◎総 説

Basophil response to antigen and anti-IgE. 1. Changes in number of basophils.

Yoshiro Tanizaki and Ikuro Kimura[1)]

Division of Medicine, Misasa Medical Branch, [1)]Second Department of Medicine, Okayama University Medical School

Abstract : Blood basophils in subjects with bronchial asthma migrate from blood stream into local allergic reaction sites after inhalation of antigen into airways. The phenomena can be observed by changes in number of basophils in the peripheral blood. The peripheral basophil count is at a normal level in non-attack stage of asthmatics as in healthy subjects. The basophil count increases in pre-attack stages, and decreases during attack stages. These findings suggest that number of basophils changes in close relation to asthma cycle, and that by observing number of basophils in the peripheral blood, it is possible to detect near future attacks.

The decrease in number of basophils and histamine release by stimulation with antigen and anti-IgE can be observed in vitro in whole blood and basophils separated by density gradient centrifugation and by counterflow centrifugation elutriation.

The decreased number of basophils in the peripheral blood represents migration of the cells into local allergic reaction sites.

Key words : basophils, bronchial asthma, asthma cycle, histamine release

Introduction

Asthma attacks are elicited by bridging of IgE receptors on mast cell membrane within 30 minutes after inhalation of antigen[1~3)]. After chemical mediators are released from mast cells, and immediate allergic reactions are induced by these mediators, various blood cells including basophils migrate into allergic reaction sites in the airways of asthmatics, accompanied with or without late asthmatic reaction.

It is well known that basophils release histamine, one of the main chemical mediators released from mast cells, on exposure to antigen and anti-IgE[4~7)]. Basophils, however, have to migrate from bloodstream into local allergic reaction sites to act as the target cells of IgE antibodies. Therefore, changes in number of basophils in the peripheral blood are observed in relation to migration of the cells into local allergic reaction sites. In the present study, changes in number of basophils were discussed in relation to IgE-mediated

allergic reaction.

Observation for changes in number of basophils in vivo

1) Staining solution for direct count of basophils

Basophils can be easily counted by a staining solution for direct count of basophils and eosinophils[8, 9]. The staining solution comprises 11 mℓ of 0.05% toluidine blue solution (0.05 g toluidine blue (Merck) in 50 mℓ of 1.8% sodium chloride, 22 mℓ of 96% ethylalchohol and 28 mℓ distilled water), 0.8mℓ of 0.03% light green solution (Merck), 0.5mℓ of saturated solution of saponin (saponin white, Merck) and 5 mℓ of 1/15 M phosphate buffer (ph 6.4). The solution is stable and basophils are well differentiated within ten hours after stained with the solution. After staining with the solution, number of basophils is counted in a Fuchs-Rosenthal chamber.

2) Increase in number of basophils in pre-attack atages

Number of basophils in the peripheral blood increases in pre-attack stages. Observation for an increase in number of basophils makes it possible to detect following attacks, and it is suggested that the future attacks will appear in almost asthmatics when the basophil count is higher than the average upper limit (60−65/cmm)[8~10]. A point, i. e. 65/cmm is designated as the 'attackthreshold' in asthmatic subjects, and when the basophil numbers exceed it, the subjects may develop an asthma attack with very near future. Furthermore, the attack-threshold is quite constant in each individual. An increase in number of basophils before asthma attacks is also observed in patients with sea squirt asthma at pre-attack stsges of working periods[11, 12]. In clinical course of natural asthma attacks, an increased number of basophils is often observed during attack season (Fig. 1).

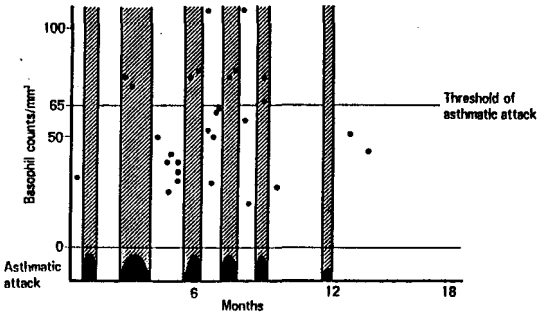

Fig. 1. Changes in number of basophils in a 13-year old boy receiving long term therapy

3) Decrease in number of basophils in attack atages

Basophils increasing in pre-attack stages show a tendency to decrease when attacks come out. When attacks improve and number of basophils still show a low level, the patient does not develop near future attack. Number of basophils, however, increases again after improvement of attacks, the patient may develop near future attack (Fig. 2).

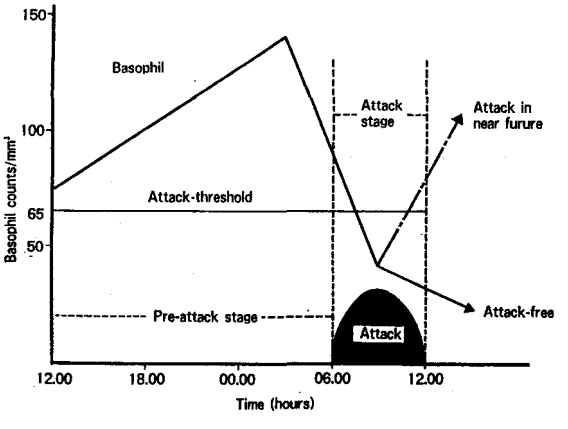

Fig. 2. Correlation between changes in number of basophils and asthma attacks (a shema).

4) Migration of basophils into local allergic reaction sites

The decrease in number of basophils during attack stages shows migration of the cells from bloodstream into local allergic reaction sites. The basophil migration is observed at skin sites of intradermal allergen injection by the application of cantharides plaster[13]. A slightly higher number (2.9%) of basophils is found when exudate is derived from an area of skin demonstrating a positive reaction to an allergen. On the contrary, analysis of vesicular fluid from areas of untreated skin shows a lower appearance of basophils (1.1%). These findings suggest that basophils tend to emerge in greater numbers at sites of intradermal allergic reactions than at other skin sites. In healthy, non-allergic subjects, fewer basophils (0.3%) are found in the vesicular fluid over areas of untreated skin.

Basophil migration into tissuse is also observed in sputum during symptomatic episodes of bronchial asthma, particularly during the last phase of attacks. At the improvement of attacks, when the patient is completely asymptomatic, basophils are no longer found in the sputum[14].

Observation for changes in number of basophils in vitro

1) Separation of basophils from peripheral blood

(1) Separation by density gradient centrifugation

Basophils can be highly separated by only one time centrifugation with density gradient using buffy coat from 6 mℓ of whole blood, and only 1 mℓ venous blood is enough to separate when the whole blood method is used. The purity of basophils averaged 22.7% in buffy coat and 15% in whole blood[15].

(2) Separation by counterflow centrifugation elutriation

Basophils can be also separated by counterflow centrifugation elutriation using a JE-6B roter (Beckman)[16, 17]. The frequency of various leucocytes collected into each fraction by this method was previously describbed[18]. The frequency of lymphocytes is higher at a low flow rate (4.5 to 8 mℓ/min), and gradually decreased as the flow rate increases. The frequency of neutrophils is low at a low flow rate, markedly increases at a flow rate of 10 mℓ/min and reachs a peak at a flow rate of 13 mℓ/min. The frequency of basophils and eosinophils increases at a flow rate of 8–10mℓ/min, and 12 mℓ/min or higher, respectively.

The purity of basophils ($0.9\pm0.5\%$) in the peripheral blood gradually increases at a low flow rate and reachs a peak ($11.8\pm2.0\%$) at a low flow rete of 9 mℓ/min. The viability of basophils is greater than 95% in all fractions, and degranulation is not observed in any basophils by the direct counting method[8~10]. The cells well moved under phase-contrast microscopic observation[19, 20].

2) Decrease in number of basophils in vitro

Basophils reacting to allergen show morphological changes, pear-shaped (oriented movement) and swollen type (degranulation), and the number of basophils decreases, accompanied with histamine release[19]. In our previous studies, reactive basophils (increase in motility) increased from 11.7% before incubation with antigen to 45.8% after stimulation with the antigen. At the same time, the number of basophils (39/cmm) decreased to a significantly lower level (10/cmm). The mean percent decrease was 74.5% and the mean histamine release from basophils was 53.4%.

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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. 1. 好塩基球数の変化

谷崎勝朗, 木村郁郎[1)]

岡山大学医学部附属病院三朝分院内科, [1)]医学部第2内科

気管支喘息では, 気道への抗原吸入後, 好塩基球は末梢血よりアレルギー反応局所へと遊走する。この現象は, 末梢血中の好塩基球数の変動により観察することができる。気管支喘息患者の好塩基球数は, 非発作時には健常人とほぼ同じで正常範囲内にあるが, 発作前段階で増加し, 発作出現とともに減少する傾向を示す。これらの所見は, 好塩基球が喘息発作と密接な関連を持ちながら変動すること, そして, これらの変動を観察することにより, 近い将来の発作を予知することが可能であることを示唆している。

抗原あるいは抗ヒトIgE刺激による好塩基球数の減少およびヒスタミン遊離は, 全血法あるいは濃度勾配法やcounterflow centrifugation elutriation法により分離された好塩基球を用いてin vitroで観察することができる。

末梢血中の好塩基球数の減少は, 好塩基球がアレルギー反応局所へと遊走したことを示している。

キーワード：好塩基球, 気管支喘息, 喘息発作, ヒスタミン遊離