◎原 著

Basophil histamine release by anti-IgE in subjects of chronic bronchitis and bronchial asthma

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Abstract : Histamine release from basophils induced by anti-IgE was studied in 8 patients with chronic bronchitis and 50 patients with bronchial asthma by analyzing doseresponse curves. As the result, there were no significant differences in maximum percent histamine release from basophils among three groups of healthy subjects(24.7±14.2%), patients with chronic bronchitis (27.7±22.1%) and those with bronchial asthma (28.4±17.0%). In the patients with bronchial asthma, the maximum percent histamine release was higher in accordance with higher serum IgE levels, and low maximum percent release was observed in patients with intrinsic asthma (14.1±7.2%).

Study of dose-response curves of anti-IgE-induced histamine release showed that a negative slope from E_2 to E_1 was observed in both healthy subjects and patients with chronic bronchitis. The majority of asthmatics with serum IgE levels of 501IU/ml or over showed a positive slope from E_2 to E_1.

Key words : Basophil response—Anti-IgE—Histamine release—chronic bronchitis—bronchial asthma

Introduction

The mechanism of histamine release induced by non-IgE-mediated stimuli(comp. 48/80[1], Ca ionophore A23187[2])is different from that caused by antigen[3)-5)], anti-IgE[6], which are IgE-mediated stimuli. Histamine release from basophils mediated by IgE is induced by bridging of IgE molecules on the cells by these stimulating agents such as antigen and anti-IgE[7), 8)]. It is considered that mechanisms of histamine release from basophils by these agents are almost slmilar. However, a recent study has indicated that there are some differences in histamine release from basophils by antigen and anti-IgE[9)].

Majority of studies on histamine release from basophils has been performed using washed leukocytes. While, there are only a few reports about the release of histamine from whole blood[10)-13)]. In the present study, basophil response to anti-IgE in chronic bronchitis and bronchial asthma was examined by measuring the release of histamine from whole blood.

Subjects and Methods

Eight patients with chronic bronchitis (4 females and 4 males, age 50−72 years) and 50 patients with bronchial asthma (34 females and 16 males, age 16−71 years) were selected for the study of histamine release. The healthy individuals were examined as controls. The mean of serum IgE levels was 221 ± 63 IU/ml in the cases with chronic bronchitis, 463 ± 67 IU/ml in the cases with bronchial asthma and 147±17 IU/ml in healthy controls.

Histamine release from basophils. It was carried out by the whole blood method as previously described[12), 13)]. Four ml of heparinized venous blood was drawn into a siliconed test tube and 0.2ml of various concentrations (10^1 −10^5 times dilution) of anti-IgE (Hoechst) was added. The mixture was incubted at 37℃ for 15 minutes. The results were expressed as a percentage of the total blood content of histamine.

Analysis of dose-response curve. Examinations were made from two viewpoints, i.e. height of dose-response curve and gradient of curve between two points. The height of dose-response curve was expressed as the maximun percent histamine release (reactivity) induced by anti-IgE. Since it was only with 100-fold and 10-fold dilutions of anti-IgE that significant release of histamine was observed in the majority of cases, expression with a positive or negative slope from 100-fold dilution (E_2) to 10-fold dilution (E_1) of anti-IgE was used in analysis of dose-response curves (Fig. 1).

Determination of histamine was made using an automated histamine analysis system[14),15)] of Technicon. As a rule, administration of any drug was stopped within 12 hours prior to blood collection for the study.

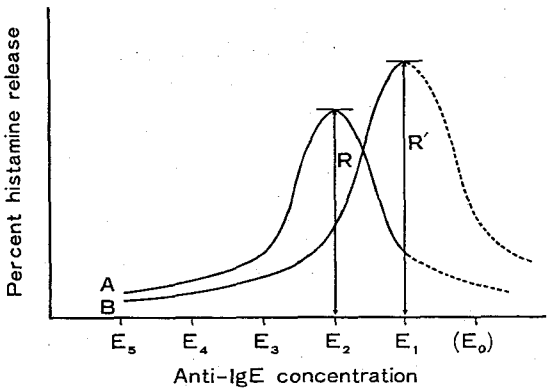

Fig. 1. A shema of dose-response curve of anti-IgE-induced histamine release. R, R′; basophil reactivity

Results

Maximum percent histamine release (reactivity) from basophils following addition of anti-IgE averaged 24.7±14.2% (mean±SD) (range:2.9−50.0%) in healthy subjects, 27.7 ±22.1% (3.5−61.4) in the subjects with chronic bronchitis and 28.4±17.0%(2.7−69.4) in the subjects with bronchial asthma. There were no significant differences in basophil reactivity among three groups. The results revealed that basophils from healthy subjects and patients with chronic bronchitis release significant amount of histamine when the cells were stimulated by anti-IgE. Among the cases with bronchial asthma, the patients with intrinsic asthma whose skin reaction to allergens is always negative showed a tendency to low maximum percent histamine release with a mean of 14.1±7.2% (Fig. 2).

In the patients with bronchial asthma, maximum percent histamine release by anti-IgE was compared according to serum IgE levels. In the cases with serum IgE levels of 0 − 100IU/ml, the maximum percent resease averaged 17.1 ± 4.4% : with 101 − 200IU/ml, 16.1 ± 3.5% : with 201 − 300IU/ml, 20.2 ±

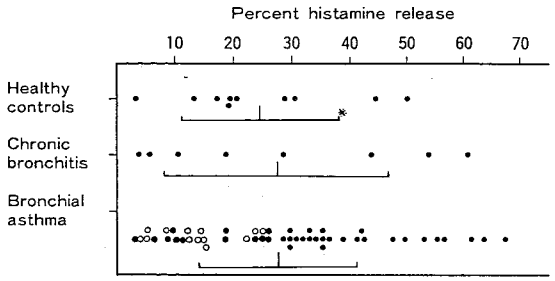

Fig. 2. Maximum percent histamine release (reactivity) in healthy controls, subjects with chronic bronchitis and bronchial asthma.(○:intrinsic asthma) *Mean±SD

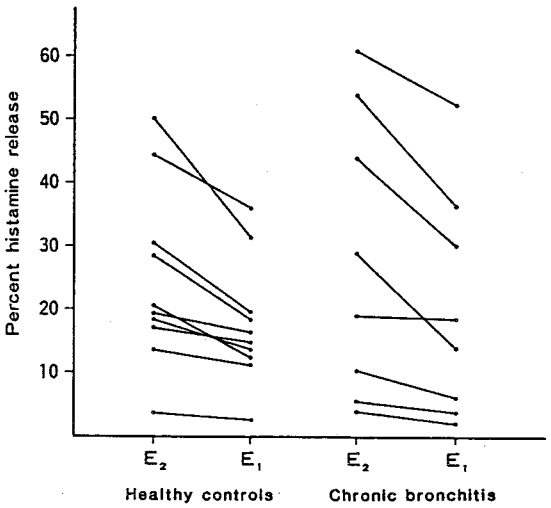

Fig. 3. Comparison between E_2-and E_1-induced histamine release in healthy controls and subjects with chronic bronchitis. Negative slope from E_2 to E_1 was shown in all cases of both subjects. E_2 : x 10^2 dilution, E_1 ; x 10 dilution of anti-IgE.

3.3% ; with301－500IU／ml, 29.6±4.4% ; with 501－1000IU／ml, 45.8±7.1% ; and with the level over 1001IU／ml, it was 47.8± 5.7%. Thus, the maximum percent release was higher in accordance with higher serum IgE levels. Especially when serum IgE levels were over than 300IU／ml, maximum percent histamine release of 20% or over was induced in all of the cases, and the difference was significantly great compared with the cases with serum IgE levels of 300IU/ml or below ($p<0.05$). Among the latter cases, however, there were considerable number of cases in which maximum percent release exceed 20%.

Dose-response slopes from 100-fold (E_2)to 10-fold (E_1) dilution of anti-IgE were studied. As the result, a negative slope from E_2 to E_1 was observed in both healthy subjects and patients with chronic bronchitis. In these cases, maximum percent histamine release was induced by the low concentration of anti-IgE (E_2) and the release of histamine was supressed by the high concentration of anti-IgE (E_1) (Fig. 3).

The majority of asthmatics with serum IgE levels of 500IU／ml or below showed a negative slope from E_2 to E_1, and maximum percent release was induced by the low concentration of 100-fold dilution (E_2) in these cases like healthy subjects and patients with chronic bronchitis. On the other hand, the majority of the cases with levels of 501IU／ml or over showed a positive slope. Thus, it became clear that dose-response slopes from E_2 to $E_1$of histamine release induced by anti-IgE would be negative in the cases with low serum IgE levels and positive in those with high serum IgE levels. The result suggests that basophils from the cases with high serum IgE levels require high concentrations of anti-IgE to cause maximum histamine release (Fig. 4).

Discussion

IgE-mediated histamine release from basophils is induced by stimuli such as antigen[3)-5)] and anti-IgE[6)]. Histamine release

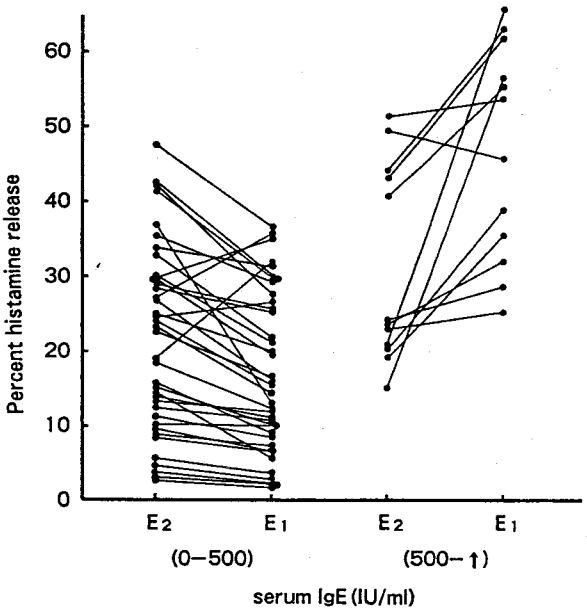

Fig. 4. Comparison between E_2- and E_1-induced histamine release in asthmatic subjects classified by serum IgE. E_2 : x 10^2 dilution, E_1 : x 10 dilution of anti-IgE.

induced by these agents have generally been studied using washed leucocytes. From the viewpoint of clinical application, simple whole blood method seems to be more useful. Siraganian et al.[11] compared washed leucocyte method with whole blood method, and suggested that the results of histamine release by these methods nearly agreed with each other. In a study of histamine release using whole blood, however, it is necesarry to consider interaction between various factors contained in serum and stimulating agents[16]. In the cases of histamine release induced by anti-IgE, it is IgE that comes into question among factors contained in serum.

In the present study, anti-IgE-mediated histamine release from basophils was investigated by analyzing dose-response curves. Lichtenstein, et al[3] observed histamine release from washed leucocytes regarding two aspects of reactivity and sensitivity of basophils. In their studies, basophil reactivity was expressed as maximum percent histamine release, and sensitivity as concentration of stimulating agents that induced 50% or 30% histamine release (HR_{50}, HR_{30}). Using the present whole blood method, it was possible to observe reactivity indicated by maximum percent histamine release in the same way as washed leucocyte method. However, basophil sensitivity indicated by HR_{50} or HR_{30} could not be observed by the present method. The first reason is that since we used commercial anti-IgE serum, maximum percent histamine release was induced only by 10-fold and 100-fold dilutions of the antiserum in any cases, and differences by various concentrations of anti-IgE were not observed well. Secondly, whole blood method did not yield high maximum percent release as with washed leucocyte method, and there were many cases in which even HR_{30} of Lichtenstein et al.[3] was not achieved. Therefore, in the present study by whole blood method, dose-response slopes from 100-fold (E_2) to 10-fold (E_1) dilution of anti-IgE were used instead of basophil sensitivity indicated by HR_{50} or HR_{30}. As the result, there was a general tendency for dose-response slopes from E_2 to E_1 to show negative gradient in the cases with low serum IgE levels and positive gradient in those with high serum IgE levels. This indicates that anti-IgE concentration required to induce maximum percent histamine release is lower in the cases with low serum IgE compared to those with high serum IgE levels. That is, basophils of the cases with low serum IgE levels appear to be able to react to lower concentration of anti-IgE. This is presumably due to interaction

between IgE in serum and anti-IgE added. In the cases with high serum IgE levels, it seems that a larger amount of anti-IgE is required to induce maximum percent histamine release because of interaction between much IgE in serum and anti-IgE. The results obtained in the present study show that observation of basophil sensitivity by washed leucocyte method is not possible by whole blood method for the interaction of IgE in serum. However, regarding basophil reactivity, similar tendency was observed by both washed leucocyte method and whole blood method.

There was no significant differences in maximum percent histamine release from basophils induced by anti-IgE among three groups of healthy subjects, patients with chronic bronchitis and those with bronchial asthma. In the cases of bronchial asthma, maximum percent histamine release was related to some extent with serum IgE levels, but there were some cases with low serum IgE levels who showed high values of maximum percent release. On the other hand, maximum percent release was always low in the cases with intrinsic asthma, who had no participation of IgE in the onset mechanism of the disease. Based on these results, it is considered that observation of anti-IgE-induced histamine release from basophils can be used as an important index to know whether IgE-mediated reaction is involved in occurrence of bronchial asthma.

References

1. Douglas, W. W. and Ueda, Y. : Mast cell secretion (histamine release) induced by 48/80 : Calcium dependent exocytosis inhibit strongly by cytochalasin only when glycolysis in rat limiting. J. Physiol., Lond. 234 ; 97−98, 1973.
2. Foreman, J. C., Mongar, J. L. and Gomperts, B. D. : Calcium ionophores and movement of calcium ions following the physiological stimulus to secretory process. Nature, Lond. 245 ; 249 − 251, 1973.
3. Lichtenstein, L. M. and Osler, A. G. : Studies on the mechanisms of hypersensitivity phenomena. IX. Histamine release from leucocytes by ragweed pollen antigen. J. Exp. Med. 120;507−530, 1964.
4. Pruzanski, J. J. and Patterson, R. : Histamine release from leucocytes of hypersensitive individuals. 1. Use of several antigens. J. Allergy 38 ; 315 − 320, 1966.
5. Radermecker, M. F. : Allergen-mediated histamine release from whole blood. Clinical evaluation. Int. Archs Allergy appl. Immun. 63;415−423, 1980.
6. Lichtenstein, L. M., Levy, D. A. and Ishizaka, K. : In vitro reversed anaphylaxis : Characteristics of anti-IgE mediated histamine release. Immunology 19 ; 831 − 842, 1970.
7. Ishizaka, T., Chang, T. H., Taggart, M. and Ishizaka, K. : Histamine release from rat mast cells by antibodies against rat basophilic leukemia membrane. J. Immunol. 119;1589−1596, 1977.
8. Ishizaka, T. : Analysis of triggering events in mast cells for immunoglobulin E-mediated histamine release. J. Allergy Clin. Immun. 67;90−96, 1981.
9. Marone, G., Kargey-Sobotka, A. and Lichtenstein, L. M. : IgE-mediated histamine release from human basophils : Differences between antigen E -and anti-IgE-induced secretion. Int. Archs Allergy appl. Immun. 63;339-348, 1981.
10. Siraganian, R. P. :Automated histamine release. A method for in vitro allergy diagnosis. Int. Archs Allergy appl. Immun. 49;108−110, 1975.

11. Siraganian, R. P. and Brodsky, B. A.: Automated histamine analysis for in vitro allergy testing. I. A method utilizing allergen-induced histamine release from whole blood. J. Allergy Clin. Immunol. 57;525－540, 1976.
12. Tanizaki, Y., Komagoe, H., Sudo, M., Morinaga, H., Kitani, H., Goda, Y., Tada, S., Takahashi, K. and Kimura, I.: IgE-mediated histamine release from whole blood in atopic asthmatics. Jpn J. Allergol. 32;1079－1083, 1983.
13. Tanizaki, Y., Komagoe, H., Morinaga, H., Kitani, H., Goda, Y. and Kimura, I.: Allergen-and anti-IgE-iuduced histamine release from whole blood. Int. Archs Allergy appl. Immun. 73;141－145, 1984.
14. Siraganian, R. P.:An automhted continuous-flow system for the extraction and fluorometric analysis of histamine. Analyt. Biochem. 57;383－394, 1974.
15. Siraganian, R. P.: Refinements in the automated fluorometric histamine analysis system. J. Immunol. Method 7;283－394, 1975.
16. Tanizaki, Y., Komagoe, H., Sudo, M., Morinaga, H., Kitani, H., Nakagawa, S., Takahashi, K. and Kimura, I.: Effect of serum factor on IgE-mediated histamine release from whole blood. Acta Med. Okayama 38;381－387, 1984.

気管支喘息および慢性気管支炎患者における抗ヒトIgEによる好塩基球からのヒスタミン遊離

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気管支喘息50例，慢性気管支炎８例を対象に，抗ヒトIgE添加時の好塩基球からのヒスタミン遊離を全血法により行ない，その臨床的評価について検討を加えた。抗ヒトIgE添加時のMax.% histamine releaseの平均は，健康人24.7±14.2%，慢性気管支炎27.7±22.1%，気管支喘息28.4±17.0%であり，３者間に有意の差はみられなかった。すなわち，抗ヒトIgE添加により健康人や慢性気管支炎患者の好塩基球からも有意のヒスタミン遊離が見られた。気管支喘息のなかでは，内因性喘息症例においてヒスタミン遊離（14.1±7.2%）の低い傾向が見られた。

Dose-response curve の検討では，健康人，慢性気管支炎症例では全例E_2からE_1へかけてのnegative slope を示した。気管支喘息症例では，血清IgE値500IU／ml以下の症例ではnegative slope を示す症例が多く，一方501IU／ml以上の症例ではpositive slopeを示す症例がより多く見られた。

キーワード：好塩基球の反応性－抗ヒトIgE－ヒスタミン遊離－慢性気管支炎－気管支喘息