EFFECT OF HIGH PRESSURE TREATMENT ON THE ALLERGENICITY OF BOVINE SERUM ALBUMIN EVALUATED BY HISTAMINE RELEASE ASSAY USING SERA FROM ALLERGIC PATIENTS AND HUMAN BASOPHILIC KU812F CELLS

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Background

In our previous study (Han *et al*., 2000), we indicated bovine serum albumin (BSA) played important role in the allergenicity of beef. Various food-processing techniques have been applied to foods in order to eliminate their allergenic proteins or to reduce levels. The effects of heat treatment on food allergenic proteins have been widely studied by many groups. Heat treatment reduced the sensitization of beef, even if the treatment was less effective on pure BSA under domestic conditions (Werefel *et al*., 1997; Fiocchi *et al*., 1998). On the contrary, in some cases, heat treatment showed negative results. Restani *et al*. (1998) reported that the heat treatment was not able to decrease the BSA capability to bind to immunoglobulin E (IgE).
High pressure treatment has recently been considered a useful food processing techniques and the efficiency of this treatment on meat has been reported by several research groups including that of Suzuki (Suzuki *et al*., 1998). However, to date studies on the effects of high pressure treatment on food allergenicity have not been done, except our previous report (Han *et al*., 2002)

Objectives

Basophils, as well as mast cells, play an important role in the induction of allergic inflammatory responses via release of inflammatory mediators. The purpose of this study is to evaluate the effect of high pressure treatment on the elimination of BSA allergenicity on the basis of histamine release from human basophilic KU812F cells sensitized with sera from allergic patients.

Materials and methods

Sera: Sera from food allergic patients were obtained from Yoshida Hospital (Niigata) for this study.
Heat or pepsin treatment: Heat or pepsin treatment of BSAwas done in captube at 100ºC for 10min and at 37ºC for 60 min, respectively.
High pressure treatment: High pressure treatment of BSA was carried by the procedure of Homma et al. (1994). The sample sealed in a polyethylene bag was pressurized under 100, 200, 300, 400, 500, and 600 MPa at 5~7ºC for 10min using NBIP (Nikkiso Isostatic Processor).
Cells and cell culture: Human basophilic KU812F cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan) and were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were cultured at 37ºC in humidified atmosphere with 5% CO_2.
Histamine release: Briefly, in order to bind IgE to high affinity IgE receptor FcεRI on the surface of KU812F cells, the cells (1.0×10^6 cells/ml) were incubated with the sera (diluted 1:100) exhibiting IgE binding activity to BSA in RPMI-1640 medium at 37ºC for 60 min. To evaluate the IgE binding activity of sera, enzyme linked immunosorbent assay (ELISA) was performed according to the method of Han *et al*. (2000). Then the cells were washed twice and resuspended in Tyrode's buffer. Histamine release was measured after stimulation of serum-sensitized cells with BSA with or without pepsin, heat or high pressure treatment at 37ºC for 60 min. After centrifugation, the amount of histamine in the supernatant was measured by means of a fluorometric assay according to the method of Shore *et al*. (1959) with slight modification. Histamine release was expressed as the percentage of the total amount of histamine; spontaneous release, occurring in the absence of any stimulus, was subtracted from all values.

Results and discussion

KU812F cells are known as human basophilic cells, which produce histamine and express high affinity IgE receptor FcεRI. The cells have a potential to release histamine upon stimulation with antigen and its specific IgE. Prior to evaluate the effect of high pressure treatment on the allergenicity of BSA, we established histamine release assay system using KU812F cells in combination with sera from allergic patients. IgE binding activity to BSA of sera used in this study was evaluated by ELISA (Table I). After sensitization of KU812F cells with sera, histamine release from the cells following addition of BSA was investigated. IgE molecules on the cell surface were confirmed by flow cytometric analysis (data not shown). Intact BSA induced histamine release from the cells sensitized with A5 serum exhibiting high IgE binding activity to BSA in a dose dependent manner (Fig, 1).

It seems that cross-linking of IgE specific to BSA on the cell surface followed by aggregation of FcεRI results in histamine release. On the contrary, no significant histamine release by BSA was observed in the cells sensitized with A4 serum exhibiting low IgE binding activity to BSA. Although we cannot eliminate a possibility that IgG specific to BSA may participate in cell activation through IgG receptor FcγRI, our data suggests that this histamine release assay system could be available to detect histamine release by BSA via its specific antibody. Using this histamine release assay system, we evaluated the effects of BSA treated with pepsin, heat and high pressure on the histamine release.

Our previous study revealed that high pressure treatments of beef extract did not show any significant changes in binding with sera from beef allergic patients (Han *et al*., 2002). In addition, SDS-PAGE and immunoblot analysis revealed that BSA treated with high pressure (600 MPa) or heat (100) is still able to bind serum IgE (unpublished data). Nevertheless, BSA pressurized at ranging from 300 to 600 MPa reduced histamine release from the cells sensitized with A5 serum with significance (Fig.2). The reducing effect of high-pressure treatment gradually increased with the increase of high pressure applied to BSA. In order to show that the result as mentioned above is not a specific case, the histamine release from the KU812F cells sensitized with 6 individual patient sera was examined here. Simultaneously, the effects of heat and pepsin treatments on the histamine release were evaluated. Almost the same results were observed in this study using A5, A17, A45, A77, A125 and P2 serum (Fig. 3). Intact BSA induced about 32 to 42% histamine release, while the BSA pressurized at 600 MPa or heated at 100 reduced the histamine release to about 6 to 14% and 5 to 19%, respectively. When the BSA treated with pepsin (1000:1 w/w), the histamine release was higher than that of intact BSA. Even though the fragmentation of BSA was confirmed on the SDS-PAGE (data not shown), the structure around the epitope recognized by anti-BSA IgE from sera seems to be retained and facilitate histamine release more easily. It is necessary to pay an attention that there are some cases where the allergenicity is more intensified by the proteolytic enzyme treatment than that of the untreated.

To our knowledge, this is the first study to report that high pressure treatment can decrease allergenicity of food components on the basis of mediator release from basophils or mast cells. Thus, food-processing by high pressure, as well as heat, raises the possibility that the technique might eliminate allergenicity of food components and contribute to attenuation of allergic responses via food allergen specific IgE. Even if high-pressure treatment fails to decrease BSA capability to bind serum IgE, pressurized BSA may undergo some changes to repress the mediator release from mast cells and basophils. Now, we are studying in detail the effect of high pressure treatment on the structure of BSA molecules responsible for this reducing effect on histamine release.

Conclusions

BSA (bovine serum albumin) is the major allergen in beef-allergic patients. SDS-PAGE and immunoblot analysis revealed that high pressure (600 MPa) treatment failed to decrease BSA capability to bind serum IgE. Nevertheless, the pressurized BSA ranging from 300 to 600 MPa lowered the percentage of histamine release from human basophilic KU812F cells sensitized with sera exhibiting high immunoglobulin E (IgE) binding activity to BSA, Under undesirable condition, enzymatic digestion of BSA by pepsin might intensify histamine release more than intact BSA. Besides industrial heat processing but domestic cooking heated nonuniformly, high-pressure treatment could also be effective food-processing technique to reduce the allergenicity of beef.

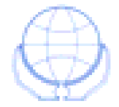

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Table 1. IgE binding activity to BSA of sera from food allergic patients evaluated by ELISA tests.

Allergic Patient's serum	ELISA test (O.D. 405 nm)	IgE binding activity to BSA
A4	0.213	low
A5	1.257	high
A17	1.040	high
A45	1.660	high
A77	1.050	high
A125	1.147	high
P2	1.632	high

ELISA was performed according to the method of Han *et al*. (2000) previously described. Values are expressed as means of two independent experiments.

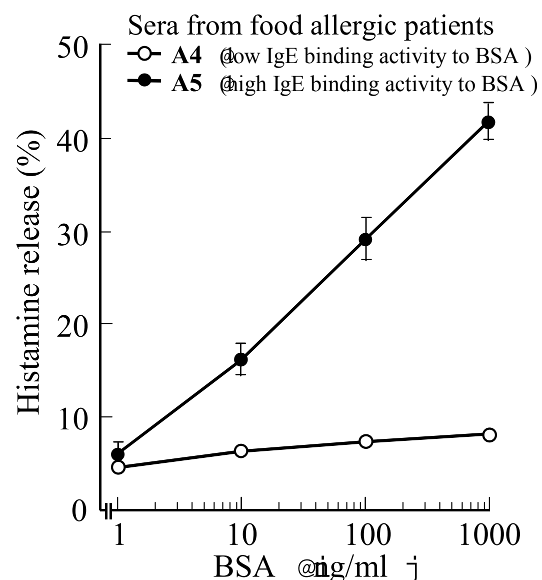
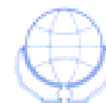


Fig. 1. Effect of BSA on histamine release from KU812F cells sensitized with sera from food allergic patients. Histamine was measured by means of a fluorometric assay. Data are expressed as means ± SE (n = 4).



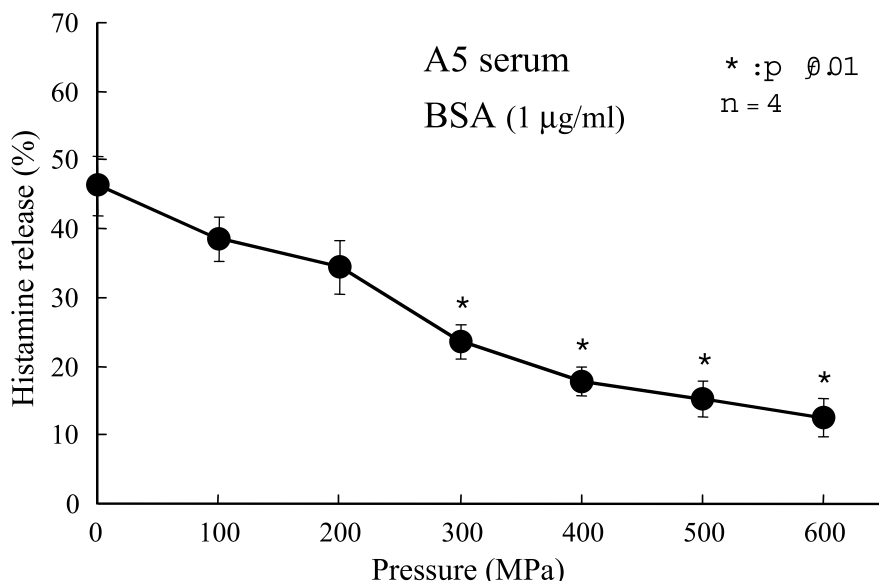


Fig. 2. Effect of BSA treated with high-pressure on histamine release from the KU812F cells sensitized with sera from allergic patients. Cells were sensitized with sera exhibiting high IgE binding activity to BSA (A5), and stimulated by adding 1 μg/ml of BSA pressurized at ranging from 100 to 600 MPa. Histamine released from the cells was measured by means of a fluorometric assay. Data are expressed as means ± SE (n = 4).

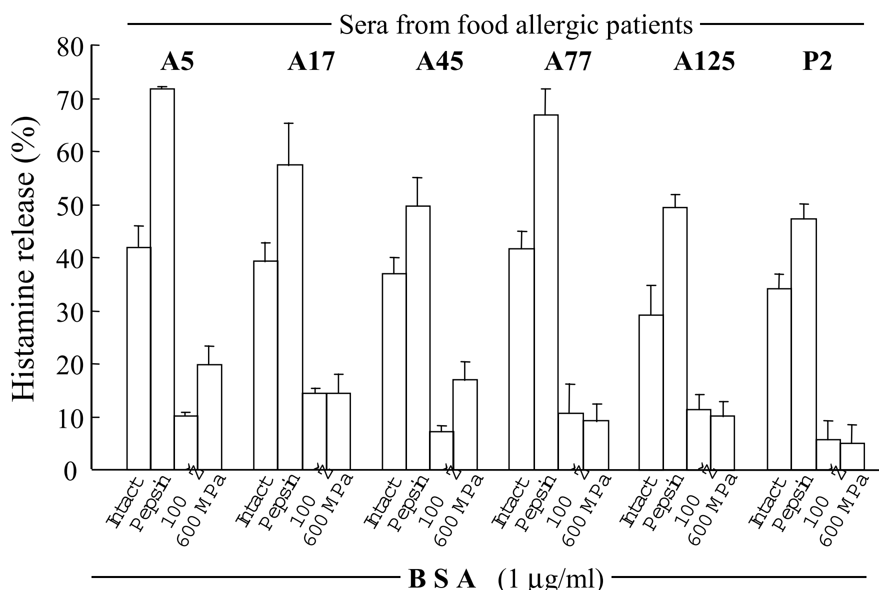


Fig. 3. Effect of digested (pepsin), heated (100 °C) and pressurized (600 MPa) BSA on histamine release from the KU812F cells sensitized with sera from allergic patients. Cells were sensitized with sera exhibiting high IgE binding activity to BSA (A5, A17, A45, A77, A125, P2), and stimulated by adding 1 μg/ml of processed or intact BSA. Histamine released from the cells was measured by means of a fluorometric assay. Data are expressed as means ± SE (n = 4).