Abstract

Over the last few decades prevalence and severity of atopic diseases has increased worldwide. Millions of people around the globe are estimated to suffer with varieties of ailments and distress on account of allergy. With the rising incidence, deteriorating quality of life and mounting expenditure in healthcare, the allergic diseases have posed a major concern to the society. Also food allergy has been reported to be fatal in highly sensitized individuals. Around 20-25% of people in Western countries suffer from IgE mediated allergy. Individuals with familial atopy are susceptible to development of allergic reactions to environmental and/or food allergens. Food hypersensitivity can be defined as "all immunologically mediated adverse reactions to food". The components of food responsible for allergic reactions are specific which set a cascade of events including release of antibodies. The current food allergy medications do not relieve underlying mechanism of food allergy but only control symptoms such as coughing, itching, wheezing, gastrointestinal disorders etc. Thus, avoidance of the causative food is the best method to prevent allergic reactions. On the other hand avoidance of food might lead to the nutritional deficiencies especially in children. Attempts are therefore required to solve the problem of food hypersensitivity in the susceptible patient groups.

Legumes are important food allergens but their prevalence may vary among different populations. The present study (chapter 2) describes pattern of sensitization to common legumes among different Indian populations, characterizes allergens of kidney bean and establishes its cross-reactivity with other legumes. Patients (n=355) with history of legume allergy were skin prick tested with 10 different legumes. Specific IgE and total IgE were estimated in sera by enzyme-linked immunosorbent assay. Characterization of kidney bean allergens and cross-reactivity was investigated by immunobiochemical methods. Identification of major allergens of kidney bean was carried out by mass spectrometry. Kidney bean exhibited sensitization in 78 (22%) patients followed by chickpea 65 (18%) and peanut 53 (15%). SPT positive patients showed significantly elevated specific IgE levels against different legumes (r=0.85, p<0.0001). Sera from 30 kidney bean sensitive individuals exhibited increased histamine release from basophils (16-54%) which significantly correlated with their, respective SPT (r=0.83, p<0.0001).
and specific IgE (r=0.99, p<0.0001) values. Kidney bean showed eight major allergens of 58, 50, 45, 42, 40, 37, 34 and 18 kDa on immunoblot and required 67.3±2.51 ng of homologous protein for 50% IgE inhibition. Inhibition assays revealed extensive cross-reactivity among kidney bean, peanut, black gram and pigeon pea. Nano LC-MS/MS analysis identified four allergens of kidney bean showing significant matches with known proteins namely lectin (phytohemagglutinin), phaseolin, alpha-amylase inhibitor precursor and group 3 late embryogenesis abundant protein. Results showed that among all the legumes, kidney bean followed by chick pea and peanut are the major allergic triggers in asthma and rhinitis patients in India. Kidney bean showed eight major allergens and cross reacted with other legumes. It was observed that a combination of SPT, specific IgE and histamine release assay is helpful in allergy diagnosis.

The chapter 3 describes the effect of different processing methods on the allergenicity of legume proteins. The extracts were processed by boiling, γ-irradiation and by combination of both the methods. The changes in soluble protein content, specific IgE binding and allergenic potential of legume proteins were assessed using immunobiological methods. Thermal processing resulted in a 3-4 fold reduction in soluble protein content. There was a significant reduction (p<0.01) in the specific IgE binding of the soluble proteins of kidney bean, black gram and peanut by 74±6.5%, 83±11.6% and 62±7.2%, respectively, after boiling. In addition, boiling decreased the IgE binding of insoluble protein fractions of kidney bean, black gram and peanut by 34±5.2%, 74±15.6% and 44±11.1%, respectively. Boiling followed by γ-irradiation also reduced IgE binding significantly (p<0.05). Biopotency of soluble protein of kidney bean, black gram and peanut was reduced by 7, 3 and 26 folds (p<0.001), respectively and that of insoluble protein decreased by 6, 4 and 8 folds (p<0.001), respectively, after boiling. Combination treatment was effective in reducing the potency of both soluble and insoluble protein significantly as compared to boiling alone (p<0.001). However, γ-irradiation alone did not bring any change in allergenicity. In conclusion, boiling followed by γ-irradiation is effective in attenuating allergenicity of legume proteins.

Enzymatic hydrolysis and further processing is commonly used to produce hypoallergenic dietary products derived from different protein sources, such as cow’s
milk. The chapter 4 describes the effect of enzymatic hydrolysis on the protein extracts of kidney bean, black gram and peanut by sequential action of two enzymes, alcalase and flavourzyme. Immunoreactivity to raw and hydrolyzed kidney bean, black gram and peanut extracts was evaluated by ELISA, IgE immunoblotting, ELISA inhibition, basophil histamine release assay and in vivo by SPT. During the hydrolysis by individual and/or sequential action of endo- and exoproteases, significant reduction of 58-79%, 61-97% and 72-93% allergenicity in the three study legumes kidney bean, black gram and peanut was observed by ELISA. The presence of intact basic polypeptide chains is responsible for the formation of IgE complexes and cross linking of IgE on sensitized basophils and mast cells. However, the digestion of these polypeptide chains by enzymatic hydrolysis caused a loss of antigenic/allergenic activity by destruction of IgE-binding epitopes suggested by histamine release assay as significant reduction in the release of histamine from basophils was observed with hydrolyzed legume extracts. In conclusion, these legume protein hydrolysates could be useful for the development of specialized hypoallergenic food products.

Purified proteins are required for component based diagnosis and therapy of allergy to obviate the drawbacks associated with the use of crude antigenic extracts. The chapter 5 is aimed to isolate and characterize a major allergen from kidney bean. Kidney bean allergen was purified using Q Sepharose column, Superdex 75 and C18 column. The protein appeared as a single band at 31 kDa on SDS-PAGE and showed IgE binding to 88% patients' sera by ELISA and immunoblotting. SPT with purified protein identified 78% kidney bean hypersensitive patients. Significant release of histamine from sensitized basophils was observed after challenge with the purified protein. PAS staining showed it to be a glycoprotein, but no change in its IgE binding was observed after periodate oxidation. The purified protein was potent and required 102 ng of self protein for 50% IgE inhibition. Mass spectrometric analysis identified the purified protein as phytohemagglutinin and it showed hemagglutination with human erythrocytes. Cross-reactivity was observed with peanut and black gram with IC50 of 185 and 228 ng respectively. In conclusion, a 31 kDa major allergen of kidney bean was purified and identified as phytohemagglutinin with cross-reactivity to peanut and black gram.