◎原 著

Microscopic observation on morphological changes of basophils induced by cross-linking of IgE receptors

Yoshiro Tanizaki, Saburo Nakagawa[1] and Ikuro Kimura[1]

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

Abstract : Differences in morphological changes of basophils between antigen and anti-IgE stimulation were examined in atopic subjects in relation to histamine release. 1. Antigen-induced release of histamine was remarkably more rapid and larger at any incubation time than the release by anit-IgE, and a significant difference was found at 3 to 9 min. 2. Decrease in number of basophils induced by antigen was significantly higher for 12 to 15 min incubation time than that by anti-IgE. 3. Morphological changes of basophils representing increased motility such as an increased ratio of short to long axis diameter (L/S ratio) and an increased incidence of basophils with localized granules (LG) were more often observed in antigen stimulation compared with anti-IgE, and the L/S ratio of the cells was significantly more increased in antigen stimulation at 6 and 15 min. 4. There was no difference in morphological changes of basophils showimg swollen type (degranulation) such as an increased mean diameter (MD) of the cells between antigen and anti-IgE. These results suggest that antigen activates basophils and induces increased motility more strongly than anti-IgE, but the action on degranulation was not different between the two agents.

Key words : Histamine release ; Morphological changes of basophils ; Antigen, Anti-IgE ; IgE receptors

Introduction

Blood basophils and tissue mast cells have IgE receptors on cell membrane[1, 2], and bridging of IgE receptors is essential for a release of chemical mediators when the release is elicited through IgE receptors[3, 4]. It has been shown that these cells have a common process for IgE-mediated release mechanisms ; an increased metabolism of membrane phospholipids, influx of calcium ions into the cells[5~8], followed by cell activation related to increased motility[9~11] and a release of chemical mediators[12~15].

IgE-mediated release of chemical mediators is characterized by following conditions ; this is (1) highly dependent on extracellular Ca^{2+}, (2) related to increased metabolism of mem-

brane phospholipids, (3) enhanced by addition of phosphatidylcholine, (4) inhibited by 2－deoxyglucose and (5) modulated by intracellular concentrations of cyclic AMP [16]. Thus, IgE-mediated release of chemical mediators have a common process and similarity among agents eliciting cross-linking of IgE receptors. Several differences have been, however, suggested in a release of histamine between antigen and anti-IgE [17].

In the present study, difference in morphological changes of basophils was examined between antigen and anti-IgE stimulation.

Subjects and Methods

Nine atopic subjects with bronchial asthma were selected for this study. All of them were sensitive to house dust mite and showed a positive radioallergosorbent test (RAST) of 2+ or more. Their mean age was 31.9 years and the mean level of total serum IgE in sera was 1143 IU／mℓ.

To 2mℓ of heparinized venous blood in a silicon-treated test tube, 0.1mℓ of house dust extract (Torii Pharmaceutical Co) at 1 : 100 dilution was added. The mixed solution was incubated at 3, 6, 9, 12 and 15 min at 37℃. After incubation, the reaction was stopped by transferring the test tube into ice bath. The smear preparations were made from the mixed solution. On the smear preparations stained with May Giemsa, morphological changes of basophils were observed under a microscope with 1000－fold magnification. After then, the mixed solution was centrifuged at 1500 rpm for 15 min at 4℃, and histamine content in the supernatant fluid and cell pellet was measured.

Basophils from 5 subjects were examined in each experiment for antigen and anti-IgE stimulation. Morphological changes were observed in 5 subjects. To compare the results between antigen and anti-IgE stimulation, the value of each observation was expressed as following formulas.

1) Histamine release (HR) : HR by antigen／HR by anti-IgE
2) Cell number : Decrease in number of basophils by antigen／decrease by anti-IgE
3) Ratio of short to long axis diameter (L／S ratio) of the cells : L／S ratio by anti-IgE
4) Frequency of basophils with localized granules (LG) : %Basophils with LG／%basophils with LG by anti-IgE
5) Mean diameter (MD) of basophils : MD of basophils by antigen／MD by anti-IgE

Histamine was measured by an automated spectrofluorometric analysis system (Technicon)[13~15,18].

Results

1. Histamine release from basophils

Basophils stimulated by anti-IgE released a significant amount of histamine. The release by anti-IgE reached the peak at 15 min after anti-IgE stimulation. Histamine release induced by antigen was more repid and larger at the early stage of incubation with the agents. The release by antigen was always higher at each incubation time than the release by anti-IgE, and sgnificant difference was present at 3, 6 and 9 min incubation between them (at 3 min ; $p<0.01$, 6 min ; $p<0.02$, and 9 min ; $p<0.05$).

The difference in histamine release between antigen and anti-IgE stimulation became smaller as the incubation time with the agents was lomger, and no significant difference was found at 12 and 15 min (Table 1, Fig. 1).

Tabale 1. Histamine release from basophils stimulated by anti-IgE

% Histamine release					
Incubation time,min					
0	3	6	9	12	15
0	4.48* ±3.53	11.69 ± 9.71	16.92 ±14.03	20.31 ±15.91	21.48 ±14.29

*Mean ± sd

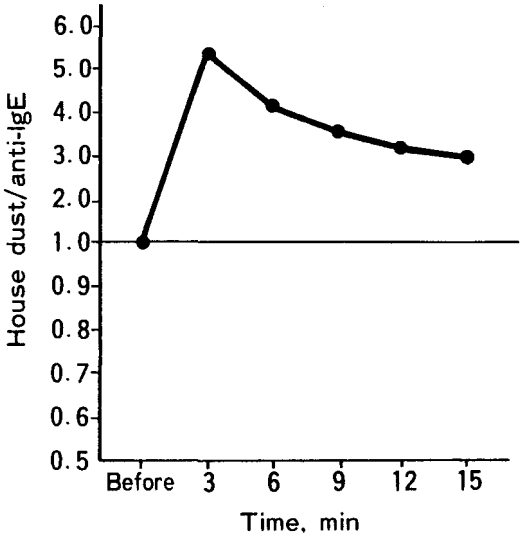

Fig. 1. Ratio of histamine release from basophils induced by house dust to the release by anti-IgE

2. Decrease in number of basophils

The number of basophils decreased by stimulation with anti-IgE. The maximum decrease was found at 15 min after addition of anti-IgE and the percent decrease was 47.2%. The number of basophils induced by antigen was more rapid and remarkably larger at 6 to 15 min of incubation time.

There was a significant difference at 12 and 15 min between the two agents (at 12 and 15 min ; $p<0.02$) (Table 2, Fig. 2).

Table 2. Decrease in number of basophils stimulated by anti-IgE

Number of basophils (%)					
Incubation time					
0	3	6	9	12	15
14.40* ±5.16	12.20 ±4.12	11.00 ±4.00	8.80 ±2.79	8.40 ±2.05	7.60 ±2.06

*Mean ± sd

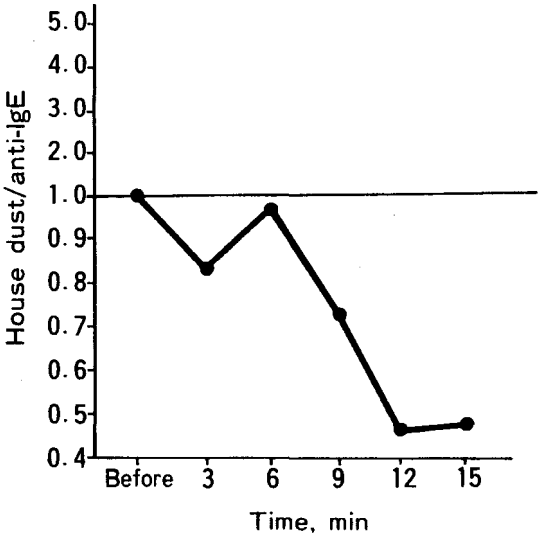

Fig. 2. Ratio of decrease in number of basophils induced by house dust to the decrease by anti-IgE

3. Ratio of short to long axis diameter of basophils (L/S ratio)

Basophils show a pear-shaped type representing an increased motility on exposure to antigen[9,10]. A L/S ratio of the cells is larger in these activated cells. The L/S ratio of basophils increased at 9 to 12 min after addition of anti-IgE. The increase in L/S ratio by anti-IgE was, however, observed for a short time. Antigen stimulation caused an increased L/S ratio of basophils. The increase in the L/S ratio by antigen was more rapid and higher, and the duration of

antigen stimulation causing an increase in L/S ratio was longer compared with anti-IgE stimulation. A significant difference was present in the L/S ratio at 6 and 15 min between the two agents (at 6 min ; $p<0.05$, and 15 min ; $p<0.01$). The results reveal that activation of basophils expressed as an increased L/S ratio was stronger and continued longer in antigen stimulation than in anti-IgE (Table 3, Fig. 3).

Table 3. Changes in ratio of short to long axis diameter (L/S ratio) of basophils stimulated by anti-IgE

Ratio of short to long axis diameter					
Incubation time					
0	3	6	9	12	15
1.16* ±0.03	1.16 ±0.03	1.16 ±0.04	1.19 ±0.02	1.19 ±0.02	1.12 ±0.02

*Mean ± sd

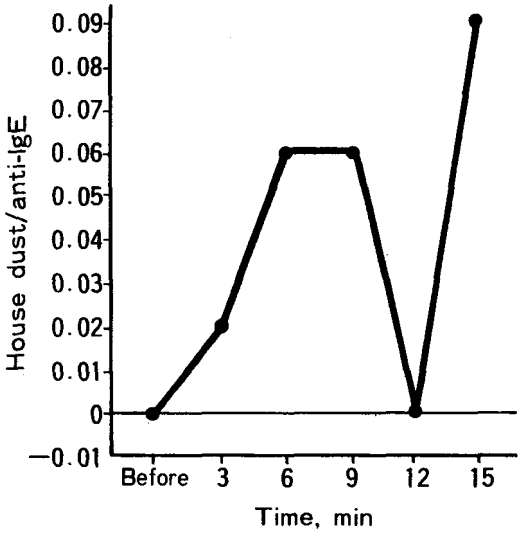

Fig. 3. Comparison of Ratio of long/short axis diameter (L/S ratio) in basophils induced by house dust with the ratio by anti-IgE

Table 4. Frequency of basophils with localized granules (LG) by stimulation with anti-IgE

Frequency of basophils with LG (%)					
Incubation time, min					
0	3	6	9	12	15
8.33* ±5.27	13.67 ±11.18	11.67 ±15.46	11.67 ± 8.50	10.00 ±10.16	11.67 ± 8.50

*Mean ± sd

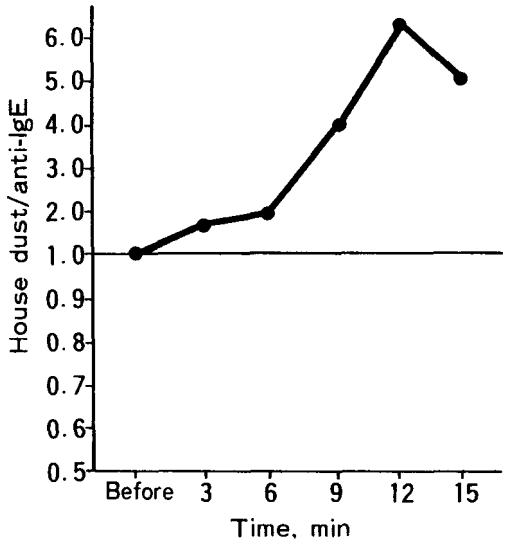

Fig. 4. Ratio of frequency of basophils with localized granules (LG) induced by house dust to the frequency by anti-IgE

4. Frequency of basophils with localized granules (LG)

Basophil granules are localized in the cells which are activated and show an increased motility. Anti-IgE stimulation induced an increase in the frequency of basophils with LG, showing an increased frequency of basophils with oriented movement[9~11,19]. The increased frequency by anti-IgE begun at 3 min after addition of the agent, and then kept almostly same increase. Antigen activat-

ed basophils and caused an increased frequency of basophils with LG. The frequency of basophils with LG more remarkably increased at 6 to 15 min incubation with antigen compared with anti-IgE. The higher increase by antigen continued from 9 to 15 min. The difference was, however, not significant between the two agents (Table 4, Fig. 4).

Table 5. Changes in mean diameter (MD) of basophils by stimulation with anti-IgE

Mean diameter (MD)(μm)					
Incubation time, min					
0	3	6	9	12	15
13.20* ±0.69	13.90 ±0.30	14.16 ±0.31	14.13 ±0.37	14.14 ±0.62	14.27 ±0.53

*Mean ± sd

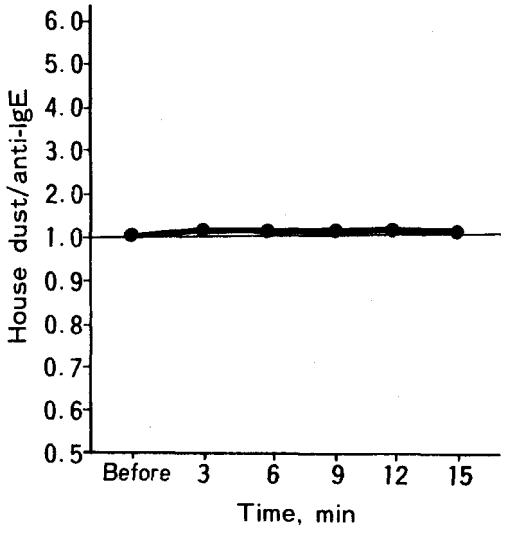

Fig. 5. Ratio of mean diameter (MD) of basophils induced by house dust to the MD by anti-IgE

5. Mean diameter of basophils (MD)

Basophils activated by these agents show an increased motility, and then degranulation phenomenon or swollen type[20, 21]. The mean diameter is larger in these cells. The MD of basophils stimulated by anti-IgE increased at 6 to 15 min after addition of the agent. The MD of the cells induced by antigen also increased. The increase in the MD by antigen was similar to the increase by anti-IgE, and any significant difference was not found between the two agents (Table 5, Fig. 5).

Discussion

It is well known that bridging of IgE receptors is essential for IgE-mediated activation of basophils[3, 4]. Activated basophils through IgE receptors show an increased motility which is observed as oriented movement (pear-shaped type)[20, 21], followed by degranulation phenomenon (swollen type). Antigen and anti-IgE cause bridging of IgE receptors, and induce morphological changes of basophils as well as histamine release. The action on histamine release and morphological changes of the cells is considered to be similar between antigen and anti-IgE. Several differences have been, however, suggested in IgE-mediated histamine release from basophils between antigen and anti-IgE[17], but there was no report about difference in morphological changes of basophils between the two agents.

Antigen-induced release of histamine is elicited over a wide range of concentrations, while release by anti-IgE occurs within a limited concentration range[17]. Our previous studies have also showed that there are some differences in dose-response curves of histamine release between antigen and anti-IgE. Anti-IgE-induced histamine release correlates to a certain extent with serum IgE levels[13,14,22]. While antigen-induced release of histamine correlates with serum levels of

specific IgE antibodies[15].

In the present study, difference in morphological changes of basophils were observed between antigen and anti-IgE stimulation. The results obtained here showed that the decrease in number of basophils was more rapidly and larger and the duration of the decrease was longer in antigen stimulation compared with anti-IgE. Morphological changes of basophils representing increased movement such as an increased L/S ratio of the cells and an increased frequency of basophils with LG were more often observed and the duration was longer in antigen stimulation than in anti-IgE, but there was no difference in changes of the MD of the cells between antigen and anti-IgE.

These results suggest that antigen activates basophils more strongly and induces an increased motility more often and longer compared with anti-IgE, but no difference was found in the action on degranulation between the two agents.

References

1. Ishizaka K, Tomioka H, Ishizaka T. Mechanisms of passive sensitization. I. Presence of IgE and IgG molecules on human leucocytes. J Immunol. 105 : 1459−1469, 1970.
2. Lichtenstein LM, Levy DA, Ishizaka K. In vitro reversed anaphylaxis : Characteristics of anti-IgE mediated histamine release. Immunology 19 : 831−847, 1970.
3. Ishizaka T. Ishizaka K, Conrad DH, Froese A. A new concept of mechanisms of IgE mediated histamine release. J Allergy Clin Immunol. 61 : 320−330, 1978.
4. Ishizaka, T. Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. J Allergy Clin Immunol. 67 : 90−96, 1981.
5. Foreman JC, Hallet MB, Mongar JL. The relationship between histamine release and ^{45}Calcium uptake by mast cells. J Physiol. 271 : 193−214, 1977.
6. Ranadive NS, Dahnari N. Movement of calcium ions and release of histamine from mast cells. Int Archs Allergy Appl Immunol. 61 : 6−18, 1980.
7. Tanizaki Y, Townley RG. Effect of BSA on Ca^{2+} influx in mast cells stimulated by ovalbumin. Int Archs Allergy Appl Immunol. 70 : 143−145, 1983.
8. Tanizaki Y, Akagi K, Lee KN, Townley RG. Inhibitory effect of nifedipine and cromolyn sodium on skin reactions and ^{45}Ca uptake and histamine release in mast cells induced by various stimulating agents. Int Archs Allergy Appl Immunol 72 : 102−109, 1983.
9. Tanizaki Y, Sasaki Y, Matsuoka T, Takahashi K, Kimura I. Antigen-induced morphological changes of basophils from atopic asthmatics, observed by phase-contrast microscopy. Jpn J Allergol. 33 : 269−274, 1984.
10. Kimura I, Tanizaki Y, Sasaki Y. In vitro antigen-induced increase in motility and degranulation of basophilic granulocytes from atopic asthmatics, studied by microscopic motion pictures. Int Archs Allergy Appl Immunol. 75 : 250−254, 1984.
11. Tanizaki Y, Matsuoka T, Maeda M, Takahashi K, Kimura I. Microscopic observation on degranulation of blood basophilic leucocytes : Relationship to different responses to antigen. Acta Haematol Jpn. 48 : 1357−1362, 1985.
12. Redermecher MF. Allergen-mediated hista-

mine release from whole blood. Int Archs Allergy Appl Immunol. 63 : 415−423, 1980.
13. Tanizaki Y, Komagoe H, Sudo M. et al. Allergen- and anti-IgE-induced histamine release from whole blood. Int Archs Allergy Appl Immunol. 73 : 141−145, 1984.
14. Tanizaki Y, Komagoe H, Sudo M. et al. Reactivity of sensitized human basophils, as expressed by histamine release. Jpn J Allergol. 33 : 463−467, 1984.
15. Tanizaki Y, Sudo M, Kitani H, et al. Release of heparin-like substance and histamine from basophilic leucoytes separated by counterflow centrifugation elutriation. Jpn J Med. 29 : 356−361, 1990.
16. Tanizaki Y. Differentiation and function in basophil-mast cell system. Release mechanisms of chemical mediators and their role. Acta Haematol Jpn. 48 : 1964−1971, 1985.
17. Marone B, Kargey-Sobotka A, Lichtenstein LM. IgE-mediated histamine release from basophils. Differences between antigen E- and anti-IgE-induced secretion. Int Archs Allergy Appl Immunol. 65 : 339−348, 1981.
18. Siraganian RP, Brodsky MJ. Automated histamine analysis for in vitro allergy testing. I. A method unilizing allergen induced histamine release. J Allergy Clin Immunol. 53 : 525−540, 1976.
19. Hastie R. The antigen-induced degranulation of basophilic leucocytes from atopic subjects, studied by phase-contrast microscopy. Clin Exp Immunol. 8 : 45−61, 1971.
20. Kimura I, Tanizaki Y, Sato S, Takahashi, K., Saito, S., Ueda, N. Supra vital observation of in vitro basophils in immunological reactions. Clin Allergy 11 : 37−41, 1981.
21. Kimura I, Tanizaki Y, Goda Y, et al. New in vitro method for detecting asthma allergen. Clin Allergy 13 : 99−105, 1983.
22. Tanizaki Y, Komagoe H, Sudo M, et al. Effect of a serum factor on IgE-mediated histamine release from whole blood. Acta Med Okayama 38 : 381−387, 1984.

IgE受容体を介する刺激に対する好塩基球の形態的変化の顕微鏡的観察．抗原と抗IgE刺激の特徴とその相違点．

谷崎勝朗，中川三郎[1)]，木村郁郎[1)]

岡山大学医学部附属病院三朝分院内科，
[1)]岡山大学医学部第二内科

抗原および抗IgE刺激時の好塩基球の形態変化の特徴について，ヒスタミン遊離との関連のもとに，顕微鏡下に経時的に観察した。1．抗原刺激時のヒスタミン遊離は，15分間のincubationのいずれの時期においても，抗IgE刺激に比べ有意に多く，incubation時間3分と9分では両者間に有意の差が見られた。2．抗原刺激時の好塩基球数の減少も抗IgE刺激に比べより高度であった。3．運動亢進により洋梨型を示す抗塩基球では，長径／短径比の増大が見られた。この長径／短径比の増大を示す好塩基球は，抗原刺激時に抗IgE刺激に比べより高頻度に見られ，特にincubation6分と15分では両者間に有意の差が見られた。4．膨化型を示す好塩基球ではその平均直径の増大が見られるが，この平均直径の増大については，抗原と抗IgE刺激との間に差は見られなかった。以上の結果より，抗原刺激では，好塩基球のactivate，運動亢進への過程が抗IgE刺激時と比べより高度であるが，膨化への過程には両者間に差が見られないことが示唆された。

キーワード：ヒスタミン遊離，好塩基球の形態的変化，抗原，抗IgE，IgE受容体