

◎総説

Basophil response to antigen and anti-IgE.

2. Morphological changes in secretory process

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Abstract : Morphological changes of blood basophils activated by antigen and anti-IgE were discussed in relation to release mechanisms of the cells. 1. Basophils activated by antigen and anti-IgE show two different types : A form(pear-shaped)and B form (swollen type), on smear preparartions. 2. Pear-shaped basophils(reactive basophils), which appear after stimulation by antigen and anti-IgE, can be observed by the staining solution for the direct count of basophils and eosinophils. 3. The incidence of reactive basophils increases after addition of antigen. Thus, observation of reactive basophils can be clinically applied for detecting allergen (DCRB ; direct connt of reactive basophils). 4. Observation of morphological basophil response by phase-contrast microscopic motion pictures demonstrates that basophils activated by antigen show two different reponses, increase in motility (oriented movement) and degranulation.

Key words : morphological changes of basophils, reactive basophils, degranulation, histamine release

Introduction

Our previous studies showed that number of basophils in the peripheral blood changes in relation to asthmatic cycle : number of basophils increases in the pre-attack stages of bronchial asthma, and decreases at the initiation of the attack stages^{1,2)}. The decrease in number of basophils during asthma sttacks is due in part to migration of the celles into the local allergic reaction sites^{3,4)}.

It is well known that blood basophils and tissue mast cells are target cells of IgE antibodies^{5,6)}. Bridging of IgE receptors on the membrane by antigen and anti-IgE activates

basophils and mast cells and induces a release of chemical mediators from the cells^{7,8)}. Basophils, being different from mast cells located at local allergic reaction sites, have to move from bloodstream to the tissues where allergic reactions take place. Therefore, the most remarkable difference in cell function between basophils and mast cells is due to whether the cells show active movement or not after stimulation with antigen and anti-IgE.

In the present study, morphological changes of basophils after activation by antigen and anti-IgE were discussed by various observing procedures.

Observation on smear preparations

Blood basophils activated by antigen and anti-IgE are classified into three morphological types, A, B and N, according to the degree of degranulation on smear preparations⁹⁾. The A form basophils are pear-shaped, have a peripherally situated nucleus and contain granules that appear to be mal-distributed (on the side of the cell opposite to the portion of nucleus). The B form basophils appear swollen and demonstrate vacuolization. The N form basophils are normal type not reactive to the stimulating agents. Microscopic observation of basophil degranulation by antigen-antibody reaction reveals a characteristic increase in the number of pear-shaped or A form basophils, which represent an increased motility of the cells with oriented movement. Swollen type or B form basophils seem to be associated with the cells showing degranulation¹⁰⁾. These observations suggest that the mode of degranulation in basophils differs from that in mast cells (Fig. 1, 2).

	Type I	Type II	Type II
A form (pear-shaped)			
B form (vacuolated and swollen)			
N form (rounded)			

Fig. 1. Morphological changes of basophils induced by antigen on smear preparation

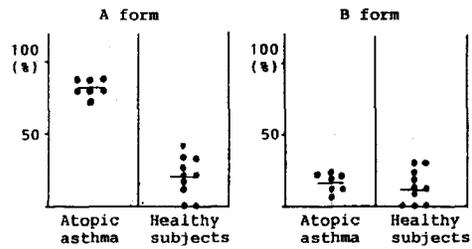


Fig. 2. Incidence of A form and B form basophils induced by anti-IgE in atopic asthmatics

Vital observation under a differential-interference microscope and a phase-contrast microscope

Under a differential-interference microscope, basophils activated by anti-IgE can be classified into three morphological types, (a) normal cells, (b) pear-shaped cells with increased movement and (c) cells showing further swelling after the initial pear-shaped changes and later showing a gradual decrease in movement^{11, 12)}. The observation reveals that basophils at first show remarkably increased movement with A form or pear-shaped when the cells are exposed to anti-IgE (Fig. 3).

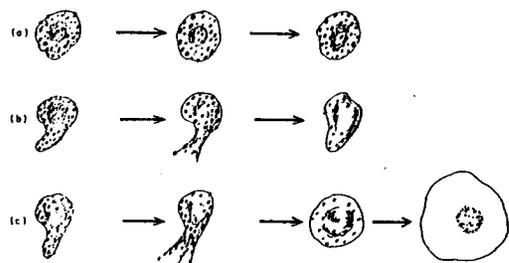


Fig. 3. Vital observation of anti-IgE-added basophils (differential-interference microscopy). (a) Normal cells; (b) pear-shaped cells with increased movement; (c) cells showing further swelling after initial pear-shaped changes and later showing a gradual decrease in movement.

An increased motility in basophils activated by antigen and anti-IgE can be shown by observing migration velocity of the cells under a phase-contrast microscope. Basophils from atopic asthmatics show increased migration velocity when the cells are stimulated by anti-IgE¹³.

An increased migration velocity of basophils stimulated by antigen is observed in atopic subjects sensitive to buckwheat¹⁴. Under phase-contrast and differential-interference microscopes, the migration velocity of basophils increases and their forms change to pear-shaped or vacuolated and swollen form with decreased or disappearance of the intracellular granules when the cells are stimulated by buckwheat extract (Fig. 4).

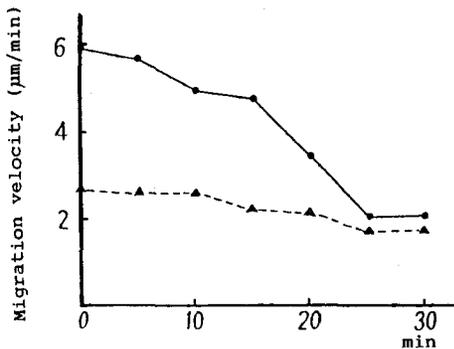


Fig. 4. Migration velocity of basophils from patients with buckwheat allergy stimulated by the allergen extract.

●—● Addition of buckwheat, ▲---▲ addition of saline.

Clinical evaluation of basophil response to allergen

When basophils are exposed to allergen, the incidence of pear-shaped basophils showing increased movement (reactive basophils) increases. Therefore, highly increased incidence of reactive basophils demonstrates that an

allergen used for basophil stimulation is specific allergen causing allergic reactions. A simple method for detecting specific allergen has been established by counting reactive basophils. In the method, the increased incidence of reactive basophils induced by an allergen shows a close correlation with skin reactivity, positive bronchial provocation test and RAST score for the same allergen.

Correlation of the incidence of reactive basophils induced by house dust mites with skin reactivity, bronchial provocation test and RAST score were examined in 20 atopic asthmatics sensitive to house dust mites. The results were as follows¹⁵.

The degree of skin reactivity to house dust mites paralleled the incidence of reactive basophils induced by the allergen. As the skin reactivity is stronger, the incidence of reactive basophils is higher.

Bronchial provocation test with house dust mites showed a close correlation to the incidence of reactive basophils by the allergen. The mean incidence of reactive basophils in cases with negative provocation test for house dust mites was 17.9% with a range of 0 to 40%. On the contrary, the incidence of reactive basophils in cases with positive provocation for the allergen was 65.0% (range, 50–80%). The incidence of reactive basophils was more than 50% in all cases with positive bronchial provocation test, and a significant difference was found in the incidence of reactive basophils between cases with negative and positive provocation tests.

A close correlation was found between RAST score for house dust mites and the incidence of reactive basophils. The mean incidence of reactive basophils induced by house dust mites was 15.9% in cases with RAST score of 0+, 35.0% in cases with

RAST score of 1+ and 65.0% with RAST score of 2+ or more. The incidence of reactive basophils increased as the RAST score for the allergen was higher.

Observation by microscopic motion pictures

The morphological types of basophils are classified into three as described above, i. e., N form (rounded type), A form (pear-shaped type with increased movement) and B form (swollen type). By observation under microscopic motion pictures, A and B further classified into three types, respectively (A_1 , A_2 , A_3 , B_1 , B_2 , B_3)¹⁶⁻¹⁸.

Basophils with random movement (A_1 type): the cells show random movement with pseudopodia from indefinite sites. The cell contour is well maintained.

Basophils with oriented movement (A_2 type): the cells show well-oriented movement with large pseudopodia from definite sites.

Pre-swollen basophils (A_3 type): the cells have pseudopodia from more extensive sites and sometimes from all the sites. No vesicles were observed in the cytoplasm. Motility is generally decreased.

Basophils with degranulation (B_1 , B_2 , B_3): the cells show a tendency towards swelling with vesicles in the cytoplasm. Motility is generally depressed; B_1 type: swelling in mild and the mean cell diameter is less than $11.9 \mu\text{m}$. Many vesicles are often noted in the cytoplasm, B_2 type: moderate swelling is noted and the mean cell diameter is $11.9 \mu\text{m}$ or larger and shorter than $14.3 \mu\text{m}$. Several vesicles are noted in the cytoplasm, B_3 type: marked swelling is noted and the mean diameter is $14.3 \mu\text{m}$ or larger. Vesicles are infrequently observed in the cytoplasm. Type B_1 and B_2 show partial degranulation and type B_3 complete degranulation (Fig. 5).

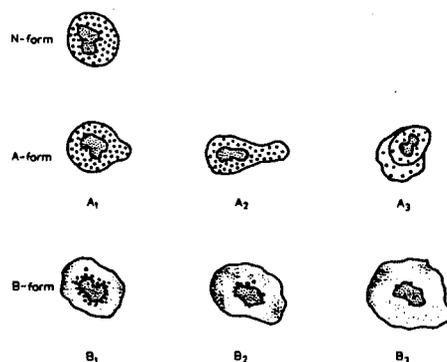


Fig. 5. Morphological changes of basophils in normal and antigen-treated cultures under phase-contrast microscopy.

Hastie observed the morphological changes of basophils after addition of antigen under a phase-contrast microscope¹⁹. He demonstrated that antigen-stimulated basophils at first show increased motility and then degranulation. Our previous studies also revealed that basophils reacting to antigen show morphological changes such as a pear-shaped and swollen form¹³.

Increase in motility and degranulation can be observed under microscopic motion pictures. The incidence of A_1 type basophils (random movement) in atopic asthmatics before addition of antigen is lower than that in healthy subjects. The incidence of A_2 type basophils (oriented movement) is clearly lower before antigen stimulation, and significantly increases after addition of antigen in atopic asthmatics compared with that of healthy subjects. The results show that motility of basophils is suppressed in atopic subjects¹⁶.

Morphological changes in secretory process

In the release mechanism of chemical mediators from basophils, which are one of the blood cells, increased movement of the cells is essential, because the cells have to migrate from bloodstream into local allergic reaction sites to play their roles. Basophils activated by antigen usually show two different responses: increase in motility (oriented movement) at the early stage of activation, and degranulation at the later stage. The results from observation by phase-contrast microscopic motion pictures were as follows^{16, 19}. The incidence of basophils showing oriented movement was significantly increased at 6–9 min after addition of antigen, being constantly increasing during observation for 15 min. While degranulation of basophils began to appear at 0–3 min after addition of antigen with the maximum response at 3–9 min. The amount of histamine release under the same condition reached a peak at 6–9 min in accordance with the maximum response of degranulation. These results reveal that degranulation of the cells closely correlates with the release of histamine, and basophils with oriented movement release little histamine²⁰.

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抗原および抗ヒトIgEに対する好塩基球の反応性.

2. 分泌機序における形態的变化

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抗原および抗ヒトIgE刺激時の好塩基球の形態的变化について, 細胞の分泌機序(ヒスタミン遊離)との関連のもとに若干の検討を加えた。1. 抗原や抗ヒトIgE刺激時には, 塗抹標本上では好塩基球の2つの形態的变化, A-form(洋梨型)とB-form(膨化型)を観察することができる。2. 抗原や抗ヒトIgE刺激時に出現する洋梨型好塩基球

(反応好塩基球)は, 好塩基球, 好酸球同時直接算定用染色希釈液で染色することにより, 容易に観察することができる。3. 抗原添加後に反応好塩基球の出現頻度は増加する。したがって, 反応好塩基球の出現頻度を観察することにより, 原因抗原を明らかにすることができる。4. 位相差顕微鏡映画による, 免疫下の好塩基球の形態的变化の観察からは, 好塩基球は2つの反応を取ることがわかる。その1つは, 運動亢進, すなわち oriented movement を示すことであり, 他の1つは, 膨化して脱顆粒現象をひき起こすことである。

キーワード: 好塩基球の形態的变化, 反応好塩基球, 脱顆粒, ヒスタミン遊離