Studies on the release of histamine from basophils

1. Determination of histamine from whole blood by an automated fluorometric histamine analysis system

Yoshio TANIZAKI, Haruki KOMAGOE, Michiyasu SUDO,
Masaaki MIFUNE and Hiroshi MORINAGA

Department of Medicine, Okayama University Medical School, Misasa Medical Branch

Jun OHTANI, Hikaru KITANI, Yoshinori GODA,
Shinya TADA and Ikuro KIMURA

The 2nd Department of Medicine, Okayama University Medical School

(Received December 28, 1982)

Introduction

Histamine is released from basophils and mast cells which are target cells of IgE when these cells are stimulated by antigen or anti-IgE (ISHIZAKA, T., et al., 1978, ISHIZAKA, T., 1981). A sensitive fluorometric method for histamine assay has been extensively utilized for diagnosis and studies of allergy. This method has some limitations for clinical application because of several reasons: it is complicated and time-consuming.

Recently, a completely automated fluorometric histamine analysis system has been developed by SIRAGANIAN, R. P. (1974, 1975). In this study, the release of histamine from whole blood elicited by antigen and anti-IgE was determined by an automated spectrofluorometric technique.

Subjects and Methods

Ten healthy subjects (3 females and 7 males aged between 18 and 45 years), 6 patients with chronic bronchitis (2 females and 4 males, 50–64 years) and 23 asthma patients comprising 12 extrinsic asthma (8 females and 4 males, 16–52 years) and 11 intrinsic asthma (8 females and 3 males, 43–70 years) were selected for the determination of histamine released from basophils following the addition of anti-IgE and house dust extract. All cases of extrinsic asthma studied here showed a positive RAST to house dust. Five cases of 12 extrinsic asthma have been treated with hyposensitization for over one year, and the others without hyposensitization. The diagnosis of intrinsic asthma was made on clinical criteria, that is a negative skin test with low or normal serum IgE levels.

Venous blood (24–25ml) was drawn into a plastic syringe containing 1 ml of heparin. The blood (4ml/test tube) was transferred into a test tube. Different concentrations of house dust extract or anti-IgE (0.2ml) were added to the tubes, and the mixed solution was then incubated for 15 min at 37°C. After 15 minutes' incubation the reaction was stopped by transferring the tubes into the ice bath, followed by centrifugation at 400 x g for 20 min at 4°C. The histamine content in the cells and supernatant fluid was pretreated with 0.8 N HClO₄ (Fig. 1), and assayed by an automated spectrofluorometric histamine analysis system which was constructed from standard Technicon autoanalyser components. The actual recorder chart from histamine analysis is shown in Fig. 2. Histamine released was calculated according to
4 ml of heparinized blood

[Diagram showing the following steps:
- Add 0.2 ml of P.S.S., anti-IgE or H.D.*
- Incubation for 15 min at 37°C
- Centrifuge at 400 G for 20 min at 4°C
- Plasma (0.8 ml) and Buffy coat
- Add 0.8 N-HClO₄ (0.8 ml)
- Add one drop of 1N-HCl
- 10 min at 100°C
- Add 1 ml of 0.8 N-HClO₄
- Centrifuge at 1600 G for 20 min
- Supernatant
- Automated fluorometric histamine assay]

Fig. 1. Method for the release of histamine from human basophils following the addition of anti-IgE or house dust extract. *House dust extract

Histamine standard (ng/ml)

Fig. 2. Recorder chart of histamine release from whole blood. Histamine released was calculated according to the value of histamine standard.
the value of the histamine standard. The results were expressed as a percent release of the total histamine content. Serum total IgE was measured by Radioimmunosorbent test (RIST) from Pharmacia.

Results

The maximum percent release of histamine from basophils of healthy subjects induced by anti-IgE varied within a wide range from 2.9 to 50.0%. The mean maximum percent release in healthy subjects was 24.7±4.5% (±SE) (Mean serum IgE level: 156 IU/ml). The markedly increased release of histamine in extrinsic asthma patients without hyposensitization (Mean serum IgE level of the seven cases: 908±264 IU/ml) was observed following the addition of anti-IgE. The mean maximum percent release from these cases was 44.7±6.8%. Basophils from extrinsic asthma patients with hyposensitization (Mean serum IgE level of the five cases: 1123±359 IU/ml) released a significant amount of histamine when they are exposed to anti-IgE. The mean maximum release from the cases with hyposensitization was 41.5±5.8%, which was less than that in the cases without hyposensitization, although no significant difference was present between them. The basophils from intrinsic asthma patients (Mean serum IgE level of the 11 cases: 197±31 IU/ml) were low reactive to anti-IgE. The mean maximum percent histamine release by anti-IgE in intrinsic asthma was 11.4±2.1%. The difference between the extrinsic and the intrinsic asthma patients was significant in respect of a serum IgE level (p <0.01) and histamine release by anti-IgE (p <0.001). The release of histamine in the patients with chronic bronchitis (Mean serum IgE level of the 6 cases: 250±81 IU/ml) was variable within a wide range from 1.9 to 53.6% with a mean of 26.3±7.9% (Fig. 3). The maximum histamine release by house dust extract in the healthy subjects was very low. The mean maximum release was 7.1±1.8% and never exceeded 17%. House dust extract caused a significantly increased release of histamine from basophils of house dust-sensitized extrinsic asthma patients with or without hyposensitization. The maximum release was 42.1±7.0% in the cases without hyposensitization and 40.8±6.5% with hyposensitization. No significant difference in the release was seen between the two groups (Fig. 4).

Discussion

Histamine release from basophils of allergic patients after incubation of washed leucocytes with specific allergen or anti-IgE has been observed for the diagnosis and study of allergy (Pruzansky, J. J., et al., 1966, Levy, D. A., et al., 1966, Lichtenstein, L. M., et al., 1966, 1970, Assem, E. S. K., et al., 1970, Marone, G., et al., 1981). To determine histamine released from leucocytes, a sensitive fluorometric method has been utilized. The method is time-consuming and must be performed by a highly trained technicians. SIRAGANIAN, R. P. (1974, 1975) has developed an automated fluorometric histamine analysis system which makes it possible to measure histamine released more easily. The method is capable of analysing 30 samples per hour. Furthermore, SIRAGANIAN, R. P., et al. (1976) has studied the release of histamine from whole blood to simplify the histamine release procedure. They reported that the maximum histamine release from whole blood correlated closely with the release from washed leucocytes. In this study, histamine released from whole blood by anti-IgE and house dust extract was determined by the automated fluorometric method. It was concluded from this study that histamine released from basophils could be easily determined using whole blood by the automated fluorometric analysis system.

We have studied basophils in bronchial asthma. Our previous results (KIMURA, I., et al., 1973, 1974, 1975) showed that basophils participate in an attack of asthma and change morphologically after the in vitro stimulation with anti-IgE and antigen (KIMURA, I., et al., 1981). We have also been much interested in the reactivity of basophils in intrinsic asthma. It is still unclear what kind of reaction participates in the mechanism
of intrinsic asthma. Generally, basophils from asthma patients show an increased reactivity to anti-IgE (Findley, S. R., et al., 1980, Conroy, M.C., et al., 1977, Marone, G., et al., 1981). It has been shown that basophils from intrinsic asthma patients also release a significantly increased amount of histamine to anti-IgE (Assem, E. S. K., et al., 1973, 1981). In this study, the release of histamine from basophils by anti-IgE in intrinsic asthma was significantly less as compared to that in extrinsic asthma. The reason why the basophil reactivity to anti-IgE in intrinsic asthma in this study was low and differed from that by Assem, E. S. K., et al. is unclear. The different reactivity of basophils to anti-IgE-high and low-might make it possible to classify asthma into two groups.

Summary

Histamine released from whole blood was determined by an automated fluorometric histamine analysis system. The increased release of histamine from basophils by anti-IgE was observed in ten healthy subjects and 12 extrinsic asthma patients, while the release in 11 intrinsic asthma patients was significantly less as compared to that
Studies on the release of histamine from basophils

in healthy and extrinsic asthma subjects. House dust extract caused a significant increase in the histamine release from basophils of the extrinsic asthma patients who are sensitive to house dust. It was concluded from this study that histamine released from basophils could be easily determined by an automated analysis system and that the method is useful for the diagnosis and study of allergy.

Acknowledgement

We wish to thank Miss Hiroi Endo for her technical assistance.

References


ASSEM, E.S.K. and ATTALLAH, N.A.: Increased release of histamine by anti-IgE from leucocytes of asthmatic patients and possible heterogeneity of IgE. Clinical Allergy 11, 367-374, 1981.


KIMURA, I., TANIZAKI, Y., SAITO, K., TAKAHASHI, K., UEDA, N. and SATO, S.: Appearance of basophils in the sputum of patients with bronchial asthma. Clinical Allergy, 1, 95-98, 1975.


好塩基球からのヒスタミン遊離に関する研究。

1 自動分析装置による全血からのヒスタミン遊離の測定

谷崎聡朗，駒越喜彬，周藤真康，御殿政明，森永寛，
大谷純，貴谷光，合田吉徳，多田慎也，木村郁男

岡山大学医学部附属病院三朝分院内科，
*岡山大学医学部附属病院第2内科

ヒスタミン自動分析装置により、健康人10名、気管支

喘息23例の全血からのヒスタミン遊離を測定した。抗ヒ

トIgEを添加した際のヒスタミン遊離は、健康人および

外因性気管支喘息症例では有意の増加傾向を示したが、

一方内因性喘息症例では遊離増加はほとんどみられなか

った。ハウスダスト抗原添加では、ハウスダストが抗原

である気管支喘息症例においてのみ全血からの有意のヒ

スタミン遊離の増加が観察された。以上の結果より、ヒ

スタミン自動分析装置による全血からの遊離ヒスタミン

の測定は、気管支喘息の病態解明の1手段として極めて

有用であると考えられる。