

Original article

Facilitated antigen presentation and its inhibition by blocking IgG antibodies depends on IgE repertoire complexity

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Background: The antibody repertoires of allergic subjects are characterized by the presence of allergen-specific IgE antibodies. We have previously shown that the composition of the IgE repertoire is critical for allergen-mediated activation of human effector cells. Activation of CD4⁺ T cells in allergic subjects is highly potentiated by the process of facilitated antigen presentation (FAP), in which allergen in complex with IgE is taken up by B cells through the low-affinity IgE receptor CD23 and presented to T cells.

Objective: We sought to investigate the influence of IgE repertoire complexity on the formation of IgE/allergen/CD23 complexes on B cells and subsequent T-cell activation.

Methods: Using defined allergen-specific recombinant IgE and IgG antibodies, we investigated the influence of individual IgE affinity, IgE clonality, specific IgE concentration, and the ratio between IgE specificities on IgE/allergen/CD23 complex formation *in vitro*.

Results: Although IgE affinity is an important factor, IgE clonality seems to be governing complex formation, especially with medium- and low-affinity IgE antibodies. We demonstrate that differences in allergen-specific IgE affinity correlate with the efficiency of subsequent T-cell activation. In addition, we show that the complexity of an IgE repertoire also affects the ability of allergen-specific IgG antibodies to block FAP.

Conclusion: The composition of allergen-specific IgE repertoires in individual patients, especially with respect to IgE clonality, might play an important role in the manifestation of allergic disease not only for the immediate allergic reaction through activation of basophils and mast cells but also for the exacerbation of allergic inflammation through recurring activation of allergen-specific T cells by FAP. (J Allergy Clin Immunol 2011;■■■:■■■-■■■.)

Key words: Allergy, allergen, rIgE, rIgG, antibody repertoire, antibody-binding affinity, antibody clonality, blocking IgG, facilitated antigen presentation, T-cell activation

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Abbreviations used

FAP: Facilitated antigen presentation
h: High-affinity antibody
l: Low-affinity antibody
m: Medium-affinity antibody
SIT: Specific immunotherapy

Allergies to inhalant allergens, such as house dust mite, tree, and grass pollen allergens, are steadily increasing in industrialized countries.^{1,2} Allergic sensitization to inhalant allergens is initiated by the uptake of allergens by antigen-presenting cells residing in the tissue of the lungs and upper airways. After migration of antigen-presenting cells to the local draining lymph nodes, processed allergen peptides are presented to naive T cells, which, in turn, undergo differentiation into mature CD4⁺ T_H2 cells, as reviewed by Romagnani.³ Subsequently, these allergen-specific T_H2 cells produce IL-4 and IL-13, which induce class switching of allergen-specific naive B cells, leading to production and secretion of allergen-specific IgE antibodies.^{4,5} After homing to the target organ, the CD4⁺ T_H2 cells further enhance airway inflammation through expression of the additional T_H2 cytokines IL-5 and IL-9, which leads to the recruitment, activation, or both of eosinophils, basophils, and airway-resident mast cells.^{6,7}

In sensitized subjects specific IgE antibodies play a pivotal role in allergic disease. In the immediate allergic reaction formation of IgE/allergen complexes cross-link FcεRI receptors on the surfaces of mast cells and basophils, leading to cell degranulation. This causes the release of different mediators, including histamine, that are mainly responsible for immediate allergic symptoms.⁸ In previously sensitized subjects, CD4⁺ T cells can be activated through the process of facilitated antigen presentation (FAP).^{9,10} Through this process, a very low concentration of allergen can drive complex formation between specific IgE, allergen, and the low-affinity IgE receptor (CD23) on antigen-presenting B cells (CD23⁺ cells).^{9,11} After internalization of complexes, the B cell presents allergen-specific peptides to T-cell receptors on CD4⁺ T cells that lead to activation on T-cell epitope recognition. It has been hypothesized that FAP in allergic subjects, through recurring activation of CD4⁺ T cells present in a T_H2-type environment, might lead to exacerbation of the allergic inflammation and increase the severity of the allergic disease.^{9,10} The IgE repertoires of individual allergic patients are expected to be polyclonal and include antibodies of different specificities and varying affinities for allergen, as demonstrated recently for the IgG response to tetanus toxoid.¹² However, functional studies on IgE repertoires

TABLE I. Der p 2-specific rIgE antibodies used in the study

rIgE affinity	K _D value	Clone	Name	Map of relative Der p 2 epitopes bound by rIgE
High	0.036 nmol/L	H10:H10*†	H10 (<i>h</i>)	
	1.18 nmol/L	H12:H12*†	H12 (<i>h</i>)	
	1.4 nmol/L	E†‡	E (<i>h</i>)	
Medium	3.99 nmol/L	H10:H1*	H10 (<i>m</i>)	
	5.14 nmol/L	H12:H7*	H12 (<i>m</i>)	
	9.43 nmol/L	F†	F (<i>m</i>)	
Low	33.2 nmol/L	H7:H12*	H12 (<i>l</i>)	
	171 nmol/L	H10:H7*	H10 (<i>l</i>)	
	291 nmol/L	C†	C (<i>l</i>)	

Combinations of clones used throughout this article

Antibody specificity	H10	H12	P4
<i>hhh</i>	H10:H10	H12:H12	E
<i>mmm</i>	H10:H1	H12:H7	F
<i>lll</i>	H10:H7	H7:H12	C
<i>hh</i>	H10:H10	H12:H12	
<i>hh§</i>	H10:H10		E
<i>mm</i>	H10:H1	H12:H7	
<i>ll</i>	H10:H7	H7:H12	
<i>hll (llh)</i>	H10:H7	H7:H12	E
<i>mml (lmm)</i>	H10:H7	H12:H7	F
<i>ml (llm)</i>	H10:H7	H7:H12	F
<i>hl (lh)</i>	H10:H7	H12:H12	

h, High-affinity antibody; *l*, low-affinity antibody; *m*, medium-affinity antibody.

*Murine/human chimeric Der p 2-specific rIgE clones (heavy chain/light chain).

†Fully human Der p 2-specific rIgE clones.²⁹

‡In addition to rIgE, clones were engineered and expressed as murine/human chimeric Der p 2-specific rIgG antibodies.

§*hh* combination of rIgE and rIgG used in Fig 5.

have mainly focused on epitope specificity/clonality,¹³⁻¹⁶ whereas the diversity in IgE affinity has only been sparsely described.¹⁷ As described above, homing and activation of T_H2 cells is a key component in the allergic inflammatory process, which makes investigations of how the complexity of the IgE repertoire influences CD23-mediated T-cell activation extremely important.

Specific immunotherapy (SIT) is a highly effective treatment of allergic disease that reduces both immediate allergic symptoms and late-phase responses.^{18,19} Allergic patients who receive SIT are characterized by a large increase in allergen-specific serum IgG antibody levels.²⁰⁻²² Several studies have demonstrated a blocking effect of allergen-specific non-IgE serum antibodies on competitive binding with IgE to the allergen, an observation that led to the so-called blocking antibody theory and the terms blocking antibodies or blocking IgG^{23,24} that were reviewed by Flicker and Valenta.²⁵ In more recent studies, a blocking effect of specific IgG on basophil activation,^{26,27} as well as FAP,^{11,27,28} has been reported. Here we examine the effects of allergen-specific IgE repertoire complexity on FAP measured as IgE/allergen/CD23 complex formation on B cells (throughout this article denoted as complex formation) and subsequent T-cell activation. In addition, we investigate the correlation between IgE repertoire complexity and the ability of allergen-specific IgG antibodies to prevent complex formation.

METHODS

Recombinant IgE and IgG antibodies

IgE antibodies binding nonoverlapping epitopes on *Dermatophagoides pteronyssinus* group 2 allergen (Der p 2; H10, H12, or P4) with high, medium, or low affinity (Table I)²⁹ were used for complex formation experiments. For

inhibition experiments with rIgG, DNA coding for the variable antibody regions from the IgE clones binding Der p 2 with high affinity was inserted into the pLNOH2 vector obtained from Norderhaug et al³⁰ containing the human constant IgG3 region. All IgE and IgG antibodies were expressed in HEK293 cells by using the Freestyle 293 expression system (Invitrogen, Carlsbad, Calif) in yields of 5 to 10 mg/L secreted into serum-free growth media. No further purification was done besides application to NAP-5 columns packed with Sephadex G-25 and sterile filtering. Antibody preparations were stored at 4°C. IgE antibody preparations were adjusted to the same concentration based on ADVIA Centaur Immunoassay analyses (Bayer, Leverkusen, Germany) with anti-IgE as the detector antibody, as previously described.³¹ IgE and IgG antibody specificities binding the same epitopes on Der p 2 with identical affinity was adjusted to the same concentration based on Biacore analyses (Biacore, Uppsala, Sweden) using rDer p 2 allergen immobilized on a CM5 chip. Concentrated preparations of rIgG antibodies in dilution series were used for the IgG inhibition studies. All IgE antibody clones were tested as single IgE controls, and no complex formation with a single rIgE was seen.

D pteronyssinus extract and rDer p 2 allergen

Purified rDer p 2 expressed in *Pichia pastoris* or *D pteronyssinus* extract (ALK-Abelló, Hørsholm, Denmark) containing 85 ng of Der p 2 per microgram of protein was used for complex formation experiments. The concentration of rDer p 2 was measured with the Advia Centaur Immunoassay system (Bayer, Leverkusen, Germany) by using biotinylated anti-Der p 2 (ALK-Abelló) as the detector antibody.

Complex formation experiments with rIgE or human serum

Complex formation was detected by means of flow cytometry (FACS). In detail, to achieve complex formation, different combinations of 2 or 3 antibody specificities (total IgE concentration, 0.4 µg/mL) in Dulbecco PBS buffer (Gibco, Invitrogen, Carlsbad, Calif) in a total volume of 10 µL or

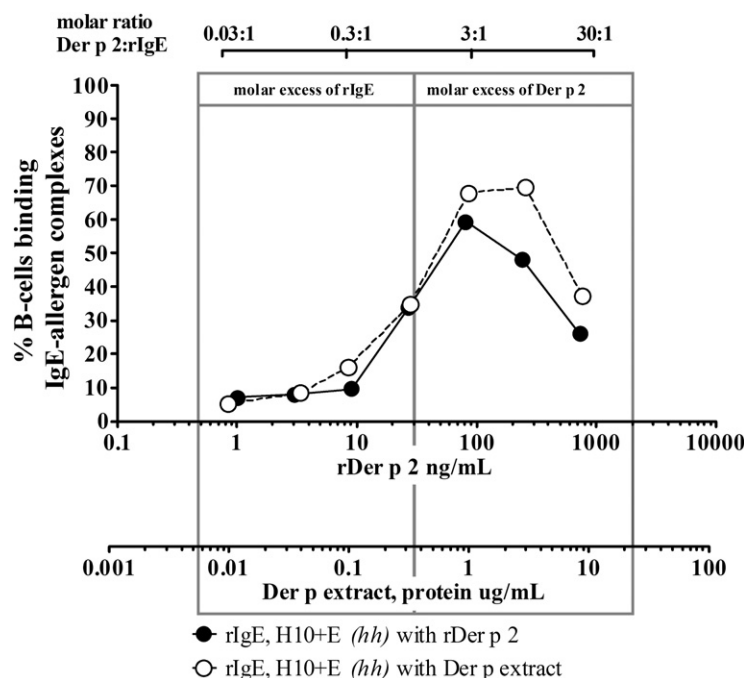


FIG 1. FACS assay. Similar levels of complex formation (percentage of B cells with bound IgE-allergen complexes) were obtained with 2 high-affinity rIgE antibody specificities (H10 plus P4E) on increasing amounts of rDer p 2 (solid circles) or *D pteronyssinus* extract (open circles) shown in the top and bottom x-axes, respectively. x-Axes were aligned according to the content of 85 ng of Der p 2 per microgram of protein in the extract.

human serum IgE (10 μ L) were added to 10 μ L of RPMI 1640 buffer (Lonza, Verviers, Belgium) and incubated with 10 μ L of serial dilutions of allergen dissolved in PBS (*D pteronyssinus* extract or rDer p 2) in microtiter (round-bottom) plates at 37°C for 1 hour. Subsequently, samples were added to 10 μ L of EBV-transformed CD23⁺ B cells (10⁵ cells) and incubated for 1 hour on ice. A buffer sample with no allergen was included as a negative control. All samples were washed in cold FACS buffer (BD, Franklin Lakes, NJ) before and after 30 minutes' incubation on ice with fluorescein isothiocyanate-labeled anti-IgE (Dako, Glostrup, Denmark), followed by detection of surface-bound complexes by means of flow cytometry on a FACS Calibur (BD). Five thousand cells per sample were measured, and results were analyzed with Winlist software, version 5.0 (Verity Software House, Inc, Topsham, Me). In all figures complex formation (as a percentage) refers to the percentage of CD23⁺ B cells with bound complexes.

Complex formation with rIgE or human serum inhibited by rIgG

IgG inhibition experiments were performed as described above, with the exception that 10 μ L of PBS buffer containing 1, 2, or 3 rIgG antibody specificities (same total [IgG], 2 μ g/mL) was mixed with rIgE or human serum IgE, replacing 10 μ L of RPMI 1640 culture medium before incubation with serial dilutions of allergen.

FAP measured as B cell-mediated T-cell proliferation

EBV-transformed CD23⁺ B cells were allowed to internalize IgE/allergen/CD23 complexes for 2 hours before use for stimulation of autologous Der p 2-specific CD4⁺ T-cell lines in a standard T-cell proliferation assay, as previously described by van Neerven et al.¹¹

RESULTS

In line with other studies,^{27,32-34} we have measured complex formation as a surrogate parameter for FAP-mediated T-cell

proliferation. All recombinant antibodies used in this study are listed with respect to the binding specificities and binding affinities shown in Table I. Based on affinity measurements, the antibodies were arbitrarily divided into different groups (ie, high affinity antibody [h], medium-affinity antibody [m], or low-affinity antibody [l]), according to the method of Christensen et al.²⁹

Complex formation with *D pteronyssinus* extract or rDer p 2

Fig 1 shows comparable complex formation (percentage of B cells with bound IgE-allergen complexes) obtained with *D pteronyssinus* extract or rDer p 2 allergen on testing the rIgE antibodies P4E and H10. In this experiment complexes were formed at molar ratios between allergen and antibody of 0.3:1 to 30:1.

Effect of individual IgE antibody affinities on complex formation

The ability of IgE affinity to affect complex formation was compared with regard to the sensitivity of the response, as well as the maximum response levels. The highest sensitivity was obtained by using 2 (hh) or 3 (hhh) nonoverlapping high-affinity IgE antibodies (same total IgE concentration), respectively (Fig 2, A). Testing lower-affinity antibodies generally led to a reduction in the sensitivity of the response as the response curve shifted to the right. Use of medium-affinity antibodies (mm) reduced the sensitivity of the response by 10- to 20-fold compared with the high-affinity combination (hh) and decreased the maximum response to approximately 50%. By assaying cells binding low-affinity IgE antibodies (ll) and allergen, the sensitivity of the response was further decreased, and the maximum response level was reduced to less than 20% of that of the

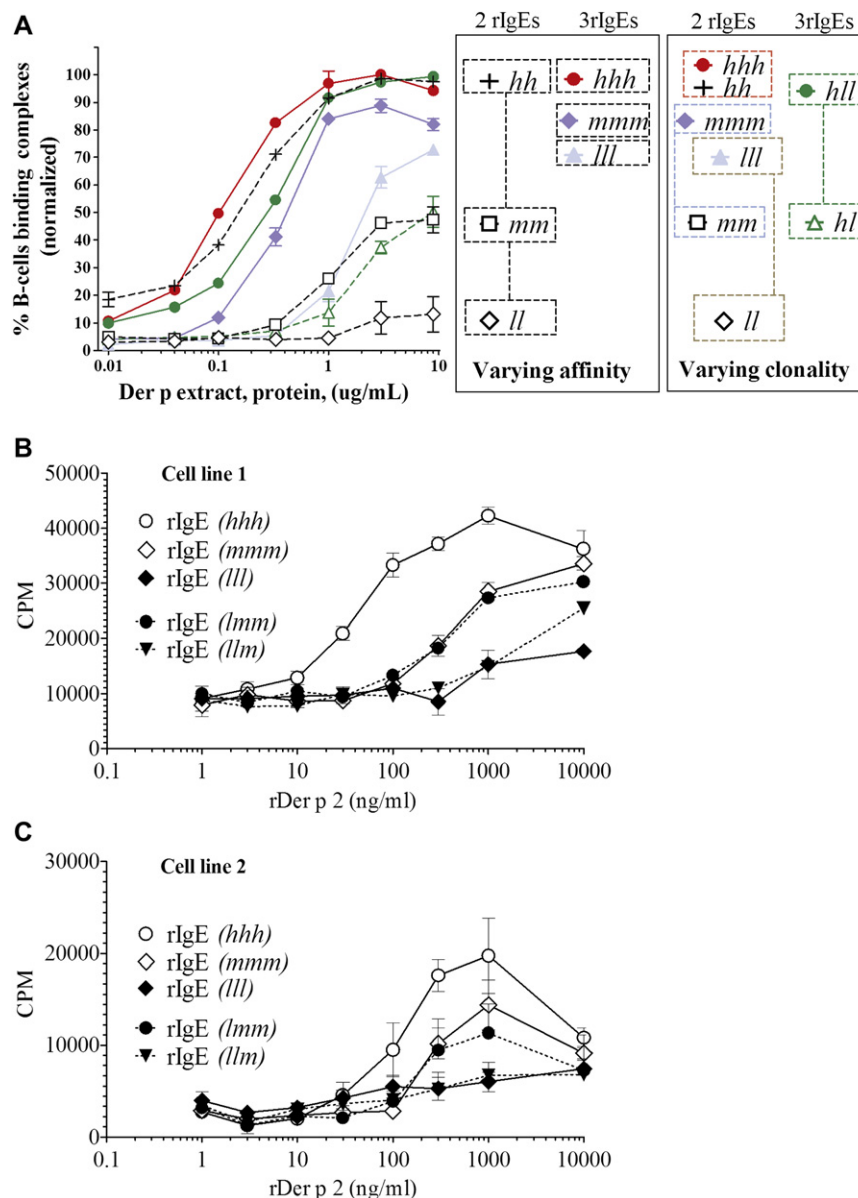


FIG 2. **A**, FACS assay. Increase in antibody affinity or clonality affects complex formation positively. rIgE antibodies binding allergen with high affinity (*h*), medium affinity (*m*), or low affinity (*l*) were tested at increasing concentrations of *D pteronyssinus* extract. For details regarding IgE specificities, see Table 1. Shown complex formation (percentage of B cells with bound IgE-allergen complexes) is normalized according to the maximum complex formation defined by 3 IgE antibody specificities (*hhh*; ie, data point: 3 μ g/mL = 89.6% and was set to 100%). **B** and **C**, T-cell activation. FAP measured as B cell-mediated T-cell proliferation in cell lines 1 (Fig 2, B) and 2 (Fig 2, C) on varying rIgE affinity to Der p 2. Decreasing the affinity of rIgE antibodies for complex formation reduces the proliferative response by T cells.

high-affinity combination (*hh*). In experiments with 3 IgE antibody specificities, the use of 3 medium-affinity IgEs (*mmm*) decreased sensitivity 2- to 3-fold and reduced the maximum response to approximately 90% of that seen with 3 high-affinity antibodies (*hhh*). Substitution of all 3 antibodies to low-affinity IgE (*lll*) decreased the sensitivity of the response by 10-fold and the maximum response to 70% to 80% of that of 3 high-affinity antibodies (*hhh*). Interestingly, substitution of only 2 IgEs to low-affinity variants (*hll*) did not affect the maximum response and produced only a small decrease (2-fold) in sensitivity compared with that seen with 3 high-affinity antibodies (*hhh*).

Effect of IgE clonality on complex formation

Decreasing IgE clonality (while maintaining the same total IgE concentration) from 3 to 2 IgEs of high affinity (*hhh* to *hh*) did not affect the sensitivity of complex formation or the maximum response (Fig 2, A). However, decreasing IgE clonality in experiments with medium- or low-affinity antibodies (ie, [*mmm*] to [*mm*] or [*lll*] to [*ll*]) considerably reduced the sensitivity of the response. In addition, the maximum response levels were greatly reduced (from approximately 90% [*mmm*] to approximately 65% [*mm*] and from approximately 70% [*lll*] to approximately 10% [*ll*]). Likewise, reducing the IgE clonality in the presence of

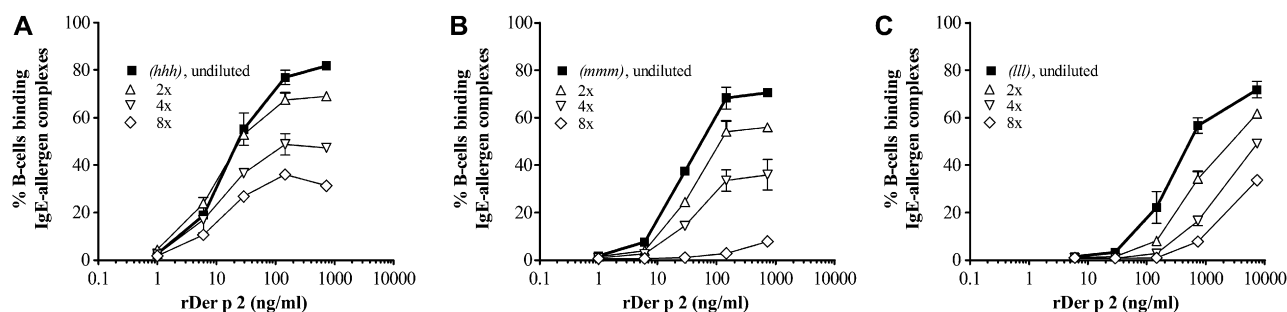


FIG 3. FACS assay. Decreasing specific IgE concentration decreases complex formation. Graphs show complex formation measured as the percentage of B cells with bound IgE-allergen complexes with rDer p 2 in dilution series on testing 1:1:1 mixes of 3 rIgE antibody specificities. Undiluted samples (■; total rIgE, 0.4 μ g/mL) and samples diluted 2-fold (Δ), 4-fold (∇), and 8-fold ($\diamond$) are shown. **A**, **B**, and **C**: High-, medium-, or low-affinity IgE, respectively.

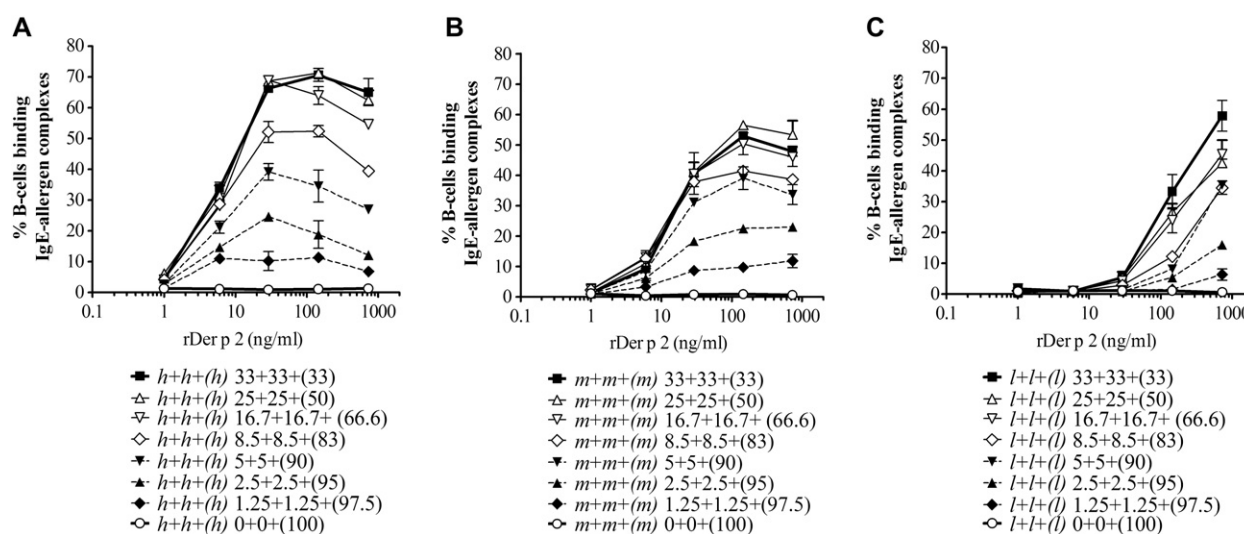


FIG 4. FACS assay. Complex formation obtained at a fixed concentration (0.4 μ g/mL) of total IgE on changing the ratio between individual antibody specificities from 33% + 33% + (33%) to 0% + 0% + (100%). Complex formation was measured as the percentage of B cells with bound IgE-allergen complexes. rIgE specificity H10 is shown in brackets. **A**, **B**, and **C**: High-affinity (*h*), medium-affinity (*m*), or low-affinity (*l*) IgE, respectively.

both high- and low-affinity IgEs from (*hll*) to (*hl*) reduced both the sensitivity of the response (approximately 10-fold) and the maximum response from a level of 100% (*hll*) to a level of less than 60% (*hl*).

Effect of IgE affinity measured as B cell-mediated T-cell proliferation

Variations in the binding affinities of individual IgE antibodies used for complex formation clearly affected B cell-mediated proliferation of the 2 T-cell lines (ie, 1 and 2; Fig 2, *B* and *C*). As with complex formation, T-cell proliferation was reduced from a high response level obtained with the high-affinity IgEs (*hhh*) to a medium response level with complexes containing medium-affinity IgEs (*mmm*) to a low response level on complex formation with low-affinity IgEs (*lll*). Combinations of low- and medium-affinity IgEs (*mlm*) induced medium responses in T-cell lines 1 and 2. The combination (*ml*) induced a response in T-cell line 1.

Effect of specific IgE concentration on complex formation

Titration of the specific IgE concentration led to decreased sensitivity, as well as reductions in the maximal complex formation level, regardless of the affinities tested (Fig 3).

Ratio between different IgE specificities affects complex formation

Regardless of the affinities tested, the most efficient formation of complexes was obtained by using an equimolar ratio of IgE antibody specificities. Fig 4 shows a reduced formation of complexes on increasing the concentration of 1 IgE specificity while decreasing the concentration of 2 other IgE specificities (same total IgE concentration). Interestingly, turning the ratio among 3 IgE specificities to 5% + 5% + 90% still induced half-maximal complex formation of that obtained with equimolar antibody ratio.

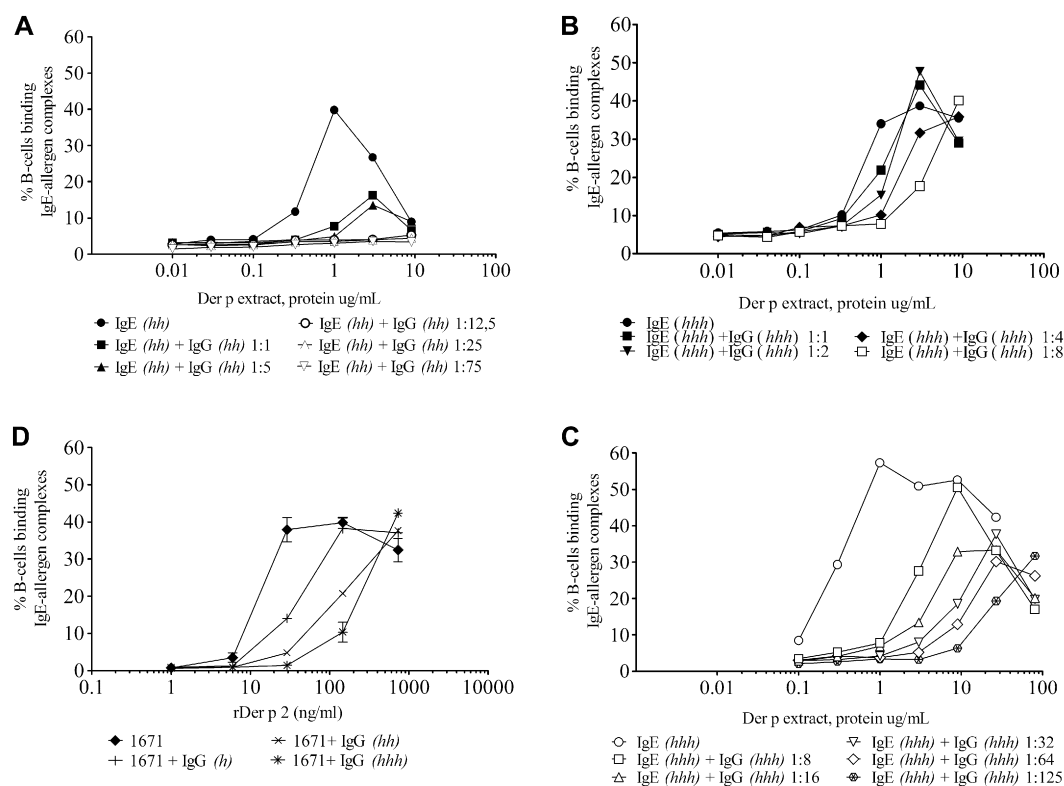


FIG 5. FACS assay. **A-C**, Complex formation (percentage of B cells binding IgE-allergen complexes) obtained with 2 or 3 rIgEs inhibited by 2 or 3 specific rIgG antibodies, respectively, in titration. Pairwise, the rIgE and rIgG antibodies bind identical epitopes on Der p 2 with high affinity. For details regarding IgE specificities, see Table I. **D**, Complex formation with serum from a patient with mite allergy inhibited by 1 (H10), 2 (H10 + E), or 3 (H10 + H12 + E) specific rIgG antibody specificities.

Specific rIgG antibodies inhibit complex formation

Fig 5, A, shows a clear reduction in complex formation obtained with 2 high-affinity rIgE antibodies on addition of 2 rIgG antibodies having the same specificity and affinity toward Der p 2 as the corresponding rIgEs. Addition of specific IgG antibodies clearly shifted the dose-response curve toward lower sensitivity and reduced the maximum level of cell-bound complexes. Similar experiments were done with 3 rIgE antibodies. Fig 5, B and C, shows the response obtained with 3 rIgEs on inhibition with increasing concentrations of 3 rIgG antibodies having the same specificity and binding affinity as the rIgEs. Again, addition of specific IgG antibodies clearly shifted the dose-response curve toward lower sensitivity. However, the maximum level of cell-bound complexes was much less affected. The ability of the same rIgG antibodies to block formation of complexes with IgE in a high-titer serum from a patient with house dust mite allergy (serum 1671; Der p 2-specific IgE, 197 kU/L) is shown in Fig 5, D. Addition of rIgG antibodies (final total [rIgG], 2 μ g/mL) to patient serum inhibited complex formation with Der p 2. Compared with allergen and patient serum alone, addition of 3 IgG antibody specificities shifted the response curve 15- to 20-fold toward lower sensitivity. Smaller shifts were seen on addition of 1 or 2 IgG antibody specificities. Again, the maximum level of cell-bound complexes was not affected on addition of 1 to 3 IgG specificities, which is reminiscent of the IgG competition of complex formation in the presence of 3 nonoverlapping IgEs and an indication of the polyclonal nature of serum 1671.

DISCUSSION

A cardinal feature of allergic sensitization is the presence of increased levels of allergen-specific serum IgE antibodies. In addition to variations in total and allergen-specific IgE concentrations, factors such as individual IgE affinity and IgE clonality are distinct properties of the allergic patient's IgE repertoire that can be expected to vary from patient to patient. Analyses of IgE V ϵ H sequences^{35,36} and phage display-established IgE libraries¹³ suggest that in allergic patients multiple epitopes on allergens are recognized by various IgE molecules. In a recent publication we demonstrated that IgE repertoire complexity is decisive for the activation of effector cells and, by inference, for the initiation of the immediate allergic response in allergic subjects.²⁹ Other studies indicate that a relationship between IgE clonality and the severity of allergic reactions in peanuts allergy exists.^{37,38} In addition, a link between individual IgE specificities and food-induced allergic reactions³⁹⁻⁴¹ and a connection between IgE affinity and the outcomes of skin prick tests⁴² have been found. These observations suggest that a further dissection of the IgE repertoire of the individual patient might help to relate IgE levels to disease severity. Currently, the understanding of the development of polyclonal IgE repertoires in sensitized subjects on their transformation from weak to stronger allergy is limited. It has been hypothesized that IgE in sensitized subjects through FAP at a very low concentration of allergen might lead to a continual activation of the immune system and thus expansion of the T_H2 cell population, inducing more B cells to switch to IgE

production.¹⁰ Still, the importance of IgE repertoire complexity on the development and maintenance or exacerbation of allergic disease is not clear. Here, using recombinant antibodies, we have investigated the dependencies of IgE repertoire complexity on the efficiency of complex formation and subsequent FAP-mediated T-cell activation.

IgE-mediated basophil activation and FAP depend on similar mechanisms (ie, the formation of complexes among IgE, allergen, and a cell-bound IgE receptor). There are, however, some distinct differences. On basophils, IgE becomes anchored to the cell surface by the high-affinity FcεRI receptor, even in the absence of allergen. This means, as previously shown,²⁹ that the first IgE interaction with allergen on the surface of the basophil, especially if the allergen is bound with high affinity, is able to facilitate a second IgE interaction to the same allergen, leading to effector cell activation even if the second interaction is of low affinity. In contrast, stable binding to CD23 requires simultaneous interactions among this low-affinity receptor, IgE, and allergen, and no cooperative binding occurs on complex formation with CD23. In line with these differences, we find that in contrast to basophil activation, the presence of a single high-affinity IgE specificity in combination with a single low-affinity IgE specificity (*hl*) was not sufficient to provide the avidity needed for obtaining high sensitivity in the complex formation assay. The presence of 2 low-affinity IgE specificities (ie, the [*hll*] combination) was necessary to obtain high sensitivity with a single high-affinity IgE antibody specificity, suggesting that IgE clonality might be a governing factor in FAP, especially with low-affinity IgE (Fig 2, A). This is further supported by the finding that even very disproportionate ratios (5% + 5% + 90%) of 3 antibody specificities result in substantial levels of complex formation (Fig 4). Although IgE clonality might be the governing factor for complex formation, the presence of high-affinity IgE was necessary for obtaining maximal complex formation (*hh*), (*hhh*), (*hll*) > (*mmm*) > (*lll*), showing a clear effect of increasing IgE affinity (Fig 2, A), and a comparable effect of IgE affinity was seen in FAP-mediated T-cell activation experiments (Fig 2, B and C), confirming that complex formation is an excellent surrogate parameter for T-cell activation.

Again, it is important to stress that medium- and low-affinity IgE at high IgE clonality by themselves were able to induce ample complex formation *in vitro* (Fig 2, A). Judging from the effect of IgE repertoire complexity on complex formation *in vitro*, we hypothesize that in the presence of high IgE clonality, low-affinity IgE antibodies in a patient's IgE repertoire alone or coexisting with a single high- or medium-affinity IgE specificity might be sufficient to drive activation of CD4⁺ T cells that in an environment dominated by T_H2 cytokines might exacerbate the allergic disease in newly sensitized allergic subjects. The low and possibly highly heterogeneous expression of CD23 on circulating B cells might add to the complexity of FAP *in vivo*. However, differences in CD23 expression of the B-cell lines investigated *in vitro* in the present study did not affect the effect of variations in IgE specificity and affinity on FAP. Finally, it was not possible to establish complex binding to the cell surface by using a combination of 1 IgE and 1 IgG specific to different epitopes on Der p 2 (data not shown), suggesting that IgG receptors are not involved in complex binding.

The process of IgE-mediated FAP by B cells provides a link between the allergen-specific humoral and cellular immune response,⁹⁻¹¹ and as demonstrated here, the extent of T-cell

activation as a result of FAP is under the direct influence of the complexity of the allergen-specific IgE repertoire. Previous studies suggest that the FAP capacity with respect to different allergens is associated with the allergen-specific IgE titer^{11,32,34} and that dilution of polyclonal serum IgE reduces complex formation.⁴³ These observations are supported by the results for complex formation presented here. Recently, murine studies were published suggesting that basophils might also function as antigen-presenting cells.⁴⁴⁻⁴⁶ However, the results of these studies have been questioned.^{47,48} Although allergen uptake and presentation and the ensuing induction of allergen-specific T_H2 cells and expression of T_H2 cytokines in all likelihood, like most other biological pathways, depend on parallel and redundant processes, it is conceivable that allergen-specific IgE plays an important role for the induction and maintenance of a proinflammatory T_H2 environment through mechanisms that depend on the properties of individual IgE repertoires described here for facilitated allergen presentation by B cells and for basophil activation, as described by Christensen et al.²⁹

In relation to allergy treatment, a marker of successful SIT is the appearance of circulating allergen-specific non-IgE antibodies (ie, blocking antibodies). The clinical effect of blocking antibodies was originally demonstrated by Cooke et al²³ and Loveless,⁴⁹ and we have recently shown that this competition between IgE and SIT-induced non-IgE affects IgE binding to allergen, basophil activation, and T-cell activation *in vitro*.²⁷ IgG, especially IgG4, has been proposed as an effective blocking antibody in SIT.⁵⁰ In the present study, being able to control the exact stoichiometry between rIgE and rIgG antibodies and allergen in the complex formation assays, we were able to examine the direct blocking capacity of IgG *in vitro* (ie, the ability to compete with IgE for allergen binding), a feature that is unrelated to the IgG subtype, allowing recombinant IgG3 antibodies to be used as a substitute for IgG4. The effect of introducing blocking IgG into the assay depended to a large extent on the complexity of the IgE/Der p 2/CD23 complexes. In competition assays with allergen-specific IgG, complex formation with only 2 IgE antibody specificities was disrupted much more easily than complex formation with 3 nonoverlapping IgE antibody specificities (Fig 5). The presence of only modest excess of 2 IgG antibodies with specificities and affinities matching the 2 IgE antibodies led to a dramatic reduction in both maximal complex formation and sensitivity. Further increase in competing IgG concentration almost abolished complex formation at 12.5-fold molar excess. In contrast, although much reduced in sensitivity, complex formation with 3 IgEs was maintained even in the presence of 125-fold molar excess of 3 IgG specificities (Fig 5, C). Similarly, testing the blocking effect of the same 3 high-affinity IgG antibody specificities on complex formation obtained with IgE in the form of a high-titer patient serum (serum 1671) showed a reduced sensitivity of the response but a maintained high level of complex formation (Fig 5, D). This result indicates that in the model system the combination of 3 rIgE antibodies binding nonoverlapping epitopes on Der p 2 form stable complexes, such as the complexes obtained with patient serum 1671, which presumably is a polyclonal serum. These effects on complex formation of IgG antibodies might be extrapolated to T-cell activation based on the correlation of the 2 assays demonstrated in previous investigations of the blocking effects of postimmunotherapy sera^{27,34} and exemplified here with 2 T-cell lines. However, it will be interesting to investigate the specific effect of the IgE/IgG competition on

T-cell activation in detail. In general, induction of regulatory T cells is thought to play a central role in the efficacy of SIT. If regulatory T cells are induced during FAP, this would be desirable. Still, T-cell activation at low allergen concentrations seems to favor T_H2 differentiation, and through FAP, mainly the existing T_H2 response will be activated, with B cells as the major antigen-presenting cell.

Assuming the IgE repertoire develops gradually in terms of clonality and affinity during sensitization (Willumsen N, manuscript in preparation), the clinical implications of our findings are that with regard to the inhibitory effect of SIT-induced non-IgE antibodies on FAP-mediated T-cell activation, **early intervention to counteract a less developed IgE repertoire might be beneficial or even essential for preventing further development of allergic sensitization.**

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Clinical implications: IgE/allergen/CD23 complex formation is increased with increasing IgE clonality, and inhibition by allergen-specific IgG is more difficult, suggesting that early intervention by SIT might be favorable to prevent disease progression.

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