Amoxicillin induced mast cell degranulation mediated by FcεRI specific IgG: a new insight to beta-lactam allergy

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To the editor,

Immediate drug hypersensitivity reactions (IDHR) to beta-lactams (BL), referred to here as beta-lactam allergy (BLA), is defined as the development of symptoms such as urticaria and bronchospasm within one hour of beta-lactam exposure (1). Classical Type 1 immunoglobulin E (IgE)-mediated allergy describes a response which occurs within minutes of allergen exposure in a previously sensitised person. It has therefore been widely accepted that BLA is mediated by beta-lactam specific IgE (BL-sIgE) (1). In other Type 1 IgE driven allergies, such as food allergy, sIgE to major allergen components can be shown to be highly specific (2, 3). However, for BLA, BL-sIgE has poor specificity, suggesting alternative mechanisms might be involved (4).

Type 1 IgE mediated reactions are mainly mediated by basophils and mast cells (MCs). Previous studies have demonstrated the use of the basophil activation test (BAT) in predicting BLA, with European allergy guidance stating that BAT can aid BLA diagnosis (5). However, BAT requires fresh whole blood which is not ideal for in depth mechanistic exploration.

In an *in vitro* BLA model using human progenitor derived MCs, we have shown that MCs sensitised with serum from a patient with confirmed BLA, demonstrated amoxicillin (AMX) induced degranulation ($CD63^+$ positivity) after 30 minutes of stimulation with AMX (Figure 1B). In contrast, sensitisation with serum from a non-BLA control demonstrated no response (Figure 1C).

The mechanism of degranulation from the BLA model is very different from the classical IgE-mediated Type 1 degranulation such as that seen in food and inhalant allergy (6). In our *in vitro* model of classical IgE-mediated Type 1 degranulation, sIgE binds to FceRI with high affinity which is not disturbed by pipetting and washing (6). In our BLA model, BLA serum sensitized MCs have a much weaker degranulation and can easily lose their response to beta-lactam stimulation under physical disturbance (Figure 1A).

We hypothesised that FceRI was involved in the degranulation process in BLA, not through the high affinity binding of IgE, but instead through IgG binding. We produced a recombinant fragment representing the extracellular portion of the FceRI receptor alpha chain, which is mainly responsible for antibody binding. When added to the serum, this fragment binds and neutralizes FceRI-specific antibody—IgE and IgG, by preventing them binding to the cells. BLA serum was treated with increasing concentrations of the FceRI fragments and then added to the MCs for sensitization. After stimulating the sensitized MCs using AMX, we observed a dosage-dependent decrease of MCs degranulation, indicating direct involvement of the FceRI

in the degranulation process of BLA (Figure 2A).

To investigate whether degranulation was mediated by IgE, we neutralized serum IgE by adding omalizumab before MC sensitisation. This showed no impact on the AMX induced degranulation (Figure 2A). In contrast, omalizumab inhibited MC degranulation in a classical IgE model, using pooled serum from cat allergic patients stimulated with cat allergen (Figure 2B).

To determine whether FcεRI-specific IgG is functionally responsible for the AMX induced MCs degranulation, we isolated IgG from BLA serum using IgG-depletion spin columns. MCs sensitized with the IgG-only fraction showed no response to anti-IgE, but retained a strong response to AMX (Figure 2B). In comparison, in the IgG-depleted serum fraction (˜60% IgG removed), we observed a strong degranulation to anti-IgE stimulation but a weakened response to AMX (Figure 2C).

A limitation of this preliminary work is it used serum from only one BLA patient.

In summary, we developed a cell model for BLA using *in vitro*differentiated human MCs. This BLA model has demonstrated the involvement of FcεRI-specific IgG in mediating AMX-induced MCs degranulation. The results are worthy of further investigation in a larger patient cohort.

Word Count 599

Figure legend (Word count 98 and 98 respectively, max 100)

Figure 1. Demonstration of *in vitro* BLA model using MCs derived from human progenitor cells. A) After washing the sensitized MCs with phosphate buffered saline (PBS), different cells responsiveness to AMX and anti-IgE. B) When adding the AMX in the presence of BL IDHR serum, cell degranulation was observed. C) No degranulation was observed from AMX stimulation when serum from a non-BL IDHR subject was used. D) Treatment of AMX in the absence of serum showed a mild increase of CD63 compared to cells without stimulation, Percentage $CD63^+$ cells is shown in the box. Representative figures from three independent experiments.

Φιγυρε 2. Ινεστιγατινγ τηε ρολε οφ ΦςεPI-σπεςιφις ΙγΓ ιν ΒΛΑ*ιν ιτρο* μοδελ A) the BLA *in vitro* model, B) classical type I IgE cat allergy model. AMX or cat allergen induced degranulation was quantified on MCs sensitized with single BLA or pooled cat allergic subject serum pre-treated with recombinant FcεRI alpha chain fragment or omalizumab. C) BLA *in vitro* model; MCs sensitized with either IgG-depleted serum fraction or IgG-only fraction. Sensitised MCs were stimulated with anti-IgE, AMX or media control. Histogram demonstrating fluorescence intensity of CD63 expression on MCs surface. Mean ±SD. *=$p<0.05$ from three independent experiments, NS=not significant.

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Figure 1

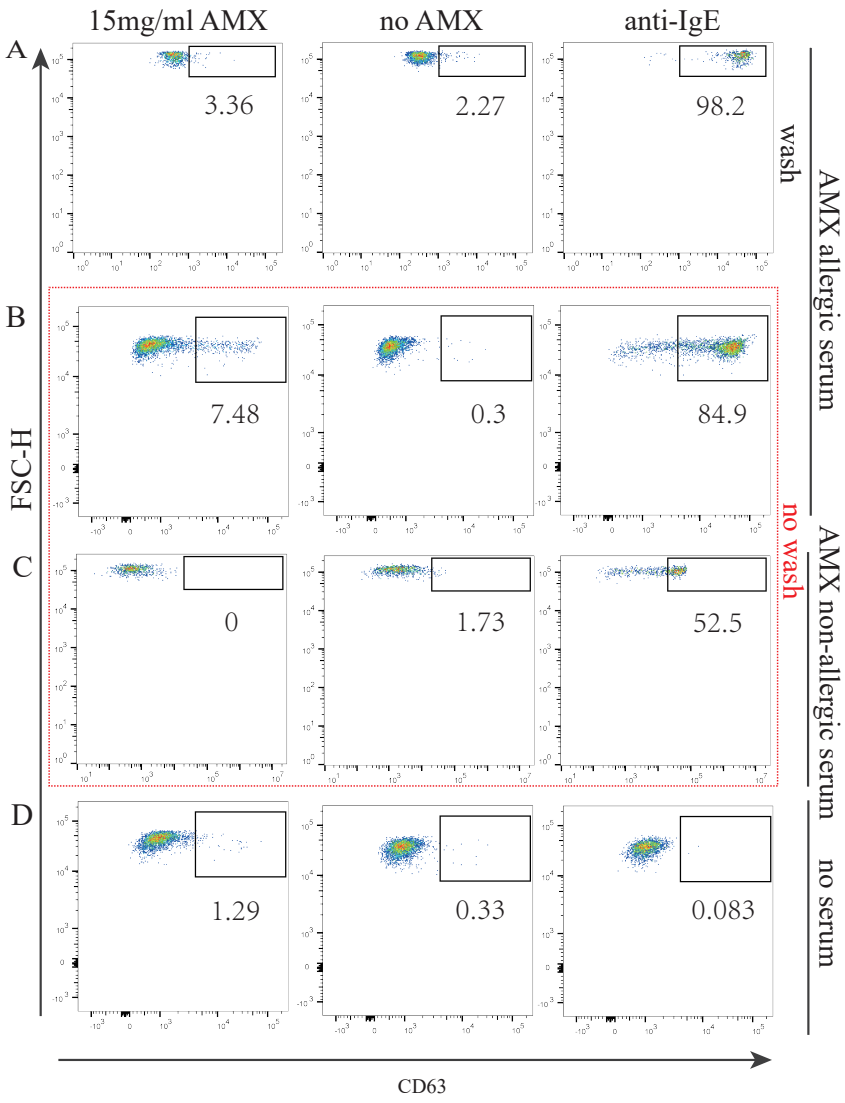

Figure 2