Mast cell reactivity depends linearly on number of productive IgE pairs and IgE affinity

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Background: Allergen specific IgE consists of individual clones with unique affinity, concentration and complexity, comprising both the number of clones binding the same allergen (clonality) and their relative ratio. We have previously shown how these characteristics of the IgE repertoire direct mast cell activation. The present data explores the correlation between mast cell activation (reactivity and sensitivity) and allergen specific IgE concentration and affinity, respectively.

Methods: Human mast cells (MC) were generated from stem cells and sensitized with combinations (2 IgE clones) of well-characterised recombinant human IgE clones specific for Dermatophagoides pteronyssinus 2 (Der p 2). Activation of mast cells was measured as upregulation of CD63 by flow cytometry. Mast cell reactivity (fraction of mast cells activated, %CD63+ MC) and sensitivity (allergen concentration triggering a half-maximal response, EC50) were estimated by non-parametric curve fitting. Statistical significance was analyzed using Kruskal-Wallis test.

Results: The total concentration of the two Der p 2 specific IgE clones was plotted against the measured MC reactivity. Similar, the net concentration of the least abundant specific IgE clone was plotted against the MC reactivity. Using a semi-log plot, a linear regression could be fitted to the points in both settings. The linear regressions were not identical but they had similar slopes of 20.7 and 20.6, respectively. Mast cells were sensitized with pairs of Der p 2 specific IgE clones with affinity ranging from $3.5 \times 10^9$ M$^{-1}$ to $2.8 \times 10^{10}$ M$^{-1}$ ($K_a$). The product of affinities ($K_a$) of the different clone combinations was calculated and plotted against the measured MC reactivity and sensitivity in a semi-log plot. A tenfold increase of $\sqrt{(\text{product of affinities})}$ of IgE for allergen (M$^{-1}$) associated significantly with a 19% increase in CD63+ mast cells, but less so with a 0.02 ng/ml decrease in EC50.

Conclusion: The concentration of specific IgE and of the least abundant specific IgE clone correlated with MC net reactivity with similar regression slopes. An excess of a single clone of specific IgE on MC thus does not increase MC reactivity. Skewing the ratio of allergen specific clones corresponds to limiting the effective concentration of IgE to that of the IgE clone at lowest concentration. When increasing affinity of the IgE pairs MC reactivity and sensitivity increase in proportion to product of the affinities. Our finding that the number of productive IgE-allergen bridges and their affinity correlate with mast cell reactivity and sensitivity, may explain how severity and allergen threshold dose are dictated by the concentration and affinity of specific IgE in the allergic individual.