博 士 論 文 の 内 容 の 要 旨

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論文題目	Immunomodulatory activity of phosphorylated buckwheat major allergen and its enzymatic hydrolysate （リン酸化ソバ主要アレルゲンとその酵素消化物の免疫調節作用）

（博士論文の内容の要旨）

The potential therapeutic effects of phosphorylated buckwheat allergens on IgE-mediated allergic reactions are sought in this dissertation. This dissertation is divided into four chapters. The background information, related literature, and objectives are presented in Chapter 1. The current medical response towards food-allergic patients is to control the symptoms of allergy without treating it. Recently, a novel approach known as “Specific Immunotherapy” (SIT) is a common alternative. Immune tolerance to allergens is established by changes in memory allergen-specific T and B cell responses, joined by changes in both mast and basophil cells activation threshold levels that would lead to prevent the occurrence of allergic symptoms. One method of SIT is “Oral Immunotherapy” (OIT), it involves the oral administration of small doses of an allergen over time, with a gradual increase in the dose. Despite its success in desensitization, OIT could carry safety risks, such as the risk of an allergic response in the initial dose setting, and the need to use epinephrine to mediate anaphylactic reactions. To override the side effects of OIT, some therapies work on modifying the allergen proteins used to decrease the potential of allergic reaction, and still induce tolerance. Such modifications include glycosylation of proteins, or phosphorylation *via* dry heating with pyrophosphate. Phosphorylation of proteins can change the conformational structure, which results in its binding capability with IgE. If phosphorylation occurred on the epitope site of antigen, it could block the binding site from the IgE.

Buckwheat plant has an IgE mediated allergen, its major allergens are Fag e 1 and Fag e 2. Fag e 1 is a 22 kDa globulin found in common buckwheat (*Fagopyrum esculentum*) and it is one of the major allergens causing severe allergic symptoms, Fag e 2, a member of 2S albumin family, reported as being resistant to pepsin digestion and appears to be the causative for an immediate hypersensitivity reaction of buckwheat allergy including anaphylaxis.

Chapter 2 deals with the expression of recombinant Fag e 1 using the *Pichia* expression system and preparing an allergen-specific hypoallergenic agent by the controlled dry-heating phosphorylation of Fag e 1 (P-Fag e 1). Then, it was investigated if P-Fag e 1 can be useful as an immunomodulator in Fag e 1-sensitized mice. The results showed a reduction in histamine release and both total and specific IgE in P-Fag e 1 treated mice. Combined with increased total IgA, increase Tfh cells, and a decrease of IL-4 from spleen and Peyer’s patches of P-Fag e 1 treated mice. The suppression of IgE production in the Fag e 1 treated group might be because of the enrichment of the Tfh cells and IgA production. Therefore, it could suggest that P-Fag e 1 is an allergen-specific immunomodulator in mice allergic to Fag e 1.

In Chapter 3, the effects of phosphorylation on the digestibility of Fag e 2 was investigated. The purpose was to investigate whether digested P-Fag e 2 (DP-Fag e 2) can attenuate allergic reactions in Fag e 2-sensitized mice. Recombinant Fag e 2, obtained using the *Pichia* expression system, was phosphorylated *via* dry-heating in the presence of pyrophosphate. Phosphorylation enhanced the peptic digestibility of Fag e 2. Mice fed DP-Fag e 2 for 6 weeks after Fag e 2 sensitization exhibited reduced allergic symptom scores

compared to those of sham-treated mice. Decreased total and specific IgE, decreased specific IgG1, and increased total IgA were observed in the serum of the DP-Fag e 2-fed group. These results showed that P-Fag e 2 was readily digested in the stomach and induced the attenuation of the IgE-mediated allergic reaction.

Lastly, Chapter 4 provides a summary and conclusion of the two studies. The results suggested that that phosphorylated buckwheat major allergen and its enzymatic hydrolysate can attenuate the allergic reactions in a mouse model of buckwheat allergy. These findings would contribute to the development of safer and more effective immunotherapy for buckwheat allergy.