Successful IgE Receptor Desensitization on Mast Cells using Heated and Digested Allergen.

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Background

Food processing and gastrointestinal digestion on allergenicity

Food processing and gastrointestinal digestion are fundamentally important for food protein allergenicity. It is usually accepted that heated 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 heated and digested allergen for IgE receptor desensitization on cultured mast cells.

1. Martons G *et al.* J Allergy Clin Immunol. 127: 990-7. 2011
2. Khodoun MV *et al.* J Allergy Clin Immunol. in press

Method

Cell and stimulation

Mouse bone marrow derive mast cells (BMMCs) were generated from C57/BL6N 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 vol% water) 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 NaCl, pH 2.0) 1 hour and pancreatin in simulated intestinal fluid (0.05M KH_2PO_4, pH 7.5) for 15 min at heat blocker at 37 degrees C (described in **Fig. 1**).

In vitro desensitization

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

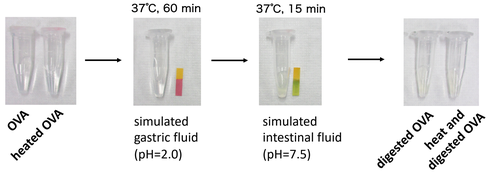

Fig. 1 Heating and artificial digestion of OVA

Result

OVA induced degranulation and desensitization of BMMCs

OVA induced BMMCs degranulation was observed with a maximum at 20 μg/ml OVA (**Fig. 2 A**). Based on this responses, we established desensitization strategy and conducted to OVA specific IgE-sensitized BMMCs. The desensization-treated cells were clearly showed decreased degranulation rate compared to untreated cells (**Fig. 2 B**).

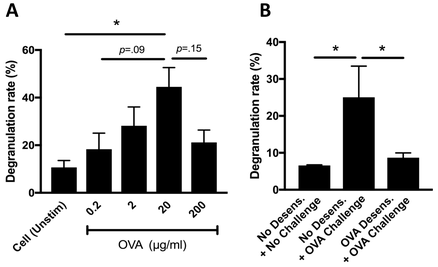

Fig. 2 OVA induced degranulation responses of BMMCs
(**A**) Sensitized BMMCs were challenged with OVA. One hour after the challenge β-hexosaminidase release was assessed. (**B**) Sensitized BMMCs were subjected to desensitization (Desens.), subsequently challenged by OVA (20 μg/ml). n=3 , mean ± SEM, **P*<0.05

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 controversially in heated-OVA, "Low-dose protocol" was successful but "High-dose protocol" was unsuitable for inducing the desensitization (**Fig. 3 B**).

A

Steps	Time (min)	Volume (μl)	Concentration (μg/ml)	
			High-dose	Low-dose
1	0	1.8	0.017	0.00017
2	10	2.0	0.026	0.00026
3	20	2.2	0.052	0.00052
4	30	2.5	0.100	0.00100
5	40	2.7	0.200	0.00200
6	50	3.0	0.410	0.00410
7	60	3.4	0.830	0.00830
8	70	3.8	1.700	0.01700
9	80	4.2	3.300	0.03300
10	90	4.6	6.600	0.06600
11 steps	100	5.2	10.00	10.00

B

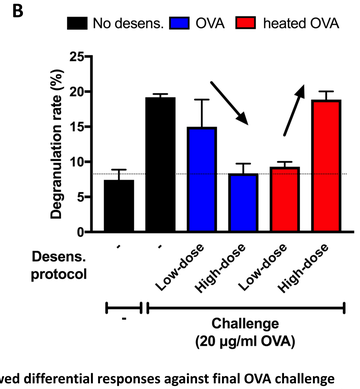

Fig. 3 Strategy to evaluate the efficacy of desensitization and result showed differential responses against final OVA challenge
(**A**) Desensitization protocol. (**B**) Sensitized BMMCs were subjected to high- and low-dose protocols using OVA (blue) and heated-OVA (red). n=2, mean ± SEM

Effect of heat and digestion on BMMC degranulation and desensitization

Sensitized BMMCs were stimulated by four types of OVAs (OVA, heated OVA, digested OVA, and heated and digested OVA) respectively. Heated OVA challenge resulted in enhanced degranulation compared to nonheated naive OVA. Artificial digestion of the heated OVA completely eliminated the increased degranulation (**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 degranulation (**Fig. 4B**).

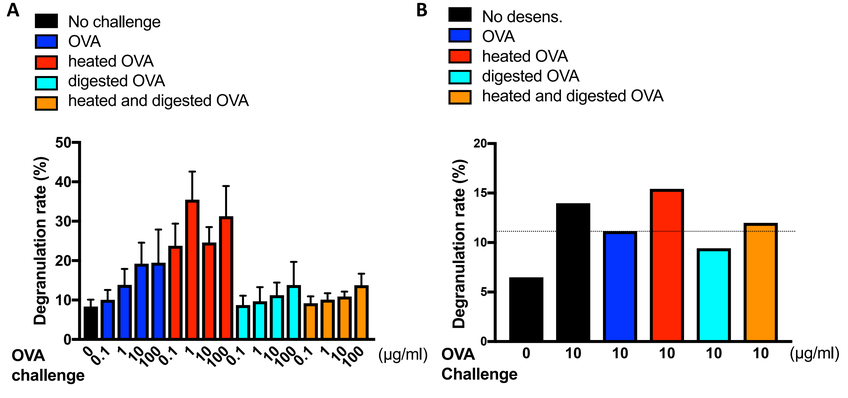

Fig. 4 BMMC degranulation and desensitization responses by OVAs.
(**A**) OVAs were challenged to the sensitized BMMCs without desensitization intervention. (**B**) Sensitized BMMCs were treated with OVAs as desensitization and subsequently challenged by nonheated naive OVA. n=2 (A), n=1 (B), mean ± SEM

Conclusion

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

Acknowledgement

This study was supported by Hokuto foundation Japan. The authors have no conflict of interest regarding the contents of this study.