Chapter 1:
Review of literature
The literature with respect to my work undertaken for PhD dissertation on food allergy to legumes has been briefly reviewed in the light of current literature in the following pages:

The human immune system has evolved by generating variety of cells and effector molecules which are capable of recognizing and eliminating a wide variety of foreign invaders. The environment contains a range of microbes and bioparticles that attacks the host continuously. The immune system uses complex protective mechanisms to control and eliminate these organisms. Normally, it generates an immune response wherein the effector molecules induce a localized inflammatory response that eliminates antigen(s) without damaging the host’s tissue. But under certain circumstances, this inflammatory response can have deleterious effects resulting in significant tissue damage, morbidity or mortality. This inappropriate immune response to an antigen/allergen is termed as hypersensitivity or allergy. These reactions may develop in the course of either humoral or cell-mediated responses and can be classified as immediate or delayed, depending on the time elapsed between the exposure to the antigen and the appearance of clinical symptoms. Exposure to allergens can give rise from mild reactions to life threatening systemic reactions (anaphylaxis). Allergic reactions may progressively transform into regular diseases such as asthma, rhinitis, atopic dermatitis, conjunctivitis, urticaria, eczema, angioedema, oral allergy syndrome and gastrointestinal allergies in pre-disposed individuals.

HISTORY OF ALLERGY

The description of an allergic reaction was made as early as 2641 BC, when Pharaoh Menes experienced anaphylactic reaction on being stung by a wasp. Some intolerance to foods such as cheese was described by Hippocrates, 375 B.C. The first classical description of allergic rhinitis was given by John Bostock in 1819 and later Blackley in 1873 demonstrated the causative agent as grass pollens. In 1902, Richet and Portier demonstrated respiratory distress and death when dogs were injected with a second small dose of toxin from sea anemone (Portier and Richet, 1902). In 1906, Clemens von Pirquet introduced the term ‘allergy’ to describe any state of altered immunological reactivity,
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giving rise to hypersensitivity and laid the foundation for modern allergology (von Pirquet, 1906). This term was proposed for the general concept of a changed reactivity with Greek origin _allos_ means “other” and _ergon_ means “work”. Portier and Richet also introduced the term ‘anaphylaxis’ to describe the increased sensitivity and the absence of protection against, the effects of the toxin. Dale and Laidlaw in 1911 discovered that histamine is the principal mediator of anaphylaxis. Immunoglobulin E (IgE) was identified as reagin or serum factor that could spontaneously sensitize skin and mediate a positive immediate-type skin reaction. Its study began in 1921 with the report of Prausnitz and Kästner and was lately discovered as a unique human immunoglobulin class by Ishizaka and Ishizaka (1967) and Johansson and Benich (1968). The term atopy was first used by Coca and Cooke (1923) to describe a tendency to develop immediate-hypersensitivity reactions to allergens that are commonly encountered in the general environment. Atopic individuals produce high amount of IgE in response to specific allergens (Mekori, 1996). Gell and Coombs classified hypersensitivity in four types in 1963 (Figure 1.1). Later, a fifth type of hypersensitivity was also added to this list (Rajan, 2003).

1. Type I hypersensitivity (Anaphylactic/Immediate): It is triggered by the interaction of IgE and allergens on the surface of mast cells or basophils resulting in the release of pharmacologically active mediators such as histamine, serotonin, leukotrienes, prostaglandins, eosinophil chemotactic factors etc. This reaction is manifested within minutes after exposure to an allergen and is therefore referred as immediate hypersensitivity. The reaction involves two phases (Figure 1.2).

   a. Sensitization: This phase sets in after first exposure to the allergen. The antigen presenting cells (APCs) internalize, process and present the allergen to T-lymphocytes. The interaction of these T-cells with B-lymphocytes signals heavy chain switching to ε type and secretion of allergen specific IgE. The released IgE binds to high affinity receptors on mast cells and basophils (Figure 1.2). This completes the sensitization phase (Descotes and Choquet-Kastylevsky, 2001; Goldsby _et al._, 2003). In this phase immune system is stimulated for IgE production, but there are no symptoms.
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Figure 1.1: Types of hypersensitive reactions: Gell and Coombs’ classification of hypersensitive reactions (Source: Goldsby et al., 2003).

Figure 1.2: Phases of allergic reaction: Allergen exposure activates B cells to form IgE secreting plasma cells. These IgE molecules bind to IgE-Fc receptors on mast cells and basophils. Subsequent exposure to the allergen leads to cross-linking of the bound IgE, thereby activating the mast cells/basophils to release pharmacologically active mediators. These mediators are responsible for the pathologic reactions of immediate hypersensitivity (Source: Goldsby et al., 2003).
b. Re-exposure: Subsequent exposure to allergen causes memory B cells to proliferate and secrete allergen specific IgE in large amount. The released IgE binds to receptors on mast cells and basophils. Interaction of multivalent allergen with receptor bound IgE causes cross-linking of receptors. This initiates intracellular signaling that leads to degranulation of cells with the release of pro-inflammatory mediators (Figure 1.2) (Goldsby et al., 2003). The mediators exert their effect on different parts of system such as smooth muscles, blood vessels etc. The reaction manifests itself in two phases:

i. Early phase response: It occurs within 5 to 30 minutes of allergen exposure. IgE receptor cross-linking following allergen exposure signals microtubule polymerization. The polymerized microtubules allow transport of cytoplasmic granules to plasma membrane for fusion (Leung, 1997). Fusion of these granules empties the preformed mediators e.g. histamine, serotonin (Table 1.1) etc. Since the constricting effects of histamine on smooth muscles last only for 1-2 hours, the changes tend to subside after most of the granules are empty (Goust and Finn, 2003).

Table 1.1: Principal mediators involved in type I hypersensitivity.

<table>
<thead>
<tr>
<th>Preformed mediators</th>
<th>Newy synthesised mediators</th>
</tr>
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<tbody>
<tr>
<td><strong>ECF-A</strong></td>
<td><strong>Prostaglandin</strong></td>
</tr>
<tr>
<td><em>Eosinophil chemotactic factor of Anaphylaxis</em> - attracts eosinophils to the site</td>
<td><em>Vasoconstriction and bronchospasm</em></td>
</tr>
<tr>
<td><strong>NCF – A</strong></td>
<td><strong>Bradykinin</strong></td>
</tr>
<tr>
<td><em>Neutrophil chemotactic factor – attracts neutrophils to the site</em></td>
<td><em>Increased vascular permeability and smooth muscle contraction</em></td>
</tr>
<tr>
<td><strong>Histamine</strong></td>
<td><strong>Leukotriene</strong></td>
</tr>
<tr>
<td><em>Vasodilation and bronchospasm</em></td>
<td><em>Vasoconstriction and bronchospasm</em></td>
</tr>
<tr>
<td><strong>Heparin</strong></td>
<td><strong>Thromboxane A2</strong></td>
</tr>
<tr>
<td><em>Promotes phagocytosis</em></td>
<td><em>Vasoconstriction and platelet aggregation</em></td>
</tr>
<tr>
<td><strong>Proteases</strong></td>
<td><strong>PAF</strong></td>
</tr>
<tr>
<td><em>Bronchial mucus secretion, degradation of blood vessel basement membrane and generation of complement split products</em></td>
<td><em>Platelet Activating Factor – bronchospasm, platelet aggregation, chemotaxis of neutrophils, eosinophils</em></td>
</tr>
<tr>
<td><strong>TNF alpha</strong></td>
<td></td>
</tr>
<tr>
<td><em>Tumour necrosis factor – transmigration of eosinophils and neutrophils</em></td>
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</tr>
</tbody>
</table>
ii. **Late phase reaction:** The reaction sets in 4 to 8 hours later without additional exposure to antigen. The mast cells and basophils continue to synthesize other mediators after early phase. The late phase mediators which are mixture of leukotrienes (Table 1.1) reach their effective concentration after few hours of challenge and have long lasting effects.

2. **Type II hypersensitivity (Cytolytic/Cytotoxic):** It occurs when antigen bound to cells is recognized by antigen specific IgG or IgM. The surface associated immune complexes are recognized by complement resulting in cell lysis. Complement is usually (but not always) necessary to affect cellular damage. The target cells can also be killed nonspecifically through antibody dependent cell mediated cytotoxicity. This mechanism involves binding of non-sensitized cells like monocytes, polymorphs and killer cells to target by their specific receptor. The reactions include mismatched blood transfusion, organ transplant rejection, etc.

3. **Type III Hypersensitivity (Immune Complex):** It involves circulating antibody (IgG or IgM) that reacts with free antigen. These circulating complexes can then deposit on tissues and leads to activation of complement. This may lead to massive inflammation due to protein C5a, influx of neutrophils discharging their lysosomes and causing tissue damage as well as inflammation. Lysis of the surrounding tissue takes place due to membrane attack complex and aggregation of platelets results in more inflammation and formation of microthrombi that block capillaries. This can lead to cell necrosis and hemorrhage. The examples are serum sickness, "Arthus" reaction, autoimmune acute glomerulonephritis, rheumatoid arthritis, systemic lupus erythematosus and allergic bronchopulmonary aspergillosis.

4. **Type IV hypersensitivity (Cell Mediated):** This is the only class of hypersensitive reaction which is mediated by T cells. The main type of cells involved are TH1 cells, originally termed T_{DH}, after the alternative name for this reaction - delayed type hypersensitivity (DTH). There are 4 subtypes, based on the T-cell subpopulation involved. Type 1 helper T cells, type 2 helper T cells, cytotoxic T cells and IL-8 secreting T cells. These cells, sensitized after contact with a specific antigen, are activated by re-exposure to the antigen; they damage tissue by direct toxic effects or through release of
cytokines, which activate eosinophils, monocytes, macrophages, neutrophils, or killer cells depending on type. Disorders involving type IV reactions include contact dermatitis, hypersensitivity pneumonitis, allograft rejection, tuberculosis and many forms of drug hypersensitivity.

5. Type V hypersensitivity: This is a recently included hypersensitivity reaction. In this case, the antibodies recognize and bind to cell surface receptors, instead of binding to cell surface components for destruction of cells. This process either prevents the intended ligand binding with the receptor or mimics the effects of the ligand, thus impairing cell signaling (Rajan, 2003). Some clinical examples are Graves' disease and myasthenia gravis.

**IMMUNOGLOBULIN E (IgE)**

In normal (non-atopic) individuals, the blood serum level of IgE is the lowest of all the antibody classes (0.004% of the total circulating immunoglobulins), falling within 0.1-0.4 µg/ml. However, it is capable of triggering the most powerful immune reactions. IgE plays central roles in type I hypersensitivity. Serum half life of IgE is 2.5 days, but receptor bound IgE may remain up to months due to high affinity binding and protection from proteolysis (Steinsvik et al., 1997).

IgE plays an important role in clinical diagnosis and management of patients with allergy and facilitating basic research involved in studying mechanisms of human allergic disease. IgE has two biological functions: (1) It is involved in Type I hypersensitive reactions. (2) It also plays a protective role in parasitic helminth diseases. Serum IgE levels rise in parasitic diseases and thus measuring IgE levels is helpful in diagnosing parasitic infections (Carvalho et al., 2006; Flohr et al., 2009). IgE molecules have high affinity for mast cells and basophils (Hasegawa et al., 1999). The antibody mediates its functions by binding to IgE receptors present on the surface of its target cells (Oettgen and Geha, 2001). There are two cell surface receptors for IgE.

1. **FcεRI-high affinity IgE receptor**: FcεRI has high affinity for IgE (Ka = 10^{-10} M). The high affinity of this receptor enables it to bind IgE despite the low serum concentration of IgE. It is usually a heterotrimer, composed of 4 subunits: the ε chain
which binds IgE, the tetra-transpanning β chain which plays a role in signal transduction and 2 γ-chain dimer (Lin et al., 1996; Gould et al., 2003). FcεRI interacts with the CH3/CH3 and CH4/CH4 domains of the IgE molecule via the two Ig-like domains of the α-chain. (Figure 1.3).

(2) FcεRII/CD23-low affinity IgE receptor: It has low-affinity for IgE (Ka= 5 × 10^{-7} M) and is expressed on B-lymphocytes, eosinophils, macrophages and airway smooth muscle cells. It belongs to the lectin family of receptors and has an unusual orientation in the membrane with its NH2-terminus directed toward the cell interior and its COOH-terminus toward the extracellular space (Figure 1.3). It presents IgE bound antigen to T_{H}2 cells and is involved in the production of pro-inflammatory cytokines such as TNF-α, IL-1 and IL-6. (Tu et al., 2005).

Figure 1.3: Structures of IgE and FcεRI. (a) The IgE domain structure is shown with each immunoglobulin domain represented by an oval. The two light chains are formed by two domains. The heavy chains are formed by a total of five domains each, with the two heavy chains forming the overall dimeric structure of the antibody. The two antigen-binding sites are located at the interface of the heavy and light chains and the receptor-binding domains (C3 and C4) are located at the ends of the two heavy chains. (b) & (c) The full IgE receptor structure is shown on the left and is composed of four protein chains: an α chain, a β chain and two γ chains anchored in the cell membrane. (Source: Goldsby et al., 2003).
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**CELLULAR KEY PLAYERS**

**T lymphocytes:** T cells or T lymphocytes belong to a group of white blood cells known as lymphocytes, play a central role in cell-mediated immunity. They can be distinguished from other lymphocytes by the presence of a T cell receptor on the cell surface. They are called T cells because they mature in the thymus. There are several subsets of T cells, each with a distinct function:

1. **CD4⁺ (T helper):** They regulate the cellular and humoral immune responses and recognize peptide presented on the membrane-proximal B2 domain of the antigen-presenting class II HLA molecule. Once activated, they divide rapidly and secrete small proteins called cytokines that regulate or assist in the active immune response. These cells can differentiate into one of several subtypes, including Th1, Th2, Th3, Th17, or Th9, which secrete different cytokines to facilitate a different type of immune response. Signalling from the APC directs T cells into particular subtypes (Gutcher and Becher, 2007).

2. **CD8⁺ T cells (cytotoxic):** They act to kill cells infected with intracellular microbes. Their receptors recognize peptide antigens presented in membrane-proximal α3 domain of major histocompatibility complex (MHC) I on the target cell i.e. APC. These cells destroy virally infected cells and tumor cells and are also implicated in transplant rejection. Through IL-10, adenosine and other molecules secreted by regulatory T cells, the CD8⁺ cells can be inactivated to an anergic state, which prevent autoimmune diseases such as experimental autoimmune encephalomyelitis (Jiang and Chess, 2004).

**B lymphocytes:** B cells are lymphocytes that play a large role in the humoral immune response. B cells are an essential component of the adaptive immune system and represent approximately 15% of peripheral blood leukocytes. B cells, which are the precursors of plasma cells, are characterized by the presence of a B-cell receptor able to bind specifically an antigen. Their principal functions are to make antibodies against antigens including IgE, perform the role of APCs and eventually develop into memory B cells after activation by antigen interaction. Recently, a suppressive function of B cells has also been discovered (Mauri and Bosma, 2012).
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Mast cells: Mast cells are a heterogeneous group of multifunctional cells that play an important role in allergy, parasitic infestation, inflammation, angiogenesis and tissue remodeling. They are present below the skin, in subserosal and submucosal layers of the airway and gastrointestinal tract and along blood vessels in the connective tissue of all the organs, except brain. They express high-affinity IgE receptor (FcεRI) and FcγRIIb (CD32), cytokine receptors (IL-3R, IL-4R, IL-5R, IL-9R, IL-10R, granulocyte-macrophage colony-stimulating factor, chemokine receptors and nerve growth factor receptors.

Basophils: Basophil granulocytes, mostly referred to as basophils, are the least common of the granulocytes, representing about 0.01% to 0.3% of circulating white blood cells. The name comes from the fact that these leukocytes are basophilic, i.e., they are susceptible to staining by basic dyes. Basophils contain large cytoplasmic granules which obscure the cell nucleus under the microscope. However, when unstained, the nucleus is visible and it usually has 2 lobes.

Eosinophils: Eosinophils function as APCs and directly amplify the Th2 immune response (Shi, 2004). In healthy conditions these cells are spread in the lamina propria of the gastric and intestinal mucosa (Mishra et al., 1999). They get activated by IL-5 cytokine and chemokines such as CCL5 and eotaxins. They also express FcεRI, bind to IgE (Janeway et al., 2001) and release various cytotoxic and pro-inflammatory mediators including reactive oxygen species. A number of cytotoxic granule and vesicular proteins such as major basic protein, eosinophil cationic protein, peroxidase, neurotoxin, cytokines, chemokines and phospholipids are released from eosinophils. These active mediators lead to mucosal damage in chronic atopic diseases.

Mediators: Cross-linking of high affinity receptors (FcεRI) by the allergen-IgE complexes results in activation and degranulation of mast cells, basophils, eosinophils and macrophages and subsequent release of mediators (Table 1.2). Preformed mediators are packaged within secretory granules of these granulocytes. Principal granule constituents include histamine, serine proteases, carboxypeptidase A and proteoglycans (heparin and chondroitin sulfate E). The major lipid mediators include prostaglandin D2 and leukotrienes.
Table 1.2: Mediators produced by mast cells, basophils and eosinophils.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Mediator category</th>
<th>Mediator</th>
<th>Function/Pathologic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mast cells and basophils</strong></td>
<td>Stored preformed in cytoplasmic granules</td>
<td>Histamine</td>
<td>Increase vascular permeability, stimulate smooth muscle cell contraction</td>
</tr>
<tr>
<td></td>
<td>Enzymes, acid hydrolases, cathepsin G, carboxypeptidase</td>
<td></td>
<td>Degrade microbial structures, tissue damage/remodelling</td>
</tr>
<tr>
<td>Major lipid mediators produced on activation</td>
<td>Prostaglandin D2</td>
<td>Vasodilation, bronchoconstriction, neutrophil chemotaxis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Leukotriene C4, D4, E4</td>
<td>Prolonged bronchoconstriction, mucus secretion, increased vascular permeability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Platelet activating factor</td>
<td>Chemotaxis and activation of leukocytes, bronchoconstriction, increased vascular permeability</td>
<td></td>
</tr>
<tr>
<td><strong>Cytokines produced on activation</strong></td>
<td>IL-3</td>
<td>Promotes mast cell proliferation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TNF-α</td>
<td>Promotes inflammation, late phase reaction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-4, IL-13</td>
<td>Promotes Th2 differentiation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-5</td>
<td>Promotes eosinophil production and activation</td>
<td></td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>Stored preformed in cytoplasmic granules</td>
<td>Major basic protein, eosinophil cationic protein</td>
<td>Toxic to helminth, bacteria, host cells</td>
</tr>
<tr>
<td></td>
<td>Eosinophil peroxidases, lysosomal hydrolases, lysocephospholipases</td>
<td></td>
<td>Degrade helminthic and protozoan cell walls, tissue damage/remodelling</td>
</tr>
<tr>
<td>Major lipid mediators produced on activation</td>
<td>Leukotriene C4, D4, E4</td>
<td>Prolonged bronchoconstriction, mucus secretion, increased vascular permeability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Lipoxins</td>
<td>Promotes inflammation</td>
<td></td>
</tr>
<tr>
<td>Cytokines produced on activation</td>
<td>IL-3, IL-5, GM-CSF</td>
<td>Promotes eosinophil production and activation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>IL-8, IL-10, eotaxin, RANTES</td>
<td>Chemotaxis of leukocytes</td>
<td></td>
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</table>

**Th1/Th2 paradigm**

The type of immune response generated to an antigen depends on the profile of cytokines released by CD4+ T-helper cells. In an atopic individual, the resulting allergen-specific T cells have a Th2 skewed phenotype with the cytokine profile dominated by IL-4, IL-5 and IL-13. In contrast, the response from the non-atopic person is characteristically Th1 skewed directed by IFN-γ. Besides, regulatory cytokines such as IL-12, IL-10 and TGF-β are important for maintaining the dynamic balance between Th1 and Th2 responses.
ALLERGENS

Antigens that elicit an allergic response are defined as allergens and possess specific peptide domain called epitopes involved in IgE binding. Allergens belong to protein families with diverse biologic functions.

Based on the route of entry to the human system the allergens can be categorized in four groups:

(i) **Inhalants**: Aeroallergens such as pollen, fungi, insects, dust mites, animal dander are prevalent in environment and enter through the airways into the nose/lungs and are grouped as inhalant allergens. They are the major triggers for respiratory allergy such as asthma and rhinitis (Guilbert et al., 2004). The food particles present in air, e.g. rye, wheat flour (implicated in Bakers’ asthma), soy dust and fumes from cooking foods (chickpea, pea, rice etc.) also induce respiratory exacerbations and act as inhalant allergens.

(ii) **Ingestants**: Allergens entering into the body through oral route or gastrointestinal tract are called ingestants. Foods such as milk, egg, peanuts, tree nuts, wheat, fish, seafood, cereals and drugs viz. penicillin, aspirin etc are common ingestant allergens.

(iii) **Contactants**: Allergens that sensitize through direct contact with the external body surface line skin eyes or mucous membranes are called contactants. The common examples are latex, poison ivy, oak, cosmetics etc. Foods can also enter into the body through contact and cause allergic reactions. Reports are there for fruit, vegetable, juices, fish, rice and peanuts etc. (Deluze et al., 1991; Strid et al., 2005; van Do et al., 2005).

(iv) **Injectants**: Allergens that enter directly into the circulation are called injectants. Drugs (injectibles), insect venoms are common examples of injectant allergens.

**IgE mediated food allergy**: It accounts for the majority of food allergic reactions mediated by antigen specific IgE antibodies. There is temporal relationship between the reaction and prior exposure to food. Such reactions can be generalized or localized to a specific organ system.
Non-IgE mediated food allergy: These reactions are caused by: (a) antibodies, other than IgE (i.e. IgG, IgM & IgA), (b) immune complexes formed by food and food antibodies and (c) cell-mediated immunity.

Pseudoallergic reactions: These are non-immunological reactions that reproduce allergic symptoms. Some of the manifestations are histamine dependent while others can be due to other mediators.

Pathophysiology of food allergy
The gastrointestinal tract is the largest immunologic organ in the body lined by a single layer of epithelium. The gastrointestinal mucosal immune system must simultaneously battle pathogens, recognize and ignore harmless food proteins and allow colonization with commensal bacteria. Disruption of this fine balance alters a normal state of oral tolerance to foods, possibly resulting in allergy. Oral tolerance may be breached directly during ingestion, or it may be bypassed altogether by presentation of proteins by alternative routes, such as via the respiratory tract or skin. In oral allergy syndrome, also known as pollen-food-related syndrome, oral tolerance is bypassed because sensitization occurs through the respiratory route (Fernandez-Rivas et al., 2006). The mechanism of oral tolerance and food hypersensitivity is presented in Figure 1.4.

Clinical manifestations of food allergy
Food allergy presents a wide spectrum of clinical manifestations, including mild rashes to urticaria, angioedema, atopic dermatitis, respiratory symptoms, gastrointestinal symptoms and life threatening anaphylaxis. The important IgE mediated food allergic manifestations are described below:

Asthma: Asthma is a chronic disease of the lung characterized by the reversible obstruction to respiratory airflow (peak flow variation ≥20%) caused by excess mucus production, narrowing of the airways and bronchial hyper-responsiveness. Inflammatory response result in mucosal oedema, smooth-muscle constriction, mucus hypersecretion and epithelial cell-shedding and thereby airway narrowing. Asthma can be subdivided into four categories - intermittent, mild, moderate and severe
persistent (Humbert et al., 2007). The diagnosis of asthma is based on the presence of parameters namely, wheezing with and without colds, dyspnea, wheeze after exertion, persistent cough and reversible airflow obstruction (American Thoracic Society, 2003).

Foods can precipitate attacks of asthma in infancy but the role of foods in asthma diminishes during childhood (4-6%) and uncommon precipitants in adults (1-4%) (Novembre et al., 1987). Food sensitivity asthma in adults is largely confined to those exposed to dusty grains, flour, coffee etc. in their work. Egg, seafood and nuts are among the foods most likely to provoke asthma in children (Aba-Alkhail and ElGamal FM, 2000).

Allergic rhinitis (AR): It is characterized by nasal itching, sneezing, watery rhinorrhea and nasal obstruction. Edema and venous engorgement of the nasal mucosa cause blockage of the eustachian tubes, cough and a sensation of pressure in the sinuses. Allergic rhinitis and its impact on asthma (ARIA) has subdivided AR into intermittent and persistent. In intermittent rhinitis, symptoms occur for <4 days per week and/or <4 weeks whereas persistent disease involves frequent symptoms lasting for >4 days per week and/or >4 weeks (ARIA, 2003). The prevalence of childhood AR shows wide variation throughout the world, ranging from 0.8-39.7% (Strachan, 1997). An overall prevalence of AR in adults ranged from 17% in Italy to 29% in Belgium (Bauchau et al., 2004). In addition, allergic rhinitis is a frequent respiratory manifestation affecting 14-20% of food allergic population (Penard-Morand et al., 2005; Wang et al., 2011; Bozkurt et al., 2005).

Cutaneous reactions: These are the most common clinical manifestations of food allergic reactions. Symptoms range from acute urticaria (most common) to atopic dermatitis.

Urticaria: Acute urticaria and angioedema generally appear within minutes of ingestion of the food allergen. The foods commonly causing these reactions in children are eggs, milk, peanut and tree nuts however, in adults fish, shellfish, tree nuts and peanuts are prevalent among US population (Sicherer, 2009).
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Atopic dermatitis (AD): Food allergy plays an important role in the pathogenesis of AD. It is characterized by pruritus, pale, erythematous and violaceous hues, vesiculation, erosion, scaling, exudation, crusting, lichenification and excoriations. In the US, prevalence of atopic dermatitis is 15-20% in children (Oranje et al., 1997). Studies show that 35-40% patients with chronic atopic dermatitis have IgE mediated food allergy.

Gastrointestinal symptoms: These often accompany allergic manifestations in other target organs (skin and lungs). Symptoms range from nausea, abdominal pain, colic, vomiting, diarrhoea to life threatening anaphylaxis.

Oral allergy syndrome (OAS): OAS is characterized by pruritus, hoarseness, tingling and angioedema of the lips, tongue, palate and throat and in the ears. The term 'OAS' was coined by Amlot (1987) to describe associations between allergy to pollen and concomitant allergic reactions to certain fruits, vegetables and spices. OAS affects up to 40% of adults with pollen allergy, especially to birch, ragweed and mugwort pollens (Bircher et al., 1994). Raw potatoes, carrots, celery, apples, hazelnuts and kiwi are often implicated in allergic reactions in birch pollen allergic patients, whereas patients allergic to ragweed pollen develop OAS with fresh melons (watermelon, cantaloupe and honeydew) and banana. OAS is also known as pollen-food syndrome, since reactions to foods may occur without pollinosis and the symptoms may range from oral and gastrointestinal to severe systemic reactions (Mari et al., 2005; Egger et al., 2006).

Anaphylaxis: It is potentially life-threatening clinical condition characterized by multiorgan involvement of the cutaneous, respiratory, cardiovascular and gastrointestinal systems resulting from mast cell degranulation and mediator release. ‘Idiopathic anaphylaxis’ describes patients who experience anaphylactic symptoms with no identifiable etiology. ‘Anaphylactic reaction’ implies occurrence of an IgE type I reaction triggered by an antigen, whereas the term ‘anaphylactoid reaction’ implies a non-IgE mediated cause, such as serum complement activation or direct mast cell degranulation by medications such as vancomycin, narcotic drugs, or radio contrast agents.
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Figure 1.4: Mechanism of oral tolerance. A. Immune responses require T-cell receptor ligation with peptide-MHC complexes in the presence of appropriate costimulatory molecules (CD80 and CD86) and cytokines. B. Low doses of antigen favor tolerance driven by regulatory cells, which suppress immune responses through soluble or cell surface-associated downregulatory cytokines, such as IL-4, IL-10 and TGF-β. C. High-dose tolerance is mediated by lymphocyte anergy or clonal deletion. Anergy can occur through T-cell receptor ligation in the absence of costimulatory signals. Clonal deletion occurs by means of FAS-mediated apoptosis (CD95). (Source: Burks et al., 2008)
Food allergy is the leading cause of generalized anaphylaxis, accounting for approximately 120-150 fatalities in the US and above 200 deaths per year in Europe (Eigenmann, 2003). Most important foods responsible for anaphylaxis are peanuts, tree nuts, fruit with shells and soy, shrimp and milk (Sicherer and Sampson, 2010). In a few reports from India, legumes such as soy bean, chickpea, pea, blackgram and pomegranate have been implicated in anaphylactic reactions (Kumar et al., 2000; Hegde and Venkatesh, 2002; Kumari et al., 2006).

**DIAGNOSIS OF FOOD ALLERGY**

There are several methods for diagnosis of suspected food allergy. Skin testing, food specific IgE, oral food challenge, atopy patch test, basophil activation test and food-specific IgG are few commonly used methods for diagnosis of allergic condition. However, double-blind, placebo-controlled food challenges (DBPCFC) represents the gold standard for diagnosing food allergy.

**Medical history:** The evaluation of food allergy begins with a detailed history, family history (atopy), a list of suspected foods, the quantity of food eliciting a reaction, the reproducibility of the reaction in relation to food ingestion, the time between exposure and reaction, the clinical manifestations produced, resolution of symptoms with elimination of the suspect food and the overall duration of symptoms after each exposure.

A clinically relevant physical examination, with particular focus on suspected target organ systems (e.g. cutaneous, respiratory and gastrointestinal) is performed. The presence of atopic disorders such as asthma, atopic dermatitis and allergic rhinitis implies an increased risk of food allergy.

**Skin prick Testing (SPT):** It is a commonly used method for clinical diagnosis of suspected food allergy and it indicates the IgE sensitization to particular protein in sensitized individual. The presence of antigen specific IgE on mast cells is evident by wheal and flare response after application of allergen extract on skin (Sampson 2001). Wheal diameter is compared with positive control and the response equal to control or more are considered as marked positive skin reactions (Aggarwal et al., 2000). It is assumed that larger wheal diameter indicates allergic condition. Inspite of being rapid
and highly sensitive method, it gives large number of false positive results as compared to other diagnostic methods. In some cases, anaphylaxis has been reported during SPT, therefore this test should be performed in the presence of doctors (Bernstein et al., 2004).

**Food specific IgE**: Increase in level of antigen specific IgE is hallmark for allergenic reaction. The presence of IgE antibody can be easily measured by many serological tests like enzyme allergosorbent test, ELISA and immunoblotting, etc. (Asero et al., 2007).

**Oral Food Challenge**: Double blind placebo controlled food challenge (DBPCFC) is the gold standard method for diagnosis of food allergy (Gellerstedt et al., 2007). Oral food challenge test is used for IgE mediated as well as non-IgE mediated allergic response. Oral food challenge is frequently used in diagnosis of non-IgE mediated allergies as other methods are not so accurate (Sicherer, 2005). In DBPCFC, the food is given at different time intervals to patients to whom the person is suspected to be allergenic and symptoms are observed (Beyer and Teuber 2005). Duration of test is generally 4 to 8 h. So far, DBPCFC is considered more accurate than other known method.

**Atopy Patch Test**: This test is based on the principle of DTH reaction IV in which late-phase immunological reactions occurs (Roehr et al., 2001). It is useful for determining the delayed responses to oral food challenges as response is cell mediated. There is still a need to develop standard guidelines for better interpretation of this assay. It is more costly and time consuming than SPT.

**Basophil Activation Test**: Cellular basophil activation test is currently used diagnostic test that is based on release of histamine and expression of CD63 antigen on outer surface. Upon allergen exposure, crosslinking of IgE antibody occurs and CD63 molecule which are normally present on inner surface of basophil exposed towards outside during degranulation process. The up-regulation of CD63 expression is accompanied by and dependent on p38MAPK phosphorylation and also related to phenomenon of basophil degranulation. Basophils which contain many granules inside the cell are released during this process and elicit allergenic symptoms (de Weck et al., 2008). However, the mechanisms of expression of various other membrane proteins present within basophil need sincere attention.
Food Specific IgG Tests: The titre of specific IgG antibodies and their subclasses fall after withdrawal of the allergen. This method estimates mainly the levels of specific IgG or IgG4. Unfortunately, this test has not much diagnostic value for food allergy as there is lack of convincing results (Atkinson et al., 2004).

Histamine release assay: This is in vitro test that detect histamine released from the patient's basophils in presence of specific allergen. The test employs anti-histamine antibody in a sandwich ELISA to detect histamine released in response to allergen. Alternatively, histamine released by basophils in response to specific allergen is combined with specific chemicals (o-phthalaldehyde) to yield fluorescent products. The measurement of fluorescence intensity gives quantitative estimation of histamine released. The histamine released is expressed as a %age of total histamine release. The cut-off point for significant release is determined in comparison with spontaneous release.

FOOD ALLERGENS

Food allergens are also classified as class I and class II food allergens as given below.

(1) Class I allergens: Class 1 food allergy results from sensitization through the GI tract (Breiteneder and Ebner, 2000). The class 1 food allergens are generally 10 to 70 kDa in size and highly stable when subjected to heat, acid, or proteases (Sampson HA, 1999). These are also called as ‘complete food allergens’. Examples of class 1 food allergens include milk (caseins), peanut (vicillins), egg (ovomucoid) and nonspecific lipid transfer proteins (Sicherer and Sampson, 2006).

(2) Class II food allergens (cross-reactive): Class 2 food allergy results from sensitization to inhalant allergens that are partially homologous to proteins in certain fruits and vegetables and principally occurs in adolescents and adults. Class 2 allergens are heat labile and susceptible to digestive processes (Breiteneder and Ebner, 2000). Consequently, symptoms occur when the food is ingested in the raw form but not in cooked form. Thus, they are often called ‘incomplete food allergens’ or non-sensitizing elicitors. They are the major culprits of adult onset of food allergy. For example, Bet v 1, a major allergen of birch pollen, shares homology with class 2 allergens in various fruits...
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and vegetables (e.g., apple, Mal d 1; carrot, Dau c 1). A similarity in the three-dimensional (3D) structures (rather than the overall sequence identity) between birch pollen epitopes and the epitopes of specific foods has been demonstrated (Jenkins et al., 2005).

Common allergenic foods

Eight foods or food groups recognized as the “Big Eight” account for more than 90% of all IgE mediated food allergies worldwide. These are milk, egg, wheat, fish, crustacean (shrimp, crab, lobster, crayfish shellfish), soybean, peanuts and tree nuts (almonds, walnuts, pecans, cashews, Brazil nuts, pistachios, hazelnuts, pine nuts, macadamia nuts, chestnuts, hickory nuts) (Cucu et al., 2012). The self-reported prevalence of food allergy to these 8 most common allergens is 2.7% among respondents with doctors’ diagnosed food allergy.

(1) Milk: Cow’s milk allergy is the most common food allergy, affecting 2-3% of infants (Branum and Lukacs, 2009), half of which is estimated to be IgE mediated and responsible for up to 13% of fatal food-induced anaphylaxis (Bock et al., 2007). The overall prognosis is favorable, although recent studies have shown a later acquisition of tolerance to cow’s milk in a subset of patients (Skripak JM et al., 2007; Cantani and Micera, 2004). It was recently reported that the majority (75%) of children with cow’s milk allergy tolerate baked milk products (e.g., muffins, waffles, cakes and breads) (Nowak-Wegrzyn et al., 2008). Cow milk contains more than 20 IgE-reactive proteins with β-lactoglobulin (Bos d 5), α-lactalbumin (Bos d 4) and caseins (Bos d 6) as major allergens (Gjesing et al., 1986, Cavagni et al., 1994 and Docena et al., 1996).

(2) Egg: Hen’s egg allergy is the second most common food allergy in infants and young children (Sicherer et al., 2010). Egg allergies are immunologic responses to proteins in foods and include IgE antibody-mediated allergy as well as other allergic syndromes such as atopic dermatitis and eosinophilic esophagitis (Hill et al., 2008). The prevalence of egg allergy confirmed by oral challenge was 1.6% of children 3 years of age in an unselected population in Denmark (Osterballe et al., 2005). A subsequent meta-analysis of the prevalence of food allergy estimated that egg allergy affects 0.5 to 2.5% of young children (Rona et al., 2007). Five major allergenic proteins from the egg of the domestic...
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chicken (*Gallus domesticus*) have been identified that are responsible for IgE mediated reactions; these are designated Gal d 1-5 (Heine *et al.*, 2006; Szépfalusi *et al.*, 1994; Quirce *et al.*, 2001).

(3) **Fish:** Fish allergy is the third most common food allergy after milk and egg in parts of Europe, US and Canada. Fish allergy is less common, affecting 0.18 per cent of children and 0.56 per cent of adults (Connett *et al.*, 2012; Sicherer *et al.*, 2004). Fish is most common causative agent of IgE mediated food allergy, in coastal countries such as Scandinavia and Japan where fish consumption is high (Pascual *et al.*, 1992; O'Neil *et al.*, 1993). A multicenter study reported that 10% of the anaphylactic reactions in the emergency departments were caused by fish allergens (Clark *et al.*, 2004). Extensive clinical cross-reactivity among fish allergens has been observed. The extensively studied fish is cod fish and the major allergen is a 12 kDa fish parvalbumin (Gad c 1) (van Do *et al.*, 2005; Untersmayr *et al.*, 2005).

(4) **Wheat:** Recent population based studies have shown that the prevalence of wheat allergy and its sensitization has increased since the last few decades. Consequently, some new allergens, including nonspecific lipid transfer protein (Tri a 14), have been identified (Inomata, 2009). Allergenic profile of wheat differs with patient age or symptoms (Battaïs *et al.*, 2005). AD with or without asthma, occurs mainly in children while urticaria and wheat-dependent exercise-induced anaphylaxis is commonly found in adults (Palosuo, 2003). The major allergens present in wheat are α-5 gliadin (Tri a 19) and LMW-glutenins.

(5) **Shellfish:** Prevalence of doctor-diagnosed “shellfish” - crustacean and mollusk-allergy, in adults was found to be 2.3% in the US population (Sampson *et al.*, 2004, Connett *et al.*, 2012). Shellfish allergy is also prevalent in Philippines (2.29%), Singapore (0.26%), Thailand (0.29%). Shellfish allergy is the most common allergy in Canada, affecting 1.42% of the population. Most sufferers are adults, 1.69% have the allergy, compared to just 0.5% of children. In Singapore and other Asian countries with vast coastal regions, the most common causative foods are seafood crustaceans especially prawn and crab and mollusks such as limpet (Thong *et al.*, 2007). Tropomyosin is identified as the major allergen from shrimp (Ayuso *et al.*, 2002).
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(6) Soybean: It is an important causative agent of IgE mediated allergy affecting about 0.6% of population based on their reported symptoms (Burks et al., 2012). To date, food allergy to soy proteins has been described mainly in young children with AD, who often outgrow their soy allergy after 1-2 years of dietary elimination (Martinez et al., 2000; Sampson and Scanlon, 1989). Food and Drug Administration (FDA) documents report that an estimated 0.2% of Americans are allergic to soy, although definitive studies assessing the prevalence of soy allergy are lacking. According to the American Academy of Asthma, Allergy and Immunology, soy is one of eight foods that account for 90% of food allergy reactions in children. In adults, soybean dust elicited respiratory allergy symptoms because of sensitization to the soybean proteins Gly m 1 and Gly m 2 (Quirce et al., 2000). However, allergic reactions to soy-derived foods in adults are less frequent (Kleine-Tebbe et al., 2002). The Kunitz trypsin inhibitor (Burks et al., 1994) glycinin, the α-subunit of β-conglycinin and a 50 kDa protein with homology to chlorophyll A-B binding protein were identified as IgE binding components in sera of subjects with soy allergy (Codina et al., 2002).

(7) Peanut: It has been postulated that more than 70% of children will outgrow milk and egg allergies by early adolescence, whereas peanut allergies may usually remain throughout life. The most serious allergic response to peanut allergy is anaphylaxis (Yawn and Fenton, 2012). Allergic reactions to peanuts are particularly common and severe in Western countries. Eleven potentially important allergens of peanut have been described so far and, of these, Ara h 1, Ara h 2 and Ara h 3 have been designated as the major peanut allergens (Burks et al., 1991; Burks et al., 1992; Rabjohn et al., 1999) and four minor allergens, Ara h 4, Ara h 5, Ara h 6 and Ara h 7 (Kleber-Janke et al., 1999), were identified. With the exception of the profilin Ara h 5, these allergens are seed storage proteins. Ara h 8 is a homologue of Bet v 1 and was identified as a major allergen in birch pollen allergic patients with concomitant peanut allergy (Mittag et al., 2004). In a recent study Ara h 2 and Ara h 6 were reported to account for the majority of the effector activity in a crude peanut extract (Zhuang and Dreskin, 2012). Another major allergen in peanut, Ara h 9 was identified among allergic patients from the Mediterranean area. This new member of the LTP allergen family seems to play an important role in peanut allergy among Mediterranean population (Lauer et al., 2009; Krause et al., 2009).
(B) Tree nut: Recently allergy to tree nuts, such as cashew, walnuts, almond, pistachio, hazel nuts and pecans have gained much attention in the United States and Europe due to their high risk of inducing severe anaphylactic reactions (Hasegawa et al., 2009). Most identified tree nut allergens belong to major protein super-families such as vicilins (7S trimeric globulins composed of 50 kDa subunits), legumins (11-13S hexameric globulins with subunits of 30-40 kDa acidic and 17-20 kDa basic peptides) and 2S albumins (15 kDa) with 9 and 5 kDa subunits (Roux et al., 2003).

Other important foods: In certain geographic regions other foods may frequently cause IgE mediated allergies, such as celery in some European countries (Wuthrich et al., 1990), buckwheat in Southeast Asia and sesame in Middle East (Kanny et al., 1996). Few other foods have also been associated with severe reactions that include certain legumes namely dry beans, peas, lentils, black gram and chickpeas and others such as poppy seeds, sunflower seeds, cottonseed, sesame and rice (Atkins et al., 1988; Kagi and Wutrich, 1993; Kalyoncu and Stalenheim, 1993; Kanny et al., 1996; Besler, et al., 2001; Dalai et al., 2002; Kumari et al., 2006).

PURIFIED FOOD ALLERGENS

The purified allergenic proteins are required for component resolved diagnosis (CRD) of food allergy. Allergenic proteins from various food sources such as peanut, milk, soybean, lentil, cowpea and wheat have been isolated and characterized (Jutel et al., 1995; Ebner et al., 1997; Durham et al., 1998). Allergens are named using the first 3 letters of the genus, followed by a single letter of the species and a number indicating the chronologic order of allergen purification (WHO/IUIS, 1994). Food allergens based on the source are mainly categorized into, 1) plant food allergens and, 2) Animal food allergens.

Plant food allergens

Plant tissues that are consumed by humans contain thousands of different proteins. Proteins are clustered together into families if they have residue identities of 30% or greater or if they have lower sequence identities but their functions and structures
are very similar. Families whose members have low sequence identities but whose structures and functional features suggest a probable common evolutionary origin are placed together in superfamilies (Murzin et al., 1995; Lo Conte et al., 2002). Most plant food allergens belong to a few protein families and superfamilies (Table 1.3) (Radauer et al., 2003).

1. The cupin superfamily: The cupins are a functionally diverse superfamily of proteins that share 2 short conserved consensus sequence motifs and a \( \beta \)-barrel structural core domain to which the term cupin was given (Dunwell, 1998). Bicupins include the globulin seed storage proteins that are major components of the human diet. The globulins have been studied in most detail in legumes, in particular soy and peanut. On the basis of their sedimentation coefficient, the globulins can be divided into the 7S vicilin-type globulins and the 11S legumin-type globulins.

1.1. Vicilins: Mature 7S globulins are trimeric proteins of about 150-190 kDa. The molecular weights of the subunits range from about 40-80 kDa. Vicilins lack cysteines and therefore contain no disulfide bonds (Shewry et al., 1995). The 3D structure of the 7S globulins canavalin from jack bean (Ko et al., 2000; 2001), phaseolin from French bean (Lawrence et al., 1994) and \( \beta \) subunit of \( \beta \)-conglycinin from soybean (Maruyama et al., 2001) have been determined. These structures illustrate that trimeric vicilins are disk shaped.

The best analyzed allergenic vicilin is the major peanut allergen Ara h 1, which is responsible for the majority of cases of fatal anaphylaxis induced by a plant food (Burks et al., 1991). In soybean approximately 50% of the 7S fraction consists of its seed storage globulins mainly composed of \( \beta \)-conglycinin, which is a 180 kDa glycoprotein. The allergen Len c 1 from lentils was identified as a \( \gamma \)-vicilin subunit (Sanchez-Monge et al., 2000).

1.2. Legumins: Mature 11S globulins are hexameric proteins that are initially assembled and transported through the secretory system as intermediate trimers (Shewry et al., 1995). In the protein storage vacuole, each subunit of the trimer is proteolytically cleaved to yield an acidic 30-40 kDa polypeptide linked by a disulfide bond to a basic
polypeptide of approximately 20 kDa. Cleavage is accompanied by the transformation of 2 trimeres into a mature hexameric 11S globulin (Muntz, 2001). The 3D structure of proglycinin from soybean, an 11S globulin precursor, has been determined (Adachi et al., 2001). Ara h 3 was identified as the N-terminal portion of a peanut glycinin subunit, an 11S legumin-like seed storage protein (Rabjohn et al., 1999).

Table 1.3: Allergens from various protein families and superfamilies.

<table>
<thead>
<tr>
<th>Protein superfamily</th>
<th>Protein family</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cupin superfamily</td>
<td>Vicilins</td>
<td>Ara h 1 (peanut), Jug r 2 (walnut)</td>
</tr>
<tr>
<td></td>
<td>Legumins</td>
<td>Ara h 3/4 (peanut), Cor a 9 (hazelnut)</td>
</tr>
<tr>
<td>Prolamin superfamily</td>
<td>2S albumins</td>
<td>Bet e 1 (Brazil nut), Ses i 2 (sesame)</td>
</tr>
<tr>
<td></td>
<td>nsLTPs</td>
<td>Pru p 3 (peach), Cor a 8 (hazelnut)</td>
</tr>
<tr>
<td></td>
<td>Cereal a-amylase/protease inhibitors</td>
<td>Rice dimeric alpha-amylase inhibitor</td>
</tr>
<tr>
<td></td>
<td>Cereal prolamins</td>
<td>Tri a 19 (wheat), Sec c 20 (rye)</td>
</tr>
<tr>
<td>Plant defense (PRs, Proteases, Protease inhibitors)</td>
<td>PR-2: endo-B1, 3-glucanases</td>
<td>Banana glucanase</td>
</tr>
<tr>
<td></td>
<td>PR-3: class I chitinases</td>
<td>Pers a 1 (avocado), Cas s 5 (chestnut)</td>
</tr>
<tr>
<td></td>
<td>PR-4: Win-like proteins</td>
<td>Brl r 2 (turnip)</td>
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<tr>
<td></td>
<td>PR-5: TLPs</td>
<td>Pru av 2 (cherry), Mal d 2 (apple)</td>
</tr>
<tr>
<td></td>
<td>PR-9: peroxidases</td>
<td>Tri a Bd 36K (wheat)</td>
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<tr>
<td></td>
<td>PR-10: intracellular PR-proteins</td>
<td>Api g 1 (celery), Mal d 1 (apple)</td>
</tr>
<tr>
<td></td>
<td>PR-14: nsLTPs</td>
<td>Pru p 3 (peach), Cor a 8 (hazelnut)</td>
</tr>
<tr>
<td></td>
<td>Papain-like cysteine proteases</td>
<td>Act c 1 (kiwi), Gly m Bd 30K (soybean)</td>
</tr>
<tr>
<td></td>
<td>Subtilisin-like serine proteases</td>
<td>Cup m 1 (melon)</td>
</tr>
<tr>
<td></td>
<td>Kunitz-type protease inhibitors</td>
<td>Soybean trypsin inhibitor</td>
</tr>
<tr>
<td>Structural proteins</td>
<td>Profilins</td>
<td>Api g 4 (celery), Pru av 4 (cherry)</td>
</tr>
<tr>
<td></td>
<td>Oleosins</td>
<td>Peanut oleosin</td>
</tr>
<tr>
<td>Storage proteins</td>
<td>Patatin</td>
<td>Sola t 1 (potato)</td>
</tr>
<tr>
<td>Enzymes</td>
<td>Phenylcoumaran benzyl ether reductases</td>
<td>Pyr c 5 (pear)</td>
</tr>
<tr>
<td></td>
<td>Cyclophilins</td>
<td>Carrot cyclophilin</td>
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<td></td>
<td>8-Fructofuranosidases</td>
<td>Lyc e 2 (tomato)</td>
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<tr>
<td></td>
<td>Flavin adenine dinucleotide-dependent oxidases</td>
<td>Api g 5 (celery)</td>
</tr>
</tbody>
</table>
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2. The prolamin superfamily: The existence of this superfamily was proposed on the basis of the presence of a conserved skeleton of 8 cysteine residues within the protein’s sequence (Kreis et al., 1985). This superfamily is named after the cereal prolamins, the major storage proteins of cereal grains (with the exception of oats and rice), which are characterized by their high contents of proline and glutamine (Shewry et al., 2002). In addition to the cereal prolamins, the broader definition of the superfamily now includes several important plant allergen families, 2S albumin seed storage proteins, nsLTPs and cereal seed inhibitors of α-amylase, trypsin, or both. All of these low molecular weight proteins are cysteine rich and have similar 3D structures that are rich in α-helices. The soybean hydrophobic protein that is responsible for respiratory allergy to soybeans also possesses the characteristic 8-cysteine residue skeleton and a similar conformation (Gonzalez et al., 1995; Baud et al., 1993).

2.1. 2S albumins: The 2S albumins are a major group of storage proteins present in many dicotyledonous plant species (Shewry et al., 1995). Several of the tree nut and seed allergens are 2S albumins. They include Ber e 1 from Brazil nut (Alcocer et al., 2002; Pastorello et al., 1998), Jug r 1 from the English walnut and 2S albumins from cashew nuts (Teuber et al., 1998; Teuber et al., 2002). Ses i 2 is the clinically most important allergen of sesame seeds (Pastorello et al., 2001). Ara h 2, 6 and 7 belong to the conglutin protein family, which is related to the 2S albumin family (Kleber-Janke et al., 1999). Ara h 2 was found to act as a weak trypsin inhibitor that protects Ara h 1 from degradation by trypsin (Maleki et al., 2003).

2.2. Nonspecific lipid transfer proteins (nsLTPs): The family of nsLTPs comprises 7-9 kDa monomeric proteins that are held together by 4 disulfide bonds to form a hydrophobic tunnel (Shin et al., 1995). The nsLTPs have a wide distribution, with sequences being available from fruits, nuts, seeds and vegetables. nsLTPs have been identified as major peach (Pru p 3) (Sanchez-Monge et al., 1999; Pastorello et al., 1999a), apple (Mal d 3) (Pastorello et al., 1999b) and apricot (Pru ar 3) (Pastorello et al., 2000) allergens in Mediterranean populations. The nsLTPs of sweet cherry and the European plum, Pru av 3 (Scheurer et al., 2001) and Pru d 3 (Pastorello et al., 2001), respectively, have been reported as the allergens. Additional allergenic nsLTPs
have been described as Zea m 14 from corn, Aspa o 1 from asparagus and Vit v 1 from grape. Although allergic reactions to lettuce are not frequent, its nsLTP was reported to cause anaphylaxis in susceptible individuals and has received the designation Lac s 1 (Pastorello et al., 2000; Diaz-Perales et al., 2002; Pastorello et al., 2003; San Miguel-Moncin et al., 2003).

2.3. The family of cereal α-amylase and protease inhibitors: Plants have evolved a certain degree of resistance through the production of defense compounds and proteins, including α-amylase inhibitors (Franco et al., 2002). Allergenic members of this family are capable of sensitizing susceptible atopic patients through ingestion or inhalation (James et al., 1997). The best characterized allergens of this group are the α-amylase inhibitors of rice grain (Nakase et al., 1996; 1998).

2.4. Cereal prolamins: The cereal prolamins, named glutenins and gliadins in wheat, secalins in rye and hordeins in barley, are the major storage proteins found in the endosperm of cereal grains (Muntz, 1998). The highest IgE reactivity was found for low-molecular-weight glutenin, followed by α-gliadin and γ-gliadin (Maruyama et al., 1998). α-5 gliadin (Tri a 19) was described as an important allergen for young children with immediate allergic reactions to ingested wheat products (Palosuo et al., 2001a). It also cross-reacts with γ-70 and γ-35 secalins from rye (Sec c 20) and with γ-3 hordein from barley (Hor v 21) (Palosuo et al., 2001b).

3. Proteins of the plant defense system: The plant defense system makes use of a wide range of compounds and proteins to resist biotic and abiotic stresses.

3.1 Pathogenesis-related (PR) proteins: PRs are defined as proteins that are induced specifically in a plant as a response to infections by pathogens, such as fungi, bacteria, viruses, or adverse environmental factors (Midoro-Horiuti et al., 2001; Hoffmann-Sommergruber, 2000).

The PR-3 family includes class I chitinases. Class I chitinases from fruits such as avocado (Pers a 1), banana and chestnut (Cas s 5) have been identified as major allergens (Sowka et al., 1998; Sanchez-Monge et al., 1999; Diaz-Perales et al., 1998).
Thaumatin-like proteins (TLPs) are members of the PR-5 family. Mal d 2, an important allergenic TLP of apple fruits, is associated with IgE mediated symptoms in individuals with apple allergy. Purified recombinant Mal d 2 displayed the ability to bind IgE from individuals with apple allergy equivalent to natural Mal d 2. In addition, the recombinant Mal d 2 exhibited antifungal activity, implying a function in plant defense against fungal pathogens. The TLP of sweet cherry, Pru av 2, was identified as a major allergen (Inschlag et al., 1998).

3.2. Kunitz-type protease inhibitors: The Kunitz family of soybean trypsin inhibitors is one of the many families of proteinase inhibitors (Laskowski and Kato 1980). It comprises plant proteins with inhibitory activity against various proteinases, such as serine proteinases from the trypsin and subtilisin families, thiol proteinases and aspartic proteinases. All members with inhibitory activity contain 2 disulfide bridges. The Kunitz soybean trypsin inhibitor was also reported to induce food anaphylaxis (Moroz et al., 1980). IgE-binding potato proteins with molecular weights of 16-20 kDa were identified as protease inhibitors that belong to the family of Kunitz-type trypsin inhibitors and were designated Sol a 2, 3 and 4 (Seppala et al., 2001).

3.3. Proteases: Proteases are grouped into families on the basis of detectable sequence similarity. Two families of proteases contain allergenic proteins, the papain-like cysteine proteases and the subtilisin-like serine proteases (Siezen and Leunissen, 1997). The papain family is named after papain from papaya. Similar proteases are found in other fruits, including bromelain from pineapple, actinidin from kiwi and ficin from fig.

4. Profilins: Profilins are 12 to 15 kDa cytosolic proteins that are found in all eukaryotic cells. The major role of profilin in plant cells is the rapid reorganization of microfilaments during processes like cytokinesis, cytoplasmic streaming, cell elongation and growth of pollen tubes and root hairs (Valster et al., 1997; Ramachandran et al., 2000). Profilin sequences are highly conserved among plants, with 70-85% identical residues in sequences of different species. Two known allergic plant profilin structures from birch pollen (Fedorov et al., 1997) and Hevea brasiliensis latex show that the sequence conservation among profilin allergens is reflected by highly similar structures.
Profilins are quite sensitive to heat denaturation and gastric digestion and thus food allergy caused by profilin is usually confined to the oral allergy syndrome elicited by raw foodstuffs (Ballmer-Weber et al., 2002).

5. Newly identified classes of allergens: The number of allergens with known sequences continuously increases. New families of storage and structural proteins and metabolic enzymes are added to the already established protein families of allergens. Sela t 1, a patatin storage protein, was described as a novel allergen of potato tuber (Seppala et al., 1999). A peanut oleosin was suggested as a new allergen (Pons et al., 2002). Oleosins are proteins of 16 to 24 kd that represent the protein components of plant lipid storage bodies called oil bodies (Murphy et al., 1991). Pyr c 5, a Bet v 6 related food allergen from pear, was identified as a phenylcoumaran benzylic ether reductase (Karamloo et al., 2001).

Animal food allergens

The most important animal food allergens are present in milk, egg and seafood. Important mammalian milk allergens are α-lactalbumin, (Bos d 4), β-lactoglobulin (Bos d 5) and the casein (Bos d 8). Ovomucoid, ovalbumin, ovotransferrin, lysozyme C and serum albumin are important egg allergens. In seafoods, two major groups of allergenic proteins: tropomyosins of crustacea and mollusks and the calcium-binding parvalbumins are present in fish and amphibians.

Cross-reactivity of food allergens

Cross-reactivity indicates the presence of epitopes shared by species that are phylogenetically related. It is the term commonly used to express similarities of different species with respect to sensitization and reactions in the human IgE system. It denotes not only the presence of shared T- and B- cell epitopes but is also different from parallel or independent sensitization to multiple fungal species. Apart from the common epitopes on proteins, carbohydrates of the glycosylated proteins can contribute towards cross-reactivity. Due to this, cross-reactivity can occur between some unrelated proteins from different genera as well. In general, proteins having >70% sequence identity are more likely to be cross-reactive. Cross-reactivity generally refers to clinical reactivity to a source (allergen) without previous exposure while ‘co-recognition’ refers to the IgE
reactivity to a number of sources bearing homologous molecules with unknown primary sensitizers (Ferreira et al., 2004).

Interestingly, apart from cross-reactivity among different legumes there also exists cross-reactivity between legumes and pollen, so pollen sensitization may be associated with increased risk of legume allergy as a result of common antigenic determinants. Pea and beans have in vitro cross-reactivity with pollens of *Lolium perenne*, *Olea europaea* and *Betula alba* (Ibanez et al., 2003). However, soybean allergens have cross-reactivity with birch pollen allergens. Also, soybean has been reported to have cross-reactivity with potato (Fu et al., 2002). In the case of peanut, there exists 25-50% cross-reactivity with tree nuts (Sicherer et al., 1998; Ewan, 1996). It was reported that the major peanut allergen Ara h 2 shares IgE-binding epitopes with almond and Brazil nut allergens, which increases the incidence of tree nut sensitization in peanut-allergic individuals (de Leon et al., 2007). The isoflavone reductase allergen from pea has a 56-80% sequence similarity with its homologue proteins from various plants, e.g., birch, apple, pear, orange, mango, litchi, carrot, banana and chickpea (Karamloo et al., 1999; Vieths et al., 1998). Cross-reactivity between allergens from mesquite tree pollen and lima bean (Dhyani et al., 2007) and allergens of latex and chickpea have also been observed (Branco Ferreira et al., 2004). These cross-reactivity studies may provide valuable information to allergic patients who may take an informed decision of avoiding the particular food that shares the IgE binding epitope to whom the person is sensitized.

**MANAGEMENT OF FOOD ALLERGY**

Four modalities are employed for the management of food allergic disorders, elimination and avoidance of specific food allergen(s), pharmacological therapies, preventive measures and immunotherapy.

(1) Elimination and avoidance of specific allergens: Currently, the best way to manage food allergy is avoidance of the allergen and prompt treatment of symptoms when they arise. Accidental exposure to food allergens is inevitable and patient and family education regarding cross-contamination, label reading and prompt recognition and treatment of food allergic reactions is a cornerstone of food allergy management.
Because avoidance of specific allergens can limit the availability of nutritious food choices, nutritional counseling and regular growth monitoring are recommended for all children with food allergy, especially during infancy.

However, elimination of causative foods should be minimized to prevent nutritional disorders and improve the quality of dietary life. Even if a food is positive for specific IgE antibodies and SPT, it should not be eliminated if an oral challenge test is negative. The National Institute of Allergy and Infectious Diseases (NIAID) guidelines recommend nutritional counseling and regular growth monitoring for all children with food allergy. (Han et al., 2012). Young children who are sensitive to multiple major food allergens are at risk for protein and calorie deficiency and may require a hypoallergenic formula to meet their needs. Various hypoallergenic formulas are available based on extensively hydrolyzed casein derived from cow’s milk or on a mixture of single amino acids. Soy protein formula is not an appropriate substitute for patients with cow milk allergy because soy allergy is highly prevalent in patients with cow milk allergy (Muraro et al., 2002). Sheep and goat milk-based formulas are also discouraged because of cross-reactivity with cow milk (Jarvinen and Chatchatee, 2009).

(2) Pharmacological therapies (Medications): Antihistamines are sometimes the only medication needed to reduce itching and rash. However, for patients with more severe symptoms of anaphylaxis, or with respiratory or cardiovascular symptoms, additional treatment is needed. Epinephrine is the ‘gold standard’ medicine for treating allergic (systemic) reactions to food. Epinephrine is the mainstay of treatment for anaphylaxis. Intramuscular injection allows more efficient absorption than the subcutaneous route. The dose is 0.01 ml/kg of 1:1000 dilution of aqueous epinephrine intramuscularly (maximum dose 0.3-0.5 ml, 0.3-0.5 mg). Premeasured doses of epinephrine for self injection allow prompt administration by patients.

Guidelines to determine who should have access to this medication are under scrutiny; candidates include food allergic patients with previous severe reactions, those with allergy to foods that commonly cause severe reactions and those with underlying asthma. Indications for administration of drugs generally include signs of respiratory or cardiovascular allergic symptoms after a known or possible exposure, but patients with a
history of severe reactions might warrant treatment after ingestion of causal food even when there are no symptoms (Sicherer, 2002). Some commonly used drugs for the treatment of allergy and their action of mechanism are given in table 1.4.

Table 1.4: Some drugs and their mechanism in treating type I hypersensitivity.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antihistamines</td>
<td>Block H1 and H2 receptors on target cells</td>
</tr>
<tr>
<td>Cromolyn sodium</td>
<td>Blocks Ca2+ influx into mast cells</td>
</tr>
<tr>
<td>Theophylline</td>
<td>Prolongs high cAMP levels in mast cells by inhibiting phosphodiesterase, which cleaves cAMP to 5' AMP</td>
</tr>
<tr>
<td>Epinephrine</td>
<td>Stimulates cAMP production by binding to β-adrenergic receptors on mast cells</td>
</tr>
<tr>
<td>Cortisone</td>
<td>Reduces histamine levels by blocking conversion of histidine to histamine and stimulates mast cell production of cAMP</td>
</tr>
</tbody>
</table>

(3) **Allergen immunotherapy (AIT):** It is the only treatment able to act on the causes and not merely on the symptoms of allergy. AIT was introduced 100 years ago by Noon but remained an empirical treatment for more than 40 years after the first controlled trial in 1954. The most important was the introduction of venom immunotherapy to prevent anaphylaxis from insect stings in 1978. Most of the immunomodulatory therapeutic approaches operate on restoring the T\(_1\)/T\(_2\) balance or activating regulatory T lymphocytes. The immunotherapy leads to the modulation of both T- and B-cell responses to allergen that includes a decline in antigen specific IgE levels (Frati et al., 2012). Immunotherapy can be classified into 4 types based on the route of administration:

i. **Oral immunotherapy (OIT):** Patients with food allergy would benefit greatly from a definitive treatment that could achieve long-term tolerance. Recent studies demonstrate that OIT can induce desensitization and modulate allergen specific immune responses. OIT holds promise as a novel approach to the definitive treatment of food allergy (Ismail and Tang, 2012). Currently, immunotherapy for cow’s milk or egg allergies is a novel approach that expands the possibility of an active treatment to improve the quality of life of patients and their families (Crisafulli et al., 2012).

ii. **Intranasal:** Allergen is given in the form of aerosols using nasal spray in the mucosa of nose. Few studies report significant improvement in rhinitis, reduction of nasal
sensitivity and local immunological responses (Tari et al., 1992) with mild adverse reactions such as rhinitis and itching in the nose.

iii. **Subcutaneous immunotherapy (SCIT):** In this immunotherapy, increasing doses of allergen are injected subcutaneously. It has been effective in patients suffering from OAS after ingestion of fresh fruits, nuts and vegetables (Asero, 2000). Rush immunotherapy (rapidly increasing doses) with peanut has also shown improvement in few patients but it resulted in systemic reactions in 39% of the cases (Nelson et al., 1997). Therefore, SCIT for food allergy is currently not recommended (Burks et al., 2001).

iv. **Sublingual immunotherapy (SLIT):** It is safer and noninvasive alternative to subcutaneous desensitization (Giovanni, 2007). SLIT appears to be very safe for respiratory allergies (Calderon et al., 2012) and therefore the same was believed to be true for food allergies. High doses of allergens are administered orally and held under the tongue for 1-2 min prior to swallowing or spitting. Although, OIT had a higher level of efficacy, but more patients had systemic reactions as compared to SLIT patients (Keet et al., 2012).

**WORLDWIDE PREVALENCE OF FOOD ALLERGY**

The rise in prevalence of food allergy in developed countries is evident from reports. Although most emerging risk factors seem related to the "modern lifestyle" the reasons for the rise in prevalence of food allergy remain poorly understood. In general population self-reported prevalence of food allergy range from 5% in Korea, 3.5% in France and 22.2% in Australia (Falcao et al., 2004). However, the perception of food allergy is much higher than the actual prevalence established by controlled oral food challenges. Food allergies affect 6% of young children and 3-4% of adults in Western countries (Sicherer and Sampson, 2009). The incidence of food allergy-related anaphylaxis is rising particularly in children younger than 5 years of age (Allen and Koplin, 2012).

The prevalence of self-reported food allergy is very high compared with objective measures. There is marked heterogeneity between studies regardless of type of assessment or food items considered and in most analyses this persisted after age
stratification. Self-reported prevalence of food allergy varied from 1.2-17% for milk, 0.2-7% for egg, 0-2% for peanuts and fish, 0-10% for shellfish and 3-35% for any food (Sicherer et al., 2004; Sampson et al., 2004; Bock et al., 2007; Branum and Lukacs, 2009; Sicherer et al., 2010; Connett et al., 2012; Sampson et al., 2004). The investigators reported an overall prevalence of self-reported food allergy in 12% for children and 13% for adults, while overall prevalence of self-reported symptoms plus sensitization by DBPCFC for any of these 5 foods was not more than 3% (Rona et al., 2007).

Over 90% of food allergy results from exposure to egg, milk, peanut, tree nut, fish, shellfish, soy and wheat (Sampson, 1999). The prevalence of food allergy is influenced by age, culture and dietary habits. Age is one of the most important factors determining the type of food allergy. The most common offending foods are egg, milk, soy and peanut in children and wheat, shellfish, tree nuts and peanuts in adults. The order of importance of specific allergens varies by country, reflecting the interaction of culture and dietary habits. Peanut, buckwheat, mustard and sesame are good examples. Despite similar levels of peanut consumption, there is a difference in the prevalence of peanut allergy between the US and China and it is generally agreed that this discrepancy stems from the effects of different cooking methods on the allergenicity of peanuts; i.e., roasting using higher temperatures apparently increases the allergenic properties of peanut proteins (Beyer et al., 2001). Buckwheat is an important food causing anaphylaxis in Korea and Japan but this is very rare in other countries (Wieslander and Norback, 2001). Sesame seed allergy is more commonly observed in Israel than elsewhere and mustard allergy is mainly observed in France (Dalal et al., 2002; Rance, 2003). The prevalence of shellfish allergy is 0.5% in Canada, but 4% in Singapore and Philippines (Shek, 2010; Han et al., 2012).

THE INDIAN SCENARIO

In India, the knowledge about food allergy/allergens is limited to only few allergens such as rice, black gram, pigeon pea and chick pea. About 2-8% population in India is estimated to suffer from bronchial asthma with or without rhinitis. Much higher prevalence of food allergy is reported in selected population like asthmatics and atopic dermatitis. There is need to study and characterize foods for allergenicity by using standard immunobiochemical methods. Oral challenges provide the scientific basis for
diagnosing the IgE mediated food allergy. SPT and specific IgE estimation demonstrate sensitization to respective food and show agreement with food challenges in many cases.

In India few systematic studies have been done to identify IgE mediated food allergy (Patil et al., 2001; Kumari et al., 2006; Kumar et al., 2007).

In India, a majority of the population consumes vegetarian diet made up of pulses (legumes), cereals, milk, egg and vegetables. Systematic studies on food allergy are limited to few clinical studies. Preliminary studies based on SPT reported that egg, milk, cereals (corn, barley) and legumes such as pea, black gram and chickpea are major sensitizers among atopic Indian population (Kumar et al., 2000; Parihar et al., 1984; Kumari et al., 2006). Anecdotal reports on fish allergy are also available. Milk, rice, banana, colocasia, refined flour, radish and citrus fruits were reported to induce respiratory symptoms in children upon open (oral) challenges (Sharman et al., 2000). Gupta et al., (1996) reported 40% prevalence of food allergy in children and around 70% in adults after surveying 7208 asthmatics in eastern India. Egg, milk, fish, banana, brinjal, curd and pulses were reported common food allergens in India.

Kumar et al., (2006) have evaluated the significance of various methods used in the diagnosis of food allergy and concluded that elevated serum total IgE, allergen specific IgE and marked positive SPT can serve as marker for atopy and food sensitization. A systemic study from Mumbai surveyed 1400 patients and reported chickpea as an important allergen in the Indian subcontinent showing actual prevalence of 2.2% chick pea allergy in atopic population (Patil et al., 2001). In another study by Kumar et al., (2007) on 1200 patients identified the prevalence of IgE mediated rice allergy in 0.8% of asthma and rhinitis cases.

Legumes are reported to be an important source of IgE mediated Type-I hypersensitivity (Gupta et al., 1996). Legumes belong to fabaceae of leguminosae which is a large food family containing beans, peas, pulses etc. Plants of this family are found throughout the world, growing in many different environments and climates. At present India’s production for pulses is 15.8 million metric tons and occupies first rank in the world for pulses production. Studies had established food allergy in cases of bronchial asthma and anaphylaxis induced by inhaling vapors from cooking legumes (Di Lernia et al., 1992;
Lezaun et al., 1994; Kumar et al., 2007). Rao et al., (2000) identified and isolated allergenic components of cowpea (Vigna sinensis) seeds and its albumin fraction was characterized by gel electrophoresis and immunoblotting. Two protein fractions of 41 and 55 kDa were identified as major allergens which are resistant to heat and proteolytic digestion. In a study by Kumari et al., (2006), 1.7% sensitization to black gram was observed in asthma and allergic rhinitis patients and its eight major allergens were identified. Prevalence of sensitization to different food including legumes was studied on a group of asthma and rhinitis patients where chick pea followed by green gram was identified as the major sensitizer among atopic Indian population (Misra et al., 2008). Pepsin resistance of chickpea, black gram and kidney bean extracts were evaluated using simulated gastric fluid digestion assay for the first time (Misra et al., 2009). The allergenic potential of red gram (Cajanus cajan) was assessed by sensitizing BALB/c mice determining the levels of specific immunoglobulins, histamine, TH2 cytokines. IgE immunoblot detected five allergens namely Caj c 1, 2, 3, 4 and 5 which showed homology to known allergens of soybean (β-conglycinin), lentil (Len c 1 and 2), peanut (Ara h 1) and pea (vicilin) (Misra et al., 2010). Four clinically relevant allergenic proteins from green gram, namely Vig r 2, 3, 4 and 5 were identified which were capable of inducing IgE mediated allergic reactions. Here, BALB/c mice were sensitized intraperitoneally with green gram proteins and levels of specific immunoglobulins, TH2 cytokines, histamine, anaphylactic symptoms and histopathological responses were studied. Four identified proteins showed significant sequence similarity with known allergens of soybean, lentil, pea, lupin, etc (Misra et al., 2011). Legumes belong to same family so high cross-reactivity is observed between different legumes. Black gram was also found to be cross-reactive to other legumes like lentil and lima bean. Humoral and cellular cross-reactivity between Prosopis juliflora pollen and lima bean was established in patients with Prosopis juliflora pollen allergy (Dhyani et al., 2007).

More recently, an international survey has been initiated for determining global variations in the prevalence of food allergies. This project has been developed to evaluate the prevalence of food allergies in China, India and Russia using the standardized methodology of the EuroPrevall protocol used for studies in the European Union. The project was aimed to estimate variations and to compare the data with different European
countries. About 37000 children were screened in the first phase of the study from the three participating countries. The data will provide more insights into the development of food allergy (Wong et al., 2010). There is a great need to conduct systemic studies to determine sensitization pattern against commonly consumed legumes in India. Identification, characterization and purification of the most frequent major allergens for better diagnosis and therapy of legume allergy is also essential. Being consumed in different processed forms the effect of various processing and hydrolyzing methods should also be evaluated for the development of hypoallergenic food formulae.

Aims and Objectives

The present study is focused on the prevalence of legume allergy in Indian population and identification and characterization of major sensitizer. The main objectives are given below:

1. To assess sensitization with common legumes (foods) in atopic Indian population.
2. To identify IgE binding components in legume extracts using hypersensitive patients’ sera.
3. To investigate effect of heat (food processing) and digestion on stability of allergenic proteins in legumes.
4. To identify allergenic cross-reactivity among important legumes and other foods.
5. To purify and characterize a major allergenic protein from most common legume allergen source.