The Electron Microscopic Study of Enzymes in Eosinophils

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ABSTRACT

The author has made the electron microscopic study of enzymes in eosinophils in order to clarify the influence of hyposensitization in allergic rhinitis to the activity of enzymes in eosinophilic granules and the following results were obtained.

1. In all 3 control, hyposensitization and allergic groups, eosinophilic granules with matrix and crystalloid core in circulating blood and tissue were observed.

2. In all 3 groups, activity of acid phosphatase was not found in neutrophil, basophil, macrophage and glands as a form of coagulated activating colony of acid phosphatase.

3. In control and hyposensitizing groups, number of eosinophils were smaller than that was counted in allergic group. Activity of peroxidase in granule was weak and granular out flowing and rupture of cell membrane were not observed.

4. In allergic group, eosinophil count was high, activity of peroxidase in granule was strong and granular out flowing and rupture of cell membrane were severe. At the same time, many vacuoles, which were suspected to be the result of phagocyte the protein as foreign substance, was observed.

Judging from the fact that eosinophil has a specific relation to allergic diseases and the activity of peroxidase that exist as an enzyme in eosinophilic granule is strong, it is believed that the major function of eosinophil is phagocytosis of antigen, or antigen-antibody complex. On the other hand, the fact that activity of peroxidase was weak in hyposensitizing group lead us to believe that the activity of peroxidase may be used as an indicator for detecting hyposensitizing status in the treatment of allergic disease.

INTRODUCTION

In the adult there are normally 5,000~10,000 white blood cells per µl of blood which are formed in the bone marrow. Of them, the granulocyte, or polymorphonuclear leucocytes (PMNS), are the numerous. And most of them contain neutrophilic granules (neutrophils), but a few contain granules that stain with acid dyes (eosinophils) and some have basophilic granules (basophils). The other two cell types formed normally in peripherial blood are lymphocytes and monocytes.

For the past one centry, a great deal of study has been made on the eosinophils which was first observed by Jone, T.W. in 1846 and first described by Paul Ehrich in 1879. Recently the nature of the eosinophil has become more clear through development of
the electron microscope.

The studies have revealed that all granulocytes contain histamine, antihistamine and three enzymes of peroxidase, acid phosphatase and ariylsulfatase. These enzymes mostly present in granules of eosinophils. The eosinophils apparently phagocytize antigen-antibody complex and the circulating eosinophil level is often elevated in patients with allergic disease.

The eosinophilic granules varies 0.7–1.3 μ in size and the outer layer matrix of eosinophil contain phospholipid that positively well stain with PAS dyes, and the inner layer crystalloid contain protein which is rich with arginine. The crystalloid cores are found in various forms of dotted line arrangement. The size of the crystalloid core is about 40 Å in adult.

It has become clear that the enzymes in eosinophil have following functions;

Peroxidase combines with hydrogen peroxide and it act as a catalyst for dehydrogenation of the organic tissue. At the same time, hydrogen peroxide is toxic to the tissue, but this toxic effect is prevented by the enzymes of peroxidase.

Acid phosphatase synthesizes protein and plays an important role in metabolism of nucleotides, phospholipid and carbohydrates in our living body.

On the other hand, Takayama et al (1975) recently reported that the out flowing of peroxidase from eosinophilic granules is closely related to the allergic diseases. But it is not yet clear how hyposensitization in allergic diseases influences the change of eosinophilic granules.

The author therefore has made the following electron microscopic study in order to clarify the influences of hyposensitization in allergic rhinitis to the activity of enzymes in eosinophilic granules.

MATERIALS AND METHODS

A. Materials

Material is classified into the following three groups.

1. Control group is consist of each 5 male and female college students of 20–25 of age who are health and had no allergic history with negative reaction in Prick test.

2. Allergic group is consist of 16 males and 15 females, 7–45 of age, diagnosed as nasal allergy at the allergic clinic of Yonsei Medical Center but who have had no hyposensitization therapy.

3. Hyposensitizing group is same patients of the allergic group, who was treated with SDV (specific desensitizing vaccine of Benedict, England).

B. Methods

The mucous membrane of 0.5 cm² has been obtained from inferior nasal conchae of all three groups and these mucous membranes were trimmed into 1 mm³ or smaller for the election microscope.

Fixed the material with 2.5% paraformaldehyde-glutaraldehyde solution at 4°C for 4 hours and washed with 0.1M cacodylate buffer solution (pH).

In order to detect the peroxidase, the material was incubated at 37°C for 1 hour in 0.05 M tris hydroxymethal aminomethane buffer solution which contains 0.05% DAB (diaminobenzidine tetrahydrochloride) and 0.01% H₂O₂.

In order to detect the acid phosphatase, the material was incubated at 37°C for 1 hour.
in Gomori medium which contains of 6 ml of 2% sodium β-glycerophosphate, 10 ml of 0.1 m acetate buffer (pH 4.7), 4 ml of 5% lead nitrate and 80 ml of distilled water.

The materials obtained through these processes were further:
1. Fixed with 1% osmium tetroxide (O₈O₄, pH 7.4) in 0.1 m cacodylate buffer;
2. Dehydrated in that order with 60, 70, 80, 90, 95 and 100% ethanol and propylene oxide;
3. Embedded on Epon 812;
4. Micromonized into 600 Å thickness with Sortvall Porter Blum MT-28 type ultramicrotome;
5. And then, the eosinophilic granule was observed by Hitachi HU-11E type electron microscope.

RESULTS

In all 3 control, allergic and hypsensitizing groups, the specific eosinophilic granules with matrix and crystalloid core in circulating blood and tissue was observed (Fig. 1).

A. The Activity of acid phosphatase

In all 3 groups, the activity of acid phosphatase was not found in basophil, macrophage and neutrophil (Fig. 2, 3).

In all 3 groups, the activity of acid phosphatase was found in epithelial cell and gland as a form of coagulated activating colony of acid phosphatase (Fig. 4, 5, 6).

B. The activity of peroxidase

1. Control group
The number of eosinophils were smaller than that was counted in allergic group and activity of peroxidase in granules was weak and granular out flowing and rupture of cell membrane were not observed (Fig. 7).

2. Allergic group
The activity of peroxidase in granules was strong (Fig. 8).
The activity of peroxidase in many vacuoles was observed clearly in high power field (Fig. 9).
The granule out flowing was observed(Fig. 10).
The rupture of cell membrane and normal cell membrane was observed and eosinophil granules and many vacuoles were also observed (Fig. 11).
The many out flowing granules were observed due to completely destroyed cell membrane (Fig. 12).

3. Hypsensitizing group
The number of eosinophils were smaller than that was counted in allergic group and activity of peroxidase in granules was weak and granular out flowing and rupture of cell membrane were not observed as control group (Fig. 13).

Table 1. The Character of Eosinophil Enzyme in three Group

<table>
<thead>
<tr>
<th>Group Enzyme</th>
<th>Control</th>
<th>Allergic</th>
<th>Hypsensitizing</th>
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<tbody>
<tr>
<td>Acid phosphatase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Eosinophil</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>2. W.B.C., Epithelium, Gland</td>
<td>#</td>
<td>#</td>
<td>#</td>
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<tr>
<td>Peroxidase</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Activity</td>
<td>+</td>
<td>#</td>
<td>+</td>
</tr>
<tr>
<td>2. Out flowing</td>
<td>−</td>
<td>#</td>
<td>−</td>
</tr>
<tr>
<td>3. Rupture of cell membrane</td>
<td>−</td>
<td>#</td>
<td>−</td>
</tr>
</tbody>
</table>

(−No response, + Mild response, # Moderate response, ♠ Severe response)

DISCUSSION

The size of eosinophil granules are about 0.7~1.3 μ (Goodman, 1957) and these granule having a matrix of medium electron density
in the center of which was a crystalloid of relatively high electron density (Bargmann, 1956; Goodman, 1957; Ghadially, 1965; Hudson, 1967). The crystalloid core of outer layer, which are variable form, has cubic lattice with a repeat of \( \sim 40 \) Å in men, \( \sim 30 \) Å in rat, and \( \sim 35 \) Å in cat (Bargmann, 1956; Miller, 1966).

The chemical composition of crystalloid core are almost hydrolytic enzymes as peroxidase and acid phosphatase (Miller, 1966; Cline, 1968; Bainton, 1970) and zinc which may play active role in activation of enzyme, was also component (Phil, 1967).

Peroxidases are demonstrable during differentiation of immature cells in bone marrow and are play role of catalyzation of organic compound.

The phagocytosis by eosinophil was more increased in present of antigen-antibody complex (Archer and Hirsh, 1963; Sabesin, 1963; Litt, 1964) and in pH 7.0 (Cline, 1968).

Eosinophil which is a kind of PMNS, was observed in tissue and blood, especially they are more numerous in the intestine wall and the lung, probably owing to the continual exposure of these two organs to foreign substance and parasites (Bainton, 1970).

The eosinophil was circulated in blood for 2~3 hours after growth in bone marrow for 3~6 days, but may persist 3~4 days in a tissue (Wintrobe, 1967).

The number of circulating eosinophils are about one thirtieth of bone marrow (Donohue, 1958). Kay (1970) and Joseph (1974) claimed that eosinophilia was occurred from bone marrow to nasal mucosa by ECF-A (Eosinophilic chemostatic factor of Anaphylaxis).

Kay (1970) claimed eosinophil infiltration was observed in 12 hours after exposed in foreign body. Connell (1968) reported that the vacuolization of eosinophil in blood of asthmatic patients was observed and already the granular change was observed in blood. It was observed in electron microscopic studies that peroxidase was released during phagocytosis (Archer and Hirsh, 1963; Zucker-Franklin, 1964; Cotran, 1970) and was observed also in author's studies.

It was suspected that acid phosphatase action in eosinophil is similar as peroxidase, but activity of acid phosphatase was lower in allergic disease and action of acid phosphatase was not clarified (Bainton, 1970; Takayama et al., 1975).

Activity of peroxidase was not observed in mature granule (Bainton, 1970) and these fact was observed in author's study.

Juding from the fact that eosinophil has a specific relation to allergic disease and the activity of peroxidase that exist as an enzyme in eosinophilic granules is strong, it is believed that the major function of eosinophils is phagocytosis of antigen or antigen-antibody complex.

On the other hand, the fact that activity of peroxidase was weak in hyposensitizing group leads us to believe that the activity of peroxidase may be used as an indicator for detecting hyposensitizing status in the treatment of allergic disease.

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Fig. 1. Eosinophil granules (X30,000). G: granule. c: crystalloid. m: matrix, Ne: eosinophil nucleus

Fig. 2. Activity of acid phosphatase in allergic group (X10,700). E: eosinophil, N: neutrophage
Fig. 3. Activity of acid phosphatase in Hyposensitizing group (X16,000). A.P.: Acid phosphatase, N: nucleus

Fig. 4. Activity of Acid phosphatase in allergic group (X16,000). V: microvilli, A.P.: Acid phosphatase
Fig. 5. Activity of Acid phosphatase in allergic group (X10,000). A.P.: Acid phosphatase. N: nucleus

Fig. 6. Activity of Acid phosphatase in hyposensitizing group (X16,000). A.P.: Acid phosphatase

Fig 8. Eosinophil in allergic group (X16,000). R: RBC CM: cell membrane, Ne: eosinophil nucleus. P: peroxidase
Fig. 9. Eosinophil in allergic group (X 30,000). Va: vacuole, P: Peroxidase Ne: eosinophil nucleus, CM: cell membrane

Fig. 10. Eosinophil in allergic group (X 22,500). Gm: granule out flowing CM: cell membrane. Va: vacuole, Ne: eosinophil nucleus
Fig. 11. Eosinophil in allergic group (X 10,700). G: granule, Ne: eosinophil nucleus, CM: cell membrane, destruction of cell membrane.

Fig. 12. Eosinophil in allergic group (X 10,700). Gm: granule out flowing, G: granule, Ne: eosinophil nucleus, destruction of cell membrane.

Fig. 13. Eosinophil in hyposensitizing group (X 16,000). R: RBC, P: peroxidase Ne: eosinophil nucleus.