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Enzyme- and immunohistochemical study of a case of histiocytic necrotizing lymphadenitis*

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Summary. A combined morphological, immunohistological, and enzyme histochemical analysis was performed on frozen and fixed lymph node tissue in a case of histiocytic necrotizing lymphadenitis (HNL) using conventional histology, a panel of monoclonal and polyclonal antibodies, and a series of common haematological enzyme reactions. Histology showed multiple paracortical necrotizing foci which, in a prominently necrobiotic background devoid of granulocytes, contained large numbers of foamy histiocytes and macrophages intermingled with cells resembling degenerating plasmacytoid T-cells. Most of the histiocytes were alpha$_1$-antichymotrypsin positive and foamy cells were also distinctly Leu-M1 positive. Strong granular acid phosphatase (AP) positivity was present in the cytoplasm of the macrophages and histiocytes. The cells with plasmacytoid features showed weaker and homogeneously diffuse AP staining. Alpha-naphthyl acetate esterase (ANAE) activity was much less striking than AP in the necrotizing foci and most of the ANAE negative cells corresponded to those with plasmacytoid features. No cells with B-cell lineage markers were present within the necrotizing foci; most of the occasional T-cells (Leu-1$^+$, Leu-4$^+$) present in the foci were Leu-2a$^+$ (OKT8$^+$) whereas OKT10$^+$ lymphoid cells were abundant and appeared to correspond with the cells with plasmacytoid features. Our combined data confirm that the special type of necrosis found in HNL develops within foci of plasmacytoid T-cells undergoing regressive changes and apparently exhibiting distinct immunohistological and enzyme histochemical features.

Key words: Histiocytic necrotizing lymphadenitis – Lymphadenitis – Lymph node – Immunohistochemistry – Enzyme histochemistry

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Introduction

Since 1972, an unusual type of self-healing lymphadenitis has been described in Japan (Kikuchi et al. 1977; Imamura et al. 1982; Wakasa and Dorfman 1983). It has been qualified as "cervical subacute necrotizing lymphadenitis", "necrotizing histiocytic lymphadenitis", and "necrotizing lymphadenitis" (Kikuchi et al. 1977). Outside Japan, 30 cases of necrotizing lymphadenitis have been collected in USA (Turner et al. 1983) and 31 in Europe (Pileri et al. 1982; Papadimitriou and Papacharalampous 1985). These European cases have been designated as "histiocytic necrotizing lymphadenitis without granulocytic infiltration". The basic histopathological features of the lymph node consist of a partial or near-complete effacement of the nodal architecture which is replaced by foci of proliferating and infiltrating histiocytes in the cortex and/or paracortex. Varying degrees of cellular regressive changes are usually seen such as pyknosis and nuclear fragmentation or marked tissue necrosis, associated with marked phagocytotic activity. Granulocytes are typically absent (Kikuchi et al. 1977; Pileri et al. 1982; Turner et al. 1983; Papadimitriou and Papacharalampous 1985).

Immunohistological studies of histiocytic necrotizing lymphadenitis are only occasionally reported in the literature (Feller et al. 1983; Turner et al. 1983).

The present study reports the clinicopathological features of a case of histiocytic necrotizing lymphadenitis without granulocytic infiltration, in which an immunohistological and enzyme histochemical analysis could be performed.

Patient and methods

Case report. A 37-year-old Italian house-wife, born in Sardinia, was admitted to another Hospital in December 1984 with mild fever, leukopaenia, and hepatosplenomegaly. Her family and past histories were unremarkable. Laboratory data together with the findings of a liver biopsy suggested the diagnosis of HB_sAg negative chronic persistent hepatitis and the patient was discharged. In April 1985, she complained of weight loss, fatigue, loss of appetite, and elevated temperature (39.5° C); she had noticed the onset of a right cervical mass and was again referred to the same Hospital. Physical examination revealed the presence of bilateral enlarged (1.5 cm) cervical lymph nodes; these were painful and moveable. Mild hepatomegaly was also recorded. Erythrocyte sedimentation rate (ESR) was elevated (65 mm). RBC were $3.9 \times 10^6/mm^3$ and haemoglobin was 10.2 g/dl. WBC were reduced ($2.6 \times 10^3/mm^3$) with a differential count of 71% lymphocytes, 26% neutrophils, and 3% monocytes. Serum protein immunoelectrophoresis yelded normal results. Test for mononucleosis was negative. Biopsy of a cervical lymph node was interpreted as "high grade malignant lymphoma" and the patient was therefore first referred to our Hospital for further evaluation. At that time, the patient complained of night sweats. Othorhinolaryngological examination was negative. Chest X-ray, abdominal computerized tomography and lymphagiography did not reveal any abnormal features. Laparoscopy with multiple biopsies showed no abnormalities. Bone marrow biopsy was negative. Laboratory data showed the persistence of leukopaenia ($2.8 \times 10^3/mm^3$) with a normal differential count. Serological tests for toxoplasmosis were negative and lymphocyte subsets in the peripheral blood were normally represented. Review of the previous biopsy did not confirm the diagnosis of lymphoma but rather suggested nonneoplastic lymphadenopathy. A repeat biopsy of two cervical nodes was performed, which showed a picture histologically identified as histiocytic necrotizing lymphadenitis. After such a diagnosis the patient was discharged with no further therapy. After 3 months the patient has regained her weight and,

at the present time, she denies night sweats and fever; her physical examination is completely negative. Laboratory data do not indicate past or present infection with Yersinia Enterocolitica and Widal-Wright reaction is negative. An appreciable increase (from 4% to 24%) in the percent of Leu-7$^+$ (killer/natural killer) cells in the peripheral blood was recently recorded. ESR and WBC count are normal.

Methods. After surgical removal, lymph nodes of the repeat biopsy were fixed in Bouin's solution, embedded in paraffin, and routinely stained with haematoxilin and eosin, Giemsa, periodic acid-Schiff (PAS) and silver impregnation (Gomori).

Fresh tissue was snap-frozen in cold isopenthane and stored at $-80°$ C for studies on cryostat sections.

For enzyme histochemical studies, tissue was treated according to the procedure of Beckstead (1983) for glycol methacrylate embedding and enzyme reactions. These latter included the demonstration of acid phosphatase (AP), beta-glucuronidase (b-gluc), ATP-ase, alpha-naphthyl acetate esterase (ANAE), and naphthol-AS-D-chloroacetate esterase (CAE). All these enzyme activities were also tested on cryostat sections.

Immunological analysis was performed with a panel of monoclonal antibodies from commercial source including: BA-1, BA-2 (Hybritech, San Diego, California), Leu-1, Leu-4, Leu-2a, Leu-3a, Leu-7, Leu-14, Leu-M1, anti-CALLA (Becton Dickinson, Milan, Italy) and OKT4, OKT6, OKT8, OKT10 (Ortho Diagnostics, Raritan, New Jersey). The list of polyclonal antisera used included those to lysozyme, alpha$_1$-antichymotrypsin, S-100 protein, kappa and lambda immunoglobulin (Ig) chains, IgD, IgM, laminin, and fibronectin. With the exception of the latter two, all the polyclonal antisera were from commercial source (Dakopatts a/s, Glostrup, Denmark). Lymph node tissue was tested with monoclonal antibodies and polyclonal antisera with the avidin-biotin immunoperoxidase procedure described by Hsu et al. (1981). The specificity is reported in the literature (Motoi et al. 1980; Barski et al. 1983; D'Ardenne et al. 1983; Hsu et al. 1983; Hsu and Jaffe 1984; Turner et al. 1984).

Results

Microscopic findings

Low-power examination revealed a multifocal partial effacement of the lymph node architecture with recognizable remnants of a few small secondary follicles and patent medullary sinuses containing a moderate number of histiocytes. The paracortex had sometimes a "mottled" appearance, especially next to the necrotizing foci (Fig. 1). These latter were multiple and were mainly located in the paracortex but, in some instances, they extended to the marginal sinus (Fig. 1). The necrotizing foci varied in size and shape and their edges were usually well defined although, sometimes, tended to become confluent. A variable proportion of different cell types composed the necrotizing foci. These cells included PAS-negative histiocytes with foamy cytoplasm and phagocytotic activity (macrophages) as well as few PAS-positive cells of histiocytic or monocytic appearance that usually lacked such an activity. All of the above cells were intermingled with a consistent amount of medium-sized cells with plasmacytoid features having a clear nucleus with a central small nucleolus and a gray-violet cytoplasm in Giemsa stained sections (Fig. 2); often these cells showed regressive changes (pyknosis and nuclear fragmentation). These cells with plasmacytoid features were also abundant in the peripheral areas of the necrotizing foci. Granulocytes, epithelioid cells, and giant cells were strikingly absent. The background of the necrotizing foci was composed by pyknotic cells

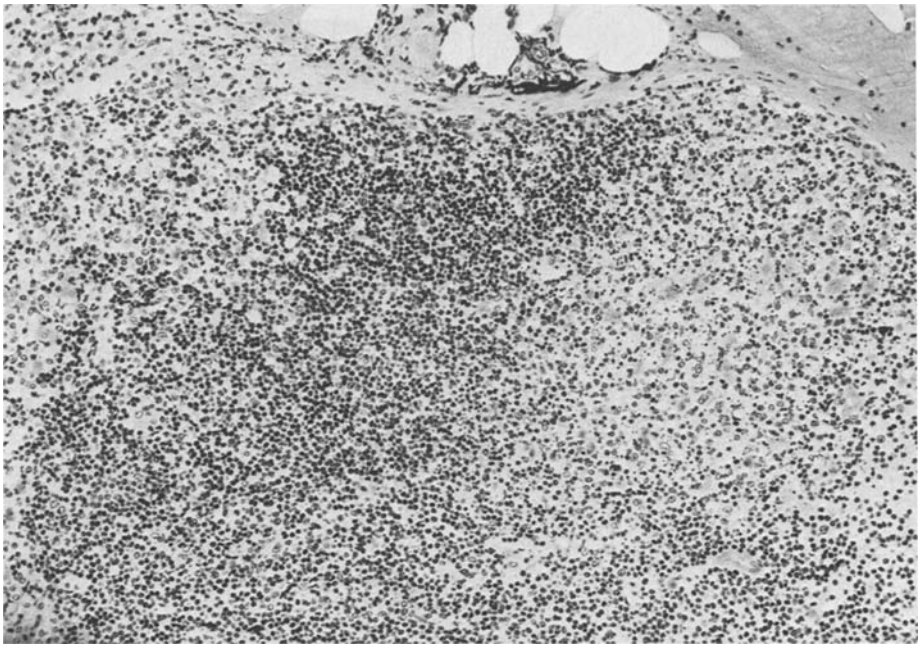

Fig. 1. A "mottled" appearance is seen in the spared paracortex between two necrotizing foci. These contain abundant kariorrhectic material. H&E ×250

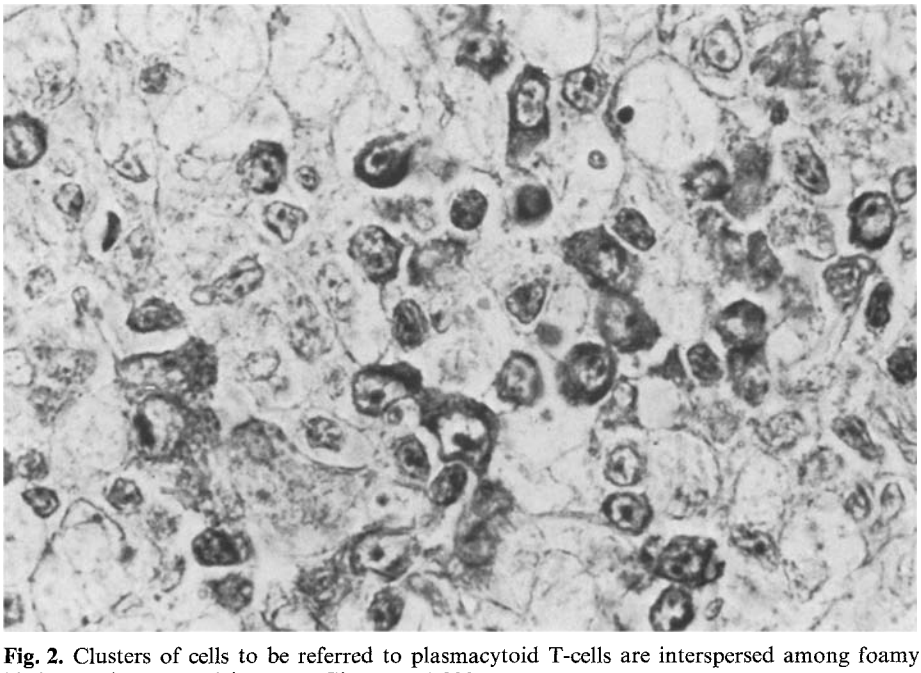

Fig. 2. Clusters of cells to be referred to plasmacytoid T-cells are interspersed among foamy histiocytes in a necrotizing area. Giemsa ×1,000

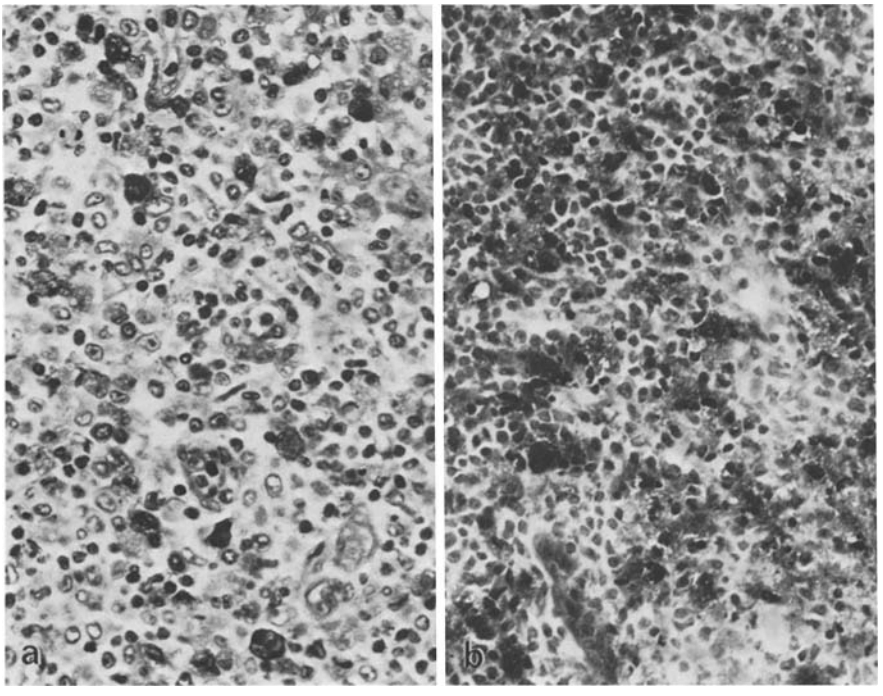

Fig. 3a, b. Strong cytoplasmic positivity for alpha$_1$-antichymotrypsin is detected in several large histiocytic cells **a**; paraffin-embedded section, avidin-biotin immunoperoxidase counterstained with haematoxylin ×250. In a necrobiotic area, cells of histiocytic nature show strong granular cytoplasmic reaction for ANAE **b**. Most of the lymphoid cells admixed with the histiocytes are negative **b**; cryostat section ×250

and kariorrhectic debris in variable proportion, associated to scanty remnants of capillary or medium-sized vessels (Fig. 1). The central portion of the necrotizing foci contained also a variable amount of argyrophilic fibres and, sometimes, a loose fibrinoid eosinophilic PAS-positive network.

Immunohistology findings

Most of the histiocytic cells located in the necrotizing areas were positive for alpha$_1$-antichymotrypsin (Fig. 3). The positivity for lysozyme appeared somewhat to be restricted to the histiocytic and monocytic cells lacking phagocytotic activity; foamy macrophages were distinctly Leu-M1$^+$ and alpha$_1$-antichymotrypsin$^+$. In the spared paracortical areas, only few cells positive for the above three markers were found; few interdigitating reticulum cells, positive for S-100 protein, were present in these spared areas.

Most of the few T-cells (Leu-1$^+$, Leu-4$^+$) located within the necrotizing foci had a cytotoxic/suppressor phenotype (Leu-2a$^+$, OKT8$^+$) whereas in the spared paracortex and the periphery of the necrotic areas most of the many lymphoid cells present had a helper/inducer (Leu-3a$^+$, OKT4$^+$) phenotype (Fig. 4). Lymphoid cells with OKT10$^+$ membrane immunostaining

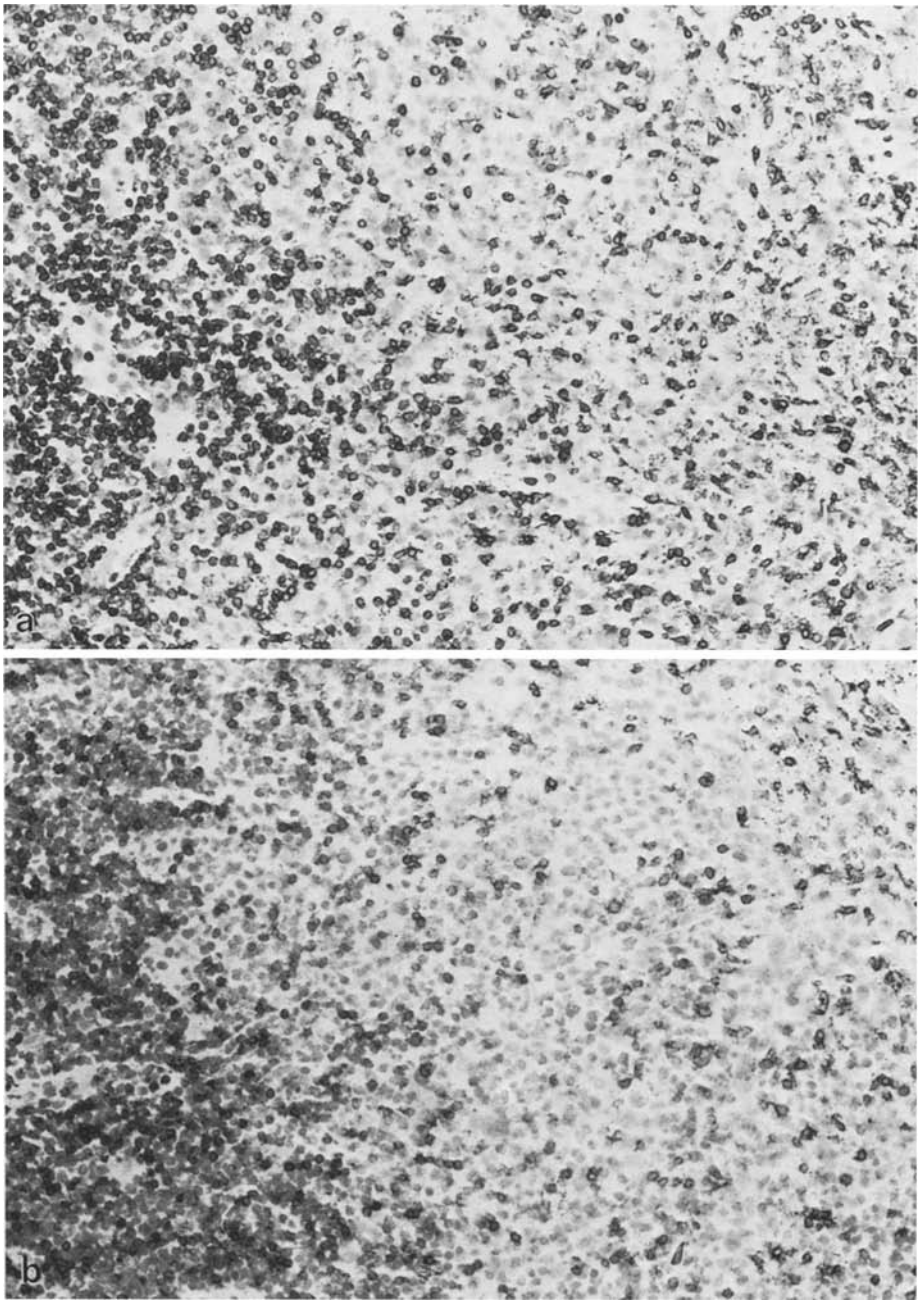

Fig. 4a, b. Cryostat sections of the lymph node stained with monoclonal antibodies for Leu-4 **a** and Leu-2a **b** demonstrate that a discrete number of T-cells (Leu-4$^+$) is present within a necrotizing focus (*on right*). Most of them appear to be Leu-2a$^+$. Avidin-biotin immunoperoxidase counterstained with haematoxylin $\times 250$

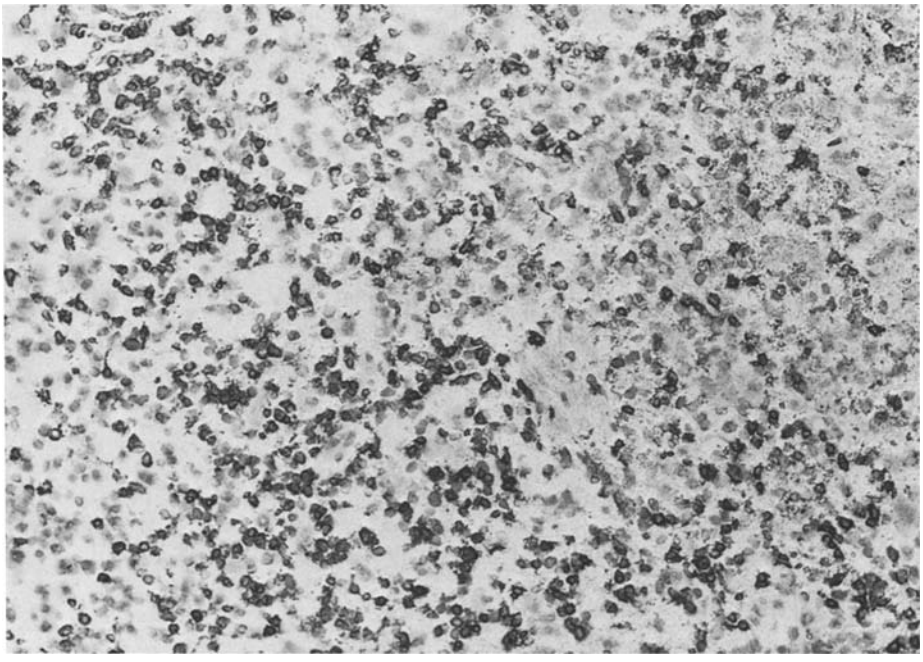

Fig. 5. Part of a necrotizing focus (*on right*) and its periphery are showed. Both areas contain numerous OKT10$^+$ cells. Cryostat section, avidin-biotin immunoperoxidase counterstained with haematoxylin ×250

were abundant within the centre of the necrotic foci and their peripheral areas and appeared to mostly correspond to medium-sized cells with plasmacytoid features (Fig. 5). Other OKT10$^+$ cells were present in the spared paracortex, often in a perivenular location.

No CALLA$^+$, Leu-7$^+$ (killer/natural killer cells), and OKT6$^+$ cells (common thymocyte phenotype) were present.

No cells with B-cell lineage markers (Ig, kappa and lambda chains, BA-1, and BA-2) were present within or around the necrotizing foci, with the single exception of very few Leu-14$^+$ (all B-cells phenotype) elements.

In the necrobiotic areas, immunoreactivity for laminin was present in the residue vessels and reticulin fibres whereas the antifibronectin antiserum stained the centrally placed fibrinoid network.

Enzyme histochemistry findings

In the necrobiotic areas, a striking AP activity was present, two distinct types of positivity being found: a strong granular cytoplasmic reaction was present in the macrophages and histiocytes (Fig. 6) whereas a weaker and homogeneously diffuse positivity was present in the cytoplasm of the cells with plasmacytoid features located within and around the necrotizing foci (Fig. 6). By contrast, ANAE activity was much less striking in the necrobiotic areas than AP and it appeared to be restricted to a lesser number

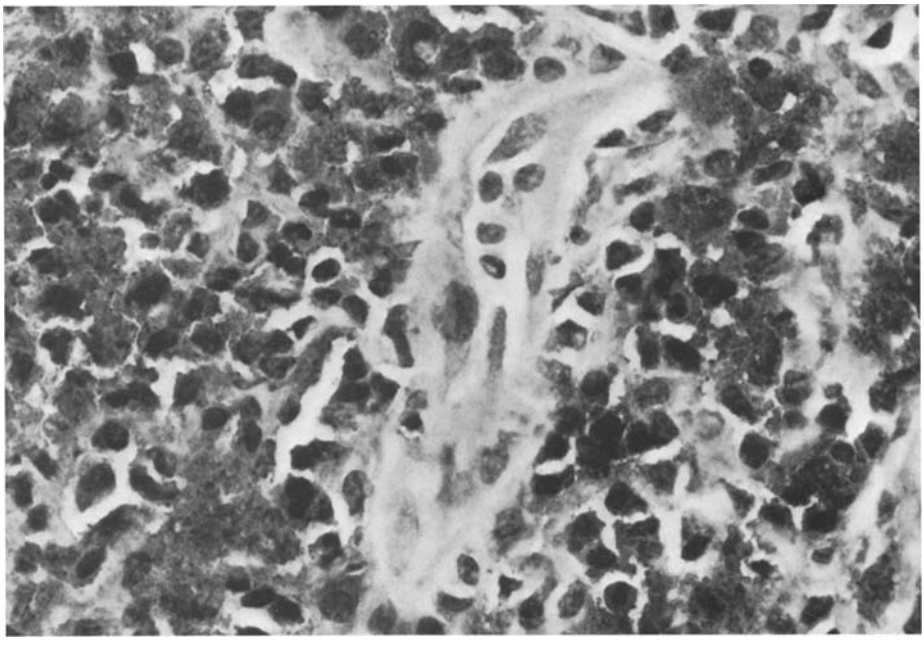

Fig. 6. All the cells present in this field are positive for AP reaction. Most of them are histiocytes and characteristically show strong granular cytoplasmic positivity. Other cells, mainly located around a central vessel, show a weaker and diffuse cytoplasmic positivity and morphologically correspond to lymphoid cells with plasmacytoid features. Cryostat section ×1,000

of histiocytes and macrophages (Fig. 3), although also few ANAE$^+$ small lymphoid cells could be detected. Most of the cells negative for ANAE appeared to correspond morphologically with those having plasmacytoid features.

No CAE positivity could be demonstrated in the necrobiotic tissue. In this latter, residue vessels were positive for ATP-ase whereas only a faint cytoplasmic diffuse positivity for b-gluc resulted to be present in the cells with plasmacytoid features, especially in those located in the peripheral portion of the necrobiotic areas. Outside the necrobiotic tissue, CAE activity was limited to single occasional neutrophils located within vascular lumina.

Discussion

Histiocytic necrotizing lymphadenitis is a fairly common disease entity in Japan (Fujimori et al. 1981; Imamura et al. 1982) whereas it appears to occur and/or to be recognized less frequently in Western countries (Feller et al. 1983; Turner et al. 1983). Among the 57 cases collected and published in USA (Turner et al. 1983) and in West Germany (Pileri et al. 1982) so far, 6 were from Italy. The clinical picture of our patient (painful cervical lymphadenopathy, fever, elevated ESR, leukopaenia, and hepatomegaly) and the self-healing clinical course are similar to those reported in the literature (Fujimori et al. 1981; Pileri et al. 1982; Turner et al. 1983).

In our patient, a diagnosis of malignant lymphoma had been made elsewhere both clinically as well as histologically. Such a misdiagnosis is commonly reported in the literature (Wakasa and Dorfman 1983; Turner et al. 1983; Papadimitriou and Papacharalampous 1985) and it is probably caused by the fact that necrotizing lymphadenitis is still a poorly recognized entity in Western countries (Turner et al. 1983).

The overall morphological findings of the repeat biopsy performed in our patient were in keeping with those reported in other European (Pileri et al. 1982) or American (Turner et al. 1983) cases of necrotizing lymphadenitis although some particular aspects seem to be worth of mention.

Regarding the histiocytic component, the presence of small collections of foamy cells has been reported as a facultative (17%) finding in the 27 cases of histiocytic necrotizing lymphadenitis without granulocytic infiltration published by Pileri et al. (1982). In our case, most of the PAS-negative macrophages present in the necrotizing foci clearly had a foamy cytoplasm and showed a distinct positivity for Leu-M1 and alpha$_1$-antichymotrypsin. The presence of foamy macrophages has been reported also by Turner et al. (1983) in 5 (16%) of their 30 cases and in a Japanese case by Kikuchi et al. (1977).

The absence of granulocytes within the necrobiotic areas in our case was confirmed by both lysozyme and CAE stainings.

Regarding the lymphoid cells present within the necrotizing foci, our immunohistological analysis showed that most of the few T-cells (Leu-1$^+$, Leu-4$^+$) present had a cytotoxic/suppressor phenotype (Leu-2a$^+$, OKT8$^+$). This feature was also found by Turner et al. (1983) in the single case of their series in which a frozen section was available whereas immune staining for Leu-3a$^+$ cells (helper/inducer phenotype) yelded weak and sparse reactivity. As in our case, in the single frozen section case tested with monoclonal antibodies by Feller et al. (1983), the centre of the necrotic focus did not stain with Leu-3a antibody; moreover these authors reported that Leu-3a$^+$ cells predominated in the more peripheral zone containing plasmacytoid T-cells and in the surrounding T region. According to the interpretation of Feller et al. (1983), more plasmacytoid T-cells might flow into the centre of the necrotic focus from peripheral areas, where they undergo pyknosis and progressive disintegration and are phagocytized by macrophages. In our case, a consistent amount of medium-sized cells with plasmacytoid features showing regressive changes (pyknosis and nuclear fragmentation) was evident within the centre of the necrotizing foci and their peripheral areas. These cells had a clear nucleus with a small central nucleolus and a gray-violet cytoplasm in Giemsa stained sections, thus corresponding to the morphology of plasmacytoid T-cells (Müller-Hermelink and Lennert 1978; Feller et al. 1983; Vollenweider and Lennert 1983). The membranes of these cells positively stained with the OKT10 monoclonal antibody. This marker is common to activated cells of both T and B lineage (Anderson et al. 1984) and, according to Hsu and Jaffe (1984) is mainly present in the membrane of cortical thymocytes and in the cytoplasm of the terminally differentiated B-cells, i.e. the plasma cells.

Plasmacytoid T-cells are usually placed in a perivenular location in the paracortex and are derived from blast cells that have strong paranuclear acid phosphatase activity (Müller-Hermelink and Lennert 1978). Plasmacytoid T-cells are terminal cells which have the usual tendency to undergo pyknosis and react with the monoclonal antibody Leu-3a (OKT4) (Feller et al. 1983; Vollenweider and Lennert 1983). No B-cell lineage markers have been demonstrated in these cells (Papadimitriou et al. 1983). A case of malignant lymphoma of plasmacytoid T-cells has been reported by Müller-Hermelink et al. (1983); in lymph node imprints these cells were found to be negative for acid nonspecific esterase and CAE but positive for AP in a granular fashion. The cells with plasmacytoid features we found were negative for ANAE and CAE but positive for AP, although with a homogeneous and diffuse cytoplasmic staining.

No data are available in the literature about the possible reactivity of plasmacytoid T-cells with the OKT10 antibody in histiocytic necrotizing lymphadenitis. Our data seem to suggest that the abundant $OKT10^+$ cells with plasmacytoid features and negative for B-cell lineage markers that we found within and around the necrotizing foci could correspond to plasmacytoid T-cells. This view seems also to be supported by the presence of $OKT10^+$ cells in a perivenular location in the spared paracortex.

In conclusion, our morphological, enzyme- and immunohistochemical data confirm that, according to Feller et al. (1983), the special type of necrosis found in histiocytic necrotizing lymphadenitis develops within foci of plasmacytoid T-cells. Some of our data are nonetheless different from those of the above authors and need confirmation as far as the immunophenotyping of these cells in such a disease is concerned.

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Note added in proof

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