SPECIFICITY OF THE IGE ANTIBODIES FROM ALLERGIC PATIENTS SENSITIVE TO MEAT PRODUCTS

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SUMMARY
To develop meat products acceptable to the allergic patients, the specificity of the IgE antibodies from 62 food-allergic patients were examined by chemiluminescence ELISA. The test antigens were the meat component proteins and the extracts from the model sausages made of five species of meat with or without foreign proteins. With the sera from the patients allergic to milk and egg, the IgE antibody levels detected were low against the extracts from the foreign protein-free products but high against those containing foreign proteins. With meat-sensitive patients, beef and turkey sausages gave higher and the lowest IgE-antibody levels, respectively. From these findings, the meat products with lower allergenicity can be developed with turkey meat and without the foreign proteins. Of the meat component proteins tested, bovine serum albumin (BSA) and collagen/gelatin were suggested to be allergens.

INTRODUCTION
Processed meat products sometimes cause food allergy. Although causative agents have not been well studied, it is speculated that the meat proteins themselves and/or various foreign proteins may cause allergy; such foreign proteins as milk, egg and soy proteins and the like are formulated in the products as binders and fillers to improve their quality and cost performances. It is, therefore, reasonable for clinicians to frequently recommend the avoidance of processed meat products to the patients allergic to milk and egg. In fact, Gern *et al.* (1991) reported the incidence of milk allergy by the consumption of the meat products containing milk protein hydrolysate. In terms of meat allergens, BSA and ovine serum albumin have been reported (Fiocchi *et al.*, 1995a). Based on the recent findings that gelatin in some vaccines (Kelso *et al.*, 1993; Sakaguchi *et al.*, 1995) and foods (Wahl and Kleinhans, 1989; Sakaguchi *et al.*, 1996) caused allergy, collagen/gelatin is also suggested to be a meat allergen. The present study was carried out to examine the allergenicity of the ingredients formulated in the meat products and the meat component proteins to develop the meat products acceptable to the allergic patients.

MATERIALS AND METHODS
Model meat products and their extracts for ELISA antigens: The model sausage was prepared by formulating beef, chicken, pork, rabbit or turkey meat with (5%) or without such binders as milk, egg and soy proteins. The sausages were homogenized in phosphate-buffered saline (PBS) (1:20, w/w), and then centrifuged at 2,500 rpm for 10 min at 25 °C. The supernatants were used as the extracts from the sausages for ELISA.
Patient sera: Sixty-two sera were obtained from food-allergic patients ranging from 8 mo to 33 yr old (mean: 7 yr old). Of them, 14 sera were from the patients allergic to milk and egg and 11 from those allergic to meats. Three sera from healthy volunteers were used as control.
Chemiluminescence ELISA: Since the conventional ELISA is not sensitive enough to measure antigen specific IgE antibody levels in sera, chemiluminescence ELISA with 96 well plates exactly (Nunc, Denmark) was employed in the present study. The antigens of either the model-sausage extracts, BSA (Sigma, MO, USA) or gelatin (Nacalai Tesque, Kyoto, Japan) were adsorbed to a 96-well microplate and blocked with 1% human serum albumin (Sigma, MO, USA). Thereafter, the patients' sera diluted 1:1000 with PBS containing 0.05% Tween 20 (PBST) were added to each well. After washing with PBST, the plates were incubated with biotin-conjugated goat anti-human IgE antibody (Zymed Lab., CA, USA) and subsequently with alkaline phosophatase-conjugated avidin (Kirkgaard & Perry Lab., MD, USA). Finally, 100 µl of 4-methoxy-4-(3-phosphate-phenyl)spiro[1,2-dioxetane-3,2'adamantane] disodium salt (Lumiphos 530, Wako Pure Chemicals, Osaka, Japan) in 1 M diethanolamine buffer (pH 9.8) containing 0.5 mM $MgCl_2$ was added to each well as a substrate. The plates were incubated for 30 min at room temperature and then the photon counts were determined with a model CT-9000D microplate reader (Dia-Iatron, Tokyo, Japan).

RESULTS AND DISCUSSION
The averages of the photon counts obtained by the reactions of the IgE antibodies to the aqueous sausage extracts in 14 sera from egg- and milk-allergic patients are shown in Fig. 1. The extracts from the products containing the foreign proteins gave significant counts irrespective of the meat ingredients. The highest levels of IgE antibodies were detected to the extracts from the products containing milk proteins. In contrast, the reactivity of the IgE antibodies was quite low to the extracts from the products without foreign proteins. Although the insoluble fraction should be taken into consideration, the results suggest that the patients examined here should not be sensitive to the meat proteins.

Avoidance of chicken meat and beef is frequently recommended to egg- and milk-allergic patients, probably due to the fear of the immunological cross-reactions between meat and milk/egg proteins. The present study, however, shows no direct corelation between the sensitivity to milk and beef, or egg and chicken meat. Such a finding implies that the meat products free from milk and egg proteins should be acceptable to the patients allergic to those proteins.

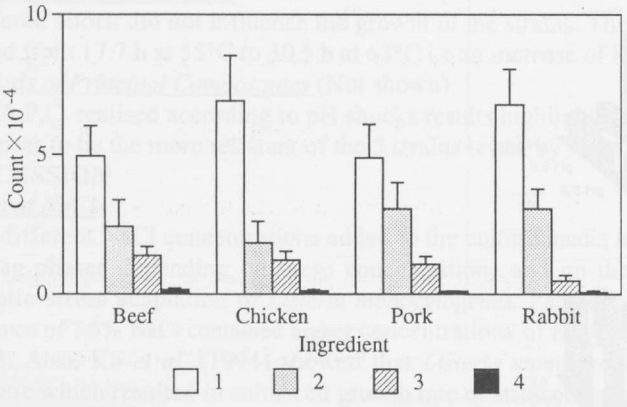

Fig. 1. Reactions of the patients' IgE antibodies to the aqueous extracts from the model sausages made of various meat species with or without foreign proteins.

All sera were from the patients clinically allergic to milk or egg. The following ingredient was added to each sausage; 1: milk protein, 2: egg protein, 3: soy protein, 4: no foreign protein. Mean ± standard error (n = 14).

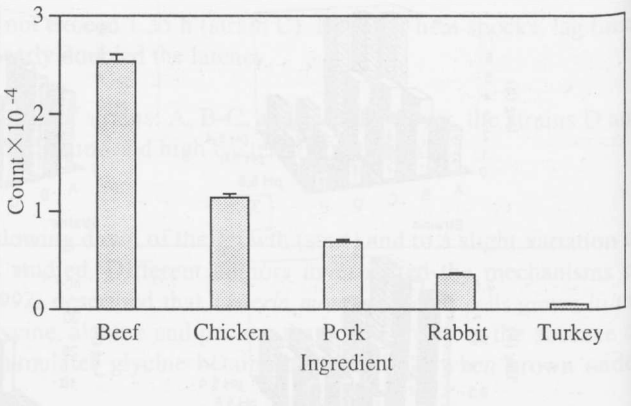

Fig. 2. Reactions of the patient's IgE antibodies to the aqueous extracts from the model sausages made of various meat species without foreign proteins.

All sera were from the patients sensitive to meat. Mean ± standard error (n = 11).

Table 1. The specificity of the IgE antibodies from meat-allergic patients to the aqueous extracts from the model sausages made of various meat species without foreign proteins.

Patients #	Ingredient				
	Beef	Chicken	Pork	Rabbit	Turkey
138	+	+	+	+	−
144	−	−	−	−	−
148	+	−	+	−	−
150	+	+	+	+	−
151	+++	+++	+	−	−
152	−	−	+	−	−
165	+++	+	−	+	+
172	+++	−	++	+	−
179	++	+	−	−	−
180	+++	+	−	−	−
181	−	−	−	−	−

All sera were from the patients clinically allergic to meat.
The IgE antibody levels were classified into 4 categories by photon counts; −, +, ++ and +++. −: < 5,000 counts; +: 5,000 - 20,000; ++: 20,001 - 40,000; +++: > 40,000.

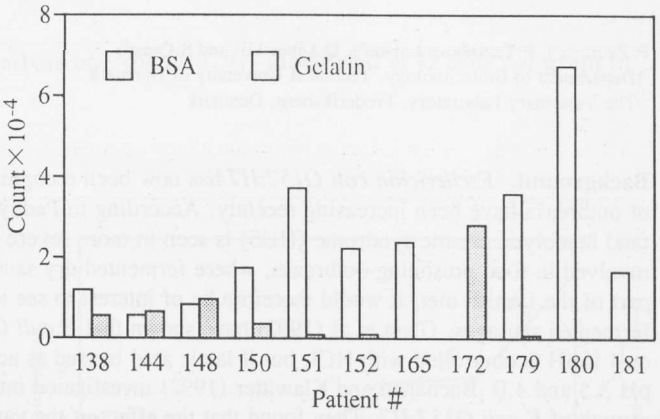

Fig. 3. Binding of the patients' IgE antibodies to bovine serum albumin and gelatin.

All sera were from the patients clinically allergic to meat.

Figure 2 shows the mean levels of the IgE antibodies from 11 meat-sensitive patients to the exracts from the sausages made of five species of meat. The reactivity to the extracts was highest for beef, intermediate for chicken and low for pork and rabbit. With the turkey extract, only one patient showed a very low count. Reasons why the IgE reactivities of the patients to the chicken and turkey extracts differed may be speculated as follows; (1) these patients had never taken turkey but chicken due to dietary customs of Japaneses, and (2) since chicken (family *Phasianidae*) and turkey (family *Meleagridae*) are genetically and taxonomically discriminated, immunological characteristics of these meat may differ. Anyhow, these results suggest that the meat products with lower allergenicity could be developed with turkey meat.

The detailed profile of the reactions of each serum specimen to the sausage extracts is shown in Table 1. To the turkey extract, only #165 was the positive patient. Patients #138 and #150 were positive to four kinds of meat but still negative to turkey. Patients #144 and #181 did not show positive reactions to any extract, suggesting their IgE antibodies should be specific to the unextractable fraction of the sausages. Although patiens #151 showed high IgE levels both to beef and chicken, theresults do not necessarily imply the cross-reactions between beef and chicken, since #172 was quite sensitive to beef but negative to chicken. Similarly, although #172 was highly sensitive both to beef and pork, #165, #179 and #180 with high levels of IgE antibodies to beef were negative to pork. Thus, the extent of the reactions to each extract differed patient to patient.

As for meat allergens, only limited information are available so far. Fiocchi *et al.* (1995a) have reported BSA as an important meat allergen. In the present study, quite high IgE antibody levels to BSA were detected with #151, #152, #165, #179 and #180 sera as shown in Fig. 3, suggesting that BSA might be allergen for the patients. In the case of #151, #165 and #180, the results were conincident with those in Table 1 on the reactivity of their IgE antibodies to the beef-sausage extract which should have containd BSA. However, some discrepancies were found between the results in Table 1 and Fig. 3: #144, #152 and #181 were negative to the beef extract but moderately possitive to BSA. This should be due to the reduction of the allergenicity by the heat-processing of the products(Fiocchi *et al.*, 1995b; Yunginger, 1991).

Recently, it has been reported that gelatin added to some vaccines as a stabilizer caused allergic reactions (Kelso *et al.*, 1993; Sakaguchi *et al.*, 1995). Moreover, the ingestion of gelatin-containing foods such as fruit gums were reported to induce allergic reactions (Wahl and Kleinhans, 1989; Sakaguchi *et al.*, 1996). It is, therefore, strongly suggested that collagen, source of gelatin, should be one of the meat allergens. The elevated level of IgE antibodies against gelatin in patient #172 serum in Fig. 3 might be related to his allergic reactions to beef.

REFERENCES

Fiocchi A., Restani P., Riva E., Qualizza R., Bruni P., Restelli A.R. and Galli C.L., 1995. Meat allergy: I- Specific IgE to BSA and OSA in atopic, beef sensitive children. J. Am. Coll. Nutr., 14, 239-244.

Fiocchi A., Restani P., Riva E., Restelli A.R., Biasucci G., Galli C.L. and Giovannini M., 1995. Meat allergy: II- Effects of food processing and enzymatic digestion on the allergenicity of bovine and ovine meats. J. Am. Coll. Nutr., 14, 245-250.

Gern J.E., Yang E., Evrard H.M. and Sampson H.A., 1991. Allergic reactions to milk-contaminated "nondairy" products. New. Engl. J. Med., 324, 976-979.

Kelso J.M., Jones R.T. and Yunginger J.W., 1993. Anaphylaxis to measles, mumps, and rubella vaccine mediated by IgE to gelatin. J. Allergy Clin. Immunol., 91, 867-872.

Sakaguchi M., Ogura H. and Inouye S., 1995. IgE antibody to gelatin in children with immediate-type reactions to measles and mumps vaccines. J. Allergy Clin. Immunol., 4, 563-565.

Sakaguchi M., Nakayama T. and Inouye S., 1996. Food allergy to gelatin in children with systemic immediate-type reactions, including anaphylaxis, to vaccines. J. Allergy Clin. Immunol., 98, 1058-1061.

Wahl R. and Kleinhans D., 1989. IgE-mediated allergic reactions to fruit gums and investigation of cross-reactivity between gelatine and modified gelatine-containing products. Clin. Exp. Allergy, 19, 77-80.

Yunginger J.W., 1991. Food allergy-adverse reaction to food and food additives. In: Food antigens. (Metcalfe D.D., Sampson H.A. and Simon R.A. ed). Blackwell Scientific Publications. Boston, pp.35-51.