

The complexity of allergic patients' IgE repertoire correlates with serum concentration of allergen-specific IgE

N. Willumsen, J. Holm, L. H. Christensen, P. A. Würtzen and K. Lund

Department of Experimental Immunology, ALK-Abelló, Hørsholm, Denmark

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Summary

Background The governing factor of both effector-cell activation and facilitated antigen presentation is IgE-repertoire complexity (IgE-clonality, -affinity and -concentration). Yet, the compositions of individual IgE repertoires and correlation between IgE-repertoire complexity and establishment of allergic sensitization remain to be determined.

Objective In complex formation assays with recombinant IgE, allergen and CD23⁺ B cells, we assess the composition of serum IgE repertoires and examine the correlation between IgE-titre and IgE-repertoire complexity.

Methods The capacity of sera, from house dust mite-sensitized individuals, to mediate IgE–Der p 2–CD23 complex formation on CD23⁺ B cells was measured. In parallel experiments, the effect of supplementing each serum with one or more Der p 2-specific monoclonal recombinant IgE antibodies on complex formation was determined.

Results Only sera with the highest concentration of Der p 2-specific IgE resulted in complex formation without supplementary recombinant IgE. Intermediately titred sera supported complex formation to various degrees when supplemented with individual recombinant IgE. The degree of complex formation depended on the affinity and epitope specificity of the recombinant IgE. Complex formation by combining serum and recombinant IgEs could not be obtained with sera of relatively low titres of specific IgE. However, these sera had the capacity to dramatically enhance the low complex formation achieved with pairs of affinity-engineered recombinant IgEs.

Conclusion and Clinical Relevance Serum IgE complexity can be indirectly assessed by combining sera with defined monoclonal IgEs in IgE–allergen–CD23 complex assays. The observed differences in epitope-coverage of Der p 2-specific serum-IgE in sera of different specific IgE titres indicate that increased IgE titres correlate with increased complexity of the IgE-repertoire. A detailed knowledge of the composition and complexity of allergen-specific IgE repertoires (and the relation to IgE titre), particularly in the early phase of sensitization, may be used to improve the prediction of the persistence and severity of allergic symptoms, as well as the progression of the Allergic March.

Keywords allergic march, allergy, antibody clonality, antibody-binding affinity, facilitated antigen presentation, IgE-repertoire complexity, IgE titre, recombinant IgE, serum IgE-repertoire, T cell activation

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Correspondence:

Kaare Lund, Department of Experimental Immunology, ALK-ABELLÓ, Bøge Allé 6–8 DK-2970 Hørsholm, Denmark.

E-mail: kludk@alk-abello.com

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Introduction

Allergy is associated with allergen-specific serum IgE antibodies that upon interaction with allergen form complexes with cell-surface expressed high-affinity (FcεRI) or low-affinity (CD23) IgE-binding receptors. The interaction between IgE, allergen and FcεRI is the basis for immediate allergic reactions through activa-

tion of mast cells and basophils [1]. In addition to effector-cell activation, interactions between IgE, allergen and IgE-receptors reduce the amount of allergen needed to activate allergen-specific Th2-cells, by >100-fold, through the process of facilitated antigen presentation (FAP). This has been shown *in vitro* on both FcεRI⁺ dendritic cells [2–4] and CD23⁺ B cells [5–11], and confirmed for the latter *in vivo* in mice [12].

Th2-cells are directly involved in the cellular influx observed during the inflammatory late-phase allergic reaction and in the B cell isotype-switch to production of IgE [13].

In everyday practice, allergen-specific serum IgE-concentrations are routinely assessed during the diagnosis of allergy. Still, despite the clear link between specific IgE titres in patients' serum and the probability of experiencing allergic symptoms [14–17], the correlation between specific IgE concentrations in serum of individual patients and the severity of their clinical manifestations is poor [18–22], suggesting that the allergen-specific IgE concentration is not a sufficiently accurate biomarker for the severity of allergic disease. Of particular interest are the events following the initial sensitization of young children. Allergen-specific IgE concentrations have been linked to the probabilities of remission or persistence, respectively, of early sensitization [23], a finding which warrants an investigation of early IgE-repertoire complexity development.

We have previously demonstrated *in vitro* that it is not only the specific IgE concentration *per se* but rather the composition and complexity of the allergen-specific IgE repertoire that determine the efficiency of basophil activation [24], as well as the formation of IgE–allergen–CD23 complexes on B cells (in this article denoted 'complex formation') and subsequent antigen presentation and T cell activation [25].

The composition and complexity of allergen-specific IgE in allergic patients' sera are also likely to be essential for allergic reactions *in vivo*. However, the composition of individual allergen-specific IgEs in patient serum is not easily measured, which presents a major barrier for assessing the overall complexity of allergen-specific serum IgE-repertoires.

To address this issue, we developed a method to indirectly assess the complexity of Der p 2-specific IgE-repertoires in sera from house dust mite (HDM) sensitized individuals. By measuring the ability of different well-defined Der p 2-specific recombinant IgEs to complement the intrinsic capacity of individual HDM-allergic patients' sera to form complexes (i.e. complexes between IgE, allergen and CD23 on the surface of CD23⁺ B cells), we were able to assess qualitative differences in the IgE repertoires of HDM-allergic individuals.

Methods

Patient sera, recombinant allergen and recombinant IgE antibodies

Sera from HDM-sensitive subjects ($n = 14$) with positive Magic Lite SQ specific IgE scores ($\geq$ class 2; Magic Lite SQ, ALK, Horsholm, Denmark) for *Dermatophagoides pteronyssinus* (Der p) were used in this study,

which was approved by the local ethical committee. Subjects included had not received allergen-specific immunotherapy (SIT).

The concentration of Der p 2-specific serum IgE was determined in an ADVIA Centaur assay (Bayer, Leverkusen, Germany), using biotinylated allergen [26].

Allergen used was recombinant Der p 2 expressed in *Pichia pastoris* [24].

A panel of recombinant IgE (rIgE) antibodies specific to the major HDM allergen, Der p 2, was characterized with regard to affinity for Der p 2 as well as relative Der p 2-epitope specificity, using surface plasmon resonance technology as described elsewhere [24]. The rIgEs used in this study were specific towards three non-overlapping epitopes on Der p 2. Each rIgE-specificity used was engineered with regard to affinity for their specific Der p 2-epitope and arbitrarily grouped as having high, medium or low affinity. An overview is given in Table 1, and throughout this article the affinity nomenclature of Christensen et al. is used as reference [24].

IgE–allergen–CD23 complex formation assay

For the assessment of the complexity of individual HDM-allergic patients' Der p 2-specific serum IgE-repertoires, we applied the FAP/Binding assay described by van Neerven et al. [8] and Shamji et al. [10], adapted for use with recombinant IgE antibodies as described in Holm et al. [25]. Here, the sources of Der p 2-specific IgE are, separately or in combination, serum from HDM-allergic individuals and a panel of Der p 2-specific recombinant IgE antibodies [24] (Table 1).

The assays were done by mixing serum from individual HDM-allergic patients with either buffer (RPMI 1640;

Table 1. The Der p 2-specific recombinant IgE antibodies (rIgEs) used in the present study

rIgE affinity	K _D value (nM)	Clone	Name	Map of relative Der p 2 epitopes bound by rIgE
High	0.036	H10:H10*	rIgE H10 (h)	
	1.18	H12:H12*	rIgE H12 (h)	
	1.4	E [†]	rIgE P4 (h)	
Medium	3.99	H10:H1*	rIgE H10 (m)	
	5.14	H12:H7*	rIgE H12 (m)	
	9.43	F [†]	rIgE P4 (m)	
	18.6	H7:H7*	rIgE H7 (m)	
Low	33.2	H7:H12*	rIgE H12 (L)	
	171	H10:H7*	rIgE H10 (L)	
	291	C [†]	rIgE P4 (L)	

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

[†]Fully human Der p 2-specific rIgE clones [24].

Gibco, Invitrogen, Taastrup, Denmark) or the indicated rIgEs (~2 µg/mL) and pre-incubated with serial dilutions of Der p 2 or without allergen at 37°C for 1 h. Subsequently, EBV-transformed CD23⁺ B cells were added in 10⁵ cells per sample (volume serum + buffer/rIgE + allergen + B cells = 1 + 1 + 1 + 1) and incubated for 1 h at 4°C. The cells were washed and incubated with FITC-labelled anti-IgE (Dako, Glostrup, Denmark) for 30 min at 4°C, prior to wash and detection of surface-bound IgE–allergen–CD23 complexes by flow cytometry. A total of 5000 cells were measured per sample and results analysed using Winlist software v5.0 (Verity Software House Inc.).

Results

The level of IgE–allergen–CD23 complex formation depends on both IgE titre and IgE complexity

Formation of IgE–allergen–CD23 complexes (henceforth 'complex formation') was in all cases mediated by CD23⁺ B cells, recombinant Der p 2 and IgE, either in the form of sera from HDM-allergic subjects or monoclonal, non-overlapping Der p 2-specific recombinant IgE (rIgE) antibodies (H10, P4 and H12, respectively) (Table 1). All sera were entirely unselected and included in this study without prior knowledge of specific IgE titre or their respective abilities to support complex formation. When measured, the Der p 2-specific IgE concentrations of the sera ranged from below quantification limit (0.35 kU/mL) to 197 kU/mL (Fig. 1a). Of the sera tested, three (Der p 2-specific IgE contents of 51, 58 and 197 kU/L, respectively) were able to form detectable IgE–Der p 2–CD23 complexes (Fig. 1b).

The ability of individual sera to form complexes in the presence of allergen and CD23⁺ B cells was tested with or without supplementation of the sera with individual high-affinity variants of the three rIgEs. The sera were divided into three groups according to their overall abilities to support complex formation.

Group 1 (serum L–N) comprises sera with the highest anti-Der p 2 serum-IgE titres (51–197 kU/L), which were able to form complexes without the addition of Der p 2-specific rIgEs. Addition of each of the three high-affinity rIgE antibodies to these serum samples, however, led to increased complex formation compared with the levels obtained with serum alone (Fig. 2a).

Group 2 (serum F–K) comprises sera with intermediate anti-Der p 2 serum-IgE titres (11.4–32 kU/L), which were unable to form detectable complexes without addition of exogenous rIgE. When supplemented with individual high-affinity variants of the three rIgEs prior to mixing with CD23⁺ B cells and allergen, these sera supported complex formation (Fig. 2b). However, the ability of the rIgEs to complement the natural IgE repertoires of these sera and hereby causing complex formation depended on the specificity of the rIgEs. Supplementing group 2 sera with high-affinity variants of rIgE H10 or rIgE P4E resulted in complex formation, whereas supplementing with a high-affinity variant of rIgE H12 gave rise to no (sera F and I) or very modest complex formation (serum J) (Fig. 2b). The significance of the specificity of the rIgE was supported by the failure of another antibody, rIgE H7, which binds to the same region as rIgE H12 (Table 1) but is clonally unrelated to H12, to induce complex formation with serum J, F or I respectively (data not shown).

Group 3 (serum A–E) comprises sera with the lowest Der p 2-specific IgE titres (≤0.35–11.4 kU/mL) where no complex formation was detected with serum alone or after supplementation with individual rIgEs (Fig. 2c).

In all experiments where patient sera and rIgE were combined, a high concentration (2 µg/mL) of rIgEs was used. From experiments with different ratios of individual rIgEs, it was clear that complex formation is not enhanced beyond the capacity of the least abundant IgE involved in the complex formation (data not shown). Therefore, experiments involving combinations of serum and rIgEs complex formation caused by rIgE supplementation reflect the properties of the natural

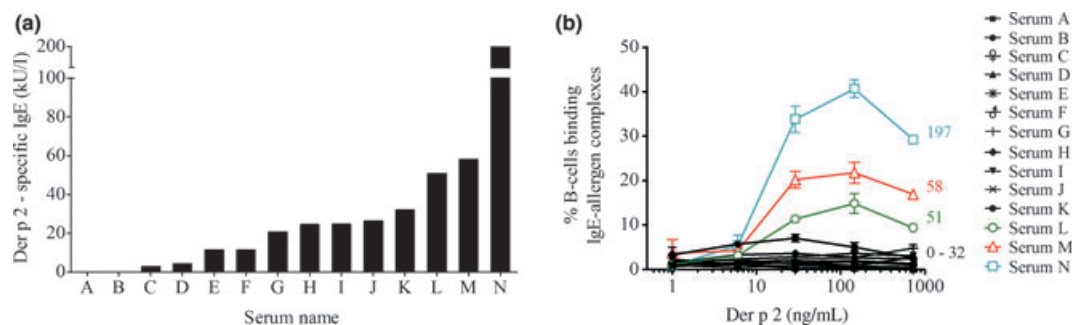


Fig. 1. Serum samples: (a) Der p 2-specific IgE concentrations (kU/L) of the human sera used. (b) Intrinsic complex formation capacity (% B cells binding IgE–allergen complexes) of individual sera. Serum IgE concentrations, determined relatively in kilo Units (kU) per litre are shown. Error-bars show SD from duplicates.

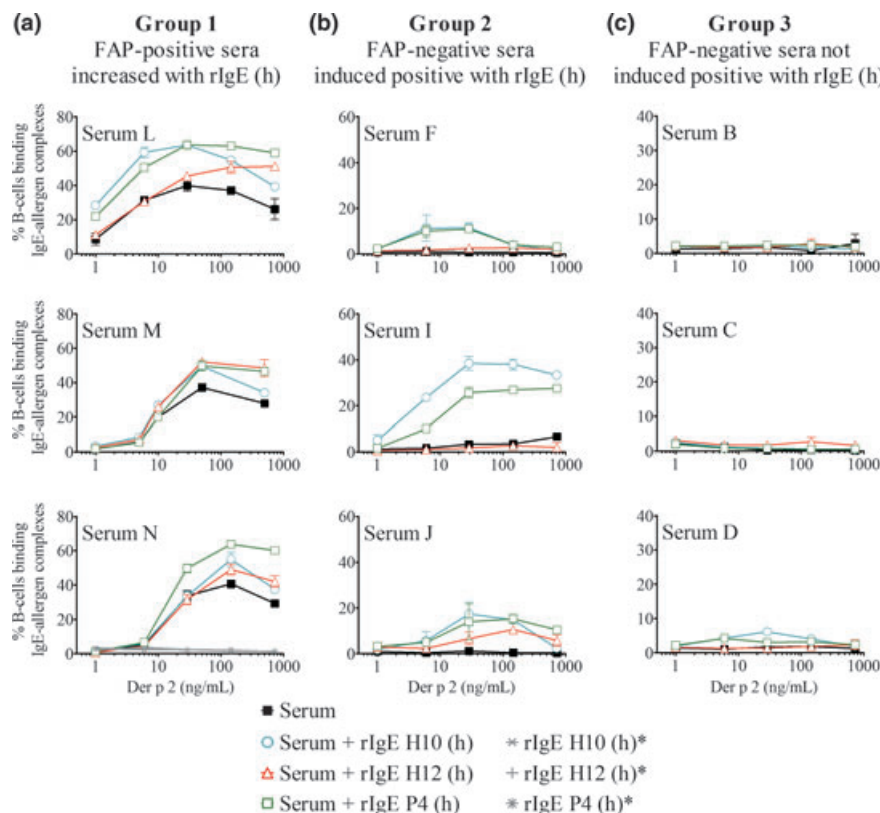


Fig. 2. Serum groups a, b and c: (a) sera with the highest specific IgE titre, (b) sera with intermediate specific IgE titres and (c) sera with relatively low specific IgE titres. Curves indicate the degree of complex formation (% B cells binding IgE–allergen complexes) obtained with the individual sera alone (black squares), when supplemented with high-affinity variants of recombinant IgEs H10 (blue circle), H12 (red triangle) or P4 (green square), respectively. Error-bars show SD from duplicates. *Control, no complex formation detected when testing individual rIgE-specificities solely (only shown for serum N, grey line).

allergen-specific serum IgE repertoire. In no case did single recombinant IgEs alone give rise to detectable complex formation (Fig. 2a bottom).

Group 3 patient sera enhance the level of complex formation obtained with two rIgE specificities

As previously shown [25], maximal complex formation is obtained with a combination of two non-overlapping anti-Der p 2 specific rIgEs of high affinity. Combinations of the high-affinity variant of rIgE P4, and the low-affinity variant of rIgE H10, (P4(h) + H10(L)), or the low-affinity variants of both P4 and H10, (P4(L) + H10(L)), consistently gave rise to only modest complex formation (<20% B cells binding IgE–allergen complexes, Fig. 3). Supplementing the group 1 sera C, D and E, respectively, with the P4(h) + H10(L) rIgE combination gave rise to marked increases in complex formation (~50–60% complex-binding B cells) compared with the levels obtained with the same pairs of rIgEs alone (Fig. 3). Likewise, while complex formation with the P4(L) + H10(L) rIgE combination was barely

detectable in the absence of serum combinations of P4 (L) + H10(L) and the three sera from group 3 gave rise to ~30% B cells binding IgE/allergen complexes (Fig. 3).

Complex formation mediated by patients' sera in the presence of rIgEs is influenced by the affinity of the individual recombinant IgE antibody

Similar levels of complex formation were obtained after supplementation of serum L (group 1) and serum I (group 2), with either high- or medium-affinity variants of rIgE H10 or P4 (Fig. 4a). Again, supplementation with rIgE H12 only affected serum L (group 1). The complex-formation abilities of serum L were not enhanced following supplementation with low-affinity variants of any of the three rIgE clones. However, low-affinity rIgE variants of H10 and P4 both enhanced the complex formation of serum I, but at lower levels than the high- and medium-affinity rIgE variants, indicating a relatively less matured and less complex IgE repertoire in the lower IgE-titred group 2 sera.

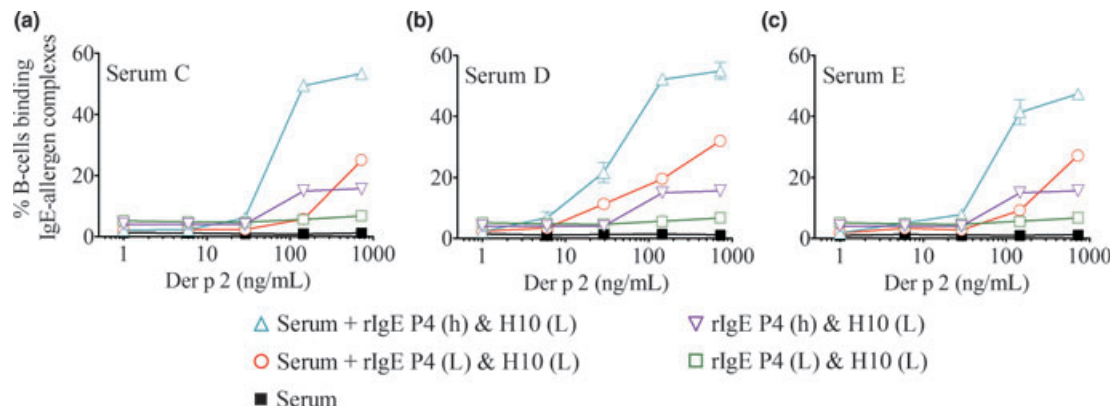


Fig. 3. Complex formation (% B cells binding IgE-allergen complexes) obtained with sera with relatively low specific IgE titres combined with pairs of affinity-engineered Der p 2-specific, monoclonal recombinant IgEs. Complex formation obtained with a combination of high-affinity variant of rIgE P4 and low-affinity variant of H10 (purple triangle), a combination of the same rIgEs and serum (blue triangle), a combination of low-affinity variants of both recombinant IgE P4 and H10 (green square), a combination of the two low-affinity IgEs and serum (red triangle). Serum alone (black square).

Discussion

We have previously demonstrated that the efficiency of allergen-mediated basophil activation [24, 27] as well as that of allergen-specific T cell activation resulting from the formation of IgE-allergen-CD23 complexes and subsequent antigen uptake and presentation on the surface of CD23⁺ B cells [25] are controlled by the complexity of the specific IgE repertoire. In both cases, the primary governing factors are IgE clonality, affinity of individual IgE antibodies and the concentration and relative ratio of allergen-specific IgEs. However, most likely due to the low-affinity association between IgE and CD23 compared with the high affinity of the FcεRI-IgE binding, CD23-mediated complex formation is demonstrably more responsive to minor changes in the IgE-repertoire composition.

In this study, we apply the insight into the mechanistic basis for IgE-allergen-CD23 complex formation described previously [25] to show how the interplay between individual patients' serum IgE and allergen-specific recombinant IgE (rIgE) affects the efficiency of complex formation. By this measure of the complexity of individual subjects' serum IgE repertoire, we are able to show, indirectly, that increased IgE titres correlate with increased complexity of the IgE repertoire. It should be noted that the IgE-titre determinations may have been influenced by the affinity of the specific IgEs (due to the dynamics of the ADVIA Centaur assay [26]), and this may contribute to the observed IgE-titre/repertoire-complexity linkage.

All the human sera used had detectable Der p 2-specific IgE concentrations, but only the sera with the highest specific IgE concentrations supported complex formation when combined with CD23⁺ B cells and Der p 2 (Fig. 1).

It is conceivable that high-titred sera represent a mature antibody response of high complexity that is based on multiple high-affinity Der p 2-specific IgEs. When diluting serum N to the same IgE concentration as sera in group 2 and 3, respectively, it lost or showed dramatically reduced capacity to support complex formation when supplemented with recombinant allergen-specific IgEs compared with the sera in groups 2 and 3 (data not shown). This result emphasizes the difference between sera of naturally low or medium titre and a diluted high-titre serum. In serum N that presumably is a serum of high IgE-repertoire complexity the relative contribution of each individual allergen-specific IgE antibody to the total allergen-specific IgE titre must be less than that in the sera of low IgE repertoire complexity. Most likely, the concentration of individual allergen-specific IgE specificities in serum N is reduced below the concentration needed to support FAP complex formation upon dilution.

In line with this, findings by Pierson-Mullany et al. [28] have demonstrated that IgE repertoires of high-titred sera from peanut-allergic patients consist of several high-affinity allergen-specific IgEs. The lack of complex formation with the lower titred sera could be due to general sub-threshold concentrations of Der p 2-specific IgE. Our results, however, support that low-titred sera, rather than just being more dilute with regard to specific IgE than higher titred sera, indeed contain less complex IgE repertoires with IgEs specific towards only part of the antigen surface, in contrast to high-titred sera with more comprehensive IgE coverage, i.e. which comprise a large number of different, most likely overlapping, IgEs specific towards all areas of the antigen surface. As Der p 2 is approximately 14 kDa, there is of course a limit as to how many IgE antibodies a single allergen molecule can accommodate simultaneously.

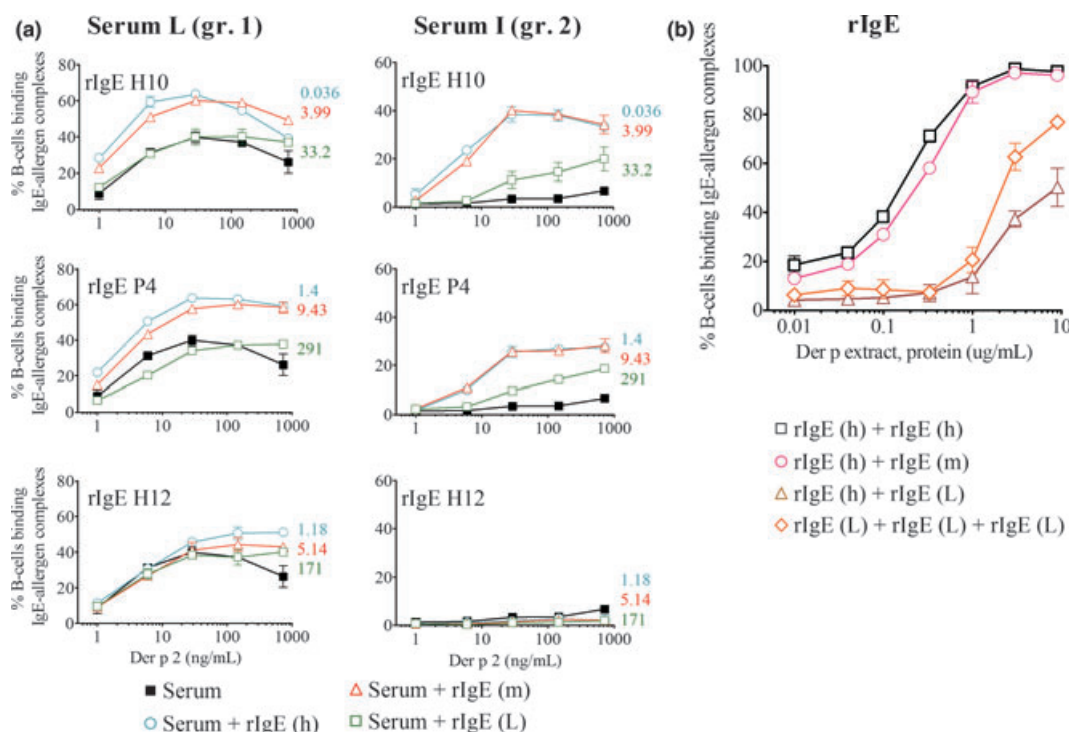


Fig. 4. Influence of IgE affinity on complex formation (% B cells binding IgE-allergen complexes). (a) Complex formation of serum L (group 1) and serum I (group 2) when combined with affinity-engineered variants of the rIgE antibodies H10, P4 or H12. h, high-affinity variant; m, intermediate-affinity variant; l, low-affinity variant. Measured affinities (nm) of rIgEs for Der p 2 are shown at the end of the curves. (b) Complex formation obtained with affinity-engineered variants of the rIgE antibodies and Der p extract, adapted from Holm et al. [25].

We [24, 25] and others [29] have demonstrated that at least three monoclonal IgE antibodies can bind independently of each other to Der p 2. Considering the bulkiness of immunoglobulins and the vast difference in size between a 14 kDa allergen (Bet v 1) and IgE, illustrated by Mirza et al. [30] by solving the three-dimensional structure of a Fab' fragment-Bet v 1 complex, it seems unlikely that stable complexes between more than three IgEs and allergens the size of Bet v 1 and Der p 2 can form. However, even though the number of IgEs that can bind an allergen simultaneously is modest, IgE repertoires of higher complexity may still have influence on the overall complex formation.

When supplementing sera with high concentration of rIgE, the concentration of natural IgE becomes the limiting factor for complex formation which cannot be compensated for by further increasing the rIgE concentration. Most likely the effect on complex formation by additional increase of the complexity of an already mature IgE response is limited. In line with this, supplementing the high-titred sera (group 1) with high-affinity variants of the recombinant anti-Der p 2 IgEs H10, P4 and H12, respectively, gave rise only to modest, incremental increases in complex formation (Fig. 2). More striking was the effect of supplementing the medium-titred sera (group 2) with recombinant IgE. All three sera in this group (F, I and J) supported complex

formation in the presence of high-affinity variants of rIgEs H10 and P4, respectively, but not in the absence of rIgE. The finding that neither serum F nor serum I supported complex formation when supplemented with rIgE H12 is taken to indicate that the Der p 2-specific IgE repertoires of these two sera are of incomplete coverage and mainly directed towards epitopes on the surface of the Der p 2 molecule that to some extent overlap with the epitope of rIgE H12. In effect, the anti-Der p 2 IgE-repertoires of sera F and I seem to be limited in IgE clonality compared with the high-titred sera of group 1.

When supplemented with rIgE H12, serum J did to some extent support complex formation and may comprise an intermediate, more complete IgE coverage for Der p 2 compared with serum F and I (Fig. 2). The specific Der p 2 IgE concentrations of e.g. serum I and J were very similar (24.6 and 26.2 kU/L, respectively), but whereas supplementation of serum I with rIgEs H10 and P4, respectively, gave rise to a dramatic increase in complex formation, the effect of the rIgEs on serum J's ability to support complex formation was more modest. We take this to indicate that despite identical IgE titres, individual sera may encompass different IgE-repertoires, and that the IgE-repertoire of serum I is more focused towards the area of Der p 2 which harbours epitopes of IgEs that complement H10 and P4 for complex formation.

Potentially, Der p 2-specific IgG present in the sera may either enhance the complex formation by antibody cross-linking two allergen molecules or reduce the complex formation by working as so-called blocking antibodies. As no IgG could be detected on the cell surface after complex formation using fluorescently labelled anti-IgG (data not shown), IgG does not seem to contribute positively to the complex formation. In addition, by measuring IgE blocking factor in serum samples as described by Wurtzen et al. [11], no inhibitory effects from non-IgE antibodies could be detected (data not shown) hereby further excluding e.g. specific IgG antibodies as a confounding factor in complex formation in the serum samples used in this study.

That the Der p 2-specific IgE-repertoire complexity is likely to correlate to the specific IgE concentration, as originally hypothesized, is supported by the experiment in Fig. 3 illustrating the ability of the three low-titred sera to boost the low-level complex formation obtained by pairs of non-overlapping rIgEs. Combinations of high + low or low + low affinity variants of rIgEs H10 and P4, respectively, reproducibly yield low but detectable complex formation (see also Holm et al. [25]). Combining these pairs of rIgEs with serum C, D or E, respectively, had a dramatic effect on complex formation which in all cases increased from <20% for the high + low and <5% for the low + low affinity rIgE combinations, respectively, to >50% (high + low IgE + serum) and >20% (low + low IgE + serum) (Fig. 3). These results indicate that not only are Der p 2-specific IgE antibodies present in the low-titred sera; they are also of specificities that can complement H10 and P4 rIgEs and, importantly, present in concentrations sufficient for efficient complex formation when combined with the two rIgEs. We propose that to support complex formation as efficiently as seen in Fig. 3, the low amount of Der p 2-specific IgEs present in the low-titred sera is likely to be limited in clonality to very few, perhaps only a single, IgE antibody clone(s) binding epitopes on Der p 2 not overlapping with the epitopes bound by H10 and P4.

The progression from low-complexity to full-coverage IgE repertoires following the increase in specific serum IgE concentrations could be explained by epitope spreading, where an established immune-response spread from the inducing epitope to other non-cross-reactive epitopes on the same antigen [31, 32]. Epitope spreading proceeds together with increasing antibody affinity in response to antigen [33] and has been theoretically linked to the progression of allergic disease via facilitated allergen presentation [34, 35]. According to this idea, complex formation on B cells between CD23, IgE and allergen efficiently lead to presentation of the whole allergen to T cells including already recognized T cell epitopes as well as new potential T cell epitopes,

simultaneously. The already-established IgE-facilitated Th2 response then directs the T cell response from the B cell receptor-mediated antigen presentation in a Th2 direction and ultimately induces IgE production towards the new epitopes.

In-vivo, IgE repertoires are expected to exist with a continuum of clonalities, concentrations and affinities. The grouping of sera into three distinct groups is of course arbitrary, but serves to illustrate the different characteristics of sera with different specific IgE titres, as discussed above.

Another aspect of IgE repertoire complexity is the affinity of individual IgE clones in the sera.

Complex formation is driven by the combined effects of IgE clonality, affinity and concentration, and it is often difficult to separate the individual contributions of the three parameters. When supplemented to the high-titred serum L, the low-affinity rIgE variants had no effect, and only small incremental effects were seen with medium- and high-affinity rIgE variants (Fig. 4a) as expected with the generally higher affinity of IgEs in a mature high-titred serum.

However, Fig. 4 also demonstrates that when a serum of intermediate specific IgE titre was supplemented with rIgEs, the extent of complex formation depended on rIgE affinity. When supplemented to serum I, a low-affinity rIgE variant gave rise to less efficient complex formation than the corresponding medium- or high-affinity variants which, in turn, gave rise to the same high level of complex formation. To interpret this finding, we assume that combinations of different IgE-affinity variants follow the same pattern described in Holm et al. [25]. Figure 4b highlights some of these findings, demonstrating that combinations of high + high and high + medium affinity rIgEs both reach maximal complex formation, and that combinations of high + low affinity rIgEs reach intermediate levels. This is the same pattern as seen in Fig. 4a, which indicates that the natural IgE in serum I could represent a single IgE specificity of high affinity. Alternatively, since the combinations of low + low + low affinity rIgEs also reach intermediate levels of complex formation (Fig. 4b), serum I could be composed of two IgEs of low affinity.

Our previous results [25] have indicated the existence of a link between IgE repertoire development and, as a result of more efficient and increasingly robust IgE-allergen-CD23 complex formation and subsequent antigen uptake and presentation, increased allergen-specific T cell activation. The data presented in this article are cross-sectional as sera were selected from a number of donors on the basis of their specific IgE concentrations. Preliminary data obtained with a limited number of sera obtained from HDM-allergic subjects indicate that IgE repertoire complexity increases over time in

individual subjects (data not shown), but due to the scarcity of serum, these data need to be corroborated with material from a larger cohort. In the literature, a number of different observations indicate that the progression of sensitization and manifestation of allergy is associated with the composition of the serum IgE-repertoire. These include studies showing (1) an overall association of IgE titre and facilitated allergen presentation capacity [6, 7, 9, 10]; (2) a relationship between IgE clonality and severity of allergic reactions in peanuts allergy [36, 37]; (3) importance of individual IgE specificities for food allergy [38–40]; (4) allergic patients' sera containing IgEs with relatively high affinity for allergen compared with other antibody isotypes [41–43]; (5) a connection between IgE affinity and the outcome of skin prick tests [44] and (6) the possibility of distinguishing between asymptomatic and symptomatic allergy in sensitized patients by the level of specific IgE [45].

Dynamic changes in IgE response during childhood have also been described in cohort studies [23, 46] including both remission and sequential development of new sensitizations. In both studies, the frequency of remissions declined with age, and in the study of Matricardi et al. [23], the remission/progression decision appeared to correlate with the strength of the initial sensitization. No remission of sensitization was found in patients with specific serum IgE levels above a critical threshold, and consequently, according to our data, level of IgE repertoire complexity. Stronger sensitization against the initial sensitizing allergen and in turn sensitizations against other allergens are in agreement

with IgE repertoires that evolve over time with regard to complexity as discussed above.

From a clinical perspective, therapies that prevent the progression of sensitization and subsequent development of allergic diseases (the 'Allergic March') are desirable. As noted previously [25], complex formation, and subsequent facilitated T cell activation, can be more easily disrupted by blocking IgG antibodies when formed with IgE repertoires of low complexity. Likewise, IgE repertoires of low complexity support allergen-mediated activation of basophils, and hence their proinflammatory impact, less efficiently than high-complexity repertoires [24]. Although IgE repertoire-complexity development and the possible increase in the associated cellular responses may not be the only drivers of the Allergic March, the mechanisms described here and elsewhere [24, 25] argues for early intervention with SIT as a means of more efficient disease prevention.

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Conflict of interest: Disclosure of potential conflict of interest: N. Willumsen was employed by ALK-ABELLÓ. J. Holm is employed by and owns stock in ALK-ABELLÓ. P. A. Würtzen is employed by and owns stock in ALK-ABELLÓ. L. H. Christensen is employed by and owns stock in ALK-ABELLÓ. K. Lund is employed by ALK-ABELLÓ and owns stock in it.

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