

Basophil immunoglobulin receptors in asthmatics under immunoscanning electron microscopy

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Summary

Immunol latex particles were used as markers for IgE receptors on basophils using electron-microscope scanning. These particles appeared to bind specifically to basophils. Basophils from atopic asthma patients showed greater binding to latex particles than those from intractable asthma patients or healthy subjects. Cap and patch formations on IgE receptors were frequently found in basophils of atopic asthmatics, and cap formation was observed only on basophils that were pear-shaped. The redistribution of IgE receptors, such as that by cap formation, may be a significant triggering mechanism for basophil activation.

The latex density on basophils pre-treated with anti-human IgG was much greater in intractable asthmatics than in atopic asthmatics or healthy subjects, and almost all basophils with increased immunol latex particles were pear-shaped, suggesting the presence of some IgG receptors on the surface, and such receptors may play an important role in intractable asthma.

Introduction

Morphological changes have been reported on human peripheral basophils of asthmatics in immunological reactions using light and electron microscopy (Hastie, 1971; Kimura, 1973; Kimura *et al.*, 1975; Hastie, 1977; Kimura *et al.*, 1981a,b). Under scanning electron microscopy an increase in reactive basophils was found in intractable asthmatics after the addition of anti-IgG, but these cells decreased after passive sensitization with IgE (Kimura *et al.*, unpublished observations). This finding suggests that some changes occur around the immunoglobulin receptors (Ishizaka, Sterk & Ishizaka, 1979). This led us to studies on the distribution and characterization of basophil immunoglobulin receptors. In this paper we report on the distribution of basophil IgE receptors detected by immunol latex in basophils in atopic patients. Tests were made for IgG receptors especially in cells from cases of intractable asthma.

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Materials and methods

Eight patients were studied. Four were atopic asthmatics with high serum IgE levels, and four were intractable asthmatics with low serum IgE levels. Four healthy control subjects with no past atopic history were also tested.

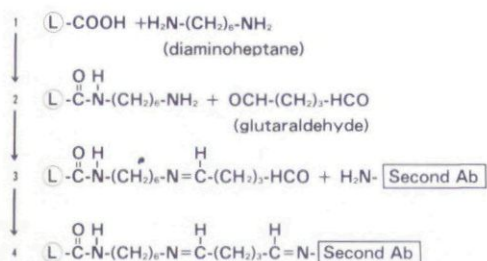


Fig. 1. A formula for synthesis and structure of immunolatex.

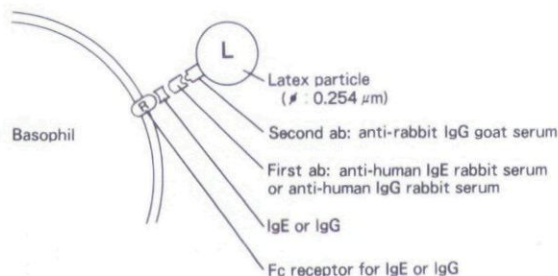


Fig. 2. A principle for immunoscanning electron microscopy using immunolatex particles as a visual marker for immunoglobulin receptors.

The basophil-rich fractions were obtained from heparinized venous blood by the Ficoll-Conray centrifugation method (Tanizaki *et al.*, 1978). Goat gamma-globulin against rabbit IgG was chemically bound to carboxylate-modified latex particles using the diaminoheptane and glutaraldehyde two-step method (Fig. 1) (Molday *et al.*, 1975). The basophils were then incubated with anti-human IgE or anti-human IgG in rabbit gamma-globulin fractions, and immunolatex particles were added to this basophil suspension (Fig. 2). The basophils were attached to poly-*L*-lysine coat glass plates, then fixed with glutaraldehyde. After identifying and mapping the basophils on glass plate under light microscopy, the cells were dehydrated and dried by critical point drying. These specimens were examined by scanning electron microscopy (JSM-U3 and HFS-2) (Fig. 3).

Results

The latex density of basophils pre-treated with rabbit anti-human IgE was much greater than the latex density of basophils pre-treated with non-immunized rabbit IgG. Thus, specific binding occurred by immunolatex particles to the IgE receptor. In order to quantify the immunolatex density on the cell surface, basophils were divided into

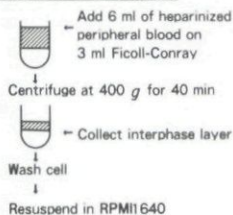
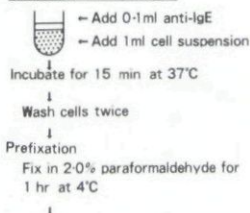
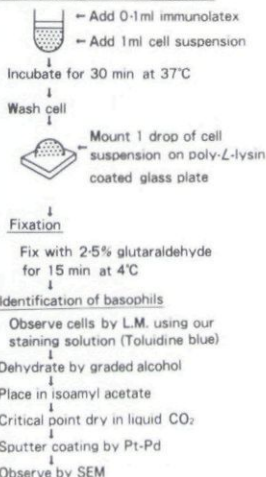
Preparation of basophils**Reaction with antigen****Cell labeling by immunolatex**

Fig. 3. A procedure for observation of immunoglobulin receptors on the basophil. In this process, preparations of basophils from human peripheral blood, reaction with first antibody to IgE, cell labelling by second antibody derivatized latex particles, detection of cells and processing for immunoscanning electron microscope are performed.

five groups according to the number of bounded immunolatex particles: from 0 to 3+. Sixty percent of basophils from atopic asthma patients were classified into the 3+ group. In contrast, intractable asthma patients had 15%, and healthy subjects 20% in the 3+ group (Fig. 4). The distribution of the immunolatex particles on the cell surface was differentiated into three types: diffuse pattern (Fig. 5a), patch formation (Fig. 5b) and cap formation (Fig. 5c). Cap and patch formations were found in 70% of basophils from atopic asthma patients. This incidence was significantly higher than in intractable asthma patients who showed 26.1%, whereas healthy subjects showed 37.5% (Fig. 6).

	Passively reacted with anti-IgE	Grade of bound immunolatex					
		(-)	(±)	(+)	(2+)	(3+)	
Atopic asthmatics	(+) (<i>n</i> =23)		●● ○○○	○	● ○	●●●●●● ○○○○○	
	(-) * (<i>n</i> =21)	○○○○○ ○○○○○	○○○○○ ○○○○○	○			
Intractable asthmatics	(+) (<i>n</i> =20)	●	○○○○	●● ○○	○○●●○○	●● ○	
	(-) * (<i>n</i> =22)	○○○○○ ○○○○○ ○○○○○	○	○	○		
Normal Subjects	(+) (<i>n</i> =20)		● ○○○	●●● ○○○	●●●● ○○○	●● ○○	
	(-) * (<i>n</i> =24)	● ○○○○○ ○○○○○	○○○ ○○○				

● pear-shaped basophil

○ sphere-shaped basophil

* Incubation with non-immunized rabbit IgG as a control

Fig. 4. A relationship between morphologic changes of basophil and the number of bound immunolatex particles on the cell surface after addition of anti-IgE in asthma patients and healthy subjects.

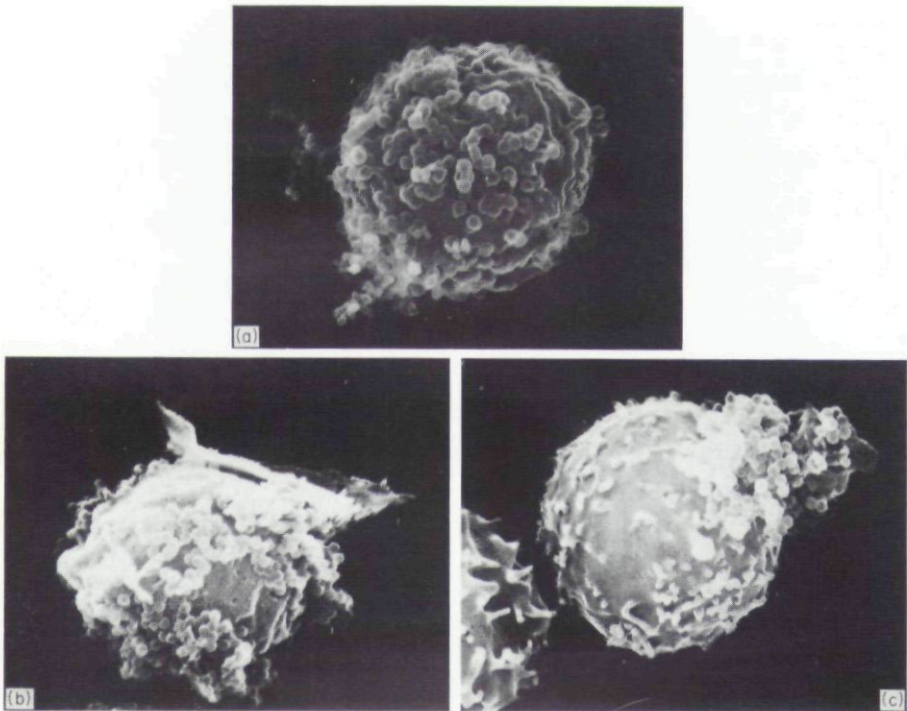


Fig. 5. A distribution of immunoglobulin receptors on basophils observed by scanning electron microscopy using immunolatex particles as a visual marker for receptors. (a) Latex particles are diffusely distributed on the surface of spherical shaped basophil ($\times 10\,000$); (b) a distribution of particles is localized on the reactive basophils after addition of appropriate allergen ($\times 10\,000$); and (c) an accumulation of particles on one pole of the pear-shaped basophil shows a cap formation ($\times 10\,000$).

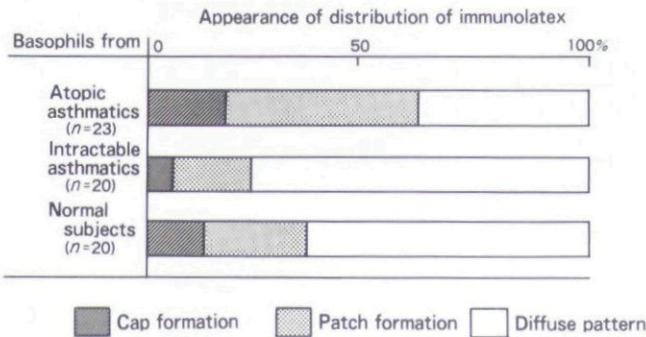


Fig. 6. An appearance of the redistribution of immunolatex particles after addition of anti-IgE in asthma patients and healthy subjects.

Pear-shaped basophils bound more immunolatex particles than spherical basophils. Cap formation of the IgE receptor was present in only pear-shaped basophils (Fig. 7).

Immunolatex markers were also used in the tests for basophil IgG receptors. The number of immunolatex particles on the basophil surface treated with anti-human IgG increased in intractable asthmatics compared to atopic asthma patients or healthy subjects. Almost all basophils with increased immunolatex were pear-shaped form

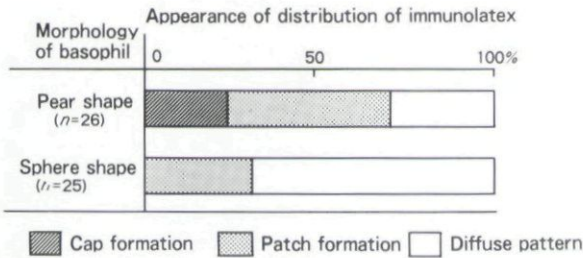


Fig. 7. A relationship between morphologic changes of basophil and redistribution of immunolates particles after addition of anti-IgE in asthma patients and healthy subjects.

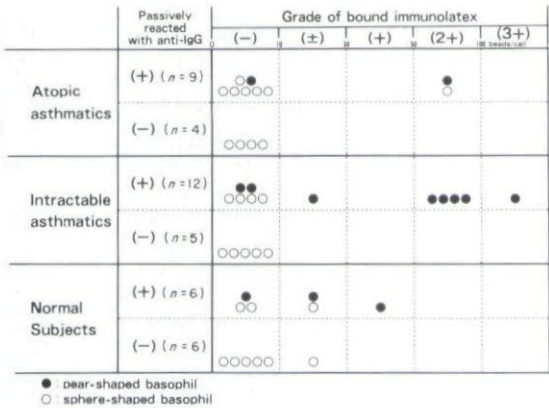


Fig. 8. A relationship between morphologic changes of basophil and the number of bound immunolates particles on the cell surface after addition of anti-IgG in asthma patients and healthy subjects.

(Fig. 8). In intractable asthma and some specific asthma subtypes the possibility exists that the basophil IgG receptors play an important role in the asthma attack, as do the IgE receptors in atopic asthma.

Discussion

Reactive basophils as pear-shaped cells in immunological reaction might help to elucidate the mechanisms involved in allergy (Kimura, 1973; Kimura *et al.*, 1975, 1981a,b). These reactive basophils initially show mobility, followed by diffuse, swollen changes when anti-immunoglobulins or specific allergens are added, as observed in motion films (Kimura *et al.*, 1984). Their degranulation probably also occurs at the site of the allergic reaction. The release of chemical mediators is triggered by the bridging of two or more IgE molecules on the basophil receptor (Ishizaka *et al.*, 1978). However, it is uncertain how the morphological changes are related to the release of chemical mediators.

Immunolates was used based on our previous studies on reactive basophils (Kimura, 1973; Kimura *et al.*, 1975, 1981a,b). Under scanning electron microscopy the distribution of IgE receptors was diffuse in normal, untreated basophils. In immunological reactions associated with IgE, basophils were often observed with patch and cap formations. Cap formation of IgE receptors produced a change in receptor distribution, and this was found only in pear-shaped basophils. Such a

redistribution of IgE receptors may be a significant triggering mechanism for the activation of basophils by allergens, followed by the release of chemical mediators.

We previously divided asthma patients into two subtypes using the basophil degranulation test (Kimura, 1973; Kimura *et al.*, 1975, 1981a): one was the IgE-dominant type, and other was the IgG-dominant type. The former type is probably atopic with a central airway obstruction, and the latter type may represent a late onset, intractable and peripheral airway obstruction-dominant type, with some histologic changes, as shown in Fig. 9. In basophil reactions by anti-immunoglobulins there were both IgE and IgG receptors on the surface. The receptors might sometimes interfere with each other (Ishizaka, Sterk & Ishizaka, 1979) as there was a tendency for a decrease in reactive basophils after the addition of anti-IgG in the IgE passive sensitization state of intractable asthmatics (Kimura *et al.*, unpublished observations).

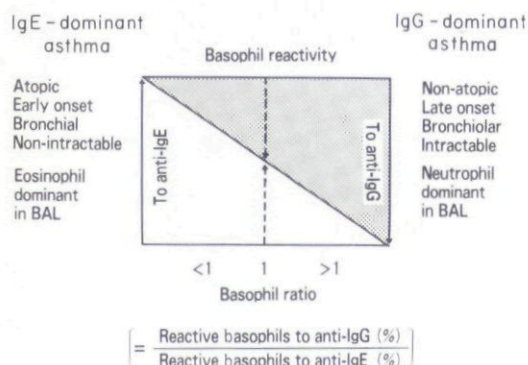


Fig. 9. A hypothesis for subtype of bronchial asthma, classified by reactivity of basophil to immunoglobulins, clinical features and a manner of cellular involvement.

In this study immunoscanning electron microscopy was used to examine the details of the distribution and the movement of the basophil receptors. In atopic asthma, cap formation of the IgE receptor could usually be observed after the addition of anti-IgE and a second antibody with immunolates, indicating the release of chemical mediators (Becker *et al.*, 1973; Lawson *et al.*, 1975). On the other hand, it seems to be clear that the IgG receptors of basophils (Parish, 1970; Bryant, Burns & Lazarus, 1973; Gwynn *et al.*, 1982) are related to severe attacks or allergic reactions in intractable asthma patients, as there was an increase of IgG receptors in intractable asthmatics. An increase of activated basophils with cap formation was uncertain in intractable asthma with increased IgG receptors, although most basophils were pear-shaped. The ratio of IgE and IgG receptors on the surface of basophils might be an index in the classification of asthma subtypes, in the same manner as previously described 'basophil ratio' (Kimura *et al.*, 1981a).

References

- BECKER, K., ISHIZAKA, T., METZGER, H., ISHIZAKA, K. & GRIMLEY, D.M. (1973) Surface IgE on human basophils during histamine release. *Journal of Experimental Medicine*, **138**, 394.
- BRYANT, D.H., BURNS, M.W. & LAZARUS, L. (1973) New type of allergic asthma due to IgG 'reaginic' antibody. *British Medical Journal*, **4**, 589.
- GWYNN, C.M., INGRAM, J., ALMOUSAWI, T. & STANWORTH, D.R. (1982) Bronchial provocation tests in atopic patients with allergen-specific IgG₄ antibodies. *Lancet*, **i**, 254.

- HASTIE, R. (1971) The antigen-induced degranulation of basophil leucocytes from atopic subjects, studied by phase-contrast microscopy. *Clinical and Experimental Immunology*, **8**, 41.
- HASTIE, R. (1977) The antigen-induced degranulation of basophil leucocytes from atopic subjects studied by electron microscopy. *Laboratory Investigation*, **36**, 173.
- ISHIZAKA, T., ISHIZAKA, K., CONRAD, D.H. & FROESE, A. (1978) A new concept of triggering mechanism of IgE-mediated histamine release. *Journal of Allergy and Clinical Immunology*, **61**, 320.
- ISHIZAKA, T., STERK, A.R. & ISHIZAKA, K. (1979) Demonstration of Fc γ receptors on human basophil granulocytes. *Journal of Immunology*, **123**, 578.
- KIMURA, I. (1973) Basophilic leucocyte—with reference to immunological reaction. *Acta Haematologica Japonica*, **36**, 755.
- KIMURA, I., TANIZAKI, Y., SATO, S., TAKAHASHI, K., SAITO, K. & UEDA, N. (1975) Morphological changes of basophils in immunological reactions—effect of sodium cromoglycate. *Clinical Allergy*, **5**, 181.
- KIMURA, I., TANIZAKI, Y., SATO, S. & TAKAHASHI, K. (1981a) Differences in response to anti-IgE and to anti-IgG in basophils from patients with bronchial asthma. *Clinical Allergy*, **11**, 31.
- KIMURA, I., TANIZAKI, Y., SATO, S., TAKAHASHI, K., SAITO, K. & UEDA, N. (1981b) Supravital observation of *in vitro* basophils in immunological reactions. *Clinical Allergy*, **11**, 37.
- KIMURA, I., TANIZAKI, Y. & SASAKI, Y. (1984) The antigen-induced increase in motility and degranulation of basophils from atopic asthmatics, studied by microscopic motion pictures. *International Archives of Allergy and Immunology* (submitted for publication).
- LAWSON, D., FEWTRILL, C., GOMPERS, B. & RAFF, M.C. (1975) Anti-immunoglobulin induced histamine secretion of rat peritoneal mast cells studied by immunoferritin electron microscopy. *Journal of Experimental Medicine*, **142**, 391.
- MOLDAY, R.S., DREYER, W.J., RENBAUM, A. & YEN, S.P.S. (1975) New immunolatespheres: visual markers of antigen on lymphocytes for scanning electron microscopy. *Journal of Cell Biology*, **64**, 75.
- PARISH, W.E. (1970) Short-term anaphylactic IgG antibodies in human sera. *Lancet*, **ii**, 591.
- TANIZAKI, Y., TAKAHASHI, K., HOSOKAWA, M., ISHIBASHI, K., ONO, H., GODA, Y., NAKAMURA, Y., SASAKI, Y., KOBAYASHI, M. & KIMURA, I. (1978) Purification of basophilic leucocytes from peripheral blood. *Acta Haematologica Japonica*, **41**, 27.

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