学 位 論 文 の 要 旨

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学 位 論 文 名	Identification of Peroxidase-1 and Beta-glucosidase as Cross-Reactive Wheat Allergens in Grass Pollen-Related Wheat Allergy
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論 文 内 容 の 要 旨

INTRODUCTION

Wheat is a common foodgrain that elicits immunoglobulin (Ig) E-mediated allergies. Wheat-induced food allergies are typically seen as immediate-type wheat allergy in young children and wheat-dependent exercise-induced anaphylaxis (WDEIA) in adolescents and adults. Water-insoluble wheat proteins, especially ω-5gliadin and high molecular weight glutenin, have been identified as major allergens for WDEIA. Recently, we experienced some adult patients with WDEIA/wheat allergy who were not sensitized to ω-5 gliadin but sensitized to water-soluble wheat proteins. Also, most of them were found to be strongly sensitized to grass pollens, such as timothy grass and sweet vernal grass. Thus, it was presumed that these patients developed an allergic reaction when they ingested wheat-containing foods due to cross-reaction of their IgE antibodies produced against grass pollen allergens to wheat allergens. This derives from the mechanism of pollen food allergy syndrome, which is caused by cross-reaction of pollen allergen-specific IgE to foods such as fruits and vegetables. However, wheat-mediated food allergy due to cross-reaction of grass pollen-specific IgE has been considered to be doubtful, because wheat-specific IgE test had been frequently shown false positive result within grass pollen allergic patients.

This study aimed to clarify clinical characteristics and wheat allergens of this undetermined phenotype of WDEIA/wheat allergy, which were tentatively diagnosed as grass pollen-related wheat allergy (GPWA).

MATERIALS AND METHODS

A total of six patients with GPWA were enrolled, and controls were 17 patients with grass pollen allergy but no episode of wheat allergy, and 29 patients with other wheat allergies: 18 with conventional WDEIA and 11 with hydrolyzed wheat protein allergy. Sensitization to wheat proteins was determined by basophil activation test (BAT). The study protocol was approved by the Ethics Committee of Shimane University.

Both water-soluble and insoluble wheat proteins were fractionated from commercial-blend wheat flour, and IgE-binding proteins in wheat flour were visualized by immunoblot assay using 10% serum from GPWA patients. An immunoblot inhibition assay was performed to confirm IgE cross-reactivity between water-soluble wheat proteins and grass pollen proteins by using sweet vernal grass pollen proteins as an inhibitor.

Wheat allergens contained in water-soluble wheat proteins were further fractionated by ammonium sulfate precipitation and cation-exchange chromatography, and two IgE-reactive proteins were identified by immunoblotting followed by mass spectrometry and database searching.

These identified wheat allergens were further purified by gel-filtration chromatography and biotinylated, and then applied to the solid-phase streptavidin ImmunoCAP^{TM} to establish wheat allergen specific IgE-tests by CAP-fluorescent enzyme-immunoassay (FEIA) system. In this study, specific IgE values ≥ 0.35 kU_A/L were determined to be positive.

RESULTS AND DISCUSSION

The highest basophil activation was induced by the phosphate buffered saline-soluble wheat proteins in five of six patients with GPWA, and immunoblotting showed that all six patients with GPWA reacted with water-soluble wheat protein fraction. These results suggested that the major culprit wheat allergens were water-soluble wheat proteins. A 60-kDa band was observed in the water-soluble wheat proteins of all six patients and a 35-kDa band was observed in three patients out of six the patients. Additionally, pre-incubation of patients' sera with sweet vernal grass pollen proteins inhibited the IgE-binding to 35-kDa and 60-kDa wheat proteins in a concentration-dependent manner. These suggest that grass pollen specific-IgE from the patients with GPWA predominantly cross-reacted with 35-kDa and 60-kDa water-soluble wheat proteins.

Next, water-soluble wheat proteins were fractionated by consecutive ammonium sulfate precipitation at 10% intervals. IgE from GPWA patients' sera bound to 35-kDa bands in the precipitate with 50, 60, and 70% ammonium sulfate and bound to 60-kDa bands in the 60 and 70% ammonium sulfate precipitates. Thus, to identify the 35-kDa and 60-kDa wheat allergen, we further fractionated the proteins in the 40–60% and 50–70% ammonium sulfate precipitate by cation-exchange chromatography, respectively. The presence of IgE-binding 35-kDa and 60-kDa

protein was confirmed by immunoblot using patients' sera, and these allergens were identified as wheat peroxidase-1 (35 kDa) and beta-glucosidase (60 kDa) by mass spectrometry analysis.

Finally, following further purification by gel-filtration chromatography and biotinylated these allergens, we established peroxidase-1- and beta-glucosidase-specific IgE tests using CAP-FEIA system. In these tests, serum IgE specific to peroxidase-1 and beta-glucosidase was positively detected in three and four of six patients with GPWA, three and five of 17 patients with grass pollen allergy, two and two of 29 patients with other wheat allergies, respectively.

Specificity of wheat-, peroxidase-1-, and beta-glucosidase-specific IgE tests to identify GPWA was 65%, 82%, and 71%, respectively, in 23 patients with grass pollen allergy (six GPWA and 17 grass pollen allergy without episode of wheat allergy). This result suggesting that sensitization to these identified allergens is a risk factor to develop wheat-induced food allergy, and indicating the potential usefulness of these tests for the diagnosis of GPWA due to reduce the false-positive result of wheat-specific IgE test within grass pollen allergic patients. Also, specificity of peroxidase-1- and beta-glucosidase-specific IgE tests to identify GPWA in 35 patients with wheat induced allergy (six GPWA, 18 conventional WDEIA and 11 hydrolyzed wheat protein allergy) was 93% and 93%, respectively, suggesting that sensitization to these allergen are specific to GPWA.

On the basic local alignment search tool analysis using the Poaceae family Poeae tribe protein database (NCBI txid147387), we found that primary structure of wheat peroxidase-1 had significant similarity to that of timothy grass uncharacterized protein (accession no. JAA00049.1) with 37.7% amino acid homology. Additionally, significant similarities were found with those of oat grass peroxidase PXC2 precursor and perennial ryegrass putative peroxidase with 44.6% and 38.9% amino acid homologies, respectively. In the same way, primary structure of beta-glucosidase had significant similarity to that of timothy grass uncharacterized protein (accession no. JAA00456.1) with 54.5% amino acid homology. Significant similarities were also found with those of oat grass beta-D-glucosidase and perennial ryegrass beta-glucosidase 31 with 45.8% and 36.8% amino acid homologies, respectively. These results support the idea that IgE raised against grass pollen proteins, such as peroxidase or beta-glucosidase family, cross-reacts to wheat peroxidase-1 or beta-glucosidase in patients with GPWA.

CONCLUSION

Specific IgE to grass pollen proteins dominantly cross-reacted with water-soluble wheat proteins that cause wheat allergies. Peroxidase-1 and beta-glucosidase are candidates of the cross-reactive wheat allergens recognized by the serum IgE of patients with GPWA. These proteins are specific wheat allergens for GPWA among grass pollen allergy and other types of wheat-induced food allergies.