

Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge

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Background: On cross-linking of receptor-bound IgE antibodies by allergens, effector cells (basophils and mast cells) involved in type I allergic reactions degranulate and release the potent chemical mediators stored inside their granules. Total and allergen-specific IgE concentrations, IgE affinity for allergen, and IgE clonality are all distinct properties of allergic patients' IgE repertoires. However, the inability to isolate individual IgE antibodies from allergic patients' sera presents a major barrier to understanding the importance of patient-specific IgE repertoires for the manifestation and severity of allergic symptoms.

Objective: We sought to investigate how individual properties of an IgE repertoire affect effector cell degranulation.

Methods: A panel of recombinant IgE (rIgE) antibodies specific for the major house dust mite allergen Der p 2 was developed and characterized in regard to Der p 2 affinity, as well as Der p 2 epitope specificity, by using surface plasmon resonance technology. Human basophils were sensitized with different combinations of rIgEs, and degranulation responses were measured by means of flow cytometry after challenge with Der p 2.

Results: A total of 31 Der p 2-specific rIgEs were produced. They bound a total of 9 different Der p 2 epitopes in the affinity range (K_D value) of 0.0358 to 291 nM.

Factors increasing human basophil degranulation were increased total IgE concentrations, increased concentrations of allergen-specific IgE relative to non-allergen-specific IgE, more even concentration of individual allergen-specific IgE clones, increased IgE affinity for allergen, and increased number of allergen epitopes recognized by the IgE repertoire (increased IgE clonality).

Conclusion: This study demonstrates how distinct properties of the IgE repertoire, such as total and allergen-specific IgE

antibody concentration, IgE affinity, and IgE clonality, affect effector cell degranulation. (*J Allergy Clin Immunol* ■■■■;■■■;■■■-■■■.)

Key words: Allergy, recombinant IgE, human IgE, chimeric IgE, antibody, affinity, epitope, allergen, Der p 2, effector cell degranulation, basophil activation test, basophils, diagnosis

Effector cells (basophils and mast cells) involved in type I allergic reactions contain high-affinity IgE receptors (FcεRI) embedded in their plasma membranes.¹ On cross-linking of receptor-bound IgE by allergens, effector cells degranulate and release the potent chemical mediators (eg, histamine) stored inside their granules, leading to immediate allergic symptoms.²

Total and allergen-specific IgE concentrations, IgE affinity, and IgE clonality are all distinct properties of an allergic patient's IgE repertoire. Most commonly seen are patient-specific variations in total and allergen-specific IgE concentrations,³ but also more subtle and less accessible parameters, such as overall IgE affinity for allergens and the number of allergen epitopes recognized by a patient's IgE repertoire (clonality), have been connected with different outcomes of skin prick tests or severity of allergic symptoms.⁴⁻⁷ Still, the extent to which these individual properties of an allergic patient's IgE repertoire contribute to effector cell degranulation remains to be established in detail. However, the inability to isolate individual IgE antibodies from allergic patients' sera presents a major barrier to understanding the importance of patient-specific IgE repertoires for the manifestation and severity of allergic symptoms.

In this study we developed a panel of 31 fully human or mouse/human chimeric recombinant IgE (rIgE) antibodies specific for the major house dust mite (HDM) allergen Der p 2, which is a monomeric, 14-kDa protein recognized by 80% of all individuals with HDM allergy.⁸ The rIgE antibodies were characterized with regard to individual affinity for Der p 2, as well as to their Der p 2 epitope specificity. Human basophils were sensitized with different mixtures of rIgE, mimicking allergic IgE repertoires of known compositions, to assess the contribution of IgE concentration, IgE affinity, and IgE clonality on allergen-mediated effector cell degranulation.

METHODS

Cloning and expression of rIgE antibodies

Previously, a set of 2 mammalian expression vectors encoding the human IgG₃ heavy chain (pLNOH2) and the human κ light chain (pLNOK) was constructed by Norderhaug et al.⁹ For expression of the rIgE antibodies described here, the human constant IgG₃ region in pLNOH2 was replaced with the human constant IgE region (Cε_{H1}-Cε_{H4}), resulting in the new vector

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Abbreviation used

HDM: House dust mite

pLNOH2IgE. C ϵ _H1-C ϵ _H4 was PCR amplified from human genomic DNA to include introns for enhanced recombinant expression yields.¹⁰

Ten hybridoma cell lines expressing murine anti-Der p 2 antibodies (IgG isotype) were made, as previously described,¹¹ from mice immunized with natural Der p 2. Variable antibody regions (V_HDJ_H/V_KJ_K) from these hybridoma cell lines were RT-PCR amplified and cloned into pLNOH2IgE and pLNOK in frame with C ϵ _H1-C ϵ _H4 and C κ , respectively.

Recombinant IgE antibodies were produced with the Freestyle 293 Expression System (Invitrogen, Carlsbad, Calif). The concentration of rIgE ranged from 150 μ g/L to 15 mg/L (mean, 6.8 mg/L), as determined in an Advia Centaur assay (Bayer, Leverkusen, Germany), by using biotinylated anti-IgE as the detector antibody.

Generation of Der p 2-specific chimeric rIgE antibodies by shuffling of antibody chains

HEK293 cells were transfected with all possible combinations of heavy- and light-chain vectors encoding the 10 hybridoma-derived chimeric rIgE antibodies mentioned above. The chain-shuffled, chimeric rIgE antibodies were screened for their ability to bind Der p 2 in ELISA: Maxisorp microtiter wells (Nunc, Roskilde, Denmark) coated with recombinant Der p 2 (rDer p 2; 1 μ g/mL) or with BSA (1 μ g/mL) as a negative control were blocked (PBS containing 2 wt/vol% skim milk) for 2 hours and incubated with rIgE-containing cell supernatant for 1 hour. Bound rIgEs were detected with horseradish peroxidase-conjugated polyclonal rabbit anti-human IgE (P0295; DAKO, Glostrup, Denmark), followed by addition of TMB One developing agent (Kem-En-Tec Diagnostics, Taastrup, Denmark). Absorbance was measured at 450 nm.

Mapping of relative positions of Der p 2 epitopes

Surface plasmon resonance mapping experiments were performed on a Biacore 2000 (Biacore, Uppsala, Sweden). Monoclonal anti-human IgE (developed in-house at ALK-Abelló, Hørsholm, Denmark) was immobilized on a CM5 chip (Biacore) by using an Amine Coupling Kit (Biacore). The cycles were run as follows. The first rDer p 2-specific rIgE clone was injected for 4 minutes, followed by injection of a blocking rIgE (anti-tetanus toxoid) for 5 minutes. This latter step was included to block free anti-human IgE sites at the chip surface not bound by the first rDer p 2-specific rIgE to avoid false-positive responses. Then rDer p 2 (10 μ g/mL) was injected for 1 minute, immediately followed by injection of the second rDer p 2-specific rIgE clone for 4 minutes.

Mapping of Der p 2 epitopes with rDer p 2 variants

Six rDer p 2 variants were developed at ALK-Abelló (Henmar et al, unpublished data). Briefly, Der p 2 (isoform 2.0101) was mutated in one of 6 surface-exposed positions: K6A, K15E, H30N, E62S, H74N, or K82N. The rDer p 2 variants were expressed in *Pichia pastoris*. Surface plasmon resonance experiments were carried out with the same anti-human IgE CM5 chip as described above. Cycles were run as follows. A Der p 2-specific rIgE clone was injected for 4 minutes, followed by injection of an rDer p 2 variant (10 μ g/mL) for 1.5 minutes with a dissociation time of 5 minutes. Controls included injection of rDer p 2 wild-type (positive control) or supernatant from *P pastoris* transfected with an empty vector (pGAPz, negative control).

Determination of rIgE affinities for rDer p 2

Surface plasmon resonance affinity measurements were carried out with a CM5 chip immobilized with anti-human IgE, as described above. Cycles were run as follows. A rIgE clone was injected for 5 minutes, followed by injection

of rDer p 2 for 5 minutes with a dissociation time of 10 minutes. The rDer p 2 concentration was varied by 2-fold dilutions between each cycle in a range from 0.349 to 5714 nM, for a complete coverage of rDer p 2 concentrations from zero response to saturation level ($R_{\max}$). The affinity constant K_D (kd/ka) was determined with BIAevaluation software (Biacore).

Basophil activation test

PBMCs were isolated on a density gradient (Lymphoprep; Nycomed, Zurich, Switzerland) from nonatopic donors whose basophils were previously tested to give equally high maximal degranulation responses (>80%). Native IgE antibodies bound to the basophil cell surface were stripped off with ice-cold lactic acid buffer, pH 3.9 (Bie & Berntsen, Herlev, Denmark), for 5 minutes. Basophils were sensitized with mixtures of rIgE (1 μ g/mL) for 1 hour at 37°C. Sensitized cells were challenged with rDer p 2 in a concentration range of 0.1 pg/mL to 1 μ g/mL in RPMI medium plus 0.5% human serum albumin plus 2 ng/mL IL-3. Degranulation was carried out for 1 hour at 37°C. After degranulation, cells were labeled with a cocktail consisting of anti-CD63-fluorescein isothiocyanate, anti-CD123-phycoerythrin, and anti-HLA-DR-peridinin-chlorophyll-protein complex (catalog no. 341068; BD Biosciences, Franklin Lakes, NJ) in addition to a non-cross-linking, biotinylated anti-IgE (developed in house at ALK-Abelló), followed by addition of 10 μ L of streptavidin-allophycocyanin (catalog no. 349024, BD Biosciences).

Basophil degranulation was measured with a FACSAria cell sorter (BD Biosciences). Briefly, PBMCs were initially gated on CD123⁺ cells (basophils and monocytes), followed by gating on HLA-DR⁺ cells (basophils but not monocytes). Nondegranulated and degranulated basophils were counted with the CD63 marker. Two hundred thousand PBMCs were counted from each sample, corresponding to 1000 to 6000 basophils.

Controls were included to confirm that native IgE was properly stripped off during the stripping step and to ensure the absence of unspecific background degranulation.

RESULTS

Cloning and expression of Der p 2-specific rIgE antibodies

Ten murine anti-Der p 2 IgG antibodies were converted into mouse/human chimeric IgE antibodies named H1 through H8, H10, and H12. In addition, 9 fully human Der p 2-specific rIgE clones, named C through K, were cloned from the peripheral IgE⁺ B cells of a patient allergic to HDM by using the phage display technique, as described in detail elsewhere (Christensen et al, unpublished data).

Mapping of antibody epitopes on Der p 2

By using surface plasmon resonance, 2 different approaches were applied to map antibody epitopes on Der p 2. First, relative positions of Der p 2 epitopes recognized by the rIgE clones were determined by the ability of pairs of 2 rIgE clones to bind rDer p 2 simultaneously, as shown in Fig 1, A and B. Seven individual binding patterns (epitopes) were identified (Fig 1, C).

In the second mapping approach, a rough estimate of Der p 2 surface areas involved in each epitope was determined by testing the capability of individual rIgE clones to bind a panel of recombinant Der p 2 variants, as shown in Fig 1, D and E. Nine different binding patterns were observed (Fig 1, F). Mutation of Der p 2 in position K15, H74, or K82 each led to abolished antibody binding by 1 or more rIgE clones (Fig 1, F), indicating a central position of these amino acids in the respective epitopes.¹² Contrarily, mutation of Der p 2 in position K6, H30, or E62 only weakly affected binding of some rIgE clones (Fig 1, F), indicating a peripheral position of these amino acids in the respective epitopes.¹²

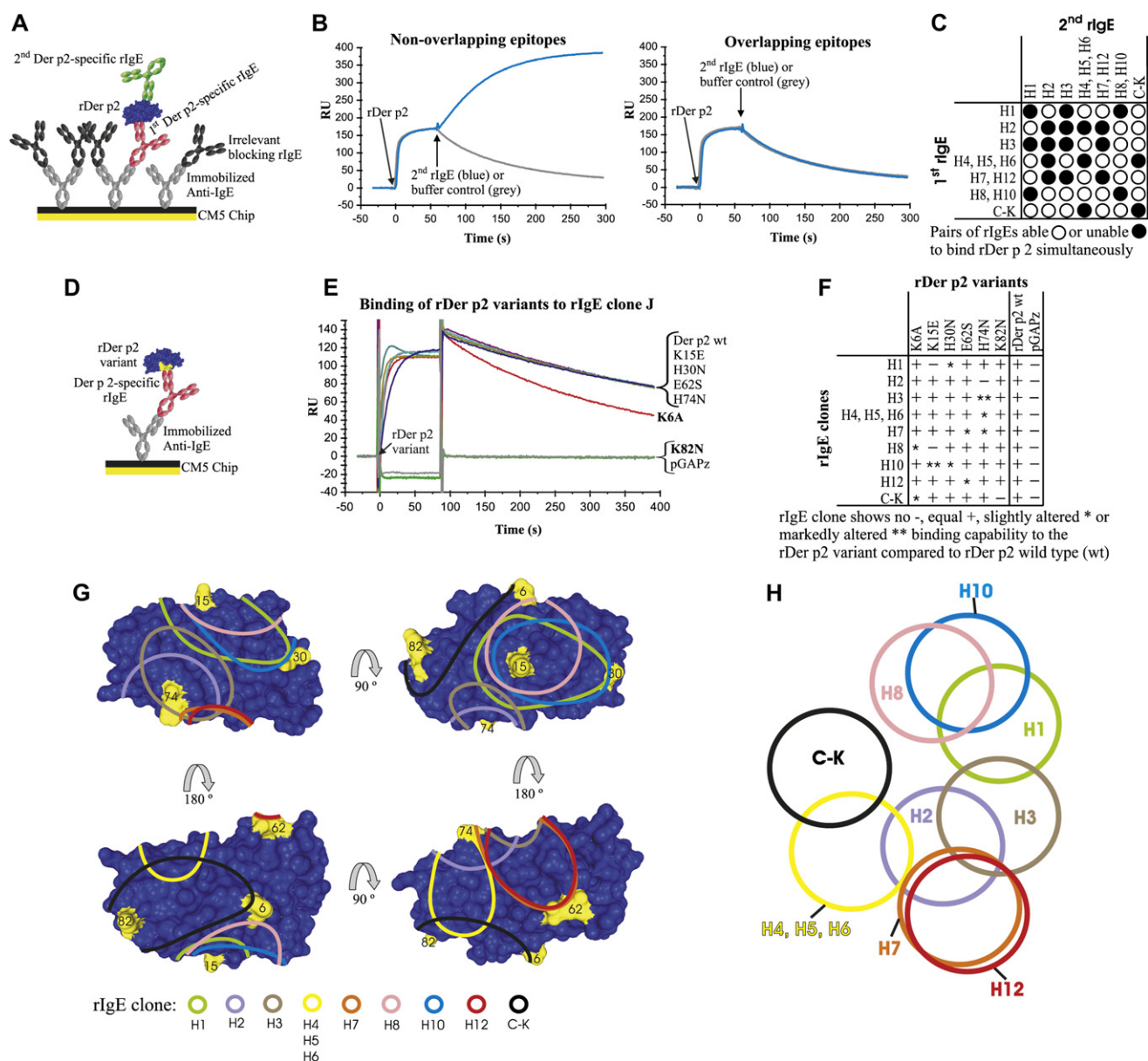


FIG 1. Der p 2 epitope mapping. Principle (A), examples (B), and summary (C) of results obtained by means of relative epitope-mapping experiments by using surface plasmon resonance are shown. Principle (D), example (E), and summary (F) of results obtained by means of epitope-mapping experiments with rDer p 2 variants by using surface plasmon resonance are shown. G and H, Three- and 2-dimensional maps of Der p 2 epitopes bound by the rIgE clones. Source of Der p 2 structure: Derewenda et al¹³; PDB ID, 1KTJ.

Based on these 2 mapping approaches, a 3- and a 2-dimensional map of epitopes recognized by the various rIgE clones was constructed (Fig 1, G and H). As can be seen from the models, several combinations of up to 3 different rIgE clones were able to bind rDer p 2 simultaneously.

Generation of additional Der p 2-specific chimeric rIgE antibodies by means of light-chain shuffling

By shuffling the light chains of the 10 hybridoma-derived rIgE clones, 12 additional Der p 2-specific rIgE clones were obtained (Table I). The chain-shuffled chimeric rIgE clones were named Hx:Hy, where Hx and Hy denote the respective heavy- and light-chain clones from which they were derived.

Der p 2 epitopes bound by these chain-shuffled rIgE clones were mapped analogous to the 2 approaches shown in Fig 1, A and D (data not shown). In all cases, each individual chain-shuffled rIgE clones exclusively bound the same Der p 2 epitope as the parent clone from which the heavy chain was derived, indicating that the overall epitope specificity was determined by heavy chains.

Determination of rIgE affinities for rDer p 2

Individual rIgE antibody affinities for rDer p 2 were determined by means of surface plasmon resonance experiments. Representative sensorgrams of rIgE clones binding rDer p 2 with high, medium, and low affinity (arbitrarily assigned categories) are shown in Figure 2, A through C, and a summary of all results is

TABLE I. Chain-shuffled rIgE clones and their ability to bind rDer p 2, as determined by means of ELISA

	Light chain clone										
	H1	H2	H3	H4	H5	H6	H7	H8	H10	H12	
Heavy chain clone											
H1	+	—	—	—	—	—	—	—	+	—	
H2	—	+	—	—	—	—	—	—	—	—	
H3	—	—	+	—	—	—	—	—	—	—	
H4	—	—	—	+	+	+	—	—	—	—	
H5	—	—	—	—	+	+	—	—	—	—	
H6	—	—	—	—	+	+	—	—	—	—	
H7	—	—	—	—	—	—	+	—	—	+	
H8	—	—	—	—	—	—	—	+	—	—	
H10	+	+	—	+	—	—	+	—	+	+	
H12	—	—	—	—	—	—	+	—	—	+	

+, Chain-shuffled rIgE clone able to bind rDer p 2; —, chain-shuffled rIgE clone unable to bind rDer p 2.

shown in Fig 2, D. Measured affinities ranged from 0.0358 to 291 nM.

Although chain-shuffled rIgE clones were all binding the same epitope as the parent rIgE clone from which the heavy chain was derived, their affinity for rDer p 2 was generally greatly altered, with most chain-shuffled rIgE clones binding rDer p 2 with lower affinity than their parent heavy-chain rIgE clone.

Higher total IgE concentrations increase both basophil sensitivity and maximal degranulation level

We used the basophil activation test (a flow cytometric method based on the CD63 marker) to assess basophil degranulation, a method now widely used as an alternative to histamine release measurements.¹⁴ On degranulation, CD63, a 53-kDa glycoprotein anchored in the granule membrane, fuses with and becomes exposed on the outside of the basophil plasma membrane,¹⁵ which perfectly correlates with histamine release, as examined by others^{15,16} and seen our own observations.

Human basophils were sensitized with different concentrations of a mixture consisting of equimolar quantities of 3 rIgE clones recognizing nonoverlapping Der p 2 epitopes. An irrelevant non-Der p 2-specific rIgE antibody (anti-tox, binding tetanus toxoid) was included in the IgE mixture as well (Fig 3) to imitate a typical allergic IgE repertoire that contains both allergen-specific and non-allergen-specific IgE. Sensitization of basophils with increasing total rIgE concentrations led to increased basophil degranulation responses observed as both increased maximal degranulation responses and increased sensitivity (the latter term was defined as the concentration of rDer p 2 triggering a half-maximal response).

Higher concentrations of allergen-specific IgE relative to non-allergen-specific IgE increase both basophil sensitivity and maximal degranulation level

The effect of changing the relative concentration between allergen-specific and non-allergen-specific IgE was examined by sensitizing human basophils with a fixed concentration of total IgE but different ratios of Der p 2-specific to non-Der p 2-specific rIgE (Fig 4).

As was also seen when increasing the total rIgE concentration, basophils sensitized with increasing relative concentrations of Der p 2-specific rIgE led to both increased maximal degranulation response and sensitivity (Fig 4).

Equimolar concentrations of individual allergen-specific IgEs result in the highest maximal degranulation level

We examined the consequences of changing the relative concentrations of individual allergen-specific IgEs in the simplest configuration. Human basophils were sensitized with different ratios of 2 rIgE clones binding nonoverlapping Der p 2 epitopes with equal affinity (Fig 5). The highest level of basophil degranulation was seen when the 2 rIgE clones were present in equimolar amounts (ratio of 50:50). In the case of uneven antibody concentrations, maximal basophil degranulation decreased when the ratio between the 2 Der p 2-specific rIgE clones was 95:5 and even more pronounced with a ratio of 99:1. Identical results were obtained with the reciprocal ratios (Fig 5). Changing the ratio of the 2 nonoverlapping IgEs affected maximal degranulation levels while leaving basophil sensitivity unchanged. Basophils monosensitized with a single Der p 2-specific rIgE (ratios of 100:0/0:100) showed no degranulation, as expected, because of the requirement for the presence of IgEs having at least 2 different epitope specificities for productive cross-linking.

Higher affinity of individual allergen-specific IgEs increases basophil sensitivity

Human basophils were sensitized with different combinations of 2 rIgE clones having different affinities for rDer p 2, still binding the same 2 epitope clusters (Fig 6), to investigate how the affinity of individual IgEs affects effector cell degranulation.

Generally, basophil sensitivity increased with increasing affinity of individual rIgE clones involved in the combinations (Fig 6). Basophils sensitized with 2 low-affinity rIgE clones (LL combination, Fig 6) required a 500- to 1000-fold higher rDer p 2 concentration than basophils sensitized with 2 high-affinity rIgE clones (HH combination, Fig 6) to reach equivalent degranulation levels.

In contrast to the low sensitivity of basophils sensitized with 2 low-affinity rIgE clones, a dramatic effect was seen when a low-affinity rIgE clone was combined with a high-affinity rIgE clone (HL combination, Fig 6). This latter combination showed a basophil sensitivity that was only 2- to 5-fold lower than that of 2 high-affinity rIgE clones (HH combination, Fig 6).

All rIgE affinity combinations resulted in similar maximal basophil degranulation levels (approximately 80%), except for the combination of 2 low-affinity antibodies (LL combination, Fig 6). It was not tested whether this combination reached the same maximal response at higher rDer p 2 concentrations, however.

Higher IgE clonality increases basophil sensitivity

The effect of IgE clonality (ie, the number of different IgE clones binding 1 allergen molecule simultaneously) was tested by sensitizing human basophils with combinations of 1, 2, or 3 rIgE clones directed toward nonoverlapping Der p 2 epitopes (Fig 7).

Basophils sensitized with 3 different rIgE clones (brown curve, Fig 7) showed 5- to 20-fold higher sensitivity than basophils

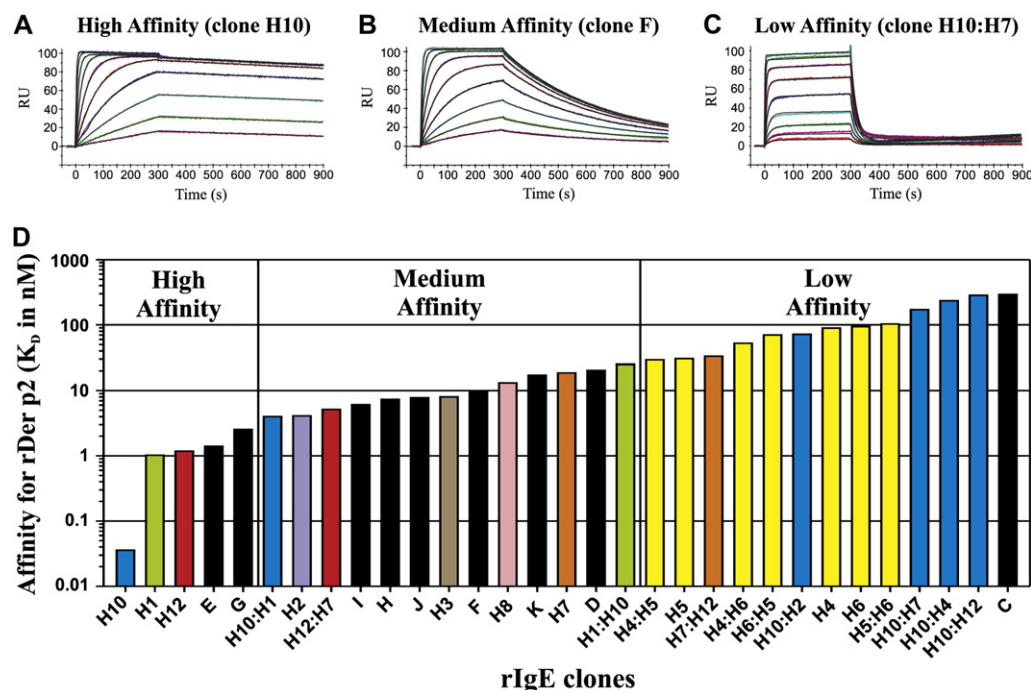


FIG 2. IgE affinities for rDer p 2. **A-C**, Examples of affinity determinations of rIgE clones binding rDer p 2 with high, medium, and low affinity, respectively, as obtained by using surface plasmon resonance experiments. Only the relevant part from where rDer p 2 is injected is shown. **D**, Summary of affinities obtained of all rIgE clones (color coded according to the epitope specificities marked in Fig 1, G and H).

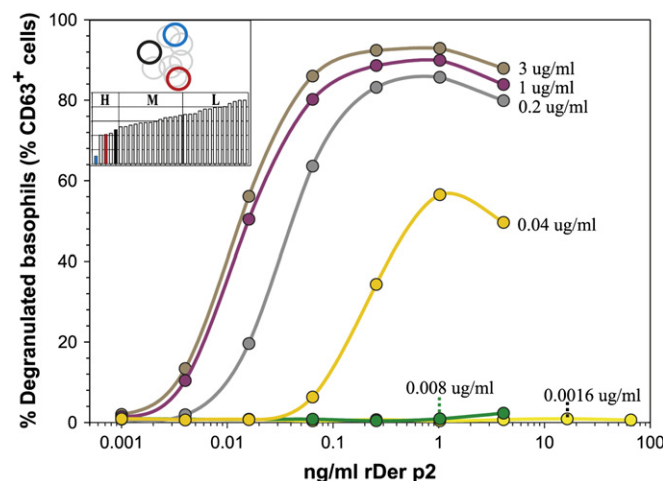


FIG 3. Basophil degranulation at different concentrations of total rIgE. FACS results of human basophils sensitized with total rIgE concentrations of 3, 1, 0.2, 0.04, 0.008, and 0.0016 μ g/mL, respectively, are shown. The composition of the rIgE mixture was kept constant with 20% rDer p 2-specific rIgE consisting of equimolar quantities (6.67% each) of 3 rIgE clones (G, H10, and H12) recognizing nonoverlapping Der p 2 epitopes and 80% non-Der p 2-specific rIgE (anti-tox). *Upper left*, Schematic representation of Der p 2 epitope specificity and affinity of rIgE clones G, H10, and H12; analog to Figs 1, H, and 2, D. H, High affinity; M, medium affinity; L, low affinity.

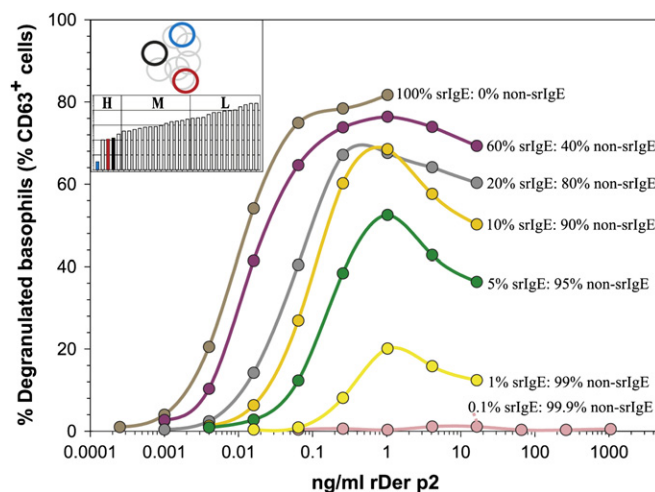


FIG 4. Basophil degranulation at different concentrations of Der p 2-specific rIgE relative to non-Der p 2-specific rIgE. FACS results of human basophils sensitized with different concentrations of Der p 2-specific rIgE (srIgE) relative to non-Der p 2-specific rIgE (non-srIgE) are shown. The composition of the rIgE mixtures consisted of equimolar quantities of 3 rIgE clones (E, H10, and H12) recognizing nonoverlapping Der p 2 epitopes and variable concentrations of non-Der p 2-specific rIgE (anti-tox). The total rIgE concentration was kept constant at 1 μ g/mL.

sensitized with only 2 different rIgE clones (purple, grey, and orange curves; Fig 7). The maximal degranulation level seemed largely unaffected by changes in IgE clonality (Fig 7). Again, basophils monosensitized with a single rIgE clone showed no degranulation, as expected.

DISCUSSION

Using a panel of well-characterized recombinant IgE antibodies, we have demonstrated how individual components of a complex IgE repertoire affect effector cell degranulation. We show that the composition of the allergen-specific IgE repertoire

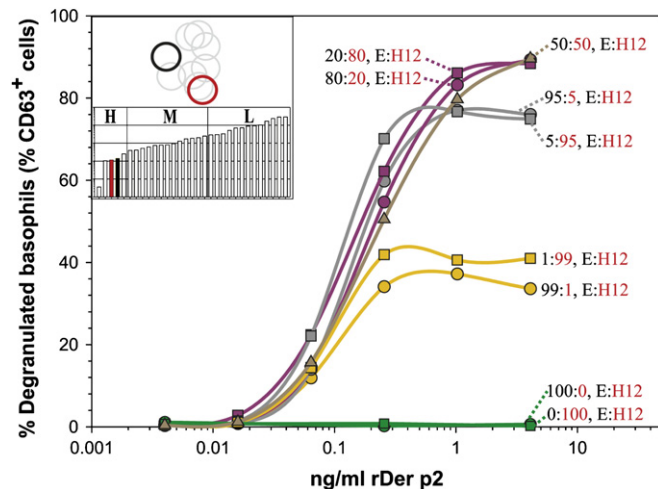


FIG 5. Basophil degranulation at different ratios between 2 Der p 2-specific rIgE clones. FACS results of human basophils sensitized with different ratios of 2 Der p 2-specific rIgE clones (E and H12) are shown. All other parameters were kept constant; that is, the total concentration of rIgE was 1 μ g/mL, and the relative concentrations were 20% Der p 2-specific to 80% non-Der p 2-specific rIgE.

is of major importance for basophil degranulation and that in particular basophil reactivity in response to allergen challenge strongly depends on the interplay between individual IgE antibodies.

The effect of Fc ϵ RI-bound allergen-specific IgE concentration on basophil degranulation was investigated both with regard to variations in the total IgE concentration at a fixed relative concentration of allergen-specific IgE and at a fixed total IgE level but with different relative concentrations of allergen-specific IgE. Both these scenarios represent naturally occurring variations in the IgE levels of allergic patients.³ Not surprisingly, these experiments showed that changes in the concentration of equimolar mixtures of nonoverlapping allergen-specific IgEs affect basophil degranulation levels. A novel finding, however, is that when we changed the relative concentrations of individual IgE specificities to levels other than equimolar, which is likely to be the case *in vivo*, the maximal basophil degranulation levels were reduced while leaving basophil sensitivity unchanged. We take this result to indicate a purely quantitative effect in that the IgE specificity present in the lowest concentration is the limiting factor in determining the maximal obtainable number of cross-linking events during allergen challenge. A direct consequence of this finding is that although allergic patients' sera might present similar titers of allergen-specific IgE, they might differ with regard to their ability to mediate effector cell degranulation in the presence of allergen.

In contrast, changes in affinity and/or clonality of the IgEs, which are both factors affecting the overall binding strength (avidity) of the IgE/allergen complexes, led almost exclusively to changes in basophil sensitivity, leaving the maximal degranulation levels largely unaffected. Interestingly, we found that the presence of only a single antibody of high affinity in the allergen-specific IgE repertoire is required for the recruitment of an IgE of even very low affinity into productive Fc ϵ RI/IgE/allergen complexes. In our experiments basophil degranulation, mediated by the high-affinity rIgE clone H10 and the low-affinity rIgE clone

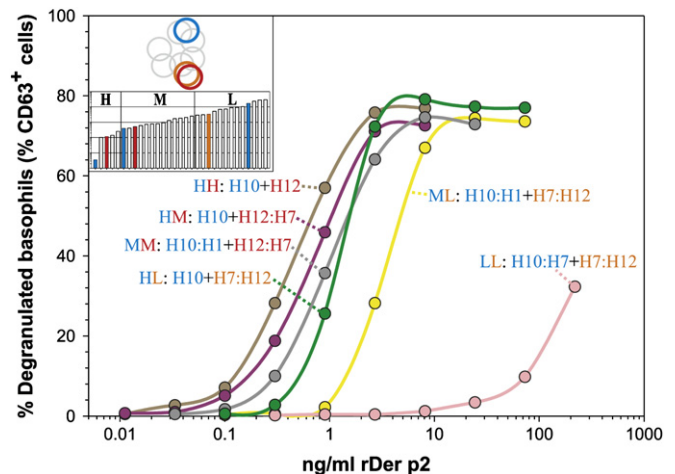


FIG 6. Basophil degranulation at different rIgE affinity combinations. FACS results of human basophils sensitized with different combinations of 2 rIgE clones having different affinities for rDer p 2 are shown. All other parameters were kept constant; that is, rIgE clones were directed against the same 2 epitope clusters, the total IgE concentration was 1 μ g/mL, the relative concentrations were 20% Der p 2-specific to 80% non-Der p 2-specific rIgE, and equimolar concentrations of the 2 Der p 2-specific rIgE clones were used. H, High affinity; M, medium affinity; L, low affinity.

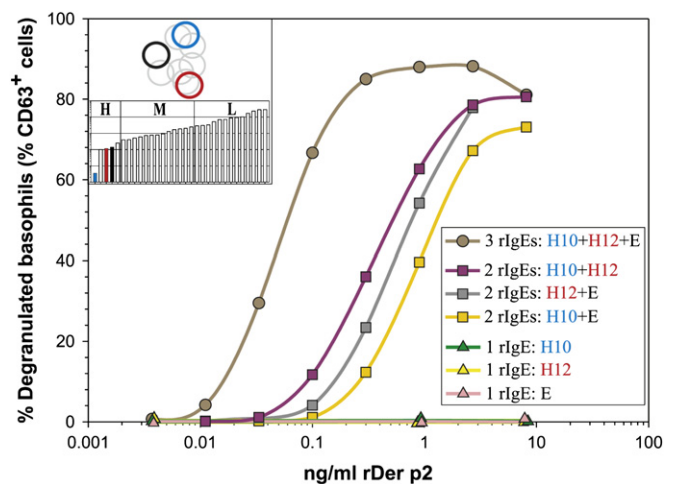


FIG 7. Basophil degranulation at different rIgE clonalities. FACS results of human basophils sensitized with combinations of 1, 2, or 3 rIgE clones binding nonoverlapping Der p 2 epitopes are shown. All other parameters were kept constant; that is, the total IgE concentration was 1 μ g/mL, the relative concentrations were 20% Der p 2-specific to 80% non-Der p 2-specific rIgE, and equimolar concentrations of the rDer p 2-specific rIgE clones were used.

H7:H12, was nearly as efficient in terms of basophil sensitivity as 2 high-affinity IgEs, despite the approximately 1000-fold difference in affinity between these 2 nonoverlapping IgE antibodies. This experimental observation is in agreement with a model previously put forth by Aalberse et al,¹⁷ who hypothesized that an allergen initially becomes captured by a high-affinity Fc ϵ RI-bound "anchor" IgE and is then dragged over the cell surface until this complex encounters other, even low-affinity, Fc ϵ RI-bound IgEs causing efficient cross-linking and effector cell degranulation. For the allergic patient, and in particular the procedures involved in allergy diagnostics, this has the direct implication that

the presence of low-affinity allergen-specific IgEs in a patient's serum should not be neglected. A prominent role of low-affinity IgE antibodies for allergic symptoms could very well be in the context of allergenic cross-reactivity, where patients react with allergic symptoms when challenged with homologous allergen molecules from related species.¹⁸

The requirements for degranulation of effector cells *in vitro* have previously been addressed by different means, including sensitization with complex sera,^{7,19,20} cross-linking with polymerized antigens/allergens,²¹⁻²⁴ cross-linking with covalently polymerized IgE,^{25,26} cross-linking with allergen extracts,⁷ and use of rat effector cell lines,^{21-25,27} in addition to a variety of other model systems. To our knowledge, the present study is the first in which factors governing human effector cell degranulation have been demonstrated in a model system consisting of an intact, clinically relevant allergen in combination with different mixtures of well-characterized, allergen-specific IgE antibodies directly mimicking allergic patients' sera of different compositions.

In current practice the IgE response in allergic patients is typically measured against complex allergen extracts at the level of polyclonal serum, either by means of skin prick tests or IgE titer measurements.²⁸ A much more detailed picture of the allergic status of individual patients would emerge if more detailed methods of analysis could be developed that accurately determine the composition of the IgE repertoire. More detailed knowledge about the development over time of IgE clonality, individual IgE antibody affinity, and concentration might furthermore lead to a better understanding of the factors determining the severity and development (ie, the atopic march²⁹) of allergic diseases.

In memory of Professor Jan Engberg, who was a great contributor and was supposed to be a coauthor of this article.

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Clinical implications: Understanding the effect of IgE/allergen interactions in relation to effector cell degranulation might contribute to the improvement of future diagnostic tests based on individual IgE repertoire compositions.

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