学 位 論 文 の 要 旨

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学 位 論 文 名　SPECIFIC IgE DETERMINATION TO EPITOPE PEPTIDES OF ω-5 GLIADIN AND HIGH MOLECULAR WEIGHT GLUTENIN SUBUNIT IS A USEFUL TOOL FOR DIAGNOSIS OF WHEAT-DEPENDENT EXERCISE-INDUCED ANAPHYLAXIS

発 表 雑 誌 名（巻，初頁～終頁，年）　The Journal of Immunology (175, 8116-8122, 2005)

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論 文 内 容 の 要 旨

INTRODUCTION

Food-dependent exercise-induced anaphylaxis is a distinct form of a common food allergy induced by the combination of causative food ingestion and physical exercise. In Japanese patients with food-dependent exercise-induced anaphylaxis, the most frequently causative food is wheat. In order to diagnose wheat-dependent exercise-induced anaphylaxis (WDEIA) we typically perform an exercise challenge test combined with wheat ingestion. However this provocation test is unsafe for patients because anaphylactic shock was sometime provoked during the test. Therefore an *in vitro* diagnostic method predicting the development of symptoms by wheat and exercise-challenge is necessary for patients with WDEIA. In the previous study, we have identified ω-5 gliadin and high molecular weight glutenin subunit (HMW-glutenin) as major allergens responsible for WDEIA. In addition we have determined sequential IgE-binding epitope sequences of ω-5 gliadin and demonstrated that patients with WDEIA had specific IgE to those epitope peptides. In this study, in order to develop a new useful *in vitro* method for diagnosis of WDEIA, we determined IgE-binding epitopes of HMW- glutenin, and then evaluated usefulness of measurement of IgE antibodies specific to combined epitope peptides of ω-5 gliadin and HMW-glutenin in diagnosis of WDEIA.

MATERIALS AND METHODS

Subjects

Thirty patients with WDEIA with recurrent episodes of anaphylaxis and a positive provocation test took part in this study. Twenty-five healthy subjects and 25 atopic dermatitis patients with wheat and/or gluten-specific IgE antibodies in their serum served as controls.

Analysis of IgE binding epitopes of HMW-glutenin.

The overlapping peptides were synthesized on a SPOTs membrane (Sigma-Genosys) in accordance with manufacturer's instruction based on the amino-acid sequence of the HMW-glutenin. The SPOTs membrane was blocked overnight at 4℃ with blocking buffer (Sigma-Genosys). The membrane was washed with TBST [50 mM Tris-bufferd saline, 1% tween 20, pH7.4] 3 times for 10 min at room temperature and then incubated overnight at 4℃ with 10% serum of patients. After washing with TBST, the membrane was incubated with horseradish peroxidase-conjugated rat anti-human IgE monoclonal antibodies (BIOSOURCE). The bound anti-human IgE antibodies were detected using ECL-Plus detection regent (Amersham Biosciences). The critical amino acids for IgE binding in the epitope sequence were determined by amino-acid substitution method.

Measurement of epitope peptide-specific IgE antibodies

The epitope peptides, KPQQQSPQQQFPQQQIPQQQ (peptide A) and PTSPQQSGQ-GQQPGQGQQ (peptide B), were synthesized to a purity of at least 80%. Specific IgE levels to epitope peptide A and B in the sera were determined using Pharmacia CAP-System (Pharmacia Diagnostics).

RESULTS AND DISCUSSION

Three IgE-binding epitopes, QQPGQ, QQPGQGQQ and QQSGQGQ, were identified in HMW-glutenin. The critical amino acids for IgE binding in these epitopes were position 1-5(QQPGQ) or 1-4(QQPG) for QQPGQ, 1-5(QQPGQ) and 7(Q) for QQPGQGQQ, and 1(Q), 3-5(SGQ), 7(Q) for QQSGQGQ, suggesting that QQPGQXQX and QXSGQXQ are major epitope sequences of HMW-glutenin.

For measurements of specific IgE levels to IgE binding epitope sequences of ω-5 gliadin and HMW-glutenin, peptide, A (KPQQQSPQQQFPQQQIPQQQ) and peptide B (PTSPQQSGQGQQPGQGQQ), were designed and chemically synthesized. Peptide A and B contain the IgE-binding epitope sequences of ω-5 gliadin (QQIPQQQ, QQFPQQQ, and QQSPQQQ) and of HMW-glutenin (QQPGQ, QQPGQGQQ, and QQSGQGQ), respectively. Peptide A inhibited serum IgE-binding to QQIPQQQ, QQFPQQQ, and QQSPQQQ, which are individual synthetic epitope peptides of ω-5 gliadin. Similarly, combined epitope peptide B inhibited IgE-binding to individual epitope peptides of HMW-glutenin. These results suggest that the IgE-binding ability of the synthetic combined peptide was equal to that of the individual epitope peptides. Specific IgE levels of peptides A, peptide B, wheat and gluten in the sera of subjects were determined using CAP-System FEIA. When a cutoff value in CAP-RAST is set at 0.35 kUa/L, none of the 25 healthy control subjects had IgE antibodies specific to wheat, gluten, peptide A and peptide B. Twenty-nine of 30 (97%) patients with WDEIA had specific IgE antibodies to peptide A and/or peptide B. In contrast, only 18 of 30 (60%) and 24 of 30 (80%) patients with WDEIA showed positive reaction to wheat and gluten, respectively. Ten of 25 (40%) patients with atopic dermatitis who had no obvious allergic reactions after ingestion of wheat products had positive CAP-RAST (>0.34 kUa/L) for epitope peptides indicating low specificity of the test. However, when the cutoff value was set at 1.0 kUa/L, sensitivity and specificity of the epitope peptide-specific CAP-RAST was satisfactory because sensitivity and specificity of the test would reach to 97% (29/30) and 98% (1/50), respectively.

CONCLUSION

Our results strongly indicate that determination of specific IgE to synthetic epitope peptide of wheat ω-5 gliadin and HMW-glutenin is useful to identify patients with WDEIA.