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DETERMINATION AND ELIMINATION OF ALLERGENS IN BEEF AND CHICKEN

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Backgrounds and Objective:

Although such major allergens as chicken eggs, cow milk, soy protein and the like frequently cause food allergy, meat rarely does so^{1,2)}. However, patients carring IgE antibodies against these major allergens sometimes carry also anti-meat IgE antibodies, resulting in meat allergy^{3,4)}. By chemiluminescence ELISA with serum samples from meat-hypersensitive patients, we previously found that beef and chicken are allergenic but pork, turkey, lamb and rabbit are rarely allergenic.

The present paper describes (1) identification of allergenic components in beef and chicken, and (2) elimination of their allergenic properties by heat treatment.

Materials and Methods:

Meat extracts: Phosphate-buffered saline (PBS) extracts of beef and chicken were prepared and subjected to SDS-PAGE to determine the allergenic molecules by immunoblotting assay. Similarly, PBS extracts heat-treated for 30 min at 63, 100 and 120°C were subjected to SDS-PAGE for immunoblotting assay.

Anti-meat IgE-containing serum: Serum samples were collected from 12 beef- or chicken-hypersensitive doners (with radioallergosorbent test: [RAST] scores larger than 2)

Determination of allergenic components in meats by immunoblotting assay with meat-hypersensitive serum samples: The meat extracts were subjected to SDS-PAGE, the gels of which were transferred to PVDF membrane. The membranes were treated with the serum samples containing anti-meat IgE antibody and then with anti-human IgE goat IgG antibody. Finally, positive bands were conventionally developed and identified.

Amino acid sequencing of the allergenic components: IgE-positive bands were cut off from the PVDF membranes and subjected to determination of N-terminal amino acids by a protein sequencer (Applied Biosystems).

Results and Discussion:

The allergenic molecules in meats: From the immunoblotting analyses with meat-hypersensitive serum samples, candidates of meat



allergens were as follow; a 67-kDa molecule for beef and 39-, 41- and 51-kDa molecules for chicken (Fig.1). From the N-terminal amino-acid sequences, the 67-kDa molecule of beef was homologous to bovine serum albumin (BSA), and 39- and 41-kDa molecules of chicken were homologous to glyceraldehydephosphate dehydrogenase (GAPDH) and fructosebisphosphate aldolase (FBA), respectively. With the 50-kDa molecure of chicken, further studies are needed.

Reduction in the allergenic property of meat: Figure 2 illustrates SDS-PAGE patterns of beef and chicken extracts heat-treated for 30 min at 63, 100 or 120° C. With increasing the temperture, SDS-PAGE bands decreased in number and color intensity. With the extracts heat-treated at higher than 100° C, no IgE-immunoblotting assay-positive band was observed (data not shown). These results suggest that the allergenic properties of meats and their component substances can be eliminated by heat treatment^{5, 6)}.

Conclusions:

We found the following; (1) the allergen in beef was BSA, and those in chiken were GAPDH, FBA and a 50-kDa component, and (2) the allergenic activities of these substances can be eliminated upon heating at a temperature higher than 100°C. Further studies are in progress.

Pertinent literature:

1. S.L.Hefle., J.A.Nordlee., S.L.Taylor., Critical Review in Food Science and Nutrition. 36, S69-S89, 1996.

2. Sampson.H.A., McCaskill.C.C., J. Pediatr. 107, 669-675, 1985.

3. A.Fiocchi., P.Restani., E.Riva., R.Qualizza., P.Bruni., A.R.Restelli., C.L.Galli., J. American Collage of Nutrition. 14, No.3, 239-244, 1995.

4. A.Fiocchi., P.Restani., E.Riva., A.R.Restelli., G.Biasucci., C.L.Galli., M.GiovanninI., J. American Collage of Nutrition. 14, No.3, 245-250, 1995.

5. Hanson.L.A., Jahansson.B.G., Chemical and Molecular Biology, 1, New York: Academic Press, 96-102, 1970.

6. Yunginger.J.W., Boston Blackwell Scientific Publications, 36-51.