Expression of TIA-1 and TIA-2 in T cell malignancies and T cell lymphocytosis

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Abstract

Objective—To investigate the reactivity with TIA-1 and TIA-2, two monoclonal antibodies that recognise, respectively, granular structures in T lymphocytes and the T cell receptor chain in cells from a variety of T cell disorders.

Methods—Cytoplasmic staining with TIA-1 and TIA-2 was carried out by the immunalkaline phosphatase anti-alkaline phosphatase technique in 67 cases with a T cell disorder: 31 large granular lymphocyte (LGL) leukaemia, nine T-prolymphocytic leukaemia (T-PLL), five Sezary syndrome, four peripheral T cell lymphoma (PTCL), 13 T cell lymphocytosis, and five T-acute lymphoblastic leukaemia (T-ALL). All had over 75% abnormal T cells which were CD2+, CD3+, CD5+, CD7+, and negative with B cell markers.

Results—TIA-1 was positive in 77% cases of LGL leukaemia and half of the PTCL and T-ALL, whereas it was negative in all Sezary syndrome and most T-PLL (8/9) and reactive T-lymphocytosis (10/13). In LGL leukaemia, TIA-1 was positive irrespective of the membrane phenotype, whether CD8+, CD4- or CD4+, CD8-, and was more often positive in cases where cells were CD16+, CD56+, or CD57+. TIA-2 was positive in 60% of cases encompassing all diagnostic types of T cell disorder. There was no correlation between TIA-2 expression and that of other T cell markers, activation antigens, and natural killer markers.

Conclusions—The pattern of TIA-1 expression in T cell malignancies may help in the differential diagnosis among LGL leukaemia (high expression), T cell lymphocytosis and other T cell diseases (low expression). As TIA-2 is expressed in over 95% mature T lymphocytes and thymic cells, its assessment may be useful to demonstrate aberrant phenotypes which can be exploited for detecting minimal residual disease.

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Keywords: T cell leukaemias, T cell lymphocytosis, TCR- ζ chain, cytotoxic, natural killer.

Malignancies of mature T cells include various entities with distinct clinical and laboratory features.^{1,2} Most cases are clonal expansions of CD4+, CD8- lymphocytes except large granular lymphocyte (LGL) leukaemia which

has usually a CD8+, CD4- phenotype. The expression of other T cell markers is variable and thus atypical profiles may be found in a proportion of cases. Identification of such phenotypes may be useful for diagnostic and follow up purposes.

New monoclonal antibodies that identify T cell functional molecules have recently become available. TIA-1 is a monoclonal antibody that recognises a 16 kDa cytoplasmic protein localised in the lysosomal granules and Golgi zone in 55% of CD8+ cytotoxic lymphocytes.³ It is also expressed in natural killer (NK) cells, in mitogen activated thymic cells, and in CD4+ and CD4+, CD8+ lymphocytes. TIA-2 identifies a 32 kDa transmembrane protein with a small extracellular domain and a large intracellular epitope which forms part of the T cell receptor (TCR) complex and is designated TCR- ζ chain.^{4,5} This molecule is linked to the CD3 complex in T lymphocytes and to the low affinity Fc γ receptor (CD16) in NK cells.^{5,7} The TCR- ζ chain has a key functional role in NK cells, being involved in antigen recognition,⁵ and it is expressed in 95% of thymic cells, circulating T lymphocytes, and NK cells but not on other cells.^{4,5}

The reactivity of TIA-1 and TIA-2 has been investigated in normal haemopoietic tissues, cell lines, and tumour infiltrating lymphocytes, where a decreased expression of TCR- ζ chain has been linked to impaired immunological surveillance.^{8,9} Decreased numbers of circulating TCR- ζ positive T lymphocytes have been documented in a B cell lymphoma and their values shown to parallel disease activity.¹⁰ In addition, the number of TIA-1 positive lymphocytes in B cell non-Hodgkin's lymphoma correlates with high grade histology, suggesting that they represent a cytolytic anti-tumour response.¹¹ There is no information concerning the expression of TIA-1 and TIA-2 in T cell disorders.

We have studied the reactivity of TIA-1 and TIA-2 in T-lymphoproliferative disorders and T cell lymphocytosis to investigate their possible role in differential diagnosis, in defining more precisely the cell nature of these diseases, and disclosing immunophenotypes which can be exploited for the detection of minimal residual disease.

Methods

PATIENTS

Peripheral blood mononuclear cells from 54 patients with a T cell malignancy and 13 with T cell lymphocytosis have been analysed. These

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Table 1 Antibodies used in this study

Antibody	CD	Source
OKT6	CD1a	Orthoclone
RFT11	CD2	Prof G Janossy (London, UK)
UCHT1	CD3	Prof P Beverley (London, UK)
Leu-3a	CD4	Beckton-Dickinson
UCHT2	CD5	Prof P Beverley (London, UK)
3A1	CD7	Sera-Lab
Leu-2A	CD8	Beckton-Dickinson
OKM1	CD11b	Orthoclone
Leu-11	CD16	Beckton-Dickinson
B4	CD19	Coulterclone
IL2R	CD25	Beckton-Dickinson
WR17	CD37	Dr J Smith (Southampton, UK)
OKT10	CD38	Orthoclone
Leu-19	CD56	Beckton-Dickinson
Leu-7	CD57	Beckton-Dickinson
anti-HLA-Dr		Prof F Garrido (Granada, Spain)
anti-TdT		Sera-Lab
TIA-1		Coulterclone
TIA-2	anti-TCR ζ	Coulterclone

were classified on the basis of clinical, morphological, and immunological features² as follows: LGL leukaemia 31, T prolymphocytic leukaemia (T-PLL) 9, Sezary syndrome 5, peripheral T cell lymphoma (PTCL) 4, and T-acute lymphoblastic leukaemia (T-ALL) 5. All cases had over 75%, usually more than 90%, circulating abnormal lymphocytes. All patients with LGL leukaemia had $>5 \times 10^9$ /litre circulating lymphocytes which persisted over one year without an underlying cause. In 14 of these cases, clonality was documented by cytogenetics or DNA analysis of the TCR β , γ , δ chain genes.

IMMUNOLOGICAL MARKERS

Immunophenotyping was performed by indirect immunofluorescence with a panel of monoclonal antibodies against B, T, and NK cells (table 1) and a fluorescein conjugated (FITC) goat anti-mouse immunoglobulin F(ab')₂ fragment as second layer. Results were analysed by flow cytometry on a FACscan (Becton-Dickinson) using a lymphocyte gate. To avoid binding of monoclonal antibodies to Fc γ receptors, 2% AB serum was added to the buffer in all incubations and washes. Controls were prepared by omitting the first layer monoclonal antibodies or replacing with mouse immunoglobulins of the various isotypes, or both. The enzyme terminal deoxynucleotidyl transferase (TdT) was analysed with a rabbit antibody by the immunoalkaline phosphatase anti-alkaline phosphatase (APAAP) as described.¹²

The reactivity with TIA-1 and TIA-2 was evaluated by the APAAP technique¹³ using a 1

Figure 1 Lymphocytes from a case with LGL leukaemia strongly positive with TIA-1. Note a negative cell (APAAP technique).

in 1000 dilution for TIA-1 and a 1 in 50 dilution for TIA-2 from a starting concentration as recommended by the manufacturers. Some samples were also investigated by flow cytometry after fixation and permeabilisation of the cell membrane.¹⁴ A case was considered positive with TIA-1 or TIA-2 when more than 30% cells reacted with either antibody.

The monoclonal antibodies TIA-1 and TIA-2 were commercially available from Coulter clone and both are mouse IgG1 ascites fluid.

Results

IMMUNOLOGICAL MARKERS

The cells in the cases studied were positive with one or more T cell markers as follows: CD2 (96%), CD3 (91%), CD5 (89%), and CD7 (78%), and they were negative with B cell markers. TdT and/or CD1a were negative in all but the five cases of T-ALL which were TdT+ and two which were CD1a+. Cells from most LGL leukaemias were CD8+, CD4- whereas a CD4+, CD8- phenotype was more frequent in Sezary syndrome and T cell lymphocytosis, and coexpression of CD4 and CD8 was more frequent in T-PLL (table 2).

Expression of NK associated markers was more common in LGL leukaemia, namely CD11b in 17/27 (63%) of cases, CD16 in 8/

Table 2 Expression of CD4 and CD8 in T cell diseases (number and proportion of positive cases)

Phenotype	LGL leukaemia	T-PLL	SS	PTCL	T-ALL	T cell lymphocytosis
CD4+ CD8-	3/31 (10%)	2/9 (22%)	4/5 (80%)	2/4 (50%)	0/3 (0%)	6/9 (67%)
CD4- CD8+	21/31 (68%)	3/9 (33%)	0/5 (0%)	1/4 (25%)	2/3 (66%)	3/9 (33%)
CD4+ CD8+	1/31 (3%)	4/9 (44%)	1/5 (20%)	0/4 (0%)	0/3 (0%)	0/9 (0%)
CD4- CD8-	6/31 (19%)	0/9 (0%)	0/5 (0%)	1/4 (25%)	1/3 (33%)	0/9 (0%)

LGL leukaemia = large granular lymphocyte leukaemia; T-PLL = T-prolymphocytic leukaemia; PTCL = peripheral T-cell lymphoma; T-ALL = T-acute lymphoblastic leukaemia.

Figure 2 FACS profile showing positive cytoplasmic staining with TIA-1 and negative TIA-2 in a case of LGL leukaemia.

Table 3 Expression of TIA-1 and TIA-2 in T cell malignancies and T cell lymphocytosis

Disease	Number of cases	Number and % of positive cases	
		TIA-1	TIA-2
LGL leukaemia	31	24/31 (77%)	17/24 (71%)
T cell lymphocytosis	13	3/31 (23%)	7/13 (54%)
T-PLL	9	1/9 (11%)	4/7 (57%)
Sezary syndrome	5	0/5 (0%)	2/4 (50%)
PTCL	4	2/4 (50%)	1/4 (25%)
T-ALL	5	2/5 (40%)	1/2 (50%)
Total	67	32/67 (48%)	32/54 (60%)

LGL leukaemia=large granular lymphocyte leukaemia; T-PLL=T-prolymphocytic leukaemia; PTCL=peripheral T-cell lymphoma; T-ALL=T-acute lymphoblastic leukaemia.

22 (36%), CD57 in 13/20 (65%), and CD56 in 8/22 (36%). These markers were rarely expressed in the other disorders: CD56 in one T-PLL, CD57+ and CD56+ in two Sezary syndrome, and CD11b+, CD16+ in one PTCL. T cell activation markers were variably expressed in all disease types: CD25 in 7/29 (24%), CD38 in 13/25 (52%), and class II HLA-Dr in 14/37 (38%).

TIA-1 AND TIA-2

The reactivity with TIA-1 and TIA-2 according to disease categories is shown in table 3. TIA-1 was positive in 48% and the proportion of cases and staining intensity was higher in LGL

Figure 3 Lymphocytes from a case of LGL leukaemia, some of which are positive with TIA-2 (APAAP).

leukaemia than in PTCL, T-ALL, and T lymphocytosis (figs 1 and 2). TIA-1 was negative in Sezary syndrome and T-PLL except for one T-PLL weakly positive in 34 % of cells.

TIA-2 was positive in 60% of cases, with no differences according to disease type except for PTCL, where this marker was rarely positive (figs 3 and 4) (table 3).

CORRELATION BETWEEN EXPRESSION OF TIA-1 AND TIA-2 AND A CD4 + AND/OR CD8 + PHENOTYPE AND OTHER T CELL AND NK MARKERS

Table 4 shows the correlation between the expression of TIA-1 and a CD8+ or CD4+ phenotype. Although it would appear that TIA-1 was more frequently positive in cases with CD8+, CD4- and CD8-, CD4- phenotypes, this was because most cases with these markers corresponded to LGL leukaemia. When considering each disease category, particularly LGL leukaemia, TIA-1 was positive regardless of the marker profile, albeit slightly more often in cases where cells were CD8+. There was no correlation between TIA-2 expression according to the reactivity with CD4 and CD8, nor with that of other T cell markers, particularly membrane CD3 and T cell activation markers (data not shown).

Table 5 shows the correlation between the reactivity with TIA-1 and TIA-2 and the expression of NK markers in LGL leukaemia.

Table 4 Correlation between TIA-1 expression and a CD4 and/or CD8 positive phenotype (number and proportion of TIA-1 positive cases)

Disease	CD4+, CD8-	CD4-, CD8+	CD4+, CD8+	CD4-, CD8-
LGL leukaemia	2/3 (66%)	17/21 (81%)	1/1 (100%)	4/6 (67%)
T-PLL	0/2 (0%)	0/3 (0%)	1/4 (25%)	
Sezary syndrome	0/4 (0%)		0/1 (0%)	
PTCL	0/2 (0%)	1/1 (100%)		1/1 (100%)
T-ALL		0/2 (0%)		1/1 (100%)
T cell lymphocytosis	0/6 (0%)	2/3 (67%)		
Total	2/17 (12%)	20/30 (67%)	2/6 (33%)	6/8 (75%)

LGL leukaemia=large granular lymphocyte leukaemia; T-PLL=T-prolymphocytic leukaemia; PTCL=peripheral T-cell lymphoma; T-ALL=T-acute lymphoblastic leukaemia.

Figure 4 FACS profile of a case of T-PLL positive with TIA-2 and negative with TIA-1.

Table 5 Correlation between TIA-1 and TIA-2 expression and NK markers in LGL leukaemia (number and proportion of cases positive with TIA-1 and TIA-2)

NK marker	TIA-1	TIA-2
CD11b+	13/17 (76%)	11/14 (78%)
CD11b-	9/10 (90%)	12/15 (80%)
CD16+	8/8 (100%)	4/6 (67%)
CD16-	10/14 (71%)	5/6 (83%)
CD56+	7/8 (87%)	5/6 (83%)
CD56-	10/14 (71%)	7/10 (70%)
CD57+	11/13 (85%)	9/12 (75%)
CD57-	5/7 (71%)	2/3 (67%)

TIA-1 was more often positive in cases where cells were positive with CD16, CD56, and CD57. In contrast, there were no differences on TIA-2 expression with respect to any of the NK markers.

Discussion

The T-lymphoproliferative disorders are heterogeneous in their clinical and laboratory features. This is also reflected on the immunological markers as there is no evidence of phenotypes unique to a particular disease except LGL leukaemia.²

We have shown here that TIA-1 is expressed in cells from the majority of cases of LGL leukaemia and, as a rule, is negative in the other T cell diseases. Although cells from some PTCL and T-ALL were TIA-1 positive, the number of cases was too small to draw conclusions. Cases with reactive T cell lymphocytosis which could be confused with LGL leukaemia were less often TIA-1 positive. The fact that TIA-1 expression did not correlate with the cells being CD8+ or CD4+ but rather with granular lymphocyte morphology suggests that it might possibly be of value in the differential diagnosis between LGL leukaemia with Sezary syndrome and T-PLL, as cells from several T-PLL and Sezary syndrome cases had

a CD8+, CD4- or a CD8+, CD4+ phenotype, the former phenotype being common in LGL leukaemia.

Our results suggest that most cases of LGL leukaemia arise from the expansion of CD8+, TIA-1+ lymphocytes with a possible cytotoxic function. Six cases (four LGL leukaemia, one PTCL, and one T-ALL) who had a CD4-, CD8- phenotype stained with TIA-1 but none had a pure NK phenotype profile and all but one were CD3+; the CD3 negative case was positive with other T cell markers. Probably none of them represented a proliferation of TIA-1 positive NK cells.

The pattern of staining with TIA-2 was more variable, without evidence of an association with a particular disease. A proportion of cases were TIA-2 negative and thus lacked the TCR- ζ chain which plays a key role as a signal transducing molecule during activation of T and NK cells. This suggests indirectly an impaired immunological function for such leukaemic cells. Indeed, a decrease in the cytotoxic and proliferative response to signalling through the TCR has been shown in mice lymphocytes which lack the TCR- ζ chain or express it poorly.¹⁵ The lack of TCR- ζ in leukaemic T cells was unrelated to the expression of CD3 or CD16, molecules to which the TCR- ζ chain is associated in T and NK cells, respectively. The lack of or weak expression of TIA-2 is also found in tumour infiltrating lymphocytes in mice and humans^{8,9,16} and it has recently been reported in non-neoplastic T cells in a case of B cell non-Hodgkin's lymphoma.¹⁰ Its absence in some T cell malignancies might be useful in monitoring response to treatment.

In conclusion, we report the spectrum of reactivity of two new monoclonal antibodies, TIA-1 and TIA-2, in a variety of T cell disorders. We have shown that TIA-1 is a useful marker for LGL leukaemia and that TIA-2 might be helpful in monitoring the disease status in patients whose cells are negative for this monoclonal antibody.

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