

◎原 著

Basophil reactivity to anti-IgE and calcium ionophore A23187 in patients with bronchial asthma

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Abstract: The release of histamine from basophils induced by anti-IgE and calcium ionophore A 23187 (CaI) was examined in 27 patients with bronchial asthma and 7 healthy subjects, using a whole blood method. (1) The release of histamine induced by anti-IgE and CaI was significantly higher in atopic asthmatics than in non-atopic cases. (2) The histamine release with anti-IgE and CaI was significantly lower in cases with long-term steroid therapy compared to the release in cases without steroid therapy. (3) The release with anti-IgE and CaI was increased in cases with age of 0-39 years and in cases with age at onset of 0-39 years. (4) Anti-IgE induced release correlated to a certain extent with the release induced by CaI.

These results show that basophil reactivity as expressed by histamine release changes under different conditions.

Key words: Histamine release, Basophils, Anti-IgE, Calcium ionophore A 23187, Bronchial asthma

Introduction

Blood basophils of patients with bronchial asthma release histamine when the cells are incubated with various stimulating agents such as antigen^{1,2)}, anti-IgE³⁻⁶⁾, concanavalin A, compound 48/80 and calcium ionophore A23187 (CaI). The former two agents stimulate basophils through IgE receptors (IgE-mediated release). The other stimulating agents also elicit histamine release from basophils, by inducing an increase in Ca²⁺ influx into the cells (non-IgE-mediated release). The release of

histamine induced by non-IgE-mediated stimuli has been reported to be different from the release elicited by IgE-mediated stimuli such as antigen and anti-IgE⁷⁻⁹⁾.

The reactivity of sensitized basophils from patients with bronchial asthma is generally increased to the allergen and anti-IgE. The amount of histamine release induced by house dust allergen parallels the amount of release induced by anti-IgE in sensitized basophils, and skin sensitivity to the allergen reveal an excellent correlation with the release of histamine⁵⁾.

In the present study, basophil reactivity

to anti-IgE and CaI was examined by observing histamine release.

Subjects and Methods

Subjects : Twenty seven patients with bronchial asthma (12 females and 15 males, their ages ranged from 18 to 62 years) and 7 healthy subjects were selected to examine basophil reactivity, as expressed by histamine release.

Histamine release : Histamine release from basophils was performed by a whole blood method¹⁰, as previously reported^{3, 4}. Venous blood (24–25ml) was drawn into a plastic syringe containing 1 ml of heparin. The blood (4 ml/test tube) was transferred into a test tube. 0.2ml of different concentrations of anti-IgE or CaI was added to the test tubes, and the mixed solution was then incubated for 15 min at 37°C. After the incubation the reaction was stopped by transferring into ice bath. The cells were separated by centrifugation at 400 g for 20 min at 4°C. The histamine content of the cells and supernatant fluid was analyzed by perchloric acid precipitation and assayed by an automated spectro-fluorometric histamine analysis system (Technicon)¹¹. The results were expressed as a percent release of the total histamine content. All medication was stopped 12 hours before the examination.

Serum IgE levels were estimated by the radioimmunosorbent test (RIST) (Pharmacia).

Results

1. Kinetics of histamine release

The kinetics of histamine release induced by anti-IgE and CaI was compared. Figure 1 shows the time course of histamine release at the concentrations of anti-IgE

or CaI causing the maximum percent release. The percent histamine release with anti-IgE reached the peak (27.4% in asthmatic subjects) after 15 min. While the release with CaI peaked (50.6% in asthmatic subjects and 34.3% in healthy subjects) after 60 min. There was a clear difference in the kinetics of the release from whole blood between anti-IgE and CaI. In the following experiments, the cells were incubated with anti-IgE for 15 min and with CaI for 60 min (Fig. 1).

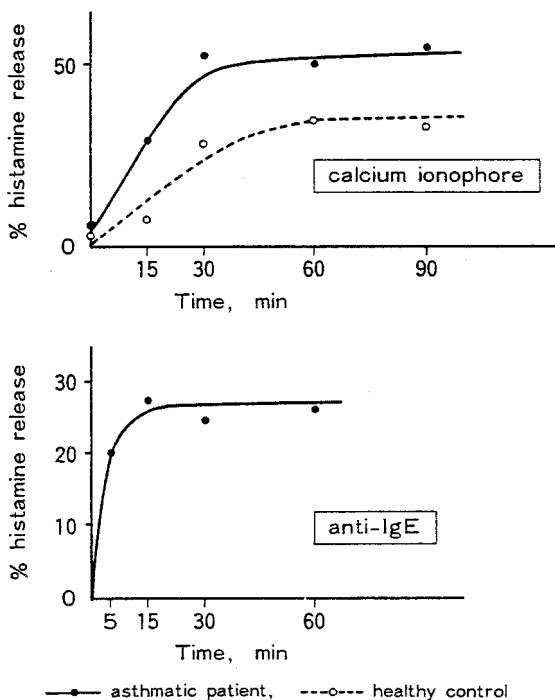


Fig. 1. Time course of calcium ionophore A23187 and anti-IgE induced histamine release

2. Dose-response curve of histamine release by CaI.

CaI caused the release of histamine from whole blood of all healthy subjects. Figure 2 shows the dose-response curve of CaI-

induced histamine release. The release with CaI reached the peak (30.6% in healthy subjects) at the concentration of 10 $\mu\text{g}/\text{ml}$ (Fig. 2). The concentration of anti-IgE causing maximum histamine release was 10- or 10² -fold dilutions, as previously reported⁴⁾

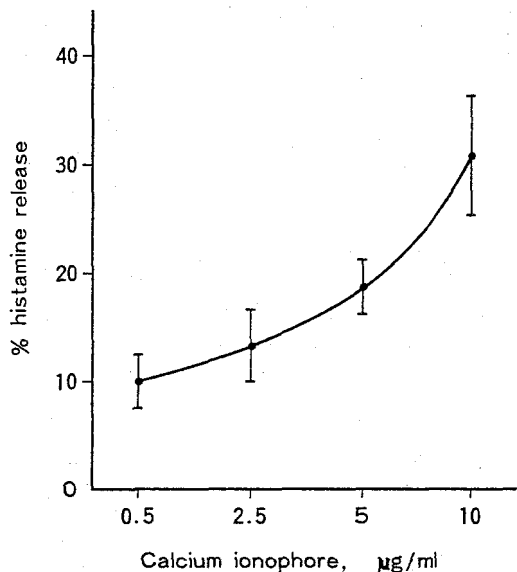


Fig. 2. Dose-response curve of calcium ionophore induced histamine release

3. Histamine release induced by anti-IgE and CaI.

The maximum release of histamine induced by anti-IgE was significantly higher in atopic asthmatics (34.0%) than in non-atopic cases (19.0%) ($p < 0.05$). The release with CaI was also significantly increased in atopic cases (53.4%) compared to the release in non-atopic cases (32.2%) ($p < 0.01$) and in healthy subjects (36.7%) ($p < 0.01$). The results show that the reactivity of basophils to anti-IgE and CaI significantly increases in atopic cases (Fig. 3).

4. Histamine release in relation to steroid regimen

The maximum histamine release induced by

anti-IgE was generally low in cases with steroid-dependent asthma (17.1%) compared to the release in cases without steroid therapy (32.3%). The release with CaI was also significantly lower in cases with steroid-dependent asthma (34.1%) than in cases without steroid therapy (50.0%) ($p < 0.05$). The percent release in healthy subjects was 26.9% with anti-IgE and 36.7% with CaI (Fig. 4).

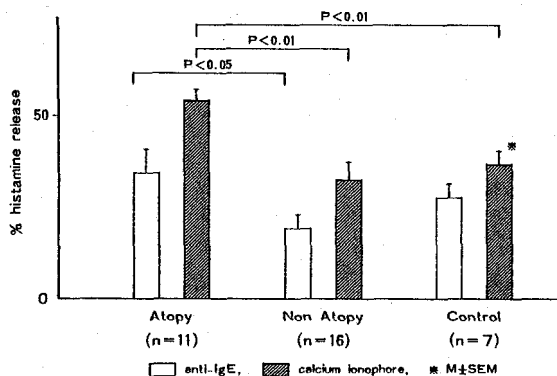


Fig. 3. Histamine release from basophils of asthmatics induced by anti-IgE and calcium ionophore A 23187

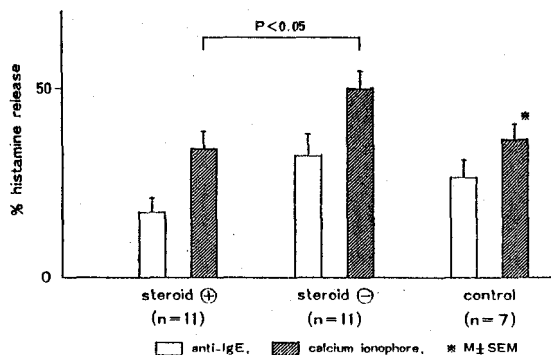


Fig. 4. Anti-IgE and calcium ionophore A 23187 induced histamine release in relation to steroid regimen

5. Histamine release in relation to patient age

The release of histamine with anti-IgE was clearly lower in cases with age of 40 years or more compared to the release in younger cases (less than 40 years of age), and the lowest (9.6%) in cases with their ages of 40-49 years. While histamine release with CaI decreased with aging, being the lowest (28.8%) in cases with age of 60 years or more (Fig. 5).

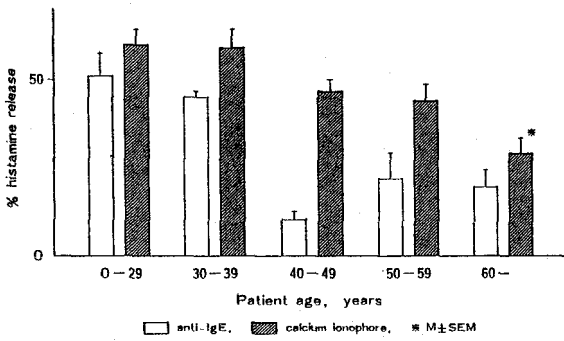


Fig. 5. Anti-IgE and calcium ionophore A23187 induced histamine release in relation to patient age

6. Histamine release in relation to age at onset

The maximum histamine release caused by anti-IgE showed a tendency to decrease as the age at onset was higher. The release in cases with age at onset of 0-29 years was significantly higher compared to the release in cases of 40-49 years ($p < 0.02$), and of 50 years or more ($p < 0.02$). The release with CaI became lower with aging. A significant difference was present in histamine release between cases with age at onset of 0-29 and of 50 years or more ($p < 0.01$). The results showed the basophil reactivity to anti-IgE and CaI tends to decrease as age at onset is higher (Fig. 6).

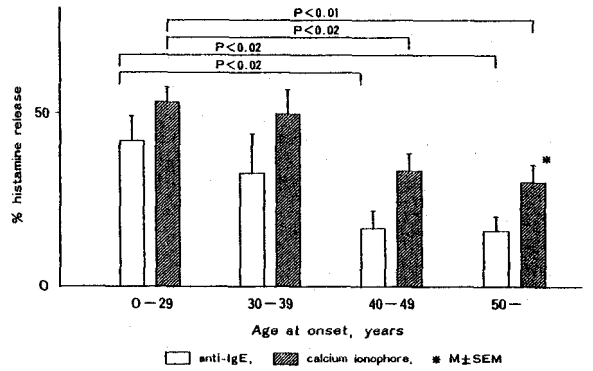


Fig. 6. Anti-IgE and calcium ionophore A23187 induced histamine release in relation to age at onset of the disease

7. Histamine release in relation to serum IgE levels

Basophils from many cases with high serum IgE levels (301 IU/ml or more) released a large amount of histamine by stimulation with anti-IgE and CaI. The histamine release was generally low in cases with low serum IgE levels (300 IU/ml or less) when the cells was incubated by anti-IgE or CaI (Fig. 7).

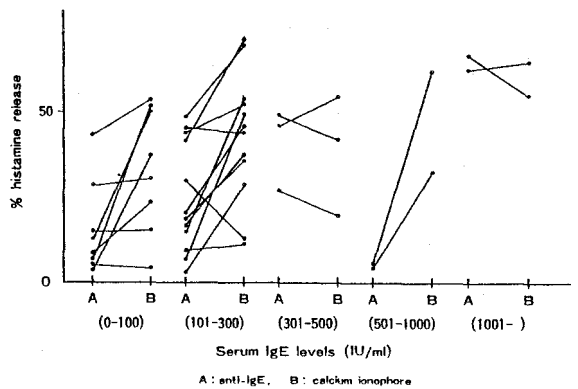


Fig. 7. Anti-IgE and calcium ionophore A23187 induced histamine release in relation to serum IgE levels

8. Correlation between histamine release induced by anti-IgE and CaI.

The release of histamine induced by anti-IgE showed to a certain extent a correlation with the release by CaI, suggesting that basophil reactivity changes similarly to anti-IgE and CaI (Fig. 8).

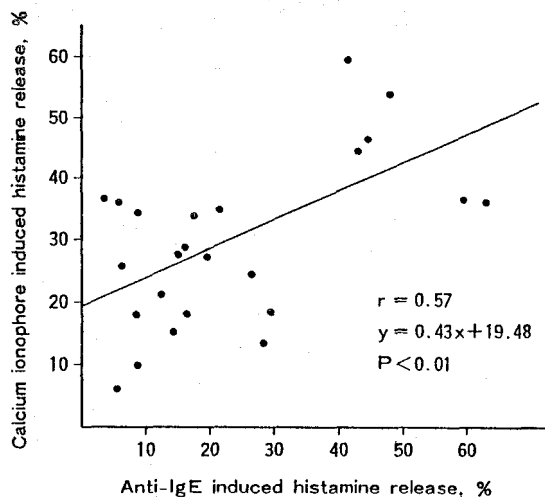


Fig. 8. Correlation between percent histamine release induced by anti-IgE and calcium ionophore A23187

Discussion

Mast cells and basophils are target cells of IgE antibodies^{11, 12)} and participate in onset mechanisms of bronchial asthma. Our previous studies showed that antigen causes an increase in Ca^{2+} uptake by sensitized mast cells¹³⁾ and that inhibition of Ca^{2+} influx leads to the inhibition of histamine release¹⁴⁻¹⁶⁾.

The release of histamine from basophils has been extensively used for in vitro studies of allergy. A number of studies using washed leucocytes demonstrated that there was a close correlation between histamine release and the severity of clinical symp-

toms^{17, 18)}. It was also shown that histamine release from basophils correlated with the results of skin test with allergen and the levels of serum IgE.

Histamine release from whole blood has been noticed by some investigators^{19, 20)} to simplify the method for measurement of histamine release. It has been reported that the maximum histamine release from whole blood correlates closely with the release from washed leucocytes. Our previous studies using a whole blood method showed that the degree of basophil reactivity (as expressed by histamine release) and skin sensitivity to the allergen reveal excellent correlation, and that age at onset of the disease was higher in the cases with less reactive basophils and lower in those with more reactive cells.

The results suggest that basophil reactivity changes in a correlation with skin sensitivity and age at onset of the disease.

In the present study, the release of histamine by anti-IgE and CaI was higher in atopic asthmatics, in cases with high serum IgE levels, in cases with age of 39 years or less, and in cases with age at onset of 0-29 years. While lower basophil histamine release with anti-IgE and CaI was observed in non-atopic asthmatics, in cases with low serum IgE levels, in cases with long-term steroid-therapy, in cases with age of 40 years or more, and in cases with age at onset of 30 years or more. The results show basophil reactivity changes under different conditions. The release induced by anti-IgE was generally similar to the release by CaI. However, some differences in basophil reactivity were observed in relation to patient age. The release with anti-IgE was the lowest in cases with age of 40-49 years, while the release with CaI

tended to decrease with aging, being the lowest in cases with age of 60 years or more. The results obtained here suggested that basophil reactivity, which correlates with onset mechanisms of bronchial asthma, can be speculated by measuring histamine release with anti-IgE and CaI.

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気管支喘息患者末梢血好塩基球の抗ヒトIgEおよびカルシウムイオノフォアA23187に対する反応性について

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気管支喘息27例および健康人7名を対象に, 抗ヒトIgEおよびカルシウムイオノフォアA23187 (CaI) 刺激時の末梢血好塩基球からのヒスタミン遊離について検討を加えた。

(1) 抗ヒトIgEおよびCaI刺激時の好塩基球からのヒスタミン遊離は, 非アトピー型喘息に比べ, アトピー型喘息において有意に高い値を示した。

(2) これらの刺激物質によるヒスタミン遊離は, 非使用例に比べ, ステロイド依存性喘息において低い傾向がみられた。また, 年齢では39才以下, 発症年齢では39才以下でヒスタミン遊離がより高い傾向がみられた。

(3) 血清IgE値の高い症例の好塩基球は, 低い症例に比べより多くのヒスタミンを遊離する傾向がみられた。

(4) 全般的には, 抗ヒトIgE刺激によるヒスタミン遊離とCaI刺激時の遊離との間にはある程度の相関がみられた。

以上の結果より, 好塩基球の反応性はいろいろの条件下でかなり異なることが示唆された。

キーワード: ヒスタミン遊離, 好塩基球, 抗ヒトIgE, カルシウムイオノフォアA23187, 気管支喘息