An Ultrastructural Study of Subacute Necrotizing Lymphadenitis

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Fifteen cases of a unique lymphadenitis called subacute necrotizing lymphadenitis were studied electron-microscopically. The large lymphoreticular cells proliferating at cortical or paracortical areas of the lymph nodes mainly consisted of immunoblasts and histiocytoid cells, which were characterized by numerous intracytoplasmic myelinlike inclusions. Such histiocytoid cells seemed to be derived from the immunoblasts. Tubuloreticular structures, which had been often noticed within endothelial cells or lymphocytes of the patients with systemic lupus erythematosus (SLE) or SLE-related diseases, were also observed with high frequency in most cases examined. They were present within the cytoplasm of immunoblasts, endothelial cells, and histiocytoid cells. Immunoblasts in mitosis occasionally contained these structures. We offer the hypothesis that subacute necrotizing lymphadenitis with still unknown etiology may reflect a self-limited SLE-like autoimmune condition induced by virus-infected transformed lymphocytes. (Am J Pathol 1982, 107:292–299)

SINCE 1972, a unique lymphadenitis called subacute necrotizing lymphadenitis has been described by many Japanese pathologists.[1-9] We have experienced 259 cases of this lymphadenitis in 2780 cases of lymph node biopsies diagnosed in our laboratory during 1969 to 1980.[10] This condition now seems to have been generally established as a disease entity in Japan, although such a disease seems not to have been described in reviews of lymph node disease published in other countries.[11-15] Recently, a similar lymphadenitis was described by an American pathologist as lymphadenopathy in influenza.[16]

This lymphadenitis mainly affects young women and has a good clinical course, healing within 2–4 weeks with no treatment. The chief complaints of the patients are cervical lymphadenopathy with or without local pain and moderate fever. Leukopenia less than 4000 may occur in some cases. The cause is unknown and the pathogenesis obscure, although some clinical and histologic characteristics of this disease suggest that it might result from any viral infection. A few investigators have proposed that toxoplasma or EB virus infections might lead to lymphadenitis of this type, because elevation of the serum antibodies against these agents was noticed in some patients.[4,17] However, these possibilities have not been agreed to by many other workers.[5,6,9]

The histologic features of subacute necrotizing lymphadenitis have been described elsewhere.[4-10] Briefly, the most striking histologic feature is the focal proliferation of pale-staining lymphoreticular cells and many dispersed tingible bodies at the cortical or paracortical areas in the lymph nodes. Thus, one of the aims of the present study was to elucidate the nature and origin of such large lymphoreticular cells with electron microscopy. Another aim was to search for a possible infectious agent, such as a virus, bacterium, or parasite, in the lymph nodes. In the course of this study, a peculiar structure of unknown

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origin, called a tubuloreticular structure,[18-20] was observed with high frequency within the lymph node cells. Such structures are well known to be present within the glomerular endothelial cells or peripheral lymphocytes of the patients with systemic lupus erythematosus (SLE) or SLE-related autoimmune diseases. Since the detection of this structure in human lymph nodes is rare except in cases of SLE, the significance of its existence in subacute necrotizing lymphadenitis will be discussed here.

Materials and Methods

Tissues from 15 patients with subacute necrotizing lymphadenitis were obtained by surgical biopsy and were placed immediately into phosphate-buffered 4% paraformaldehyde solution. Paraffin sections stained with hematoxylin and eosin were studied in all cases. For electron microscopy, small pieces were cut, were postfixed in osmium tetroxide, and were embedded in Epon. Semithin sections, cut at 1 to 2 μm on the ultramicrotome, were stained with toluidine blue. Thin sections cut from the selected areas and stained with uranyl acetate and lead citrate were studied with a Hitachi H-500 electron microscope.

Results

Clinical Data

Table 1 summarizes the clinical features of this series. Fifteen patients were studied, 4 males and 11 females aged 15 to 31, all Japanese residents in Hokkaido. Cervical lymphadenopathy was a major clinical symptom in all cases, although systemic lymphadenopathy was noticed in 2 cases (Patients 1 and 4). Moderate fever and local pain were common complaints in many cases. In all cases cervical lymph nodes were surgically removed for pathologic diagnosis. The interval from the onset of the symptoms to lymph node biopsy was 1-3 weeks for all patients except patient 4, who had suffered from the same disease 2 years before. All patients are well from 1 to 3 years after excision without any treatment.

Pathologic Findings

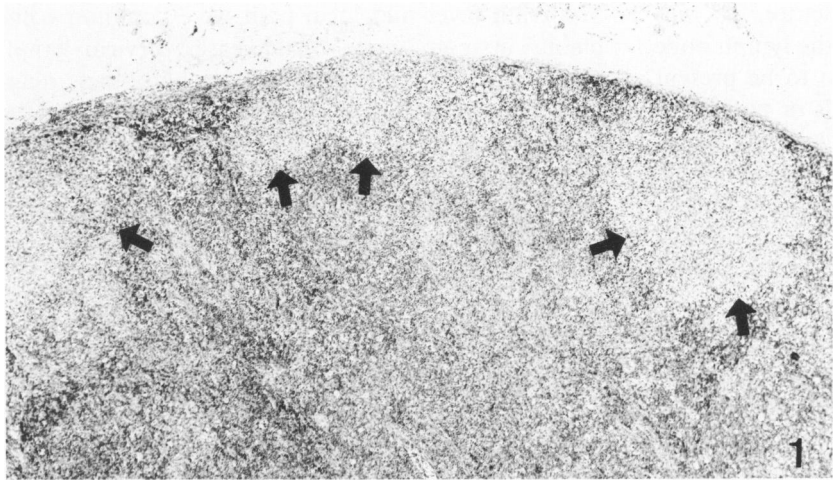
Surgically removed lymph nodes were 1.0 to 2.5 cm in diameter and were normal in hardness. Usually the surface was smooth and not conglomerated. The cut surface was gray-white and occasionally partially hemorrhagic. Yellowish necrotic foci were sometimes present.

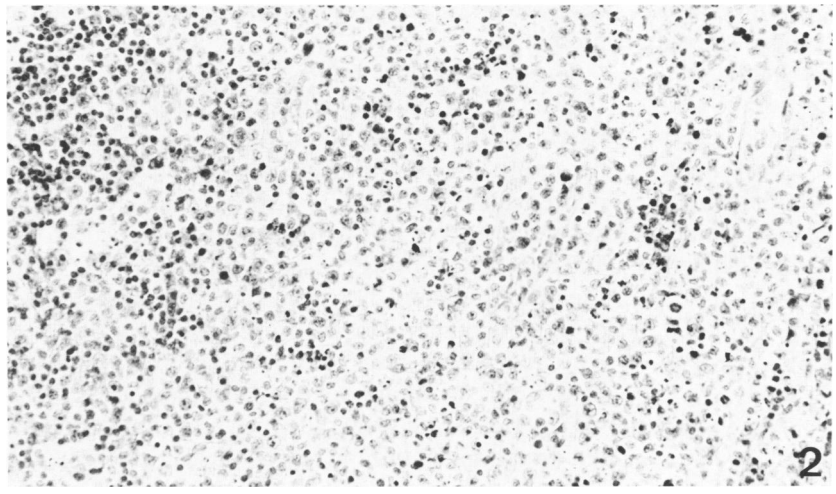
The sections of lymph node from the 15 patients showed similar histologic pictures and exhibited apparent partial obliteration of the normal architecture by a focal proliferation of pale-staining large lymphoreticular cells (Figures 1 and 2). Such focal areas at the cortical or paracortical regions varied in size and number, showing occasionally fusion. They were usually accompanied by deposition of a large number of tingible bodies derived from the nuclear debris of lymphocytes. The exact nature of the large lymphoreticular cells could not be easily identified at the light-microscopic level. Generally they had abundant pale-red cytoplasm and round to oval nuclei, which showed reticular scanty chromatin patterns with one or two distinct nucleoli. Mitosis was frequently observed. There was occasionally coagulation necrosis of the large lymphoreticular cells, a part of which often contained intracytoplasmic phagosomes, including the tingible bodies. Neutrophil infiltrations were not

Table 1—Patients with Subacute Necrotizing Lymphadenitis Examined by Electron Microscopy

Patient	Sex	Age	Clinical diagnosis	Duration of Symptom	Lymphadenopathy	Fever
1	M	17	Lymphadenitis	2 weeks	Systemic	+ +
2	F	17	Tuberculosis	2 weeks	R-cervical	–
3	M	24	Lymphadenitis	1 week	R-cervical	+
4	F	26	Malignant Lymphoma	2 years	Systemic	+ + +
5	F	15	Lymphadenitis	3 weeks	R-cervical	–
6	F	17	Tuberculosis	2 weeks	B-cervical	+ +
7	F	15	Lymphadenitis	3 weeks	L-cervical	–
8	F	25	Unclear	1 week	B-cervical	+ +
9	M	22	Tuberculosis	3 weeks	L-cervical	+
10	F	31	Malignant lymphoma	?	B-cervical	+ +
11	F	20	Lymphadenitis	2 weeks	R-cervical	–
12	M	28	Lymphadenitis	2 weeks	L-cervical	+ +
13	F	15	Lymphadenitis	2 weeks	R-cervical	+
14	F	24	Tuberculosis	4 weeks	R-cervical	–
15	F	26	Lymphadenitis	1 week	B-cervical	+

M = male, F = female, R-cervical = right-cervical, L-cervical = left-cervical, B-cervical = both-sides, cervical.





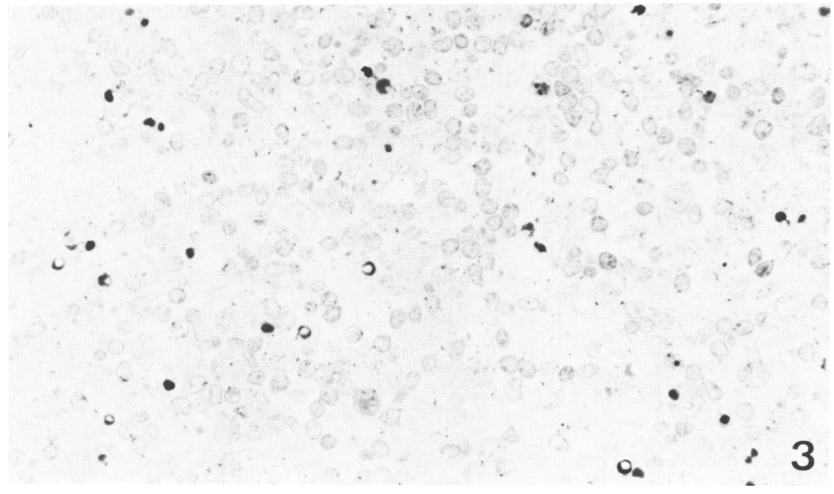


Figure 1—Low-power photomicrograph of subacute necrotizing lymphadenitis. Focal areas at paracortical regions are shown by arrows. (H&E, ×40) **Figure 2**—Photomicrograph of representative focal lesion consisting of large lymphoreticular cells and many tingible bodies. (H&E, ×100) **Figure 3**—Photomicrograph of the semithin section of Epon-embedded tissue. Large lymphoreticular cells and tingible bodies derived from destroyed lymphocytes are visible. (Toluidine blue, ×200)

seen. Diffuse hemorrhage was encountered in some cases.

Parafollicular histiocytosis with a starry-sky appearance was usually seen, but sinus histiocytosis was not prominent. Lymphoid follicles with a germinal center were generally hypoplastic or had disappeared. In several cases a considerable capsular infiltration of lymphocytes was seen. Semithin sections stained with toluidine blue showed that the cytoplasm of the large lymphoreticular cells frequently

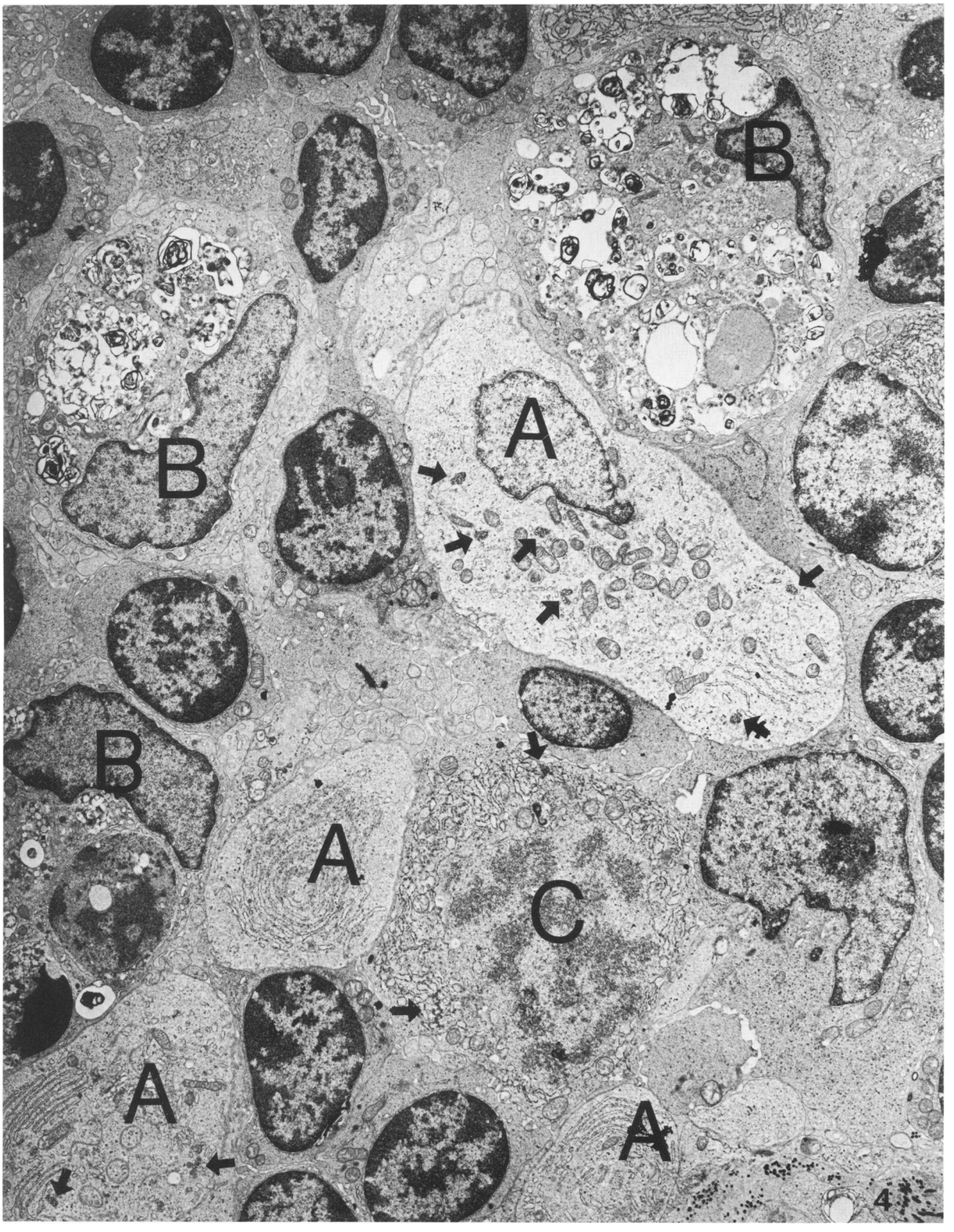


Figure 4—Low-power electron micrograph of representative focal lesion consisting of immunoblasts (*A*), histiocytoid cells (*B*), plasmacytoid cells (*C*) in mitosis, and small lymphocytes. Tubuloreticular structures are indicated by *arrows*. (×5300)

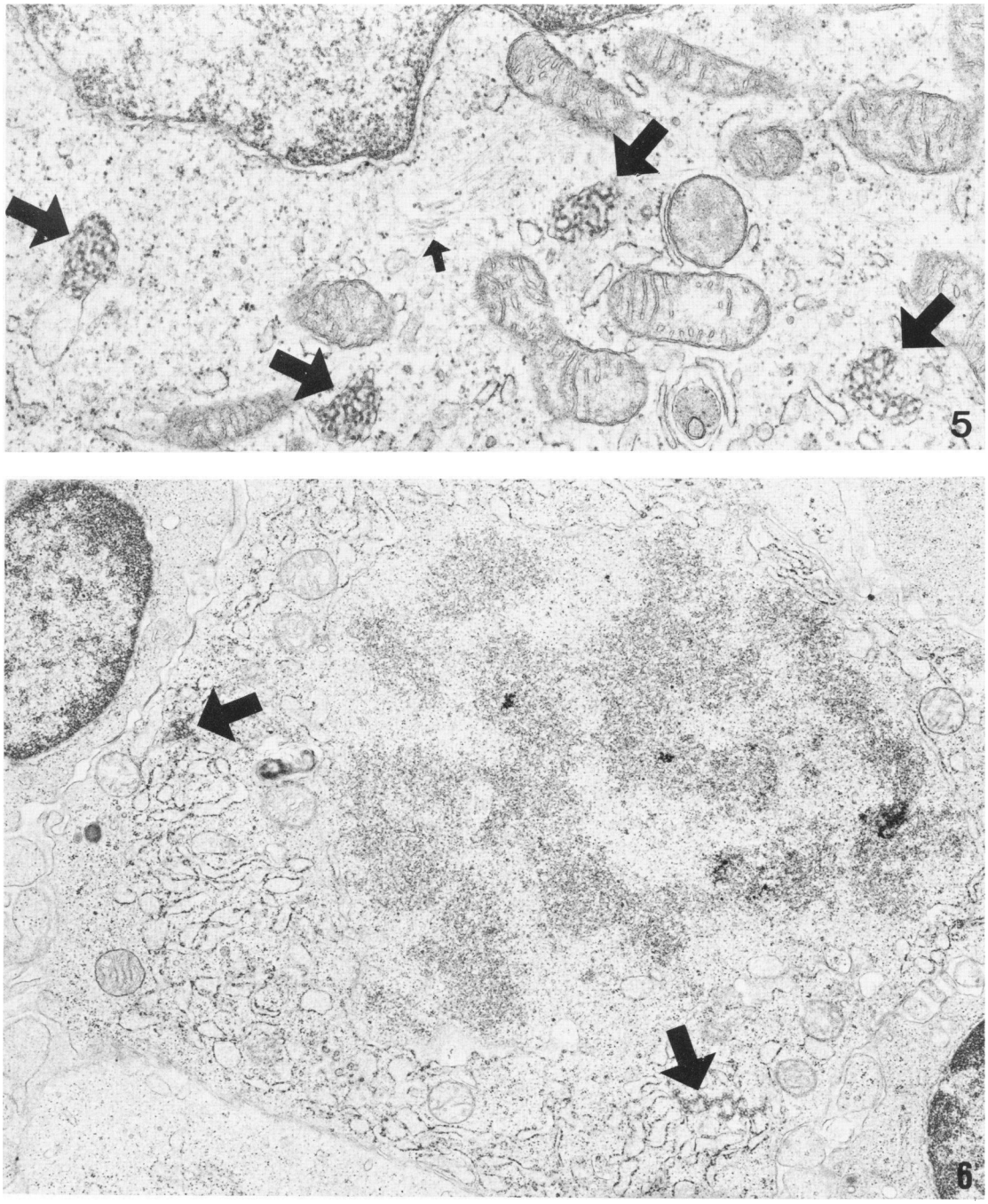


Figure 5 — Higher magnification of the cytoplasm of the immunoblast shown at Figure 4. Four tubuloreticular structures are indicated by *large arrows*. A cluster of rodlike structures 20 nm in diameter is seen near the nuclear membrane (*small arrow*). (×22,000) **Figure 6** — Higher magnification of a plasmacytoid cell in mitosis shown in Figure 4. Two tubuloreticular structures are seen at the periphery of the cytoplasm (*arrows*). (×11,000)

contained numerous extracted lipidlike vacuoles, in addition to the phagocytized tingible bodies in various numbers (Figure 3).

Ultrastructural Findings

All 15 cases were studied by electron microscopy, and no significant difference was noted in the ultrastructural characteristics of the cell and tissue from one lymph node to the next. At first, nature of the large lymphoreticular cells was examined. A typical feature is intensively shown at Figure 4, which contains two types of large lymphoreticular cells. Ultrastructurally, one of them was defined as activated lymphocytes, that is, immunoblasts, although their cytoplasmic features fairly varied from cell to cell. They were of round configuration with smooth cell membranes and measured 10–15 μ in diameter, with an abundant clear cytoplasm containing poly-

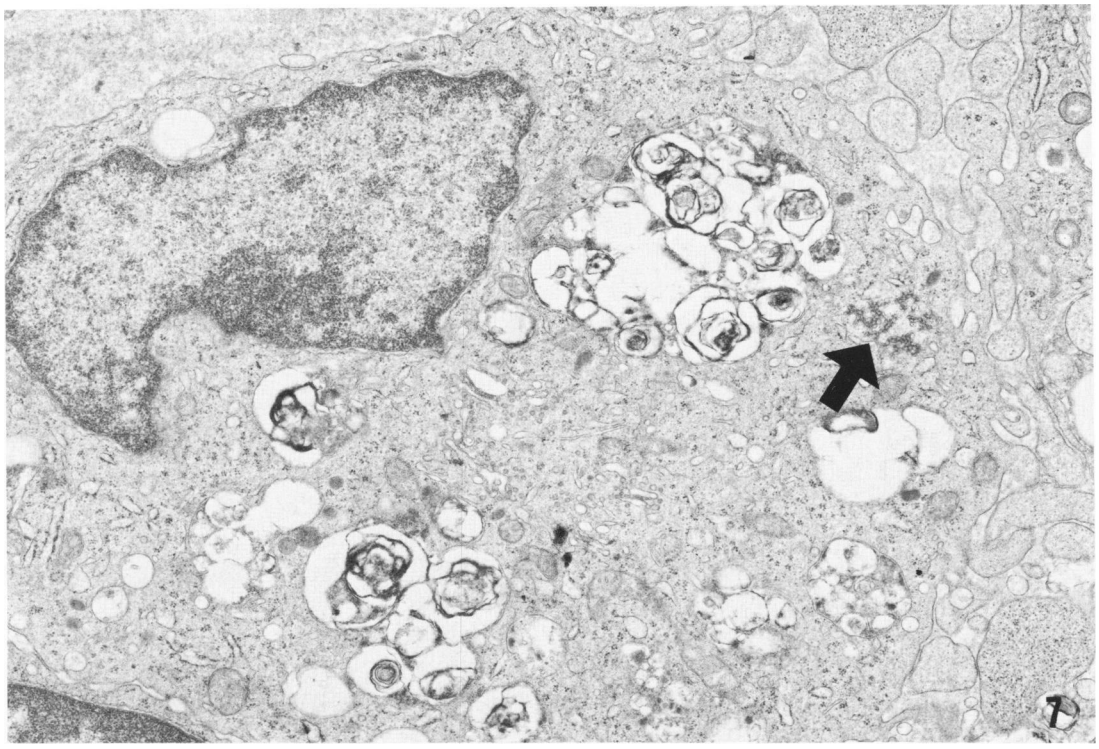


Figure 7—Electron micrograph of a histiocytoid cell containing vacuole-associated myelinlike inclusions and a tubuloreticular structure (*arrow*). (×11,000)

somes, vesicles, and well-developed Golgi apparatus. They also contained rough endoplasmic reticulum and were often characterized by well-developed lamellar rough endoplasmic reticulum located at one side in the cytoplasm. The nucleus, with finely dispersed chromatin, showed a round to ovoid configuration with a prominent nucleolus.

Another type of large lymphoreticular cell was the histiocytoid cell, which was predominantly occupied by numerous large intracytoplasmic myelinlike inclusions associated with cytolysosomes or vacuoles, varying in size. These cells often contained some phagocytized nuclear debris and a few lysosomal granules. Many polysomes and short-size rough endoplasmic reticulum were present, but Golgi apparatus was not so prominent. The nucleus, with relatively dense perinuclear heterochromatin, was usually compressed at one side of the cell, causing a flat configuration. The cellular size did not differ from that of the above-mentioned immunoblasts, and the cellular membrane was generally smooth, with only a few short foot processes.

Besides, many mature lymphocytes and a few plasma cells were seen among the proliferating immunoblasts or histiocytoid cells. Another type of cell with abundant cytoplasm, which was thought to be a typical macrophage, with many cytoplasmic projections and numerous intracytoplasmic lysosomal granules, was only occasionally seen at the periphery on the affected lesions.

Tubuloreticular structures (TRSs), which were characterized by osmiophilic, membrane-bound intertwined tubules about 20 nm in diameter were frequently found in 14 of 15 cases we examined (Figures 5–7). In only 1 case was this structure not seen (Patient 3), in spite of extensive searchings. TRSs were observed within various types of cells, and were most present within the cytoplasm of the immunoblasts, endothelial cells, and immature plasma cells. Two to eight TRSs were often noticed within one cell (Figure 5), and the cells in mitosis occasionally contained TRSs (Figure 6). The cells with numerous myelinlike inclusions, that is, histiocytoid cells, also occasionally contained this structure (Figure 7).

Discussion

Subacute necrotizing lymphadenitis as described here possesses a histologic appearance somewhat similar to that of other lymph node lesions with immunoblastic proliferation,[11–13] but it has additional unique clinical and pathologic characteristics. Details of the clinical and light-microscopic findings and a differential diagnosis have been described elsewhere.[4–10] Briefly, the affected lesions in the lymph nodes consist of numerous immunoblasts and histiocytoid cells,

usually mixed with various numbers of tingible bodies derived from degenerated lymphocytes. Massive coagulation necrosis is often noticed in parts of such lesions. Neutrophils are not seen. This lymphadenitis mainly affects young women of 15 to 35 years and generally has a good clinical course, healing within 2–4 weeks with no treatment. Evidence for toxoplasma or EB virus infection cannot be found in most cases except in a few patients showing a high serum titer against them.[4-6,9,17]

One of the most characteristic histologic features of this lymphadenitis is a diffuse proliferation of the large lymphoreticular pale cells at the cortical or paracortical areas of the lymph nodes. A similar reaction is seen in lymphoid tissues in certain types of infections, including vaccinial lymphadenitis, toxoplasma lymphadenitis, and infectious mononucleosis.[11,13,16] Although there is much controversy and speculation about the origin and nature of such large pale cells, many investigators are of the opinion that they are derived from lymphocytes following reactive changes.[11,13] The present electron-microscopic study has indicated that the proliferating large lymphoreticular cells were largely composed of two types of cells, immunoblasts and histiocytoid cells. The former occasionally showed a tendency to differentiate into plasma cells with well-developed rough endoplasmic reticulum.

Here, the question is whether the cell tentatively described as a histiocytoid cell in the present paper is a true histiocyte or not. Some investigators have pointed out that most of the large lymphoreticular cells characteristic of subacute necrotizing lymphadenitis might be of histiocytic origin, because the lysozyme was present in their cytoplasm.[7] Recently we also reconfirmed this by the immunoperoxidase method.[10] On the other hand, the fine structure of the histiocytoid cells was different from that of mature histiocytes; that is, their size was not so different from that of the immunoblasts, and the cellular membrane was generally smooth, without distinct projections. Their cytoplasm contained many polysomes and short-size rough endoplasmic reticulum in addition to many myelinlike inclusions, but they were only rarely occupied by phagocytosed materials (tingible bodies) or lysosomal granules. A transitional cell between the immunoblasts and the histiocytoid cells was occasionally observed. Thus, we believe that the histiocytoid cells presented here at least differ from mature histiocytes (macrophages) and might originate from activated lymphocytes or immunoblasts. Recent works indicate that T-lymphocyte-derived lymphoma cells can possess many lysosomal granules and often phagocytize cellular and other materials.[18] The possibility that the lysozyme appears within the transformed lymphocytes (immunoblasts) is under active consideration in our laboratory.

A large number of myelinlike inclusions appearing within the cytoplasm of the histiocytoid cells was characteristic of the present study. Similar structures have been observed within lymphoid cells in some other pathologic conditions, including lysosomal storage diseases and sarcoid granuloma (Schaumann's body),[19,20] but their ultrastructural form is somewhat different from that of those in the present study. Although there have been only a few electron-microscopic studies on lymphadenitis showing prominent proliferation of the large lymphoreticular cells,[13] the appearance of the myelin figures has not been described. They seem to be a kind of autophagic body, probably showing a stage of cellular destruction. Here, it might be noted that the cultured cells infected with picornavirus have revealed numerous autophagic vacuoles similar morphologically to the present finding.[21]

The clinical and histologic characteristics of subacute necrotizing lymphadenitis strongly suggest that the disease may result from any infection, but the etiology remains to be resolved. Attempts to separate or identify virus or other causative agents from the lymph nodes with the disease has not been successful.[7-9] No virus or other infectious agent has been seen in the papers mentioning the fine structures of this disease.[5,7,9] Although we found some kinds of the structures simulating virus particles, they were considered nonspecific in every case. Thus, the finding of the tubuloreticular structure, previously associated with the group of autoimmune collagen diseases, may be significant. It should be emphasized that TRSs were frequently observed in every case except 1 patient. We could easily find 10 or more in one field of vision under low magnification during electron-microscopic examination. They were found within the cytoplasm of a wide variety of the cells in focal lesions in the lymph nodes. They were noted with the highest frequency within immunoblasts and were also found within endothelial cells, immature plasma cells and histiocytoid cells. TRSs have been previously reported within the endothelial cells, lymphocytes, and some tumor cells from a variety of human and animal conditions, including SLE and SLE-related autoimmune diseases, neoplasia, and virus infections.[22-25] It is well known that they occur with high frequency in the glomerular endothelium and peripheral lymphocytes of patients with SLE.[24,25] In the lymph nodes, on the other hand, they have been reported only in rare cases of histiocytosis X,[26] malignant lymphoma, and SLE.[24]

It is now widely agreed that the TRS is distinct from any known virus agent.[24,27-31] However, induc-

tion of this structure in human lymphoid cells by exposure to halogenated pyrimidines, which activate latent viruses, has revitalized the question of a viral origin.[29,32] Furthermore, there is a speculation that the TRS could be a widely distributed infectious agent that manifests itself in response to immunologic or related stimuli.[24] It is also known that the antigenicity of the surface membrane of cells infected with the virus could often undergo a change.[33,34] The occasional existence of TRSs in subacute necrotizing lymphadenitis strongly suggests that this disease reflects any SLE-like immunologic event occurring in the lymph nodes. Subacute necrotizing lymphadenitis as presented here might be a reflection of virus-induced autoimmunity and originate from a self-limited host reaction induced against the lymphocytes infected with any lymphocytotropic virus.

Note Added in Proof

Since completion of this work, Fujimoto et al (Acta Pathol Jpn 1981, 701–797) have described the clinical and pathologic findings of 21 patients with subacute necrotizing lymphadenitis.

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