
◎原 著

Difference in motility of basophilic, granulocytes from atopic subjects following antigen- and Ca ionophore A23187-stimulation

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Abstract : We examined histamine release and morphological changes in basophilic granulocytes from atopic subjects, in response to stimulation with antigen and Ca ionophore A23187. 1. Histamine release and a reduction in the number of basophils were more rapid and greater in extent at an early stage of antigen stimulation compared with Ca ionophore A23187 stimulation. 2. Morphological changes in basophils, represented by increased motility, in terms of an increased ratio of short to long axis diameter (L/S ratio), as well as the increased frequency of basophils with localized granules and those with pseudopods, were more often observed antigen stimulation than with Ca ionophore A23187 stimulation. In contrast, morphological changes in which basophils appeared swollen, showing an increased mean cell diameter and an increased frequency of cells with 5 vacuoles or more were more predominant with Ca ionophore A23187 than with antigen stimulation.

The results obtained here show that bridging of IgE receptors is essential to activate basophils and induce increased motility in these cells.

Key words : histamine release, increased basophil motility, bridging of IgE receptors, antigen, Ca ionophore A23187

Introduction

Blood basophils and tissue mast cells are target cells for IgE antibodies (1, 2). These cells release chemical mediators in response

to stimulation with antigen (3-5), anti-IgE (6,7), and other stimulating agents such as concanavalin A (8) and Ca ionophore A23187 (9). Calcium ions play an important two-step role in the mechanisms responsible for the

release of chemical mediators from these cells, activating the cells during the first stage of IgE-mediated reactions, in which the bridging of IgE receptors is essential for the release of chemical mediators; calcium ions also participate in a late calcium-requiring step that is common for all stimuli (10).

IgE-mediated histamine release and morphological changes in basophils have been observed when antigen (11, 12) or anti-IgE (13, 14) is added to these cells. Basophils in the bloodstream are different from tissue mast cells and have to migrate to allergic reaction sites to exert their effect. The migration of basophils to allergic reaction sites in the skin (15) and bronchi (16) has been observed. We have made several reports showing the increased motility and degranulation of basophils following stimulation with antigen (17-19).

In the present study, we observed the morphological changes in basophils following stimulation with antigen or Ca ionophore A23187 in relation to histamine release.

Subjects and Methods

Our subjects were 10 atopic asthmatics (4 females and 6 males) who were sensitized by house dust mite. Their mean age was 32.6 years and the mean level of total IgE in sera was 876 IU/ml.

To a test tube containing 2ml heparinized venous blood, 0.1ml of house dust extract, at 1:100 dilution (Torii Pharmaceutical Co), or 0.1ml of Ca ionophore A23187, at 5 µg/0.1 ml, was added. The mixed solution was incubated for periods of 3, 6, 9, 12, and 15min at 37°C, and then the reaction was stopped by placing the test tube in an ice bath. Smear preparations were made from the mixed solution. After they had been air-dried, the

smear preparations were stained with May Giemsa for microscopic observation. The mixed solution was centrifuged at 1500 rpm for 15min at 4°C. The content of histamine in the supernatant and cell pellet was estimated.

Morphological changes in 12 basophils from each subject were observed in 5 atopic subjects for each experiment with antigen and Ca ionophore A23187 stimulation. The morphological changes were classified into three types, as described in our previous reports (14, 17, 18), i. e., N-form (round form), A-form (pear-shaped type with increased movement), and B-form (swollen type). In this study, following antigen or Ca ionophore A23187, the ratio of the short to the long axis diameter and the frequency of basophils with localized granules or with pseudopods were observed as morphological changes associated with the pear-shaped type, and changes in mean diameter of the cells and the frequency of basophils with 5 vacuoles or more were observed as changes related to the swollen type.

The number of basophils was calculated by observing 1000 white blood cells, and the results were expressed as a percentage of total cells.

Histamine was measured with an automated histamine analysis system (4, 5, 20). The results were expressed as a percentage of total histamine content.

Results

I. Histamine release from basophils

1. Relation to basophil count

Basophils stimulated by antigen rapidly released histamine, and the number of the cells decrease rapidly, as shown in Fig. 1. The time course of histamine release seemed to be compatible with that of the decrease in cell

number. Ca ionophore A23187 also caused histamine release, accompanied by a reduction

in cell number. The release of histamine and the reduction in cell number were more rapid and greater in extent in antigen stimulated cells than in those stimulated with Ca ionophore A23187 (Fig. 1, 2).

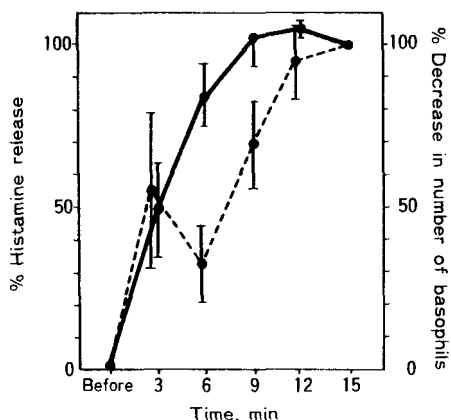


Fig. 1. Time course of histamine release (●—●) and decrease in number of basophils (●--●) induced by stimulation with antigen (house dust extract). Histamine release and decrease in basophil number were taken to be 100% at 15min after the addition of antigen.

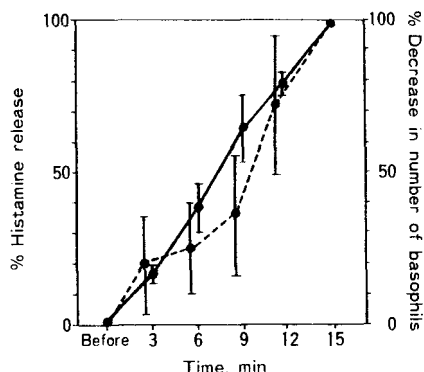


Fig. 2. Time course of histamine release (●—●) and decrease in number of basophils (●--●) induced by stimulation with Ca ionophore A23187. Histamine release and decrease in basophil number were taken to be 100% at 15 min after the addition of Ca ionophore A23187.

2. Relation to number of granules

The number of granules in each basophilic leucocyte was calculated. The number of granules decreased more rapidly on antigen stimulation, and a significant difference between antigen and Ca ionophore A23187 was found at 6 min following addition of the agents ($p < 0.01$). After incubation with the agents for 9 min or longer, no definite tendency was observed with either antigen or Ca ionophore A23187 stimulation (Fig. 3).

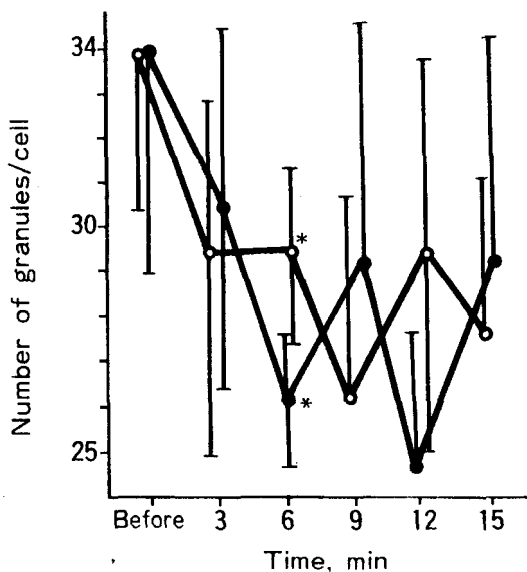


Fig. 3. Changes in number of granules in basophils stimulated with antigen (●—●) and Ca ionophore A23187 (○—○). Vertical bars represent mean \pm SD. * $p < 0.01$.

II. Morphological changes of basophils

1. Changes associated with increased movement

1) Ratio of short to long axis diameter (L/S ratio)

The L/S ratio of basophils increased when the cells were stimulated with antigen. The L/S ratio showed a marked increase at 6 min after the addition of antigen, and this increase was then maintained up to 15 min following the antigen stimulation. However, the L/S ratio did not change at any incubation time with Ca ionophore A23187. The value for the ratio following antigen stimulation was significantly higher at 6, 9, and 15 min of incubation time than the ratio with Ca ionophore A23187 (at 6, 9 and 15 min ; $p < 0.001$) (Table 1).

Table 1. Changes in ratio of short to long axis diameter (L/S ratio) in basophils stimulated by antigen and Ca ionophore A23187

Agent	No of cases	Ratio of short to long axis diameter					
		Before	3	6	9	12	15
Antigen	5	1.0	1.02 ± 0.05	1.06 ^a ± 0.05	1.10 ^b ± 0.06	1.04 ± 0.09	1.05 ^c ± 0.04
Ca Iono.	5	1.0	0.98 ± 0.02	0.98 ^a ± 0.02	0.98 ^b ± 0.02	0.99 ± 0.03	0.99 ^c ± 0.02

Twelve basophils were observed in each subject. Ca Iono; Ca ionophore A23187. The ratio before stimulation was taken to be 1.0. a, b and c; $p < 0.001$.

2) Frequency of basophils with localized granules

When basophils were activated by antigen, the frequency of pear-shaped cells showing an increased motility was higher. In these activated basophils, the granules were localized on one side of the cell. We observed changes in the frequency of basophils with localized granules (LG) after the agents were added. The frequency of basophils with LG increased

linearly as the incubation time was increased, reaching a peak at 12 min after the addition of antigen. In contrast, the frequency of basophils with LG increased at 6 to 9 min after the addition of Ca ionophore A23187. After that time, the frequency did not increase, as shown in Fig. 4. A significant difference was found in the frequency of such cells at 12 min after stimulation by antigen and Ca ionophore A23187 (Fig. 4).

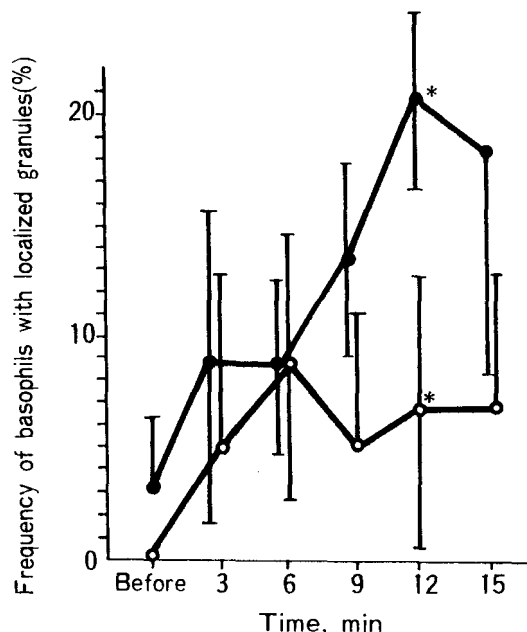


Fig. 4. Changes in frequency of basophils with localized granules induced by stimulation with antigen (●—●) and Ca ionophore A23187 (○—○). Vertical bars represent mean \pm SD. * $p < 0.001$.

3) Frequency of basophils with pseudopods

Pseudopod formation is often observed in activated basophils. The frequency of basophils with pseudopods increased at 3 to 9

min following the addition of antigen, and at 3 to 6 min after Ca ionophore A23187 stimulation. Thereafter, the frequency with both stimulating agents tended to decrease. There was no significant difference between the frequency of antigen- and Ca ionophore A23187-stimulated basophils with pseudopods (Fig. 5).

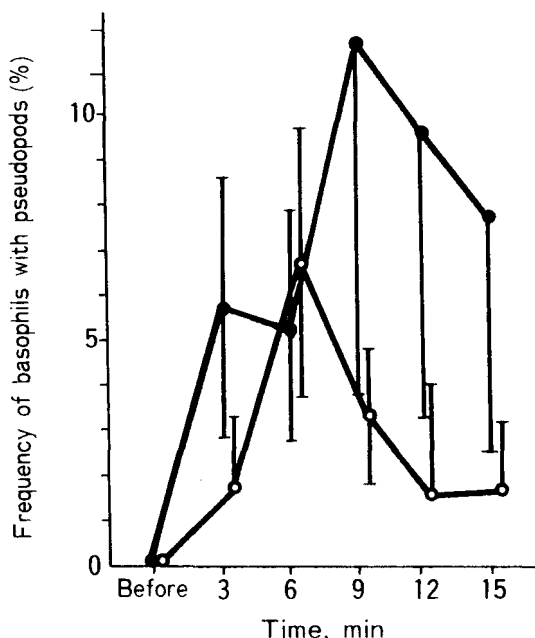


Fig. 5. Changes in frequency of basophils with pseudopods induced by stimulation with antigen (●—●) and Ca ionophore A23187 (○—○). Vertical bars represent mean \pm SD.

2. Changes associated with swollen type basophils (degranulation)

1) Mean cell diameter

The mean diameter of the basophils clearly increased at 6 to 15 min following the addition of Ca ionophore A23187. An increased diameter was also observed in basophils stimulated with antigen. The increase in mean diameter induced by antigen was,

however, not as high as that induced by Ca ionophore A23187. The increase in mean diameter induced by Ca ionophore A23187 was significantly higher than that induced by antigen at 6 to 15 min of incubation time (Table 2).

Table 2. Changes in mean diameter of basophils stimulated by antigen and Ca ionophore A23187

Agent	No of cases	Mean diameter (μ m)					
		Before	3	6	9	12	15
Antigen	5	12.5 ± 0.3	13.4 ± 0.5	13.9 ^a ± 0.5	13.9 ^b ± 0.5	13.9 ^c ± 0.5	13.9 ^d ± 0.2
Ca iono.	5	12.5 ± 0.5	13.9 ± 0.7	14.5 ^a ± 0.7	14.7 ^b ± 0.5	14.6 ^c ± 0.8	14.6 ^d ± 0.9

Twelve basophils were observed in each subject. Ca iono: Ca ionophore A23187. The mean diameter of the cells before stimulation with Ca ionophore A23187 was the same value as the diameter of the cells before stimulation by antigen. a; $p < 0.05$, b; $p < 0.001$, c and d; $p < 0.02$.

2) Frequency of basophils with 5 vacuoles or more

Basophils stimulated by antigen showed increased motility, and a later degranulation phenomenon, in which the number of granules decreased and large vacuoles appeared in the stimulated cells. The frequency of basophils with 5 vacuoles or more was increased to a greater extent by stimulation with Ca ionophore A23187 than by stimulation with antigen; the difference was not significant.

Discussion

The process by which chemical mediators are released is common to both basophils and mast cells. Calcium ions are required for this process, and their influx into the cell causes a release of chemical mediators. It has been shown that antigen stimulation leads to increased phospholipid metabolism in the cell membrane, followed by calcium ion influx into the cell (21, 22). The authors have observed histamine release and calcium ion

uptake in mast cells induced by various stimulation agents, including antigen and Ca ionophore A23187 (23, 24). The uptake of calcium ions by basophils and mast cells was observed following antigen stimulation (25, 26) and following stimulation with a calcium ion carrier, Ca ionophore A23187 (24).

Atopic asthma patients show immediate asthmatic reaction (IAR) and late asthmatic reaction (LAR) after bronchial challenge with antigen. It has been reported that LAR is associated with airway inflammation, in which lymphocytes, neutrophils, eosinophils and basophils migrate from the bloodstream (27, 28). In the LAR and nasal late phase response (LPR), basophils are responsible for the persistent release of histamine during stimulation with antigen. Reshef et al., using a skin blister technique, showed that histamine was continuously released into the skin of antigen-stimulated subjects in a pattern characterized by a rapid peak, followed by diminishing, but significant, levels of the mediator during the initiation of the clinical LPR (29). A similar trend of progressively diminishing, but significant, amounts of histamine was observed in the cutaneous LPR (30). An initial peak of histamine release, followed by lower level plateau, was also observed in immediate to late phase allergic reactions (31). These findings suggest that basophils are source of the persistent histamine release in the LAR and LPR.

In this study, we examined differences in histamine release and morphological changes in basophils induced by antigen and Ca ionophore A23187 stimulation. Histamine release from basophils was more rapid and greater in amount at the early stage of antigen stimulation than with stimulation by Ca ionophore A23187. The maximum percent

histamine release was, however, similar for the two agents, the most striking difference between their effects being observed in the morphological changes in basophils.

Changes representing increased motility, i. e., the increased L/S ratio of the cells and the increased frequency of basophils with localized granules, were more often observed on stimulation with antigen than with Ca ionophore A23187 stimulation. In contrast, changes in which swollen-type basophils (in terms of increased mean cell diameter) were observed, were predominant with Ca ionophore A23187 stimulation.

The results of this study show that, at first, antigen stimulation caused increased motility, which seems to be essential for IgE-mediated allergic reactions, while stimulation with Ca ionophore A23187 did not induce increased basophil motility. The results suggest that the bridging of IgE receptors is required to activate basophils and induce their increased motility. The degranulation phenomenon and swollen-type basophils observed at the late stage of antigen and Ca ionophore A23187 stimulation were common for the two agents.

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抗原および Ca ionophore A23187 刺激時におけるアトピー型気管支喘息末梢血好塩基球の運動能

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アトピー型気管支喘息末梢血好塩基球を用い, 抗原および Ca ionophore A23187 刺激時のヒスタミン遊離およびその形態的变化について比較検討した。

1. 抗原刺激時には, Ca ionophore A23187 刺激時に比べ, 好塩基球からのヒスタミン遊離および好塩基球数の減少の程度は, より急激でか

つ高度であった。

2. 好塩基球の形態的变化, すなわち, 長径/短径比の増大, 限局性顆粒あるいは偽足を有する好塩基球の出現頻度の増加などの運動能の亢進を示唆する好塩基球の形態的变化は, Ca ionophore A23187 に比べ抗原刺激時により高度であった。一方, 平均直径の増大, 5個以上の空胞を有する好塩基球の出現頻度の増加などの, むしろ膨化傾向を示唆する好塩基球の形態的变化は, 抗原刺激時に比べ, Co ionophore A23187 刺激時により高度であった。

これらの結果より, 抗原刺激による IgE 受容体の bridging が, 好塩基球を活性化し, 運動能の亢進をひき起こすものと判断された。

キーワード: ヒスタミン遊離, 好塩基球の運動能の亢進, IgE 受容体, 抗原, Ca ionophore A23187