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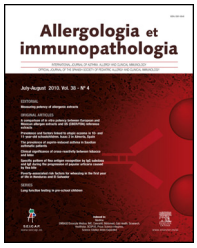

ORIGINAL ARTICLE

Modulation of IgE immunoreactivity to broad bean proteins after food processing in a Moroccan population

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Abstract
Background: The aim of this study was to assess the sensitivity profile of the population of Fez and Casablanca in Morocco to dry broad bean (*Vicia faba*), and to investigate the effect of food processing (heat and/or enzymatic hydrolysis by pepsin) on the human IgE binding capacity to broad bean proteins (BBP).
Methods: Sera samples from 146 patients with atopic hypersensitivity were recruited in order to evaluate specific IgE levels to native and processed broad bean proteins by ELISA. Under the same conditions, we assessed the immunoreactivity of rabbit IgG obtained by immunisation with native BBP.
Results: High IgE levels to BBP were found; in fact, 79.3% of children and 80.4% of adults had positive values. The heat treatment (70 °C during 60 min) of dry beans proteins showed slight reduction in recognition of these antigens by rabbit IgG (22%) and by human IgE (12%). Pepsin hydrolysis decreased rabbit-IgG recognition by 55% in the first 30 min of treatment. In contrast, and under the same conditions, pepsin increased human-IgE recognition with an average of 143% for all patients. However, the combination of the two treatments (heating and pepsin digestion) showed a decrease of 16% in BBP recognition for all patients.
Conclusions: This study demonstrates a high sensitivity of a Moroccan population to broad bean proteins which was resistant to heat and digestion by pepsin.

Introduction

Food allergies are a pressing worldwide problem, and their prevalence seems to be on the rise. They can induce serious systemic symptoms that affect the quality of life. They are found in 5% of young children and 4% of adults in Western countries.[1]

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Food legumes play an important role in the human diet, providing a high proportion of proteins. Their consumption is very frequent in the Mediterranean area. Broad beans (*Vicia faba L.*), which belong to the Fabaceae family, are protein-rich legume seeds that are typically used for animal feed and human food. Broad beans are among the most consumed food in the Moroccan diet. Many case reports have indicated that legumes are responsible for allergic reactions. In Spain, lentils and chickpeas are the most frequent cause of allergic reactions to legumes in children.[2,3] In India, allergies to kidney beans, chickpeas and peanuts are common.[4] Peanut and soy are the legumes most frequently involved in human food allergies in countries such as the United States, the United Kingdom and Japan.[5,6] In Morocco, peanuts are identified as an important food causing allergies.[7,8] Allergic reactions to broad beans have rarely been reported in the literature, but a few cases have been observed previously in Italy[9,10] and Spain.[11]

In order to manage and to minimise the risk of food allergies, investigations have studied the effect of food processing on food allergenicity. In legumes, it has been shown that heating or enzymatic hydrolysis processing may enhance, reduce, or eliminate their allergenic potential.[12–17]

The objective of this study was to evaluate the sensitivity profile of the Moroccan population from the two cities of Fez and Casablanca to broad bean proteins, and to investigate the modulation of this sensitivity by heat and/or enzymatic hydrolysis.

Methods

Patients

The work was conducted on a sera-bank, composed of samples obtained from 155 volunteers. From these patients, nine were non-atopic, and 146 atopic. The atopic-patients were consulted for hypersensitivity, addressed by dermatology and pneumology services to laboratories to measure their total IgE. The patients were recruited from July 2010 to December 2010 after ethical approbation.

After formal consent from each patient, a serum sample was collected at the University Hospital Centre of Fez as well as from biomedical laboratories in Fez and Casablanca. The collected sera were stored at −20 °C until used. These patients had not been sensitised to dry broad bean, nor were they challenged orally.

Extraction and treatment of the broad bean proteins

Bean seeds were very finely ground. The powder or flour obtained was defatted with chloroform and then dried before proteins were extracted by suspending the samples in PBS (phosphate buffer solution pH 7.4) at 20% (w/v). The mixture was stirred for 2 h, filtered and then centrifuged at 3000 rpm for 15 min at 4 °C. The collected supernatant, considered as native broad beans proteins (BBP), was frozen at −20 °C until use.

The native BBP was then treated in four different ways. It was either (1) heated at different temperatures (70, 80, 90 and 100 °C) for 60, 120 and 180 min; (2) treated in an acidic (pH 2) or basic (pH 11) medium for 60, 120, 180, and 210 min at 37 °C; (3) digested by pepsin (hog stomach, 3354 U/mg) at a concentration of 50 U/ml in an acidic environment (pH 2) during 30–210 min at 37 °C; or (4) processed by a combination of the two treatments (heating and enzymatic digestion; 1 and 3).

Production of polyclonal antibodies against the BBP

To study the immunoreactivity of antibodies with BBP, we prepared IgG antibodies against native BBP. These antibodies were obtained after immunisation of rabbits against the native BBP using Freund's adjuvant.

The BBP were injected subcutaneously at several points on the animals' back in combination with complete Freund's adjuvant for the first injection and with incomplete Freund's adjuvant in subsequent immunisations at one week intervals. After one month, animals were sacrificed and blood samples were collected in dry tubes. After centrifugation for 15 min at 3000 rpm at 4 °C, the sera were supplemented with sodium azide 0.02% and frozen at −20 °C.

IgE determinations

Total IgE was evaluated by direct ELISA as described before.[18] Briefly, diluted human sera were placed in 96 micro-titration plate wells and incubated overnight at 4 °C. The non-specific sites were saturated with bovine serum albumin (BSA) 0.25% (200 μl/well). Then, 100 μl of human anti-IgE peroxidase conjugate was added and immune complex revealed after the addition of 0.05% of orthophenylenediamine (OPD). Absorbance was measured at 490 nm by an ELISA reader (LabsystemsMultiskan MS). Positive and negative controls were included in each plate to check the specificity and sensitivity of each measure.

For specific IgE, the BBP diluted at 0.5 mg/ml in PBS was deposited on a micro-titration plate (100 μl/well) indirect ELISA was used. After overnight incubation at 4 °C, wells were washed, saturated with bovine serum albumin 0.25% and the plate was treated the same way as for the total IgE determination.

The binding of rabbit IgG to BBP was determined by ELISA similarly to that of specific IgE.

Polyacrylamide gel electrophoresis of BBP

Beans proteins were separated by 12% (w/v) polyacrylamide gel electrophoresis under denaturing or non-denaturing conditions. The native and treated proteins were denatured by boiling for 3 min in the presence of SDS 10% and β-mercaptoethanol 0.8% (denaturing conditions). The migration was done under a 25 mA current and the gel was stained with 0.1% Coomassie blue R250.

Ethics

This study was approved by the ethics committee at The University Hospital Centre of Fez.