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## Abstract

Using the peroxidase antiperoxidase (PAP) method, lysozyme (LZM) was shown to exist in normal, reactive and neoplastic cells belonging to the mononuclear phagocyte system (MPS), but was not detected in histiocytosis X cells. Immunostaining for cytoplasmic LZM by the PAP method is useful for identification of mononuclear phagocytes and for diagnosis of the diseases in which these cells participate.

**KEYWORDS:** lysozyme, PAP method, mononuclear phagocyte system

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## IMMUNOHISTOCHEMICAL DEMONSTRATION OF LYSOZYME IN NORMAL, REACTIVE AND NEOPLASTIC CELLS OF THE MONONUCLEAR PHAGOCYTE SYSTEM

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*Abstract.* Using the peroxidase antiperoxidase (PAP) method, lysozyme (LZM) was shown to exist in normal, reactive and neoplastic cells belonging to the mononuclear phagocyte system (MPS), but was not detected in histiocytosis X cells. Immunostaining for cytoplasmic LZM by the PAP method is useful for identification of mononuclear phagocytes and for diagnosis of the diseases in which these cells participate.

*Key words :* lysozyme, PAP method, mononuclear phagocyte system.

In the past certain cellular systems having defence activity were designated as the "macrophage system" of Metchnikoff (1) and the "reticulo-endothelial system" of Aschoff (2). In 1972, R. van Furth *et al.* (3) proposed a new system of phagocytic cells under the name "mononuclear phagocyte system (MPS)". According to this concept (3), the tissue macrophages, which include histiocytes in the connective tissue, Kupffer cells in the liver, alveolar macrophages in the lung, free and fixed macrophages in the lymph nodes and spleen, macrophages in the bone marrow, pleural and peritoneal macrophages in the serous cavities, osteoclasts in the bone tissue and microglial cells in the nervous system, are derived from monocytes which in turn arise from the promonocytes in the bone marrow (Fig. 1).

Mononuclear phagocytes participate in various disorders such as inflammatory processes, neoplastic conditions and storage diseases (4). By morphology alone, reliable identification of these cells in various disorders is not always easy, because they often change their shape during each maturation and functional phase, and also in neoplastic conditions. Besides tissue macrophages (histiocytes), LZM has been detected in myeloid cells and mucus-producing epithelial cells but not in lymphoid cells (5).

The immunoperoxidase method recently proposed by Sternberger *et al.* (7) can be successfully applied to the identification of LZM in various tissues, even in formalin-fixed paraffin sections (5, 7-10).

The purpose of the present study is to systematically analyze mononuclear

phagocytes in normal tissues and in tissues of various disorders using this method for identifying LZM.

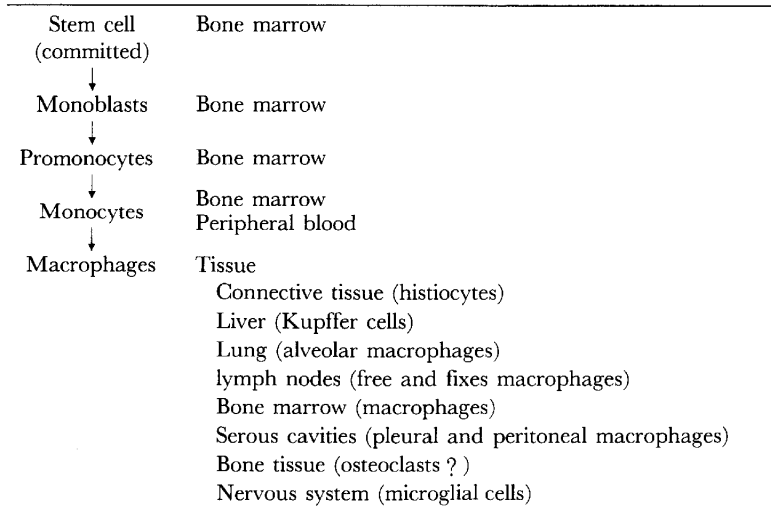


Fig. 1. The mononuclear phagocyte system proposed by van Furth *et al.* (3).

#### MATERIALS AND METHODS

Paraffin-embedded blocks of several different tissues fixed in formalin were drawn from the surgical histology files of the Second Department of Pathology, Okayama University Medical School (Table 1).

The blocks were cut into 3  $\mu$  sections which were stained for the presence of LZM using the immunoperoxidase technique (PAP method) described previously (11). Rabbit anti-human LZM sera, PAP complex, normal sheep serum and sheep anti-rabbit IgG were obtained from

TABLE 1. DIAGNOSES MADE ON LYMPH NODE BIOPSIES

| Diagnosis                                  | No. of cases |
|--|--------------|
| Sarcoidosis                                | 2            |
| Tuberculosis                               | 2            |
| Piringer's lymphadenitis                   | 2            |
| Wegener's granulomatosis                   | 1            |
| Massive hemophagocytic sinus histiocytosis | 1            |
| Gaucher's disease                          | 1            |
| Niemann-Pick's disease                     | 2            |
| Monocytic leukemia                         | 5            |
| Histiocytic reticulum cell sarcoma         | 7            |
| Hodgkin's disease                          | 8            |
| Histiocytosis X                            | 10           |
| Malignant fibrous histiocytoma             | 5            |

Dakopatts, Denmark (Japan agent : Kyowa Medix, Tokyo). Anti-LZM serum was diluted 1 : 200 , anti-rabbit IgG, 1 : 50, and the PAP-complex, 1 : 40. Trypsinization was carried out according to the method described by Curren *et al.* (12). Undiluted normal sheep serum was used for pretreatment to reduce nonspecific binding of subsequently applied test antisera.

## RESULTS

### *Distribution of LZM-positive Cells in Normal Tissues.*

*Bone marrow.* Sections of bone marrow revealed a large number of LZM-positive cells. All morphologically recognized neutrophils, eosinophils and macrophages were positive for LZM. Other positive mononuclear cells were difficult to identify, but it was supposed that they included some mature or immature myeloid cells (Fig. 2). Megakaryocytes were negative for LZM.

*Connective tissue (histiocytes).* A small number of cells positive for LZM were scattered throughout the dermis of the skin. From their size and shape they seemed to be histiocytes inactively distributed in normal skin. Fibroblasts were negative as were Langerhans' cells in the epidermis.

*Liver (Kupffer cells).* In the liver, Kupffer cells were strongly positive for LZM (Fig. 3). Endothelial cells of the sinusoidal capillaries and Itoh's cells were negative.

*Lung (alveolar macrophages).* Most alveolar macrophages which appeared in the lungs of various inflammatory processes or circulatory disturbances were weakly positive for LZM.

*Lymph nodes and spleen (free and fixed macrophages).* LZM was detectable in macrophages in the sinus of hyperplastic lymph nodes (sinus histiocytosis), but negative in inactive lymph nodes. Macrophages in the germinal centers were occasionally weakly positive for LZM (Fig. 4). Sinus lining cells showed a negative or faintly positive reaction.

In a case of a ruptured spleen by trauma, LZM was detected in fixed and free macrophages (Fig. 5). Eosinophils and neutrophils were positive for LZM.

*Serous cavities (pleural and peritoneal macrophages).* Pleural and peritoneal macrophages, which adhered to the serosal surface of organs were examined. These macrophages in serous cavities were weakly positive or negative for LZM.

*Bone tissue (osteoclasts).* Non-neoplastic parts of the bone destroyed by infiltration of malignant lymphoma were examined. Osteoclasts were easily recognized around degenerated bone tissue. These cells showed faintly positive staining for LZM.

*Nervous system (microglial cells).* Six specimens of non-neoplastic brain cortex taken from 3 cases of astrocytoma were examined. In all cases, LZM-positive cells were recognized mainly around the small blood vessels and hemorrhagic areas (Fig. 6). A small number of positive cells were also found around the nerve cells. Besides neutrophils, two kinds of cells were recognized : small cells with irregular nuclei, and scanty cytoplasm, and large macrophages. The former appeared immature and reacted for LZM stronger than the large macrophages.

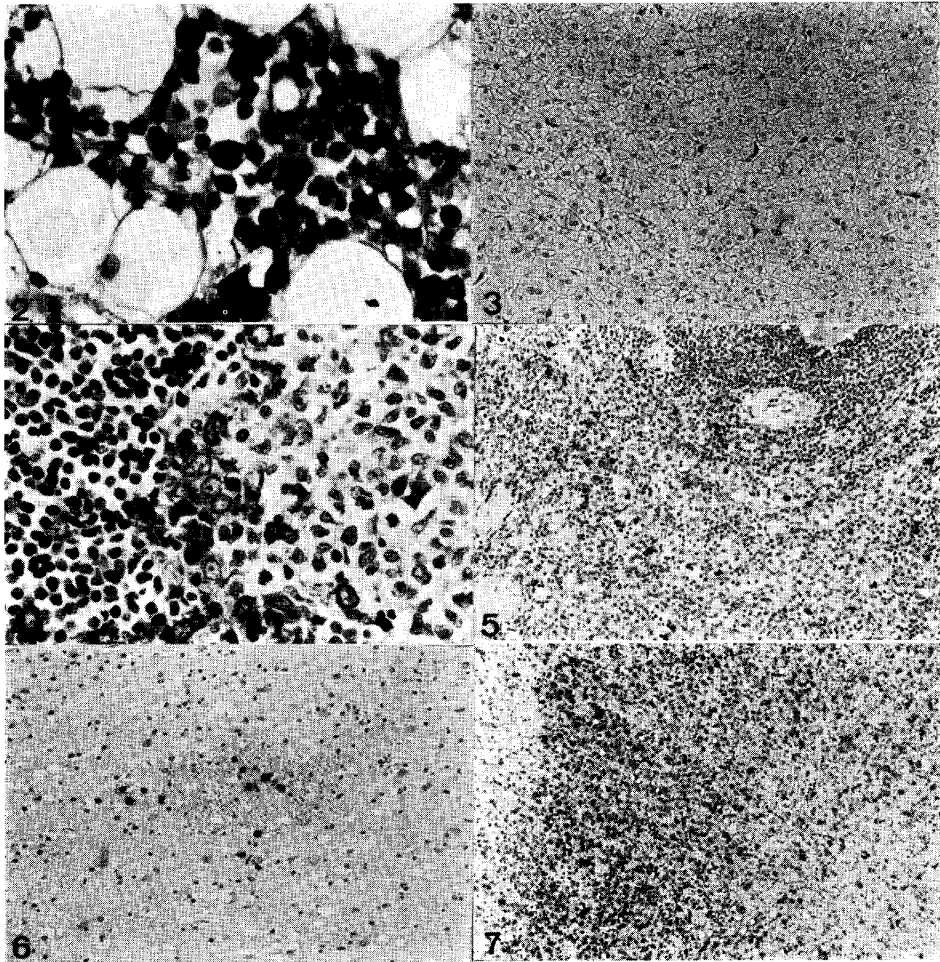


Fig. 2. Most mature and immature cells in the bone marrow are positive for LYM.  $\times 400$ .

Fig. 3. LYM-positive Kupffer cells.  $\times 100$ .

Fig. 4. A small number of histiocytes in the germinal center of a lymph node are positive for LYM.  $\times 400$ .

Fig. 5. LYM-positive histiocytes are scattered in the red pulp of the spleen.  $\times 100$ .

Fig. 6. LYM-positive histiocytes (so-called microglia) are present around the hemorrhagic area of the brain cortex.  $\times 100$ .

Fig. 7. Epithelioid cells in a tuberculous lesion are positive for LYM.  $\times 100$ .

#### *Distribution of LYM-Positive Cells in Pathological Tissues.*

*Reactive proliferation in inflammatory diseases.* In lymph nodes affected with sarcoidosis and with tuberculous lymphadenitis, most of the epithelioid histiocytes and multinucleated giant cells of Langhans type in or around the tubercles were slightly or moderately positive for intracytoplasmic LYM (Fig. 7).

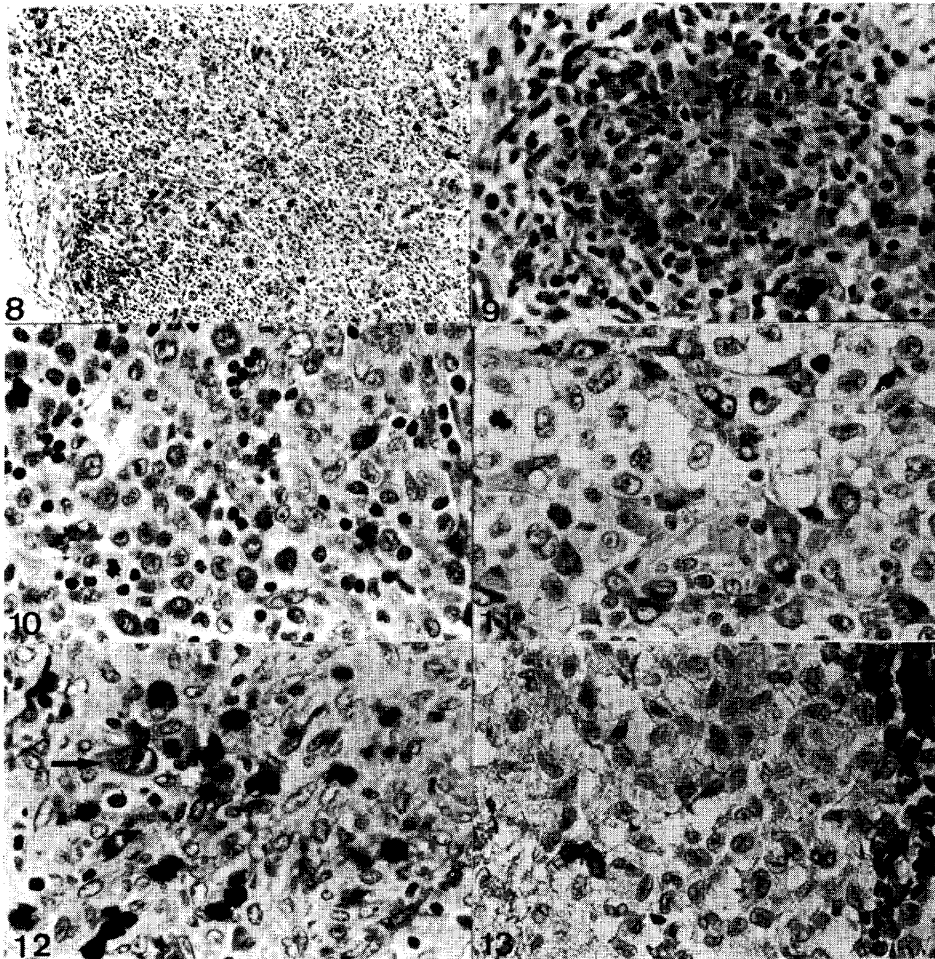


Fig. 8. Many clustered epithelioid cells in Piringir's lymphadenitis are positive for LZM.  $\times 100$ .

Fig. 9. Histiocytes in Wegener's granuloma are positive for LZM.  $\times 200$ .

Fig. 10. Leukemic cells of monocytic leukemia are positive for LZM.  $\times 400$ .

Fig. 11. Some tumor cells in histiocytic reticulum cell sarcoma are positive for LZM.  $\times 400$ .

Fig. 12. Histiocytes in Hodgkin's disease are positive for LZM, but a Hodgkin's cell (arrow) is negative.  $\times 400$ .

Fig. 13. Histiocytosis X cells are negative for LZM.  $\times 400$ .

Epithelioid type histiocytes in lymph nodes with Piringir's lymphadenitis, which were present in small clusters in the interfollicular areas, showed moderate to strong staining for cytoplasmic LZM (Fig. 8). Sinus histiocytes were slightly positive for LZM. Most of the reactive germinal centers contained a small number of LZM-positive macrophages.

In one patient with Wegener's granuloma, histiocytes in the granulomatous

lesions were also positive for LZM (Fig. 9).

Large, prominently erythrophagocytized histiocytes in the lymph node with "sinus histiocytosis with massive lymphadenopathy" (ROSAI and DORFMANN) were negative or faintly positive for LZM. Coexistent histiocytes, which showed no phagocytosis, were moderately positive.

*Storage diseases.* Most of lipid-containing phagocytes and a few giant cells in 1 case of Gaucher's disease and 2 cases of Niemann-Pick's disease were negative for LZM, but a few were diffusely positive. On the contrary, lipid-free histiocytes were strongly positive for LZM.

*Neoplastic proliferation.* In lymph nodes from patients with monocytic leukemia, immature and mature forms of both monocytic and myeloid cells were positive for LZM (Fig. 10). The number of positive leukemic cells varied from case to case.

In seven cases of histiocytic reticulon cell sarcoma or malignant histiocytosis (MH) of the lymph nodes, medium-sized neoplastic histiocytes had intracytoplasmic LZM, but large-sized neoplastic histiocytes were negative (Fig. 11). In 2 cases of the large cell type of MH (histiocytic medullary reticulosis by Robb & Smith), tumor cells were negative or faintly positive for LZM. The number of LZM-positive cells varied from case to case.

Eight cases of Hodgkin's disease of various histologic subtypes had many LZM-positive cells, such as eosinophils, neutrophils, epithelioid cells and histiocytes, which were positive for LZM (Fig. 12), in contrast to Hodgkin's and Reed-Sternberg's cells which were negative.

In many cases of histologically proved histiocytosis X (eosinophilic granuloma, Hand-Schüller-Christian disease, or Letter-Siwe disease) the lymph nodes were frequently involved with other organs. The histological structure of the affected lymph nodes was partially preserved but widely occupied by characteristic histiocytosis X (HX) cells. These cells were of large or intermediate size and had round and invaginated nuclei. Infiltration of eosinophils and giant cells as well as necrosis were found in many cases. By the PAP technique, LZM could not be detected in these histiocytosis X cells (Fig. 13). In addition, LZM-positive cells, larger than histiocytosis X cells were observed around necrotic foci intermingled with eosinophils or other inflammatory cells. These cells had abundant eosinophilic cytoplasm and frequently showed phagocytosis, suggesting that these cells were histiocytic reticulum cells.

Five cases of malignant fibrous histiocytoma (MFH) included both fibroblast-like and histiocyte-like cells. A small number of tumor cells in these cases were slightly positive for LZM. LZM was detected only in histiocyte-like cells having pleomorphic nuclei and eosinophilic cytoplasm, but not in fibroblast-like cells, some of which were arranged in a striform pattern.



## DISCUSSION

The mononuclear phagocyte system (MPS) (3) includes promonocytes and their precursors in the bone marrow, monocytes in the peripheral blood, and macrophages in various tissues (Fig. 1).

Among these cells, the presence of LZM in promonocytes and their precursor cells, monocytes in the peripheral blood, Kupffer cells and free and fixed macrophages in the lymph nodes has been well established (5, 9, 11). We confirmed the existence of LZM in such cells. However, as to the other cells belonging to the MPS, an examination for the presence of LZM has not been done.

In macrophages in connective tissue (histiocytes), LZM was detectable. This finding supports the evidence that histiocytes in connective tissue belong to the MPS. However, it remains obscure whether histiocytes arise from monocytes of the blood or from unidentified precursors in the connective tissue itself.

Alveolar macrophages in the lung and macrophages in serous cavities showed a faintly positive reaction for LZM. LZM was also faintly positive in the starry sky cells in the germinal center of lymph nodes. In mature macrophages it may be considered that LZM has been exhausted or the cellular content of LZM has decreased with maturation.

Osteoclasts as well as myeloid cells in decalcified specimens usually showed faintly positive staining for LZM. The procedure for decalcification using acidic solution may be not suitable for LZM detection.

In Kupffer cells of the liver, LZM was strongly positive, but endothelial cells of the sinusoids and Itoh's cells were completely negative for LZM.

The reticulum cells which constitute lymphoid tissues were recently classified by morphology and cytochemical examination into 4 types: histiocytic, fibroblastic, interdigitating and dendritic (13). In these reticulum cells, LZM was detectable in histiocytic ones, but not in the other 3 types (14). This result suggests that the former may be fixed macrophages which migrated from the bone marrow into the lymphoid tissues in the early developing stage.

There is a variety of diseases in which mainly monocytes and tissue macrophages participate. Mononuclear phagocytes may proliferate appropriately in response to various kinds of stimuli (reactive proliferation), increase in number and accumulate intracellularly abundant phagocytized materials in storage diseases, and proliferate autonomously (neoplastic proliferation).

Reactive histiocytes in inflammatory states and storage diseases showed varying stainability for intracytoplasmic LZM according to their functional activity. Especially, epithelioid histiocytes proliferating in Piringer's lymphadenitis, tuberculosis and sarcoidosis were strongly positive for LZM. However, histiocytes in the fully phagocytizing phase had generally little or no LZM, indicating that LZM is nearly exhausted in such histiocytes.

The presence of LZM positive tumor cells in monocytic leukemia, histiocytic reticulum cell sarcoma and some cases of MFH have already been reported by

several authors (5, 6, 9, 10, 14-16). Also in our study, demonstration of LZM in the tumor cells was very useful for the distinction of these tumors from others having similar histological findings.

The origin of Hodgkin's cells or Reed-Sternberg's cells is controversial at present, but that LZM is not detectable in these cells suggests that they do not originate from histiocytes.

Until recently, various types of HX have been considered to be histiocytic disorders (17). However, HX cells are characterized by having no LZM and thus can be distinguished from the usual type of histiocytes. In addition, S100 protein specific for neuroepithelial cells has recently been demonstrated in HX cells, but not in usual histiocytes (18). This evidence indicates that histiocytes can be categorized into two kinds : LZM-positive, S100-negative histiocytes and LZM-negative, S100-positive histiocytes.

The results obtained in our study show that the demonstration of LZM by the PAP method is valuable in identifying histiocytes derived from MPS and clarifying the pathogenesis of various disorders of MPS, including histiocytic neoplasma.

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