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Angiocentric lymphoma with granulomatous panniculitis in the skin expressing natural killer cell and large granular T-cell phenotypes

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Abstract We investigated three patients suffering from angiocentric lymphoma with granulomatous panniculitis in the skin. All three patients presented with multiple purple subcutaneous nodules. Immunohistologically, the lymphoma cells in all three patients expressed CD2 (T-11), CD56 (neural cell adhesion molecule), and Mik-β1 (interleukin-2β receptor). CD3s (CD3, Leu-4)-positive lymphoma cells were found in two patients. A pore-forming protein (perforin) was detected in the lymphoma cells of all three patients. Perforin-possessing lymphoid cells were focally scattered in 2 of 15 patients with CD56-negative cutaneous lymphomas who served as controls. By the Southern blot method, one patient showed a rearranged T-cell receptor (TcR)β gene in the biopsied specimen, and the other two patients had germline configurations of TcRs and immunoglobulin heavy chain genes. One patient had serum anti-human T-cell lymphotropic virus (HTLV)-I antibody, but showed no integration of its proviral DNA. Ultrastructurally, membrane-bound azurophilic granules were detected in the atypical lymphoid cells of all three patients. Angiocentric lymphoma with panniculitis in three patients showed the characteristics of natural killer and large granular T-cells. The histological features might be due to the characteristics of the neoplastic cells with azurophilic granules and perforin.

Key words Angiocentric lymphoma
Natural killer cells · Large granular cells · Perforin

Introduction

The spectrum of angiocentric immunoproliferative lesions includes lymphocytic vasculitis, lymphomatoid granulomatosis, polymorphic reticulosis and angiocentric lymphoma [6]. Angiocentric lymphoma frequently affects extranodal sites, especially the skin and upper respiratory tract. Chan et a. [2] demonstrated that 19 patients with cutaneous angiocentric T-cell lymphoma showed perivascular and periadnexal infiltration by the lymphoma cells mainly in the mid and lower dermis, and in the subcutis when included in a biopsy. Wong et al. [21] reported that 5 patients with CD3-negative and CD56 [neural cell adhesion molecule (NCAM)]-positive lymphoid malignancies of the skin showed the characteristics of natural killer (NK) cells. However, they did not describe detailed histology in the subcutis and performed no genotypic examination of T-cell receptor (*TcR*) genes by Southern blot analysis. Gonzalez et al. [4] reported 8 patients with subcutaneous T-cell lymphoma with granulomatous panniculitis, among whom rearranged *TcR*β gene was detected in 1 of 3 patients. Burg et al. [1] reported that a case of subcutaneous CD56 (NCAM)-positive lymphoma with lobular panniculitis showed clonal rearrangement for *TcR*δ gene.

We now report that angiocentric lymphoma with granulomatous panniculitis in 3 patients possessed azurophilic granules in the cytoplasm, and show the pheno- and genotypic characteristics of NK cell and large granular α/β T-cell. We also demonstrated that the tumour cells had a pore-forming protein (perforin), which is one of the characteristic enzymes in NK cell and large granular lymphocyte [8, 15].

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Table 1 Panel of antibodies used

Enzymes and antibodies	CD no.	Source
OKT-6	CD 1	Ortho
T-11	CD2	Coulter
CD-3	CD3 ε	Dako
Leu-4	CD3$\varepsilon\gamma/\varepsilon\delta$	Becton Dickinson
OKT-4A+4	CD4	Ortho
Leu-9	CD7	Becton Dickinson
OKT-8	CD8	Ortho
MY-7	CD13	Coulter
Leu-11	CD16	Becton Dickinson
B-4	CD19	Coulter
B-1	CD20	Coulter
Interleukin-2α receptor	CD25	Dako
MY-9	CD33	Coulter
Human progenitor cell antigen (HPCA)-1	CD34	Becton Dickinson
Leucocyte common antigen (LCA)	CD45	Dako
Leu-19 [neural cell adhesion molecule (NCAM)]	CD56	Becton Dickinson
Leu-7	CD57	Becton Dickinson
Mik-β1 (interleukin-2β receptor)		Nichirei
Perforin (pore-forming protein)		Sumitomo Electronics

Patients and methods

We selected three patients with angiocentric lymphoma from the 90 registered patients with cutaneous lymphoma in the Department of Pathology, Fukuoka University. The histological findings of angiocentric lymphoma were based on the descriptions of Jaffe [6] and Lipford et al. [11].

Histology and immunohistochemistry

The tissue specimens examined were fixed with B-5 solution, embedded in paraffin, and stained with haematoxylin-eosin and Giemsa stains for the routine histological examination. The formalin-fixed and liquid-nitrogen-frozen tissue specimens were used for immunostaining with monoclonal and polyclonal antibodies (Table 1). The immunostaining procedure was performed by the alkaline-phosphatase-conjugated avidin-biotin complex method (Vectastain). To confirm the specificity of antibody perforin, we examined CD56 (NCAM)-negative cutaneous lymphomas including 3 of anaplastic large cell type, 3 of mycosis fungoides, 5 of human T-cell lymphotropic virus (HTLV)-I positive and 4 of HTLV-I negative pleomorphic type, 3 of centroblastic type and 5 of nonspecific dermatitis.

Cell genotype

DNA analysis was performed using standard methods described in previous reports [7, 14]. DNA was extracted from the frozen subcutaneous tissue. Ten micrograms of DNA was digested with each restriction endonuclease, subjected to electrophoresis in 0.8% agarose gel, transferred on a nitrocellulose filter by the method of Southern, and hybridized to ^{32}P-labelled DNA probes. Rearrangement of the *TcR*δ was examined with a 1.0-kb germline *Pst-Eco*RI fragment containing the first *J* region (*J*δ*1*) after digestion with *Eco*RI, *Bam*HI and *Hin*III endonucleases. A probe of the *TcR*β gene specific for the constant regions (*C*β) was used. Analysis of immunoglobulin gene rearrangement was performed using a *JH* probe. The integration of HTLV-I proviral DNA was examined with its full-length DNA probe (*LTR*, *gag*, *pol*, *env* and *pX*) after the DNA digestion with *Eco*RI.

Electron microscopy

The cut tissue specimens were fixed in 3% phosphate-buffered glutaraldehyde, pH 7.4, and postfixed in 1% OsO4 solution. After the specimens had been embedded in epoxy resin, ultrathin sections stained with uranyl acetate and lead citrate were examined with a JEM 100CX electron microscope (Nihon Densi, Japan).

Results

Clinical findings

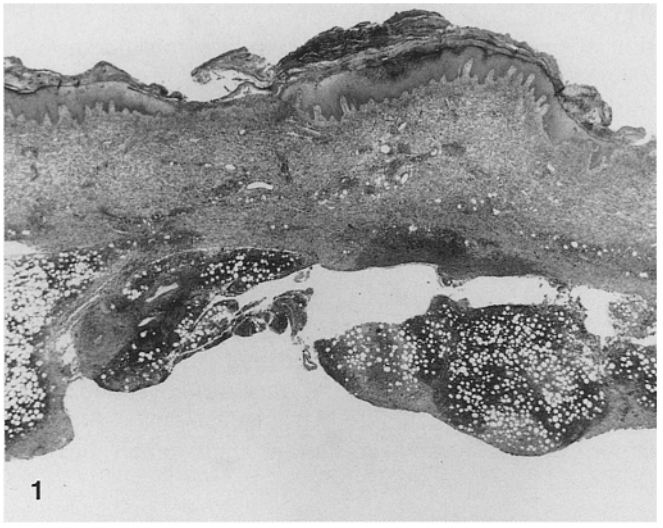
All three patients were female; their ages were 53, 57 and 74 years, respectively. Two patients (cases 1, 3) presented systemic purple nontender hard subcutaneous nodules. The remaining patient (case 2) showed multiple subcutaneous nodules in the left hand and forearm. No lymphadenopathy was found in each patient. Two patients (cases 1, 3) had mild hepatosplenomegaly and sinonasal necrotic lesion. Neither haematological abnormality nor bone marrow invasion was seen. Two patients (cases 1, 3) showed a slight elevation of lactate dehydrogenase. Case 3 had serum anti-HTLV-I antibody. Each patient received multiple cytotoxic drugs. Despite immediate medication, two patients (cases 1, 3) suffered from infection by methicillin-resistant *Staphylococcus aureus* either in the lung or intestine, disseminated intravascular coagulopathy (DIC) and pancytopenia. The patients (cases 1, 3) died of sepsis 4 and 5 months after the initial administration, respectively. Case 2 is presently alive without relapse of the lymphoma for 28 months.

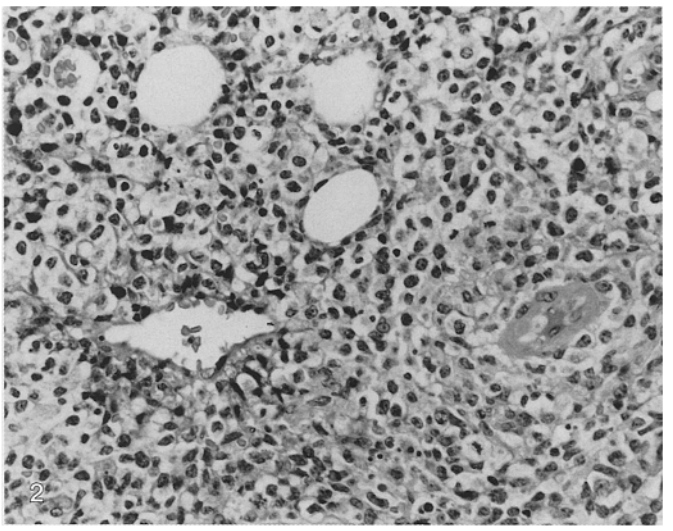
Histology

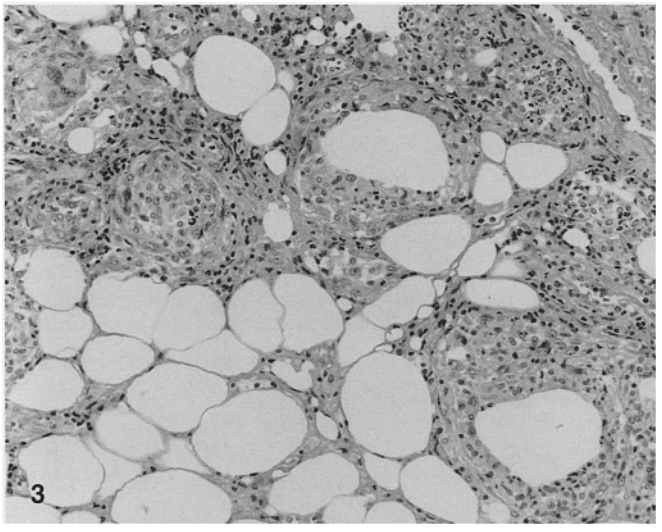
All three patients showed a patchy and diffuse infiltration of atypical lymphoid cells in the deep dermis and subcutaneous tissue and partly in the upper dermis (Fig. 1). The atypical lymphoid cells were composed of medium-sized and large nuclei with slightly indented contours and frequent mitotic figures. Each patient showed angiocentric and angiodestructive features by the lymphoma cells (Fig. 2), prominent histiocytic infiltration and some epithelioid cell granulomas (Fig. 3). Erythrophagocytic macrophages were found in case 3. Extensive fibrinoid and coagulative necrosis was found in the subcutis in all three patients. Cases 1 and 3 showed diffuse infiltration of atypical lymphoid cells with angiocentric and angiodestructive features in the biopsy specimens of the nose and ethmoid sinus.

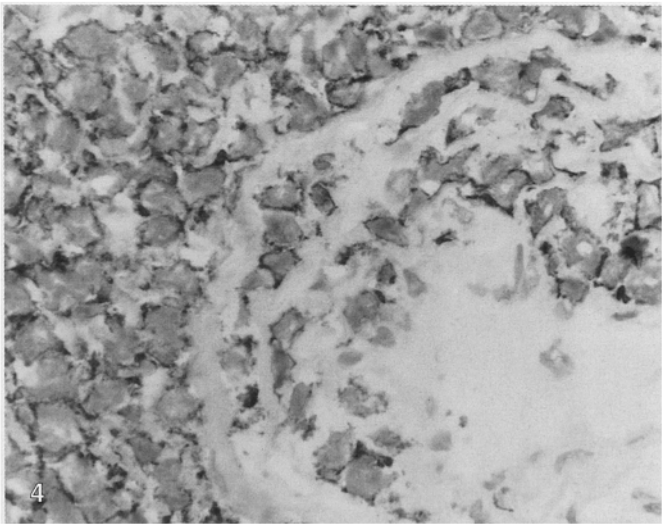
Immunohistochemistry

The lymphoma cells in three patients showed positive reactions to CD45 (leukocyte common antigen), CD2 (T-11), CD56 (NCAM, Fig. 4) and Mik-β1 (interleukin-2β receptor), but were negative for CD57 (Leu-7), CD1 (OKT-6), CD25 (interleukin-2α receptor), CD19 (B-4),









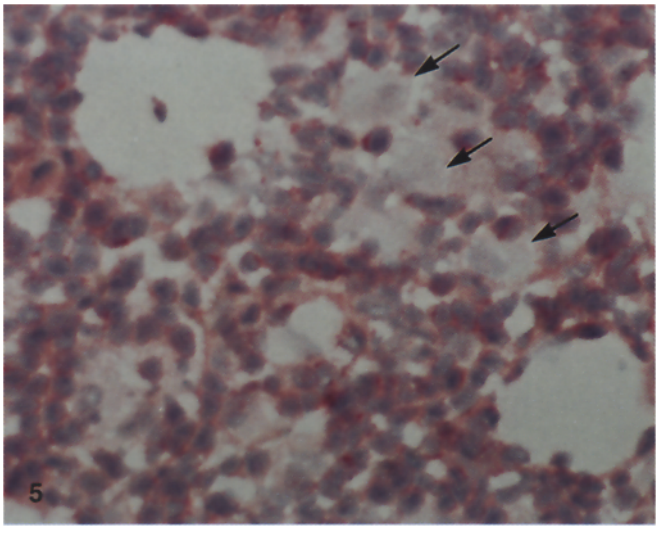


Fig. 1 Case 2. Perivascular and adnexal infiltration of mononuclear cells in the deep dermis. Septal and lobular infiltration of mononuclear cells in the subcutaneous adipose tissue. H & E stain, ×20

Fig. 2 Case 3. Diffuse invasion by medium-sized lymphoma cells in the subcutis. Lymphoma cells have slightly irregular nuclei and stippled chromatin. Angiocentric features in lymphoma cells are present. H & E stain, ×400

Fig. 3 Case 1. Some epithelioid cell granulomas are present in the involved subcutaneous tissue. H & E stain, ×250

Fig. 4 Case 2. CD56 (NCAM)-possessing cells infiltrate in and around vascular wall. ABC technique, ×500

Fig. 5 Case 3. Many lymphoid cells possess a positive reaction to perforin (pore-forming protein) in the cytoplasm, while some histiocytes (*arrows*) are negative for perforin. ABC method, ×400

CD20 (B-1), CD13 (MY-7), CD33 (MY-9), and CD34 (HPCA-1) (Table 2). The neoplastic cells showed expression of CD16 (Leu-11) in case 2. CD7 (Leu-9)-positive lymphoma cells were detected in two patients. In cases 1 and 3, CD3s [CD3ε (CD-3), CD3$\varepsilon\gamma/\varepsilon\delta$ (Leu-4)] were positive, but CD4 (OKT-4A+4) and CD8 (OKT-8) were negative for the lymphoma cells. The lymphoma cells in case 2 were negative for CD3s, but were CD4 and CD8 double-positive. Perforin-possessing neoplastic cells were diffusely found in case 3 (Fig. 5), and were partly

Table 2 Immunohistological and genetic findings of angiocentric lymphoma with granulomatous panniculitis (*IL* interleukin, *TcR* T-cell receptor, *JH* immunoglobulin heavy chain, *R* rearranged, *D* deletion, *G* germline

Patient no.	1	2	3
CD45 (LCA	+	+	+
CD2 (T-11)	+	+	+
CD56 (NCAM)	+	+	+
Mik-β 1 (IL-2β receptor)	+	+	+
CD16 (Leu-11)	–	+	–
CD57 (Leu-7)	–	–	–
CD7 (Leu-9)	+	+	–
CD1 (OKT-6)	–	–	–
CD3ε (CD-3)	+	–	+
CD3$\varepsilon\gamma/\varepsilon\delta$ (Leu-4)	+	–	+
CD4 (OKT-4A+4)	–	+	–
CD8 (OKT-8)	–	+	–
CD25 (IL-2α receptor)	–	–	–
CD19 (B-4)	–	–	–
CD20 (B-1)	–	–	–
CD13 (MY-7)	–	–	–
CD33 (MY-9)	–	–	–
CD34 (HPCA-1)	–	–	–
TcRs β/δ/JH genes	G/G/G	D/D/G	G/R/G

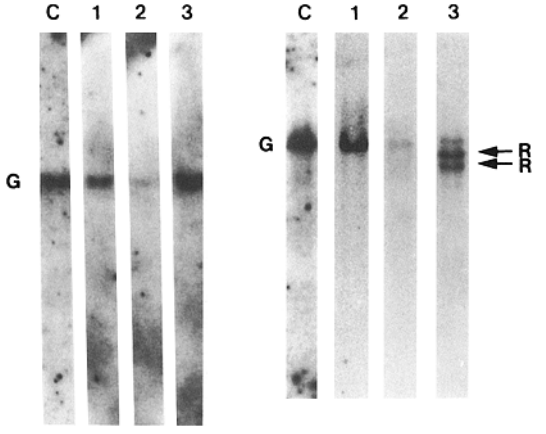


Fig. 6 A gene analysis of *TcRs* δ and β by the Southern blot method. Rearranged *C*β gene is detected in case 3. The other two patients show germline (*G*) configurations of *J*δ1 and *C*β or depletion of germline. DNA was digested with *Hin*dIII and *Bam*HI. *C* Placental DNA. The *lane numbers* correspond to the patient number in the text

clustered in the remaining two patients. Among CD56 (NCAM)-negative lymphomas and nonspecific dermatitis, perforin-possessing lymphoid cells were focally scattered near the necrosis in two patients with mycosis fungoides and HTLV-I negative pleomorphic T-cell lymphoma. Histiocytes, neutrophils and endothelium were negative for perforin in examined skin tissues.

DNA analysis

One patient (case 3) showed the germline of *J*δ*1* gene and the rearranged *C*β gene in the biopsied specimen after the digestion of *Bam*HI, while the other two patients had germline configurations of *TcR*δ and *TcR*β, or deletion of germline (Fig. 6). The germline configuration of *JH* gene was noted in all three patients. One patient (case 3) with serum anti-HTLV-1 antibody revealed no integration of HTLV-1 proviral DNA in the involved tissue and peripheral blood mononuclear cells.

Electron-microscopy findings

The infiltrating atypical lymphoid cells in each patient possessed scattered electron-dense membrane-bound azurophilic granules (160–280 nm), some endoplasmic reticulum and mitochondria in an abundant cytoplasm, slightly irregular nuclear contour, and small nucleoli (Fig. 7).

Discussion

We report three patients with angiocentric lymphoma with granulomatous panniculitis in the skin. The neoplastic cells in two patients possessed the following characteristics of NK cells; expression of CD2 (T11), CD56 (NCAM), CD7 (Leu-9) and Mik-β1 (interleukin-2β receptor), the presence of perforin and azurophilic granules on electron microscopy, and germline configurations of *TcRs* and *JH* genes in the involved tissue [2, 9, 15]. The remaining patient had almost the same histology and immunohistological findings, but revealed T-cell characteristics with a rearranged *TcR*β gene. Genetically, subcutaneous angiocentric α/β and γ/δ T-cell lymphomas have been reported in one patient each [1, 4]. Medeiros and coworkers [13] demonstrated that one patient revealed a rearranged *TcR*γ gene among three patients with angiocentric lymphoma of the nasopharynx and lung. Suzumiya et al. [18] reported that nasopharyngeal lymphoma with angiocentric features showed the pheno- and genotypic characteristics of NK cell and α/β T-cell in eight and two patients, respectively. These findings and our results support the notion that angiocentric lymphoma with granulomatous panniculitis in the skin has the characteristic pheno- and genotypes of NK cell, α/β and γ/δ T-cells, as described in other organs.

Among our 3 patients, case 1 showed expression of CD3ε (CD3) and CD3$\varepsilon\gamma/\varepsilon\delta$ (Leu-4) by the lymphoma cells, but had no rearranged *TcRs* genes. Lanier et al. [10] and Phillips et al. [16] demonstrated that fetal CD56-positive and CD16-negative NK cells without rearranged *TcRs* genes had positive reactions to Cd3γ, δ, ε complexes. Suzumiya et al. [18] proposed that sinonasal CD56-positive lymphoma with expression of CD3ε (CD3) or CD3$\varepsilon\gamma/\varepsilon\delta$ (Leu-4) and without rearranged *TcRs* genes should be considered as neoplasms of a fetal NK-cell lineage. We demonstrate here that two patients with angiocentric lymphoma with granulomatous panniculitis in the skin expressed the fetal and adult NK-cell characteristics.

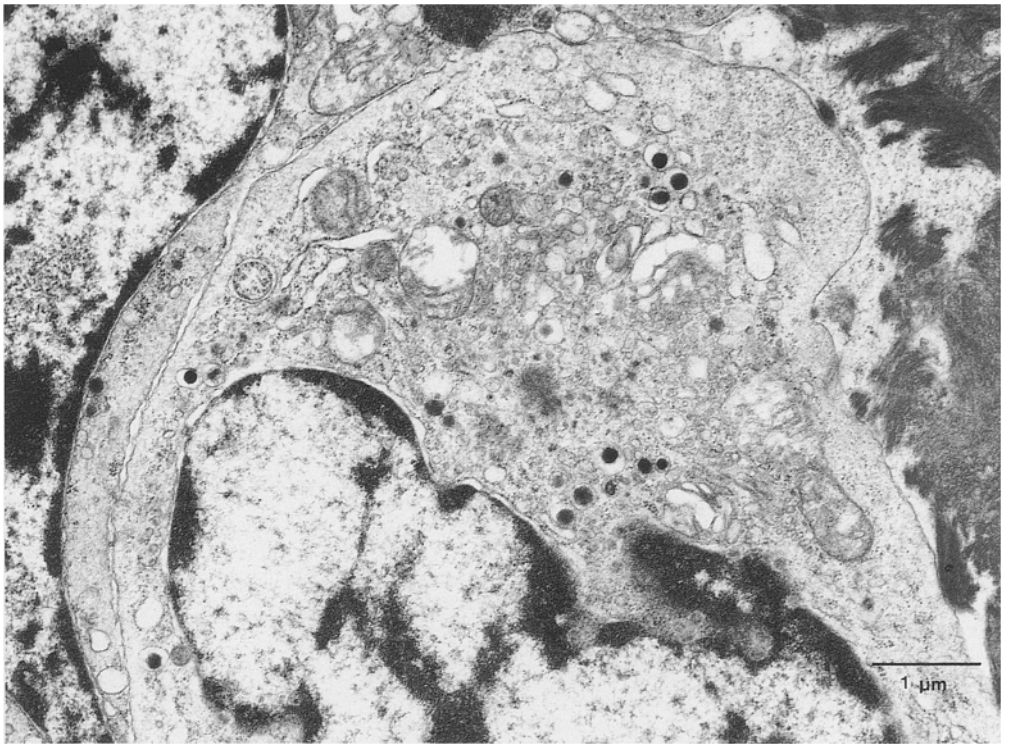


Fig. 7 Case 2. Ultrastructural findings of atypical lymphoid cell. Some electron-dense and membrane-bound granules are scattered. Irregular nuclear contour and marginal chromatin in the nuclei

Umehara and Bloom [19] demonstrate that NK and lymphokine activated killer activities and proliferation of large granular lymphocytes are mediated by the interaction between interleukin-2 and interleukin-2β receptor. NK cells and large granular T-cells have major histocompatibility complex unrestricted cytotoxicity, and show target cell-lysis by the enzymes of perforin and granzyme A etc. in the azurophilic granules [8, 23]. Perforin shows functional homology with the ninth component of complement [22], and is a good marker of activated cytotoxic lymphocytes [5]. In the patients examined, the lymphoma cells showed expression of Mik-β1 (interleukin-2β receptor) and possessed perforin in the cytoplasm. In the CD56-negative cutaneous lymphomas examined, only two patients with T-cell lymphoma showed focally scattered perforin-possessing lymphoid cells. Cutaneous T-cell lymphomas such as mycosis fungoides, Sézary syndrome and adult T-cell leukaemia/lymphoma (ATL) show less angiocentric and angiodestructive features by the lymphoma cells [20]. Angiocentric lymphoma with azurophilic granules and perforin might possess some cytolytic function against the blood vessels and parenchymal cells, and thus reveal panniculitis and massive necrosis. Angiocentric lymphoma of the skin frequently presents an aggressive clinical course with severe infection, liver dysfunction, DIC and pancytopenia [2]. These clinicopathological findings in angiocentric lymphoma are also different from those of other types of malignant lymphoma.

McNutt et al. [12] reported two patients with angiocentric lymphoma and small dense granules in the cytoplasm and serum anti-HTLV-I antibody. One patient showed no rearrangement of *TcRs* genes in the peripheral blood or involved tissue. There are no patients with angiocentric and large granular cell lymphomas which show an integration of HTLV-I proviral DNA by Southern blot analysis [12, 17]. Ultrastructurally, lymphoma cells in ATL possess focally clustered dense bodies, but have no membrane-bound azurophilic granules in the cytoplasm [3]. In this study, one patient (case 3) with a large granular T-cell lymphoma had serum anti-HTLV-I antibody, but showed no integration of HTLV-I proviral DNA in the involved tissue. The cytologic, pheno-, genotypic and clinical findings in case 3 were not consistent with those of ATL.

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