Importance of IgE affinity for the cytokine and chemokine response of human mast cells

Charlotte Hjort[1], Lars Harder Christensen[2], Peter Adler Würtzen[2], Hans Jürgen Hoffmann[1]

[1]Allergy Center West, Department of Respiratory Diseases and Allergy, Aarhus University Hospital, Denmark
[2]ALK-Abelló A/S, Global Research, Horsholm, Denmark

INTRODUCTION

We have shown that reactivity and sensitivity of the early response of cultured human mast cells (MCs) increase with increasing IgE affinity[1].
Evidence of molecular editing of cellular responses by the high affinity IgE receptor, FcεRI, on mast cells, suggests that affinity of IgE for allergen directs the type of response mounted[2]. The late phase response of murine bone marrow derived mast cells (BMMCs) through low affinity IgE induced a chemokine response recruiting monocytes in vivo, whereas the response through high affinity IgE induced a cytokine response recruiting neutrophils[2].

AIM

This study explores whether differences in the affinity of IgE for allergen result in a distinct pattern of mediator release from cultured human mast cells.

METHODS

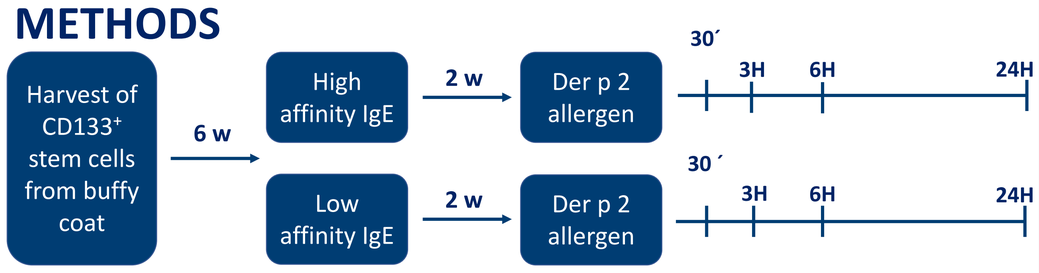

Figure 1: Human mast cells cultured for 8 weeks in StemSpan medium with IL-6, stem cell factor, penicillin/ streptomycin, IL-3, IL-4 and 10% FBS. Sensitization with IgE antibodies for 2 weeks[3]. Activation for 30 minutes and for 3, 6 and 24 hours.

IgE: 80 kU/L recombinant IgE containing pairs of non-overlapping IgE clones with either high or low affinity for *Dermatophagoides pteronyssinus* antigen 2 (Der p 2) in combination with non-allergen-specific monoclonal IgE. The allergen-specific IgE clones are well characterised regarding Der p 2 affinity and epitope specificity (figure 2+3)[4]. IgE clones H10:H12 and C were used for low affinity sensitization and clones H12 and E were used for high affinity sensitization. The difference in affinity of the Der p 2 clones was 224-fold.

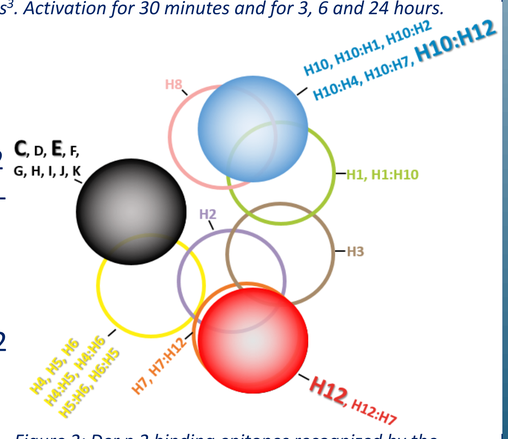

Figure 2: Der p 2 binding epitopes recognized by the different Der p 2 specific IgE clones. (Christensen LH et al (2008), JACI 122 p298)[4].

Activation with log dilutions of Der p 2 allergen for 30 minutes and for 3, 6 and 24 hours. Activation of the MCs after 30 minutes was measured as upregulation of the activation marker CD63 by flow cytometry. Mast cell reactivity (fraction of mast cells activated) and sensitivity (Der p 2 concentration triggering a half-maximal response, EC_{50}) were estimated by non-parametric curve fitting. Statistical significance was analyzed using Mann-Whitney test or 2way ANOVA.

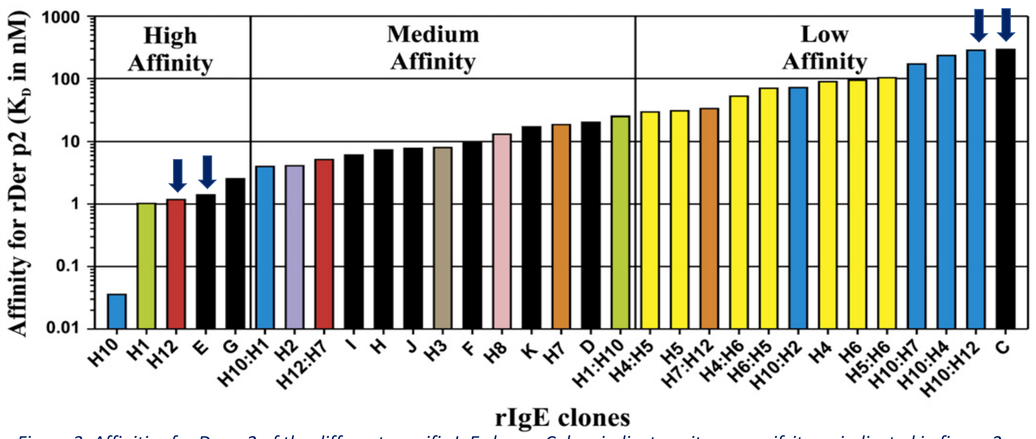

Figure 3: Affinities for Der p 2 of the different specific IgE clones. Colors indicate epitope specifcity as indicated in figure 2. Clones used in the present study are marked with arrows. (Christensen LH et al (2008), JACI 122 p298)[4].

Release of cytokines from MCs activated at optimal Der p 2 concentrations for 3, 6 or 24 hours compared with baseline was measured using a multiplex immunoassay based on the Proximity Extension Assay (PEA) technology (Olink Proteomics, Uppsala, Sweden) (figure 4).

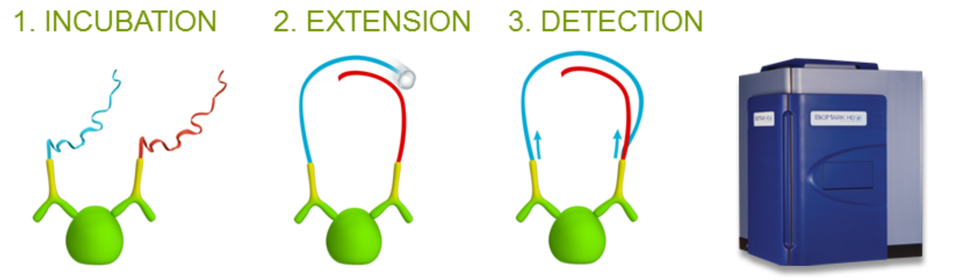

Figure 4: In the PEA technology, each biomarker is addressed by two DNA-tagged antibodies that bind in close proximity to the same protein. This allows for a new PCR target sequence to be formed by the action of a DNA polymerase. The resulting sequence is subsequently detected and quantified using standard real-time PCR. (Olink Proteomics, Uppsala, Sweden).

REFERENCES

1. Hjort C, Schiøtz PO, Ohlin M, Würtzen PA, Christensen LH, Hoffmann HJ. The number and affinity of productive IgE pairs determine allergen activation of mast cells. *J Allergy Clin Immunol*. 2017;140(4).
2. Suzuki R, Leach S, Liu W, et al. Molecular editing of cellular responses by the high-affinity receptor for IgE. *Science*. 2014;343(6174):1021-1025.
3. Holm M et al. Seven week culture of functional human mast cells from buffy coat preparations. J Immunol Methods 2008;336;213–21
4. Christensen LH et al. Several distinct properties of the IgE repertoire determine effector cell degranulation in response to allergen challenge. J Allergy Clin Immunol 2008 Aug;122(2);298–304.

RESULTS

Affinity of the clones ranged from 3.4 x 10^6 M to 8.5 x 10^8 M (K_A) (figure 3). Mast cell reactivity increased with increasing IgE affinity from 20% (IQR = 12 – 25) $CD63^+$ mast cells with two low affinity clones to 65% (IQR = 47 – 80) with two high affinity clones (p=0.0286) (figure 5). Mast cell sensitivity (EC_{50}) increased 13000-fold from 0.9 ng/ml (IQR = 0.3 – 1.1) Der p 2 allergen when the cells were sensitized with two low affinity clones to 0.07 pg/ml (IQR = 0.02 – 7.2) Der p 2 allergen when two high affinity clones were used for sensitization (p=0.0286) (figure 5).

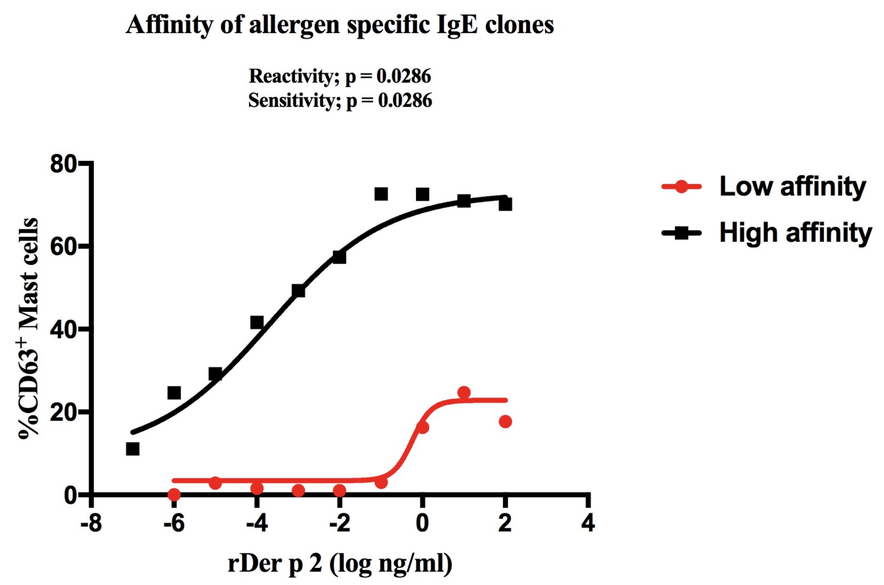

Figure 5: Mast cell activation at high (black) and low (red) IgE affinity.

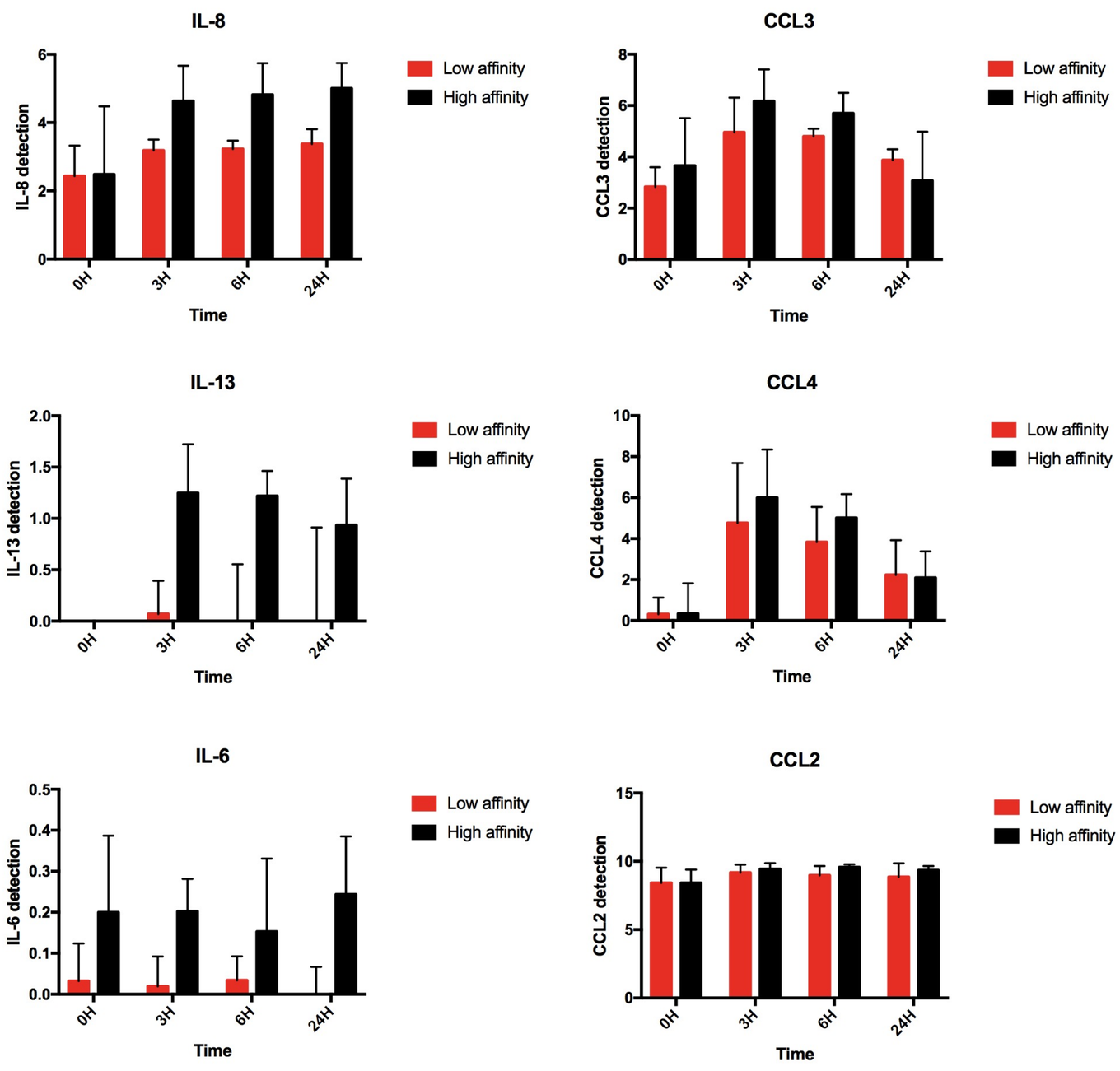

Figure 6: Expression of cytokines and chemokines from human mast cells after optimal Der p 2 activation for 3, 6 and 24 hours (H). 0H is the baseline detection (no allergen activation).

A quantative measurement of the cytokine and chemokine response was obtained from the supernatant samples (n = 4). Secretion of the cytokines interleukin(IL)-8 and IL-13 was significantly increased at high IgE affinity compared with baseline and with low affinity stimulation (IL-8; p=0.006, IL-13; p=0.0018) (figure 6). Secretion of IL-6 was significantly increased at high IgE affinity compared to low affinity (p=0.0011). However, the secretion did not differ from baseline expression (0H, no allergen activation). Secretion of the chemokines CCL3 and CCL4, but not CCL2, was significantly increased at both high and low affinity stimulation compared with baseline (CCL3; p<0.0001, CCL4; p<0.0001, CCL2; ns). However, the responses were not affected by IgE affinity (figure 6).

CONCLUSIONS

We hereby confirm our previous results showing a significant increase in mast cell reactivity and sensitivity with increasing avidity of the IgE-allergen complexes. Furthermore, we have observed a difference in mediator release, which is partly comparable to the results from BMMCs:

- High IgE affinity results in an enhanced cytokine release compared to low IgE affinity stimulation.
- Chemokine expression is elevated even in a low affinity IgE system.
- We do not see a difference between high and low affinity IgE induction of the chemokine response.

A change in the cytokine response from mast cells may influence the severity of the late phase response and may create a possible new target for anti-asthmatic treatment.

CONTACT INFORMATION

Charlotte Hjort, Stud. Med.: charlotte.hjort@post.au.dk
Hans Jürgen Hoffmann, Ph.D.: hjh@clin.au.dk

In relation to this presentation, I declare that there are no conflicts of interest.