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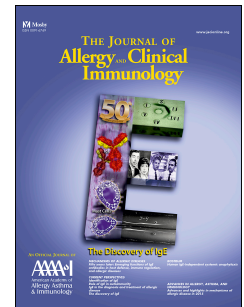
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The number and affinity of productive IgE pairs determine allergen activation of mast cells

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26 **Capsule summary**

27 IgE composition (number and concentration of IgE pairs, IgE clonality, and IgE affinity)
28 determines number of mast cells reacting and allergen threshold for activation and may
29 dictate the threshold allergen dose and severity of allergy symptoms in the individual
30 allergic patient.

31

Key words

- Mast cell activation
- CD63
- Mast cell reactivity
- Allergy severity
- Allergen threshold
- Allergen specific IgE
- IgE composition
- IgE clonality
- IgE affinity

42 **Abbreviations**

43	MC	Mast cells
44	spIgE	Allergen specific IgE
45	Der p 2	Dermatophagoides pteronyssinus allergen 2
46	Phl p 5a	Phleum Pratense allergen 5a
47	rIgE	Recombinant IgE
48	BAT	Basophil activation test
49	scFv-Fcε	Single chain fragment variable IgE

To the editor

Allergen cross-linking of IgE bound to the high-affinity IgE-Fc receptor, FcεRI, on human mast cells (MC) results in a type I allergic reaction. The positive correlation between FcεRI density and plasma IgE concentration underlines the importance of IgE antibodies in activation of mast cells (1). Human MC degranulation, histamine release and PGD₂ secretion increase with increasing concentration of specific IgE (spIgE) (2). As MC from allergic asthmatic patients and healthy controls respond similarly when cultured, sensitized and activated under identical conditions, the microenvironment, including circulating IgE, may be more important than atopic donor status (3). Blood basophils share the FcεRI degranulation pathway with MC. A well-characterised panel of recombinant human IgE created previously to characterise how IgE composition influences the allergen-specific response of basophils was used here to investigate the contribution of concentration, relative ratio of spIgE clones, IgE clonality and affinity for allergen on degranulation of cultured, monosensitized human MC.

MC were cultured from CD133⁺ progenitors, and mature, eight week old MC, cultured with 80 kUa/L total recombinant human IgE containing allergen specific IgE (spIgE) (4)(5) during the last two weeks, were activated with allergen (2). Percentage CD63⁺ MC was plotted against log of allergen Der p 2 or Phl p 5 concentration. MC reactivity (%CD63⁺ mast cells) and MC sensitivity (EC₅₀) were estimated by non-linear curve fitting. Statistical significance was analysed using the non-parametric Kruskal-Wallis or Mann-Whitney U-test.

The lowest spIgE fraction tested (0.2%; 0.16 kUa/L) did not activate MC. Increasing the fraction of spIgE from 0.3% to 20% increased net reactivity from 5.6%±1 (SE) CD63⁺ MC to 62.5%±2.5 (SE) (n>4, p=0.0006) (Figure 1A). Sensitivity increased 180-fold from 90 (IQR 11–220) to 0.5 (IQR 0.2–1.3) pg Der p 2/ml (p=0.0008). Optimising the ratio of two high affinity antibodies to Der p 2 (H12 and E) in five steps from 1:99 to 1:1 of 14% spIgE, increased net reactivity from 30.2%±3 (SE) CD63⁺ MC to 70.1%±2 (SE) (p=0.038, n=4, Figure 1B). Increasing clonality by activating MC cultured with 7% of each of three high affinity antibodies with recombinant Der p 2 that bind two of the antibodies, or natural Der p 2, that binds all three, increased net reactivity from 38%±1.4 (SE) CD63⁺ MC to 49.5%±1.6 (SE) (p=0.0039, n=8) (Figure 1C). Stimulating MC sensitized with 7% of two or three Phl p 5 specific IgE clones with Phl p 5, (5) (Figure 1D + Table S1)

increased net reactivity from $49.6\% \pm 4.3$ (SE) CD63⁺ MC to $77\% \pm 4$ (SE) ($p=0.0286$, $n=4$). In contrast to basophils (4), clonality did not affect MC sensitivity. The concentration of sIgE (Figure 1A) and of the least abundant sIgE clone (Figure 1B) correlated with MC net reactivity with similar regression slopes, 20.7 and 20.6 (Figure 1E). An excess of a single clone of sIgE on MC (Figure 1B) thus does not increase MC reactivity. Increasing clonality from two to three (Figure 1C+D) might disproportionately increase reactivity (Figure 1E).

Reactivity of MC sensitized with pairs of clones of Der p 2 sIgE with affinity ranging from $3.5 \times 10^6 \text{ M}^{-1}$ to $2.8 \times 10^{10} \text{ M}^{-1}$ (K_A) (Table S1) increased from $26.4\% \pm 3.6$ (SE) CD63⁺ MC to 74.8 ± 5.3 (SE) ($p=0.0068$) (Figure 2A), and sensitivity increased 15000-fold from 12 ng Der p 2/ml (IQR=11–16) to 0.8 pg Der p 2/ml (IQR=0.2–1.6) ($p=0.0005$). A tenfold increase of $\sqrt{(\text{product of affinities})}$ of IgE for allergen (M^{-1}) associated significantly with a 19% increase in CD63⁺ MC, but less so with a 0.02 ng/ml decrease in EC_{50} (Figure 2B).

We used MC sensitized with 80 kUa/L total IgE for two weeks to simulate the condition in allergic patients. Skewing the ratio of allergen specific clones corresponds to limiting the effective concentration of IgE to that of the IgE clone at lowest concentration (Figure 1D). Increasing affinity of one of the clones present had a significant effect on MC reactivity and sensitivity. The effect on sensitivity was more pronounced on MC (Figure 2A) than on basophils (4). As one high affinity clone in the mixture is of major importance and the combination of a high and a low affinity clone results in higher MC reactivity and sensitivity than combination of two medium affinity clones (Figure 2A), affinity maturation of the IgE population is likely to substantially enhance the extent of MC response *in vivo* and thus the threshold and severity of the clinical response upon allergen encounter.

Allergy may begin with one clone sufficient for IgE mediated antigen presentation leading to increased Th2 activation. The response becomes clinically relevant as additional IgE clones with non-overlapping epitope specificities develop (6). Increasing the number of epitopes recognized by IgE on either Der p 2 or Phl p 5 significantly increased MC reactivity. There may be at least three nonoverlapping epitopes on Der p 2 (5), and more than four on Phl p 5 (7). Evolution of the allergic response may thus

115 arise from both affinity maturation and generation of additional IgE producing B-cell
116 clones.

117 As basophil activation (8) obeys the same rules as mast cell activation, increased
118 severity of symptoms that associate with increased reactivity (9) may be explained by
119 increases in IgE affinity or clonality. The threshold of sensitivity in allergen
120 provocations may depend on the number and affinity of productive IgE-allergen
121 bridges.

122 Our data explain why, even though positive allergen-specific serum IgE and skin prick
123 tests increase the likelihood of allergic disease, a positive basophil test conveys more
124 reproducible and quantitative information about allergic disease without reexposing
125 the patient to allergen, and may be useful for diagnosis and monitoring where first line
126 tests fail (9)(10). To facilitate this some allergy centers are starting to perform BAT on a
127 routine basis. Our finding that the number of productive IgE-allergen bridges and their
128 affinity correlate with mast cell reactivity, while specific IgE concentration and affinity
129 correlate with allergen threshold, may explain how severity and allergen threshold dose
130 are dictated by the clonality, concentration and affinity of specific IgE in the allergic
131 individual.

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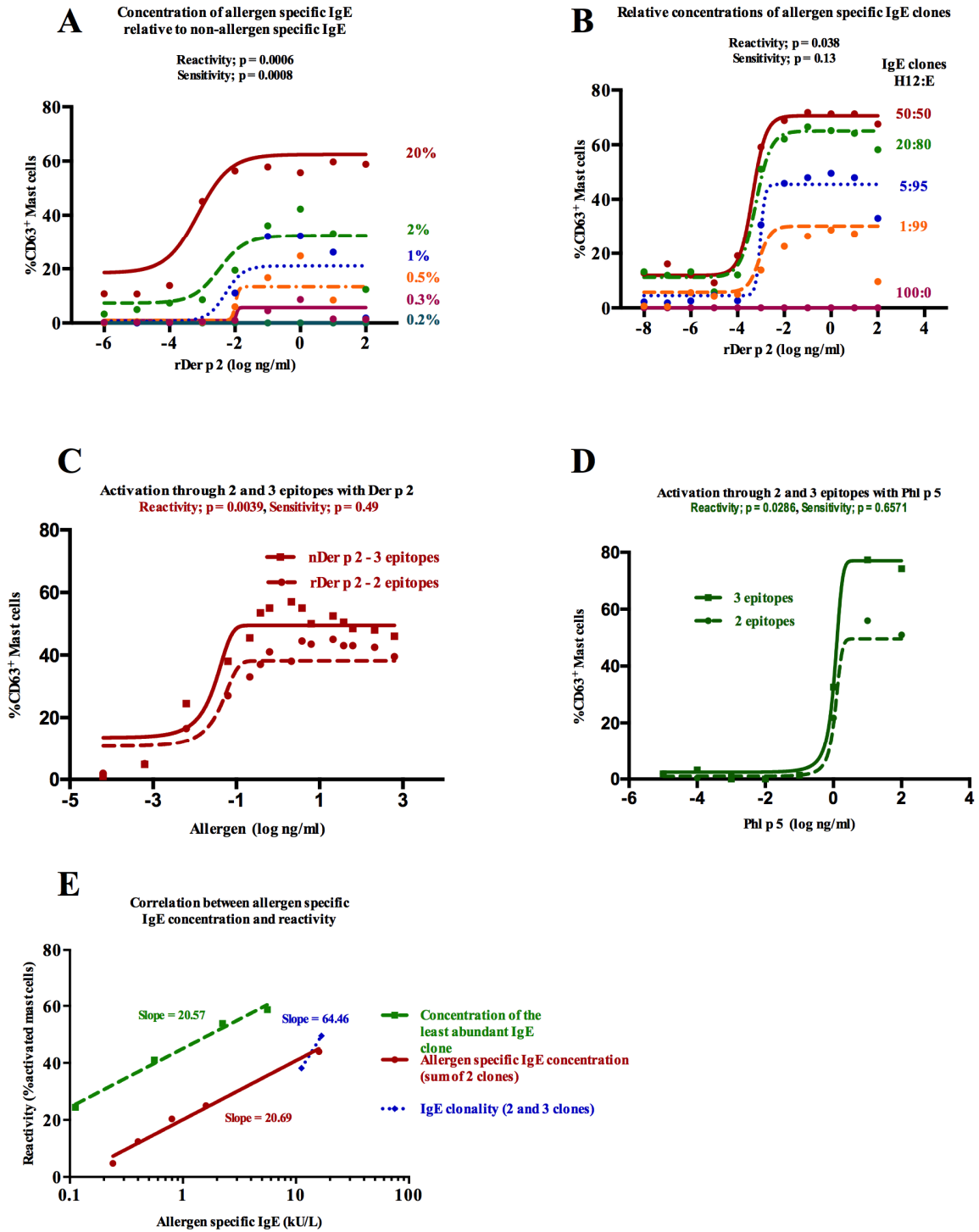
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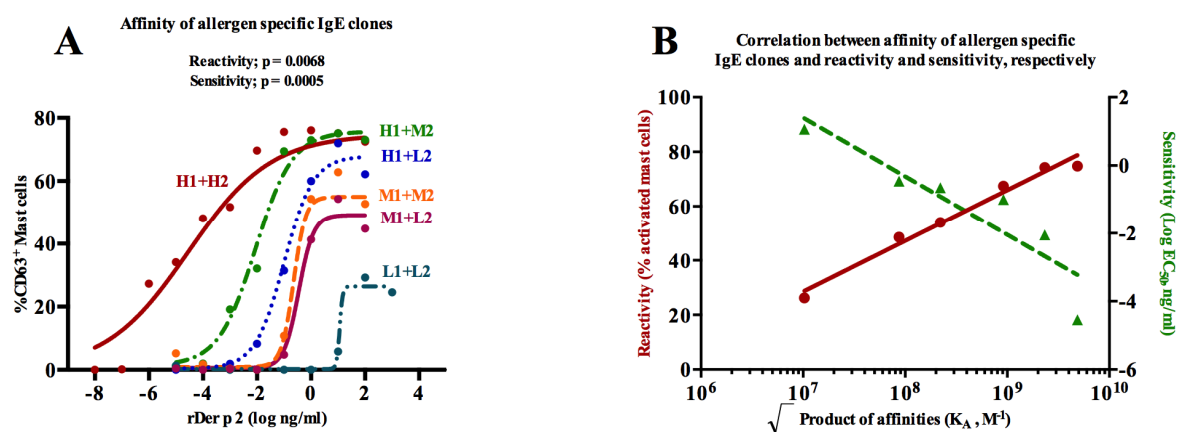
Figure 1:

Mast cell activation at (A) increasing concentrations of specific IgE (clone H12 and E), (B) at different relative concentrations of IgE clones (ratios given as H12:E), (C) increasing clonality of Der p 2, and (D) Phl p 5, (E) Correlation of specific IgE concentration and mast cell reactivity. Data from A (red), B (green) and C (blue). Dots indicate median mast cell activation.

Figure 2:

(A) Mast cell activation increases with increasing IgE affinity (H: High affinity, M: Medium affinity, L: Low affinity, clones listed in the online supplement), (B) Correlation of $\sqrt{(\text{product of affinities})}$ in (A) and mast cell reactivity (red) and sensitivity (green). Dots indicate median mast cell activation.





Online Supplement:

Methods: IgE composition

Specified amounts of recombinant human IgE specific for Der p 2 (7%, 14% or 21%) or single chain variant IgE specific for Phl p 5 (14% or 21%) as detailed in table S1 below were combined with a single clone of recombinant human IgE specific for Tetanus toxin to make up 80 kU/L of IgE in Stem span medium containing FBS, Stem Cell factor, IL6 and IL4. Mast cells were cultured in this medium for two weeks before activation as described¹.

Table S1 – IgE binding characteristics

<i>scFv-Fcε clone</i>	<i>Affinity, K_A (M^{-1}), for Phl p 5.0101</i>	<i>Non-over-lapping epitope</i>
p5-AB5	9.4×10^8	Phl p 5-#1
4.3	3.4×10^8	Phl p 5-#2
4.13	3.9×10^6	Phl p 5-#3
<i>rIgE clone</i>	<i>Affinity, K_A (M^{-1}), for Der p 2.0101</i>	<i>Non-over-lapping epitope</i>
High affinity clones		
H10	2.8×10^{10}	Der p 2-#1
H12	8.5×10^8	Der p 2-#2
E	7.1×10^8	Der p 2-#3
Medium affinity clones		
H10:H1	2.5×10^8	Der p 2-#1
H12:H7	1.9×10^8	Der p 2-#2
Low affinity clones		
H7:H12	3.0×10^7	Der p 2-#2
H10:H12	3.5×10^6	Der p 2-#1

Table 1: Summary of properties of Phl p 5.0101² and Der p 2.0101³ specific rIgE.

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