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*.
ResultsAlthough 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;127:1029-37.)

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

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 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

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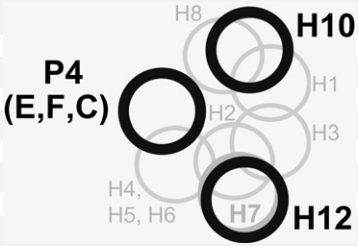
TABLE I. Der p 2–specific rIgE antibodies used in the study

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 *(h)*	
	1.18 nmol/L	H12:H12*‡	H12 *(h)*	
	1.4 nmol/L	E†‡	E *(h)*	
Medium	3.99 nmol/L	H10:H1*	H10 *(m)*	
	5.14 nmol/L	H12:H7*	H12 *(m)*	
	9.43 nmol/L	F†	F *(m)*	
Low	33.2 nmol/L	H7:H12*	H12 *(l)*	
	171 nmol/L	H10:H7*	H10 *(l)*	
	291 nmol/L	C†	C *(l)*	
Combinations of clones used throughout this article				
Antibody specificity	H10	H12	P4	
hhh	H10:H10	H12:H12	E	
mmm	H10:H1	H12:H7	F	
lll	H10:H7	H7:H12	C	
hh	H10:H10	H12:H12		
hh§	H10:H10		E	
mm	H10:H1	H12:H7		
ll	H10:H7	H7:H12		
hll (llh)	H10:H7	H7:H12	E	
mml (lmm)	H10:H7	H12:H7	F	
mll (llm)	H10:H7	H7:H12	F	
hl (lh)	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.

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 al[30] 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 human serum IgE (10 μL) were added to 10 μL of RPMI 1640 buffer (Lonza,