Journal of Korean Medical Science
Vol. 9, No. 1, 9-15 February 1994

Extrafollicular Reticulum Cells in Pathologic Lymph Nodes

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Extrafollicular reticulum cells in lymph nodes are heterogeneous. They express cytokeratins, desmin, and/or vimentin as their intermediate filament profile. Using those markers, we undertook an immunohistochemical study of human lymph nodes under various pathologic conditions. Samples included 15 simple reactive lymph nodes, 7 follicular hyperplasia, 1 necrotizing lymphadenitis, 4 tuberculous lymphadenitis, 13 malignant lymphoma (9 non-Hodgkin's and 4 Hodgkin's lymphomas), and 11 metastatic adenocarcinoma. In lymph nodes with follicular hyperplasia, cytokeratin and/or desmin expressing reticulum cells displayed a characteristic dendritic meshwork in the subcapsular, perisinusoidal, and paracortical regions. In other forms reactive lymph nodes, they were similarly distributed but were less prominent. By SDS-PAGE and immunoblotting, cytokeratin polypeptides were identified. In necrotizing lymphadenitis, they were increased and the pattern of distibution was disturbed. In tuberculous lymphadenitis, they were also increased and located at nongranulomatous as well as in perigranulomatous areas. In lymphomas the reticular meshwork was entirely obliterated. Cytokeratin or desmin expressing reticulum cells were rarely seen within tumors. The reticular meshwork was also obliterated in metastatic carcinoma. However, the meshwork was maintained in uninvolved areas. In conclusion, extrafollicular reticulum cells displayed characteristic patterns of distribution under various pathologic conditions, and may be implicated in the pathogenesis of those pathologic conditions in human lymph nodes.

Key Words : *lymph nodes, extrafollicular reticulum cells, intermediate filaments*

INTRODUCTION

The lymph nodes contain diverse nonlymphoid reticulum cells which apparently provide a supportive meshwork for lymphoid cells (Swartzendurber, 1965 ; De Sousa, 1969 ; Barclay, 1981 ; Tykocinski et al., 1983). In addition to architectural support of lymph nodes, they may be involved in various functions such as lymphocytic stimulation or homing. The nonlymphoid reticulum consists of follicular dendritic reticulum cells and extrafollicular reticulum cells. Extrafollicular reticulum cells form a meshwork at the subcapsular, paracortical, perisinusoidal, and perivascular spaces while follicular dendritic reticulum cells constitute a follicular meshwork. They are distinct from each other not only by their locations but also by their biochemical characteristics. Histochemically, the extrafollicular and follicular reticu-

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This work was partly supported by an Asan Life Science Research Grant.

lum cells are characterized by their relatively high expression of 5'-nucleotidase and alkaline phosphatase activities, respectively (Müller-Hermelink et al., 1981).

As their intermediate filament profile, follicular dendritic cells express vimentin whereas extrafollicular reticulum cells heterogeneously express cytokeratin, desmin, and/or vimentin. Follicular dendritic reticulum cells express desmosomes while extrafollicular reticulum cells do not (Swartzendruber, 1966; Franke and Moll, 1987). No matter what the functional implication of the heterogeneity, the intermediate filaments provide valuable markers to analyze extrafollicular reticulum cells in lymph nodes.

In this article, we have analyzed extrafollicular reticulum cells under various pathologic conditions. The distribution patterns were characteristically disturbed under these conditions, and the possible implication is discussed.

MATERIALS AND METHODS

Tissue

Fifty one lymph nodes were obtained from freshly resected surgical pathology specimens, and are summarized in Table 1. They included perigastric, axillary, pericolic, and pulmonary lymph nodes, and ranged from 5-20mm in diameter. They consisted of 15 simple reactive lymph nodes, 7 follicular hyperplasias, 1 necrotizing lymphadenitis, 4 tuberculous lymphadenitis, 13 malignant lymphomas (9 non-Hodgkin's and 4 Hodgkin's lymphomas), and 11 lymph nodes with metastatic adenocarcinoma. Among non-Hodgkin's lymphomas, 1 follicular mixed, 4 diffuse mixed and 4 diffuse large cell types were included. In Hodgkin's lymphomas, 1 lymphocyte predominance, 2 lymphocyte depletion and 1 nodular sclerosis types were included.

Table 1. Diagnosis of Various Lymph Nodes

Diagnosis	No. of LNs
Reactive Lymph Nodes	22
with Follicular Hyperplasia	7
without Follicular Hyperplasia	15
Pathologic conditions	29
Tuberculous Lymphadenitis	4
Necrotizing Lymphadenitis	1
Malignant Lymphoma	9
follicular, mixed	1
diffuse, mixed	4
diffuse, large cell	4
Hodgkin's lymphoma	4
lymphocytic predominence	1
lymphocytic depletion	2
nodular sclerosis	1
Metastatic adenocarcinoma	11
Total	51

All tissue samples were snap frozen in isopentane cooled in liquid nitrogen, and were kept in a deep freezer at-70℃.

Antibodies

Monoclonal antibodies against various cytoskeletal peptides were used; they inculded anti-cytokeratin 8,18 (CAM 5.2 Becton Dickinson) (Makin et al., 1984), anti-cytokeratin 10, 17, 18 (Dakoppats), anti-desmin (BioGenex) (Debus et al., 1983), and anti-vimentin (BioGenex) (Osborn et al., 1984) antibodies. Biotin-labeled goat anti-mouse immunoglobulin (Dakoppats) was used as secondary antibody.

Immunohistochemical staining

Four μm frozen sections were fixed in cold acetone, and were stained using immunoperoxidase technique. Following preincubation with 0.5% normal horse serum for 20 min. at room temperature, they were covered with the adequately diluted antibodies and were incubated in a moist chamber for 40 min. After washing in cold PBS, sections were incubated with biotin-labeled goat anti-mouse IgG (1 : 200) for 30 min. at room temperature, washed thoroughly in cold phosphate buffered saline (PBS), and then avidin horseradish peroxidase conjugate was applied for 45 min. at room temperature. They were then washed and stained for peroxidase activity using 3,3-diamino-benzidine-tetrahydrochloride (DAB) (Sigma Diagnostics, ST Louis) (Hsu et al., 1981).

Preparation of Cytoskeletal Fractions from Tissue Sample

Cytoskeletal fractions were made from tissue samples following the method of Achstatter et al. (1986). Samples of reactive lymph nodes, necrotizing lymphadenitis and renal cell carcinoma were minced in cold PBS with a scalpel and centrifuged for 5 min at 3000 rpm in the cold. The supernatant

was discarded and the pellet was resuspended in PBS. The pellet was vigorously homogenized with low-salt buffer (10mM Tris-HCl, pH 7.4, 1 Triton X-100, and 150mM NaCl) by Dounce homogenizer and centrifuged. Then the pellet was homogenized again in high-salt buffer (10mM Tris-HCl, pH 7.4, 0.5% Triton X-100, and 1.5M KCl) using a Dounce homogenizer and incubated for 30 min. in the cold room. The pellet obtained after centrifugation was resuspended and was extracted once more as described above. The final pellet was washed and centrifuged, and was used directly for gelelectrophoresis and immunoblotting.

SDS-Polyacrylamide Gel Electrophoresis and Western Blotting

For electrophoretic separation of cytoskeletal proteins, 10% polyacrylamide gel was used. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and protein transfer onto nitrocellulose paper were carried out. Following preincubation in PBS containing 0.05% Tween 20, the nitrocellulose paper was incubated in the same solution containing monoclonal antibodies against cytokeratin and desmin, respectively. After thorough washing, alkaline phosphatase-conjugated goat anti-mouse antiserum (Sigma, 1 : 10000) was applied, and was visualized with alkanine phosphatase substrated (Pierce, USA).

RESULTS

Immunolocalization in Hyperplastic Lymph Nodes

Numerous cytokeratin-expressing reticulum cells were identified in all extrafollicular regions, including the subcapsular, perisinusoidal, and paracortical areas. They were particularly prominent along small blood vessels (Fig. 1A). They displayed various dendritic shapes with long, slender, sometimes branched processes (Fig. 1A). However, no cytokeratin-positive reticulum cells were present in the follicles. In addition to the reticulum cells, some smooth muscle cells in blood vessel wall were also focally positive for cytokeratin. Desmin-positive reticulum cells were relatively less and distributed mainly in paracortical areas away from sinuses and blood vessels (Fig. 1B). These cells tended to have more slender cytoplasmic processes than those of cytokeratin-positive cells. The nuclei of both types of reticulum cells were slightly larger than those of lymphocytes. Most reticulum cells displayed vimentin-immunoreactivity diffusely. The number of those reticulum cells varied considerably among the cases or among different lymph node samples from same patients. Despite the variation, the reticulum

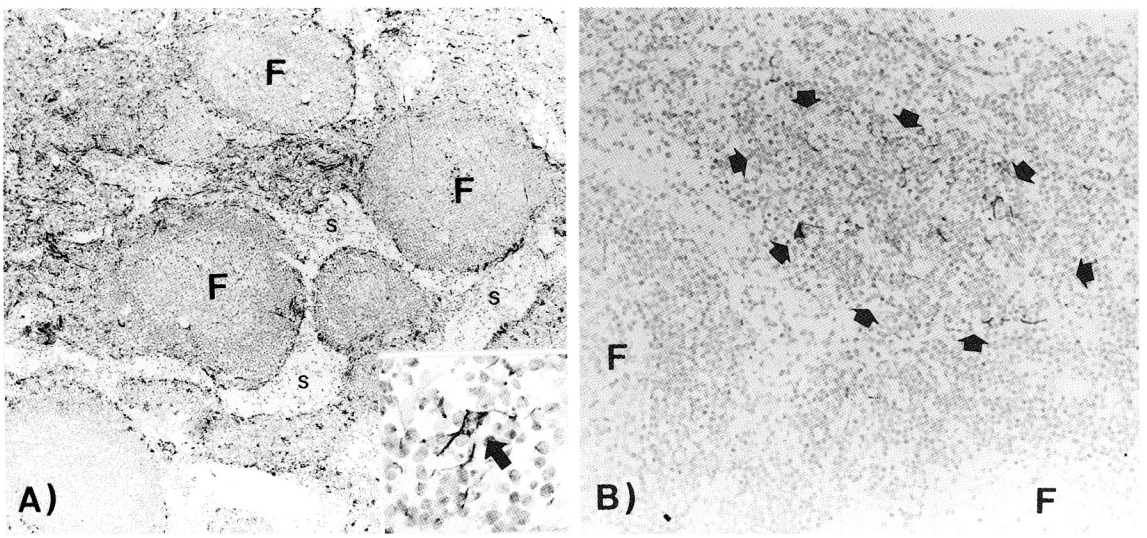

Fig. 1 A-B. The distribution of cytokeratin-and desmin-positive reticulum cells in follicular hyperplasia. A : Cytokeratin-positive cells were prominently seen around the follicles (F) and sinusoids (S). Note the cytokeratin-positive reticulum cells (arrows) showing elongated shape and cytoplasmic processes (inset) ; ABC, ×60 ; inset : ABC, ×400. B : Desmin-positive cells (arrows) in paracortical areas away from sinuses and follicles (F) ; ABC, ×90.

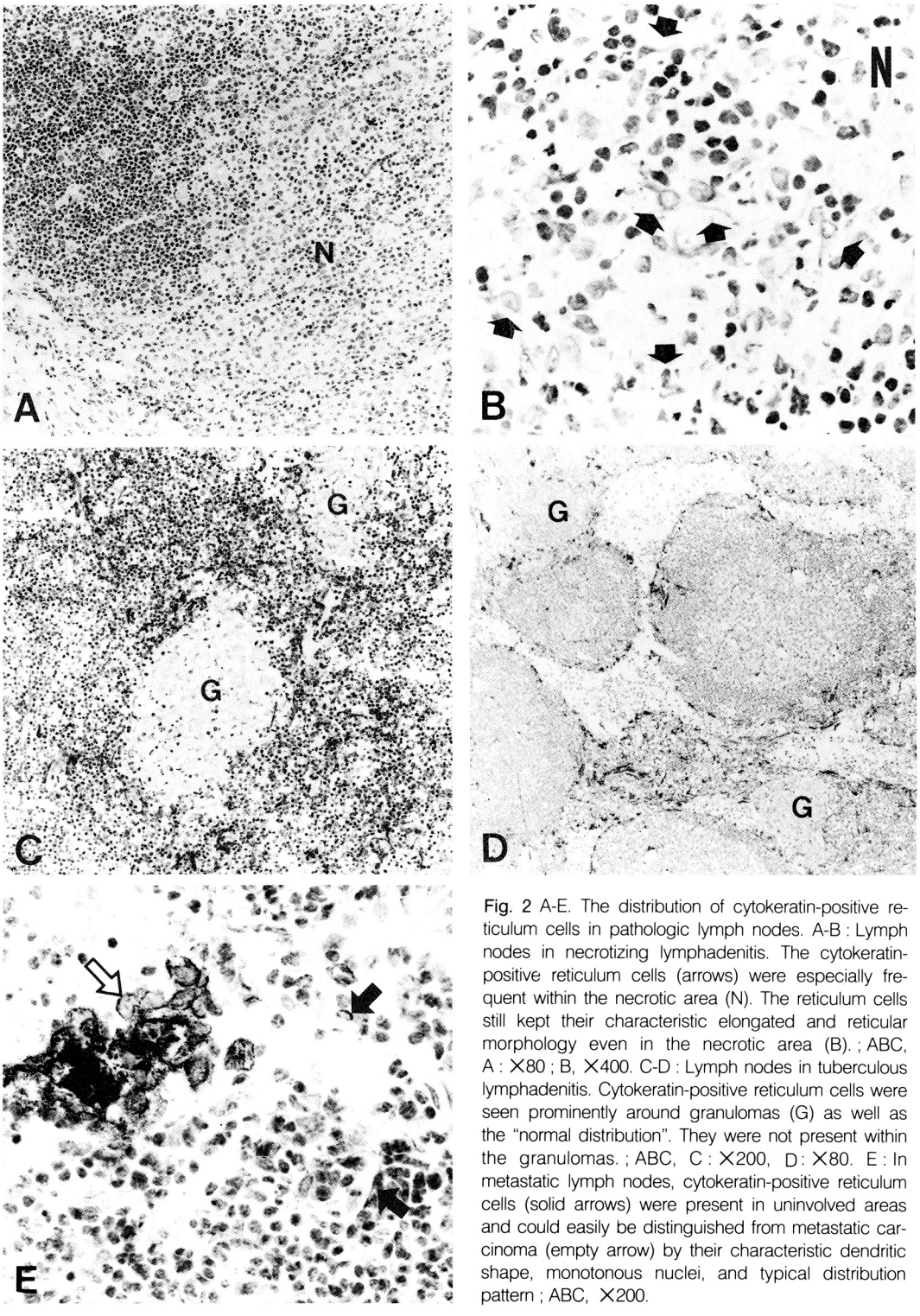

Fig. 2 A-E. The distribution of cytokeratin-positive reticulum cells in pathologic lymph nodes. A-B : Lymph nodes in necrotizing lymphadenitis. The cytokeratin-positive reticulum cells (arrows) were especially frequent within the necrotic area (N). The reticulum cells still kept their characteristic elongated and reticular morphology even in the necrotic area (B). ; ABC, A : ×80 ; B, ×400. C-D : Lymph nodes in tuberculous lymphadenitis. Cytokeratin-positive reticulum cells were seen prominently around granulomas (G) as well as the "normal distribution". They were not present within the granulomas. ; ABC, C : ×200, D : ×80. E : In metastatic lymph nodes, cytokeratin-positive reticulum cells (solid arrows) were present in uninvolved areas and could easily be distinguished from metastatic carcinoma (empty arrow) by their characteristic dendritic shape, monotonous nuclei, and typical distribution pattern ; ABC, ×200.

cells were present more prominently in follicular hyperplasia than in simple reactive lymph nodes.

Immunolocalization in lymphadenitis and tumors

Cytokeratin expressing reticulum cells were also prominently seen in necrotizing lymphadenitis. They displayed typical dendritic shape as in hyperplastic lymph nodes. While they were distributed diffusely, they were especially frequent within the necrotic area (Fig. 2A-B). Desmin-positive cells were also present, especially in the peripheral zone of the necrosis. As were the cytokeratin-expressing cells, they were more prominent than those of reactive lymph nodes.

Both cytokeratin-and desmin-positive reticulum cells were found in the tuberculous lymphadenitis (Fig. 2C-D). Cytokeratin-positive cells were seen prominently around granulomas as well as the "normal distribution". Usually they were not present within the granulomas. Only large granulomas contained a few scattered cytokeratin-or desmin-positive reticulum cells. While cytokeratin-positive cells were increased in number compared to those of reactive nodes, desmin-positive cells were rather sparsely distributed in interfollicular areas.

In both non-Hodgkin's and Hodgkin's lymphomas, the normal meshwork of reticulum cells was entirely obliterated. Only a few cytokeratin-positive reticulum cells were distributed around periphery of the blood vessels. Desmin-positive reticulum cells were also rarely seen.

In 11 metastatic lymph nodes (Fig. 2E) extrafollicular dendritic cells were also present in uninvolved areas. There was great variation in the number of positive cells among samples. The cytokeratin-positive reticulum cells could be easily distinguished from metastatic carcinoma by their characteristic dendritic shape, monotonous nuclei, and typical distribution pattern. Desmin-positive cells appeared to be decreased and were rarely observed in the paracortical areas.

Gel Electrophoresis and Immunoblotting

To identify the specific cytokeratin and desmin polypeptides, cytoskeletal fractions of reactive lymph nodes, and necrotizing lymphadenitis were analyzed by SDS-PAGE and immunoblotting with anti-cytokeratin and anti-desmin antibodies. Cytokeratin polypeptides were classified following Moll et al., (1982). Cytokeratin polypeptide was detected in both samples as were in a positive control sample (renal cell carcinoma) (Fig. 3). Desmin was also detected in every fraction.

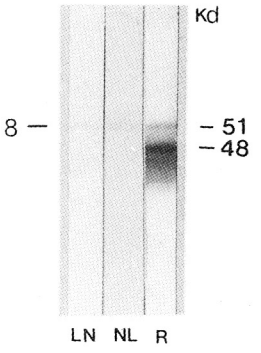

Fig. 3. Immunoblotting detection of reactive lymph nodes (LN) and necrotizing lymphadenitis (NL) for cytokeratin protein using monoclonal anti-cytokeratin 8 and 18. Cytokeratin 8 (left hand side) was detected in both reactive lymph nodes and necrotizing lymphadenitis. Tissue from renal cell carcinoma (R) was used for positive control.

DISCUSSION

Extrafollicular reticulum in lymph nodes consists of a meshwork of nonlymphoid cells. They have been described as fibroblastic reticulum cells (Tykocinski et al., 1983), reticular fibroblasts (Van Vilet et al., 1986), or stromal cells (Toccanier et al, 1987). With the advent of various phenotypic markers, it became obvious that they comprised a variety of heterogeneous cells. Recently, extrafollicular reticulum cells expressing markers of myoid differentiation such as desmin and alpha smooth muscle specific actin have been described (Folse et al., 1975 ; Tykocinski et al., 1983 ; Pinkus et al., 1986 ; Norton et al., 1987 ; Toccainer et al., 1987). Furthermore, extrafollicular reticulum cells expressing cytokeratin have also been described (Franke and Moll, 1987).

A subset of extrafollicular reticulum cells expressed cytokeratin in all lymph node samples. The expression of cytokeratin has been regarded as a hallmark of epithelial differentiation (Schlegel et al., 1980 ; Franke et al., 1982 ; Gown et al., 1984). Embryologically, however, extrafollicular reticulum cells can hardly be regarded as epithelial cells. Furthermore, they do not express desmoplakins while most true epithelial cells do. In addition to extrafollicular reticulum cells, there were scattered

vascular smooth muscle cells expressing cytokeratin in many lymph nodes. However, it was focal and limited, and may be regarded as a reactive process under various pathologic conditions.

Cytokeratin-positive reticulum cells may coexpress desmin or vimentin. However, cytokeratin and desmin-positive cells displayed different patterns of distribution in the same lymph nodes. Also, their cytological appearances were slightly different. Based on this evidence, it could be concluded that they are distinct cell populations despite some overlap.

Lymph nodes are one of the most dynamic organs. Cell populations may vary considerably under various stimuli. It was not easy to study "truly normal" lymph nodes. Among the reactive lymph nodes, however, extrafollicular reticulum cells in follicular hyperplasia were more prominent than those in lymph nodes with sinus histiocytosis or paracortical hyperplasia. In follicular hyperplasia, there was prominent proliferation of cytokeratin and/or desmin expressing reticulum cells at perifollicular areas. Similarly, they were present prominently at perigranulomatous areas in tuberculous lymphadenitis. It may be assumed that they are involved in formation of new structures such as follicles or granulomas. It was of interest that, in necrotizing lymphadenitis, the reticulum cells expressing cytokeratin and/or desmin were prominently seen not only in normal extrafollicular spaces but also in "necrotic areas". In necrotic areas, the reticulum cells still kept their characteristic elongated, reticular morphology. It may be suggested that in necrotizing lymphadenitis only lymphoid cells undergo necrosis, at least initially, while the reticulum cells survive for a while.

Lymphomas represent malignant transformation of lymphoid cells. Interestingly, however, the normal extrafollicular reticulum was entirely obliterated in all lymphomas as it was in metastatic carcinoma. It may be that extrafollicular reticulum is a delicate structure and is maintained by a close and steady collaboration between lymphoid cells and reticular cells themselves.

Metastatic carcinomas also expressed cytokeratin. However, they usually formed solid nests of epithelial cells, and could easily be differentiated from extrafollicular reticulum cells. They existed in reticulum, and even though the normal reticular pattern was disrupted by various pathologic conditions, they maintained a characteristic thin, elongated appearance. It should be mentioned that mere presence of cytokeratin-positive cells in lymph nodes does not necessarily represent a metastatic carcinoma.

In conclusion, extrafollicular reticulum cells display characteristic reactive patterns under various reactive, inflammatory, and neoplastic conditions. It still remains to be understood how the reticulum cells are involved in the pathogenesis of those diseases.

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