

TIA-1 expression in hairy cell leukemia

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We measured T-cell intracellular antigen-1 (TIA-1) expression in neoplastic cells from patients with hairy cell leukemia. Five of nine cases were positive for cytoplasmic TIA-1, with a small, dot-like, granular expression pattern. However, neoplastic cells were granzyme B- and perforin- negative in all cases. Other positive markers were CD20 in 9/9 cases, CD19 in 9/9 cases, DBA44 in 8/9 cases, LeuM5(CD11C) in 8/9 cases, IL-2R(CD25) in 7/9 cases, CD103 in 7/9 cases, FMC7 in 6/9 cases, and tartrate-resistant acid phosphatase in 5/7 cases. We also analyzed TIA-1 expression in 94 B cell lymphomas, including 19 diffuse, large cell lymphomas, 19 mantle cell lymphomas, six follicular lymphomas, two extranodal marginal zone B-cell lymphomas, 13 nodal marginal zone B-cell lymphomas, one mediastinal large-cell lymphoma, 19 diffuse small-cell lymphomas, 14 myelomas, and one splenic lymphoma with villous lymphocytes. All cases were negative for TIA-1 expression. Based on these findings, TIA-1 expression in neoplastic cells of low-grade B-cell lymphomas may be a good diagnostic marker for hairy cell leukemia. Moreover, TIA-1 reactivity in lymphomas does not necessarily indicate a T- or NK-cell derivation.

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The predominant cytotoxic molecules are perforin, granzyme, and T-cell intracellular antigen-1 (TIA-1).^{1–3} Expression of these proteins has been reported in cytotoxic T cells^{4–6} and natural killer cells (NK cells).^{6,7} These proteins are also expressed in T-cell or NK-cell neoplasms,^{8–11} including large granular lymphocyte leukemia, NK-cell leukemia/lymphoma,^{12–14} hepatosplenic T-cell lymphoma,^{15–17} subcutaneous panniculitic T-cell lymphoma,^{18,19} enteropathy-type T-cell lymphoma,²⁰ and anaplastic large-cell lymphoma.²¹ Perforin functions by making a hole in the target cell membrane. Then, TIA-1 and granzymes enter the cytoplasm through the pore made by perforin and activate apoptosis-related proteins.^{9,10,21} Perforin and granzymes are only expressed in cytotoxic cells upon activation and are associated with induction of apoptotic activity. TIA-1, however, is expressed in cytotoxic T cells, regardless of their activation status, and in myeloid cells.^{6–8}

Cytotoxic molecules are also expressed in some cases of Hodgkin's lymphoma.²¹ However, no

reports have detected cytotoxic molecule expression in B-cell lymphomas. We incidentally detected TIA-1 expression in some cases of hairy cell leukemia. The aim of our study, therefore, is to analyze TIA-1 expression in hairy cell leukemia and to determine whether other B cell lymphomas express TIA-1.

Materials and methods

Fresh-frozen tissues were available from nine cases of hairy cell leukemia. Of these, four splenic tissues, three bone marrow clot sections, and two peripheral blood samples were obtained. Paraffin tissue sections were also available in six cases. Three bone marrow biopsy specimens, one bone marrow clot section, and four splenic tissues were available in paraffin tissue specimens. TIA-1 expression was also studied in paraffin tissue sections from 94 cases of various B-cell lymphomas.

Peripheral Blood Analysis

Flow cytometric analysis was performed in four cases. Peripheral blood was taken in heparin-containing tubes, and mononuclear fractions were collected by gradient centrifugation method using Ficoll-Paque (Pharmacia, Uppsala, Sweden). Cells

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were washed twice with phosphate-buffered saline (PBS), and incubated with each antibodies according to the instructions of the manufacturers. After 30 min incubation on ice, cells were washed twice with PBS containing albumin and azide. FACScan and software (Consort 30 or Cell Quest) were used for analysis (Becton Dickinson, San Jose, CA, USA). All antibodies for flow cytometry were purchased from Becton Dickinson. Fluorescent labeled antibodies were for CD5, 10, 19, 20, 21, 22, 23, 24, 25, 38, 11a, 11c, and FMC7 and these clones were L17F12, H10a, HIB19, 2H7, B-ly4, S-HCL-1, M-L233, ML-5, M-A251, HIT-2, G43-25B, B-ly6, and FMC7, respectively. These antibodies were labeled with fluorescein isothiocyanate (FITC), or phycoerythrin (PE). Negative control cells were stained with each isotype antibodies labeled with adequate fluorescent dyes (mouse IgG1, or IgG2 labeled with FITC, or PE). Hairy cells were targeted on front scatter and side scatter plotgraph if hairy cells were lower than 95% of mononuclear cells.

Frozen Tissue Analysis

Fresh frozen tissues were fixed with periodate-lysine-paraformaldehyde,²² frozen, cut with a cryostat to a thickness of 6–8 μ m, fixed with acetone for 10 min, reacted with primary antibodies, and stained with an avidin–biotin–peroxidase complex method.²³ Primary antibodies used were CD5(Leu1) and CD11c(LeuM5) (Becton Dickinson, San Jose, CA, USA), CD25(IL-2R) (Becton Dickinson, Mountain View, CA, USA), FMC7 (Sera-Lab, Sussex, UK), CD103 (Immunotech, Marseille, France), DBA44 (DAKO, Copenhagen, Denmark), CD20(B1) and CD19(B4), (Coulter, Hialeah, FA), TIA-1 and perforin (T Cell Diagnostics, Cambridge, MA, USA), and granzyme B (Monosan, Uden, Netherlands).

For immunoelectron microscopy, after performing the immunoperoxidase protocol, specimens were fixed with 1.25% glutaraldehyde for 15 min and washed three times with cold phosphate-buffered saline. Specimens were then reacted with diaminobenzidine solution containing 0.01% hydrogen peroxide for 5 min at room temperature. They were

postfixed with osmium tetroxide, dehydrated, embedded, and observed under an electron microscope without counterstain. Details of immunoelectron microscopy have been described elsewhere.²³

Paraffin Tissue Analysis

For paraffin sections, tissues were fixed with 10% formaldehyde and embedded in paraffin. Sections 2–4 μ m thick were prepared and stained with hematoxylin and eosin (H&E). The avidin–biotin–peroxidase complex method²⁴ was used for antibody staining. Antibodies used were CD20 (L26) and CD79a (mb-1) (DAKO, Copenhagen, Denmark), TIA-1 and perforin (T Cell Diagnostics, Cambridge, MA, USA), granzyme B (Monosan, Uden, Netherlands), CD43(MT1) (Immunochemicals, Biochemicals and Diagnostics, Emmerbruecke, Switzerland), and CD45RO (UCHL-1) (Nichirei Corp., Tokyo, Japan).

Results

In all, nine cases of hairy cell leukemia were studied. The average age of the patients was 65 years, and of these, six were males and three were female patients. All cases had some degree of splenomegaly and some had marked splenomegaly (Table 1). One patient had liver involvement. In the four cases where bone marrow specimens were available, the bone marrow was diffusely infiltrated with tumor cells in all cases.

Wright staining of a bone marrow smear revealed atypical lymphoid cells with abundant and pale cytoplasm (Figure 1a). Hairy projections were identified in almost all cases investigated with electron or phase contrast microscopy (Table 1). Most patients had leukopenia, although two cases had leukocytosis, suggesting them to be the Japanese type variant.

Flow cytometric analysis was performed in four cases (Case No. 3, 4, 5 and 8). As a result, CD19, 20, and CD11c were all positive (4/4). CD25 was positive in 3/4 cases, FMC7 in 2/2, CD21 in 2/3, CD22 in 2/2, CD23 in 1/3, CD24 in 0/2, CD38 in 0/3, CD11a in 1/2, CD5 in 0/4, and CD10 in 0/4,

Table 1 Clinical features of the cases

Case	Age (years)	Sex	Hematological findings	Splenomegaly	Therapy	Prognosis
1	76	M	Non-remarkable	+	Splenectomy	CR (1)
2	72	F	Pancytopenia, hairy projections (PC)	+	Splenectomy	CR (11)
3	44	M	Pancytopenia, hairy projections (PC)	+	Splenectomy	PR (5)
4	56	M	Leukocytopenia (2900), hairy projections (PC)	+	Splenectomy	Unknown
5	69	M	Leukocytosis (24 600), hairy projections (PC)	+	IFN	PR (20)
6	72	F	Leukopenia	+	Unknown	Unknown
7	53	M	Leukocytosis (9200)	+	Splenectomy	Unknown
8	65	M	Pancytopenia, hairy projections (EM)	+	IFN	CR (13)
9	79	F	Leukopenia, hairy cells	+	Unknown	Unknown

PC, phase contrast microscopy; EM, electron microscopy; CR (11), complete remission (11 years); PR, partial remission; IFN, interferon.

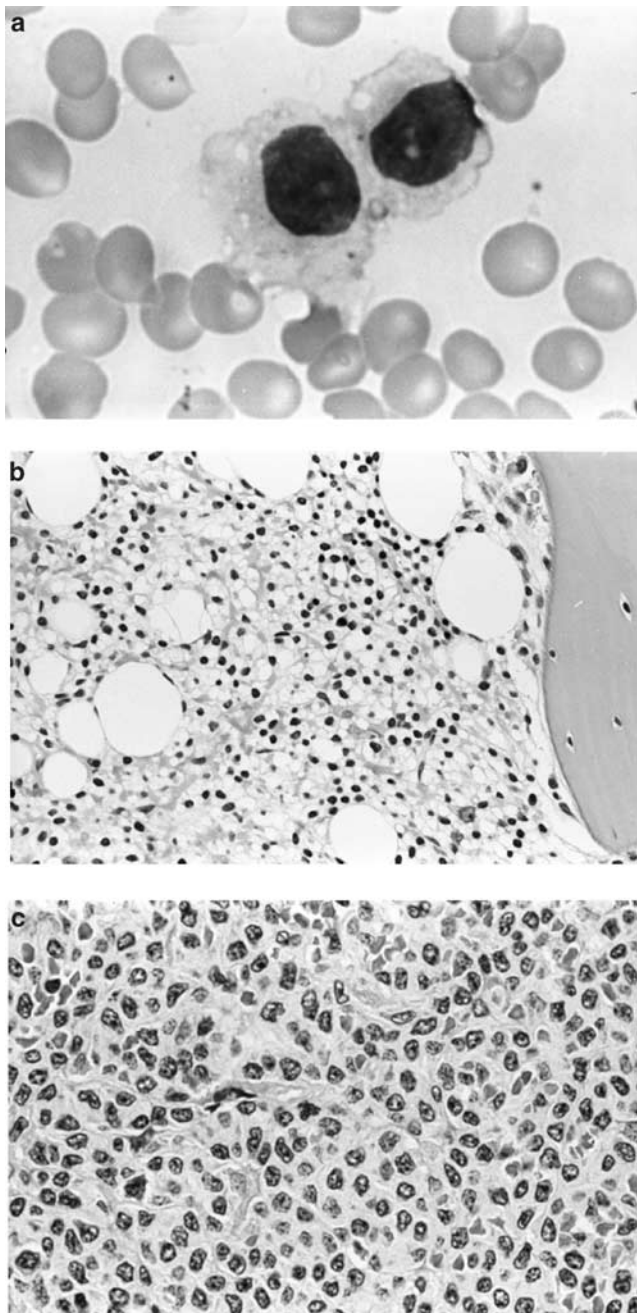


Figure 1 (a) Wright staining of a bone marrow smear reveals atypical lymphoid cells with abundant and pale cytoplasm (case 3) and (b), (c) H&E section of hairy cell leukemia; (b) Bone marrow ($\times 340$), (c) spleen ($\times 680$). The neoplastic cells have an ample, pale cytoplasm and a medium-sized, round, sometimes slightly irregular nucleus.

respectively. Positivity was defined when more than 60% of hairy cells were positively stained.

In paraffin tissue sections, neoplastic cells were small to medium-sized with round nuclei and ample, pale cytoplasm in the bone marrow and spleen specimens (Figure 1b and c). Blood lakes were occasionally observed in spleens. Five of seven cases investigated were tartrate-resistant acid phosphatase (TRAP)-positive. Of these, frozen tissue

specimens were available in all cases, and paraffin tissues were available in six cases. In all, 3/6 paraffin tissue sections and 5/9 frozen tissue specimens were TIA-1 positive (Figure 2a and b). Granzyme B and perforin were not positive in any cases. Pan B cell markers, such as CD19 (B4) and CD20 (B1), were positive in all cases (Figure 3). Other positive markers were DBA44 in 8/9, LeuM5(CD11c) in 8/9, IL-2R(CD25) in 8/9, CD103 in 7/9, FMC7 in 6/9 (Figures 4–6), and TRAP in 5/7 cases. CD5 was not expressed in any cases (Table 2). Immunoelectron microscopy revealed TIA-1 expression in the perigranular region of neoplastic cells (Figure 7). The positive reactivity was not localized in the ribosome lamellar complexes we examined so far.

Discussion

Hairy cell leukemia, formerly known as leukemic reticuloendotheliosis,^{25–31} is characterized by bone marrow³² and splenic involvement by small to medium-sized neoplastic cells with abundant cytoplasm and hairy projections.^{33–35} In our present study, hairy cytoplasmic projections were observed in the peripheral blood in most cases (7/9) by electron or phase-contrast microscopy.^{34,35} Furthermore, TRAP was positive in most cases (5/7 cases studied).^{26,31,34,35} In the bone marrows and spleens studied, neoplastic cells had a small to medium-sized, round nucleus and ample, pale cytoplasm. All patients had splenomegaly, and in some cases, blood lakes were observed by light microscopy.³⁶ Therefore, we considered these cases to fit the category of hairy cell leukemia. Patients with hairy cell leukemia usually have low peripheral blood leukocyte counts.^{34,35} However, the leukocyte count in case 5 was 24 600 and may represent the Japanese variant,^{37–39} as opposed to the Western type hairy cell variant.⁴⁰ In this case of hairy cell leukemia, the neoplastic cells were CD5–, CD11c+, and CD25–, a phenotype consistent with a Japanese variant.^{37–39} In hairy cell leukemia, in addition to pan-B-cell markers such as CD19(B4), CD20(B1 or L26), and CD79a, various markers have been reportedly positive, including DBA44, CD11c(LeuM5), CD25(IL-2R), CD103, and FMC7.^{34,35,41–44} In this study, these markers were largely positive, with DBA44 positivity in 8/9 cases, CD11c in 8/9 cases, CD25 in 7/9 cases, CD103 in 7/9 cases, and FMC in 6/9 cases, respectively. None of the cases were positive for CD5. Cytotoxic proteins including perforin, granzyme B, and TIA-1 are expressed in various cytotoxic T-cell and NK cell lymphomas.^{10,11,13,14} These lymphomas generally express all of these proteins. Nasal lymphoma shows strong perforin,¹² granzyme B, and TIA-1 expression. Cytotoxic proteins are also reportedly expressed in certain cases of Hodgkin's lymphoma.²¹ It has been reported that neoplastic cells from patients with hepatosplenic T-cell lymphoma only express TIA-1.¹⁵

Table 2 Histochemical and immunohistochemical findings of hairy cell leukemia

Case	TIA-1	grzB	pf.	CD20	CD19	DBA44	LeuM5	IL-2R	CD103	FMC7	CD5	TRAP
1	+	-	-	+	+	+	+	+/-	+	+	-	ND
2	+	-	-	+	+	+	+	+	+	+	-	+
3	+	-	-	+	+	+	+	+	+	+	-	+
4	+	-	-	+	+	-	+	+	-	-	-	+
5	+	-	-	+	+	+	+	-	+	+	-	+
6	-	-	-	+	+	+	-	+	-	-	-	-
7	-	-	-	+	+	+	+	-	+	+	-	-
8	-	-	-	+	+	+	+	+	+	+	-	+
9	-	-	-	+	+	+	+	+	+	-	-	ND

GrzB, granzyme B; pf, perforin; TRAP, tartrate-resistant acid phosphatase; ND, not done.

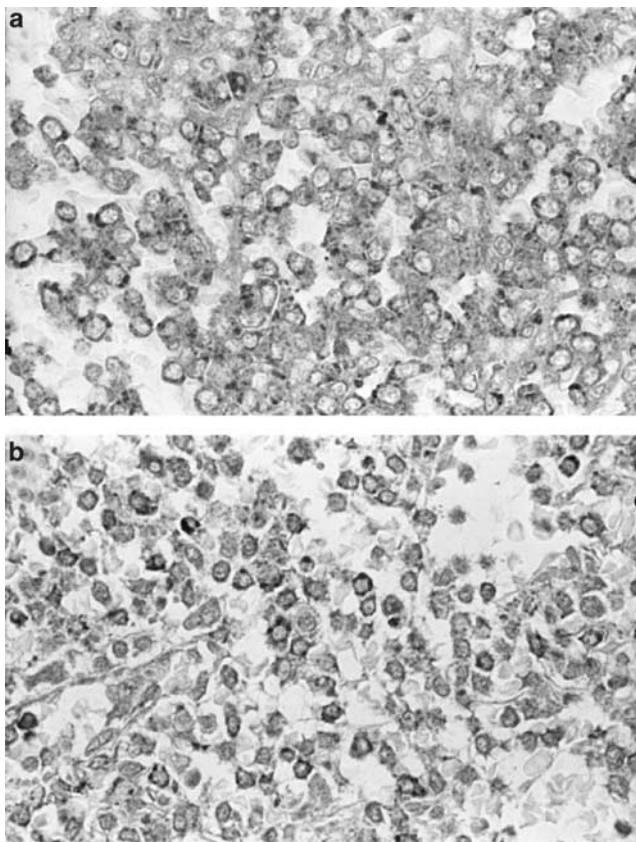


Figure 2 TIA-1 immunostaining reveals positive granular reactivity in the cytoplasm of most neoplastic cells. (a) case 1, (b) case 2 ($\times 340$).

Cooke *et al*¹⁵ reported that hepatosplenic T-cell lymphoma is a distinct and rare entity, with distinct clinicopathologic features characterized by its extranodal hepatosplenic presentation and sinusal/sinusoidal tropism of the neoplastic T cells. These tumors reportedly expressed TIA-1, although only one of eight cases expressed perforin.¹⁵ They therefore considered that hepatosplenic T-cell lymphoma cells may be functionally immature and not yet capable of cytotoxic function.¹⁵ Although TIA-1 expression, in the absence of granzyme and perforin expression, is similar between the cases of hairy cell leukemia and hepatosplenic T-cell lymphoma,^{15,16}

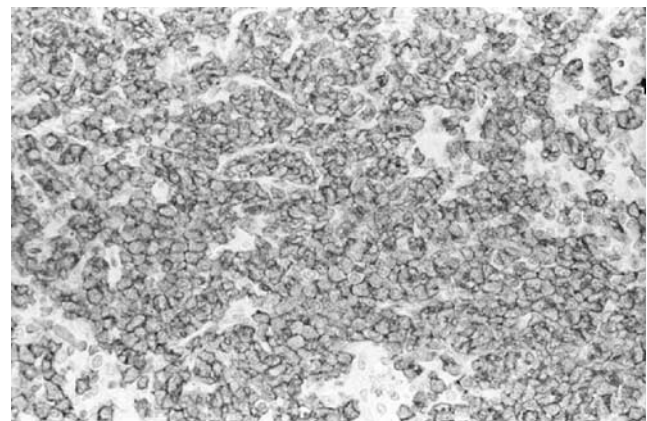


Figure 3 CD20 immunostaining reveals positive reactivity in the cytoplasm of neoplastic cells ($\times 340$).

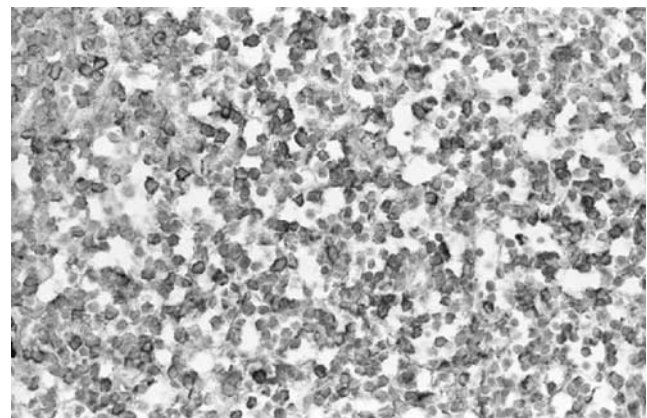


Figure 4 DBA44 immunostaining reveals positive reactivity in the neoplastic cells ($\times 340$).

the mechanism underlying their expression might be different. Since hairy cell leukemia is a B-cell lymphoma, it is not expected to have a role in cytotoxic function.

Recent studies indicated that cytotoxic T cells possess azurophilic cytoplasmic granules containing perforin, granzyme A and B, and TIA-1.³ Perforin has a role in forming pores in target cell membranes. Then, granzymes and TIA-1 trigger a

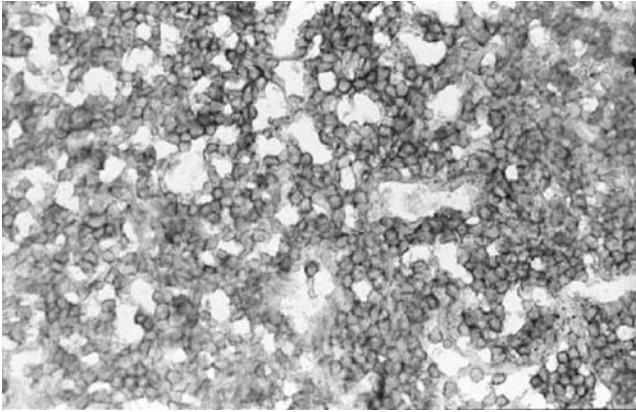


Figure 5 IL2-R (CD25) immunostaining reveals positive reactivity in the neoplastic cells ($\times 340$).

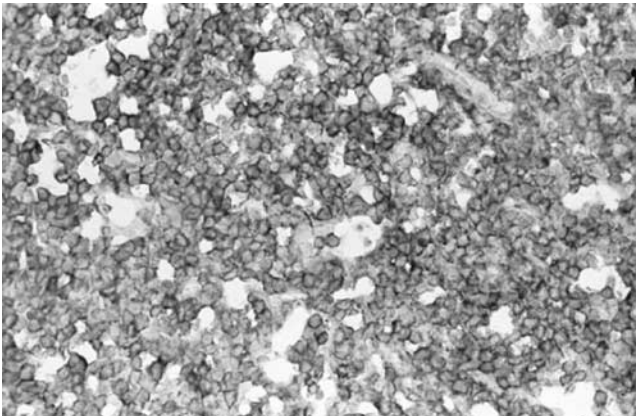


Figure 6 LeuM5 (CD11c) immunostaining reveals positive reactivity in the neoplastic cells ($\times 340$).

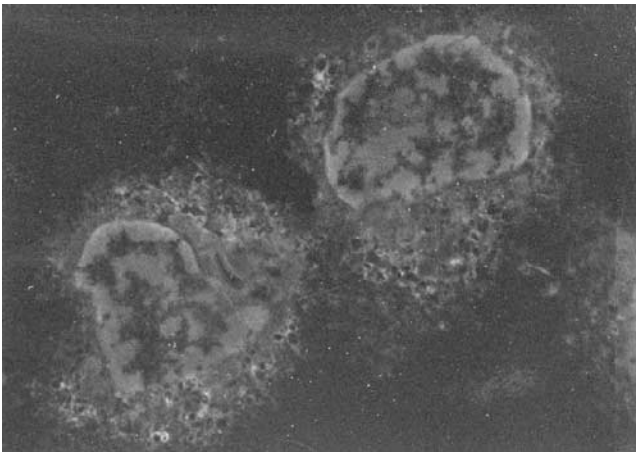


Figure 7 Immunoelectron microscopy reveals positive reactivity in the perigranular spaces of the neoplastic cells ($\times 5000$).

process leading to apoptotic DNA fragmentation of target cells.^{8,9,21} Medley *et al*⁶ localized TIA-1 to the membrane of cytotoxic granules in NK cells by immunoelectron microscopy. They, therefore, renamed this protein granule membrane protein of 17 kDa, GMP-17, given its subcellular localization.⁶

Our immunoelectron microscopy results support their finding of TIA-1 reactivity in the perigranular spaces of neoplastic cells.

Azurophilic granules have also been observed in neoplastic hairy cell leukemia.^{28,29} We, therefore, speculate that TIA-1 expression may account for the presence of azurophilic granules in hairy cells. TIA-1 is also expressed in granulocytes,^{6,7} in addition to cytotoxic T cells and NK cells. Therefore, TIA-1 expression is not necessarily related to the induction of apoptosis-related proteins.

Beck *et al*² reported that murine TIA-1 is predominantly expressed in brain, testis, and spleen. TIA-1 has been suggested to be incorporated in hairy cells, rather than being endogenously expressed, because hairy cells have a role in phagocytosis³⁰ as well as a receptor for cytophilic antibodies.²⁷ However, this mechanism is unlikely because most neoplastic cells contain TIA-1 granules in their cytoplasm, suggesting endogenous TIA-1 production rather than exogenous intake.

Although the nature of the neoplastic cells of hairy cell leukemia still remains to be clarified, the neoplastic cells do not seem to be restricted to a defined lineage.⁴⁵ In addition, various cytokines such as fibroblast growth factor^{45,46} or TNF-alpha,^{47,48} are reportedly produced in hairy cells, showing their unique nature. TIA-1 finding in the neoplastic cells of hairy cell leukemia may be helpful for elucidating its nature.

Although the mechanism of TIA-1 expression in hairy cell leukemia neoplastic cells is unknown, TIA-1 is a good marker for diagnosing hairy cell leukemia among low-grade B-cell lymphomas.

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