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Immunohistological study of histiocytic necrotizing lymphadenitis*

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Summary. Immunohistological study of 18 cases of histiocytic necrotizing lymphadenitis (HNL) demonstrated numerous helper/inducer cells (OKT-4) and suppressor/cytotoxic cells (OKT-8) with activation (Tac) and proliferation (OKT-9) markers, and histiocytes (lysozyme, α-1 antichymotrypsin, OK-M1) in the affected areas. However, B cells (B-1), NK cells (Leu-7 and Leu-11), complement proteins and receptor (C4 and C3d receptor), and neutrophils (chloroacetate esterase) were scanty or absent in these foci. Activity of NK cells was also decreased in the peripheral blood of 2 cases examined. The results suggest that HNL might be induced by the abnormal T cell-histiocyte response against some causative agents which induce a similar reaction of delayed hypersensitivity type.

Key words: Lymphadenitis – T cell proliferation

Introduction

Since the first description of histiocytic necrotizing lymphadenitis (HNL) (Kikuchi et al. 1972), many cases have been reported in Japan (Fujimoto et al. 1972; Kikuchi et al. 1977, 1983; Kikuchi 1978; Fujimori et al. 1981). The lesion was also found in Europe (Pileri et al. 1982; Feller et al. 1983; Ali and Horton 1985) and the U.S.A. (Turner et al. 1983), recently. The histogenesis and pathogenesis of the lesion are not yet known. Immunohistological study revealed that the proliferating cells in the affected foci consisted both of T cells and histiocytes (Feller et al. 1983; Turner et al. 1983; Kikuchi et al. 1983), but the subsets of T cells were not yet exactly characterise,

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because only a few cases have been examined immunohistologically. In this paper we have described the immunohistological results of 18 cases with examination of NK activity in the peripheral blood in 2 cases and discussed on the nature of the lesion.

Materials and methods

The study group consisted of 18 cases of HNL collected at the department of pathology, Fukuoka University School of Medicine. Questionnaires were sent to the clinicians to obtain the main clinical data: age, sex, occupation, presenting symptoms and signs, sites of adenopathy, laboratory findings, serological titers performed, treatment, and follow-up. All nodes biopsied were from the neck. The tissue was divided in 3 parts for light microscopy, immunohistochemistry and electron microscopy.

Paraffin sections of the cases for light microscopy were fixed in B5 solution (10% neutral formalin with 6% w/v mercuric chloride). In addition to routine stains of H&E, Giemsa, PAS and silver impregnation the sections were stained for intracytoplasmic immunoglobulins, α-1 anti-chymotrypsin, lysozyme, chloracetate esterase, and S-100 protein.

The fresh tissues for immunohistochemical study were embedded in OCT compound and kept in fluid nitrogen until examination. Cryostat sections (5 micron) were fixed in cold acetone, subsequently washed in phosphate buffered saline (PBS pH 7.2), incubated with horse serum, then incubated with a monoclonal mouse antihuman antibody, biothinylated horse anti-mouse $F(ab')_2$ fragments and avidine horseradish peroxidase, and mouse peroxidase anti-peroxidase complex. After each incubation, sections were washed in PBS. The reaction product was produced by a 15 minutes incubation with diaminobenzidine (DAB) in Tris buffer (pH 7.6) and 5.0% H_2O_2 and counterstained with Mayer's haematoxylin. Control sections involved substitution of the first-stage reagent with PBS.

The anti-human monoclonal antibodies and enzymes that we employed are tabulated in Table 1.

For electron microscopy a portion of each lymph node was divided into 1 mm^3 fragments, fixed in 2.5% glutaraldehyde, and postfixed in 1% OsO_4. The tissue was then embedded in EPON, sectioned, stained with uranyl acetate and lead citrate, and examined on a JEM 100CX electron microscope.

The peripheral blood of 2 cases of HNL and 20 healthy control subjects was examined to detect the levels of NK activity. Mononuclear cells were separated from heparinized peripheral blood by the standard technique with Ficoll-Hypaque. NK cell activity was studied by the ^{51}Cr release cytotoxicity assay with modification (Yoda et al. 1982). Briefly, 2.5×10^6 effector cells were incubated for an hour in 1 ml of RPMI 1620 with 10% fetal calf serum. The cells were washed three times with the culture medium, and 2.0×10^5 cells in 100 µl of the culture medium were assayed in tripicate using 1×10^4 ^{51}Cr-labelled K562 target cells. The percentage of cytotoxicity after 4 hour incubation at 37° C was calculated as

$$(E\text{-}SR/(max\text{-}SR)) \times 100,$$

where E is experimental ^{51}Cr release; SR, spontaneous release; max, maximum release by distilled water.

Results

The main clinical findings are summarized in Table 2. The results are consistent with those of the previous reports (Kikuchi et al. 1972, 1983) except for a male predominance in this series. The patients were distributed between 12 and 47 years old with a mean age of 29.4. All patients had neck involvement, with 2 cases of adenopathy in the axilla. Biopsies were performed from 5 to 60 days after the onset (two were at a recurrence). Leukocytopaenia under 4,000/µl was present in 9. Serum levels of complement proteins

Table 1. Antigenic and enzymatic profile of mononuclear cells

Reagent	Cell population recognized	Source
T-11	E rosette forming T cells	Coulter Clone
OKT-4A	Helper/inducer T cells	Ortho Diagnostic System
OKT-8	Suppressor/cytotoxic T cells	Ortho Diagnostic System
Leu-8	Subset of peripheral T cells	Becton Dickinson
Tac	Receptor for interleukin II	Dr. Uchiyama, Kyoto Univ.
Leu-7	NK cells	Becton Dickinson
Leu-11	Activated NK cells	Becton Dickinson
B-1	All B cells	Coulter Clone
CIg (κ, λ, M, G, A, D, E)	Cytoplasmic immunoglobulins	Dako Patts
Lysozyme	Macrophages and granulocytes	Dako Patts
α-1 anti-chymotrypsin	Macrophages and granulocytes	Dako Patts
OK-M1	Monocytes and myeloid cells	Ortho Diagnostic System
S-100 protein	Interdigitating reticulum cells	Dako Patts
OKT-6	Interdigitating reticulum cells and cortical thymocytes	Ortho Diagnostic System
OKT-9	Proliferating cells and transferrin	Ortho Diagnostic System
C_3bR	Receptor for complement C_3b component	Dako Patts
C_4c	Complement C_4c component	Dako Patts
Chloroacetate esterase (Leder's stain)	Granulocytes	Sigma, St. Louis, MO, USA

were within normal in 8 of 9 for C3d, and increased in 8 of 9 for C4 and 2 of 3 for CH_{50}. An increased value of LDH was seen in 5 of 14. Serum titers for toxoplasmic infection were increased in 1 of 13. Positive reactions against EB virus were found in 1 of 9 for IgG antigen and 1 of 5 for EBNA, but no positive reaction for IgM. Serum titers for varicella were positive in 1 of 2 and for cytomegalovirus in 1 of 2, but negative in 2 for herpes simplex and in 1 for yersinia enterocolitica. All patients recovered from their symptoms within 1.5 months.

Light microscopic study

Histological features were similar to those of the previous reports (Kikuchi et al. 1972, 1983; Pileri et al. 1982; Turner et al. 1983). A proliferation of transformed lymphocytes which had oval or slightly indented vesicular nuclei, small but distinct nucleoli, and obvious pale or amphophilic cytoplasm was focally or partially found mainly in the paracortex and subcapsular cortex (Figs. 1, 2). Histiocytes and nuclear debris were intermingled with these lymphocytes. Neutrophilic and plasma cell infiltration were absent

Table 2. Clinical findings of histiocytic necrotizing lymphadenitis

No. of cases	Age	Sex	Site	Duration (days)	Fever (over 38° C)	Skin rash	WBC (/μl)	AL	CRP	C3[a] (mg/dl)	C4[a] (mg/dl)	CH_{50}[a] (U)	LDH[a] (IU/l)
1	29	M	N	20	−	−	5,400	−	−	ND	ND	ND	ND
2[b]	17	M	N	15	+	−	2,300	−	+	ND	ND	ND	991
3	25	F	N	20	−	−	10,800	−	ND	111	58	ND	125
4	30	M	N	5	−	−	5,400	−	−	ND	ND	ND	127
5	34	M	N	20	+	−	4,100	−	−	ND	ND	ND	137
6	26	M	N	60	+	+	3,000	+	+	162	120	70	ND
7	22	M	N, Ax	40	+	−	4,900	+	−	104	54	ND	604
8	30	F	N	60	−	−	5,400	−	−	ND	ND	ND	ND
9[b]	47	F	N	30	+	+	3,000	+	−	ND	ND	ND	ND
10	33	M	N	40	+	−	5,500	−	+	112	72	ND	430
11	20	M	N, Ax	15	+	+	6,800	+	+	67	169	31	364
12	30	M	N	10	+	−	2,400	−	ND	ND	ND	ND	ND
13	37	F	N	60	−	−	2,800	−	−	101	45	48	546
14	26	F	N	50	+	−	2,000	−	−	ND	ND	ND	120
15	43	F	N	20	−	−	3,100	+	−	ND	ND	ND	148
16	29	F	N	20	+	−	2,800	+	−	67	37	ND	ND
17	40	F	N	50	+	−	4,500	+	−	119	28	ND	359
18	12	M	N	60	+	−	3,500	−	±	108	84	ND	1,011

AL = Atypical Lymphocytes in peripheral blood, N = Neck, Ax = Axilla, ND = Not Done

[a] Normal range (C3 male 120-40, female 140-50; C4 male 59-13, female 20-7; CH_{50} 30-45; LDH under 400)

[b] Recurrent case

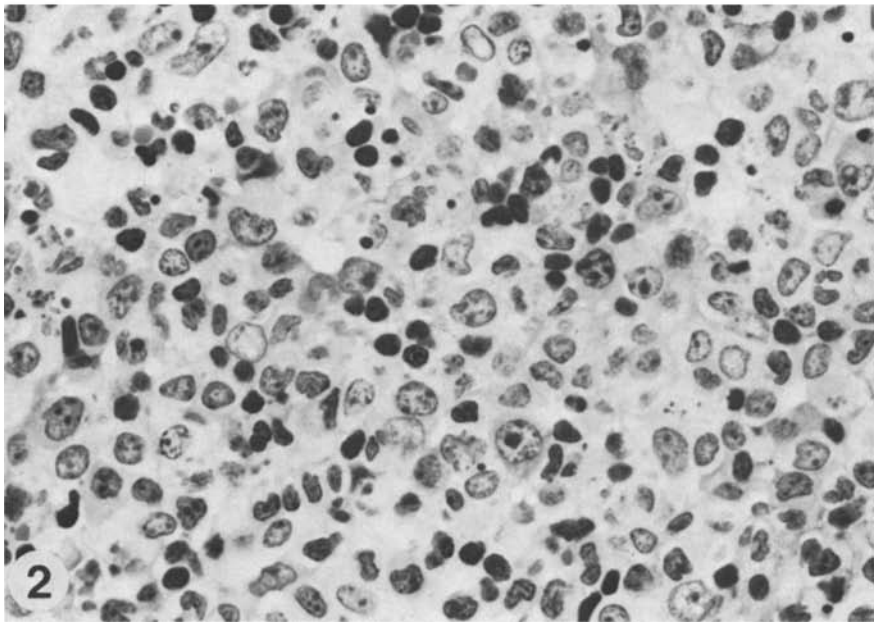

Fig. 1. Well-demarcated affected area in the left. No lymph follicles are found at the non-affected area in the right. H&E stain, × 60. Case 3

Fig. 2. High manification of the affected area. Many transformed lymphocytes, macrophages and histiocytes are present, accompanied by individual cell necrosis. A few immunoblasts are seen in places. H&E stain, × 750. Case 18

Table 3. Quantitative analysis of proliferating cells in affected areas in histiocytic necrotizing lymphadenitis

No. of cases	Cells with surface or cytoplasmic markers (%)															
	T -11	OKT -4	OKT -8	Leu -8	Tac	Leu -7	Leu -11	B -1	CIg	Lyso-zyme	α-1 ACT	OK -M1	S-100	OKT -6	OKT -9	C_3bR	C_4c
1	45	20	20	30	10	<5	ND	5	<5	40	40	40	5	<1	20	<1	0
2	55	20	30	30	<5	<1	<1	5	5	45	40	40	<5	1	40	<1	0
3	50	10	40	ND	ND	<1	<1	10	<5	40	40	40	<5	1	ND	ND	ND
4	60	20	40	20	30	<1	<1	<5	<5	35	40	30	<5	<1	40	<1	<1
5	ND	10	40	ND	ND	<1	ND	5	<5	40	40	40	<5	<1	ND	ND	ND
6	30	20	10	<5	<5	<1	<1	<5	10	40	40	60	<5	1	ND	ND	ND
7	40	20	20	20	ND	<1	<1	<5	<5	35	40	ND	5	<1	ND	ND	ND
8	40	10	30	10	<5	<1	<1	<5	<5	50	50	ND	<5	<1	ND	ND	ND
9	40	20	20	20	<5	<1	<1	<5	5	50	50	ND	0	<1	ND	ND	ND
10	50	30	20	20	ND	<1	ND	<5	5	40	40	ND	10	<1	ND	ND	ND
11	60	30	30	ND	5	<1	<1	<5	<5	35	35	ND	15	<1	ND	ND	ND
12	50	25	25	20	ND	<1	<1	<5	<5	40	40	ND	5	<1	ND	ND	ND
13	50	30	20	30	30	<1	<1	<5	5	40	40	40	5	<1	40	<1	0
14	50	10	40	15	5	<1	<1	5	<5	40	40	40	<5	<1	ND	ND	ND
15	50	30	20	20	<5	<1	<1	<5	<5	40	40	30	10	<1	40	<1	0
16	70	30	40	40	10	<1	<1	<5	<5	40	40	30	<5	<1	ND	ND	ND
17	55	40	10	40	5	<1	<1	5	5	40	40	30	5	<1	ND	ND	ND
18	50	25	25	20	10	<1	ND	<5	<5	40	40	40	5	ND	ND	ND	ND

α-1-ACT = α-1-anti-chymotrypsin; ND = Not Done

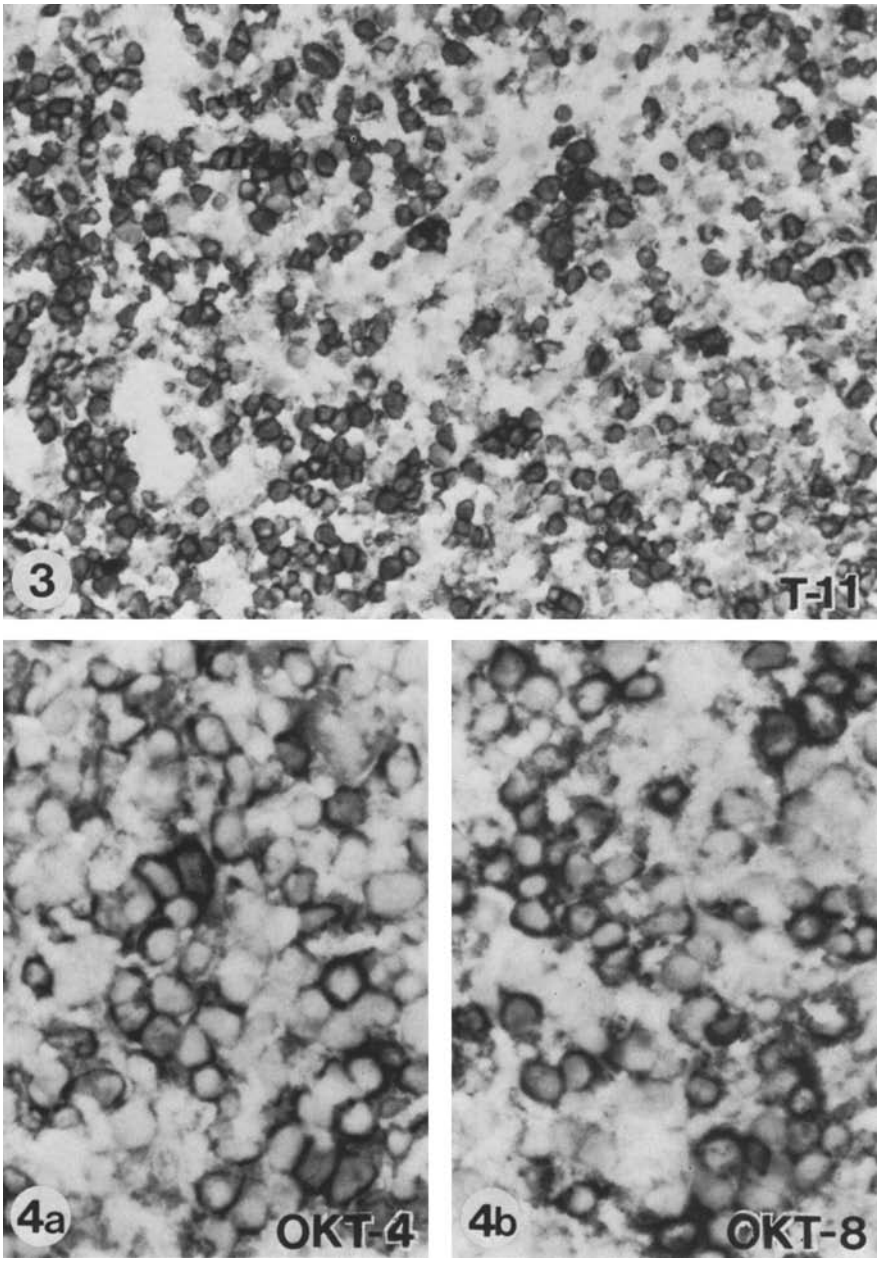

Fig. 3. Many T-11 positive cells (pan-T cells) are seen in the affected area. Frozen tissue, × 380. Case 16

Fig. 4. Both OKT-4 positive (helper/inducer) and OKT-8 positive (suppressor/cytotoxic) T cells are intermingled in the affected area. Frozen tissue, × 750. Case 13

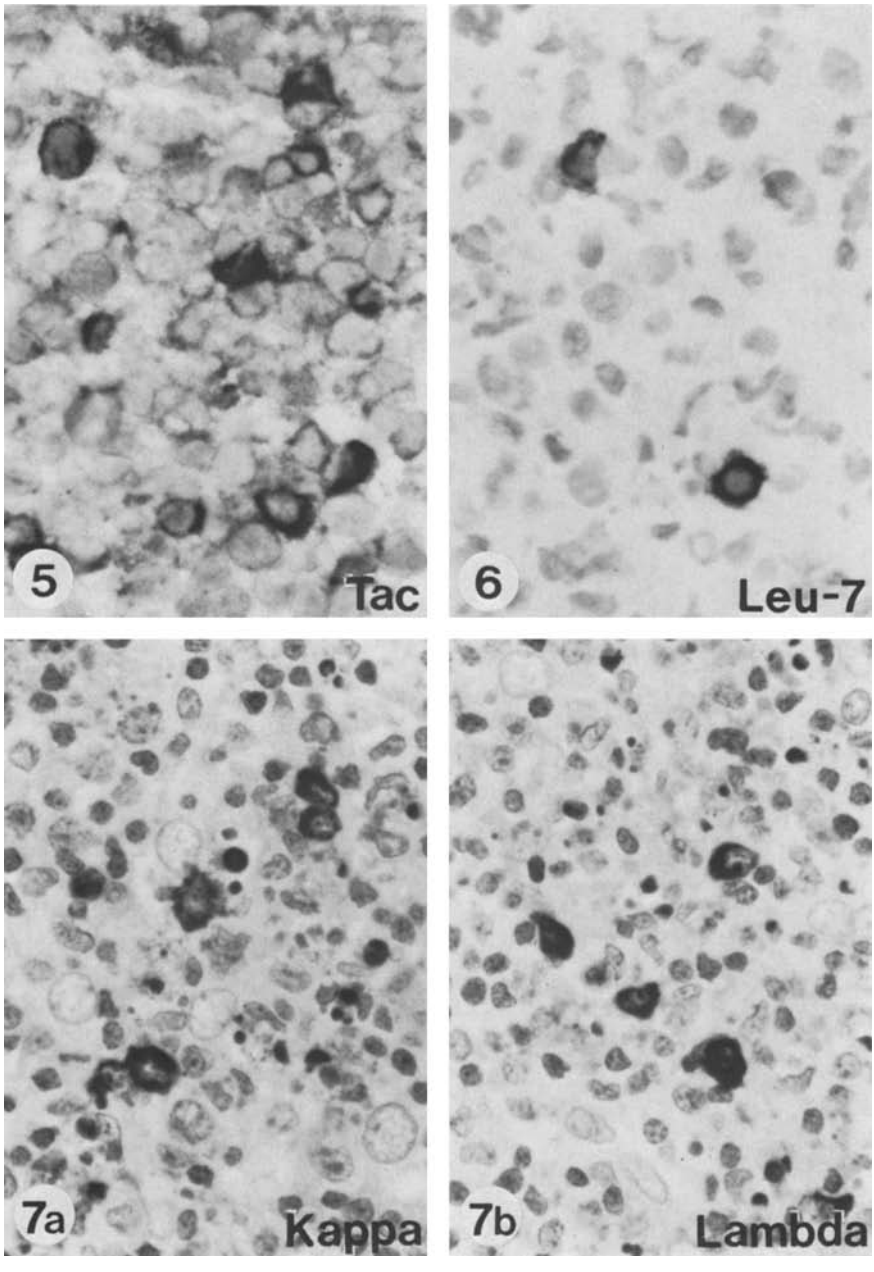

Fig. 5. Some anti-Tac positive cells (activated T cells with interleukin II receptor) are present in the affected area. Frozen tissue, × 750. Case 16

Fig. 6. Two Leu-7 positive cells (NK cells) with medium-sized nuclei and abundant cytoplasm are found the affected area. Frozen tissue, × 750. Case 3

Fig. 7a, b. Some cytoplasmic immunoglobulin possessing cells are scatered in the affected area. **a** Kappa chain. **b** Lambda chain. Paraffin section, × 750. Case 5

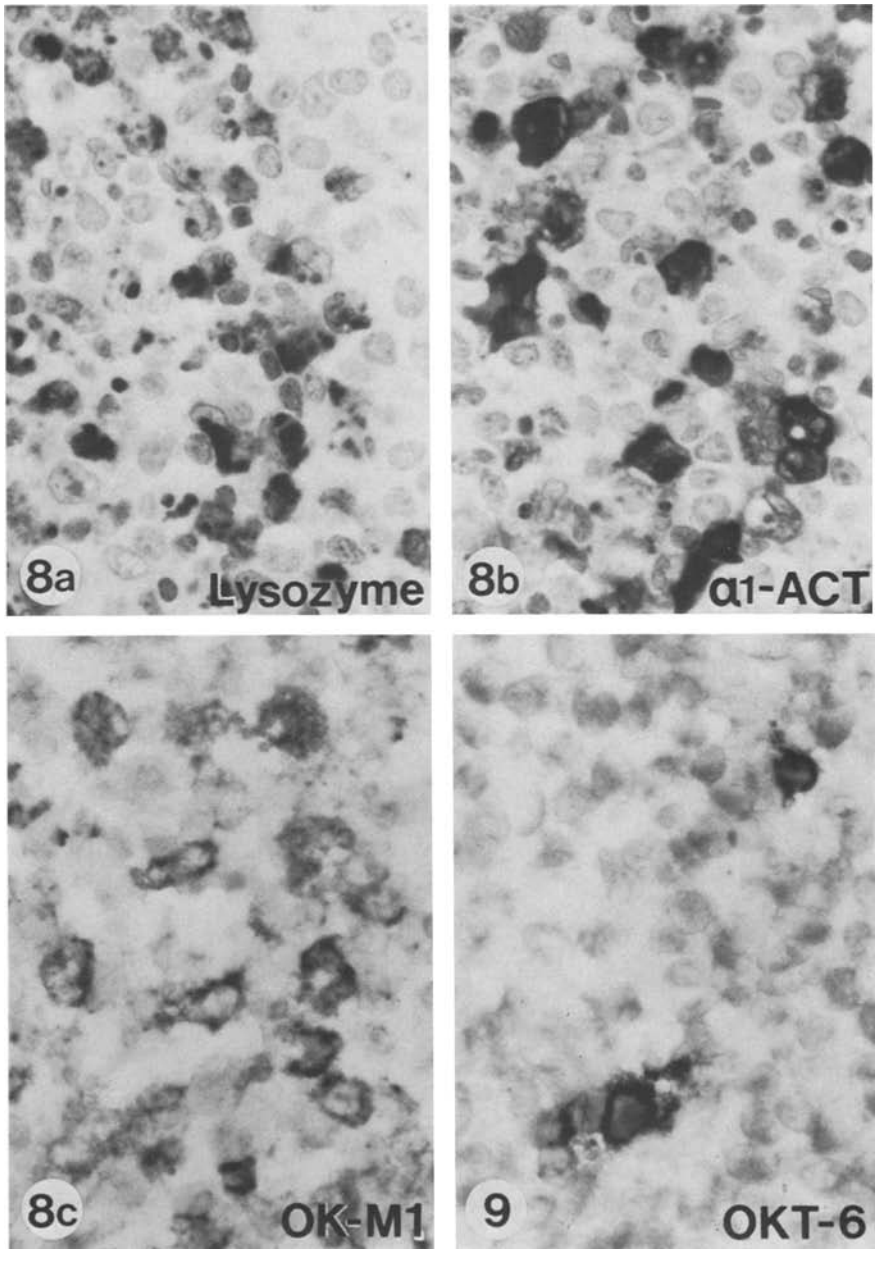

Fig. 8a–c. Numerous macrophages/histiocytes are distributed in the affected area. **a** Lysozyme. Paraffin section. Case 2. **b** α1-anti-chymotrypsin. Paraffin section. Case 18. **c** OK-M1. Frozen tissue, × 750. Case 15

Fig. 9. Two OKT-6 positive cells (interdigitating reticulum cells) are seen the affected area. Frozen tissue, × 750. Case 15

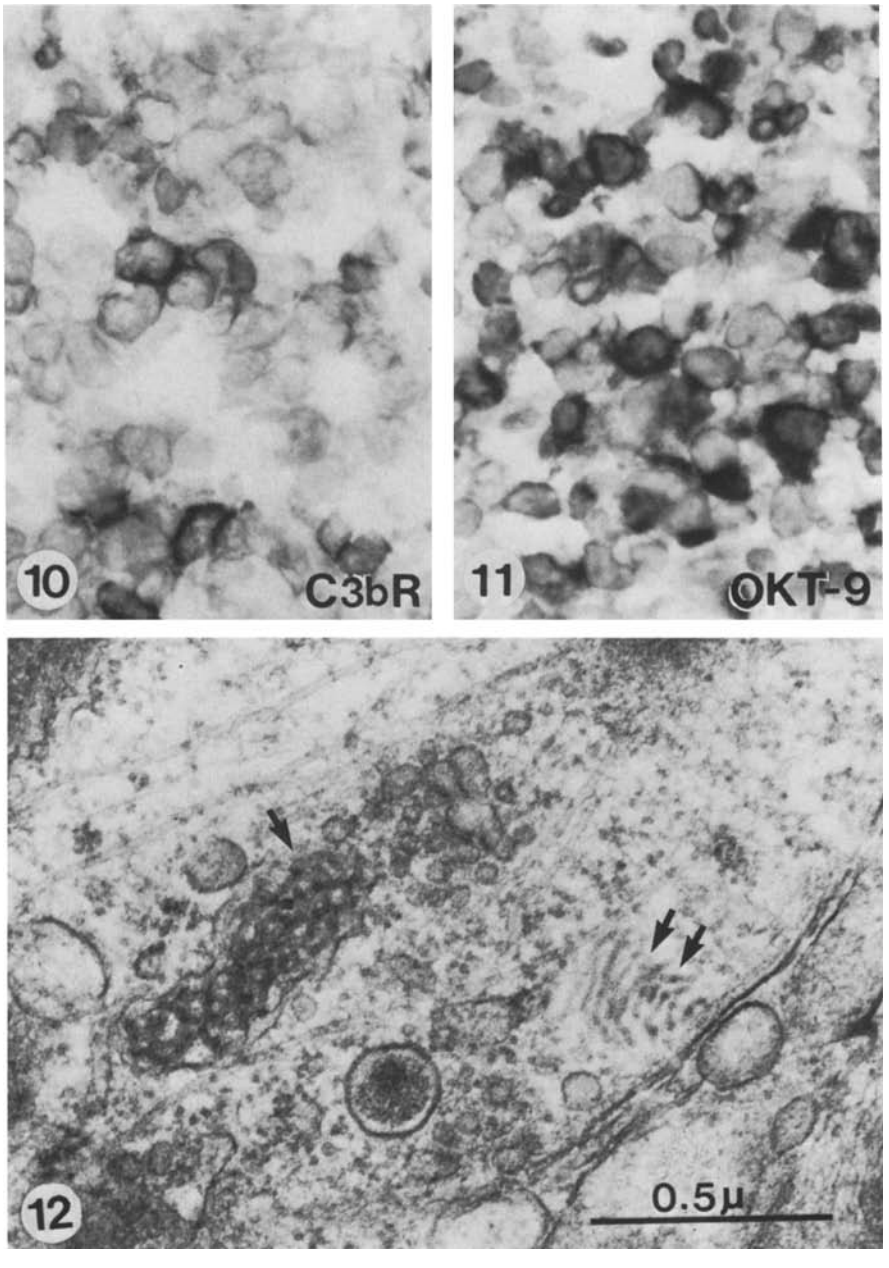

Fig. 10. Some C3b receptor possessing cells are present in the affected area. Frozen tissue, ×750. Case 2

Fig. 11. Many OKT-9 positive cells (Proliferating cells) are found in the affected area. Frozen tissue, ×750. Case 2

Fig. 12. Tubuloreticular structures (↑) and intracytoplasmic rodlets (↑↑) in the affected area in a transformed lymphocyte. Electron microscopy, ×64,000. Case 1

Table 4. NK Activity in histiocytic necrotizing lymphadenitis (2 cases)

Reaction time (hours)	E:T ratio	% lysis		
		Case 15	Case 16	Control (20 cases)
4	32	26.1	10.8	28.8+ 7.1
4	16	14.8	7.7	19.6+ 6.1
16	32	18.8	7.9	44.2+14.5
16	16	15.1	2.8	31.3+11.2

E: Effector cells; T: Target cells

or scanty in all cases. In 3 cases necrotic changes were extensive with aggregates of foamy macrophages at the marginal zone in 2. Fibrin thrombi were present in small vessels in the affected areas with obvious necrosis in 2. Lymph follicles were diminished in 14 cases, but in 2 cases some enlarged follicles were found. Scattered swollen reticulum cells were present in the enlarged paracortex in 8 cases.

Immunohistological study

The immunohistological findings of the affected foci are summarized in Table 3.

About 50% of nucleated cells in the foci were positive for T-11 (Fig. 3), which reacted and were distributed throughout all the foci equally and consisted usually of the cells positive with OKT-4 or OKT-8 (Figs. 4a, b). Some of these T cells were positive for Tac (receptor for interleukin II) (Fig. 5). Reactions for Leu-7 and Leu-11 (NK cells and activated one) were positive in a few cells in all cases (Fig. 6). Intracytoplasmic immunoglobulins and B-1 (B cells) were detected in only occasional lymphocytes (Figs. 7a, b). About 40% of cells reacted for lysozyme, α-1 anti-chymotripsin and M-1 (monocytes/macrophages) (Figs. 8a, b, c), but the cells with OKT-6 and S-100 protein (reticulum cells) were only encountered occasionally (Fig. 9). Both the 4 c component of complement and receptor for 3 b component were negative or weakly positive on the surface of only a few cells in each of the 5 cases examined (Fig. 10). OKT-9 (proliferating cells) were positive in all the 5 cases examined with over 40% of the cells in 4 (Fig. 11). In the non-affected parenchyma which showed an enlarged paracortex with scattered swollen reticulum cells and diminished lymph follicles were there numerous T cells with T-11, but OKT-9 positive cells were occasionally encountered.

Electron microscopy

All the nodes examined evealed tubuloreticular structures and intracytoplasmic rodlets in the transformed lymphocytes (Fig. 12) and/or endothelial cells or histiocytes in the affected areas as reported previously (Eimoto et al. 1983).

NK activity of peripheral blood

The levels of NK activity of peripheral blood in 2 cases are tabulated in Table 4. Both cases showed a decreased percentage of lysis.

Discussion

The nature of HNL has been discussed repeatedly but a definite view of the lesion is not yet available. The cells in the foci have been thought to be reticular cells (Kikuchi et al. 1972) or lymphocytes (Fujimoto et al. 1972), but a solution had to wait until the recent development of immunohistological methods using monoclonal antibodies. A predominance of T cells was confirmed by several authors recently (Feller et al. 1983; Turner et al. 1983; Kikuchi et al. 1983) but the results of these authors on the T cell subsets were not in agreement each other, because only a few cases were examined immunohistologically. Our result in this series indicated that the proliferating T cells consisted of both helper/inducer and suppressor/cytotoxic types with a ratio from 4:1 to 1:4 and in 6 cases the ratio was 1:1 (Table 3) (Figs. 4a, b). This result documented that the cells proliferating were not monoclonal but rather polyclonal T cells. In addition, the affected areas contained no or few neutrophils. Naito et al. (1985) reported that granulocytopaenia of HNL may be related to the presence of a humoral factor in serum. They also suggested that an absence of neutrophils in the affected foci seemed to be related to some lymphokines from the proliferating T cells. Kawauchi et al. (1984) described depressed NK activity and the dissociation of NK activity from antibody-dependent cell-mediated cytotoxicity in 3 cases of HNL. From these results they suggested that the disturbance of cellular immunity might have an important role on the aetiology of HNL. Only a few Leu-7 and Leu-11 positive cells (NK cell and its activated one) in the affected foci (Fig. 6) and decreased NK activity in peripheral blood in this series also support their speculation (Table 4). A large number of tubuloreticular structures and intracytoplasmic rodlets in lymphocytes and other cells in the affected areas (Imamura et al. 1982; Eimoto et al. 1983; Ali and Horton 1985) as like as SLE, also supported the immunological disturbances (Fig. 12). In spite of some resemblance of clinical and histological features between SLE and HNL, in the latter normal levels of C3 component and increased C4 and CH50 were usually observed and only a few positive cells with C_4c component and receptor for C_3b component were present in the affected foci (Table 3) (Fig. 10). The results demonstrated no important role of the complement system in the HNL lesion. In addition to T cells there were numerous histiocytes and nuclear debris of destroyed cells in the foci (Figs. 8a–c). The presence of histiocytes and appearance of abundant nuclear debris in the affected foci suggested that the lymphocytes had also macrophage migration inhibition factor and cytotoxic effects on the proliferated cells. These histological features seem to be related to delayed-type hypersensitivity reaction as well as defence mechanism against some agents such as viruses, protozoa and fungi. De-

layed-type hypersensitivity with cutaneous response to tubeculin in sensitized individuals was charaterized by early enrichment of the OKT-4 positive subpopulation of T lymphocytes, subsequent T lymphocytes activation (an increase of Tac- and OKT-9 positive cells with an increase of OKT-8 positive cells), and appearance of monocytes and histiocytes but sparse neutrophils (Platt et al. 1983). The findings are similar to those in the lesion of HNL. Thus HNL may be induced by sensitized T cells against some antigens, and then activated macrophages to destroy causative agents including microorganisms. In our cases no histological or serological evidence of specific infection was found but some antigens which induce delayed-type hypersensitivity reaction may have arrived in the affected nodes.

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