**Background**

Food processing and gastrointestional digestion on allergenicity

Food processing and gastrointestional digestion are fundamentally important for food protein allergenicity. It is usually accepted that heat and/or digested proteins changes their conformation and alters its allergenicity, which can be either enhanced or reduced depending on the certain allergen [1]. IgE receptor desensitization is an effective intervention strategy for the rapid inhibition of IgE-mediated anaphylactic responses. Recently, it is increasingly recognized that its efficacy appears to be variable depending on the allergen protein conformation [2]. However, the underlying mechanisms for IgE receptor desensitization on mast cells remain to be elucidated. Particularly, the impact of heating and/or digestion of food on the desensitization of mast cells is still not well studied. Here we investigated effects of heat and digested allergen for IgE receptor desensitization on cultured mast cells.

**Method**

Mouse bone marrow derived mast cells (BMMCs) were generated from C57/Bl6J mice in RPMI-1640 medium with recombinant mouse IL-3 and SCF. BMMCs were sensitized with anti-OVA-IgE (1 µg/ml) and challenged with each dose of OVA. Degranulation response was determined by β-hexosaminidase release assay according to an established method.

**Heating and simulated digestion**

OVA was dissolved in water (final 0.01% v/v sorbitol) and boiled at heat blocker at 100 degrees C for 1 min. Artificial digestion of OVA was performed by pepsin in simulated gastric fluid (35 mMol/l HCl, pH 2.0) 1 hour and pancreatin in simulated intestinal fluid (0.5M K_HPO_4 pH 7.5) for 15 min at heat blocker at 37 degrees C (described in Fig. 1).

**In vitro desensitization**

OVA specific IgG-sensitized BMMCs were subjected to the allergen-specific desensitization of IgE receptor with increasing doses of heated or digested OVA at 10 min intervals. Timing and stress doses were described in Fig. 3A. To evaluate IgE receptor desensitization in mast cells, the degradation (i.e. β-hexosaminidase) responses were observed after naive OVA challenge.

**Result**

**OVA induced degranulation and desensitization of BMMCs**

OVA-induced BMMCs' degluation was observed with a maximum at 20 μg/ml OVA (Fig. 1A). Based on these responses, we established desensitization strategy and conducted to OVA specific IgG-sensitized BMMCs. The desensitization-treated cells were clearly show decreased degluation rate compared to untrreated cells (Fig. 2B).

**Differential desensitization efficacy toward high- and low-dose protocol**

To examine whether the in vitro allergen-induced desensitization rate is correlated with the allergen doses, we tested high- and low-dose desensitization protocol to the sensitized BMMCs (Fig. 3A). Low-dose protocol is 1/100 times lower compared to the normal protocol conducted in Fig. 1 (named as High-dose protocol). OVA-induced desensitization was tended to be failed at "low-dose protocol", whereas controversely in heated-OVA, "low-dose protocol" was successful but "High-dose protocol" was unsuitable for inducing the desensitization (Fig. 3B).

**Effect of heat and digestion on BMMC degranulation and desensitization**

Sensitized BMMCs were simulated by four types of OVA (DWA, heated OVA, digested OVA, and heated and digested OVA) respectively. Heated OVA challenge resulted in enhanced degluation compared to nonheated naive OVA. Artificial digestion of the heated OVA completely eliminated the increased degluation (Fig. 4A). OVA-induced desensitization was conducted using "High-dose protocol" as described in Fig. 3A. Allergen-induced desensitization tended to be established approximately related to the capacity to potentiate degluation (Fig. 4B).

**Conclusion**

- Heated or digested allergen changed food protein allergenicity.
- Digestion may abolish heat-induced higher allergenicity.
- Digested allergens showed a tendency toward a reduced degluation responses, however it definitely have the potential to induce desensitization for IgE receptor.

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