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ORIGINAL ARTICLE

IgE cross-reactivity of peanut with walnut and soybean in children with food allergy

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Abstract
Background: Peanut allergies are common and can be life-threating for sensitised individuals. Peanut allergens share significant amino acid homology with those of other legumes and tree nuts, but their cross-reactivity still remains unclear.
Objective: We sought to determine the clinical significance of the cross-reactivity of peanut allergens with those of walnut and soybean.
Methods: Pooled sera from eight subjects with both peanut and walnut specific IgE were investigated in an inhibition test. After the sera were incubated with either peanut or walnut protein extracts, the quantity of IgE antibodies against the peanut and walnut was measured using an immunoCAP test. Likewise, pooled sera from 18 subjects with both peanut and soybean specific IgE antibodies were incubated with either peanut or soybean protein extracts and evaluated with a peanut and soybean immunoCAP test. SDS-PAGE and immunoblotting were also performed with peanut, walnut and soybean protein extracts and relevant sera.
Results: Peanut specific IgE was inhibited up to 20% and 26% by walnut and soybean protein extracts, respectively. In reverse, walnut and soybean specific IgE were inhibited up to 21% and 23% by peanut protein extracts, respectively. In the immunoblot analysis, pooled serum from the subjects with peanut specific IgE antibodies reacted with walnut protein extracts significantly.
Conclusion: Although the clinical significance of the cross-reactivity of peanut specific IgE with walnut and soybean protein extracts has not been established, we believe that individuals who are allergic to peanuts need to be cautious about consuming walnuts and soybeans.

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Introduction

The prevalence of food allergies has been increasing in recent years and can affect up to 10% of young children and 2–3% of adults globally.[1,2] Food allergies can cause urticaria, angio-oedema, rhinitis, wheezing, and in some cases can be life threatening due to anaphylactic shock. A recent meta-analysis study reported that fatal food anaphylaxis has an incidence rate of 1.81 per million person-years.[3]

Peanut is one of the primary allergenic foods. It contains at least 18 proteins that have been shown to cause specific IgE reactivity and have a higher potential to cause anaphylactic shock compared to other foods.[4–6] The peanut allergens, vicilin (Ara h 1), 2S albumin (Ara h 2), and 11S globulin (Ara h 3), share significant amino acid homology with their respective allergens from other legumes and tree nuts.[7–9]

Tree nut, despite its botanical taxonomic distance, is believed to be cross-reactive with peanut, due to the high number of individuals reported to be co-sensitised or co-allergic to peanut and tree nut.[10] A study of the prevalence of peanut and tree nut allergies among 1218 newborns in the United Kingdom revealed that 1 in 200 children could have adverse reactions to either peanuts or tree nuts by the age of four.[11] In another study among individuals with nut allergies, 65% reacted specifically to peanuts of which 30% also reacted with other tree nuts.[12]

Soybean, belonging to the same legume family as the peanut, has also been investigated as a cross-reactive food with peanuts. Of 69 legume-sensitive individuals, 43% showed sensitivity to soybean and 87% to peanut.[13] It has also been reported that soybean immunotherapy in a peanut allergy mouse model resulted in a significant reduction of clinical symptoms following peanut challenge compared with placebo-treated mice.[14]

However, despite the above findings, the clinical significance of cross-reactivity between peanut allergens and those of walnut and soybean remains unclear. With this in mind, we sought to determine the cross-reactivity of peanut allergens with those of walnut and soybean in the sera of allergic patients.

Materials and methods

Subjects

Pooled sera from eight subjects with allergic sensitisation to both peanut and walnut were used to investigate cross-reactivity of peanut and walnut in an inhibition test (Supplemental Table 1). For the peanut and soybean cross-reactivity test, the pooled sera from 18 subjects with both peanut and soybean specific IgE antibodies were evaluated (Supplemental Table 2). Allergic sensitisation, the presence of specific IgE, was evaluated using an immunoCAP test (Phadia AB, Upsala, Sweden). The clinical data of the subjects used for the immunoblot analysis are summarised in Table 1. The pooled sera from eight subjects without an allergic sensitisation were used as a normal control. All sera were obtained from Severance Children's Hospital of Yonsei University, Seoul (South Korea). This study was approved by the institutional Review Board of Severance Children's Hospital of Yonsei University.

Protein extraction

To obtain protein extracts, peanuts, walnuts and soybeans were purchased and finely ground. The ground meals were defatted with cold (4 °C) acetone (1:5, w/v) under constant stirring for one hour and then filtered with filter paper. The defatting procedure was repeated until the filtrate became clear. The defatted meals were completely air dried at ambient temperature and then stirred in phosphate buffered saline (PBS, pH 7.4) (1:4, w:v) for 48 h at 4 °C. The extracts were centrifuged twice at 13,000 × *g* for 30 min at 4 °C. The supernatants were filtered and dialysed against several changes of distilled water for 48 h at 4 °C. The extracts were centrifuged once again, lyophilised, and stored at −20 °C until use.

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The extracts were resuspended in PBS and the protein concentration of each of the extracts was measured by the Bradford assay. Equal concentration of the samples was mixed with loading buffer (60 mM Tris–HCl, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue) and heated for 5 min in a heating block at 4 °C. The samples were then analysed by SDS-PAGE. Following electrophoresis, the gels were stained with Coomassie brilliant blue or used for immunoblotting.

IgE immunoblotting

Proteins separated by SDS-PAGE were electrotransfered onto a polyvinyl-difluoride membrane. After blocking with 5% skimmed milk in PBS containing 0.1% tween 20, immunodetection of IgE-binding proteins was performed by sequential incubation with subjects' sera (diluted 1:10) and alkaline phosphatase-conjugated goat anti-human IgE (ε-chain specific) (Sigma Chemical, MO, USA). IgE reactive protein bands were visualised using 5-bromo-4-chloro-3′-indolyphosphate p-toluidine salt/nitro-blue tetrazolium chloride (Promega, USA).

Inhibition test

Pooled sera, which were positive for both peanut and walnut by ImmunoCAP, were incubated with 0, 0.1, 1, 5, 10, 50, 100 μg of either peanut or walnut protein extracts (9:1, w:v) over night at 4 °C. The IgE-reactivity of the inhibited sera against peanut and walnut was then measured by immunoCAP. Pooled sera of both peanut and soybean ImmunoCAP positive sera were also inhibited with either peanut or soybean protein and measured by a CAP radio allergosorbent test against peanut and soybean. House dust mite extract (*Dermatophagoides pteronyssinus*; Yonsei University, Seoul, Korea) was used as a negative control inhibitor.