

論文の要旨

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論文題目 Identification and immunochemical characterization of novel major allergens from the house dust mite, *Dermatophagoides farinae*
(室内塵ダニ *Dermatophagoides farinae* からの新規主要アレルゲンの同定と免疫生化学的性状の解析)

Chapter 1. General introduction

Asthma is a common chronic respiratory disease. The prevalence of asthma is about 5%-16% worldwide. House dust mite (HDM) is one of the important allergens, and caused nearly 50% of asthmatic diseases. The allergen-specific immunotherapy (SIT) using HDM allergens is recognized to be the only curable treatment for HDM-related allergic asthma. However, it is technically difficult to produce large amounts of pharmaceutical grade of natural HDM allergens for SIT. In addition, the crude extracts of HDM comprise complex mixtures of proteins, and usually cause additional allergic responses and/or adverse effects including systemic anaphylaxis. Therefore, the use of recombinant allergens is suggested to be an alternative strategy for SIT, and the standardization and characterization of major component allergens are crucial for the development of safer and more effective SIT vaccines for HDM allergy. Objective of this study is to identify novel major HDM allergens with clinical importance, and to elucidate their immunochemical properties for drug applications.

Chapter 2. Cloning and characterization of Der f 34, a novel major HDM allergen homologous to a highly conserved Rid/YjgF/YER057c/UK114 family of imine deaminases

In the present chapter, I identified a novel HDM allergen Der f 34, belonging to the Rid/YjgF/YER057c/UK114 family (Rid family), by immunoscreening of HDM cDNA library with specific antibodies against a HDM feces fraction. The Rid family protein is highly conserved from bacteria to mammals, and their enzymatic function is to deaminate reactive intermediates of branched chain amino acids, and catalyze hydrolysis of the imines to 2-oxo acids that are relatively stable. Structural analysis confirmed that the predicted Der f 34 polypeptide sequence contained the seven conserved amino acid residues that are essential for imine deaminase activity. I prepared recombinant Der f 34 (rDer f 34), and analyzed its imine deaminase activity using leucine, methionine and phenylalanine as substrates. I found that rDer f 34 showed imine deaminase activity especially acting on leucine and methionine in cooperation with L-amino acid oxidase

while it poorly digested phenylalanine, suggesting that the enzymatic properties of rDer f 34 were similar to those of the Rid family members. I also found that native Der f 34 purified from HDM feces extract showed a high IgE-binding frequency (62.5% on two-dimensional IgE immunoblotting, and 68% assessed by ELISA), and that Der f 34 cross-reacted with another important indoor allergen source, *Aspergillus fumigatus*. These results suggest that the Rid family imine deaminase represents an additional important pan-allergen that is conserved across organisms, and that Der f 34 would also be an important HDM allergen component for the development of SIT vaccine.

Chapter 3. Potent IgE-binding and cytokine-induction capacities of a newly identified N-terminal region of Der f 14, an apolipophorin-like major HDM allergen

Objective of this chapter is to identify full length of Der f 14/M-177, an apolipophorin-like high molecular weight HDM allergen, and to elucidate allergenic and immunological properties by comparative analysis of its N-terminal (Der f 14N), middle (Mag3), and C-terminal (Mag1) subdomains. IgE ELISA using 34 HDM allergic patients sera revealed that the N-terminal domain of Der f 14 (Der f 14N) showed the highest IgE-binding frequency (61.8%) as compared to that of the middle Mag 1 (38.2%) and the C-terminal Mag 3 (14.7%) subdomains. I also found that Der f 14N worked as the most potent Der f 14 subdomain to induce lymphocyte proliferation and proinflammatory cytokine production not only in HDM-sensitized mice, but also in HDM-allergic patients. These results suggest that the newly identified N-terminal domain (Der f 14N) is critical to achieve the high sensitization rate of Der f 14, and that the Der f 14N would be taken into consideration for the component of HDM SIT vaccine.

Chapter 4. General conclusion

In this study, I have identified novel major HDM allergens (Der f 34/Rid family of imine deaminase and Der f 14/apolipophorin) and elucidated their immunochemical properties. First, I provide evidence that Der f 34 represents a new class of pan-allergen that is highly conserved among the species. I also have demonstrated that Der f 14 fulfills its high allergenicity and proinflammatory property through immunological exposure of newly-identified N-terminal subdomain. My results strongly suggest that both of those new major HDM antigens would be clinically important, and that they should be nominated as additional SIT vaccine components. Data presented here provide valuable information for the development of diagnostic and therapeutic strategies for HDM allergy.