学 位 論 文 の 要 旨

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学 位 論 文 名	Usability of Fag e 2 ImmunoCAP in the Diagnosis of Buckwheat Allergy
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論 文 内 容 の 要 旨

INTRODUCTION

Buckwheat (*Fagopyrum esculentum*) is a popular traditional food in Asian countries including Japan, China, and Korea. The prevalence of buckwheat allergy in Japan is 0.22% among school children and 0.45% among adults with asthma. Thus, buckwheat allergy is not highly seen in clinical practice, however, ingesting, inhaling, or mucous membrane contact with only a small amount of buckwheat proteins can cause serious immediate hypersensitivity reactions such as anaphylactic shock. Currently, the detection of crude buckwheat extract-specific IgE by ImmunoCAP (f11) (Phadia AB) is widely used to diagnose buckwheat allergy. However, the results of this test do not always correlate with the development of allergic symptoms. This study aimed to evaluate the diagnostic usefulness of specific IgE antibody titers for the major buckwheat allergens, Fag e 1 and Fag e 2.

MATERIALS AND METHODS

The buckwheat allergic patients were diagnosed on the basis of a history of recurrent immediate hypersensitivity reactions after ingestion of buckwheat noodles and a positive buckwheat ImmunoCAP (f11) result. This study enrolled 10 buckwheat allergic patients, 14 atopic dermatitis patients with positive buckwheat ImmunoCAP (f11) who had not experienced hypersensitivity reactions to buckwheat, and 15 healthy subjects.

Native Fag e 1 (nFag e 1) was purified as a complex of a legumin-like protein α subunit by using an anion-exchange column (HiTrap Q HP). Native Fag e 2 (nFag e 2) was purified by an anion-exchange column and gel-filtration column (Superdex 75 10/300 GL) chromatography. Recombinant Fag e 1 and Fag e 2 (rFag e 1, rFag e 2) were produced in *E. coli* as a histidine tagged protein and purified by affinity chromatography (His-Trap HP). The allergen-specific IgE levels were determined using the ImmunoCAP method.

RESULTS AND DISCUSSION

We successfully purified native and recombinant buckwheat allergens, nFag e 1, nFag e 2, rFag e 1 and rFag e 2 and confirmed that these allergens to be target proteins by using N-terminal amino acid sequence data. Specific IgE antibody titers to these purified buckwheat allergens were determined by ImmunoCAP in the sera derived from patients and controls. With a cut-off value of 0.35 kUa/L, the nFag e 2-specific IgE test results were positive in 100% of buckwheat allergy patients, 57.1% of atopic dermatitis patients, and 6.7% of healthy subjects. The nFag e 1-specific IgE test result was positive for 100% of buckwheat patients, 64.2% of atopic dermatitis patients, and 6.7% healthy subjects. The sensitivities of the rFag e 1- and rFag e 2-specific IgE test were lower than those of native proteins. The low allergenicity of recombinant preparations may be attributable to differences in the three-dimensional structures of the

recombinant and native proteins.

Receiver operating characteristic curve (ROC) analysis was conducted with buckwheat (fl1), nFag e 1, and nFag e 2 ImmunoCAP values, and the area under the curve (AUC) of buckwheat (fl1), nFag e 1, and nFag e 2 was determined to be 0.823, 0.890, and 0.967, respectively. This result indicated that the nFag e 2 ImmunoCAP test had the highest precision. The sensitivity and specificity of nFag e 2 ImmunoCAP test at the optimal cut-off value of 2.74 kUa/L was 90% and 89.6%. This was higher than the commercially available buckwheat ImmunoCAP (fl1) test, which demonstrated a sensitivity of 80% and a specificity of 72.4% in this study.

Buckwheat extract (fl1) contains a variety of allergenic, non-allergenic components, and, cross-reacting carbohydrate determinants (CCD). It has been reported that the binding of IgE with CCD affected in an allergen-specific IgE measurement. In fact, 7 of 15 patients with atopic dermatitis had IgE antibodies specific to horseradish peroxidase that included CCD and the sera from 2 of them cross-reacted with buckwheat proteins (fl1). In addition, we found a strong association between the nFag e 1- and nFag e 2-specific IgE titers in buckwheat allergy patients but not in atopic dermatitis patients. These results may partly explain the discrepancy in the data of our atopic dermatitis patients that showed positive buckwheat ImmunoCAP (fl1) test results but no clinical symptoms associated with buckwheat ingestion.

CONCLUSION

We have provided evidence that the allergen-specific IgE test with nFag e 2 is more reliable than the buckwheat ImmunoCAP (fl1) for predicting buckwheat allergy.