Chapter 1

Review of literature
1. Food allergy

Food allergy, a worldwide health problem, is caused by abnormal immunological responses to certain foods, usually proteins (Longo et al., 2013). It is a form of adverse reaction to food in which the cause is an immunological response (Fig. 1.1). Though diversity of the human diet is enormous, only a small number of foods account for the majority of food allergies around the world. Milk, egg, and peanut account for the vast majority of food-induced allergic reactions in children, whereas peanut, tree nuts, fish, and shellfish account for most of the food-induced allergic reactions in adults (Kumar et al., 2013a). Food allergies are ranked sixth human health problem by the World Health Organization (WHO). Globally, 220-250 million people may suffer from food allergy and the incidence of food allergy (often life-threatening) is commonly estimated to be 5-8% in children and 1-2% adults (WAO report, 2013). Incidence of food allergy has been increasing in developed as well developing countries of the world. In the United States, it is estimated that 125-150 people die each year as the result of food anaphylaxis (Kumar et al., 2013b).

In the last decade, great efforts have been undertaken to identify the characteristics of nontoxic food proteins that evoke IgE-mediated allergic response in predisposed individuals and many hundreds of allergens have been identified in a variety of animal and plant-derived foodstuffs. As a consequence, in the recent past a number of allergen databases have been set up to collect and curate the existing data on allergens, their physicochemical properties and their allergenic relevance.
Fig. 1.1. **Common allergic foods.** The vast majority of food-induced allergic reactions have been reported to induce by shellfish, tree nuts, peanut, soybean, milk and eggs (Source of the image: http://www.therootofhealth.com/food-allergies-intolerances/).
2. Plant foods with allergenic proteins

The Pfam protein family database is a large collection of protein families (Bateman et al., 2004). The Pfam database classifies plant protein sequences into families on the basis of sequence homology, which is related to conserved three-dimensional structures and possibly function. It has become increasingly obvious that almost all plant food allergens are either storage or defense-related proteins. Strikingly, only three dominating plant food allergen protein families/super families have been identified as prolamin super family, cupin super family, and Bet v 1 family. Common plant foods having allergenic proteins include peanuts, tree nuts, and soybeans etc. Peanut (Arachis hypogaea) and soybean (Glycine max) have long been recognized as highly allergenic and are responsible for IgE-mediated clinical reactions in humans (Verma et al., 2013). The following sections provide details of major plant food allergens families:

2.1. Prolamins

The prolamin super family comprises three major groups of plant food allergens that include 2S albumins, nonspecific lipid transfer proteins (nsLTPs) and cereal alpha-amylase/trypsin inhibitors (Bublin et al., 2013; Le et al., 2013). These are low-molecular weight, cysteine rich proteins, have similar three-dimensional folds with abundance of alpha helices, and are stable to thermal processing and proteolysis.
2.2. 2S albumins

The 2S albumins are a major group of storage proteins present in many mono- and dicotyledonous plants (Pascal et al., 2013). 2S albumins can also play a protective role in plants as defensive weapons against fungal attack. Some members of this protein family have been described as major food allergens demonstrating their ability to bind IgE from the sera of allergic patients (Moreno & Alfonso, 2008). Examples include Arabidopsis-albumin, Radish-albumin, Oilseed rape-albumin, Sunflower-albumin, Caster bean-albumin, Walnut-albumin, Brazil nut-albumin, Sunflower albumin (SFA8), Cotton seed albumin, Arachis hypogaea 2 (Ara h2), Arachis hypogaea 6 (Ara h6), Soybean albumin 1 (soy alb1) and Soybean albumin 3 (soy alb3).

2.3. Nonspecific lipid transfer proteins (nsLTPs)

The nsLTPs play an important role in plant defense against fungi and bacteria. These have been identified as major fruit allergens in patients from the Mediterranean area (Schulten et al., 2011; Le et al., 2013). The typical structure of nonspecific LTPs is based on four disulfide bridges, which contribute to the overall stability of these proteins against enzymatic digestion or thermal denaturation, although the stability is pH-dependent. Under acidic conditions, thermal denaturation of allergen of peach (Prunus persica) Pru p 3 was reversible, whereas under neutral conditions Pru p 3 was unable to refold after cooling (Scheurer et al., 2004). Examples are wheat LTP, Rice LTP1, maize LTP.
2.4. α-amylase and protease inhibitors

The family of cereal alpha-amylase and protease inhibitors mediates a certain degree of resistance to insect pests that feed on plant tissues (Radauer & Breiteneder, 2007). Just like the 2S albumins and the nsLTPs, the members of this protein family are capable of sensitizing susceptible atopic individuals through ingestion or inhalation. This group of proteins is found in cereals and sensitizes individuals via the lungs, giving rise to occupational allergies such as bakers’ asthma, or via the gastrointestinal tract, resulting in wheat, barley and rice allergies (García-Casado et al., 1996).

2.5. Cupins

Cupins (Latin cupa means small barrel) vary widely in sequence but are characterized by two short consensus sequence motifs and a core structural feature - a barrel-like, double-stranded beta helix (Breiteneder & Radauer, 2004). The cupin super family comprises the major globulin storage proteins mainly from legumes and nuts (Misra et al., 2011). The globulins are divided into the 7S vicilin-like globulins and the 11S legumin-like globulins (Dam et al., 2013). Globulins have been found to be highly relevant allergens in plant foods including peanuts, soybean, lentils, walnut, hazelnut and sesame.

2.6. Bet v 1 family

Bet v 1 family was the first of many allergens published that showed homology to the family of the pathogenesis-related proteins (Pauli & Metz-Favre, 2013). Bet v 1-type allergens are rather unstable to heating and digestion. Consequently,
symptoms are mostly restricted to the oral cavity. In general, Bet v 1 from birch pollen acts as the primary sensitizing agent. The overall high levels of conserved surface residues between the members of the Bet v 1 family play an important role in conservation of IgE-binding epitopes and underlie the fruit–vegetable-pollen cross reactive syndromes (Hemmer et al., 2010). The majority of these reactions are caused by allergens of Rosaceae fruits (examples include apple, cherry, apricot and pear) and Apiaceae vegetables (examples include celery, carrot), which cross-react with allergens that are present in birch pollen, particularly the major birch pollen allergen Bet v 1.

2.7. Profilins

Profilins are small (12 to 15 kDa) proteins present in all eukaryotic cells and located in the cytosol and act as actin binding proteins (Santos & Van Ree, 2011). Sequence similarity among profilins from lower eukaryotes, plants and animals is low, whereas profilins of higher plants share 75% and more of their sequence (Kumar et al., 2012a). However, at lower pH in SGF assays profilins get readily degraded and display no further allergenic activity, as has been shown for allergens of apple (Malus domestica), Mal d 4 and muskmelon (Cucumis melo), Cuc m 2 (López-Torrejón et al., 2005). Profilins from various botanical species such as apple, hazelnut, peanut, celery and wheat have been included in the EuroPrevall allergen library.
2.8. Oleosins, pathogenesis-related proteins and glycoproteins

Oleosins are considered to contribute towards stabilizing plant lipid storage bodies and represent the proteinaceous component (Jolivet et al., 2013). Recently, oleosins with allergenic activity were identified from legumes, nuts and seeds (Sáiz et al., 2013).

Pathogenesis-related proteins are up regulated within plants upon pathogen attack or exposure to abiotic stress factors (Nakai et al., 2013). Surprisingly, a considerable number of food allergens have been identified from various families of pathogenesis-related proteins, such as the β-1, 3-glucanases, various types of chitinases and the thaumatin-like proteins (Hoffmann-Sommergruber, 2000). Food allergens of the pathogenesis-related protein family consisted several fruits, such as Pru av 2 from cherry, Mal d 2 from apple, Act d 2 from green kiwi, Act c 2 from gold kiwi, orange, grape and Cap a 1 from bell pepper (Breiteneder, 2004). Some of the glycoproteins have been also reported as allergenic (Maria et al., 2013). The binding of the peptides to IgE antibodies was suggested to be predominantly dependent on their glycan moiety (Hiemori et al., 2004).

3. Molecular properties of proteins and allergenicity

Foods contain a wide variety of proteins, yet only a few are allergens. The reason why some proteins are highly allergenic and others are not remains poorly understood, but certain chemical and physical properties appear to be associated with allergenicity. Most allergens are typically stable to changes in heat and pH, and to enzymatic digestion (Foster et al., 2013). They generally have an acid
isoelectric point (pI) and are soluble therefore, get easily absorbed across the gastrointestinal tract (Misra et al., 2011; Misra et al., 2010). However, many nonallergenic proteins also show these properties and many allergens do not have these properties; e.g., profilins are not stable to digestion and lipid transfer proteins do not have an acid pI.

3.1. Abundance

Seeds and nuts contain storage proteins that may account for 50% or more of the total proteins in the organ. Most major food allergens that sensitize via the gastrointestinal tract are present in at least 10% of the total protein content of plant foods (DBT, 2008). However, some proteins that are present in all plants in large quantities, such as the enzyme ribulose-1, 5-bisphosphate carboxylase, which accounts for 30–40% of total leaf protein, have never been reported as allergens (Takagi et al., 2003). In contrast, nsLTPs are potent allergens, but are not very abundant (Asero & Pravettoni, 2013). Thus, the amount of protein alone does not explain its allergenicity. While abundance is an important factor, it is probably secondary to protein stability.

3.2. Stability to processing and digestion

A compact three-dimensional structure, ligand-binding, disulphide bonds, and glycosylation contribute to protein stability. These factors are relevant to both the resistance of proteins to denaturation by food processing and the harsh conditions of the gastro-intestinal tract. Ligand-binding can have the overall effect of reducing mobility of the polypeptide backbone, increasing both thermal stability
and resistance to proteolysis. Some proteins form a cavity while others possess a tunnel into which a ligand can fit. One of the structural features clearly related to stability is the presence of disulphide bonds (Huang et al., 2013). Both inter- and intra chain disulphide bridges constrain the three-dimensional fold such that perturbation of the structure by heat or chemicals is limited and frequently reversible. Important plant food allergens that have high numbers of disulphide bonds include members of the prolamin superfamily (nsLTPs, 2S albumins, cereal alpha-amylase/trypsin inhibitors) as well as of the pathogenesis-related proteins (class I chitinases, thaumatin-like proteins). N glycosylation can also have a significant stabilizing effect on protein structure (Shental-Bechor & Levy, 2008).

3.3. **Gastric and intestinal digestion**

Digestion assays with simulated gastric fluid have been introduced for characterization of food proteins to predict the effect of stomach proteolysis on dietary compounds *in vitro* (Toomer et al., 2013). Gastric digestion substantially decreases the potential of food proteins to bind IgE, which increases the threshold dose of allergens required to elicit symptoms in patients with food allergy. Stability to digestion is considered by many as one of the properties shared by food allergens (Lee et al., 2013). Resistance of proteins to pepsin digestion has been proposed as a marker for potential allergenicity because it does appear to be a characteristic shared by many food allergens. A number of food allergens have been shown to be stable to conditions simulating human gastrointestinal digestion. Examples are β-lactoglobulin A (milk), β-conglycinin (β-subunit), Gly m1, trypsin inhibitor, soy lectin (soybean), tropomysin (shrimp), Ara h1, Ara h2 and
Pn lectin (*Arachis hypogaea*) and ovalbumin and conalbumin from egg (Fu et al., 2002).

### 3.4. Thermal stability

Thermal processing may alter (increase or decrease) the allergenicity of a protein, but the overall effect on a complex food allergen cannot be predicted (Yu et al., 2013). In addition, interactions with other constituents of the food matrix may occur and have no major effect on the overall allergenicity of the food. Pastorello et al. (2004) did not observe any loss of IgE-binding capacity in a lipid transfer protein (LTP) of maize after a thermal treatment at 100°C for 160 min (Pastorello et al., 2004). It has been also shown that dry processing at 100°C for up to 90 min had no effect on the allergenicity of some hazelnut proteins, suggesting the existence of very heat stable allergenic proteins with molecular weight less than 14 kDa (Wigotzki et al., 2001).

### 3.5. Interaction of protein with lipid structures and aggregation

Many plant food allergens are able to attach with cell membranes or other types of lipid structures found in food or show a propensity to aggregate as a result of food processing (Yazicioglu et al., 2013). The allergenic 2S albumin from mustard has been demonstrated to interact with phospholipid vesicles (Breiteneder & Mills, 2005a). This led to the proposition that such interactions might affect the uptake and processing of the allergen in the gastro-intestinal tract, indicating that the biologic activity of these proteins may play a role in attenuating their allergenic potential. Similarly, there is evidence that nsLTPs can interact with lipid
structures as well (Breiteneder & Mills, 2005b). A propensity of certain proteins to aggregate might affect their ability to sensitize by generally enhancing their immunogenicity. Both 7S and 11S globulins are highly thermostable and it seems that the cupin barrel remains intact during heating, but the unfolding of other regions of the protein results in a loss of structure, leading to formation of large aggregates (Breiteneder & Mills, 2005a). Peanuts are often subjected to thermal processing at low water levels such as roasting (Verma et al., 2012a). Thus, peanut proteins become more thermo-stable in low water systems, while at the same time glycation reactions cross-link individual molecules and increase their allergenic activity. Interaction with lipids and the formation of larger aggregates contribute to the allergenicity of plant food proteins in conjunction with the amount of protein ingested and the stability to processing and digestion.

4. Cross allergies between different allergens

The term cross allergy refers to cross-reaction between different foods and cross-reactions between foods and non-food items (Abramovitch et al., 2013). Most studies of cross-reactivity are based on skin prick test and IgE antibody test results (Amoah et al., 2013). In terms of cross reactivity, patients allergic to lentil presented skin reactions to chickpea (67-80%), garden pea (22–54%) and 11% to green bean while patients allergic to chickpea had clinical signs when orally challenged with lentil (84%), pea (68%) and 10% with peanuts (Bernhisel et al., 1989). Another protein family known to cross-react with pollen allergens is the profilin family (Wang et al., 2013). Fruits, such as cherry (Pru av 4), pear (Pyr c 4) and celery (Api g 4), cross-react with the birch pollen Bet v 2 and may cause
allergic symptoms in pollen-sensitized patients (Hoffmann-Sommergruber & Clare Mills, 2009). Profilin Mus xp1 from banana showed a high IgE cross-reactivity with birch pollen profilin, Bet v2, and latex profilin, Hev b8 (Alenius et al., 1996). The marked antigenic similarity between the proteins in the milk of cows, goats, sheep and horses means that almost all subjects who are allergic to cow's milk protein are allergic to the milks from other animals. The eggs from turkeys, duck, goose and seagull all contain ovalbumin, ovomucoid and ovotransferrin, the major allergens in hens' eggs. Mesquite tree (Prosopis juliflora) and lima bean (Phaseolus lunatus) belong to the family Leguminosae. There are reports suggesting that lima bean cross-reacts with other allergenic legumes, such as soya, peanut and black gram based on skin test reactivity (Bernhisel et al., 1989; Kumar et al., 2006). Cross-reactivity to tropomyosin from other molluscan shellfish species has been observed with sera from patients allergic to oysters, suggesting that individuals with allergies to molluscan shellfish should avoid eating all species of molluscan shellfish (Taylor, 2008). The hevein domain has been identified as a cross-reactive determinant between hevein, from Hevea brasiliensis latex, and food allergens from avocado- Pers a 1, chestnut- Cas s 5, grape- Vit v 5, and banana. All these allergens account for the latex-fruit syndrome. The catalytic domain of these proteins displays rather low IgE-binding capacity (Diaz-Perales et al., 2002).

Another type of plant defense is conducted by β-1, 3 glucanases. These glycosyl hydrolases share an (αβ) 8 TIM barrel structure and are usually 25 to 35 kDa in size. They catalyse hydrolysis of 1, 3-β-D-glucosidic linkages in β-1, 3- glucans,
and are abundant in plant cell walls (Blanco et al., 1994). Allergens from this protein family have been identified from avocado, banana, chestnut, fig, and kiwi and inhalant allergens are known from olive pollen and latex contributing to the latex-fruit syndrome.

5. Molecular mechanisms of food allergy

Food allergens are usually proteins capable of eliciting allergic symptoms in predisposed individuals, including severe, even life-threatening allergic reactions. These proteins are from our daily dietary intake and they are often difficult to avoid. Proteins may either be from plants or animal food sources (Kumar et al., 2012a). Allergens are specific antigens having capacity to elicit IgE mediated reactions. The allergenic proteins have certain site (s) which can recognize the specific antibody to provoke immune response called epitope (Perez-Gordo et al., 2013).

5.1. Immunoglobin E (IgE), mast cells and basophils in food allergy

The IgE, mainly associated with allergic reactions is one of the five immunoglobulins. These antibodies are majorly found in the lungs, skin and mucous membranes. IgE antibody levels are often high in people suffering from allergic symptoms (Rispens et al., 2013). They cause the body to react against foreign substances such as pollen, fungal spores, food allergens and animal dander. Total IgE test in allergy has a good predictive value in children less than 3 years of age and may be used as a screening test in this group (Kumar et al., 2012a). Due to the high impact of IgE in food allergy, the total and specific IgE
are useful tool to diagnose allergic patients. A value of specific IgE more than 0.35 kUA/L can be considered important regarding the prevalence of allergy due to any food (Mirabelli et al., 2009).

Mast cells were first time identified by Paul Ehrlich in 1878 in human connective tissues on the basis of the metachromatic staining properties of their cytoplasmic granules (Ribatti & Crivellato, 2011). Mast cells arise from pluripotential hematopoietic cells (CD34+, CD13+, and CD117+ cells) in the bone marrow and found at perivascular sites in tissues that directly get exposed to the environment and function as first line of defense (Kraneveld et al., 2012). After activation, mast cells release cytokines, chemokines, proteases, leukotrienes, and bioactive polyamines (Shea-Donohue et al., 2010). Mast cells also possess high-affinity IgE receptors (FcεRI) that participate in the allergic reactions (Bounab et al., 2013).

Basophils are other leukocytes containing cytoplasmatic granules that stain with basophilic dyes constituting less than 1% of peripheral blood contributing their similarity to mast cells, and also termed as circulating mast cells (Ohmori et al., 2009). Like mast cells, basophils also possess high-affinity IgE receptors FcεRI (Falcone et al., 2000). Basophilic activation test is one of the common tests for the prediction of food allergy in susceptible patients (De Knop et al., 2010).

5.2. Transcription factors: The key regulators in food allergy

Transcription factors are key molecules involved in the determination of Th1/Th2 balance (Stelmaszczyk-Emmel et al., 2013). The shift towards Th2 determines allergic responses, while Th1 cytokines supposed to suppress these reactions.
Many important transcription factors like Signal transducer and activator of transcription 6 (STAT6), Trans-acting T-cell-specific transcription factor GATA-3, T-bet, NFAT, transcription factor Maf or c-maf, Mast/stem cell growth factor receptor (SCFR) also known as proto-oncogene c-Kit and NF-kB are actively involved in Th1/Th2 balance.

STAT-6 plays an important role in signal transduction pathway used by interleukin-4 (IL-4) and interleukin-13 (IL-13) as well as in class switch to IgE and Th2 cytokines production (Cheng et al., 2013). It has been reported that mice deficient in STAT6 show reduced IL-4-mediated functions (Davey et al., 2000). STAT6 also regulates the production of Th2-type cytokines through CD4+ as well as CD8+ T cells, indicating its vital role of STAT6 in allergic reactions (Wang et al., 2011). It has been reported that IL-13, having very similar biological activities to IL-4, can also account for the development of allergic response in the absence of IL-4 in the same pathway, using STAT6 activation (Wurster et al., 2000).

The suppressor of cytokine signaling (SOCS) proteins, especially SOCS-2 regulates cytokines like IL-4 which is a major driver of allergic diseases (Gong et al., 2013; Knosp et al., 2011). Suppressor of cytokine signaling-1, an IL-4-inducible gene in macrophages has been found but its functional role in mast cells and food allergy is yet to be explored (Gardo, 2003).

GATA-3 is a trans-acting T-cell-specific transcription factor in humans encoded by the GATA3 gene (Hwang et al., 2002). The GATA-3 contains two GATA-type zinc fingers and regulates T cell development (Ho et al., 2009). GATA-3 promotes the secretion of IL-4, IL-5, and IL-13 cytokines from activated Th2 cells.
(Soyer et al., 2013; Yamashita et al., 2004). It has been reported that GATA-3 along with T-bet plays an important role in the shift of Th2 or Th1 reactions (Kiwamoto et al., 2006). The GATA-3/T-bet ratio is an important factor while looking at Th2 shift or allergic manifestation.

Transcription factor musculoaponeurotic fibro sarcoma oncogene Maf (c-Maf or V-maf) is a transcription factor encoded by the MAF gene in human (Sakai et al., 2001). It has been reported that IL-4 gene transcription is critically regulated by c-Maf (Schulz et al., 2013; Yang et al., 2005). IL-4 is important in the food allergic reactions as it enhances the differentiation of naive CD4+ T cells into IL-4-producing Th2 cells. It has been shown that in c-maf−/− mice, CD4+ T cells had significantly reduced capacity of IL-4 production, while the levels of IL-13 and IgE were normal (Dent et al., 1998). The direct correlation of IL-4 with c-maf gave a clue to use c-maf for diagnostic as well as therapeutics purpose.

Mast/stem cell growth factor receptor (SCFR, proto-oncogene c-Kit or tyrosine-protein kinase kit or CD117) is encoded by the KIT gene in humans (Buka et al., 2013). The c-kit proto-oncogene produces the receptor for stem cell factor (CD117). Multiple transcript variants encoding different isoforms have been found for c-kit (Sperling et al., 1997). The Asp816Val is a well-characterized product of the c-kit proto-oncogene due to point mutation within exon 17. This is highly evident in the patients with mastocytosis (Nagata et al., 1995). These receptors activate mast cells for IgE-dependent activation, and improve the probability of anaphylaxis in patients with mastocytosis.
The nuclear factor of activated T cells (NFAT) transcription factors may play an important role in allergic events especially in the Ca++ signaling during the degranulation of mast cells. Its role in protein kinase C (PKC) activation has been revealed but complete exploration regarding its involvement in food allergy is yet to be done (Hermann-Kleiter et al., 2010).

5.3. Exposures of allergen and allergic reaction

5.3.1. Primary exposure of allergens

Allergens have enzymatic or irritating factor that help them to penetrate the mucosa. After primary exposure, food allergens are captured by antigen presenting cells especially dendritic cells (DCs) of lamina propria in the intestine (Li et al., 2007). The allergens are internalized by DCs due to receptor-mediated endocytosis process, macropinocytosis, or phagocytosis or by incorporation of microvesicles shed from the surface of neighboring cells, and by their interaction with nanovesicles or exosomes with a size less than 100 nm (Morelli et al., 2004). The allergens are detected by ubiquitin, a 76-residue protein that is highly conserved in all eukaryotes. This is present within the cytosol of DCs. The
Fig. 1.2. Priming of IgE on mast cells. The IgE immunoglobulins bind to the specific receptor Fragment, crystallizable region epsilon R1 (FceR1). The FceR1 is an IgE receptor that contains four subunits α, β, γ1 and γ2. The α and β subunits are found to be involve in attachment of IgE, while γ1 and γ2 are involve in phosphorylation process. Allergen cross link to two IgE molecules that leads to degranulation of mast cell via a cascade of reaction.
Selective attachment of ubiquitin to allergens is the initial signal for targeted protein degradation (Nandi et al., 2006). These ubiquitinized allergens move to proteosomal complex and ultimately get degraded to peptide fragments (Wang et al., 2006). The degraded peptide fragments are presented by major histocompatibility complex class-II (MHC-II) and recognized by naïve CD4+T cells. The T helper cells or CD4+T cells, have been divided into two broad classes Th1 and Th2, basically based on the type of cytokines they produce. These CD4+T cells differentiate into Th2 cells especially in the presence of adequate amount of IL-4 (Lipscomb et al., 2007). The differentiated Th2 cells secret cytokines IL-4 and IL-13 which induce class switching to IgE. The class switching of immunoglobulin is a biological phenomenon that changes a B cell's production of antibody from one class to another. Class switching causes change in the constant region portion of the antibody heavy chain, but the variable region of the heavy chain stays the same, which does not affect antigen specificity (Matthias et al., 1995). The B cells activation signal and class switching to IgE are mainly induced by IL-4 and IL-13 (signal 1) and interaction of CD40 on B cells and CD40-ligand (signal 2) on Th2 cells. The IgE immunoglobulin attaches with FcεR1 (Fragment, crystallizable region epsilon R1) of mast cells or basophil cells. FcεR1 is an IgE receptor in human and is composed of four subunits α, β, γ1 and γ2 (Abramson et al., 2007). The α and β subunits are found to be involve in attachment of IgE, while γ1 and γ2 are involve in phosphorylation process. The FcεRI is found on epidermal Langerhans cells, eosinophils, mast cells and basophils, due to its
Fig. 1.3. **Primary exposure of allergens.** Following primary exposure allergens processed into small peptides and presented by MHC-II to Th2 cells that ultimately release cytokines. Where, MHC-II= major histocompatibility complex II; IL-4= Interleukin -4; CD= Cluster of differentiation; Th2= Type 2 helper T-cell.
cellular distribution on several cell types. A diagrammatic representation of primary exposure of allergens and its subsequent events has been given in Fig.1.2 and Fig.1.3.

5.3.2. Secondary exposure of allergens

The IgE and its cell surface receptors, the high affinity receptor FceRI and the low affinity receptor FceRII (CD23), are key components of immediate-type allergic reactions (Gould & Sutton, 2008). IgE activates the allergic cascade effectively via the high affinity receptor, FceRI, on blood- and tissue cells. As a member of the immunoglobulin receptor super family, FceRI consists of a ligand-binding immunoglobulin domain-containing protein (α-chain) which binds the Fc-part of IgE and signaling subunits that regulate cellular activation (β- and γ-chains) [Kraft & Kinet, 2007]. Humans express a tetrameric FceRI (FceRIαβγ2) on mast cells and basophils and a trimeric form (FceRIαγ2) on antigen presenting cells (Call & Wucherpfennig, 2005). The FceRI expression is regulated by IgE since binding of monomeric IgE to FceRIα stabilizes the receptor at the cell surface as an IgE-FceRI complex (Kubota et al., 2006; MacGlashan, 2005). After secondary exposure of same or similar (having same epitope) allergens occur, they cross-link to the IgE and form allergen-IgE-FceRI complex. When allergen-IgE-FceRI complex is formed, it phosphorylates tyrosines of immunoreceptor tyrosine activation motifs or ITAMS (Hübner et al., 2011). On the cellular level, the granules present in the cytoplasm migrate to the cellular membrane and spill out their contents into the surrounding area. The mechanisms for mast cell activation and mediator release are dependent on the binding of IgE to FceRI (Kubo et al.,...
Upon antigen binding to IgE, these receptors aggregate and initiate the signaling cascade. The ITAMs components of FceRI have specific sequences, containing tyrosine residues that are phosphorylated by Src family member tyrosine kinases, including liver tyrosine kinase (Lyn) and Spleen tyrosine kinase (Syk), activated after receptor aggregation (Johnson et al., 1995). Phosphorylated ITAMs serve as high-affinity docking sites for Lyn and Syk. The Lyn, is highly important in initial tyrosine phosphorylation that ultimately launches the signal cascade, and recruits adaptor proteins responsible for regulating downstream events (Gilfillan & Tkaczyk, 2006). Signal complex recruit Syk which gets further activated by tyrosine autophosphorylation and phosphorylation by Lyn (Gilfillan & Rivera, 2009). The activation of these tyrosine kinases results in phosphorylation of the transmembrane adaptor molecules, linker for activation of T cells (LAT) and non-T-cell activation linker (NTAL). The LAT and NTAL provide scaffold for direct and indirect interaction for additional adaptor molecules including Grb2, Gads, Shc, and SLP76, the guanine nucleotide exchange factors and adaptor molecules Sos and Vav, and the major signaling enzymes phospholipase Cγ (PLCγ) and PI3 kinase (Metcalf et al., 2009). The releases of calcium ions from intracellular components and activation of protein kinase C (PKC) is facilitated due to the activation of PLCγ and PI3K, which lead to mast cell degranulation (Okkenhaug et al., 2006). The Ras-Raf-mitogen-activated protein (MAP) kinase cascade is also activated through Sos and Vav, which lead to PLA2 activation, arachidonic acid metabolism and production of lipid mediator generation and release (Siraganian et al., 2002). These events lead
to activation of the transcription factors, AP-1, nuclear factor of activated T cells (NFAT) and NF-κB. Activated transcription factors induce secretion of cytokine and chemokine production. The role of another tyrosine kinase Fyn has been also reported in mast cell degranulation through another signaling cascade to phosphorylate the adaptor protein Gab2 and activate PI3K, Bruton's tyrosine kinase (Btk), PLCγ, and sphingosine kinase and the generation of (S1P) or sphingosine-1-phosphate (Tkaczyk et al., 2004). The Hck, a tyrosine kinase has been reported to show positive regulation of mast cell degranulation by inhibition of Lyn and by phosphorylation of FcεRI (Hong et al., 2007).

5.3.3. Mediators release and symptoms

Prostaglandins, cytokines, leukotrienes, histamine, slow reacting substance of anaphylaxis (SRS-A), heparin, platelet-activation factor (PAF), eosinophil chemotactic factor of anaphylaxis, proteolytic enzymes and other mediators are secreted by degranulated mast cells or basophils. These mediators may cause smooth muscle dilation, capillary disruption, local swelling and other allergic symptoms. In some individuals, these reactions may occur very vigorously leading to anaphylaxis or sometimes death.

Histamine acts on histamine 1 (H1) and histamine 2 (H2) receptors to cause contraction of smooth muscles of the airway and gastrointestinal (GI) tract, increased vasopermeability and vasodilation, enhanced mucus production, pruritus, cutaneous vasodilation, and gastric acid secretion (Jutel et al., 2001). Tryptase is a major protease released by mast cells, though its exact role is
uncertain, but it can cleave C3, C3a as well as C5 (Fukuoka et al., 2008). Tryptase is found in all human mast cells and some other cells and is a good marker of mast cell activation. Proteoglycans include heparin and chondroitin sulfate. Though, the role of the latter is unknown, heparin seems to be important in storing the preformed proteases and may play a role in the production of $\alpha$-tryptase (Brunnée et al., 1997; Noga et al., 1999). An eosinophilic chemotactic factor of anaphylaxis causes eosinophil chemotaxis while an inflammatory factor of anaphylaxis results in neutrophil chemotaxis. Eosinophils release eosinophil major basic protein (EMBP) and, together with the activity of neutrophils, can cause significant tissue damage in the later phases of allergic reactions. Degranulation fluids also contain IL-4 that stimulates and maintains Th2 cell proliferation and switches B cells to IgE synthesis (Hart, 2001). The IL-5 is key molecule in the maturation, chemotaxis, activation, and survival of eosinophils. The IL-5 primes basophils for histamine and leukotriene release while IL-6 promotes mucus production. The IL-13 cytokine has many of the same effects as IL-4. Tumor necrosis factor-\(\alpha\) activates neutrophils, increases monocyte chemotaxis, and enhances production of other cytokines by T cells (Pearlman, 1999).

6. Activation of innate immunity by food allergens

Along with acquired immunity, the role of innate immunity has been also important in food allergy. The innate immunity involved in food allergy encompasses several cells including DCs, epithelial cells, basophils, nuocytes, NK cells and TLRs. Allergens can directly interact with DCs through pattern
recognition receptors (Ruiter & Shreffler, 2012). Epithelial cells play a vital role as it is sites of allergen entry into the body and also have close interaction with DCs in situ. Epithelial cells secret TSLP, a cytokine after exposure of allergens which stimulates DCs to produce the Th2 cell–attracting chemokines and cytokines. Due to its properties like an adjuvant it is also referred as natural adjuvant (Hammad & Lambrecht, 2008). The role of basophil is not limited to acting as an effector cells and degranulation during food allergy but, it can also act as APCs in the induction of Th2 responses which have been evident in papain and ovalbumin (Karasuyama et al., 2010; Bourgeois et al., 2009). The invariant natural killer T (iNKT) cells have also been reported to involve in food allergy but, limited to the allergens with sphingolipids nature. The iNKT cells have found to proliferate and secret Th2-type cytokine secretion after allergen exposure (Barlow & McKenzie, 2011). The nuocytes or natural helper cells, innate immune cells initially discovered in mice and found to be engaged in allergic reactions via induction of Th2 responses. These cells are stimulated by IL-25 and IL-33. The stimulated nuocytes have been reported to secret cytokines IL-5 and IL-13 which may enhance the Th2 reaction (Williams et al. 2012).

7. Common diseases due to food allergy

There are many diseases reported due to food allergy, including allergic rhinitis, bronchial asthma, atopic dermatitis, and gastrointestinal disorder. Moreover, the allergenic prevalence in each person varies significantly, depending on genetic disposition and environmental factors, which makes a person allergic to one protein but not to another. The different atopic diseases have been reported due to
intake of foods like peanut, soybeans, red kidney beans, red grams, green grams, eggs and fishes.

7.1. Nasobronchial asthma and allergic rhinitis

Nasobronchial asthma and allergic rhinitis are two most common complications that occur during an allergic attack (Misra et al., 2008). There is a clear association between allergic rhinitis and asthma. Cases of developing asthma and nasal symptoms at or about the same time have been documented (Prasad et al., 2009). Patients with above conditions can expect to suffer more severe asthmatic attacks and require stronger medications to treat their asthma. Example of such foods causing nasobronchial asthma and rhinitis are peanut, soybeans, red gram, green gram and chickpea (Misra et al., 2010; Misra et al., 2011).

7.2. Atopic dermatitis

Allergic contact eczema is a red, itchy and weepy reaction where the skin has come into contact with allergens. Atopic dermatitis is a very common, often chronic, skin disease that affects a large percentage of the world's population. It is also called eczema, dermatitis, or atopy. The term atopic refers to diseases that are hereditary, tend to run in families, and often occur together. In atopic dermatitis, the skin becomes extremely itchy and inflamed, causing redness, swelling, cracking, weeping, crusting, and scaling. Atopic dermatitis often accompanies asthma, allergies or hay fever, and eczema. Examples of some food causing atopic dermatitis are egg, wheat, milk and soy (Werfel & Breuer, 2004).
7.3. Oral allergy syndrome (OAS)

Oral Allergy Syndrome (OAS) is an allergic reaction to food that is limited to the lips, mouth and throat. The major symptoms of OAS include itching and swelling of the lip or throat. These symptoms generally start within minutes of eating and settle down within an hour. OAS is caused by cross-reactivity between proteins in fresh fruits and vegetables and pollens. The proteins in the fruits and vegetables causing OAS are easily broken down with cooking or processing. Therefore, OAS typically does not occur from eating cooked or baked fruits and vegetables, or processed fruits. Kiwi fruit is now one of the most common causes of OAS. Although patients often present with mild OAS, severe systemic reactions are not uncommon, particularly in children (Lucas et al., 2004). Examples of some foods causing OAS are raw fruits like cherry and vegetables.

7.4. Eosinophilic esophagitis

Eosinophilic esophagitis is inflammatory condition in which the wall of the esophagus is filled by large numbers of eosinophils. Although, the cause of eosinophilic esophagitis is unknown, allergic responses including food allergies have been implicated. The esophagus is a muscular tube utilized for propelling swallowed food from the mouth into the stomach. Esophagitis refers to inflammation of the esophagus. The most common cause of esophagitis is acid reflux, which most frequently results in heartburn, although acid reflux also can cause ulcers in the inner lining of the esophagus. Examples of foods involved in induction of eosinophilic oesophagitis are egg, milk, and soy. These foods were
identified most frequently with skin prick testing. Corn, soy and wheat were identified most frequently with atopy patch testing (Spergel et al., 2005).

7.5. Edema of the uvula

The uvula is a tiny organ in the oral cavity attached to the soft palate. It is involved in the articulation of human voice, swallowing and prevents the entry of foods into the nasal cavity. Swelling of the uvula occurs in different medical condition including food allergy. Usually, edema manifests as fullness of the oropharynx, difficulty in talking, difficulty in breathing and, since it affects the vocal cords, dysphonia (Alcoceba et al., 2010). Seafoods, hazelnuts and walnuts are some examples of foods that can induce edema of the uvula.

7.6. Recurrent aphthous stomatitis (RAS)

RAS is one of the most common oral lesions induced by milk, gluten and other allergens. RAS can occur either in single or multiple forms in oral mucosa. This chronic, incurable condition can be painful to the patient, making it uncomfortable to speak, eat or drink (Wardhana et al., 2010). RAS can be caused by multiple foods.

7.7. Food protein-induced enterocolitis syndrome (FPIES)

FPIES is a non-IgE-mediated, gastrointestinal food-mediated hypersensitivity (Nowak-Wegrzyn & Muraro, 2009). Vomiting, followed by an elevation of the peripheral blood polymorphonuclear leukocyte number, diarrhea, and possibly
lethargy and hypotension are characteristic of FPIES (Sicherer, 2005). Rice is the most common solid food causing FPIES.

7.8. Neonatal diarrhea

Diarrhea represents a major condition responsible for pediatric mortality worldwide. Diarrhea in children due cow milk and other foods have been very frequently reported. The onset of neonatal diarrhea may rapidly lead to life threatening dehydration and malnutrition (Passariello et al., 2010). Milk, soy and grains can cause diarrhea in children.

8. Diagnosis of food allergy

There are several diagnostic methods for determination of food allergies. A brief overview of each different diagnostic method for detection of food allergy is given below.

8.1. Skin prick test (SPT)

Skin prick test can be performed with pollens, insects, danders, dust, fungi and food extracts in bronchial asthma, allergic rhinitis, allergic dermatitis and other atopic patients. Glycerinated buffer saline and glycerol histamine acid phosphate can be used as negative and positive control, respectively. Comparison against a histamine-induced weal determines the allergic reaction. If the SPT weal is smaller than the histamine-induced weal, a score of +1 is given. If the two weals are equal, the score is +2. If the test weal is larger than the histamine weal, the score is +3 and if it is larger with pseudopodia, then the score is +4. The results are read 20 minutes after the allergen prick (Misra et al., 2008). The presence of
allergen-specific IgE on cutaneous mast cells results in a positive skin test in the form of a transient “weal-and-flare” reaction (Horsmanheimo et al., 1996). When possible, allergy skin testing is the preferred method in comparison to various *in vitro* tests for assessing the presence of specific IgE antibodies because it is more sensitive and specific, simpler to use, and less expensive (Ten et al., 1995).

### 8.2. Oral food challenge

A placebo control food challenge is a very common test for allergy, often considered the gold standard in the diagnosis of food allergy (Sicherer & Teuber, 2004; Beyer & Teuber, 2005). The aim of a food challenge is to study the consequences of a food or food additive ingestion. In a double-blind, placebo-controlled, oral food challenge, the specific food is masked in a vehicle food and then administered in a graded fashion. The active food and an equivalent amount of placebo are given in random order and both tests are performed in a controlled manner. A single-blinded challenge is when the patient is unaware but the physician is aware of the content of the challenge. It is sufficient as a screening tool for reactivity.

### 8.3. RAST (*Radioallergosorbent* test)

The RAST is a radioimmunoassay test to detect specific IgE antibodies to suspected or known allergens. This *in vitro* test using the blood of a susceptible individual is useful for detection of allergy with good reproducibility (Balatsouras et al., 2011). One of the major advantages of this test is that it is not necessary to stop antihistamine medications. It is also used if skin conditions (such as eczema) are so widespread that allergy skin testing cannot be done. A commercially
available, radio-labeled anti-human (an antibody directed against human) IgE antibody can be used to detect reactivity. The amount of radioactivity is proportional to the serum IgE for the allergen.

8.4. In-vitro specific immunoglobulin test

In vitro tests for food-specific antibodies may also be used to screen patients suspected of IgE-mediated food allergies (Sampson, 2001). Enzyme linked Immunosorbant assay (ELISA) has been used as quantification tool to determine the IgG1, IgG2a and IgE levels in the serum of allergic patients. This in vitro test is very sensitive and requires only a small amount (4-5 µL) of serum (Misra et al., 2011).

8.5. Bryan’s Test

Cytotoxic food testing, also known as “Bryan’s Test”, involves observing changes in the shape of white cells when a specific antigen is added to whole blood. It is prone to bias as it depends on subjective interpretation (Lieberman et al., 1975).

8.6. Sublingual/intradermal provocation tests

Here, the allergen is applied sublingually or intradermally, followed by an observation period for a local response. The application of allergen is progressively increased until a weal appears on the skin (intradermal provocation dose), and the dosage is then decreased until the weal disappears (Teuber & Vogt, 1999).
8.7. Western blotting

Western blotting can be used as a diagnostic method for identification of a culprit food using human serum. With this method, the interaction between IgE presented in the serum of the patient and food proteins transferred on the polyvinyl diflouride (PVDF) membrane can be easily observed. Western blotting also determines allergenic proteins among whole food proteins since the IgE only binds to the proteins having an epitope on their surface.

9. Genesis of the study

The Red kidney bean or RKB (*Phaseolus vulgaris* L.) is a commonly consumed bean worldwide due to its delicacy and high protein contents along with the presence of antioxidants, minerals and polyphenols (Moma, 2006). RKB are grown as a staple food source for humans and other animals throughout the tropical and subtropical regions of the world. The production of red kidney beans have been reported from China, Indonesia, Turkey, India, Thailand, Egypt, Morocco, Italy, Spain, Mexico, Brazil, Myanmar, United States, Tanzania, Uganda, Kenya, Argentina and other countries (FAOSTAT, 2010). RKB are consumed in raw as well as cooked forms in several dishes including salad, casserole, macaroni and rajma-chawal. In spite, of its higher culinary utilization, questions are always raised on its toxicity as well as allergenicity potentials of RKB. Considering these issues, the RKB consumption is always a matter of dilemma in a large population of the world. There are some reports available in regard of the toxicological manifestations induced after kidney beans
consumption (Fitches et al., 2001; Pusztai & Palmer, 1977; Banwell et al., 1983). Legumes are a rich and important source of proteins for a large vegetarian population. But, at the same time frequently encountered IgE binding proteins (allergens) from legumes cannot be ignored. The allergenic response induced by legumes may range from mild skin reactions to a severe anaphylactic manifestation (Vaza et al., 2011). Legume allergens are playing a big role in prevalence of food allergy throughout the world. Allergic reactions to peanuts in children have become a significant medical and legal concern worldwide, with a rising incidence of this potentially fatal condition (Vaza et al., 2012). The prevalence of peanut, lentil, green gram, red gram, chickpea, fenugreek, lupin and other legumes allergy have been reported in a large population in several parts of the world including USA, Spain, UK, India and Sweden (Verma et al., 2013). In the 90’s it was reported that RKB does not commonly induce symptoms of food allergy in sensitized individuals, but as with other legumes, allergic reactions are possible which indicate that there is presence of same or similar epitopes (Alizadeh et al., 2011). The detection of IgE antibodies against red kidney beans has also been reported (Fang et al., 2011). Further, contact dermatitis to RKB, along with skin reactivity, was found in a former group indicating a vital chance of kidney bean induced allergic manifestations (Sharma et al., 2010). The pepsin resistant is a recognized property of allergenic proteins. The digestibility of RKB proteins studied by in vitro pepsin digestibility assay which revealed a 20-kDa polypeptide identified as a basic subunit of legumin, remained stable after thermal
processing. This pepsin and thermal resistance properties are probably found due to presence of rigid intramolecular disulfide bonds (Moma, 2006).

As food allergy varies according to the food habits of different regions/countries and Indian population being major consumer of RKB as protein source, therefore cases of allergy to this crops may be of very serious concern. There are hardly any studies in India on allergens and allergenicity of RKB. Therefore, study on prevalence of RKB in human subjects, allergenicity assessment, identification, characterization and purification of allergens is required in order to develop appropriate strategies. So, three major objectives for research work have been proposed as:

(A) Evaluation of the allergenicity of red kidney beans crude protein extract.

(B) Purification of allergenic protein/s from red kidney bean.

(C) Evaluation of allergenic potential of purified red kidney bean protein/s.