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IDENTIFICATION AND ELIMINATION OF BEEF ALLERGENS

Gi dong Han[1], Masatomo Matsuno[4], Giichi Ito[3], Yoshihide Ikeuchi[2], and Atusi Suzuki[2]

[1]Doctor's Program in Functional Biology, Graduate School of Science and Technology, [2]Dept of Applied Biol Chem., and [3]Health Administration Center, Niigata University, Niigata 950-2181, Japan
[4]Department of Pediatrics, Yoshida Hospital, Yoshida 959-9213, Japan

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Background and Objectives:

In recent years, the number of food-allergic patients has been increasing. Food allergy usually appears in infants or children, and it might be often outgrown with age (Ogle & Bullock, 1980). Nowadays, food allergies in adults have begun to be prevalent. Although meat allergy has been considered as a rare pathology, it could not be ignored due to its nutritional and functional importance. Little information about meat allergy has been published so far. In particular, there are conflicting reports about beef allergy. Ogle & Bullock (1980) reported that beef was hypoallergenic. In contrast, there are several reports that beef was one of the common allergenic foods (Chandra et al., 1986; Fiocchi et al., 1995a, b; Werfel et al., 1997). Fiocchi et al. suggested that bovine serum albumin (BSA) was the primary beef allergen. Several reports showed that technological treatment could reduce the allergenic potential of meat products.

This study describes the recent situation of meat allergy through questionnaires in large groups. The identification of allergenic proteins of beef and effects of heat and high-pressure treatment on the allergenicity of beef were discussed.

Material and Methods:

Questionnaire: Items of questionnaire about food allergy were made by G. Ito. 2,331 subjects (1092 male and 1239 female) whose ages ranged from 18 to 24 years answered in detail to the questionnaire on their allergic history.

Sera of food allergic patients: Sera of beef- and/or cow's milk-allergic patients were donated from M. Matsuno. The sera of 10 individuals with no food allergy were used as negative-control in this study.

Preparations of beef, pork and chicken extracts and muscle protein: The meat extracts and residues were prepared by centrifugation following homogenization with 100ml of 20mM sodium phosphate buffer (pH 7.4).

Heat and high-pressure treatment on beef: Beef was treated by heat (60℃,100℃) for 10 min and by high pressure (100,200, 300, 400, 500, 600 MPa) for 5min using NBIP (Nikkiso Isostatic Processor).

Enzyme-linked immunosorbent assay (ELISA) and immunoblots: ELISA and immunoblots were performed according to the method of Engval & Perlmann (1972) and Towbin et al. (1979) with slight modifications, respectively.

Results and discussion:

As a result of questionnaire, 12.8%[299] of the subjects answered that they had a history of food allergies, and some of them are still suffering from the allergies. 9%[27] of these subjects had a history of meat allergies. In the meat allergies, the prevalence of chicken-meat allergy (40%[11]) was higher than that of other meat allergies; complex meat allergy*, including more than one species of meat as causative one, was 26%[7], beef allergy 19%[5] and pork allergy 15%[4] (Fig.1). 4 beef- and/or cow's milk-allergic patients from Yosida Hospital and 40 of the subjects who had a history of food allergies in the questionnaires were subjected to immune-assay. As a result, 13 had positive reactions with beef extracts in ELISA (Fig. 2), If the value of absorbance is > 0.6*, it is regarded as positive reaction; 0.6 means three-fold value of median negative-control values). These 13 were further subjected to immunoblots to identify the allergen of beef. 12 patients reacted to ~60 kDa in beef extract (Fig.3 B-2, 3, Fig.4 B, C-2) and in skim milk (Fig.4 B, C-3), but not to BSA (Fig.3-1). ~60 kDa was suggested to be bovine gamma globulin (BGG) from the result that the patients reacted to the purified commercial BGG (Fig.4 B, C-1). 1 patient reacted to ~66.2 kDa in beef extract (Fig.5 B-3) and skim milk (Fig.5 B-5). From the Fig.5 B-1, ~66.2 kDa is considered to be BSA. Restani et al. (1997) suggested that actin was one of the beef allergens. Werfel et al. (1997) suggested that 17.8 kDa component in beef extract was another beef allergen. However, no our patients had a specific IgE-bindings to actin (Fig.5 B-4) and ~17.8 kDa component (Fig. 5 B-3). Control individuals had no specific reaction with the beef extract (Fig. 3 C-1, 2). BSA and BGG disappeared on SDS-PAGE when the extract of beef mass heated (100℃). In immunoblots, however,

the sera of patients were reacted to residue of the beef mass heated. From these results, it was seemed that heat treatment caused aggregation in the meat protein. No changes in their allegenicity when the beef-extract heated in the same way as beef mass. Werfel et al. (1997) reported that BSA and BGG were heat-labile in beef, but they might not take protein aggregation by heating into consideration. When the beef-extract was pressured, there were no changes in comparison with non-treated beef-extract. While as beef mass was pressured, BGG became to disappear on SDS-PAGE with increasing pressure, but no changes were seen in BSA (Fig.6). These results suggested that BGG was degraded by a proteolytic enzyme existing in the meat under high-pressure. In immunoblots, the specific IgE binding between serum of patient and BGG gradually weaken as the pressure was raised, but the reaction did not disappear completely. Further studies are needed to establish the techniques for eliminating allergenicity of beef.

Conclusions:

From the results, it was clarified that not only BSA but also BGG in beef was primary beef allergen. A cross-reactivity between beef- and cow's milk-allergens were recognized. Heat treatment (60℃,100℃) on beef did not reduce its allergenicity. While, high-pressure treatment on beef was suggested to be one of the techniques for reducing its allergenicity (BGG), even if further studies are needed.

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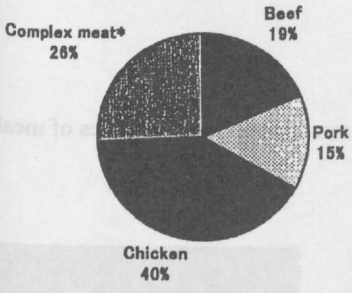

Fig. 1. Distribution of meat allergies

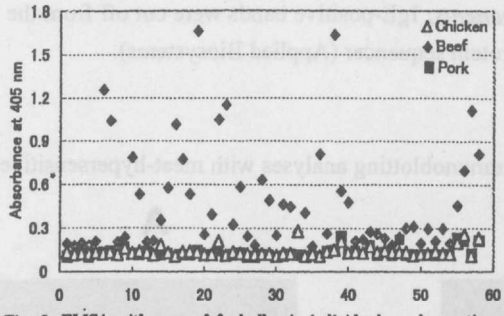

Fig. 2. ELISA with sera of food-allergic individuals and negative-control individuals. 1~4, sodium carbonate buffer; 5~44, sera of food-allergic individuals; 45~54, sera of negative-control individuals; 55~58, sera of beef- and/or milk-allergic patients in Yoshida Hospital.

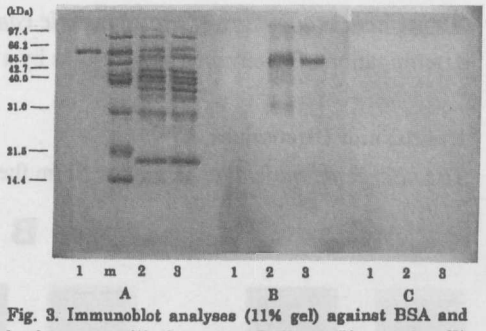

Fig. 3. Immunoblot analyses (11% gel) against BSA and beef extracts. (A) Coomassie Brilliant Blue stain; (B) immunoblots with the serum of allergic patient; (C) immunoblots with serum of negative-control individual. Lane m shows molecular weight markers. Lane 1~3 show samples from BSA, beef 1, and beef 2 respectively.

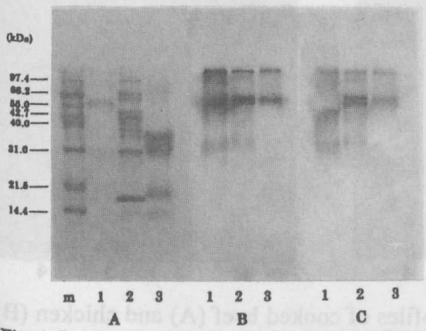

Fig. 4. Immunoblot analyses (11% gel) against BGG, beef extract and skim milk. (A) Coomassie Brilliant Blue stain; (B) and (C) immunoblots with the sera of allergic patients. Lane m shows molecular weight markers. Lane 1~3 show samples from BGG, beef and skim milk respectively.

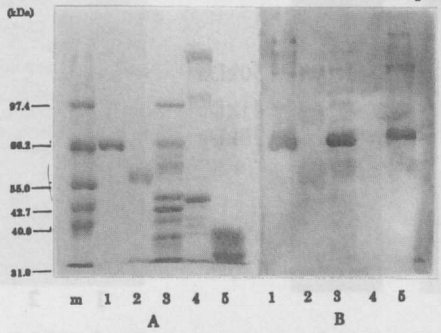

Fig. 5. Immunoblot analyses (8% gel) with the serum of patient (positive about BSA). (A) Coomassie Brilliant Blue stain; (B) immunoblots with the serum of allergic patient. Lane m shows molecular weight markers. Lane 1~5 show samples from BSA, BGG, beef, muscular protein of beef and skim milk, respectively.

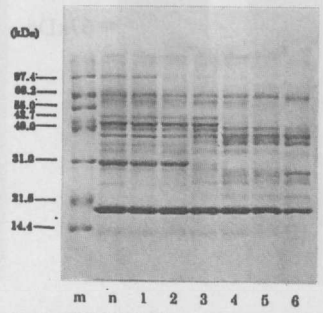

Fig. 6 SDS-PAGE (11%) patterns of the beef treated by high-pressure. Lane m shows molecular weight markers. Lane n shows non-treated beef, Lane 1~6 show samples from extract of beef mass pressured at 100 ~600 respectively.