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Clinical, immunohistochemical and phenotypic features of aggressive nodal cytotoxic lymphomas, including α/β, γ/δ T-cell and natural killer cell types

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Abstract Cytotoxic cells include natural killer (NK) cells and cytotoxic αβ and γδ T lymphocytes (CTLs). These cells express cytotoxic molecules of T-cell restricted intracellular antigen(TIA-1), and activated cytotoxic molecules of perforin, granzyme B, and FasL. Recent studies suggest that most extranodal T-cell lymphomas are derived from CTLs, and that NK cell lymphomas are extranodal. However, only a few nodal NK and cytotoxic lymphomas have been described so far. We present here the clinicopathological features of seven cases of nodal cytotoxic T and NK cell lymphomas. The study excluded anaplastic large-cell lymphomas expressing cytotoxic molecules. The neoplastic cells of all cases contained activated cytotoxic molecules of TIA-1, granzyme B, Fas ligand, and/or perforin. Phenotypically and genotypically, four cases showed αβ T cell type [CD2+, CD3+, T-cell receptor (TCR) δ-1–, βF1+, and *TCR* gene rearrangement], two cases showed γδ T-cell type [CD2+, CD3+, T-cell receptor (TCR)δ–1+, βF1–, and TCR gene rearrangement], and one case showed NK cell type [CD2+, CD3-, CD56+, T-cell receptor (TCR)δ-1–, βF1–, and *TCR* gene germline]. Using Southern blot analysis, Epstein-Barr virus (EBV) sequences were detected in six cases, and monoclonal terminal repeat proliferation was confirmed. In addition, in situ hybridization (ISH) studies for EBV showed EBV infection in almost all neoplastic cells. Clinically, all patients presented with peripheral lymphadenopathy in high clinical stages and showed an aggressive course. Hepatosplenomegaly was detected in six cases. During the course of the disease, bone marrow and extranodal invasion were noted in five cases. The nodal type showed an aggressive clinical course in all cases but one, as did the extranodal type. The nodal type varied in phenotype, but was closely associated with EBV infection.

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Introduction

Lymphomas with T-cell phenotype represent a heterogeneous group of diseases differing in histopathological features, tumour site, and cell origin. They include peripheral T-cell lymphomas (PTCLs) derived from αβ-cells and also some recently recognised entities, such as γδ-hepatosplenic lymphomas and natural killer (NK) cell lymphomas [23]. Few studies have investigated the possible origin of PTCLs from lymphocytes with cytotoxic potential.

Cytotoxic cells include NK cells and cytotoxic αβ- and γδ-T-lymphocytes (CTLs). These cells play a major role in defence against neoplastic processes and viral infections [25]. Expression of T-cell-restricted intracellular antigen (TIA-1) is characteristic of cytotoxic cells irrespective of their activation status. Furthermore, expressions of perforin and granzymes are much increased in activated cytotoxic cells, and their levels correlate with the induction of cytolytic activity [1, 37]. Thus, antibodies to TIA-1 might be useful in the identification of specific subsets of lymphoid neoplasms derived from CTLs or NK cells [15]. NK cell lymphomas frequently express TIA-1, perforin, and granzyme B [5, 34]. Furthermore, all γδ-PTCLs show expression of TIA-1 protein in most tumour cells, with a different cytotoxic antigen profile in hepatosplenic γδ-PTCL (TIA-1+, perforin-, granzyme B-) and in nonhepatosplenic γδ-PTCLs (TIA-1+, perforin+, granzyme B+) [2, 14]. In addition, the presence of TIA-1 indicates that the following are cytotoxic neoplasms; most large granular lymphocytic leukaemias, 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 [15]. The expression of the cytotoxic protein seems to correlate with

the localisation site, and especially with extranodal sites [15]. In lymph nodes, anaplastic lymphoma expresses cytotoxic proteins, but other types of lymphomas rarely do [15]. Identification of cytotoxic lymphoid neoplasms (excluding anaplastic lymphomas) may be important, because many of these neoplasms are clinically aggressive [12, 22, 30].

γδ-T-cells have rarely been implicated in neoplastic lymphoproliferative disorders. Among peripheral T-cell lymphomas, hepatosplenic γδ–T-cell lymphomas have been identified as a distinct entity [14, 16]. Several cases of nonhepatosplenic γδ-T-cell lymphomas have recently been reported [2, 3]. Most have been cutaneous γδ–T-cell lymphomas [2, 3]. In addition, rare cases of nasal, gastrointestinal, and pulmonary γδ-T-cell lymphomas have also been reported [18, 26]. Epstein-Barr virus (EBV) sequences were detected in nonhepatosplenic γδ-T-cell lymphoma by in situ hybridisation (ISH), as they have been in nasal NK cell lymphomas [2, 3]. However, in hepatosplenic γδ-lymphoma, EBV infection has not been detected [14, 16].

This study presents the clinicopathological features of seven consecutive cases of nodal cytotoxic T and NK cell lymphomas (we excluded anaplastic large cell lymphoma expressing cytotoxic molecules). We investigated (1) whether nodal cytotoxic cell lymphoma includes αβ- and γδ-T and NK cell lymphomas, (2) the presence of specific clinical features, (3) the expression of cytotoxic molecules, and (4) the presence of EBV in tumour cells.

Materials and methods

Tissue samples

We examined seven cases of nodal cytotoxic cell lymphomas from the lymph node samples available in the Department of Pathology, Fukuoka University School of Medicine. Case selection was based on fulfilment of the following two inclusion criteria. (1) On their initial admission, patients showed peripheral lymphadenopathy with or without hepatosplenomegaly, together with available biopsied lymph nodes. (2) Cases expressed cytotoxic molecules of TIA-1, granzyme B, perforin, and/or FasL. Cases with anaplastic large-cell lymphoma were not included in the study. Each specimen was divided into three parts. Paraffin-embedded sections for light microscopy were fixed in buffered formalin then stained with haematoxylin and eosin (H&E), Giemsa, periodic acid–Schiff (PAS), and silver impregnation. Fresh specimens were kept in liquid nitrogen until examination. For chromosome analysis, metaphase chromosomes were obtained from the suspended lymph node material.

Immunohistochemical staining

Fresh tissue specimens for the immunohistochemical study were embedded in OCT compound and then kept in liquid nitrogen until examination. Serial cryostat sections were prepared for immunohistochemical staining using the APPLP method. The following antibodies were used: T11 (CD2) and 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), CD16 and CD56 for natural killer cells (Becton-Dickinson), CD19 and CD20 for B cells (Coulter, Hialeah, Fla.), and Leu 1 (CD5) and Leu 2 (CD7) (Becton-Dickinson). In addition, the following antibodies were also used on fresh materials and/or paraffin-embedded materials. Antibodies of perforin and granzyme B for cytoplasmic granules of cytotoxic T or NK cells (T Cell Diagnostic, Cambridge, Mass.; Pharmacell, Paris, France, respectively), TIA-1 for cytotoxic T or NK cells (Coulter), and TCR βF1 and δ1 (T Cell Diagnostic). Furthermore, the anti-Fas ligand (FasL) antibody of FasL (N-20) (Santa Cruz Biotechnology, Santa Cruz, Calif.) was used for immunostaining of fresh materials and/or paraffin-embedded materials, while anti-multidrug resistance P-glycoprotein (mdr-1) antibody (Oncogene Science, Uniondale, N.Y.) was used on paraffin-embedded materials. Monoclonal antibodies for EBV-encoded latent membrane protein 1 (LMP1; Dakopatts) and nuclear antigen 2 (EBNA2; Novocastra Laboratories, Newcastle upon Tyne, UK) were also used on paraffin-embedded materials.

In situ hybridisation studies

For detection of EBV RNA, the tissue was hybridised with an oligo-probe (EBER-1 region), (AGACACCGTCCTCACCACC-CGGGACTTGTA), and a sense probe (negative control) labelled with digoxigenin (DIG), using the method reported by Weiss et al. [38]. Briefly, after dewaxing, dehydration, and proteinase K digestion, sections were hybridised with DIG-labelled probes. After washing, detection was achieved using an avidin–alkaline phosphatase conjugate.

DNA analysis

For Southern blotting analysis, part of the frozen material was used for DNA isolation and gene analysis. The T-cell receptor gene (*TCR*) Cβ, Jγ, immunoglobulin heavy chain (*JH*) gene, *EBV* gene (*Bam*HI W region) (Enzo, Hudson, N.Y.) and EBV-TR (EBV terminal repetitive sequence), kindly provided by Prof. K. Hirai, Department of Virology and Immunology, Tokyo Medical and Dental University, Tokyo, Japan, were used as probes. DNA was digested with the restriction enzymes *Eco*RI, *Hind*III, or *Bam*HI. The proviral DNA of human T-lymphotropic virus type I (HTLV-I) (full length; gag, pol, env, pX, LTR) was also examined by Southern blot analysis. The isolated DNA was used for polymerase chain reaction (PCR). Specific primers were synthesised based on the published DNA sequence. For EBV, we synthesised primers and probes corresponding to the *Bam*HI W region of EBV (EBW-1, CCAGAGGTAAGTGGACTT; primer EBW-2, GACCGGTGC-CTTCTTAGG; probe EBW, TTCTGCTAAGCCCAC) [36]. For the EBV subtype, we synthesised primers based on a previously reported method [6]. Primers (EBNA2A-5', AACTTCAACCCA-CACCATCA; EBNA2A-3', TTCTGGACTATCTGGATCAT; EBNA2B-5', TACTCTTCCTCAACCCAGAA; EBNA2B-3', GGTGGTAGACTTAGTTGATG) were synthesised. For LMP-1 deletion, two 20-base oligonucleotide primers flanking the site of the characteristic 30-bp deletion were used, as previously described [9]: primer LMP-1–1 (CGGAGGAGGTGGAAAACAAA) and primer LMP-1–2 (GTGGGGGTCGTCATCATCTC). The probe (LMP-1–3, GGCGGGCCCTGGTCACCTCC) was synthesised and α-^{32}P-labelled probe was used for the Southern blot of PCR products.

Results

Clinical features

The clinical features are summarised in Table 1. There were three male and four female patients with nodal cytotoxic cell lymphomas, with a median age of 15 years (range 1–60 years). All these patients were Japanese, and

Table 1 Clinical features of patients with nodal cytotoxic T-cell (*T*)/natural killer cell (*NK*) lymphoma (*WBC* white blood cells, *LDH* lactate dehydrogenase, *LN* lymph nodes, *Hep-Spl* hepatosplenomegaly, *HPS* hematophagocytic syndrome, *D* dead, *A* alive, *BM* bone marrow, *GIT* gastrointestinal tract, *CT* chemotherapy, *D* dead, *A* alive)

Patient no.	Age	Sex	WBC	LDH	LN	Hep-Spl	HPS	Stage	Involvement during course	VGA-IgG	IgM	EBNA	Treatment	Duration	D/A
1	1	M	1900	1820	Neck	+/+	+	3	–	320	<10	20	Steroid	1 week	D
2	1	F	1100	1240	Neck, inguinal	+/+	+	4	BM, GIT	160	<10	<10	CT (CHOP)	2 months	D
3	4	F	1100	1720	Neck	+/+	+	3	–	20	<10	40	CT (CHOP)	14 months	D
4	15	F	2500	2160	Neck	–/–	+/–	3	Gingiva, leukemic	1280	<10	20	CT (CHOP)	2 years	A
5	20	F	2900	1939	Neck	–/–	+/–	4	BM	640	10	10	CT (CHOP)	2 weeks	A
6	46	M	2800	4290	Axillary, inguinal	+/+	+	4	BM, stomach	ND	ND	ND	CT (CHOP)	5 months	D
7	60	M	1020	2693	Inguinal	+/+	+	4	BM, chest wall	40	<10	40	CT (CHOP)	2 months	D

Table 2 Histological and immunohistochemical findings (*Pleo* pleomorphic type, *M* medium sized, *L* large, *Histio* histiocyte reaction, *ND* not done, *TCR* T-cell receptor gene, *G* germline, *R* rearrangement, *D* deletion)

Case no.	Histology	Necrosis	Histio	Immunohistochemical staining										DNA		Type
				CD2	CD3	ββ	δ	CD5	CD7	CD4	CD8	CD56	CD30	TCR-β	TCR-γ	
1	Pleo M&L	+	+	+	+	+	–	+	+	–	+	–	–	G	R	αβ
2	Pleo M&L	+	+	+	+	+	–	ND	ND	–	+	+	–	G	R	αβ
3	Pleo M&L	+	+	+	+	–	+	–	–	–	+	–	–	G	R	γδ
4	Pleo M:–	+/–	+	+	+	–	+	ND	ND	–	–	–	–	R	R	γδ
5	Pleo L	+	+	+	+	+	–	+	–	–	+	–	–	G	R	αβ
6	Pleo L	+	+	+	–	–	–	–	–	–	–	+	–	G	G	NK
7	Pleo L	+	+	+	+	+	–	+	+	–	+	–	–	D	R	αβ

were negative for human immunodeficiency virus and human T-lymphotrophic virus type I (HTLV-I) and were free of primary immunodeficiency syndrome.

All cases demonstrated local or systemic lymphadenopathy, fever, wasting, and hepatosplenomegaly at presentation. High levels of lactate dehydrogenase (LDH) and haemophagocytic features including pancytopenia were detected in almost all cases. Variable titres of EBV antibody of VCA-IgG were detected in these patients. The clinical stage was advanced (stages 3 or 4) in all cases, and four patients had bone marrow infiltration. In addition, extranodal involvement was detected, including gastrointestinal tract, chest wall, and leukaemic changes, during the disease course in four cases. All patients received chemotherapy or steroid therapy; however, five of the seven cases died within 14 months of diagnosis.

Histological findings

Histological findings are summarised in Table 2. All specimens showed a diffuse infiltrate of atypical lymphoid cells and were classified as pleomorphic medium and large-cell (pleo M&L; three cases), pleomorphic large-cell (pleo L; three cases), and pleomorphic medium-cell (pleo M; one case). Examination of the lymph node specimens revealed nonsuppurative necrosis with numerous apoptotic cells, histiocytes, and haemophagocytic histiocytes in all cases but one, but neutrophils and plasmacytes were not detected (Table 2, Figs. 1–3). Features of angiocentrism were rarely present. In the stump samples, azurophilic cytoplasmic granules appeared in almost all cases (Fig. 4).

Phenotypic and genotypic analysis

Complete immunophenotypic characterisation was performed in all cases using frozen and paraffin sections (Table 2). All cases expressed CD45RO and CD2. Based on our definition, phenotypic and genotypic analyses identified four cases of αβ T-cell type [CD2+, CD3+, T-cell receptor (TCR)δ–1-, βF1+, *TCR* gene rearrangement], two cases of γδ-T-cell type [CD2+, CD3+, T-cell receptor (TCR)δ–1+, βF1-, *TCR* gene rearrangement] and one case of NK cell type [CD2+, CD3–, CD56+, T-cell receptor (TCR)δ–1–, βF1–, *TCR* gene germ line] (Figs. 1–3). The αβ–T-cell type expressed CD5 and CD7, but the other types lacked CD5 and CD7. All cases lacked CD4 and CD30. CD8 was expressed in all four cases with αβ-T-cell type and in one of the two cases with γδ–T-cell type. CD8 was not expressed in the other case with γδ-T-cell type or in the cases with NK cell type (Table 2). All αβ-T-cell types and one γδ–T-cell type

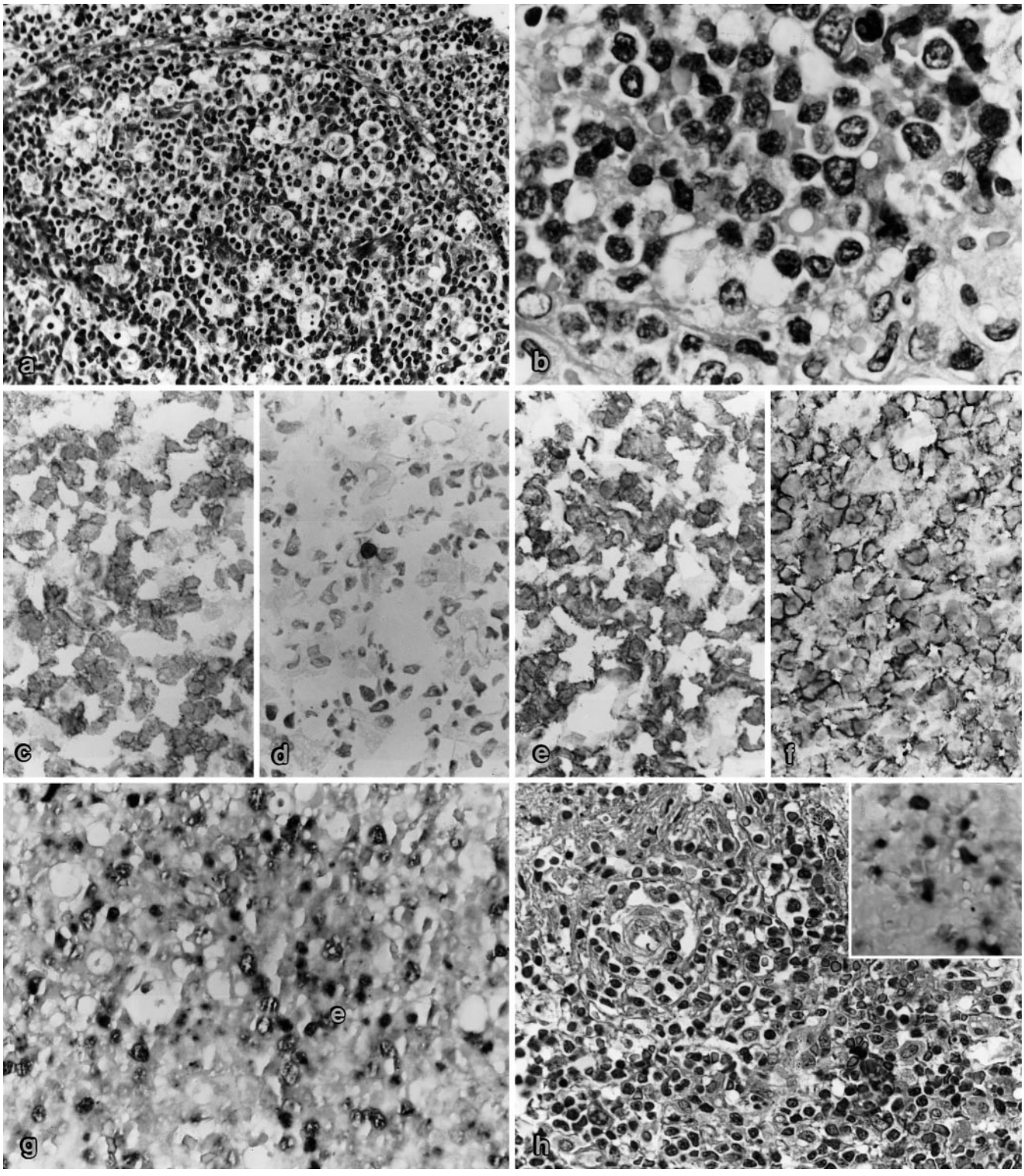

Fig. 1a–h Histological features, immunohistochemical staining, and in situ hybridisation using EBER-1 antisense oligonucleotide of γδ T-cell type (case 3). The lymph node shows necrosis with numerous apoptotic cells and histiocytes (**a**) and a diffuse infiltrate of atypical lymphoid cells, and is classified as pleomorphic with medium and large cells (**b**). Atypical cells are positive for T-cell receptor (TCR)-δ-1 (**c**), but negative for TCR-βF1 (**d**). Tumour cells are also positive for CD3 (**e**) and CD8 (**f**). In situ hybridisation using EBER-1 antisense oligonucleotide shows a positive reaction of the nuclei (**g**). Section of the spleen shows some scattered atypical cells (**h**), in which the presence of EBV is seen (*inset*)

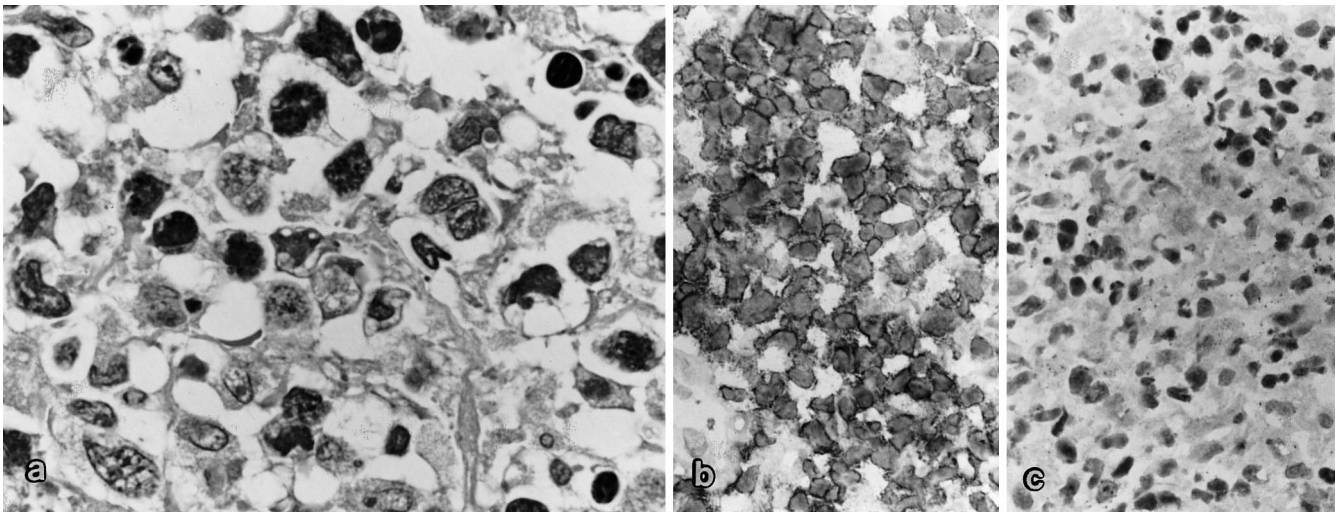

Fig. 2a–c Histological features and immunohistochemical staining of αβ T-cell type (case 7). Note the diffuse infiltrate of atypical lymphoid cells in the lymph node, which was classified as pleomorphic large-cell type (**a**). Lymphoma cells are positive for TCR βF1 (**b**), but negative for TCRδ–1 (**c**)

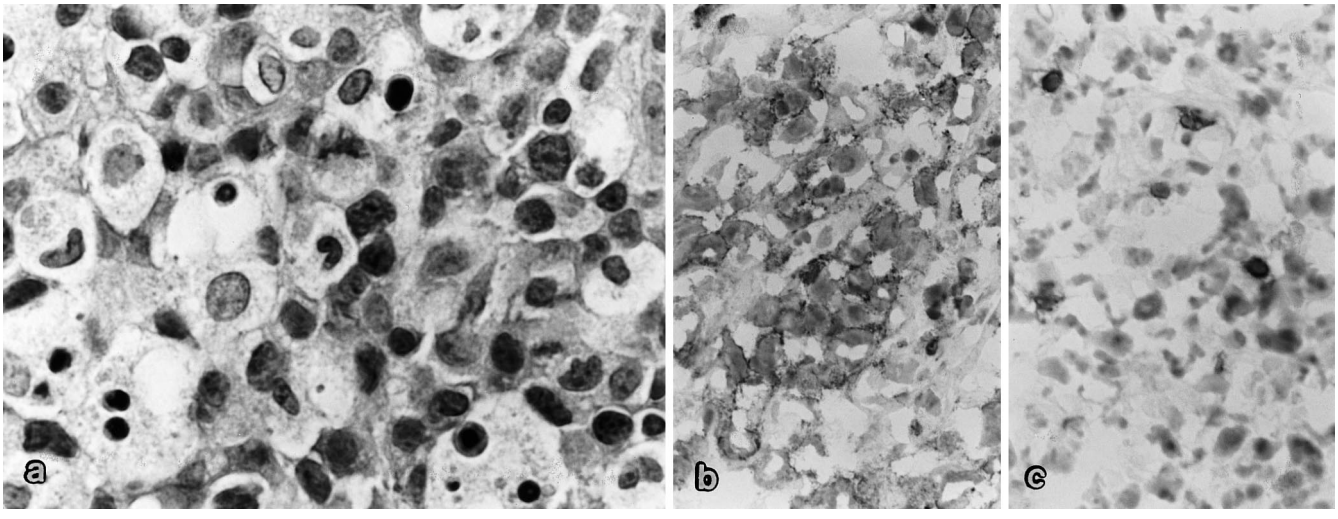

Fig. 3a–c Histological features and immunohistochemical staining of NK cell type (case 6). The lymph node shows diffuse infiltration of atypical lymphoid cells, which were classified as pleomorphic large-cell type(**a**). Lymphoma cells are positive for CD56 (**b**), but negative for CD3 (**c**)

showed rearranged bands of TCR Cβ and/or Jγ. However, the rearranged bands were not detected in the NK cell type (Table 2, Fig. 5). Expression of mdr-1 was detected in all cases examined.

Detection of cytotoxic molecules

All cases demonstrated a strong granular cytoplasmic staining for the cytotoxic granule-associated protein TIA-1. The proportion of neoplastic cells positive for granzyme B varied from case to case, and the reaction for FasL also varied in intensity and proportion of neoplastic cells. However, a positive reaction for perforin was detected in six of the seven cases (Table 3, Fig. 4).

EBV analysis

Immunological staining showed a lack of expression of EBV-associated protein, LMP and EBNA-2 in all cases (Table 3), although a few LMP-positive cells were detected in case 2 (Table 2, Fig. 1). Using Southern blot analysis, EBV sequences were detected in six cases of αβ-T-cell type and γδ-T-cell type, but not in NK cell type. Monoclonal terminal repeat (TR) proliferation was also confirmed in six cases (Table 3, Fig. 5). ISH showed the presence of EBV in almost all tumour cells of the six cases. However, reactive histiocytes, fibroblasts, and endothelial cells did not contain EBV (Fig. 1). PCR analysis showed EBV genomes in six cases, and further subtype analysis of EBV showed all six cases were of type

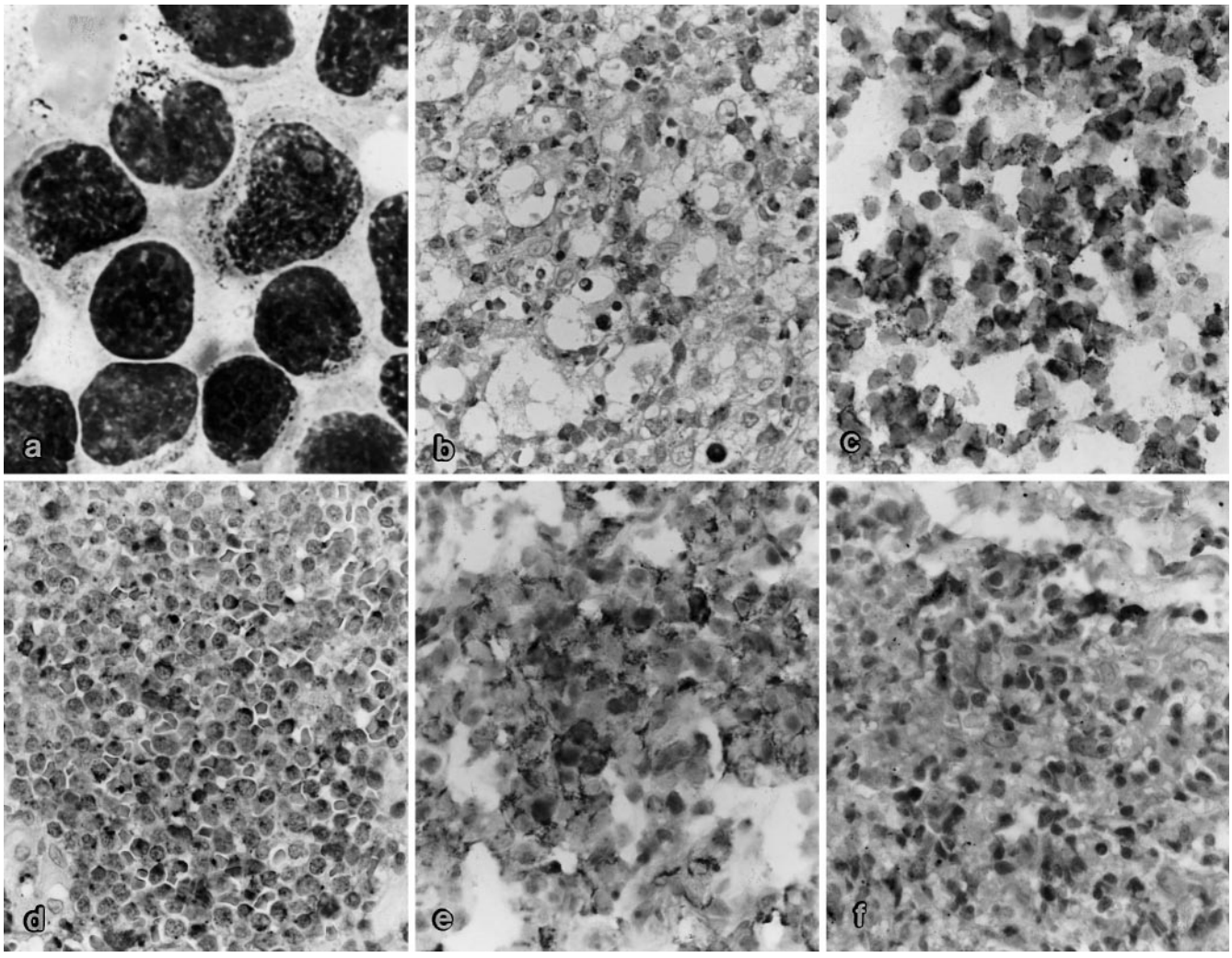

Fig. 4a–f Immunological staining of cytotoxic and other molecules (case 3). In the stump samples, note the presence of azurophilic cytoplasmic granules (**a**). The lymphoma cells are positive for cytotoxic granule-associated protein TIA-1 (**b**) and for granzyme B (**c**), perforin (**d**) and FasL (**e**), but the intensity of staining and proportion of stained neoplastic cells varied from one tumour to another. Note also the expression of mdr-1 (**f**)

Table 3 Cytotoxic molecules and Ebstein-Barr virus (*EBV*) analysis (*mdr* , *TIA-1* T-cell restricted intracellular antigen

Case no.	Cytotoxic molecules				mdr	EBV						HTLV-1
	TIA-1	Perforin	Granzyme B	FasL		LMP/EBNA2	EBER	W	TR	Subtype	LMP	
1	+	–(+)[a]	+	–(+)	+/–	–/–	+	+	M	A	D	–
2	+	–(+)	+	+/–	+	–/–	+	+	M	A	D	–
3	+	+/–(+)	+	+/–(+)	+	–/–	+	+	M	A	W	–
4	+	–(–)	–	(+)	ND	–/–	+	+	M	A	D	ND
5	+	+	+	+	+	–/–	+	+	M	A	D	–
6	+	+/–	+	+/–	+	ND	–	–	–	–	–	–
7	+	–(+)	+	+/–	+/–	–/–	+	+	M	A	D	–

[a]Use of round brackets indicates that frozen material was used

A. Analysis of LMP sequences demonstrated a deleted type in five cases and wild type in one case (Table 3). HTLV-I proviral DNA was not detected in any of the cases.

Chromosomal analysis

Chromosomal analysis was performed in two cases. Case 2 showed [46, XX, del(6)(q21q23), add(8)(q24)], and case 3, [45, X, -X, t(4;6)(q35;q14), add(22)(p10)].

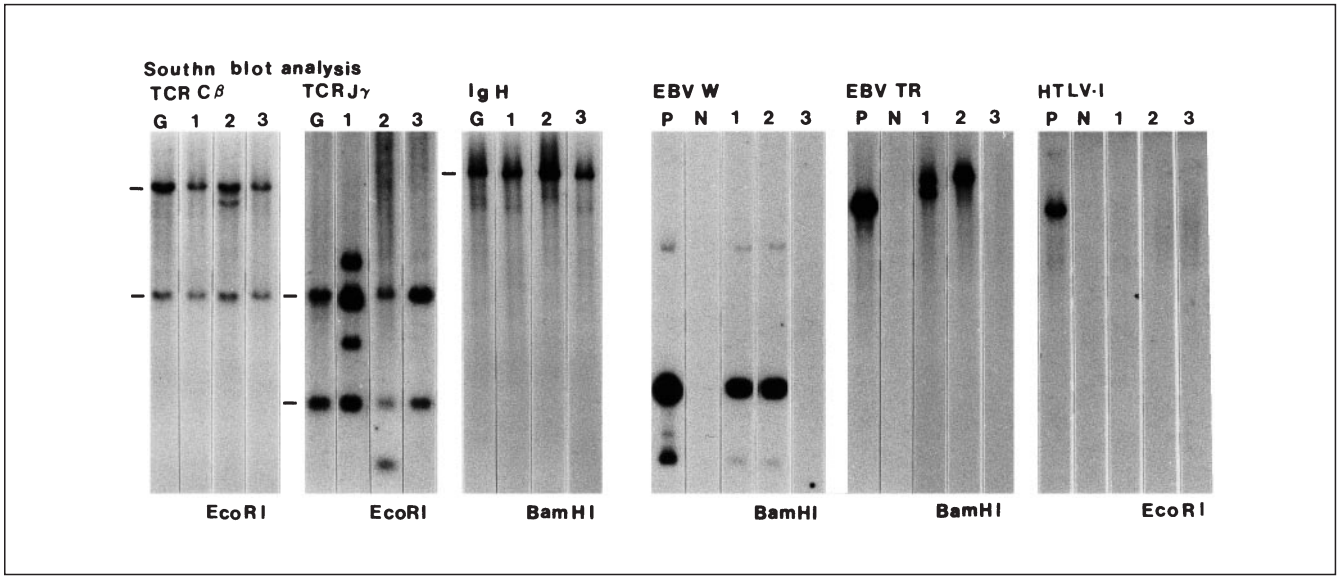

Fig. 5 Southern blot analysis of the receptor genes. *Lane 1* case 1 (αβ-T-cell type), *lane 2* case 4 (γδ-T-cell type), and *lane 3* case 6 (NK cell type). *Lanes 1* and *2* show rearranged bands of TCR-Cβ and/or Jγ, and a germline band of Ig JH. *Lane 3* shows no rearrangement of receptor genes. In EBV and HTLV-I analysis, *lane 1* case 2 (αβ T cell type), *lane 2* case 4 (γδ T cell type), and *lane 3* case 6 (NK cell type). *Lanes 1* and *2* show dense EBV-W bands and clonal EBV-TR bands. *Lane 3* shows no such bands. In HTLV-I analysis, note lack of monoclonal integration of HTLV-I DNA

Discussion

In this study we have presented the clinicopathological features of seven patients with nodal cytotoxic T and NK cell lymphomas. Histologically, they were classified as pleomorphic tumours. The lymph nodes in all cases but one showed nonsuppurative necrosis with numerous apoptotic cells. All tumours contained cells that were positive for cytotoxic molecules of TIA-1, granzyme B and FasL, although perforin was detected in six of the seven cases. Phenotypic and genotypic analyses showed four cases of αβ T-cell type [CD2+, CD3+, TCR δ–1-, βF1+, *TCR* gene rearrangement], two cases of γδ T-cell type [CD2+, CD3+, TCR δ–1+, βF1–, *TCR* gene rearrangement], and one case of NK cell type [CD2+, CD3–, CD56+, TCR δ–1–, βF1-, *TCR* gene germline].

TIA-1 is a 15-kb cytotoxic granule-associated protein and is expressed in NK cells and CTLs [1, 37]. Felgar et al. [15] recently evaluated TIA-1 expression by paraffin immunohistochemistry in about 200 malignant lymphomas. Based on TIA-1 expression, the cytotoxic lymphoma could be divided into major and minor categories, with the former consisting of entities in which most or all cases were derived from CTL or NK cells. The major categories included LGL leukaemia, NK-like T cell lymphoma (hepatosplenic γδ-T-cell lymphoma, intestinal and subcutaneous αβ T-cell lymphoma), NK cell lymphoma, nasal NK/T-cell lymphoma, subcutaneous NK/T-cell lymphoma, pulmonary angiocentric lymphoma and CD30-positive anaplastic large cell lymphoma (ALCL). The minor categories included nodal peripheral T-cell lymphoma and cutaneous T-cell lymphoma [15]. The present study included the minor categories of nodal cytotoxic lymphoma, but excluded ALCL.

Phenotypically and genotypically, these cytotoxic lymphomas could be divided into NK cell type and T-cell type, including αβ– and γδ-T-cell types [24]. With the exception of ALCL, cytotoxic lymphomas are usually extranodal and show an aggressive clinical course [12, 22, 30]. On the other hand, ALCL show a favourable clinical behaviour, somewhere between those of Hodgkin disease and low-grade T-cell lymphoma [33]. As an example of extranodal T-cell lymphomas, intestinal T-cell lymphoma (ITCL) is an uncommon entity. The phenotypic profile of ITCL (CD3+, CD4–, CD8+/–, and CD103+) suggests that this lymphoma is derived from intraepithelial T-cells [32]. The consistent expression of cytotoxic molecules introduces ITCL to a growing family of usually aggressive extranodal lymphomas of cytotoxic T-cells (αβ and γδ types) and NK cell origin. In contrast to putative NK cell lymphoma of the sinonasal region, intestinal NK cell lymphomas are very rare [11]. Nodal T-cell lymphomas do not usually express cytotoxic molecules [15]. Nodal cytotoxic lymphomas are considered rare [15]. However, in the present study, nodal cytotoxic lymphomas included NK cell type, αβ– and γδ-T-cell type, as did the extranodal cytotoxic lymphomas such as ITCL. In addition, nodal cytotoxic lymphomas showed an aggressive clinical course similar to that of extranodal cytotoxic lymphomas. Clinically, nodal cytotoxic lymphomas should be differentiated from nodal anaplastic large cell lymphomas, which are also considered to be derived from cytotoxic T-cells.

Two molecular mechanisms of T-cell- and NK cell-mediated cytotoxicity have been suggested. The first is a perforin-based mechanism, while the other is Fas based

[24]. Both mechanisms independently induce apoptotic cell death [24]. The perforin-based pathway seems to involve granule exocytosis, whereas the Fas pathway involves a cell-bound ligand–receptor interaction. In support of CTL and NK cell origin, the presence of cytoplasmic azurophilic granules, expression of NK-cell-associated antigens such as CD56, and expression of other cytolytic effector proteins, such as perforin, granzyme B, and FasL (CD95L), have been reported [8, 13, 28]. The present nodal cases showed CD56 in two cases, and azurophilic granules in almost all cases. In addition, the expressions of granzyme B and FasL were detected in all cases, and perforin was detected in six cases.

TIA-1 is expressed in both activated and nonactivated CTLs and NK cells [1], whereas the expression of perforin and granzyme B is limited to activated CTLs and NK cells [29]. Angiocentric NK cell lymphoma is a distinct clinicopathological entity that is closely associated with EBV [7, 19]. The most common clinical presentation is with a destructive nasal or midline facial tumour, while NK cell lymphomas are commonly reported in other extranodal sites, including skin, soft tissue, testis, upper respiratory tract, and gastrointestinal tract [17, 21, 22]. Kägi et al. [34] examined the expressions of perforin and TIA-1 in 24 NK cell lymphomas, 18 peripheral T-cell lymphomas (1 case CD8+ CD56+ and three ALCL) and reported a strong correlation between activated perforin and massive tumour cell apoptosis. In the present study, activated perforin and granzyme B were expressed on all but one of the nodal cytotoxic lymphomas. Perforin- and granzyme B-positive cases contained numerous apoptotic cells and showed an aggressive clinical course. In contrast, the single case negative for perforin and granzyme B showed very little or no apoptosis and was associated with a relatively indolent clinical course. The presence of numerous apoptotic cells in the lymph nodes might be caused by the activated cytotoxicity of cytotoxic lymphoma cells.

Kanavaros et al. [27] indicated that the putative role of EBV in the development of T-cell lymphomas was site dependent and was probably related to the site of the viral reservoir. They also demonstrated that all cases of sinonasal lymphomas originated from activated cytotoxic cells (either NK or γδ T- and/or αβ T-cell origin) and that all of them contained abundant EBV-positive tumour cells [27]. On the other hand, EBV sequences were not present in hepatosplenic γδ-T-cell lymphoma [14], but were detected in half of a series of cases of nonhepatosplenic γδ-T-cell lymphoma [2]. These findings reinforce the role of EBV in the pathogenesis of such lymphomas, since EBV, in addition to its oncogenic properties, can also induce activation and proliferation of cytotoxic cells [20]. While the lymph node was not a reservoir for EBV in the present study, nodal cytotoxic lymphomas were closely related to EBV infection.

Cheng et al. [10] have previously reported a poor prognosis in recurrent PTCLs and EBV-associated PTCLs. The survival-after-recurrence curve in their study correlated significantly with a negative expression of mdr-1 in recurrent lymphomas. In another study, mdr-1 expression also correlated with EBV infection [10]. In this regard, expression of mdr-1 is thought to be a significant prognostic factor in recurrent lymphomas, and a high expression of the glycoprotein is observed in recurrent EBV-associated PTCLs [10]. In the present study, mdr-1 expression was detected in all cases, suggesting that it might be related to the aggressive clinical course and EBV association. In addition to more work on EBV-dependent oncogenesis, further studies are necessary to determine the functional role of EBV in the clinical behaviour of these lymphomas.

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