

RESEARCH REPORT - No. 1404 FY 2014–2015

Title of research project	Newly emerging allergy/allergy-like reactions provoked by food intake.
Research project no.	(1404)
Research period	FY 2014–2015
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【Abstract】

In recent years, new allergy/allergy-like reactions due to food intake, which are hard to explain by the classic food-protein allergy concept, have been increasingly reported. In addition, the importance of sensitization via routes other than oral, such as skin or airway, has been attracting attention. In this project, we intended to collect the results of domestic and international epidemiological information of new allergy/allergy-like reactions, and then, based on these results, conduct fundamental studies on the mechanisms and biomarkers of these reactions.

First, we conducted a nationwide questionnaire survey among 3093 allergists certified by Japanese Society of Allergy to understand the current situation of new allergy/allergy-like reactions. We then asked for their cooperation to collect samples from patients with these reactions.

The obtained specimens were analyzed for sensitization of basophils by the basophil activation test (BAT). IgE crosslinking-induced luciferase expression in cultured mast cells (RS-ATL8) sensitized with patients sera (EXiLE) was also performed to evaluate the allergenicity.

Food antigens contained in supplements and cosmetics were analyzed by Western blot using patients' sera or allergen-specific animal antibodies.

The effect of erythritol on transepithelial electrical resistance (TER) was determined using cultured Caco-2 cells. The effect of the erythritol intake on mouse fecal mucin levels were analyzed using the Fecal Mucin Assay Kit. Mouse fecal microbiota was investigated by next-generation sequencing analysis.

The mechanisms of immediate-type allergy to low-molecular-weight compounds contained in food are speculated as follows: (1) compounds bound to other proteins act as haptens; (2) compounds enhance the allergic reaction to other proteins by modifying the functions of mast cells and basophils; and (3) protein contamination.

In case of sugar alcohol allergy, sugar alcohol bound to an unknown protein carrier might act as a hapten. Seven patients with erythritol allergy, one with xylitol allergy, and one with sorbitol allergy were analyzed by BAT. Three patients with erythritol allergy and one with xylitol allergy showed a positive reaction, which was consistent with the results of the skin prick test. Basophil activations were inhibited by wortmannin, and washed blood cells containing basophils were not activated by sugar alcohols. These results suggested that the activation of basophils from patients with sugar alcohol allergy are through crosslinking of FcεR1s and are dependent to non-cell components of the patients' blood.

Next, we determined the effects of erythritol on intestinal absorption using a mouse model. The TER of high dose erythritol group was lower than that of control and low dose xylitol group. The lucifer yellow flux was

higher in the cells incubated with high dose of erythritol than in the control cells and those treated with low dose of xylitol. These results suggest that the addition of high doses of erythritol affect the absorption through the paracellular pathway.

Mouse fecal mucin levels were significantly decreased by erythritol intake. Next-generation sequencing analysis showed that the oral consumption of erythritol affected the population of fecal microbiota, including an increase in *Bifidobacterium*.

Tartrazine has previously been reported to enhance basophil activation. In this study, one patient with tartrazine allergy was examined by BAT; however, the basophils from this patient did not respond, even to anti-IgE antibody. Hence, tartrazine did not induce direct basophil activation, nor enhance the activation by crosslinking FcεR1s in this low BAT responder patient.

Most of patients with a cochineal allergy are thought to be allergic to contaminants. The measurements of protein content revealed that carminic acid contain 0.4%, a raw material for cosmetics contain 29.7%, cosmetics (containing 2-3% of carmine) contain 0.52%, cosmetics (containing 5% of carmine) contain 1.3% and carmin for food additives contain 26% of protein, respectively. Three patients with cochineal allergy were analyzed by BAT and EXiLE. One patient showed a positive result to cochineal dye that included protein contaminants, but not to purified carminic acid, suggesting reaction to the protein contaminants.

We could not obtain blood samples from the patients with chitosan or glucosamine allergy. Instead, we assessed supplements containing chitosan or glucosamine for the presence of contaminating crustacean proteins. Crustacean proteins were not detected in all of these materials. The characteristics of chitosan and glucosamine as allergens and mechanism of onset of the allergy should be investigated in future.

To investigate allergy/allergy-like reactions due to environmental allergens sensitized through skin or airway tracts, we focused on three allergies as models. Gly m 4 is regarded as a major allergen for the systemic type of soy milk allergy; however, not all of the allergies can be explained by Gly m 4. Hence, we attempted to identify the allergens that cause soymilk anaphylaxis. Using patients' sera, we measured IgE titer to a new peach allergen (GRP) by ELISA, and results were negative. In the future, we plan to repeat this experiment using a soy GRP extract.

In Japan, patients with red meat allergy are accumulated in Shimane Prefecture, and analysis of the patients living in the area has been progressing. We analyzed seven patients from an area other than Shimane by BAT to detect clinically relevant, polyvalent, α-gal-specific IgE. All patients showed negative results. All, except one, were negative for α-gal-specific IgE with the CAP System FEIA, and the patients positive for α-gal specific-IgE were low responders for BAT. These results highlight the difficulty in diagnosing red meat allergy in regions other than Shimane.

We analyzed the distribution of rice allergens and found a 19-kDa globulin in polished rice and a 52-kDa globulin in rice bran. We also found the 52-kDa globulin in several cosmetics and health foods and identified the globulin as the causative allergen of a patient with rice bran allergy. In the case of papaya allergy, we found a major 22-kDa allergen in cosmetics and health foods.

We conducted a nationwide questionnaire survey among 3093 allergists on newly emerged allergy/allergy-like reactions. These included allergies to low-molecular-weight compounds contained in food

and food allergy sensitized by environmental allergen through skin or airway. Analysis of blood samples from patients found in the survey suggested that sugar alcohol activates basophils as a hapten, and a patient with a cochineal allergy reacted to a contaminating protein other than carminic acid. In the mouse model, it was suggested that erythritol might affect intestinal permeability, intestinal mucin levels, and fecal microbiota populations. These effects could explain why erythritol allergies are more common than allergies caused by other sugar alcohols. We also found the 52-kDa globulin from rice and a major 22-kDa allergen from papaya in several cosmetics and health foods. Furthermore, we identified the 52-kDa globulin from rice as the causative allergen for a patient with rice bran allergy.