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A histochemical study of hydrolytic and oxidative enzymes in an eosinophilic granuloma of parotid gland region*

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Abstract

We experienced a case of eosinophilic granuloma in soft tissue, and demonstrated its patterns of hydrolytic and oxidative enzymes histochemically. Neutrophils were rich in acid phosphatase and glucose-6-phosphate dehydrogenase. Eosinophils had much acid phosphatase and less other hydrolytic and oxidative enzymes. Lymphocytes showed weak reaction in all enzymes. Lymph follicles and histiocytes or fibrocytes had moderately oxidative enzymes. Small blood vessels and collagen fibers were rich in alkaline phosphatase and had a moderate amount of oxidative enzymes and acid phosphatase.

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**A HISTOCHEMICAL STUDY OF HYDROLYTIC AND OXIDATIVE
ENZYMES IN AN EOSINOPHILIC GRANULOMA
OF PAROTID GLAND REGION**

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Since KATAYAMA reported a case of eosinophilic granuloma in soft tissue in 1909, we have 47 cases reported in Japan. As far as we know, there is no report of this disease in other countries. Recently the eosinophilic granuloma of soft tissue was histologically distinguished from Miculicz's disease, eosinophilic granuloma of the bone and the skin by IZUKA,² WATANUKI *et al.*³ and TOKORO.⁴ According to WATANUKI *et al.*³ over 90 per cent of the disease occurred in male, mostly in the age range of 10 to 30 years. Usually it develops at salivary gland or lachrymal gland region. Moreover, it does occur at the soft tissues of neck, supraclavicular region, axillary region, inguinal region, elbow, upper leg, cheek, temporal part, occipital part, breast and ear, and at the superficial lymph nodes of these regions. The chief complaint is tumor or swelling. And other complaints are sometimes of itching, or exophthalmus or the complaint which might be caused by purulent inflammations. In our case purulent inflammation occurred twice. In laboratory study, a slight increase in erythrocyte sedimentation reaction and leucocytosis were often observed, and more often eosinocytosis.

The treatment for such cases is complete removal of tumor. WATANUKI *et al.*³ recommends radiation therapy, adrenocorticoid hormones, antitumor agents and parotin as local recurrence is apt to occur. Even if the disease escapes detection and is left untreated, the tumor does not affect physical condition and does progress to malignant tumor.

KATAYAMA¹ and SHOJI⁵ believe that the pathogeny is related to pseudoleukemia, but UMEMURA *et al.*⁶ consider the pathogeny as allergy of bacteriological or parasitic inflammation. KIMURA *et al.*^{7,8} think one of its causes as an endocrine disorder of parotid gland. From the two facts that the disease occur mainly in male, and that from 6 month of pregnancy in TANAKA's case the swelling began to decrease but after child birth the tumor increased again, we suspect some

relationship between this disease and sex hormones.

According to histopathological findings KIMURA *et al.*^{7,8} designated the disease as "eosinophilic lymphoid granuloma" while TSUKAMOTO⁹ as "eosinophilic lymphadenitis". Recently TOKORO⁴ precisely has observed its histology in detail and proposed to name it "eosinophilic lymphfolliculo-hyperplastic panniculitis (or panniculosis)" or "eosinophilic folliculohyperplastic panniculolymphadenitis". IZUKA² is of the opinion that the disease had better be called "KIMURA's disease".

Large numbers of histochemical observations on tumors have been reported, however, there is no report on an enzymatic histochemical study of this rare entity. In the present study various kinds of hydrolytic and oxidative enzymes have been studied, and the activity of these enzymes in eosinophilic granuloma is comparable to that of lymphnode and connective tissue.

MATERIALS AND METHODS

After the surgical extirpation, without fixation the granuloma specimen was quickly frozen with dry ice, then it was cut at 15μ in a -20°C cryostat using a sliding microtome. For the routine histologic observation a part of tumor was fixed in 10% formol and paraffin sections were prepared. For the histochemical demonstration of hydrolytic enzymes, the tissue sections were fixed in 10% formalin and rinsed in distilled water, then they were incubated by the following procedures.

Alkaline phosphatase: 10 mg of sodium alpha-naphthyl phosphate were dissolved in 20 ml of CLARK and LUB's buffer at pH 9.2 and 20 mg of fast blue B were added. The sections were incubated at 20°C for 30 minutes dehydrated and mounted in balsam.

Acid phosphatase: 10 mg of sodium alpha-naphthyl phosphate were dissolved in 20 ml of acetate buffer at pH 5.8, to which 20 mg of fast blue B were added. Incubation time was one hour at 20°C . Sections were dehydrated and mounted in balsam.

Esterase: 5~10 mg beta-naphthyl acetate were dissolved in 1 ml of acetone, and 20 ml of Michaelis buffer at pH 7.2 and 20 mg of fast blue B were added. Incubation was carried out at 20°C for 30 minutes. The slides were mounted in glycerin.

Beta-Glucuronidase: The method of Seligman *et al.* (1954) was applied, in which 6-bromo-2-naphthyl-beta-D-glucuronide was used as a substrate.

Aminopeptidase: The method of NACHLAS *et al.* (1957) was used.

For the demonstration of oxidative enzymes, the sections dried at room temperature were incubated with the following substrate solutions.

Succinic dehydrogenase: Incubation mixture was composed of 5 ml of

0.2 *M* sodium succinate, 5 ml of 0.2 *M* phosphate buffer at pH 7.6, to which 10 ml of Nitro BT aqueous solution (1 mg/1 ml) were added. The sections were incubated in the mixture at 37°C for 30 minutes, fixed in 10 % formalin and mounted in glycerin without dehydration.

Lactic, glutamic, alpha-glycerophosphate and beta-hydroxybutyric dehydrogenase: Incubating solutions used were consisted of 4 ml of 1 *M* substrate solution, 3 ml of Nitro BT solution (5 mg/3 ml), 11 ml of 0.1 *M* phosphate buffer at pH 7.6, 2.5 mg of DPN (100 %), 2 ml of 0.1 *M* KCN and adjusted at pH 7.6 with 0.5 *M* HCl.

Malic dehydrogenase: Incubating solution was composed of 5 ml of 1 *M* sodium malate, 3 ml of Nitro BT solution (5 mg/3 ml), 10 ml of phosphate buffer (0.1 *M*) at pH 7.4, 2.5 mg of DPN, 2 ml of 0.1 *M* KCN and adjusted at pH 7.4 with 0.5 *M* HCl.

Glucose-6-phosphate dehydrogenase: Incubating solution was consisted of 4 ml of 0.02 *M* disodium glucose-6-phosphate, 3 ml of Nitro BT solution (5 mg/3 ml), 11 ml of 0.1 *M* Veronal buffer at pH 7.6, each 1 ml of 0.01 *M* MgCl₂ and 0.5 *M* MnCl₂ solution and with 7 mg of TPN. For the lactic and malic dehydrogenases, incubation was carried out at 37°C for 30 minutes, and for the other DPN- and TPN- linked dehydrogenases one hour.

CLINICAL FINDINGS OF THE PATIENT

Unmarried sailor of 29 years old. Family history: non-contributory. Past history: He had appendectomy by acute appendicitis in 1952, and nephritis in 1959. However, he had no history with such diseases being accompanied by eosinocytosis, as anchylostomiasis or allergic disease.

History of present illness: In 1957 he noticed a painless mass at the right parotid gland region without any apparent cause, and it was removed in a hospital in the summer of 1957. Pathological examination revealed a tumor, suspected to be of a mixed tumor of parotid gland. There were local recurrences in October, 1953 and in July, 1959 and the recurrent tumor had been removed but it recurred each time. In September, 1961 he noticed again a diffuse swollen mass at the right parotid region. And in September, 1962 the mass became painful, tender and red and it was incised resulting in discharge of pus. Four months before admission, a similar inflammation developed again and it was incised. The patient was admitted to our hospital on October 3, 1963. After admission he appeared to be in a good condition. No similar tumor or swelling of lymph nodes was observed.

Local views: Diffuse smooth-surfaced and child-hand size elevation with two operation scars was observed between the angle of the right jaw and the

lobe of the ear. It was firm, not tender, somewhat movable, relatively indefinite in outline, and closely attached to skin. Two retroauricular lymph nodes were swollen. Function of right facial nerve was well preserved and parotid orifice was normal.

Laboratory studies: Blood pictures, as summarised in Table 1, show leucocytosis with marked eosinocytosis. The result of urinalysis, stool examination and blood serum examination showed no abnormalities.

Table 1.

| examination | | Blood Examinations | | |
|-------------------------|-------------|--------------------|------------------|-------------------|
| | | preoperation | 9 days after op. | 18 days after op. |
| R. B. C. per cumm | | 4,860,000 | 3,680,000 | 3,800,000 |
| Hb. per cent | | 112 | 75 | 85 |
| W. B. C. per cumm | | 11,400 | 5,900 | 5,650 |
| haemogram (per cent) | neutrophils | 35 | 65 | 50 |
| | ensinophils | 37 | 19 | 20 |
| | basophils | 0 | 3 | 0 |
| | monocytes | 4 | 4 | 4 |
| | lymphocytes | 24 | 9 | 26 |

X-ray examination: The X-ray examinations of bone and lung were normal.

Operation record: The mass removal was performed on November 18, 1963. The tumor was found in the right parotid gland and seemed to have infiltrated into the surrounding tissues, especially densely adherent to the skin and its underlying tissue. Two swollen retroauricular lymph nodes were removed and the histologic observation of frozen sections revealed an eosinophilic granuloma. The mass was removed completely without any injury to the facial nerve. Appearance of the excised tumor was an oval, pink, hard and had no special capsule, and 8.2 cm × 4.3 cm × 3.7 cm in size and 55 g in weight. The cut-surface was gray white.

Postoperative course: After removal of the tumor the patient has become healthy and the blood picture has improved as shown in Table 1.

Histopathological findings on hematoxylin eosin stained specimen: The tumor which was distinctly separated from the normal parotid gland by proliferated connective tissue showed typical findings of chronic inflammatory granuloma of marked proliferation of fibrous tissue, newly-formed capillaries and prominent proliferation of inflammatory cells, especially of abundant eosinophils and some lymphocytes. Although moderate infiltration of these inflam-

matory cells was demonstrated in the stroma of the normal parotid gland, panniculum was generally involved, and this presented an appearance of panniculitis (Fig. 1).

In the tumorous panniculum, some gatherings of lymphocytes with their clear centers and with comparative demarcations, i. e., the newly-formed lymph follicles, were scattered sparsely. These centers or germinal centers were mainly made of some lymphogonia-like cells. Around these follicles, marked infiltration of eosinophils and lymphocytes were present, but poor in reticulum cells and plasma cells. These were well said to present a figure of lymphfolliculitis. Most of these eosinophils were of bi-nuclear and some are uni-nuclear (Fig. 2).

From these findings this granuloma was diagnosed as the eosinophilic lymphfolliculo-hyperplastic panniculitis (TOKORO).

HISTOCHEMICAL FINDINGS

The activity of each enzymes is summerised in Table 2. Alkaline phosphatase activity was moderate or strong in fibroblasts, fibrocytes, neutrophils and collagen fibers, and strong in capillary endothelium (Fig. 3).

Table 2.

| enzymes | neutrophil n | neutrophil c | eosinophil n | eosinophil c | lymphocyte n | lymphocyte c | lymphfol- licle | histiocyte fibrocyte | blood vessel | collagen |
|--------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|--------------------|-------------------------|--------------|----------|
| alkaline phosphatase | | + | | | | | | + | + | + |
| acid phosphatase | | + | | + | | | | + | + | + |
| beta-esterase | | + | | + | | | | + | + | + |
| aminopeptidase | | + | | + | | | | + | + | + |
| beta-glucuronidase | | + | | + | | | | + | + | + |
| succinic dehydrogenase | | + | | + | | | | + | + | + |
| lactic dehydrogenase | | + | | + | | | | + | + | + |
| malic dehydrogenase | | + | | + | | | | + | + | + |
| alpha-glycerophosphate dehydrogenase | | + | | + | | | | + | + | + |
| glutamic dehydrogenase | | + | | + | | | | + | + | + |
| beta-hydroxybutyric dehydrogenase | | + | | + | | | | + | + | + |
| glucose-6-phosphate dehydrogenase | | + | | + | | | | + | + | + |

n : nucleus c : cytoplasma

That of acid phosphatase was slightly positive in lymph follicles, histiocytes and fibrocytes, moderate in collagen fibers and small capillary wall and strong in neutrophils and eosinophils (Fig. 4). Deposition of pigment granules was observed in eosinophilic granules of eosinophils. Beta-esterase and aminopeptidase reactions were slightly positive or negative in this granuloma. Beta-glucuronidase showed weak reactions in stromal elements and moderate in neutrophils but negative in the other elements of this tumor tissue. Generally, the activity of dehydrogenases was moderate in eosinophilic tumor tissue and localized in lymph follicles, especially germinal center and small vascular wall, and weak in histiocytes, fibrocytes, collagen fiber, neutrophils and eosinophils (Figs. 5, 6, 7). But only glucose-6-phosphate dehydrogenase reaction (Fig. 8) was weaker than other dehydrogenase reaction except for neutrophils and proliferating histiocytes which revealed strong reactions.

DISCUSSION

ACKERMAN *et al.*¹⁴ have reported that alkaline phosphatase activity is positive in lymphocyte by GOMORI's technique, but BRAUNSTEIN *et al.*¹⁰ and TAKEUCHI *et al.* have noticed no activity in lymphocytes and eosinophils. In the present study the enzymatic activity was negative or slightly positive in lymphocytes, and eosinophils. FELL *et al.*¹² and FISCHER *et al.*¹³ have stated that alkaline phosphatase activity is positive in fibroblasts, young collagen fibers, fibrocytes and round inflammatory cells in new formation of connective tissue during wound healing at skin and subcutaneous tissue, and according to BRAUNSTEIN *et al.*¹⁰ it is also positive in capillary endothelium. In the present case the activity was moderate or strongly positive in fibroblasts, fibrocytes neutrophils and collagen fibers, and strong in capillary endothelium.

On the demonstration of acid phosphatase, ACKERMAN *et al.*¹⁴ have noted that this enzyme is distributed in nucleus of lymphocytes, but BRAUNSTEIN *et al.*¹⁰ and TAKEUCHI *et al.*¹¹ state that lymphocytes manifest a slight but distinct activity in cytoplasmic granules. And according to TAKEUCHI *et al.*¹¹ neutrophils and eosinophils show strong activity in cytoplasm. In the present study acid phosphatase was slightly positive or weak in lymph follicles, histiocytes and fibrocytes, moderately positive in collagen fibers and small capillary walls, and strongly positive in neutrophils and eosinophils.

On the demonstration of esterase, BRAUNSTEIN *et al.*¹⁰ have reported that the activity is negative in lymphocytes and lymph nodes using azo-coupling method, and SAKAMOTO¹⁵ observed no activity in inflammatory cells infiltrating into connective tissue during wound healing process of oral cavity, but positive in inflammatory cells floating out on the surface of wound and in connective

tissue under crust formation. According to TAKEUCHI *et al.*¹¹ neutrophils, eosinophils and lymphocytes are positive in cytoplasm. Moreover, SAKAMOTO¹⁵ and ARGIRIS¹⁶ state that esterase reveals the activity similar to that of alkaline phosphatase but reverse of succinic dehydrogenase. Esterase reaction in eosinophilic granuloma was weak in lymph follicles, neutrophils and eosinophils, and negative in connective tissue.

Aminopeptidase is demonstrated to be almost negative in lymphocytes, by TAKEUCHI *et al.*¹¹ and BRAUNSTEIN *et al.*¹⁰ and to be positive in histiocytes of human and animal lymph nodes by BURSTON *et al.*¹⁷ In the present case it was weak only in stromal elements and negative in all other elements of tumor tissue.

MONIS *et al.*¹⁸ and TAKEUCHI *et al.*¹¹ have found a moderate or an intense activity of beta-glucuronidase in neutrophils and no activity in lymphocytes and lymph nodes. In the eosinophilic granuloma, beta-glucuronidase activity was weak in only collagen fibers and negative in other elements.

According to TAKEUCHI *et al.*¹¹ the activity of most of dehydrogenases is negative in lymphocytes, slightly positive in cytoplasm of eosinophils and positive in cytoplasm of neutrophils.

On the demonstration of succinic dehydrogenase in normal and hyperplastic lymph nodes, BRAUNSTEIN *et al.*¹⁰ have reported that activity is strong in germinal center, proliferating histiocytes and small capillary wall and moderate in lymphocytes. In the present study the activity was moderately positive in germinal center and small vascular wall and slightly positive in histiocytes, fibrocytes, collagen fibers, neutrophils and eosinophils, i. e., succinic dehydrogenase in this tumor was weaker than that in normal and hyperplastic lymph nodes.

With respect to DPN-linked dehydrogenases such as lactic, malic, glutamic, alpha-glycerophosphate, and beta-hydroxybutyric dehydrogenase, there have been reported a few studies toward the demonstration of these dehydrogenases in lymph node. BRAUNSTEIN *et al.*¹⁰ have mentioned of these dehydrogenases in normal and hyperplastic lymph nodes and they state that lactic, glutamic and malic dehydrogenases have almost the same activity and distribution, i. e., the strongest activity is demonstrated in germinal center and vascular wall and strong in histiocytes and lymphocytes. And they have also observed negative activity of alpha-glycerophosphate dehydrogenase and beta-hydroxybutyric dehydrogenase in each element of these lymph nodes except for a weak or moderate alpha-glycerophosphate dehydrogenase activity in proliferating histiocytes. In the present case a tendency of enzymatic activities almost similar to BRAUNSTEIN's work was observed, however, generally these activities were not so strong as that found by BRAUNSTEIN *et al.*¹⁰

Of TPN-linked dehydrogenases only glucose-6-phosphate dehydrogenase was employed. According to BRAUNSTEIN *et al.*¹⁰ the activity of glucose-6-phos-

phate dehydrogenase is strong in neutrophils and proliferating histiocytes and moderate in germinal center, vascular wall, histiocytes and lymphocytes. The present result was almost similar to that of BRAUNSTEIN *et al.*,¹⁰ i. e. the activity was from moderate to weak in histiocytes and vascular wall and weak in collagen fibers and lymph follicles.

SUMMARY

We experienced a case of eosinophilic granuloma in soft tissue, and demonstrated its patterns of hydrolytic and oxidative enzymes histochemically. Neutrophils were rich in acid phosphatase and glucose-6-phosphate dehydrogenase. Eosinophils had much acid phosphatase and less other hydrolytic and oxidative enzymes. Lymphocytes showed weak reaction in all enzymes. Lymph follicles and histiocytes or fibrocytes had moderately oxidative enzymes. Small blood vessels and collagen fibers were rich in alkaline phosphatase and had a moderate amount of oxidative enzymes and acid phosphatase.

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- Fig. 1. Hematoxylin-eosin counter staining
The tumor is chronic inflammatory granuloma with eosinophils and is clearly separated from parotid gland by connective tissue.
- Fig. 2. Hematoxylin-eosin counter staining
Many newly-formed lymph follicles and lymphocytes infiltration are observed in the granuloma.
- Fig. 3. Alkaline phosphatase staining
The activity is moderate or strong in fibroblasts, fibrocytes, neutrophils, collagen fibers and capillary endothelium.
- Fig. 4. Acid phosphatase staining
Note the strong activity in eosinophils and neutrophils.
- Fig. 5. Lactic dehydrogenase
- Fig. 6. Malic dehydrogenase
- Fig. 7. Glutamic dehydrogenase
Note the strong activity in lymph follicles in Figs. 5, 6, 7.
- Fig. 8. Glucose-6-phosphate dehydrogenase
Histiocytes and neutrophils show a strong activity.

