

Affinity of IgE antibody to antigen influences allergen-induced histamine release

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Summary

Background Although affinity of an antibody for an antigen is recognized to be an important factor in determining its biological effects, little is known about the relevance of such affinity of IgE antibodies to the functional response.

Objectives To investigate the effect of IgE antibody affinity to Der p 2 on Der p 2-induced histamine release from human basophils.

Methods The most probable value of the dissociation constant (K_d) of IgE antibody to Der p 2 was calculated and histamine release by Der p 2-challenged leucocytes was used to evaluate the biological efficacy of the IgE antibody.

Results The most probable K_d value of IgE antibody to Der p 2 ranged from 5.6 to 177.8 pM in 14 asthmatic patients sensitive to Der p 2. A significant correlation was observed in Der p 2-induced histamine release between the sensitivity and the K_d value for Der p 2-specific IgE antibody ($r_s = 0.797$, $P = 0.0040$), suggesting that the higher the affinity, the lower the amount of allergen required for the release of a specific level of histamine.

Conclusion Apart from the changes associated with the reactivity, the sensitivity of histamine release is closely related to the affinity of IgE antibody for its antigen.

Keywords: histamine release, histamine, IgE

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Introduction

Affinity of an antibody for its antigen has been shown to be an important determinant of the biological efficacy of the antibody. However, measurement of the affinity or avidity (functional affinity) of an antibody in serum has been difficult particularly in the case of IgE antibodies, because (1) the serum concentrations of total IgE are on the order of ng/mL even in patients with allergic diseases and allergen-specific IgE antibody constitutes only a small proportion of the total IgE (2) it is impossible to use a simple 'law of mass action' to calculate the affinity of an antibody to multivalent allergens and (3) human antibodies are polyclonal in nature, i.e. serum antibodies are the products of a number of clones producing and secreting antibodies with different affinities. Although a variety of methods, including ammonium sulphate

precipitation [1–3], a solid phase immunoassay [4,5] or a technique using chaotropic agents to disrupt antigen-antibody complexes [6–8] have been proposed to measure the antibody affinity or avidity, the methodologies have not led to a resolution of the problems associated with estimation of the affinity of human IgE antibodies. There is as yet no satisfactory experimental method to adequately describe the distribution of affinities of the antibodies in the serum, and a simple method for the measurement of the affinity of IgE antibodies is obviously needed. A method for calculating the affinity distribution of IgE antibodies has recently been reported [9], in which increasing concentrations of biotin-labelled allergen are bound to IgE immobilized on magnetic particles and subsequently detected with avidin-alkaline phosphatase conjugates. The affinity distribution of the IgE antibodies was calculated from the amount of biotin-labelled allergen bound to IgE antibodies with a computer. The method allows measurement of the affinity distribution of IgE antibodies without the need for any complicated

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procedure or special reagents. We used this assay technique to study the affinities of IgE antibodies for antigens in patients with allergic disorders.

To evaluate the efficacy of IgE antibodies *in vitro*, we performed a test of histamine release from basophils, which is an established *in vitro* model of IgE-dependent allergic reactions. Basophils release histamine in an IgE-dependent manner after incubation with the relevant allergen. Thus, it is assumed that the histamine release may be dependent on the affinity of the IgE antibody for the allergen because IgE-mediated degranulation is a consequence of binding of the allergen to the IgE antibody on basophils. Two parameters, i.e. sensitivity and reactivity, have been used to assess the extent of histamine release. The lowest concentration of allergens that evokes a given percentage of histamine release is regarded as an indication of the cell sensitivity, whereas reactivity is defined as the maximum percentage of histamine release evoked by an optimal concentration of the allergen. These concepts correspond to the potency and efficacy of drugs in drug studies. However, there is as yet no experimental evidence regarding the relationship between allergen-induced histamine release and the affinity of the IgE antibody for the allergen.

The aim of the present study is to investigate the relationship between the affinity of IgE antibody to Der p 2 and allergen-induced histamine release, in order to better understand the factors involved in the regulation of allergen-induced histamine release from basophils.

Materials and methods

Chemicals

Dynabeads M-450 coated with goat antimouse IgG (DynaL AS, Norway), antihuman IgE (De2) (clone E124.2.8, Immunotech, Marseille, France), bovine serum albumin (fraction V, Bayer Corporation, USA), streptavidin- β -galactosidase (Roche Diagnostics GmbH, Mannheim, Germany), 4-methylumbelliferyl β -D-galactoside (Sigma, St Louis, MO, USA), polyclonal antihuman IgE (KPL, Gaithersburg, MD, USA), horseradish-peroxidase (HRP) labelled antihuman IgE or antihuman IgG (Biosource International, Camarillo, CA, USA).

Histamine release from patient basophils

After obtaining informed consent, venous blood samples were drawn from 21 asthmatic patients (mean age 46.5 ± 14.6 , 13 females, eight males) who were selected on the basis of positive skin tests and the presence of specific IgE antibodies for mite allergens in the serum. None of the patients had received immunotherapy and all medication was stopped for at least 12 h prior to the blood collection. The histamine release test was performed using the same

technique as described previously [10]. In brief, heparinized blood supplemented with EDTA was layered on top of three discontinuous gradients of Percoll solution. After centrifugation, plasma was collected from the top layer of the tube and the fraction containing basophils was retrieved from the Percoll layer with a density of 1.079 g/mL and the interface pellicle between the Percoll layers with the densities of 1.070 and 1.079 g/mL. After washing in saline, the cells were incubated with various concentrations of Der p 2 (0.01, 0.04, 0.1, 0.4, 1, 4, 10 and 40 ng/mL), or anti-IgE (0.01, 0.1, 1 and 5 μ g/mL) as a positive control. The supernatants were collected and assayed for histamine by an automatic fluorometric technique. Cells incubated in buffer alone were included to determine spontaneous histamine release, which was subtracted from all experimental value. The spontaneous histamine release was below 7% in all experiments. Results were expressed as the percentage release by the total histamine, which was determined by lysis of the cells in 0.4 N perchloric acid, with correction of spontaneous release.

Isolation of IgE from plasma

After mixing the plasma sample (0.5 mL) with an equal volume of saturated ammonium sulphate, the tube was centrifuged at 1200 g for 10 min. The precipitate was dissolved in a small volume of water and dialysed against phosphate-buffered saline. The dialysate was incubated with magnetic beads coated with anti-IgE (2×10^7 beads), which had been prepared by mixing of Dynabeads M-450 coated with goat antimouse IgG and mouse antihuman IgE (De2) according to the manufacturer's instructions, at 4 °C overnight. The magnetic beads were washed three times by resuspension in phosphate-buffered saline containing 0.05% Tween 20 (PBS-Tween), followed by centrifugation. The magnetic beads were suspended in 1.5 mL of phosphate-buffered saline containing 0.2% bovine serum albumin and 0.1 mL of the suspension was transferred into each well of a 96-well microtitre plate (Nunc 267245, Denmark). The magnetic beads in the wells were incubated with various concentrations of biotin-labelled Der p 2. We incubated 14 concentrations of biotin-labelled Der p 2 ranging from 0.1 to 400 ng/mL with the magnetic beads at room temperature for 2 h under continuous shaking. After washing the magnetic beads in each well three times with PBS Tween, 0.1 mL of 0.1 mM streptavidin- β -galactosidase was added to each well, followed by incubation at room temperature for 2 h. The plate was washed three times and 0.1 mL of 4-methylumbelliferyl β -D-galactoside was added to each well. After incubation at 37 °C for 1 h, the reaction was stopped by the addition of 0.05 mL of 1.0 M glycine (pH 10.3). An aliquot of the supernatant was transferred to the wells of a 96-well microtiter plate (FluoroNunc, 475515, Denmark). Fluorescence intensities were measured at 360 nm excitation and

460 nm emission wavelengths using a microplate reader (Spectra Fluor, Tecan GmbH, Salzburg, Austria).

Calculation of affinity distribution of specific IgE antibody for Der p 2

The procedure for determining the affinity distribution was based on the method of Pierson *et al.* [9]. Briefly, the fluorescence intensity for each concentration of biotin-labelled Der p 2 was converted to the degree of saturation (r) after confirmation of a saturation value. Binding of biotin-labelled Der p 2 usually reached saturation at around 300 ng/mL, which corresponds to $10^{-7.7}$ M. When r was plotted on the ordinate and the corresponding concentration of biotin-labelled Der p 2 on the abscissa, the binding curve for biotin-labelled Der p 2 was obtained. The relation was expressed as a function r by a polynomial regression. To simplify the calculation, the curve was approximated by an equation of the third degree for concentrations ranging up to $10^{-8.5}$ M by a computer. The accuracy of the final results depended on that of the approximation. Substituting the values calculated from the regression line in the equation described by Hunston [11] and Pierson *et al.* [9] yielded the affinity distribution function $N(K)$. The pattern of affinity distribution was shown when $N(K)$ was plotted against $\log K$, which was the reciprocal of the concentration of biotin-labelled Der p 2. The maximum value of the dissociation constant was determined from the correlation and was considered as the measure for antibody affinity. As the affinity value was calculated from an approximation, we have expressed it as 'the most probable K_d '.

Data analysis

All data were expressed as the mean $\pm$ SD. Correlation between parameters was determined by means of Spearman's rank correlation test. P -values less than 0.05 were regarded as denoting statistical significance.

Results

Histamine release from basophils

The basophil sensitivity was defined as the minimum concentration required for 40% histamine release in response to Der p 2 stimulation in this study. Basophils from five of the subjects did not exhibit greater than 25% histamine release even at the optimal concentration of Der p 2. In addition, the basophils from two individuals were completely unresponsive to Der p 2 and anti-IgE, indicating that they were nonreleasing basophils. These seven subjects were excluded from the subsequent analyses.

Incubation of the mixed leucocytes with various concentrations of Der p 2 or anti-IgE induced histamine release in a dose-dependent manner. The mean maximal histamine release (reactivity) was $87.5 \pm 7.2\%$ in response to Der p 2 and $71.8 \pm 16.7\%$ in response to anti-IgE stimulation. The basophil sensitivity to Der p 2, defined above, ranged from 0.013 to 0.9 ng/mL (geometric mean; 0.061 ng/mL, 1 SD; 0.020–0.185).

The Spearman's rank correlation coefficient between the reactivity and the sensitivity of histamine release was $r_s = -0.917$ with $P = 0.0059$ in the anti-IgE-induced histamine release, indicating a highly significant correlation between the two parameters, but no such relationship was observed between the reactivity and the sensitivity in histamine release induced by Der p 2 ($r_s = -0.514$, $P = 0.064$).

Isolation of IgE from plasma

After the addition of various concentrations of myeloma IgE to a plasma sample containing a small amount of endogenous IgE (<40 IU/mL), the amounts of IgE not adsorbed on the solid phase were measured. IgE could not be detected in the supernatant up to the addition of about 200 IU/mL, but thereafter, increased gradually with an increase in the amount of IgE added, suggesting that less than 200 IU/mL of IgE was completely adsorbed on the magnetic beads coated with anti-IgE under the conditions employed.

The specific adsorption of IgE was confirmed by detection of the IgE and IgG retained on the beads. After incubation with plasma, the solid support was allowed to react with either HRP-labelled IgE or HRP-labelled IgG and then detected with a reagent containing o-phenylenediamine and H_2O_2 . In a control experiment, bovine serum albumin was covalently linked to tosyl-activated Dynabeads according to the procedure recommended by the manufacturer and the beads were incubated with plasma. As shown in Fig. 1, the control beads bound to neither IgG nor IgE, suggesting that nonspecific binding on the magnetic particles is negligibly low. On the other hand, IgG, but not IgE, could be detected on the magnetic beads when the plasma was incubated with commercially available Dynabeads, which had been coated with goat antimouse IgG as a second antibody. Considering these observations, it is inferred that IgG bound on the solid support in linkage with goat antibody could be a heterophile antibody and not an allergen-specific IgG antibody.

Measurement of the affinity distribution of IgE antibody to Der p 2

First, we examined whether biotin-labelled Der p 2 behaves in the same manner as unlabelled Der p 2. Biotinylation did not alter the allergenicity of Der p 2 because the reactivity

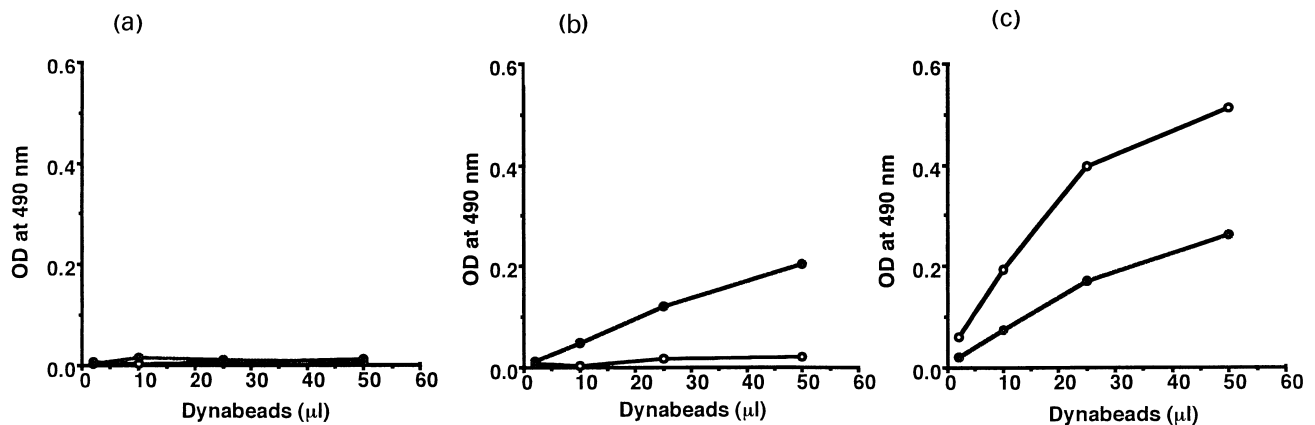


Fig. 1. Binding of IgE (○) and IgG (●) from human serum to Dynabeads M-280 coated with bovine serum albumin (a), Dynabeads M-450-goat antimouse IgG (b) and Dynabeads M-280-goat antihuman IgE (Fc) (c).

and the sensitivity of Der p 2-induced histamine release were quite similar to those of biotin-labelled Der p 2-induced histamine release (Fig. 2).

The fluorescence intensity in the absence of biotin-labelled Der p 2 was consistently about a half of that measured at the lowest concentration of biotin-Der p 2. The fluorescence intensity of a control plasma which did not contain specific IgE antibody was minimal even at the highest concentration of biotin-Der p 2. These results suggest that nonspecific binding to the solid support is negligibly small.

To check the reproducibility of the data, we measured the affinity distribution in two plasma samples on three different occasions. The interassay variation which may arise from the measurement in binding studies and in calculation of the data was about 20% as determined by the coefficient of variation (27.3 ± 3.8 pM and 80 ± 16.3 pM).

The affinity distribution of the IgE antibodies was

unimodal or skew-unimodal in nine out of the 15 patients and bimodal in the remainder. An example of each case is shown in Fig. 3. As shown in Fig. 3, the most probable K_d was evaluated from the log K corresponding to the maximum value for the affinity distribution. The most probable K_d ranged from 5.6 to 177.8 pM (geometric mean 31.4 pM; 95% confidence interval, 21.2–179.0), not correlated with either the IgE antibody titre or the total IgE levels.

Relationship between affinity of IgE antibody and histamine release

To determine the relationship between the most probable K_d value for the affinity distribution and the sensitivity or the reactivity, the individual data were analysed by means of Spearman's rank correlation test. As shown in Fig. 4, a highly significant correlation was observed between the K_d value and the sensitivity ($r_s = 0.797$, $P = 0.0040$), but not

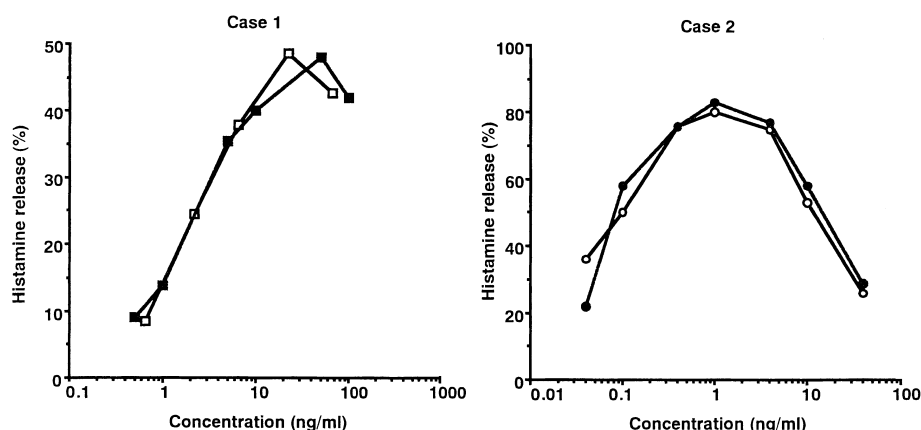


Fig. 2. Der p 2- or biotin-labelled Der p 2-induced histamine release from peripheral blood basophils of two patients with mite allergy. The filled symbols represent histamine release induced by biotinylated Der p 2.

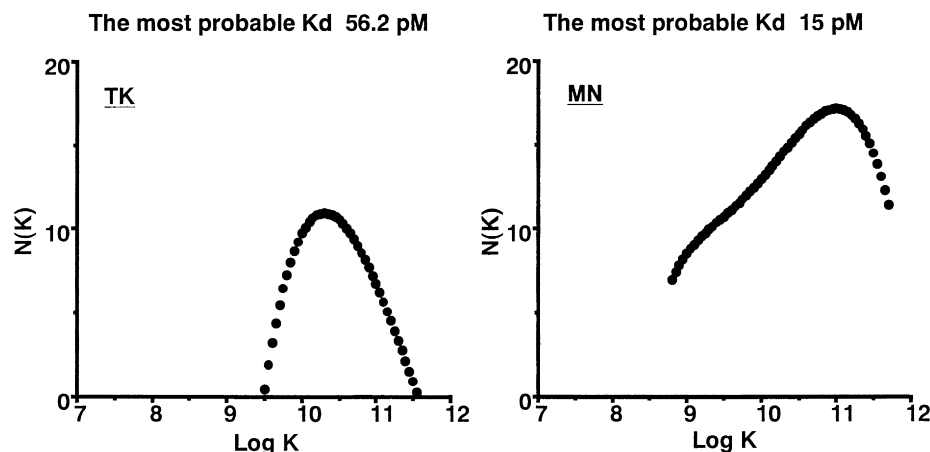


Fig. 3. Pattern of affinity distribution of IgE antibody to Der p 2.

between the Kd value and the reactivity ($r_s = -0.359$, $P = 0.165$).

When the relationship has been examined in detail, basophil sensitivity diminished with decreasing the affinity of IgE antibody, reaching plateau and did not change thereafter. There was a strong linear correlation between basophil sensitivity and affinity of IgE antibody for up to the most probable Kd of around 30 pM. Sensitivity was dependent on the affinity of IgE antibody within the range. Regardless of the affinity, basophil sensitivity was almost constant over the affinity value of about 30 pM.

Discussion

Immediate-type allergic response is associated with an antibody response to the allergen. Affinity for the antigen is the most important factor determining the effector function of an antibody. High-affinity antibodies have been shown to be more effective than low-affinity antibodies in a number of biological reactions and *in vitro* assays. Studies on the effect of antibody affinity on immunoassay measurements reveal that the binding constant directly influences the detection limit in immunoassays [12,13]. In infectious diseases, low-affinity antibodies are less efficient at immune clearance of the antigen than high-affinity antibodies. In many clinical studies, the variations in antibody avidity in infections or allergic disease have been measured by the ammonium sulphate precipitation technique, competitive immunoassay or determination of the concentration of chaotropic ions that disrupt the hydrophobic bond between an antigen and an antibody [6,7,8,14,15]. On the other hand, in some reports, antibody avidity was determined by Scatchard analysis using linear approximation within a narrow concentration range [16]. In studies which measured the affinity of an antibody only macroscopically, only the relative affinity value was obtained, and the true affinity value was

not obtained. On the other hand, it has been acknowledged that other than the true binding force, the number of aromatic amino acid residues in the paratope also influences the avidity of antibodies when chaotropic ions are present [8]. These aforementioned methods cannot provide any information on the heterogeneity of affinity of an antibody. In this study, we calculated the probability of affinity distribution based on the method of Pierson *et al.* there were a few cases in which the distribution of affinity of IgE antibodies showed a complete Gaussian distribution, while in most cases either a skew-Gaussian or a bimodal distribution was observed. Thus, various distribution patterns of IgE antibody affinity were observed. However, an appropriate method for evaluation of the relationship between the distribution patterns of IgE affinity and the biological response has not been reported yet. Therefore, in order to simplify this evaluation, we used the most probable Kd as the affinity value.

The IgE antibody affinities to Der p 2 vary by approximately 30-fold among patients. An interesting question is whether or not such variations of affinity are reflected in the biological functions. We addressed this question using the histamine release test. This test, using washed leucocytes induced by allergens, is widely used for the corroboration of sensitization to allergens and evaluation of the allergic status of patients, because the ability of basophil to release histamine could be of relevance in the pathogenesis of allergic disorders. Our previous detailed study of the dose-response curve of histamine release revealed a highly significant correlation between the reactivity and sensitivity of histamine release evoked by polyclonal anti-IgE. However, no or poor correlation was observed between these parameters of histamine release evoked by allergens [10]. These findings indicate that the dose-response curve can shift not only up or down, but also to the right or left. Accordingly, the allergen concentration which evokes the

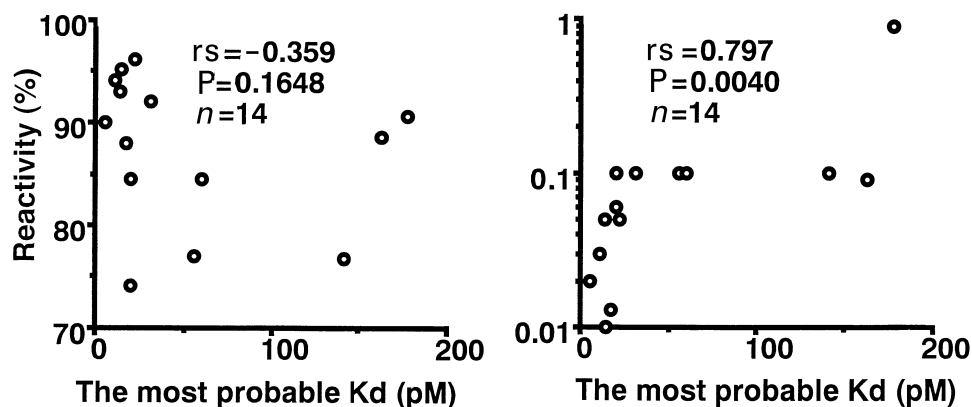


Fig. 4. Correlation between the most probable Kd value of IgE antibody specific for Der p 2 and the reactivity and sensitivity of Der p 2-induced histamine release.

maximum release was different depending on the basophils in each patient. As one cause of differences in the latter, it is assumed that the degree of affinity of the IgE antibodies to the allergens is different among patients. In other words, it is considered that a greater amount of allergen is required to evoke histamine release in the presence of IgE antibodies with low affinity.

The IgE antibody affinity for Der p 2 ranges from 5.6 to 177.8 pM. As shown in Fig. 4, a highly significant correlation is observed between the most probable Kd value and the sensitivity of histamine release. The greater the most probable Kd value, which implies a lower affinity, the greater the sensitivity value. The sensitivity reached a plateau value when the most probable Kd value was approximately 30 pM. On the other hand, no correlation was observed between the most probable Kd value and the reactivity of histamine release. It has already been reported that the sensitivity is linked to the reactivity of histamine release, since the greater the reactivity, the lower the sensitivity. The aforementioned results indicate that the difference in the affinity of the IgE antibody to the allergen greatly influences the right or left movement of the dose-response curve of histamine release evoked by allergens from the patient's basophils, i.e. there are fluctuations in the concentration of the allergens which evokes maximum release.

Since IgE antibodies can play the role of effectors of an allergic response at extremely low concentrations, it can be considered that IgE antibodies can bind to allergens with high affinity. The most probable Kd value ranges from 5.6 to 177.8 pM. This Kd value range almost corresponds to the Kd value range of 6.7 pM to 1 nM [16], which was measured for the IgE antibodies for cow's hair and dander using crude allergens, and also to the Kd value range of 3.8 pM to 111 pM [17] which was measured for the IgE antibodies for Amb a V using a reciprocal plot. In the case of monoclonal antibodies, according to the law of mass action, the concentration of

ligands required for saturation of half of the antibody binding sites (the antigen concentration required for half-maximum binding) corresponds to the Kd value. When the concentration of ligands is assumed to follow the law of mass action and the molecular weight of Der p 2 is assumed to be 14000, the concentration of Der p 2 when it is bound to half of the IgE antibodies can be calculated to range from 78.4 pg/mL to 2.5 ng/mL. The sensitivity of histamine release, i.e. the concentration of Der p 2 which produces 40% histamine release, ranged from 13 pg/mL to 0.9 ng/mL. The concentration of Der p 2 which produces maximal histamine release ranged from 400 pg/mL to 40 ng/mL. These concentration ranges overlap; therefore, we believe that the most probable Kd value obtained by calculation reflects the biological functions of the IgE antibodies. We consider that the sensitivity value being lower than the most probable Kd value is attributable to the following causes; (1) histamine release occurs by the cross-linking of only a limited number of IgE molecules (the number of IgE molecules ranges from several hundreds to 10 000 and averages 2500) on the basophil cell surface. Maximal histamine release takes place when either 10–15% or less than 10% IgE antibodies are cross-linked [18,19] (2) one Der p 2 molecule needs to be bound to at least two IgE antibody molecules to evoke histamine release (3) unlike the affinity distribution of other monoclonal antibodies, that of human IgE antibodies rarely shows a Gaussian distribution, and more frequently shows a skew-Gaussian or bimodal distribution [20].

Our data indicate that the affinity of IgE antibodies for allergens is one of the primary factors influencing the pattern of histamine release. To date, various events have been reported to influence the reactivity and sensitivity of histamine release. One study reported that IgE promotes immunological histamine release through its expression of FcεRI [21]. However, no correlation was clinically observed

between the IgE levels in patients' sera and the histamine release [22,23]. Secondly, it has been reported that the IgE antibody titre is correlated to the parameters of histamine release, in particular, to reactivity. However, there are some reports which refute the correlation between the IgE antibody titre and the parameters of histamine release [22,24–26]. Thirdly, correlation between the density of IgE antibodies on the surface of a cell and the parameters of histamine release was observed [10,22]. In this case, the IgE antibody density was determined by the ratio of the level of specific IgE to that of total IgE antibody in the serum. Fourthly, intrinsic factors other than the IgE antibody density on the surface of the basophils were reported to control the extent of histamine release [10]. Although a series of biological processes with activation of various tyrosine kinases are known to be involved in histamine release [27], intrinsic factors which directly control the histamine release remain to be identified. Finally, a relationship between histamine release and genetic factors has been speculated [28], in particular, the relationship between histamine release and polymorphism of the β chain of Fc ϵ RI has been studied. However, no mutations associated with abnormal patterns of histamine release have been observed so far [29].

The aforementioned results reveal that the affinity of IgE antibodies to allergens is one of the primary factors influencing the sensitivity of histamine release. We believe that the measurement of this affinity, as well as of the IgE antibody levels is important to understand the biological functions of the IgE antibody.

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