

Differences in response to anti-IgE and to anti-IgG in basophils from patients with bronchial asthma

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Summary

Peripheral blood basophils of thirty-eight patients with bronchial asthma were examined for their reactivity to anti-IgE and anti-IgG antisera. Basophils of patients with serum IgE levels of more than 1001 i.u./ml reacted strongly to anti-IgE. Basophils of patients with serum IgE levels of less than 100 i.u./ml had a tendency to react more strongly to anti-IgG. An index (basophil ratio) was devised to compare the patient basophil reactivity to anti-IgE and anti-IgG. This basophil ratio was lower (IgE dominant) in the atopic cases which usually exhibited a high serum IgE level. Most cases with low serum IgE exhibited a high basophil ratio (IgG dominant). The basophils of seven intractable patients reacted more strongly to anti-IgG than to anti-IgE regardless of the serum IgE level.

Introduction

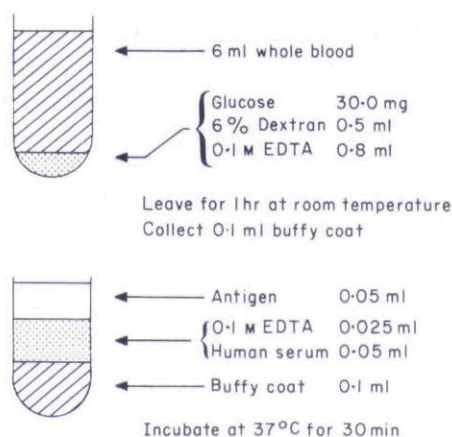
We previously reported that peripheral blood basophils increase in the pre-attack stage of bronchial asthma and decrease at the initiation of the attack stage (Kimura *et al.*, 1968; Kimura, Moritani & Tanizaki, 1973). The decrease of peripheral basophils is due in part to the extravascular movement of cells toward the local allergic site (Kimura *et al.*, 1974, 1975a). The IgE target cell is identified as the basophil (Ishizaka, Tomioka & Ishizaka, 1970). The cells appear not only in the IgE reacting reagin-type of allergic reaction, but in other types of allergic reactions and may be involved in all types of allergies. It is, however, uncertain whether the basophil is sensitized by only IgE or whether IgG also sensitizes the cells even in the immediate type of allergic reaction. The variations in therapeutic success indicate that there are several subtypes of bronchial asthma that may reflect differences in the classes of antibodies-sensitizing basophils. In the present study the *in vitro* basophil reactivity to anti-immunoglobulins was examined in relation to patient serum IgE level.

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Materials and methods

The subjects were thirty-eight bronchial asthma patients including five cases of buck-wheat asthma and seven cases of intractable asthma. Healthy subjects without allergic disorders were selected as control subjects. Serum IgE was measured by the RIST method. The patients were divided into five groups according to serum IgE levels, as follows: patients with less than 100 i.u./ml ($n=9$), patients between 101 and 300 i.u./ml ($n=5$), patients between 301 and 700 i.u./ml ($n=5$), patients between 701 and 1001 i.u./ml ($n=3$) and patients with levels more than 1001 i.u./ml ($n=16$).

The morphological reactivity of basophils to anti-immunoglobulins was described previously as the basophil degranulation or disruption test (Kimura, 1973; Kimura *et al.*, 1975b). Six millilitres of venous blood was collected. The leucocyte layer was separated from peripheral blood. A small amount of anti-immunoglobulin (anti-IgE [ϵ -chain specific, Hyland] or anti-IgG [H- and L-chain specific, Hyland]) was added to the leucocyte suspension. No anti-IgE was present in anti-IgG samples in pre-examination by RIST. After incubation for 30 min at 37°C, smear specimens of the leucocytes were prepared and later stained by May-Giemsa (Fig. 1). The morphology of a total of fifteen basophils was examined from each subject. Two types of 'reactive basophils' were recorded: (a) pear-shaped cells—designated A-form and (b) swollen cells with high intracytoplasmic vacuolation with diameters of more than 15 μm —designated B-form. An index of reactive basophils (A-form cells and B-form cells) was later computed (basophil ratio).



Discard supernate and prepare smear using 1 drop of the buffy coat and dry quickly. Stain with May-Giemsa and study microscopically.

Fig. 1. The method of basophil degranulation test.

Results

Reactivity of basophils to anti-IgE

The addition of anti-IgE to peripheral blood basophils of patients with serum IgE levels of more than 1001 i.u./ml (high IgE group) resulted in two-thirds of the basophil population being reactive (Fig. 2). In patients with serum IgE levels of less than 100

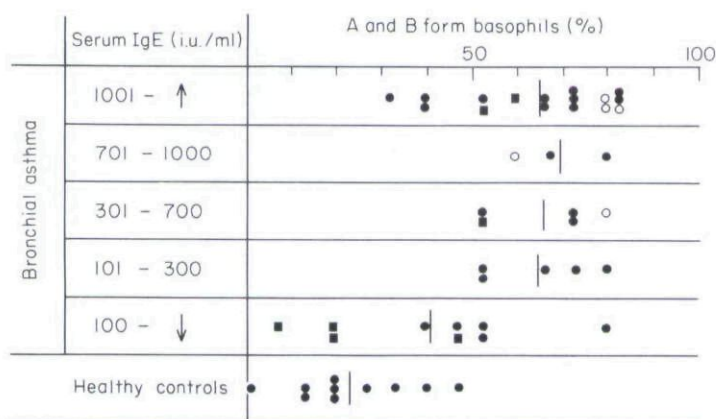


Fig. 2. The incidence of reactive basophils (A & B form) after the addition of anti-IgE. ■, Intractable asthma; ○, buckwheat asthma.

i.u./ml (low IgE group), 41% of basophils became changed. The difference in basophil reactivity between these two patient groups was statistically significant ($P < 0.02$). The reactive value for the control group was 23.6%. The difference between the control group and the high IgE group was highly significant ($P < 0.001$). A statistically significant difference was not present between the control group and the low IgE group. The three groups with serum IgE levels between 101 and 1001 i.u./ml showed distributions of A-form and B-form basophils that were similar to the high IgE group.

Reactivity of basophils to anti-IgG

The addition of anti-IgG to peripheral blood basophils of the high IgE group resulted in 26.2% of basophils being reactive. The comparable figure for the low IgE group was 65.6% (Fig. 3). The difference between the two groups was statistically significant ($P < 0.001$). The low IgE group showed a significantly higher percentage of reactive basophils compared to the control group ($P < 0.01$). There was no statistical difference between the high IgE group and control group. A linear reactivity was suggested for

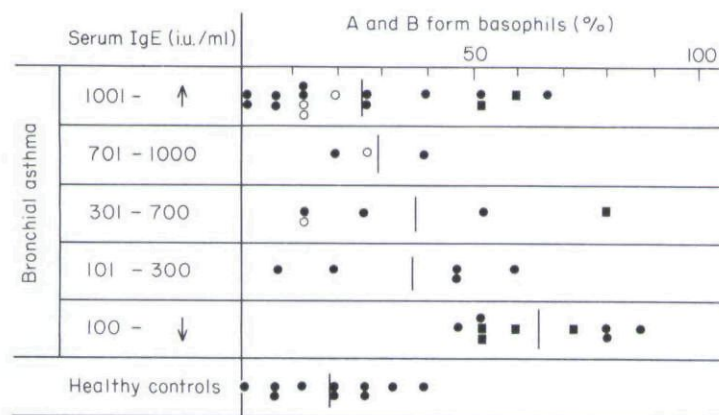


Fig. 3. The incidence of reactive basophils (A & B form) after the addition of anti-IgG. ■, Intractable asthma; ○, buckwheat asthma.

the three intermediate serum IgE groups, but the differences were not statistically significant.

Comparison of basophil reactivity (basophil ratio)

The frequency of basophils reacting to anti-IgG and anti-IgE was compared by the following formula:

$$\text{Basophil ratio} = \frac{\text{Frequency of reactive basophils to anti-IgG}}{\text{Frequency of reactive basophils to anti-IgE}}$$

A basophil ratio of less than one demonstrates a stronger reaction to anti-IgE (IgE dominant type); a stronger reaction to anti-IgG would be indicated by a ratio greater than one (IgG dominant type). As shown in Fig. 4, twelve, out of sixteen patients from the high IgE group demonstrated a ratio of less than one, but in contrast only one of nine patients from the low IgE group demonstrated a ratio of less than one. Most patients with serum IgE levels between 101 and 1000 i.u./ml exhibited a ratio less than one.

Buckwheat asthma

The five cases of buckwheat asthma had serum IgE levels of more than 301 i.u./ml. All these cases showed an IgE dominant basophil ratio.

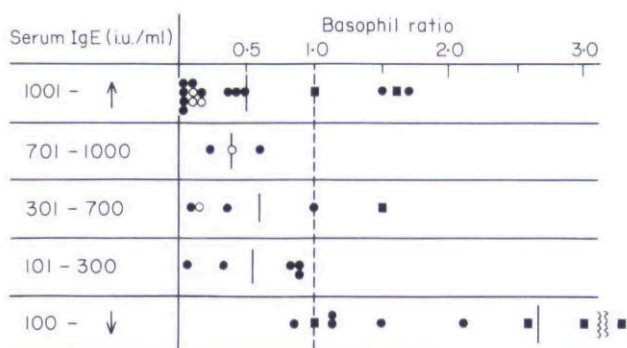


Fig. 4. Comparison of basophil reactivity against anti-IgE and anti-IgG (basophil ratio). ■, Intractable asthma; ○, buckwheat asthma.

Intractable bronchial asthma

Intractable bronchial asthma is defined as 'perennial bronchial asthma which is not improved by general treatment and requires steroids to maintain patients' normal activity'. Seven of thirty-eight patients were in this category: two with serum IgE levels higher than 1001 i.u./ml, one between 301 and 700 i.u./ml and four patients with levels less than 100 i.u./ml.

All seven intractable bronchial asthma patients showed basophil ratios of one or more than one and were therefore the IgG-dominant type. The intractable population constituted half of the strong anti-IgG reactive cases of the present sample and all these patients were either in the high or low IgE group.

Discussion

Peripheral basophils of low serum IgE asthma patients (less than 100 i.u./ml) exhibited a stronger reaction to anti-IgG than to anti-IgE. This is in sharp contrast to peripheral basophils of the atopic bronchial asthma cases with high serum IgE levels (more than 1001 i.u./ml) that exhibited almost no reaction to anti-IgG and reacted intensely to anti-IgE. This suggests the possibility that IgG may play a role in the allergic sensitization of the low IgE asthma patients. In the seven intractable asthma cases examined, the basophils of all seven patients reacted strongly to anti-IgG irrespective of the serum IgE level. Even in cases with high serum IgE levels, it is possible that IgG as well as IgE are involved.

It is generally acknowledged that in the atopic type of bronchial asthma, serum IgE levels are high and have a tendency to decrease with successful hyposensitization. Hyposensitization, however, is often not successful, especially in adult cases of bronchial asthma. In some cases of adult bronchial asthma a precise allergen cannot be found and in others even after allergen identification and hyposensitization treatment, the clinical signs worsen.

In failures of hyposensitization, we observed a tendency for the IgE level to rise as the clinical symptoms worsen. These intractable patients are difficult to treat, and in spite of recent advances in basic immunology, little progress has been made in treatment methods.

The nature and role of antibodies in bronchial asthma need to be reconsidered. It is not known if IgE is the only sensitizing antibody. There are various reports on the anaphylactic sensitizing properties of IgG (Loveless, 1964; Perelmutter, Rose & Goodfriend, 1965; Reid, Minden & Farr, 1966; Malley & Perlman, 1966; Parish, 1970; Malley, Baecher & Crossley, 1973; Bryant, Burns & Lazarus, 1973). IgG has been claimed to have properties similar to IgE to sensitize skin for long periods and to be heat-labile (Reid *et al.*, 1966; Malley & Perlman, 1966; Malley *et al.*, 1973). Another possibility is that some IgG exists as a heat-stable anaphylactic antibody that sensitizes lung or skin for short periods only (Parish, 1970, 1973, 1974; Bryant *et al.*, 1973). This short-term sensitizing IgG has been shown to sensitize human basophils *in vitro* for the release of histamine on challenge with anti-IgG (Parish, 1973); a phenomenon similar to our results when basophils from the asthmatic patients reacted with anti-IgG. The nature of the IgG demonstrated in the basophil reactivity described here is uncertain but we believe that it has clinical relevance.

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