Early And Late Phase Responses Of Human Mast Cells Increase With Increasing IgE Affinity

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Introduction
We have previously shown that reactivity and sensitivity of the early response of cultured human mast cells (MCs) increase with increasing IgE affinity. Similar results have been obtained with bone marrow-derived murine mast cells (BMMCs) activated with antigens with different relative affinities for binding to FcεRI-bound specific IgE. The late phase response was surprisingly different in BMMCs; the low affinity interaction gave rise to enhanced chemokine expression, whereas the high affinity interaction resulted in an enhanced cytokine expression. Here we explore whether differences in the affinity of IgE for allergen result in a similar pattern of mediator release from human mast cells.

Methods
Human MCs generated from CD133+ stem cells were sensitized with pairs of recombinant human IgE clones with either high or low affinity for Dermatophagoides pteronyssinus group 2 allergen (Der p 2). Activation of MCs was measured as upregulation of CD63 by flow cytometry. MC reactivity (fraction of MCs activated, %CD63+ MC) and sensitivity (allergen concentration triggering a half-maximal response, EC50) were estimated by non-parametric curve fitting. The release of cytokines and chemokines from activated MCs was measured 3, 6 and 24 hours after activation using a multiplex immunoassay based on the Proximity Extension Assay (PEA) technology (Olink, Uppsala, Sweden).

Results
The combination of two high affinity IgE clones significantly increased MC reactivity ($p=0.0286$) and MC sensitivity ($p=0.0286$) relative to a pair of low affinity IgE clones ($n=4$). Interleukin(IL)-13 ($p=0.0018$) and IL-8 ($p=0.003$) secretion was significantly increased at high IgE affinity compared with low affinity stimulation, while CD5 ($p=0.0016$) and CD6 ($p=0.030$) secretion was significantly increased at low IgE affinity compared to high affinity stimulation. Secretion of the chemokines CCL3 ($p<0.0001$) and CCL4 ($p<0.0001$), but not CCL2 (p; ns), was significantly increased at both high and low affinity stimulation compared with baseline. However, the concentration of chemokines produced did not differ between high and low IgE affinity.

Conclusions
Increased IgE affinity for the allergen increased MC reactivity and sensitivity, and enhanced MC cytokine, but not chemokine, response. This suggests that affinity maturation of the IgE population is likely to substantially enhance the MC response in vivo and thus the extent and characteristics of the clinical response upon allergen encounter.