学 位 論 文 の 要 旨

氏名　　千貫　祐子

学 位 論 文 名　CD203c Expression-Based Basophil Activation Test for Diagnosis of Wheat-Dependent Exercise-Induced Anaphylaxis

発 表 雑 誌 名
(巻，初頁～終頁，年)
著　　者　　名

Refer to the attached document

論 文 内 容 の 要 旨

INTRODUCTION

Wheat-dependent exercise-induced anaphylaxis (WDEIA) is a specific form of wheat allergy typically induced by exercise after ingestion of wheat products. Wheat ω-5 gliadin is a major allergen associated with conventional WDEIA (CO-WDEIA), and detection of serum IgE specific to recombinant ω-5 gliadin is a reliable method for its diagnosis. Recently, an increased incidence of a new subtype of WDEIA, which is likely to be sensitized via a percutaneous and/or rhinoconjunctival route to hydrolyzed wheat protein (HWP), has been observed (HWP-WDEIA). All of the patients with HWP-WDEIA had used the same brand of soap, which contained HWP-A. The predominant observed symptom of the HWP-WDEIA was angioedema of the eyelids; a number of patients developed anaphylaxis. These patients has little serum ω-5 gliadin-specific IgE .

Flow cytometry-based tests of basophil activation status have been described to diagnose or confirm sensitization in allergic patients. Recently, the CD203c ectoenzyme was reported to be a more suitable basophil marker that is not only expressed constitutively on resting basophils but also upregulated at high levels on activated

basophils.

To establish a predictive *in vitro* test for differentiating these 2 subtypes of WDEIA (HWP-WDEIA and CO-WDEIA), we measured basophil CD203c expression induced by different types of wheat proteins and evaluated the diagnostic efficiency of the reactions in the patients.

MATERIALS AND METHOD

Ten patients with WDEIA were enrolled in this study: 5 patients with HWP-WDEIA (patients 1–5; all women; age range, 44–54 years) and 5 patients with CO-WDEIA (patients 6–10; 2 men and 3 women; age range, 39–73 years). Sensitization to wheat proteins was confirmed by skin prick testing, detection of serum specific IgE, challenge testing, and IgE-immunoblotting.

The Allergenicity Kit (Beckmann Coulter, Fullerton, CA, USA) is a commercial kit for quantification of basophil CD203c expression. This kit identifies basophils as CD3-negative and CRTH2-positive fractions from whole blood samples and measures fluorescence intensity of CD203c that is enhanced by cross-linking of surface-bound IgE molecules. Expression of CD203c on basophils was analyzed by flow cytometry using ω-5 gliadin and HWP. To establish allergenicity of HWP, 6 preparation of HWPs with different sources (HWP-A-F) were evaluated with IgE immunoblotting, basophil CD203c expression test, and size exclusion chromatography (SEC).

RESULTS AND DISCUSSION

HWP-A was found to enhance CD203c expression of the basophils in a concentration-dependent manner in the HWP-WDEIA patients. The maximum reaction reached over 60% at a concentration of 0.1 μg/mL. No significant enhancement of CD203c was observed with purified ω-5 gliadin. Instead, purified ω-5 gliadin induced enhancement of CD203c expression of the basophils in a concentration-dependent manner in the CO-WDEIA patients. The maximum reaction reached up to 60% at a concentration of 0.01 μg/mL. No significant enhancement of CD203c was observed in the presence of HWP.

SDS-PAGE showed smear staining that ranged the entire length of the gel for HWP-A and HWP-B. These values largely exceeded the molecular weights (MWs) of native proteins, gliadins and glutenins, and indicated the presence of large polypeptide

aggregates. HWP-D and HWP-E showed intense staining at the lower part of the gel with several bands. HWP-C and HWP-F showed no remarkable staining. The IgE of all the 5 patients with HWP-WDEIA reacted to several protein hydrolysates. HWP-A and -B showed MW 15000-250000 or higher smears. HWP-E showed several individualized bands in 3 of the 5 patients. HWP-C, -D, and -F showed no reaction. All the 5 patients had almost identical reaction patterns.

When basophil CD203c expression was measured using these 6 HWPs, HWP-A and HWP-B were found to induce significant enhancement comparable to soap solution supplemented with HWP-A, which had been used by the patients with HWP-WDEIA. In contrast, other HWP preparations and the supplement-free soap solution showed no significant enhancement.

The SEC analysis showed dominant peaks of HWP-A and HWP-B at an elution volume corresponding to MWs of approximately 669000 and 158000, respectively. In contrast, peaks of HWP-D, -E, and -F were observed at elution volumes corresponding to MWs between 13700 and 6500, and peak of HWP-C was observed at elution volume less than 6500. The SEC of HWP-C showed only small peaks despite loading about 200 μg of the sample.

In the present study, we showed that the *in vitro* wheat protein-induced basophil activation test for quantifying CD203c expression is highly useful for diagnosing the subtypes of WDEIA, HWP-WDEIA and CO-WDEIA, since the determination of CD203c expression clearly differentiated the sensitization conditions of the both types of WDEIA in well accordance with the results obtained with the skin prick testing, the determination of serum allergen-specific IgE, and the immunoblotting. HWPs composed of large polypeptide aggregates showed predominant binding to IgE from the patients with HWP-WDEIA, indicating higher allergenicity than lower MW HWPs.

CONCLUSION

Measurement of basophil CD203c expression induced by various preparations of wheat proteins is highly useful in predicting causative allergens in the patients with WDEIA. It is also useful to evaluate the allergenicity of different kinds of proteins such as HWP.

別紙

氏名　　千　貫　祐　子

論文名

1. CD203c Expression-Based Basophil Activation Test for Diagnosis of Wheat-Dependent Exercise-Induced Anaphylaxis

2. Higher Allergenicity of High Molecular Weight Hydrolysed Wheat Protein in Cosmetics for Percutaneous Sensitization

発表雑誌名
(巻,初頁～終頁,年)

1. Journal of Allergy and Clinical Immunology, (**129**, 1404-1406, 2012)

2. Contact Dermatitis, (doi: 10.1111/j.1600-0536.2012.02168.x. [Epub ahead of print])

著者名

1. Yuko Chinuki, Sakae Kaneko, Itaru Dekio, Hitoshi Takahashi, Reiko Tokuda, Mizuho Nagao, Takao Fujisawa, Eishin Morita

2. Yuko Chinuki, Hitoshi Takahashi, Itaru Dekio, Sakae Kaneko, Reiko Tokuda, Mizuho Nagao, Takao Fujisawa, Eishin Morita