

Characterization of novel allergens in peanut

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BACKGROUND

Peanut allergy is characterized by a higher frequency of severe and fatal reactions than found in other food allergies and represents a lifelong health problem often starting in early childhood. Peanut allergy represents the most common cause of fatal and near fatal food-related anaphylaxis and affects with an incidence of around 1% of the children a relevant number of individuals. Moreover, this particular food allergy is complicated by the common use of peanuts as additive hidden in various foods. Hidden allergens are not obviously recognizable as such by allergic patients and therefore difficult to avoid.

OBJECTIVE

In order to understand the relationship between clinical manifestation and the severe and complex immune responses, the allergenic components of peanuts need to be identified and fully characterized. Peanuts have a complex protein chemistry containing over 30 different proteins and more of the half of these are able to bind serum IgE from sensitized individuals. Therefore, crude peanut extracts contain a mixture of non-allergenic and allergenic proteins

not quantifiable at single protein level. Peanut allergic individuals may experience symptoms ranging from mild urticaria, facial swelling, abdominal cramp to hypertension with anaphylactic shock. The contribution of specific proteins to the potency of peanuts for provoking severe symptoms is not understood and there are no current *in vitro* tests that can distinguish between patients experiencing mild or severe reactions, or predict the severity of a reaction. The identification of further minor and major allergenic components and the subsequent establishment of individual patients' IgE-reactivity profiles is necessary to throw light upon this matter. The quantitative determination of specific IgE raised against a single allergen in serum requires the availability of highly pure protein which also can substantially contribute to improve the development of sensitive methods for the detection of hidden allergens added to foods and to study their stability.

METHODS

To isolate a panel of peanut allergens efficiently we have applied a recently described technology based on phage surface display of cDNA expression products. The major advantage of the

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cloning system termed pJuFo [1] results from the physical linkage between gene products displayed on the phage coat and the genetic information required for their production. The direct linkage of cDNA gene products via the phage surface to the DNA encoding the product itself, allows screening of as much as 10^{11} single clones on a single well of a microtitre plate coated with a ligand. This semifluid screening approach which keeps the library in a fluid phase allows the specific enrichment of clones interacting with the solid-phase immobilized serum IgE from allergic patients by repeated rounds of phage affinity selection.

RESULTS

In addition to the known allergens Ara h 1 and Ara h 2 we were able to identify four allergens with estimated MW of 36,16,14.5 and 14 kDa [2]. Three of them formally termed Ara h 4, Ara h 6 and Ara h 7 show significant sequence similarities to the family of seed storage proteins and the fourth (Ara h 5) corresponds to the well known plant allergen profilin. Immunoblotting of the six expressed recombinant allergens with 40 sera of peanut allergic individuals selected according to case history, RAST and IgE-immunoblot analysis

shows 14 individual recognition pattern and the following frequency of specific IgE-binding: Ara h 1 was recognized by 65%, Ara h 2 by 85%, Ara h 4 by 53%, Ara h 5 by 13%, Ara h 6 by 38% and Ara h 7 by 43% of the selected sera. Antigen-specific IgE ELISA assays of selected sera confirm the immunoblot analysis results.

CONCLUSION

The frequency of the specific IgE-binding as well as the individual recognition pattern of 40 patients' sera towards the six allergens leads to the conclusion that the allergenic potential of the four newly identified allergens may also play an important role compared to the already known major allergens Ara h 1 and Ara h 2. Therefore this panel of allergens can be used to develop a highly sensitive diagnostic system based on recombinant allergens. All of the selected peanut-positive sera can detect at least one of the six identified recombinant allergens which can be used to establish individual patients' reactivity profiles. A comparison of these profiles with the clinical data will possibly allow a further insight into the relationship between clinical severity of the symptoms and specific IgE-levels towards the six peanut allergens.

REFERENCES

1. Crameri R., Suter M. – Display of biologically active proteins on the surface of filamentous phages: A cDNA cloning system for selection of functional gene products linked to the genetic information responsible for their production. *Gene*, 1993, 137, 69-75.
2. Kleber-Janke T., Crameri R., Appenzeller U., Schlaak M., Becker W.M. – Selective cloning of peanut allergens, including profilin and 2S albumins, by phage display technology. *Int. Arch. Allergy Immunol.*, 1999, in press.

