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Absence of cytotoxic molecules in CD8- and/or CD56-positive adult T-cell leukaemia/lymphoma

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Abstract Adult T-cell leukaemia/lymphoma (ATLL) cells usually exhibit a $CD4^+$ (helper/inducer) phenotype ($CD4^+/8^-/56^-$), and only a minority of tumours express the CD8 (cytotoxic/suppressor) or CD56 (natural killer [NK]-associated) antigens. TIA-1 is a cytotoxic granule-associated protein expressed in NK cells and cytotoxic T lymphocytes (CTLs). Granzyme B, perforin and Fas ligand (FasL) are also expressed in activated CTLs and NK cells. To clarify the cytotoxic potential of ATLL cells, immunohistochemistry was performed in $CD8^+$ and/or $CD56^+$ ATLL cells, using anti-TIA-1, anti-granzyme B, anti-perforin and anti-FasL antibodies. We studied nine cases of $CD8^+$ and/or CD56+ ATLL, all of which exhibited monoclonal integration of human T-cell leukaemia virus type 1 (HTLV-1) proviral DNA. Four cases exhibited a $CD8^+/CD56^-$ phenotype, four others had a $CD8^-/CD56^+$ phenotype, and one was $CD8^+/CD56^+$. All but one case also expressed the surface antigens CD3, TCR αβ, and CD4. Expression of granzyme B and TIA-1 were demonstrated in three and two cases, respectively, but none expressed perforin or FasL. In the control study, 10 cases with typical $CD3^+/4^+/8^-/56^-$ ATLL demonstrated no expression of those cytotoxic-associated proteins. Our findings suggest that CD8 and/or CD56 positivity probably confer(s) no cytotoxic function on ATLL cells, and it is possible that CD8 and CD56 may be simply aberrant surface markers in ATLL.

Key words ATLL · CD8 · CD56 · Cytotoxic molecules

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Introduction

Adult T-cell leukaemia/lymphoma (ATLL) is a human malignancy associated with human T-cell leukaemia virus type I (HTLV-1), which is a type C retrovirus [18]. It can be diagnosed by characteristic clinicopathological features and the presence of integrated proviral HTLV-1 DNA in tumour cells with a helper/inducer ($CD4^+$) phenotype [19, 21]. In rare cases, ATLL cells express CD8 (cytotoxic/suppressor) or CD56 (natural killer [NK]-associated) antigens [4, 6, 9].

Expression of T-cell restricted intracellular antigen (TIA-1) is a characteristic feature of cytotoxic cells, including cytotoxic T-cells (CTLs) and NK cells. Thus, antibodies to TIA-1 should be useful in distinguishing specific subsets of lymphoid neoplasms derived from CTLs or NK cells [5]. Expression levels of perforin and granzyme proteins are highly elevated in activated cytotoxic cells and correlate with the induction of cytolytic activity [2, 20]. Two molecular mechanisms of T-cell-mediated cytotoxicity, one perforin based, the other Fas based, have previously been demonstrated [8]. The perforin-based mechanism seems to require molecules other than perforin, including certain serine esterases, and the granzymes [16]. The Fas-based mechanism has been molecularly defined independently by the involvement of the cell death-transducing molecule Fas at the target cell level and at the effector cell level of the Fas ligand (FasL) [15, 17].

This study was designed to investigate the expression of cytotoxic cell-associated molecules (TIA-1, perforin, granzyme B and FasL) in $CD8^+$ and/or $CD56^+$ ATLL.

Materials and methods

This study comprised nine cases of molecularly confirmed ATLL expressing surface CD8 and/or CD56 antigens, obtained from lymph node biopsies from the Department of Pathology, Fukuoka University School of Medicine. Specimens were divided into three sections. Paraffin-embedded sections for light microscopy were fixed in buffered formalin. As the control study, we analysed ten

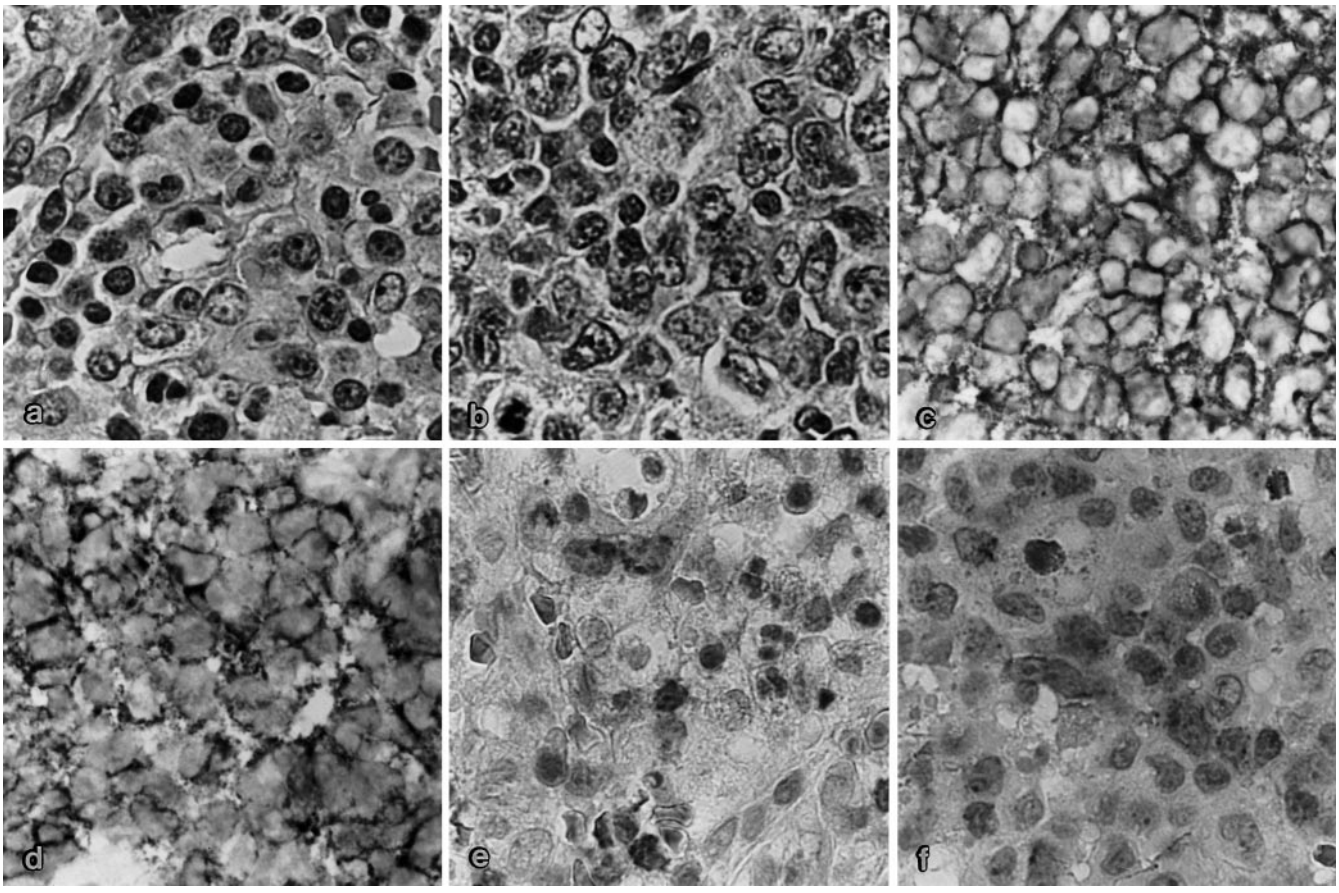

Fig. 1a–f Histological features and immunohistochemical staining. **a** The lymph node shows a diffuse infiltrate of atypical medium-sized to large lymphoid cells with irregular nuclei, which was classified as a pleiomorphic medium-sized and large-cell ATLL. **b** A pleiomorphic large-cell ATLL. **c**, **d** The lymphoma cells were positive for **c** CD8 and/or **d** CD56. **e**, **f** Immunological staining of cytotoxic molecules in lymphoma cells revealed weak granular cytoplasmic positivity for **e** the cytotoxic granule-associated protein TIA-1 and **f** granzyme B

patients with typical $CD3^+/4^+/8^-/56^-$ ATLL. The histology of these ten patients was variously pleomorphic, medium-sized and large-cell type.

Immunohistochemistry

Serial cryostat sections were prepared for immunohistochemical staining. The following distinguishing antibodies were used: T11 (CD2), Leu 4 (CD3) for T-cells (Ortho, Raritan, N.J.; Becton-Dickinson, Mountain View, Calif.), Leu 3 (CD4) for helper/inducer T-cells (Becton-Dickinson), Leu 2 (CD8) for suppressor/cytotoxic T-cells (Becton-Dickinson), CD68 for histiocytes (Dakopatts, Glostrup, Denmark), CD56 for NK cells (Becton-Dickinson), CD19 and CD20 for B cells (Coulter, Hialeah, Fla.), and Leu 1 (CD5) and Leu 2 (CD7) (Becton-Dickinson). In addition, antibodies to perforin and granzyme B were used for staining of cytoplasmic granules in CTLs or NK cells (T Cell Diagnostic, Cambridge, Mass.; Pharmacell, Paris, France), and anti-t-TIA-1 antibody for CTLs or NK cells (Coulter); anti-TCR βF1 and δ1 (T Cell Diagnostic) were also used on fresh materials and/or paraffin-embedded materials. The anti-FasL antibody (N-20: Santa Cruz Biotechnology, Santa Cruz, Calif.) was used for immunohistochemical staining, which was done on fresh and/or paraffin-embedded materials.

DNA analysis

One section of the frozen material was used for DNA isolation and Southern blotting gene analysis. Before the DNA analysis, the samples were confirmed to consist of lymphoma cells, which occupied >70% of the nucleated cells, using HE and immunological stainings of the frozen samples. By means of Southern blot analysis of the clonal HTLV-I integration, we were able to detect 3% clonal HTLV-I integrated cells (data not shown). The details of the examination methods have been reported previously [13]. The T-cell receptor (*TCR*), *Cβ* and *Jγ* genes were used as probes with the restriction enzymes *Eco*RI, *Hind*III, or *Bam*HI. Proviral HTLV-1 DNA (full length; gag, pol, env, pX, LTR) was also examined by Southern blot analysis. The monoclonal integration of HTLV-I DNA was examined by the digestion of the enzymes *Eco*RI, as have already reported [13].

Results

Clinical findings

The patients' ages ranged from 42 to 75 years, with a median age of 65; there were five men and four women. All nine cases showed peripheral lymph node swelling. Laboratory data recorded in the peripheral blood showed a few atypical lymphocytes (1–2%) in five patients and an elevation of lactate dehydrogenase (LDH) in six. All patients received chemotherapy with CHOP or modified CHOP after the nodal biopsy, but seven patients were dead within 8 months (Table 1). The clinical features

Table 1 Clinical findings, Non of these patients showed hepatosplenomegaly or haemophagocytic syndrome, and all received chemotherapy (*WBC* white blood cells, *ALPB* abnormal lymphocytes in peripheral blood, *LDH* lactate dehydrogenase, *Pleo* pleomorphic type, *M* medium sized, *L* large, *A* abnormal, *N* normal)

Case no.	Age (years)	Sex	WBC	ALPB[a]	LDH	Stage	Status	Histology
1	68	F	15500	2%	A	2	5 months, dead	Pleo M&L
2	46	M	4500	0	A	3	8 months, dead	Pleo M&L
3	64	F	2600	0	A	3	5 months, alive	Pleo M&L
4	75	M	5900	0	A	4	8 months, dead	Pleo M&L
5	63	M	9700	0	A	4	2 months, dead	Pleo M&L
6	65	M	3100	1%	N	4	8 months, dead	Pleo L
7	69	M	5800	2%	N	2	5 months, alive	Pleo M&L
8	66	F	3600	1%	A	2	6 months, dead	Pleo M%L
9	42	F	4600	2%	N	3	5 months, dead	Pleo M&L

[a] Proportion of abnormal lymphocytes in peripheral blood

Table 2 Phenotype of the neoplastic cells in nine cases diagnosed as ATLL with CD8 and/or CD56 positivity

Case no.	Immunochemical stainings														DNA analysis		
	CD2	CD3	β	δ	CD5	CD7	CD4	CD8	CD56	CD30	TIA-1	Perforin	Granzyme B	FasL	TCR-β	TCR-γ	HTLV-I
1	+	+	+	–	+	+	+	+	–	+	+/–	–	+	–	R	G	+
2	+	+	+	–	+	–	+	+	–	+	–	–	–	–	R	R	+
3	+	+	+	–	+	–	+	+	–	+	–	–	–	–	R	R	+
4	+	+	+	–	+	–	+	+	–	+	–	–	–	–	R	R	+
5	+	–	ND	ND	ND	ND	–	–	+	+	+/–	–	+	–	R	R	+
6	+	+	+	–	ND	ND	+	–	+	–	–	–	–	–	R	R	+
7	+	+	+	–	+	–	+	+	+	–	–	–	–	–	R	R	+
8	+	+	+	–	+	+	+	–	+	+/–	–	–	+/–	–	R	G	+
9	+	+	+	–	+	–	+	–	+	–	–	–	–	–	R	D	+

were not so different from those of typical ATLL with $CD3^+/8^-/56^-$ phenotype.

Histology and DNA analysis

Eight cases were diagnosed morphologically as having pleomorphic medium-sized and large cell types, and one case (case 6) was pleiomorphic with a large cell type (Fig. 1). Necrotic lesions with apoptotic cells and histiocyte reaction were not obvious, and angiocentric features were not present. The histological features of $CD8^+/56^+$ ATLL were no different from those of typical $CD4^+/8^-/56^-$ ATLL. In addition, $CD8^+/56^+$ ATLL showed no necrosis and angiocentricity, which are characteristic findings in nasal NK cell lymphoma.

All cases exhibited monoclonal integration of HTLV-1 proviral DNA and TCR rearrangements on Southern blot analysis (Table 1).

Phenotype and cytotoxic-associated proteins

All cases except one exhibited a $CD2^+$, $CD3^+$, $CD4^+$, $CD5^+$, TCR-$\alpha\beta^+$, and TCR-$\gamma\delta^-$ phenotype (Table 2). Only one case had a $CD3^-$ and $CD4^-$ phenotype. Four of nine cases exhibited $CD8^+/CD56^-$ surface antigens, four cases were $CD8^-/CD56^+$, and one was $CD8^+/CD56^+$ (Table 2). All cases examined were $CD5^+$, and five of seven cases were $CD7^-$. CD30 positivity was detected in six of the nine cases.

Granzyme B expression was detected in three cases, but the reaction was weak in one case and granzyme B-positive neoplastic cells were a few in number overall (Fig. 1). TIA-1 was demonstrated in only two cases: the reaction was very weak and TIA-1-positive neoplastic cells were few in number. No tumours expressed perforin or FasL.

Discussion

TIA-1 was previously described as a cytolytic granule protein of CTLs and NK cells [2]. However, TIA-1 has recently been renamed GMP-17, and it has been demonstrated that this protein is not actually released but leads translocation from cytotoxic granules to the plasma membrane [12]. Felgar et al. [5] evaluated TIA-1 expression by paraffin-embedded immunohistochemistry in 115 T or NK cell neoplasms: $TIA\text{-}1^+$ granules were identified within the cytoplasm of neoplastic cells in almost all extranodal cytotoxic T and NK cell lymphomas and nodal anaplastic large-cell lymphomas (ALCL). In contrast, nodal peripheral T-cell lymphomas, especially $CD4^+$ lymphoma, rarely expressed TIA-1. However, $CD4^+$ ALCLs were $TIA\text{-}1^+$, suggesting that some AL-

CLs may be derived from $CD4^+$ CTLs [5]. In the present study, we analysed 10 patients with typical $CD3^+/4^+/8^-$ ATLL, and TIA-1 was not expressed.

Two molecular mechanisms of T-cell-mediated cytotoxicity, one perforin based and the other Fas based, have been demonstrated [8]. Both mechanisms independently induce cell death by apoptosis [8]. The perforin-based pathway seems to involve granule exocytosis, whereas the Fas pathway involves a cell-bound ligand (FasL)–receptor interaction.

Expression of TIA-1 is characteristic of cytotoxic cells regardless of their activation status, whereas expression levels of perforin and granzymes are highly elevated in activated cytotoxic cells [3]. For example, the $CD3^+/CD8^+/CD56^+$, NK-like (cytotoxic) T-cell lymphoma and $CD3^-/CD56^+$ NK cell leukaemia/lymphoma express cytotoxic-associated proteins, including TIA-1, perforin, granzyme B and FasL [3, 11, 14].

Thymus-independent T-cells may be αβ- or γδ-types, and also $CD4^-/CD8^+$, $CD4^+/CD8^-$, $CD4^+/CD8^+$, or $CD4^-/CD8^-$ [1, 10]. All these phenotypes have been observed among NK-like T-cell lymphomas.

ATLL is a human malignancy associated with HTLV-1 [18]. It can be diagnosed clinicopathologically by characteristic features and molecularly by the presence of integrated proviral HTLV-1 in tumour cells with a helper/inducer ($CD4^+/8^-/56^-$) phenotype [19, 21]. Occasionally, ATLL cells express CD8 (cytotoxic/suppressor) or CD56 (NK-associated) antigens [4, 6, 9].

Clinical presentation is very important in the classification of T-cell malignancies. For T-cell lymphomas, cytological features alone are not sufficient to distinguish among disease entities. For example, ATLL often cannot be distinguished morphologically from HTLV-1-negative T-cell lymphomas. Most extranodal T-cell lymphomas express TIA-1 and granzyme B [7]. In the present control study, the typical $CD4^+/8^-/56^-$ ATLL expressed no cytotoxin-associated proteins of TIA-1, granzyme B, perforin or FasL. In addition, the rare type of $CD8^+/CD56^+$ ATLL tumours also rarely express cytotoxin-associated proteins, and therefore CD8 and CD56 surface markers seem to have no association with cytotoxic function in ATLL. ATLL is probably the single most common subtype of T-cell lymphoma having no association with CD8 and CD56 expression.

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