasms may be important because many of these neoplasms are clinically aggressive.[17,18,26,28–32] To determine the spectrum of cytotoxic lymphoid neoplasms, we stained a broad range of lymphoid neoplasms, representing most major categories within the REAL classification, with a commercially available, paraffin-reactive antibody to TIA-1. Our data suggest that LGL leukemia, hepatosplenic T-cell lymphoma, intestinal T-cell lymphoma, subcutaneous T/NK-cell lymphoma, NK-cell lymphoma, NK-like T-cell lymphoma, nasal T/NK-cell lymphoma, pulmonary angiocentric lymphomas of T- and NK-cell type, and most T-cell ALCLs are cytotoxic lymphoid neoplasms derived from CTL or NK cells, whereas B-cell neoplasms, most $CD4^+$ T-cell neoplasms, and nodular lymphocyte predominance Hodgkin's disease are not. Furthermore, TIA-1 staining supports CTL or NK-cell origin for Reed-Sternberg cells in some cases of classical Hodgkin's disease.

Materials and Methods

Case Selection and Controls

A total of 205 lymphoid malignancies, including 115 T- or NK-cell neoplasms, 45 B-cell neoplasms, and 45 cases of Hodgkin's disease were retrieved from the hematopathology consultation and surgical pathology files of the Hospital of the University of Pennsylvania and from the hematopathology files of Vanderbilt University Medical Center. Only well documented examples of recognized entities that had been adequately immunophenotyped and had paraffin blocks available for TIA-1 staining were included in the study. Histological sections and immunophenotyping studies were reviewed to confirm the diagnosis, immunophenotype, and classification of all lymphoid neoplasms in the study.

T- or NK-cell neoplasms included in the study were 6 LGL leukemias (3 T and 3 NK); 11 hepatosplenic T-cell lymphomas (6 γδ, 1 αβ, and 4 undetermined subtype), 15 intestinal T-cell lymphomas, including enteropathy-associated cases; 6 NK-like T-cell lymphomas of no specific type; 2 NK-cell lymphomas; 9 nasal T/NK-cell lymphomas; 8 subcutaneous T-cell lymphomas (6 αβ and 2 γδ); 5 pulmonary angiocentric lymphomas (lymphomatoid granulomatosis, LyG) of T- or NK-cell phenotype; 19 $CD30^+$ ALCLs (T-cell phenotype); 12 node-based peripheral T-cell lymphomas (PTCLs); 9 cutaneous T-cell lymphomas (CTCLs; 6 $CD4^+$ and 3 $CD8^+$); 3 $CD4^+$ adult T-cell leukemia/lymphomas (ATLLs); 3 T-cell chronic lymphocytic/prolymphocytic leukemias (1 $CD8^+$ T-CLL and 2 $CD4^+$ T-PLLs); and 7 T-cell acute lymphoblastic leukemia/lymphomas (T-ALLs or T-LBLs).

B-cell neoplasms and Hodgkin's disease cases included in the study were chosen to represent most of the commonly recognized entities and subtypes. B-cell neoplasms studied were two precursor B-cell ALLs, five B-cell CLLs or small lymphocytic lymphomas, six follicular lymphomas, four low-grade gastric B-cell lymphomas of mucosa-associated lymphoid tissue, two nodal parafollicular (monocytoid/marginal zone) B-cell lymphomas, nine diffuse large-B-cell lymphomas, two small noncleaved cell (Burkitt's) lymphomas, four hairy-cell leukemias, and five multiple myelomas. We also included one hepatosplenic B-cell lymphoma, one intestinal B-cell lymphoma, two pulmonary angiocentric B-cell lymphomas, and two $CD30^+$ B-cell ALCLs. Hodgkin's disease cases included 26 nodular sclerosis (NSHD), 9 mixed cellularity (MCHD), 3 lymphocyte depletion (LDHD),

Table 1. *TIA-1 Expression by Lymphoid Neoplasms*

Lymphoid neoplasms studied	Positive cases
T-Cell and NK-cell neoplasms	
LGL leukemia (3 $CD8^+$ T and 3 NK cell)	6/6 (100%)
Hepatosplenic TCL (6 $\gamma\delta$, 1 $\alpha\beta$, 4 undetermined)	11/11 (100%)
Intestinal TCL (including enteropathy-associated TCL)	15/15 (100%)
NK-like TCL, no special type	6/6 (100%)
NK-cell lymphoma	2/2 (100%)
Nasal T/NK-cell lymphoma	8/9 (89%)
Subcutaneous TCL	7/8 (88%)
Pulmonary angiocentric lymphoma (LyG) of T- or NK-cell type	4/5 (80%)
$CD30^+$ ALCL (T-cell phenotype)	12/19 (63%)
PTCL, nodal	2/12 (17%)
CTCL, $CD8^+$	1/3 (33%)
CTCL, $CD4^+$	0/6 (0%)
Adult T-cell leukemia/lymphoma, $CD4^+$	0/3 (0%)
T-cell CLL/PLL (1 $CD8^+$ T-CLL, 2 $CD4^+$ PLLs)	0/3 (0%)
T-cell ALL/T-cell LBL	0/7 (0%)
Hodgkin's disease	
Classical Hodgkin's disease (3 NSHD, 1 MCHD, 1 LDHD cases TIA-1^+)	5/38 (13%)
Lymphocyt-predominance Hodgkin's disease, nodular	0/7 (0%)
B-cell neoplasms	
ALL, precursor B cell	0/2 (0%)
CLL/SLL, B cell	0/5 (0%)
Follicular lymphoma	0/6 (0%)
MALT lymphoma, low grade	0/4 (0%)
Parafollicular (monocytoid) B-cell lymphoma	0/2 (0%)
Diffuse large-B-cell lymphoma	0/9 (0%)
Small noncleaved cell (Burkitt's) lymphoma	0/2 (0%)
Hairy-cell leukemia	0/4 (0%)
Multiple myeloma	0/5 (0%)
Hepatosplenic B-cell lymphoma	0/1 (0%)
Intestinal B-cell lymphoma	0/1 (0%)
Pulmonary angiocentric B-cell lymphoma	0/2 (0%)
$CD30^+$ ALCL, B cell	0/2 (0%)

TCL, T-cell lymphoma; CLL, chronic lymphocytic leukemia; PLL, prolymphocytic leukemia; ALL, acute lymphoblastic leukemia; SLL, small lymphocytic lymphoma; MALT, mucosa-associated lymphoid tissue.

and 7 nodular lymphocyte-predominance (LPHD) cases.

Nineteen specimens from various normal and reactive tissues (mostly of hematolymphoid type) were studied to determine the normal distribution of TIA-1-positive lymphocytes. These included reactive lymph nodes (five cases) and normal tonsils (three cases), spleens (five cases), bone marrows (three cases), and liver biopsies (three cases).

Immunohistochemical Staining for TIA-1

Immunohistochemical staining for TIA-1 was performed by automated methodology (BioTek, Santa Barbara, CA) on 4-μm paraffin sections of formalin-, B5-, or Bouin's-fixed tissue using the avidin-biotin complex-peroxidase method and horseradish peroxidase reacted with 2′,5′-diaminobenzidine (DAB), according to the manufacturer's protocol. Monoclonal anti-TIA-1 (Coulter Immunology, Hialeah, FL) was run at a 1:1000 dilution and did not require protease digestion or antigen retrieval. Normal tonsil and spleen were used as positive controls, but internal positive controls were usually present. After staining for TIA-1, all cases were evaluated independently by two hematopathologists (R. E. Felgar and K. E. Salhany); Vanderbilt cases were also reviewed by W. R. Macon and M. C. Kinney. TIA-1 staining of lymphoid cells was visually estimated and scored as follows: 1+, <25% positive; 2+, 25 to 50% positive; 3+, 51 to 75% positive; and 4+, >75% positive. Neoplasms were considered to be TIA-1 positive when labeled granules were present in cytologically malignant lymphocytes or Reed-Sternberg cells and variants or when >75% (4+) of lymphocytes contained labeled cytoplasmic granules in those neoplasms that lacked significant cytological atypia. In some cases, TIA-1-positive tumor-infiltrating lymphocytes were also identified.

Results

The distribution of TIA-1^+ lymphoid neoplasms is summarized in Table 1. TIA-1^+ lymphoid neoplasms

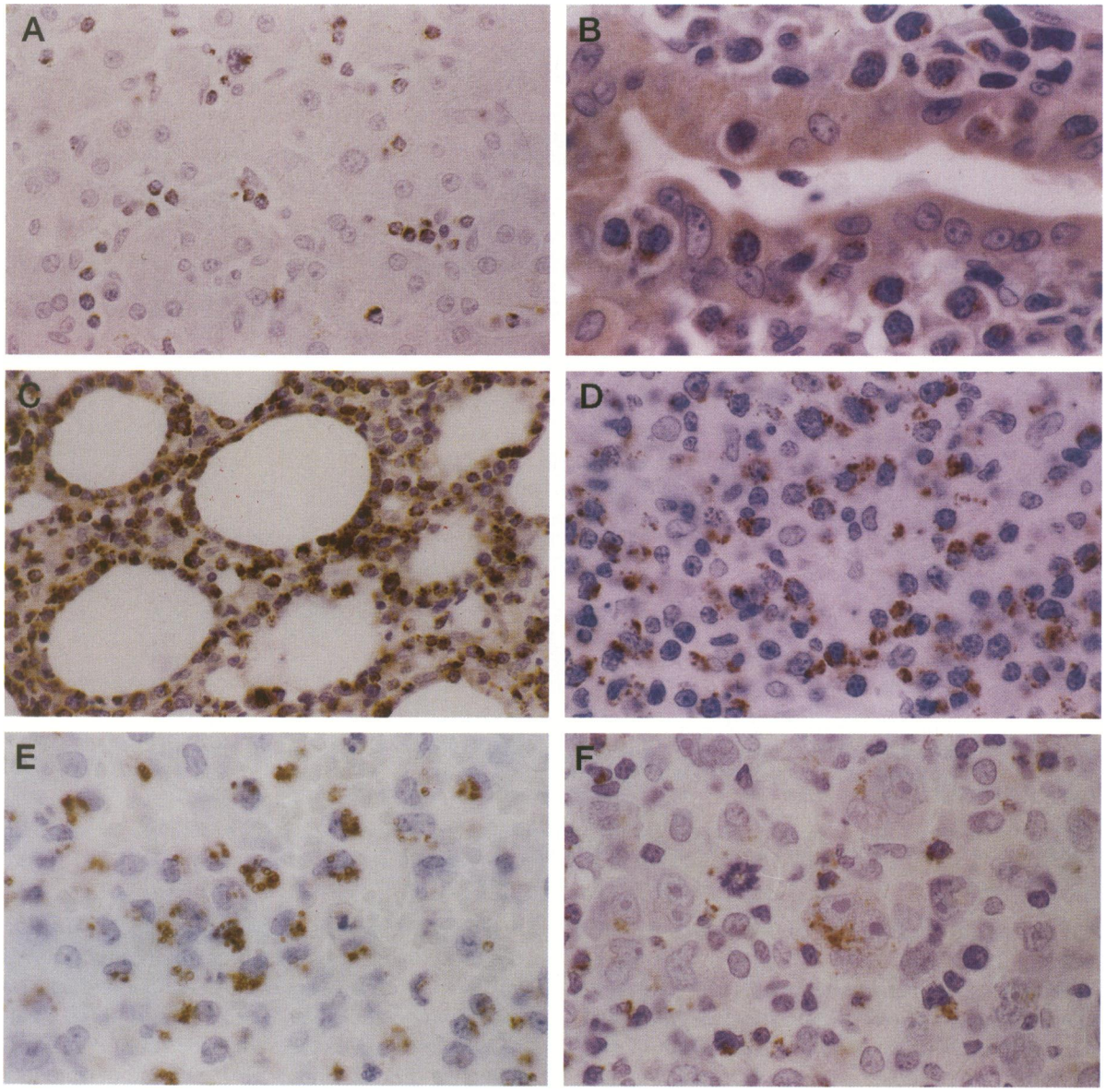

Figure 1. *Representative examples of TIA-1 expression in lymphoid neoplasms. Anti-TIA-1 labels cytoplasmic granules.* A: *Hepatosplenic γδ T-cell lymphoma, mixed small- and large-cell type, liver biopsy. Intrasinusoidal small lymphocytes and occasional large transformed cells are TIA-1$^+$. DAB-hematoxylin; magnification, ×300.* B: *Enteropathy-associated intestinal T-cell lymphoma. Medium to large intraepithelial and lamina propria T cells are TIA-1$^+$. DAB-hematoxylin; magnification, ×500.* C: *Subcutaneous panniculitic T-cell lymphoma, αβ subtype. Numerous TIA-1$^+$ small to medium, pleomorphic T cells are shown infiltrating subcutaneous fat. DAB-hematoxylin; magnification, ×300.* D: *Nasal T/NK-cell lymphoma, cytoplasmic CD3 and EBER-1 positive (not shown). Most pleomorphic small to medium and large cells contain TIA-1$^+$ cytoplasmic granules. DAB-hematoxylin; magnification, ×500.* E: *CD30$^+$ anaplastic large T-cell lymphoma. Anaplastic large cells have clusters of TIA-1$^+$ granules concentrated in the region of cytoplasmic/nuclear invagination. Note localization of TIA-1 staining to the granule membranes. TIA-1$^+$ small lymphocytes represent tumor-infiltrating lymphocytes. DAB-hematoxylin; magnification, ×500.* F: *Nodular sclerosis Hodgkin's disease. Binucleate Reed-Sternberg and mononuclear Hodgkin cells show perinuclear clustering of TIA-1$^+$ cytoplasmic granules. Small TIA-1$^+$ tumor-infiltrating lymphocytes are also present DAB-hematoxylin; magnification, ×500.*

were restricted to the T and NK cell and classical Hodgkin's disease categories; 74/115 (64%) of T- or NK-cell neoplasms and 5/38 (13%) of classical Hodgkin's disease cases were positive for TIA-1, whereas no B-cell neoplasms (0/45) or cases of nodular lymphocyte-predominance Hodgkin's disease (0/7) were TIA-1$^+$. Immunopositive tumor cells exhibited a granular cytoplasmic pattern compatible with localization of TIA-1 to cytolytic granules (Figure 1). TIA-1$^+$ tumor-infiltrating lymphocytes could be distinguished from tumor cells in B-cell neoplasms, Hodgkin's disease, and some T- or NK-cell neoplasms and were distributed in a scattered reactive pattern. The number of TIA-1$^+$ tumor-infiltrating lymphocytes was variable and was not further evaluated in this study.

T- and NK-Cell Neoplasms

TIA-1$^+$ granules were consistently seen in neoplastic cells from several subsets of T- and NK-cell lymphomas and leukemias, supporting their derivation from CTLs and NK cells. All LGL leukemias, hepatosplenic T-cell lymphomas (Figure 1A), intestinal T-cell lymphomas (Figure 1B), NK-like T-cell lymphomas of no special type, and NK-cell lymphomas studied were TIA-1$^+$. Most subcutaneous T-cell lymphomas (Figure 1C), nasal T/NK-cell lymphomas (Figure 1D), phenotypically T- or NK-cell LyG, and phenotypically T-cell ALCL (Figure 1E) were also positive for TIA-1 (Table 1). All documented NK-cell neoplasms (three NK-LGL and two NK-cell lymphomas), which were CD2$^+$, surface CD3$^-$, CD56$^+$ and lacked T-cell receptor (TCR) framework determinants or clonal TCR gene rearrangements, were strongly and uniformly TIA-1$^+$ (4+). All post-thymic $\gamma\delta$ T-cell lymphomas, six hepatosplenic and two subcutaneous, were strongly TIA-1$^+$ (3 to 4+), but the only documented $\gamma\delta$ T-lymphoblastic lymphoma (T-LBL) studied was TIA-1$^-$. Many extranodal $\alpha\beta$ T-cell lymphomas were also TIA-1$^+$, particularly intestinal T-cell lymphomas, subcutaneous T-cell lymphomas, at least one $\alpha\beta$ hepatosplenic T-cell lymphoma, and other NK-like T-cell lymphomas of no specific type. Most lymphomas of undefined T- or NK-cell origin, including eight of nine nasal and four of five pulmonary LyG were TIA-1$^+$ (3 to 4+), as were many CD8$^+$ T-cell leukemias and lymphomas. However, two CD8$^+$ nodal PTCL, two CD8$^+$ CTCL, and one CD8$^+$ T-CLL were TIA-1$^-$, suggesting that some CD8$^+$ T-cell lymphomas and leukemias are derived from suppressor T cells. Interestingly, all CD4$^+$ T-cell lymphomas or leukemias, which are generally thought to be derived from T helper/inducer cells (such as CTCLs and ATLLs) were TIA-1$^-$; this category included six of six CD4$^+$ CTCLs, three of three CD4$^+$ ATLLs, and two of two CD4$^+$ T-PLLs. However, three CD4$^+$ ALCLs were TIA-1$^+$, suggesting that some ALCLs may be derived from CD4$^+$ CTLs. All T-LBL and T-ALL cases were also TIA-1$^-$.

Hodgkin's Disease

TIA-1 expression in Hodgkin's disease was restricted to classical variants; 3/26 NSHD, 1/9 MCHD, and 1/3 LDHD. TIA-1 expression in Hodgkin's disease was manifested as fine to coarse cytoplasmic granules in Reed-Sternberg cells and Hodgkin variants. Cytoplasmic granules were often concentrated in clusters in the perinuclear region (Figure 1F). TIA-1 staining was often less intense with fewer positive tumor cells (2 to 3+) than for NK- or cytotoxic T-cell neoplasms (3 to 4+). TIA-1 expression was not seen in the lymphocytic and histiocytic cells of nodular LPHD.

Distribution of TIA-1$^+$ Lymphocytes in Normal or Reactive Hematolymphoid Tissues

TIA-1$^+$ cytoplasmic granules were identified within small lymphocytes of all normal and reactive hematolymphoid tissue and liver controls. TIA-1$^+$ lymphocytes were most numerous (2 to 3+) in sections of normal spleen and were predominantly distributed within the red pulp and in the outer zones of the periarteriolar lymphoid sheaths. In most normal and reactive lymph nodes and tonsils, TIA-1$^+$ lymphocytes were rare to occasional (1+) and primarily localized to germinal centers, mantle zones, and intervening lymph node sinuses. Numerous TIA-1$^+$ lymphocytes were identified within the germinal centers in one case of HIV-related follicular hyperplasia. Fine granular cytoplasmic staining with TIA-1 was seen within reactive histiocytes in some lymphomas, but histiocytes were usually negative. Bone marrow biopsies also contained rare to occasional TIA-1$^+$ small lymphocytes (1+) scattered through the interstitium. Unexpectedly, however, fine granular to diffuse cytoplasmic staining of myeloid precursors and mature granulocytes was observed, but this pattern could be easily distinguished from the large TIA-1$^+$ granules in cytotoxic lymphocytes. In the liver, TIA-1$^+$ small lymphocytes (1 to 2+) were localized within hepatic sinusoids.

Discussion

We present data supporting a cytotoxic lymphocyte (CTL or NK cell) origin for several major categories of T-cell and NK-cell leukemias and lymphomas, as indicated by their expression of the cytotoxic granule-associated protein TIA-1. The overall high percentage (64%) of T- or NK-cell neoplasms that were TIA-1$^+$ in this study reflects selection bias, as our study was designed to address the utility of TIA-1 antibodies in detecting lymphoid neoplasms accepted as having an NK or CTL phenotype or possible origin. TIA-1 expression also supports CTL or NK-cell derivation for Reed-Sternberg and Hodgkin's cells in some cases of classical Hodgkin's disease. However, TIA-1 expression was not seen in B-cell lymphomas, nodular LPHD, CD4$^+$ CTCL, CD4$^+$ ATLL, or CD4$^+$ T-PLL.

Table 2. *Classification of Cytotoxic Lymphoid Neoplasms*

Major categories (most cases have cytotoxic phenotypes)	Minor categories (occasional cases have cytotoxic phenotypes)
LGL leukemia ($CD8^+$ T cell and NK cell)	Peripheral TCL, nodal
NK-like TCL	Cutaneous TCL, $CD8^+$
Hepatosplenic TCL ($\gamma\delta$ and $\alpha\beta$)	Classical Hodgkin's disease (NSHD, MCHD, LDHD)
Intestinal TCL (including enteropathy-associated TCL)	
Subcutaneous panniculitic TCL ($\alpha\beta$ and $\gamma\delta$)	
NK-cell lymphoma	
Nasal angiocentric T/NK-cell lymphoma	
Subcutaneous angiocentric T/NK-cell lymphoma	
Pulmonary angiocentric lymphoma (LyG) of T- or NK-cell type	
$CD30^+$ ALCL (T cell and null cell)	

TCL, T-cell lymphoma.

TIA-1 was consistently expressed by most lymphoid neoplasms within several categories of T- or putative NK-cell neoplasms recently recognized by the REAL classification[27] as well as other recently proposed NK and NK-like T-cell lymphomas and leukemias.[18,26,31] Based upon the apparent level of TIA-1 expression, our data suggest that cytotoxic lymphoid neoplasms can be divided into major and minor categories, with the former being composed of entities in which most or all cases are derived from CTL or NK cells (Table 2). In support of this, we and others have shown that tumor cells within the major category often exhibit other features of CTLs or NK cells, including the presence of cytoplasmic granules on Wright's- or Giemsa-stained preparations or by electron microscopy, expression of NK-cell-associated antigens such as CD56, and expression of other cytolytic effector proteins such as perforin, granzyme B, and Fas ligand (CD95L).[14–24,26,28,29,32] Moreover, functional cytolytic activity has been documented in some of these neoplasms.[14,16,32,33]

Recently, Chan et al[34] and Macon et al[18] have proposed the general term NK-like T-cell lymphoma for peripheral T-cell lymphomas that express NK-cell-associated antigens such as CD11b, CD11c, CD16, CD56, or CD57 and/or have cytoplasmic granules by routine light or electron microscopy.[18] Most thoroughly studied hepatosplenic T-cell lymphomas,[14,15] intestinal T-cell lymphomas,[17] and subcutaneous panniculitic T-cell lymphomas[21,35] fulfill these criteria and can be regarded as specific subsets of NK-like T-cell lymphomas (Table 2). This subgrouping is also supported by the presence of $TIA\text{-}1^+$ cytoplasmic granules in virtually all of these lymphomas in our study. Furthermore, TIA-1 positivity in a high percentage of T-cell ALCLs suggests that these may also be NK-like or cytolytic T-cell lymphomas. It should be noted, however, that few cases that may fall within the spectrum of NK-like T-cell lymphomas have been studied for NK-like functional activity. Falcao et al[33] demonstrated spontaneous, NK-like non-MHC-restricted cytolysis of K562 cells in a $\gamma\delta$ T-cell lymphoma of probable hepatosplenic origin. Although we have recently demonstrated MHC-restricted, TCR-mediated cytolytic activity and antibody-dependent cellular cytotoxicity in hepatosplenic $\gamma\delta$ T-cell lymphomas,[14] we were unable to show spontaneous NK-like cytotoxicity against K562 cells in the only case we have studied thus far (Salhany, unpublished observations). Therefore, it remains to be confirmed whether NK-like T-cell lymphomas generally have NK-like functional activity.

Most nasal, subcutaneous, and pulmonary angiocentric lymphomas (of T or NK phenotype) in our study were also $TIA\text{-}1^+$, supporting their derivation from NK cells or CTLs. Additional support of CTL or NK-cell origin for these lymphomas has been provided in other studies demonstrating perforin, granzyme B, and/or TIA-1 expression in nasal T/NK-cell lymphomas,[17,19,20,26] subcutaneous angiocentric T/NK-cell lymphomas,[21] and pulmonary T-cell lymphomas.[17]

Interestingly, we found $TIA\text{-}1^+$ cytoplasmic granules in more than 60% of T-cell ALCLs but in neither of the two B-cell, $CD30^+$ ALCLs studied, suggesting that most T-cell ALCLs are derived from CTLs or NK cells. Our findings are supported by the recent demonstration of perforin and/or granzyme B expression in 92% of T- or null-cell ALCLs by Foss et al[23] and expression of perforin and/or TIA-1 in 76% of ALCLs by Krenacs et al.[24] Foss et al[23] confirmed CTL origin for most of their ALCLs by demonstrating clonal TCR-β gene rearrangements; TCR gene rearrangement studies were not available in most of our cases, but a CTL origin was supported by surface membrane CD3 staining and $\alpha\beta$-TCR expression in five $TIA\text{-}1^+$ cases.

Furthermore, our data suggest that Reed-Sternberg and Hodgkin cells are derived from CTLs or NK

cells in a minority of classical Hodgkin's disease cases. TIA-1$^+$ granules were found in Reed-Sternberg and Hodgkin's cells in 13% of our cases of classical Hodgkin's disease (three nodular sclerosis, one mixed cellularity, and one lymphocyte depletion) but in none of the cases of nodular LPHD in which the lymphocytic and histiocytic cells are of B-cell lineage. These findings are consistent with other recent studies that have demonstrated granzyme B and/or perforin expression in Reed-Sternberg and Hodgkin's cells of 9 to 18% of classical Hodgkin's disease cases.[23,25]

The commercially available TIA-1 antibody employed in this study is a useful reagent for identification of CTL and NK-cell neoplasms. We obtained equally strong staining of cytoplasmic granules in formalin-, B5- and Bouin's-fixed paraffin sections without requiring antigen retrieval or protease digestion. Our study suggests that anti-TIA-1 may detect a higher percentage of cytotoxic lymphoid neoplasms than antibodies to perforin and granzyme B. Granzyme B expression was detected in only 43% of intestinal T-cell lymphomas by de Bruin et al,[17] whereas we found TIA-1 expression in all 15 intestinal T-cell lymphomas studied. Cooke et al[15] also found more frequent expression of TIA-1 than perforin in their study of hepatosplenic $\gamma\delta$ T-cell lymphomas (6/6 TIA-1$^+$, 1/6 perforin positive); we also found TIA-1 expression in all 11 of our hepatosplenic T-cell lymphomas but observed perforin and granzyme B expression in only 3 of 5 $\gamma\delta$ T-cell cases studied.[14] More consistent expression of TIA-1 by these lymphomas may reflect their state of activation; TIA-1 is expressed by both activated and nonactivated CTLs and NK cells,[10,12] whereas perforin and granzyme B are expressed only by activated CTLs and NK cells.[36]

Thus, TIA-1 is a useful antibody for the identification of certain lymphoid neoplasms of probable NK-cell or cytolytic T-lymphocyte origin. Its routine use in combination with other antibodies may allow for more rapid identification and subclassification of cytolytic lymphocyte-related neoplasms, many of which have an aggressive clinical course.

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