INTRODUCTION

The human immune system has evolved by generating variety of cells and effector molecules capable of specifically recognizing and eliminating a wide variety of foreign invaders. 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 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 to systemic (anaphylaxis) and or localized reactions such as rhinitis, conjunctivitis, urticaria, eczema, asthma, angioedema, oral allergy syndrome and gastrointestinal allergies in pre-disposed individuals.

ALLERGY

The word allergy was derived from Greek word ‘alol’ meaning change/altered and ‘ergos’ meaning reactivity. Allergy was recognized in modern era after John Bostock’s description of ‘catarrus aestivus’ or hay fever in 1819 (Bostock, 1828). Later, Charles Blackley (1873) identified pollen grains as potential causative agents for such a phenomenon i.e. hay fever (Blackley, 1873). However, Viennese pediatrician Baron Clemmens Von Pirquet (1906) was first to use the term ‘allergy’ to describe the strange, non disease related symptoms that some diphtheria patients developed when treated with a horse serum antitoxin (Von Pirquet, 1906). The term ‘atopy’ was coined (Coca and Cooke, 1923) to describe a tendency for immediate hyperreactivity reaction to allergens. The development of suitable technology, led to the identification of IgE, earlier known as ‘reagin’ (Ishizaka, 1966; Bennich and Johansson, 1971). Atopic individuals produce high amount of IgE in response to specific allergens (Mekori, 1996).
Studies suggest that over 20% individuals worldwide suffer with IgE-mediated allergic diseases such as asthma, rhino-conjunctivitis, eczema, and anaphylaxis (Linhart and Valenta, 2005). The incidence of allergic diseases has increased over the past two decades in many countries. Allergies manifest as mild reactions to life threatening systemic reaction (anaphylaxis). These diseases occur due to complex interplay of various immune cells, which is initiated upon exposure of predisposed individual to normally innocuous triggers called allergens. At the first exposure, allergen(s) induce the formation of antibodies belonging to class ‘IgE’. Subsequent exposure to allergen sets off a cascade of events leading to inflammation, hyper-reactivity and manifestation of allergic disease. Such specific ‘IgE-mediated reactions’ to environmental substances/allergens are termed as ‘Type I Allergy’ or ‘immediate hypersensitivity’ (Kay, 2001, 2006).

HYPERSENSITIVITY REACTIONS

Immune response to any antigen is generally directed to eliminate infectious agent. However, in some cases it gets misdirected, becomes hyper-reactive and leads to tissue injury. These reactions can be categorized as ‘immediate’ or ‘delayed’ based upon the time taken for symptoms to appear after antigen exposure. These may be divided into ‘humoral’ or ‘cell mediated’ immune response based upon type of components. A four-group classification of hypersensitivity reactions was expounded by British immunologists Gell and Coombs (Gell and Coombs, 1963; Figure 1.1). A fifth group was also recognized later (Rajan, 2003).

Type I (Immediate) hypersensitivity or anaphylaxis

Cross-linking of mast cell-bound IgE by the allergen triggers histamine release and synthesis of other inflammatory mediators such as platelet-activating factor, leukotrienes, bradykinins, prostaglandins, and cytokines which contribute to allergic inflammation. These mediators cause the early phase of allergic reaction that appears within minutes after exposure to the allergen and is therefore referred as immediate hypersensitivity. Allergic rhinitis, asthma and urticaria are some of the examples of type I hypersensitivity. These immunoglobulin E mediated allergic reactions are triggered upon re-exposure of predisposed individuals to allergens (Goust and Finn, 2003). The reaction involves two phases (Figure 1.2)
**Figure 1.1: The four types of hypersensitive responses:**

**Type I or Immediate hypersensitivity:** Ag induces cross-linking of IgE bound to mast cells and basophils with release of vasoactive mediators.

**Type II or Cytotoxic Hypersensitivity:** Ab directed against cell surface antigens mediates cell destruction via complement activation or antibody dependent cell cytotoxicity (ADCC).

**Immune Complex-Mediated Hypersensitivity:** Ag-Ab complexes deposited in various tissues induce complement activation leading to inflammation and damage to endothelium.

**Type IV or Delayed Hypersensitivity:** Receptors on the sensitized T lymphocytes (T\(_{H1}\), T\(_{H2}\) or Cytotoxic T cells (CTL) combine with the target cell antigens, release inflammatory cytokines, resulting in cell death.

*Source: Janeway et al., 2001*
Figure 1.2: The sequence of events in immediate hypersensitivity: Allergen exposure triggers synthesis and release of specific IgE by B-cells. IgE binds to receptors (FcεRI) on mast cells, and subsequent exposure to the allergen activates the mast cells to secrete the mediators that are responsible for the pathologic reactions of immediate hypersensitivity.

Source: Abbas and Lichtman, 2004
**Sensitization:** This phase sets in upon first exposure to allergen. The antigen presenting cells (APCs) in the airway epithelium internalize process and present the allergen to T-lymphocytes. The interaction of these T-cells with B-lymphocytes signals heavy chain switching to IgE specific to the allergen. Released IgE binds to high affinity receptors on mast cells and basophils (Descotes and Choquet-Kastylevsky, 2001; Goldsby et al., 2003). In this phase immune system is stimulated for IgE production, but no symptoms occur.

**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 (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:

**Early phase response:** It occurs within 5 to 30 minutes of allergen exposure. IgE receptor cross-linking following allergen exposure signals microtubule polymerization, which allows transport of cytoplasmic granules to plasma membrane for fusion (Leung, 1997). Fusion of these granules empties the preformed mediators e.g. histamine, serotonin 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). Such reactions e.g. sneezing, mucus secretion and vasodilatation in upper respiratory tract, leads to nasal blockage and to wheezing, oedema and broncho-constriction in the lungs (Leech, 2002).

**Late phase reaction:** The reaction sets within 2 to 8 hours without additional exposure to antigen. The mast cells and basophils continue to synthesize other mediators after early phase. The late phase mediators e.g. prostaglandin D2, leukotriene C4 and slow reacting substances of anaphylaxis (SRS-A) reach their effective concentration after few hours of antigen challenge and have effects lasting for several hours. This phase is characterized by intense infiltration of inflammatory cells such as eosinophils, neutrophils, basophils, monocytes and CD4+ T cells as well
as tissue destruction in the form of mucosal epithelial cell damage (Leung, 1997). In the lung, late phase response is characterized by cellular infiltration, fibrin deposition and tissue destruction (Leech, 2002).

**Type II hypersensitivity (Antibody-mediated cytotoxic reactions)**

Type II reactions are characterized by antigen-antibody interactions. These reactions are triggered when IgG and/or IgM antibodies produced by the immune system bind to antigens (intrinsic as well as extrinsic) on the patient's own cell surface. The antibody mediates destruction by activating complement system or by antibody-dependent cell-mediated toxicity (ADCC) (Goldsby et al., 2003; Rajan, 2003).

The most common clinical manifestations of type-II hypersensitivity reactions are autoimmune haemolytic anemia, Goodpasture's syndrome, pernicious anemia and mismatched blood transfusion reactions.

**Type III hypersensitivity (Immune complex-mediated reactions)**

Type III reactions cause tissue injury by immune complexes. Such reactions occur when antigen-antibody reaction forms micro-precipitates in and around small blood vessels. Such circulating immune complexes are generally cleared by phagocytic systems. When clearance capacity of phagocytic systems is exceeded (Goust and Finn, 2003), soluble immune complexes (aggregations of antigens and IgG and IgM antibodies) are deposited in the endothelial lining of blood vessel walls and tissues (typically the skin, kidney and joints) triggering complement activation. Macrophages, neutrophils and platelets are subsequently attracted to the deposition site and contribute to the tissue damage. Typical manifestations include glomerulonephritis, rheumatoid arthritis, serum sickness and systemic lupus erythematosus (Rajan, 2003).

Many molds/fungal spores (particles) are known to evoke antigen-antibody complex (IgG) mediated hypersensitivity. Such response may manifest as allergic bronchopulmonary aspergillosis (ABPA), allergic fungal sinusitis (AFS), hypersensitivity pneumonitis (HP), etc. Mold particles that reach lower airways may manifest as pulmonary hypersensitivity syndrome/extrinsic allergic alveolitis due to massive occupational exposure. HP may present as flu-like illness with cough (acute
HP), recurrent pneumonia (sub-acute HP) or as productive cough, lung scarring (fibrosis), weight loss and dyspnea (chronic HP). It is characterized by diffuse inflammation of lung parenchyma and airways in sensitized individuals. *Aspergillus spp.* (*A. fumigatus*) are primarily responsible for afflicting ABPA in asthmatic or other predisposed individuals (Kumar et al., 2003). Fungi such as *Curvularia, Epicoccum* and *Fusarium* are also involved in HP or ABPA like fungal diseases (Travis et al., 1991; Noble et al., 1997; Lee et al., 2000).

**Type IV- Delayed or cell-mediated hypersensitivity**

Type IV reactions are triggered when the antigen is presented to T-lymphocytes by antigen-presenting cells (APCs). This results in lymphocyte stimulation and cytokine release (Rajan, 2003). The released cytokines activate macrophages or cytotoxic T cells, which mediate direct cellular damage. CD 8+ cytotoxic T cells and CD 4+ helper T cells recognize antigen in a complex with either type I or II major histocompatibility complex (MHCs). The symptoms usually develop within 2-14 days after exposure to the allergen depending upon the sensitization status of the patient. Type IV reactions typically manifest as contact dermatitis (skin eruptions in response to drugs, cosmetics and environmental chemicals), transplant rejection, insulin dependent (type I) diabetes mellitus etc.

**Type V - Stimulatory hypersensitivity**

These reactions are similar to type II reactions. Here, instead of binding to cell surface components, the antibodies recognize and bind to the cell surface receptors, which either prevents the intended ligand binding with the receptor or mimics the effects of the ligand, thus impairing cell signaling. Some clinical examples are Graves' disease, Myasthenia gravis etc. (Descotes and Choquet-Kastylevsky, 2001).

**Limitations and validity of the Gell and Coombs’s classification**

The classification does not account for all hypersensitivities. Moreover, multiple components may be simultaneously or subsequently involved in a given reaction (Descotes and Choquet-Kastylevsky, 2001) e.g. T cells also play an important role in the pathophysiology of allergic reactions.
A classification system proposed by Sell (1996) divides immune-pathological responses into seven categories viz. inactivation/activation antibody reactions, cytotoxic or cytolytic antibody reactions, immune-complex reactions, allergic reactions, T-cell cytotoxic reactions, delayed hypersensitivity reactions and granulomatous reactions.

**ALLERGIC DISORDERS**

During the last few decades, a rapid upsurge has been observed in incidence of allergic ailments. Allergic diseases affect an individual in terms of personal sufferings i.e. quality of life as well as the society in terms of work performance and productivity (Bolin and Lindgren, 2002). In the global scenario, inhabitants in western countries are the most affected with allergies i.e. >10% compared to approximately 5% population in India (Masoli et al., 2004; Figure 1.3).

**Allergic asthma:** It is a major health problem among children and adults worldwide. It involves inflammation of lower airways and is characterized physiologically by variable airflow obstruction and airway hyper-responsiveness (Brightling et al., 2002; Nelson, 2000). Asthma affects over 150 million people worldwide and as per estimates of American Association of Asthma and Allergy foundation (2005), nearly 50% of asthma cases are of ‘allergic-asthma’ affecting ~60 million people every year in US with approximately 58% increase per year. The incidence of asthma in India is reported in the range of 2.3-16.6 % (Chhabra et al., 1998; Gaur et al., 2006; Aggarwal et al., 2006; Jindal, 2007), which is higher than reported (< 1 %) earlier (Viswanathan et al., 1966). Asthma prevalence was reported to be 3.5% with diagnosis, whereas it was 9-12% of symptomatic subjects without diagnosis (Chowgule et al., 1998). The reported prevalence of asthma was 2.3-3.3% in the children from Lucknow (Awasthi et al., 2004) whereas it was 2.6% in rural children from Ludhiana (Singh et al., 2002). In Bangalore, prevalence of asthma was 9% in 1979 that reached to 29.5% in 1999 (Paramesh, 2002). This may be attributed to environmental pollution, urbanization and change in demography of the city. A study in Tamil Nadu on urban and rural children of 6-12 years showed 18% prevalence of wheeze (Chakravarthy et al., 2002).
Figure 1.3: Global prevalence of allergy and asthma: Mapping the prevalence of allergy and asthma on world map. Red colored zones (Western industrialized nations) represent most affected population (≥10% population affected). India represents relatively lesser affected region (2.5-5.0% population affected).
Source: Masoli et al., 2004
**Allergic rhinitis (AR):** It involves inflammation of the upper airways. This condition is characterized by sneezing, runny nose (rhinorrhea) and/or nasal blockade, post nasal drip etc. It is considered as first step leading to asthma in many cases. The estimates based upon questionnaires, parental or self-report, physician's examination, skin tests and serological IgE assays, from eight countries in Europe, encompassing over 100,000 subjects indicate a prevalence of over 10-15% (10.9-18.6%) (European allergy white paper. www.theucbinstittuteofallergy.com). About 40% of the world’s population suffers with atopic disorders and allergic rhinitis is commonest of them (Weinmayr et al., 2008). It affects 24% of the population in UK, 20.6% Norway and 19.6% in Germany (Weinmayr et al., 2008). In Asia, approximately 10-50% subjects are afflicted with allergic rhinitis (Wong et al., 2004). In India, 50% of atopics are afflicted with allergic rhinitis (Gaur et al., 2004). In children, incidence of allergic rhinitis has been reported as 7.3% whereas in industrial workers it was 17.5% (Gaur et al., 2004, 2005)

**Atopic dermatitis (AD):** Dermatitis is allergic condition of the skin that includes itching, reddening and flaking or peeling of the skin. It usually affects young infants, but can occur in atopics even in later age. Studies show that the persistence rate of atopic dermatitis first occurring in infants and continuing into adulthood range from 45-60% and the risk of developing respiratory symptoms in later years is around 40-60% (pollen allergy 41.5%, perennial rhinitis 25% and asthma 25%). Urticaria/Hives are itchy, red bumps that appear on the surface of the skin. They can occur in clumps, and can be ‘chronic’- appearing and disappearing for no reason, or ‘acute’.

**Allergy: Gene-Environment Interplay**

**Genetic factors:** In last two decades, several studies have indicated that allergy is a complex genetic disorder involving number of genes and environmental inputs viz. allergen exposure, cigarette smoking, low birth weight, pollution, immunocompromised state of individual etc (Falliers et al., 1971; Hanson et al., 1991). Previous studies suggest that allergic subjects had significantly higher incidence of family history of allergy or atopy compared to the control subjects. This has made family history an important parameter in preliminary diagnosis of allergy (Steinke et al., 2003).
Various genes and markers associated with allergies have been identified by ‘positional cloning’ and ‘candidate gene approach’ (Thomas et al., 1997; Stienke et al. 2003). The former includes scanning the entire genome for the presence of linkage between highly polymorphic markers and the known disease gene. The latter approach is directed towards the known biochemical markers or candidate genes that play a role in allergy regulation or etiology. Based on these, numerous genes linked to atopy and asthma have been identified (Table 1.1). Candidate genes of allergic disease and asthma include genes that regulate IgE production, proliferation and maturation of effector cells including eosinophils and mast cells. Genes responsible for atopic conditions are linked to a genetic marker on the long arm (q) of chromosome 11 at band 13 (11q13) along with γ chain of high affinity IgE receptor (Cookson, 1998). Cytokine gene cluster affecting allergic inflammation is located on chromosome 5 (5q31-33) including the genes encoding for IL-3, IL-4, IL-5, IL-9 and IL-13 (Le Beau et al., 2004). Polymorphisms have been observed in cytokine genes (IL-4, IL-9 and IL-13) responsible for modulating the intensity of allergic response and receptors of IL-13 and IL-4 (Hegab et al., 2004). IFN-γ gene and stem cell factor on chromosome 12q contribute to the pathogenesis of atopic disorders.

Huang and Marsh (1991) demonstrated HLA influence on the IgE response with antigen derived from ragweed pollens. This association was due to restriction of the response to a minor component of ragweed antigen (Amb a 5) by HLA-DR5 allele (Huang and Marsh, 1991). Antigen presentation of the immunodominant T-cell epitope of the major mugwort pollen allergen (Art v 1) has been associated with the expression of HLA-DRB1*01 (Jahn-Schmid et al., 2005). Similarly, type I hypersensitivity to allergens such as Fel d 1, Alt a 1 and bee venom has been associated with different HLA class II molecules (Faux et al., 1997; Soriano et al., 1997). Recently maturity of monocytes in response to external stimuli, an important predictor of allergen-specific T-cell reactivity, has been linked to the differential expression of HLA-DR (Upham et al., 2004). Understanding the genetic basis of disease will lead to new therapeutic approaches for allergy and asthma (Howard et al., 2003).
Table 1.1: Genes linked with atopy identified by positional cloning and candidate gene approach

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Putative genes or products</th>
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<tbody>
<tr>
<td>1p</td>
<td>IL-12 receptor</td>
</tr>
<tr>
<td>2q</td>
<td>IL-1, cytotoxic T lymphocyte-associated antigen, CD28</td>
</tr>
<tr>
<td>3p24</td>
<td>B-cell lymphoma-6 (STAT-6 binding inhibition), Chemokine cell receptor 4</td>
</tr>
<tr>
<td>5q23-35</td>
<td>IL-3, IL-4, IL-5, IL-9, IL-13, GM-CSF, LTC4S, Macrophage colony-stimulating factor receptor, β2-Adrenergic receptor, Glucocorticosteroid receptor</td>
</tr>
<tr>
<td>6p21-23</td>
<td>MHC, TNFs, Transporters involved in antigen processing and presentation (TAP1 and TAP2) Large multicatalytic proteolytic particles</td>
</tr>
<tr>
<td>7q11-14</td>
<td>T-cell receptor γ chain, IL-6</td>
</tr>
<tr>
<td>11q13</td>
<td>High-affinity IgE receptor (FcεRI) β chain, Fibroblast growth factor 3</td>
</tr>
<tr>
<td>12q14-24</td>
<td>IFN-γ, Stem cell factor, Nitric oxide synthetase (constitutive), β Subunit of nuclear factor Y (transcription factor for HLA genes) Insulinlike growth factor 1 Leukotriene A4 hydrolase STAT-6 (IL-4 STAT)</td>
</tr>
<tr>
<td>13q21-24</td>
<td>Cysteinyl leukotriene 2 receptor</td>
</tr>
<tr>
<td>14q11-13</td>
<td>T-cell receptor α and δ chains Nuclear factor κB inhibitor</td>
</tr>
<tr>
<td>16p11-12</td>
<td>IL-4 receptor</td>
</tr>
</tbody>
</table>
Environmental factors: Environmental factors such as tobacco smoke, pollution levels, etc. also contribute to the development of allergic diseases. The epigenetic changes make an individual respond to the changes in the environmental allergens skewing the immune response to Th2 type. Environmental factors are important regulators of development of Th2 cells/or their functions. Weak Th2-skewed priming to transplacental allergen occurs in foetus, which may syncital virus, para influenza virus, rhinovirus and adenovirus have been reported to induce asthma by release of granulocyte monocyte-colony stimulating factors and other inflammatory cytokines (McCunney., 2005; Myers and Maynard., 2005; Trasande and Thurston., 2005).

COMPONENTS OF ALLERGIC REACTIONS

Immunoglobulin E: Following exposure to specific allergens, atopic individuals produce high amount of IgE whereas non-allergic individuals have low IgE levels in the serum (0.0003 mg/ml) that is less than 0.002% of the total immunoglobulin (Dolan et al., 2004). Individuals with allergic conditions have high IgE levels up to 1% of total immunoglobulin (Dolan et al., 2004). Genetic pre-disposition, age, immune status and race determine the serum IgE levels. The widely used unit for measuring IgE is the international unit (IU-2.4 ng/ml). IgE levels in normal individuals range from 40.9 to 200 IU/ml (Dolan et al., 2004). In Indian population due to helminthic infestation, the levels of IgE could be comparatively higher i.e. 700 to 1025 IU/ml (Prussin and Metcalfe., 2003). Sharma et al. (2006) reported serum IgE levels 75 to 1500 IU/ml in allergic rhinitis (AR), 100 to 4000 IU/ml in bronchial asthma and 102 - 3300 IU/ml in asthma with AR group, while 10–725 IU/mL was in the control group (Sharma et al., 2006). A study on asthma patients recorded serum total IgE in the range of 6 to 800 IU/ml whereas it was 6-231 IU/ml in healthy controls (Kumar et al., 2006). Inspite of high IgE levels in Indian allergic patients, its diagnostic significance seems to be limited due to wide overlap of IgE levels in patients and healthy subjects. IgE antibodies provide protection against parasitic (helminthic) infestation.

Bennich and Johansson (1971) described the structure of IgE antibody that consists of two heavy and two light chains linked by disulphide bonds (Figure 1.4). Presence of ε chain in the constant region of the heavy chain of immunoglobulin is the characteristic feature of IgE antibody. Serum half-life of IgE is 2.5 days but
receptor bound IgE may remain for months due to protection from proteolysis (Steinsvik et al., 1997).

**IgE receptors:** Two classes of receptors based on affinity to IgE were identified and designated as FcεRI and FcεRII (Figure 1.5). They are expressed by various cell types and differ by 1000-fold for affinity to IgE.

**High-affinity receptor (FcεRI):** Mast cells and basophils express FcεRI, a high affinity receptor for IgE ($k_d = 1-2 \times 10^{-9}$ M). It is a multimeric protein containing four polypeptide chains of $\alpha$, $\beta$ and $2\gamma$ chains. It interacts with IgE molecule via two Ig-like domains of the chain. The $\beta$ chain spans the plasma membrane four times. The two-$\gamma$ chains are disulphide-linked and extend a considerable distance and extend into the cytoplasm. Each $\gamma$ chain has a conserved sequence in the cytosolic domain known as an immunoreceptor tyrosine based activation motif (ITAM). Allergen-mediated cross-linkage of the bound IgE results in the aggregation of the FcεRI receptors and rapid tyrosine phosphorylation leads to mast cell degranulation. This role of IgE receptor was confirmed by knockout mice experiments lacking FcεRI (Knol, 2006).

**Low-affinity receptor (FcεRII):** It has lower affinity for IgE ($k_d = 1-2 \times 10^{-6}$ M) and is present on lymphocytes, eosinophils, platelets, macrophages and dendritic cells (de la Salle et al., 1997). Allergen cross-linking of IgE bound to FcεRII activates B cells, alveolar macrophages and eosinophils. Atopic individuals have higher levels of CD23 (FcεRII) on lymphocytes and macrophages (Conrad et al., 1997).

**IgE synthesis and regulation:** The IgE response is under the control of various regulatory factors, the most important being T cells. IgE levels are regulated by the cytokines released by Th1 and Th2 cells. The production of IgE is regulated by complex interaction between B and T cells cytokines (Figure 1.6). Allergen is recognized by specific IgM present on the B-cell surface. This leads to internalization, endosomal processing and subsequent presentation of allergen in the form of peptide fragments associated with HLA class II to specific T cells. Recognition of HLA
Figure 1.4: **Schematic representation of IgE**: The molecular features of IgE antibody showing variable and heavy regions of light and heavy chains. **V L**: Light chain, variable region, **V H**: Heavy chain, variable region, **C L**: Light chain, constant region, **C H**: Heavy chain, constant region. *(Source: Arnold et al. 2006)*

Figure 1.5: **Schematic representation of IgE receptors**: High affinity (FceRI) and low affinity (FceRII) receptors depicted as transmembrane proteins in association with IgE. *(Source: Goldsby et al., 2003)*
Figure 1.6: Regulation of IgE synthesis via T-cell B-cell interactions: Binding of allergen to allergen specific B cell leads to 1) Internalization of allergen, its processing and presentation with MHC II on cell surface. 2) Recognition of the complex by T cell receptor (TCR) induces CD40 ligand expression. 3) CD40-CD40L interaction leads to CD80 up-regulation. 4) CD80-CD28 interaction provides stimulus for 5) Cytokine synthesis and T cell proliferation. 6) Binding of cytokines to B cell receptor causes 7) IgE synthesis and B cell proliferation.

Source: Miescher and Vogel, 2002; Larche, 2006
associated allergen fragment by the T-cell receptor (TCR) complex leads to two events:

☑ The secretion of lymphokines especially IL-4 and/or IL-13 (Binding of these cytokines to their receptors on B-cells provides first signal for isotype switching to Cε heavy chain gene).

☑ The expression of the CD40 ligand (CD40L) by the T cell. (Interaction of CD40L with CD40 expressed on B-cell surface provides second signal for switch to IgE).

**1) IL-4/IL13 interaction with receptors:** The interaction between IL-4 and IL-13 with their receptors initiate a signaling cascade that results in binding of STAT6 to the promoter regions to initiate expression of precursor mRNA for ε-chain. Other cytokines, including IL-5, IL-6, IL-12 and IFN-γ have modulatory effects on IgE synthesis. IL-5 and IL-6 increase IL-4 dependent IgE synthesis whereas IFN-γ inhibits the IL-4-dependent IgE response (Romagnani et al., 1989).

**2) CD 40/ CD40 L interaction:** The co-stimulatory interaction between CD40 ligand with CD40 leads to activation of nuclear factor kappa B (NF-κB) that binds to the ε-germline gene promoter and synergizes with STAT6 for ε-chain transcription and IgE synthesis. Also, CD80 (B7-1)/CD86 (B7-2), interaction with CD28 provides signals for T-cell survival and IL-4 secretion and subsequent Th1/Th2 development. Interaction of CD23 with CD21 on B cells induces IgE synthesis in an isotype specific manner (Figure 1.7). It has also been observed that IL-4 induction enhances IgE synthesis (Yssel et al., 1998) that is further enhanced by IL-5 and IL-6. IgE synthesis is also independently regulated by IL-13 in human and murine B cells (Vercelli et al., 1989). A balanced action of these lymphokines regulates IgE synthesis. The cytokines secreted by Th1 cells viz. IL-2, IFN-γ, tumor necrosis factor (TNF-α) etc. antagonize the IgE response. IgE synthesis is suppressed by IL-2 and IFN-γ cytokines by their direct action on the B cells. In addition to IL-2 and IFN-γ, TGF-β and PGE2 also inhibit IgE synthesis (Miescher and Vogel, 2002).
Figure 1.7: **Th1-Th2 interplay**: Schematic representation of factors regulating Th1 and Th2 responses.

*Source: Lee, 2008.*
Cells of immune system

The cells participating in the allergic response differentiate from pleuripotent hematopoietic stem cells that develop into the two distinct progenitor lineages: one for lymphoid and the other for myeloid cells. From the progenitor lymphoid cells, the T-cells develop under the environmental influence of the thymus and differentiate into T-cell subpopulations, including the T-cytotoxic (CD8), T-helper (T\(_h\)/CD4), and T-suppressor cells (Kay, 2006). Other lymphoid progenitor cells develop into B-cells that further differentiate into Ig-secreting plasma cells. Lymphoid cells also develop into large, granular lymphocytes, the so-called non-T, non-B (null), or natural killer (NK) cells (Trottein et al., 2006). The myeloid cells differentiate into the neutrophils, eosinophils and basophils (Blanchard and Rothenberg, 2009). The salient features of these cells are depicted in Table 1.2 (Goldsby et al., 2003).

Mediators

Mediators produced by human mast cells are divided into preformed mediators (packaged within secretory granules) and newly synthesized lipid mediators, which are the product of extracellular peptidolytic processing of LTC4. Lately, cytokines were also considered important mediators. The important properties of these mediators are listed in Table 1.3 (Goldspy et al., 2003).

Allergens

Allergen from various sources such as pollen, fungi, animal dander, insects, dust mites, drugs and foods act as sensitizing agent inducing type I hypersensitivities (Linhart and Valenta, 2005). Most of the allergens characterized are proteins or their modified forms eg. glycoprotein, lipoprotein, or proteins conjugated with chemical or drug haptens (Wagner et al., 2000; Beezhold et al., 2003). The role of certain carbohydrates as sole allergen has also been demonstrated (Aalberse et al., 1981; Jappe et al., 2006). Based on the route of exposure, allergens are classified into four categories i.e. inhalants (aeroallergens), ingestants (food), injectants (insect bite, stings etc) and contactants (cosmetics).
### Table 1.2: Cells of the immune system

<table>
<thead>
<tr>
<th>Cell Type Precursor (factors)</th>
<th>Activity (cells /μl)</th>
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<tbody>
<tr>
<td><strong>B cell</strong> Lymphoid stem cell (IL-7, IL-3); B progenitor (IL - 4, IL - 2, IL - 5, IL - 6).</td>
<td>Plasma cell; secrete antibodies. Memory cell; immunity. (total lymphocyte: 2750 cells /μl or 20-40% of WBC)</td>
</tr>
<tr>
<td><strong>T cell</strong> Lymphoid stem cell; B progenitor (IL-4); Thymocyte (IL -7, IL -2, IL-4).</td>
<td>T –helper cell (CD4+): secrete cytokines. Cytotoxic T lymphocytes (CD8+) eliminate altered self cells. Memory cells; long-term immunity.</td>
</tr>
<tr>
<td><strong>Monocyte</strong> Myeloid stem cell (IL -3, GM –CSF,IL -6); Granulocyte - monocyte progenitor (GM –CSF, M –CSF).</td>
<td>Circulate in blood streams, migrate to tissue and differentiate into macrophages. (Average of 540 cells /μl 1-6% of WBC).</td>
</tr>
<tr>
<td><strong>Macrophage</strong> Myeloid stem cell (IL -3, GM –CSF,IL -6); Granulocyte - monocyte progenitor (GM –CSF, M –CSF) monocyte (IL-8).</td>
<td>Tissue macrophage: phagocytic secrete hydrolytic enzymes, soluble factors and cytotoxic proteins.</td>
</tr>
<tr>
<td><strong>Dendritic cell</strong> Myeloid stem cell; Granulocyte - monocyte progenitor? monocyte?</td>
<td>Potent antigen presenting cell (no activation needed). Found in tissue; migrate to blood. (0.1% of WBC.)</td>
</tr>
<tr>
<td><strong>Neutrophil</strong> Myeloid stem cell (IL -3,GM –CSF,IL - 6); Granulocyte - monocyte progenitor (GM –CSF, M –CSF) monocyte (IL-8).</td>
<td>Polymorphonuclear leukocyte (PMN); phagocytic, first response to inflammation; moves from blood into tissue through extravasation. (Average of 5400 cells/μl or 50-70% of WBC.)</td>
</tr>
<tr>
<td><strong>Eosinophil</strong> Myeloid stem cell IL -3, GM –CSF EosinPhil progenitor GM – CSF,IL 5</td>
<td>Migrate from blood into tissue, anti parasitic, somewhat phagocytic (average of 275 cells/μl or 1-3% of WBC.)</td>
</tr>
<tr>
<td><strong>Basophil</strong> Myeloid stem cell (IL -3, GM –CSF); Basophil progenitor (GM – CSF, IL - 4).</td>
<td>Along with MAST CELLS, releases active substance; major role in development of allergenic response, Non phagocytic (average of 35 cells/μl or&lt;1% of WBC.)</td>
</tr>
</tbody>
</table>
Table 1.3: Mediators of allergic reaction and their effect.

<table>
<thead>
<tr>
<th>MEDIATOR</th>
<th>EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stored Mediators</strong></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>Smooth muscle contraction (leads to bronco-constriction in case of inhalant allergens increased peristalsis in case of ingested allergens). Vasodilatation (causes increased blood flow through dilated vessels manifesting as redness and local hyperthermia). Mucous secretion.</td>
</tr>
<tr>
<td>Proteases: Tryptase, Chymase, Carboxypeptidase, Cathepsin G.</td>
<td>Cleave C3 and C3a, activate fibroblasts and promote accumulation of inflammatory cells. Cause tissue damage.</td>
</tr>
<tr>
<td>Chemotactic factors: Neutrophil chemotactic factor Eosinophil chemotactic factor</td>
<td>Chemotactic for neutrophils Chemotactic for eosinophils</td>
</tr>
<tr>
<td>Proteoglycans: Heparin, Chondroitin sulfate</td>
<td>Aid in storage of preformed molecules.</td>
</tr>
<tr>
<td><strong>De-novo formed Mediators</strong></td>
<td></td>
</tr>
<tr>
<td>Cyclooxygenase products: Prostaglandins- PGD2, PGE2α, PGF2α, Thromboxane A2 (TXA2)</td>
<td>Vasodilatation, Smooth muscle contraction Chemotactic for neutrophils and eosinophils Severe and prolonged bronchoconstriction, mucus secretion and increased vascular permeability.</td>
</tr>
<tr>
<td>Lipoxygenase products: Leukotrienes- LTB₄, LTC₄, LTD₄</td>
<td></td>
</tr>
<tr>
<td>Platelet activating factor (PAF)</td>
<td>Platelet aggregation, accumulation of infiltrating leukocytes, bronchoconstriction. Activates serotonin release from platelets.</td>
</tr>
<tr>
<td>Cytokines: TNFα, IL-4,1L-5</td>
<td>Various effects (Section 1.3.3)</td>
</tr>
</tbody>
</table>

Generated using data from Broide, 2001; Abbas and Lichtman, 2004; Goldsby et al. 2003; Leech, 2002
**Inhalants:** The suspended biogenic material from pollens, spores, mites, insects and animals belong to inhalant allergens responsible for allergic triggers (Kay, 2006).

**Ingestants:** Ingestants include foods or drugs that can cause nausea, vomiting, stomach upset, diarrhea as well as rhinitis, asthma or skin rashes (Linhart and Valenta, 2005).

**Contactants:** They can result in rashes, itching, weeping blisters or other skin allergies on contact. The examples are cosmetics, metal, chemicals etc (Kay, 2006).

**Injectants:** This group includes injectible drugs, insect sting/bites, bee/fire ant venom etc. Exposure to them can cause severe systemic reactions (anaphylaxis) and could be fatal (Kay, 2006).

Based on the prevalence of an allergen in a given subset of population, they are classified as major and minor allergens. An allergen is considered ‘major’ when it elicits a reaction in 50% or more of an allergic population and ‘minor’ when less than 50% of the allergic population responds to the allergen. Lipid transfer protein (LTP) of hazelnut is classified as a minor allergen in northern and middle Europe, while the PR-10 protein is acknowledged to be major allergen in this region (Pastorello et al., 1999). LTP is suspected to cause more severe reactions than the PR-10 protein, which usually induces milder reactions.

**Purified allergens**

A large number of native proteins from different sources like mites (e.g. Der p 1, Der p 2), fungi (e.g. Alt a 1, Alt a 13, Asp f 1, Cur l 1, Epi p 1, Pen ch 13), pollens (e.g. Bet v1, Phl p 1, Phl p2, Phl p3), animal dander (e.g. Fel d 1), etc are purified and characterized in recent years. Despite the knowledge available, the native proteins could not become popular for clinical use due to certain disadvantages. The cost of protein purification is high and the yield is low with most of the methods. The purified samples from different chromatographic techniques show variation from one batch to another. The purified proteins did not show adequate biologic activity sometimes because of degradation or conformational changes due to harsh
conditions used for purification. Partially purified allergens get degraded during long time storage due to inherent proteases.

**Recombinant allergens**

Conventionally whole allergen extracts of the source materials are used as diagnostic agents or therapy. The majority of such extracts are complex mixtures of proteins, and the actual allergen content is usually a small fraction of all proteins. The diagnosis of type I allergic disorders using crude allergen extracts allows only the identification of source but not the disease-eliciting molecules. With the introduction of recombinant DNA technology, a panel of allergenic molecules has become available (Allergome database: [www.allergen.org](http://www.allergen.org)). The application of these recombinant allergens for *in vitro* tests has led to new forms of component-resolved diagnosis (CRD) and allowed the establishment of a patient's individual reactivity profile (Asturias et al., 2005; Crameri, 2006). Recombinant allergens can be produced as defined molecules in consistent quality and large amounts (Chapman et al., 2000; Bohle and Veiths, 2004). Furthermore, they can be modified to reduce their allergenic activity and to foster advantageous immunologic properties (Niederberger and Valenta, 2004; Bonura and Colombo, 2009).

Over the past few years, recombinant allergens from mites, pollens, animal dander, insects, moulds and foods have been cloned, sequenced, purified and characterized (Chapman et al., 2000; Valenta, 2002; Singh and Bhalla, 2003; Jeong et al., 2006). They can be used to dissect immune responses, elucidate allergen structure, determine structure–function relationships, cross-reactivity and generate products with novel immunologic or immunochemical properties (Valenta et al., 1999; Slater, 2004, Zhang et al., 2009). In addition, these can be used to enhance the therapeutic index of allergen vaccines substantially over their natural counterparts (Chapman et al., 2000; Lowenstein and Larsen, 2001; Valenta and Kraft, 2002; Niederberger and Valenta, 2004; Linhart and Valenta, 2005; Tonnel, 2005). Bet v 1 was the first reported allergen, whose structure was solved by X-ray crystallography and nuclear magnetic resonance (NMR) (Fedorov et al., 1997). Later, three dimensional structure of other recombinant allergens such as Der p1, Der p 2, Bla g 2, Equ c1, Bos d 2 and Ara h 1 were determined by these methods (Mueller et al.,...
Review of literature

1998; Rouvinen et al., 1999; Lascombe et al., 2000; Meno et al., 2005). At present more than 1200 proteins or nucleotide allergen sequences are deposited in Genbank and other databases. Recombinant allergens have been suggested for patient tailored specific allergy therapy (Bousquet et al., 1998; Chapman et al., 2000; Mothes et al., 2004; Egger et al, 2009).

Food allergens: IgE-mediated food allergy is a significant health problem affecting 6-8 % children and 3-4 % adults in general population (Roehr et al., 2004; Sampson, 2004). Various food allergens have been produced in recombinant form and have improved the diagnostics of food allergy (Bohle and Vieths, 2004). Recombinant Api g 1, Ara h 1, Mal d 1 and Gly m 1 demonstrated similar IgE activity to their natural counterpart and the diagnostic tests with these allergens were more sensitive than whole mass extracts. Recombinant food allergens have also been used as tools to investigate IgE-cross reactivity of profilins, tropomyosins, chitinases and Bet v 1 proteins (Scheurer et al., 1997, Leung et al. 1998). Allergenic fragments were used to map the IgE-binding regions of Pru a 1 and Pen a 1 (Reese et al., 1999). Mutants of food allergens showed low IgE reactivity hence suggested for safe and specific immunotherapy (Lorenz et al., 2001; Burks et al., 2008).

Fungal allergens: Airborne fungal particles have been implicated as causative factors in respiratory allergy, particularly asthma (Kurup et al., 2000). Fungi produce complex repertoire of allergens due to which cross-reactivity occurs between different fungal species (Crameri et al., 2006). At present, more than 70 fungal allergens have been cloned, sequenced and some of the proteins are commercially available (Kurup et al., 2002; Yasueda and Takeuchi, 2004). Many allergic proteins from fungi namely Aspergillus, Alternaria, Cladosporium and Penicillium were characterized (Vijay and Kurup, 2008). Recently, NADP-dependent mannitol dehydrogenase from Cladosporium herbarum, 11S globulin from yellow mustard seeds and Anis 4, a cysteine protease inhibitor from Anisakis simplex have been cloned, expressed and characterized (Trevino et al., 2004; Simon Nobbe et al., 2006). Cross-reactivity between Alternaria, Cladosporium and Aspergillus was established using NTF2, manganese superoxide dismutases and enolase (Wagner et al., 2000;
Fluckiger et al., 2002; Weichel et al., 2003). Asp f 2 was expressed and residues involved in IgE-binding were delineated using mutational studies (Banerjee and Kurup, 2003). These allergens have potential for diagnosis and therapy of fungal allergic disorders (Helbling and Reimers, 2003). The high-throughput cloning of fungal allergens revealed that fungi produce extremely complex repertoire of species-specific and cross-reactive allergens (Gupta et al., 2002; Shankar et al., 2005). The assessment of the clinical relevance of each cloned protein is a bigger challenge at present than cloning and production of these molecules.

**House dust mite allergens:** They are major source of indoor allergens with high frequency of IgE-mediated sensitization in many countries. House dust mites namely *Dermatophagoides farinae*, *D. pteronyssinus* and *Blomia tropicalis* play crucial role in triggering allergic diseases (Thomas et al., 2002). Efforts have been made to characterize allergens from mite sources (Bircher, 2005). At present 17 different groups of mite allergens have been recognized. Most of the allergens characterized from mite sources are enzymatic (proteases) in nature (Kawamoto et al., 2002). Der p 1 and Der p 5 cleaves the cell surface markers of dendritic cells, T cells and B cells, skewing the immune response towards Th2 type (Schulz et al., 1998; Kauffman et al., 2006). Recombinant tropomyosin from *D. pteronyssinus* was used to establish cross-reactivity between mite, cockroach and shrimp (Arruda et al., 2001). X-ray crystallography of Der p 1 elucidated a cysteine protease fold typical of the papain family, a magnesium-binding site and forms dimers with a large interface. Such an assembly appears ideal to interact with cell surface and trigger allergic inflammation. Native and recombinant glutathione–S-transferase (GST) from house dust mite possess similar allergenicity and was cross-reactive with GST from cockroach (Huang et al., 2006). Recombinant mite allergens have enabled better understanding of the biology of house dust mites and their role in allergic diseases (Milian and Diaz, 2004).

**Animal allergens:** According to the American Academy of Allergy Asthma and Immunology, approximately 3 million children in United States have asthma triggered by allergens e.g., animal saliva, dander, dust mites and pollen. Pet allergies are caused by type I immune response to proteins present in the animal saliva,
dander, or urine. Molecular characterization of a major cat allergen (Fel d 1), was done using the recombinant form of protein. T-cell epitopes of Fel d 1 were identified and hypoallergenic variant of allergen showed reduced IgE-binding (Saarne et al., 2005). Studies suggest that recombinant major dog allergens (Can f 1 and Can f 2) can be used for diagnosis/therapy of dog allergy (Immonen et al., 2005). Crystal structure of a major horse allergen, (Equ c 1) has helped in identification of B-cell determinants present on this allergen (Lascombe et al., 2000).

**Insect allergens:** IgE-mediated hypersensitivity has been reported to insects such as, cockroaches, hornets, bumblebees, ants, mosquitoes, flies or kissing bugs (Bircher, 2005). However, a majority of allergic reactions to insects are due to bites and stings. Stings from bees, wasps and ants produce a variety of clinical manifestations. But, most of the studies have been performed with whole-body insect extracts to detect IgE mediated sensitization (Pomes, 2008b). Serine protease, phospholipase, troponin C, hyaluronidase and tropomyosin are well-characterized allergens from different insect sources (Pomes, 2008b). Phospholipase A2, hyaluronidase and acid phosphatase are the three most potent honeybee venom allergens responsible for IgE-mediated allergic reactions (Grunwald et al., 2006). But expression of these allergens in E. coli reduced IgE-binding as compared to native allergens (Soldatova et al., 1998). These allergens are glycoprotein in nature and their expression in mammalian system yield protein(s) with immunoreactivity similar to native allergen (Soldatova et al., 1998). Crystal structure of recombinant phospholipase A2 and hyaluronidase led to identification of residues involved in IgE-binding. Allergens of B. germanica and P. americana were well-characterized among insect species (Jeong et al., 2004, 2006). A high prevalence of cockroach hypersensitivity was documented in atopic (20-55%) and asthmatic (49-60%) populations (Wu and Lee, 2005). Per a 1, 3 and 7 of P. americana and Bla g 2, 4, 5 and 6 of B. germanica are recognized as major allergens (Arruda et al., 2001). Epitopic region of Per a 1 and Per a 3 was identified using deletion mutants (Wu et al., 2003). Recombinant proteins derived from mosquito, honeybee, Blattella germanica, Periplaneta americana, Chironomus thummi, wasps and ants were demonstrated as potent allergens (Jeong et al, 2006).
Pollen allergens: Pollen grains of weeds, trees and grasses are important sources of inhalant allergens. The advent of molecular biology techniques has led to isolation and cloning of genes encoding major pollen allergens (Bhalla and Singh, 2004). Three-dimensional structure of recombinant Bet v 1 (Betula verrucosa), Ole e 6 (Olea europea) and Amb t 5 (Ambrosia trifida) revealed that they consist of mixed α-helical and β-sheet fold whereas Phl p 5 (Phleum pratense) consist mainly of α-helices and Phl p 2 consists of β-sheet structure (Spangfort et al., 2003; Trevino et al., 2004; Westritschnig et al., 2004). Epitope mapping of major pollen allergens became possible because of sequence availability of allergens. Overlapping peptides prepared as per the primary amino-acid sequences were used to identify the epitopic region of pollen allergens such as Cry j 1, Cry j 2 (Cryptomeria japonica), Bet v 1 (Betula verrucosa), Ole e 1 (Olea europea) (Sone et al., 1998; Gonzalez et al., 2006). Clinical trials with variants of Par j 1 (Perietaria judaica) and Bet v 1 having reduced allergenicity showed lower sensitization (Bonura et al., 2001; Mahler et al., 2004). Fusion of epitopes of major timothy allergens induced a Th1 type of immune response in mice model (Kussebi et al., 2005).

Recombinant allergens were analyzed with respect to structure, epitopes or cross-reactivity (Valenta, 2002). Their clinical potential has also been explored by generating genetically engineered hypoallergenic derivatives (Kraft et al., 1999; Chapman et al., 2000) with reduced allergenicity. The modified allergen should retain T cell-stimulating activity along with reduced IgE binding and induce blocking antibodies. Niederberger et al. (2004) had shown that recombinant trimeric constructs of Bet v 1 have ability to modify IgE and IgG antibody production, skin test reactivity and symptom score. A new generation of hypoallergens can be produced based on the detailed knowledge of the tertiary structures of the allergens and their T-cell and B-cell epitopes (Chapman et al., 2000). Many hypoallergenic variants were produced from pollen, insect venom, food and latex with promising characteristics determined in preclinical studies (Kraft et al., 1999; Kazemi-Shirazi et al., 2002; Bohle and Vieths, 2004; Niederberger and Valenta, 2004).
Cytokines (Th1/Th2 paradigm)

Cytokines are small secretory proteins that mediate and regulate immunity, inflammation, and hematopoiesis. They generally act over short distances (paracrine), have short half life and are required at low concentration. They act by binding to specific membrane receptors, which signals via secondary messengers to alter gene expression. Cytokines can modulate expression of membrane proteins, proliferation, and secretion of effector molecules (Ngoc et al., 2005). Cytokines can be categorized as lymphokine, monokine, interleukin and chemokine based on source of synthesis and activity. Th1 and Th2 subsets develop from the same precursor cells and the pattern of differentiation is determined by environmental stimuli present early during immune responses (Figure 1.8 and 1.9).

Following cytokines have been implicated in allergic conditions:

- **IL-4**: It is produced primarily by Th2 cells, NK cells, mast cells, basophils and eosinophils (Seder et al., 1991; Ying et al., 1997). It stimulates growth and differentiation of B-cells, enhances surface expression of CD23, MHC class II and IL-4R on B cells and monocytes. It promotes synthesis of IgG1, IgE, IgA and drives initial differentiation of T cells to the Th2 phenotype and ε-type switch. IL-2, IL-5 and IL-6 are synergistic with IL-4 to increase IgE secretion (Banchereau et al., 1994). Release of IL-4 within airways also increases goblet cell metaplasia, mucus hypersecretion and eosinophil recruitment (Hamid and Minshall, 2000). IL-25 stimulates IL-4 secretion.

- **IL-5**: It is secreted by activated Th2 cells and eosinophils. It enhances IL-4 induced IgE synthesis and CD23 expression. It stimulates growth and differentiation of eosinophils precursor in bone marrow and acts as an eosinophil chemotactic factor (Chevailler, 1992).

- **IL-6**: IL-6 is secreted by Th2 cells, eosinophils and macrophages. It differentiates B lymphocytes into mature plasma cells and secrete immunoglobulins (Borish and Steinke, 2003).
Figure 1.8: Schematic overview of T-cell phenotypes in allergic disease: Th1, Th2, Th17, and Treg cells are characterized by cytokines, which mediate specific functions in different tissue cells such as dendritic cells (Dc), keratinocytes (Ker), epithelial cells (Epit), B cells, eosinophils (Eos), mast cells, fibroblasts (Fib) and neutrophils (Neut).

Source: Schmidt-Weber et al., 2007.
Figure 1.9: Development of Th1 and Th2 lymphocytes: Antigens enter through the endobronchial tree, cross the epithelial surface, and interact with naive Th cells and DCs. As a result of signals from the surrounding microenvironment, they differentiate into Th1 cells, which produce IFN-γ, IL-2, and lymphotoxin (LT), or Th2 cells, which produce IL-4, IL-5, IL-9, IL-13, and IL-10. Polarization into Th1 cells occurs via a STAT-1 and T-bet–dependent pathway under the influence of CD8α+ DCs and macrophage-derived cytokines such as IFN-γ, IL-12, and IL-18. Differentiation into Th2 cells occurs via a pathway that involves STAT-6, GATA-3, nuclear factor of activated T cells-c (NFATc), and c-maf under the influence of CD8α– DCs and IL-4, which may come from mast cells. Source, Elias et al., 2003.
**IL-8:** It is a pro-inflammatory cytokine present along cytoplasmic membranes and in intracellular granules that induces chemotaxis of neutrophils and T lymphocytes (Cheng et al., 2007).

**IL-9:** IL-9 was described initially as mast cell growth factor (Renauld, 2007). It contributes to allergy by stimulating production of mast cell proteases and FCERI α chain. It also promotes growth and survival of T lymphocytes (Borish and Steinke, 2003).

**IL-10:** IL-10 inhibits production of pro-inflammatory cytokines (IL-4 and IL-5) and chemokines (eotaxin I &II), expression of HLA-DR, and some co-stimulatory molecules by Th1 macrophages, neutrophils, and eosinophils. Those with asthma have a decreased expression of IL-10 illustrating IL-10’s important role in regulating allergic responses (Yssel et al., 2001).

**IL-12:** It is produced by B cells and macrophages. It induces IFN-γ and IL-2 production and down regulates Th2 cytokines (Kroenke et al., 2008).

**IL-13:** It is produced by activated Th2 cells and mast cells (Hoshino et al., 1999). The biological effects of IL-13 overlap with IL-4 except that IL-13 does not activate human T cells (Hamid and Minshall, 2000).

**IL-17:** It is produced by T-cells and eosinophils, and induces bronchial fibroblasts to increase expression of several mediators, such as IL-6, GM-CSF which helps in amplification of airway inflammation and remodeling (Bloemen et al., 2007).

**IL-23:** It inhibits IgE isotype switching (Ju et al., 2008)

**IL-25:** It is a novel Th2 cytokine of the IL-17 family that plays a key role in allergic inflammation (Ballantyne et al., 2007; Wang et al., 2007). Tamachi et al., have reported that over-expression of IL-25 in mouse induces eosinophilia (Tamachi et al., 2006).
 IFN-γ: IFN-γ inhibits the IL-4-dependent IgE response *in vitro* and promotes Th1 response (Yoshimoto et al., 1997).

 Tumor necrosis factor-α: TNF-α, lymphotoxin and other cytokines activate macrophages for cell-mediated immunity against intracellular microbes and antagonize the IgE response (Abbas et al., 1996).

 Transforming growth factor-β: It is the most potent pro-fibrotic cytokine which correlates with disease severity. It is a chemoattractant for many inflammatory cells and helps in tissue remodeling (Bloemen et al., 2007).

**Allergic Reaction: Signalling Pathway**

Mast cells and basophils are granulated cells that play a pivotal role in allergy and inflammation. The activation of mast cells induces exocytosis and fusion of cytoplasmic granules with the plasma membrane, followed by the release of inflammatory mediators within minutes of stimulation. FceRI-stimulation initiates a signaling cascade that includes activation of tyrosine kinases, such as syk, lyn, fyn, and btk, and phosphorylation of adapter proteins (Oliver et al., 2000). These adapters include the linker for the activation of T-cells (lat), SH2 domain-containing leukocyte protein of 76 kDa (slp-76), grb2-associated binder2 (gab2), mist/clnk, 3bp2, and adhesion-and degranulation-promoting adapter protein. The role of microtubules in degranulation has been shown by Nishida et al., 2005.

Allergen cross-linking of IgE leads to FcγRI aggregation initiating synergistically acting *calcium dependent* (Lyn/Syk/PLC/Ca^{2+} mediated) and *calcium independent* (Fyn/GAB2/PKC mediated) degranulation of mast cell vesicles (Nishida et al., 2005) (Figure 1.10)

**Calcium dependent signaling:** Clustering of FcγRI initiates cellular response by three ways:

a. Phosphorylation of tyrosines in ITAMs of β and γ chains by protein tyrosine kinase (PTK) ‘Lyn’ (Cambier, 1995), which signals recruitment and activation of cytosolic kinase ‘Syk’, (Oliver et al., 2000; Bruhns et al., 2005). This signaling recruits cytosolic phospholipase Cy1 in membrane catalysing the formation of inositol-1, 4, 5-
Figure 1.10: **Allergic cascade and signaling events**: Diagrammatic representation of events occurring in course of mast cell degranulation. Cross-linking of IgE antibodies by allergen signals calcium dependent (yellow arrows) or calcium independent (purple arrows) events resulting in mediator release. *(Source: Nishida et al., 2005, Goldsby et al., 2003)*
trisphosphate (IP3) and diacylglycerol (DAG) from membrane phospholipids. IP3 opens calcium channels on the surface of intracellular calcium stores. DAG activates protein kinase C (PKC) which causes microtubule assembly and granule fusion in presence of calcium ions.

b. Phosphatidylserine (PS) is converted to phosphatidylethanolamine (PE) which is methylated to phosphatidylcholine (PC). Accumulation of PC on membrane surface increases membrane permeability and helps in formation of Ca^{2+} channels (Goldsby et al., 2003).

c. Activation of membrane adenylate kinase, leading to transient increase in cAMP that activates protein kinases. These kinases phosphorylate some membrane proteins to modulate permeability of granular membrane to water and Ca^{2+}. This leads to swelling and fusion of granules to plasma membrane. Sustained rise in cytosolic Ca^{2+} leads to formation of arachidonic acid (AA) that is converted into leukotrienes and prostaglandins by action of lipoxygenase and cyclo-oxygenase, respectively.

**Calcium independent signaling:** Aggregation of FcεRI by allergen induces phosphorylation of adaptor protein Gab2 by ‘Fyn’ activating a GTP binding protein of Rho family ‘Rho-A’. This signals microtubule stabilization and greater access of granules to plasma membrane. The consequent restructuring of cytoskeletal network causes translocation of granules to plasma membrane in microtubule-dependent and calcium-independent manner (Nishida et al., 2005).

**THERAPEUTIC STRATEGIES**

The key elements of allergy management are, 1) preventing the exposure of sensitized individuals to allergen and, 2) treating these individuals with therapeutic agents appropriate to the disorder. Precisely, the management of allergic diseases includes allergen avoidance, medication (pharmacological treatment), immunotherapy and education (Figure. 1.11).
Figure 1.11: Therapeutic strategies for allergy management.
Allergen avoidance: Allergen avoidance or reduction in exposure whenever possible is the best form of therapy (Malling and Weeke, 1993; Chan-Yeung et al., 2007). Allergen avoidance can provide considerable benefit to patients with demonstrated allergies. However, this is not always possible with inhalant allergens e.g. pollen or mold. Animal dander allergens can be removed from the patient’s home by removing the pets. But unless the patient’s social life is to become severely restricted, it is not possible to eliminate totally the indirect allergen contact of the patient by exposure to animal allergens. In theory, elimination of house-dust mites is possible, but, in practice, it has proved difficult to reduce the number of mites by ordinary cleaning. Immunotherapy should be considered in patients with allergic diseases when allergen reduction is impossible, or when the disease responds insufficiently to allergen reduction, even when symptomatic relief can be achieved with drug therapy (Van metre and Adkinson, 1993). The latter point is controversial and needs further documentation. Total allergen avoidance appears to be effective e.g. patients are symptom-free out of the pollen season or away from occupational exposure. However, patients are often sensitized to many allergens and the degree of exposure varies within indoor and outdoor environments. Thus, avoidance measures are still recommended but more studies are required in this aspect.

Pharmacotherapy

**Antihistamines:** Antihistamines counter the effects of histamine released by the mast cells or basophils and have proved useful in relieving sneezing and other rhinitis symptoms. Patients on antihistamines experience some distressing side effects such as, drowsiness, loss of alertness and coordination (Blaiss, 2005), and hence antihistamines with fewer side effects were suggested. These non-sedating antihistamines are effective in preventing histamine- induced symptoms but at higher doses it has risk of cardiovascular disease (Howarth, 2002; Walsh, 2002).

**Topical nasal steroids:** Topical nasal steroids are anti-inflammatory drugs in reducing allergic manifestation (Barnes et al., 1999; Mygind et al., 2001). In addition to other beneficial actions, they reduce number of mast cells, mucus secretion and nasal swelling. The combination of antihistamines and nasal steroids is effective in
Chapter 1

Review of literature

Reducing moderate or severe allergic rhinitis. The topical nasal steroids may cause side effects, but they are safe when used in recommended doses for short period.

**Cromolyn sodium:** Cromolyn sodium is a non-steroidal anti-inflammatory drug to prevent allergic reactions. It acts as mast cell stabilizer by inhibiting the release of histamine and leukotrienes from the mast cell. It has a few side effects such as bronchospasm, nasal congestion etc (Konig, 1997; Ratner et al., 2002; Kotaniemi-Syrjanen et al., 2005; Rosner, 2006).

**Decongestants:** Decongestants relieve nasal congestion and swelling by narrowing the blood vessels. Some of the decongestants include oxymetazoline (Afrin) and pseudoephedrine (Sudafed, Actifed). These act by suppression of multiple inflammatory genes for cytokine adhesion molecules and inflammatory mediator receptors. However, side effects such as headache, dizziness, and hallucinations limit their scope (Barnes et al., 2000).

**Corticosteroids:** Steroids are the most effective treatment for atopic diseases and asthma. Inhaled steroids are preferred and known to improve bronchial hyper-responsiveness and symptoms of allergies and asthma. However, systemic side effects limit the dose and use for prolonged periods. New-generation inhaled corticosteroids including budesonide, fluticasone propionate and mometasone furoate have a high level of anti-inflammatory action with minimal side effects, as the swallowed fraction of drug is largely removed by hepatic metabolism. However, these drugs are absorbed from the lung or nasal mucosa and may have some systemic effects. They act by binding to cytosolic glucocorticoid receptor, which translocates to the nucleus and binds as a homodimer to DNA to activate genes. Their effectiveness in treating complex inflammatory conditions is mainly because corticosteroids can suppress multiple inflammatory genes like cytokines, inflammatory enzymes, adhesion molecules, transcription factors, such as activator protein-1 (AP-1), nuclear factor-kB (NF-kB) and nuclear factor of activated T cells (NFAT), that regulate inflammatory gene expression and inflammatory mediator receptors (Barnes et al., 2000).
Immunotherapy

Allergen immunotherapy (IT) is effective in controlling symptoms of allergic rhinitis/asthma and is the only method demonstrated to modify the long-term course of the disease (Des Roches et al., 1997). However, studies have also demonstrated low degree of clinical efficacy (Fernández-Caldas et al., 2006). Meta analysis of clinical trials of allergen immunotherapy has shown benefits for the treatment of allergic rhinitis/asthma, but the major risk associated is systemic reactions (anaphylaxis) leading to death (Rogala, 1998; Karaayvaz et al., 1999). The use of IT decreased because the risk-to-benefit ratio was not acceptable, compared to pharmacologic management of allergic disorders (Rogala, 1998). Specific IT requires extracts with consistent allergenic activity and composition. But the whole mass extract with undefined allergenic potency is still used in vaccines limiting the scope of IT.

Allergen immunotherapy

Allergen specific immunotherapy (IT) is an efficacious treatment that prevents progression of allergic disease. It involves administration of gradually increasing quantities of an allergen or allergen extract. The efficacy of therapy has been proved by number of studies with several allergens. The therapy has been successfully used to treat allergic rhinitis (Durham and Till, 1998), allergic asthma and insect venom anaphylaxis (Muller et al., 1998). Immunotherapy is also suggested as a secondary preventive measure for those with allergic rhinitis (Malling, 2004).

Types of Immunotherapy

Based on the routes of antigen administration, immunotherapy has been classified as sublingual, subcutaneous, intranasal and oral.

- **Subcutaneous immunotherapy (SCIT):** It is performed by subcutaneous injection of increasing doses of allergen.

- **Sublingual immunotherapy (SLIT):** In sublingual swallow immunotherapy, allergen is kept under the tongue for 1-2 minutes and then swallowed. Studies with SLIT showed a reduction in asthma symptoms and requirement for β-agonists in treated patients (Bousquet et al., 1998).
**Oral IT**: Allergens are placed in beads/tablets which are then coated. The enteric coating of allergen preparation dissolves in high pH of intestine making allergen available for the immune response (Umetsu, 2004). Administration of rye grass pollen extract by oral IT led to significant reduction in symptoms and medication score of asthmatic subjects (Tepas et al., 2004).

### Mechanism of Immunotherapy

The exact mechanism that operates in immunotherapy to yield allergen specific non-responsiveness is unknown (Bousquet, 2005). Studies suggest two types of immunological changes that ameliorate the disease symptoms.

**a) Switching/skewing of T cell response to Th1 type**: Immunotherapy acts on T cells to modify peripheral and mucosal Th2 responses to allergen in favor of Th1 responses (Till et al., 2004). This is paralleled by increase in allergen-specific IgG (mainly IgG4), (Benjaponpitak et al., 1999; Majori et al., 2000; Wachholz et al., 2002; Fu et al., 2003; Wachholz et al., 2003; Gardner et al., 2004a).

**b) Generation of CD4+CD25+ T regulatory cells**: These cells secrete inhibitory cytokines IL-10 and TGF β (Till et al., 2004).

### OXIDATIVE STRESS

Oxidant generation is part of the normal metabolism of many types of cells and is critical for cell homeostasis. To protect against exposure to reactive oxidants, the lung has a well developed antioxidant defense system (Comhair and Erzurum, 2002). Oxidative stress is said to occur, when there is imbalance between oxidants and antioxidants (in favor of oxidants). The experimental and clinical data suggest that oxidants play a role in the pathogenesis of several respiratory disorders, including bronchial asthma (Caramori and Papi, 2004). There is increasing evidence that the chronic airway inflammation typical of asthma results in an increased oxidative stress to the airways (Figure 1.12). Also, many of the triggers for asthma exacerbations, including viral infection and air pollutants, may activate the production of oxidants, resulting in increased inflammation that leads to asthmatic symptoms.
Figure 1.12: Mechanism of oxidant mediated lung inflammation: The inflammatory response is mediated by oxidants which are inhaled and/or released by the activated neutrophils, alveolar macrophages (AMs) and epithelial cells leading to depletion of the antioxidant reduced glutathione (GSH). Activation of transcription of the pro-inflammatory cytokine and chemokine genes, upregulation of adhesion molecules and increased release of pro-inflammatory mediators are involved in the inflammatory responses. GSSG: oxidized glutathione; ↓: decrease; ↑: increase.
Source, Rahman and MacNee., 2000
Sources: The inflammatory cells recruited to the asthmatic airways have an exceptional capacity for producing oxidants. Once recruited in the airspaces, inflammatory cells may become activated and generate reactive oxidants in response to various stimuli. Activated eosinophils, neutrophils, monocytes, macrophages, and also resident cells such as bronchial epithelial cells, can generate oxidants (Dworski, 2000; Henricks et al., 2001; Bowler et al., 2002; Rahman et al., 2002). Eosinophils possess several times greater capacity for generating oxidants than neutrophils, and the EPO content of eosinophils is several times higher than that of MPO in neutrophils (Dworski, 2000; Henricks et al., 2001; Aldridge et al., 2002; Bowler et al., 2002; Rahman et al., 2002). MPO and EPO derived reactive oxygen species can also interact with nitrite (NO$_2^-$) and H$_2$O$_2$ leading to the formation of reactive nitrogen species (RNS; nitrosants). A powerful oxidant, the radical peroxynitrite (ONOO$^-$), is produced from the reaction between O$_2^-$ and nitric oxide (NO) which is increased in the asthmatic airways (Dworski, 2000; Henricks et al., 2001; Bowler et al., 2002; Rahman et al., 2002; Ricciardolo, 2003). As in many other inflammatory conditions, the oxidative ‘burst’ in asthma is a non-specific process initiated by the concurrent action of numerous inflammatory pathways. Indeed, several asthma mediators including lipid mediators, chemokines, adhesion molecules, and eosinophil granule proteins are potential promoters of oxidant production (Barnes et al., 1998).

Oxidants and Asthma: The studies suggest that oxidative stress is increased in children and in adults with asthma, not only in lungs but also in the circulation. Excessive production of oxidants occur spontaneously or after stimulation in blood leucocytes of stable asthmatics compared to normal subjects (Dworski, 2000; Henricks et al., 2001; Bowler et al., 2002; Rahman et al., 2002; Nadeem et al., 2003). Levels of EPO and/or MPO are increased in the peripheral blood, induced sputum, and bronchoalveolar lavage (BAL) fluid from patients with stable asthma (Dworski, 2000; Henricks et al., 2001; Monteseirin et al., 2001; Aldridge et al., 2002; Bowler et al., 2002; Rahman et al., 2002). Recently, increased levels of direct and indirect markers of oxidative stress (including malondialdehyde, thiobarbituric acid reactive products (TBARs) and H$_2$O$_2$ have been found in the urine, plasma, sputum, BAL fluid,
and lung tissues of patients with asthma. Their level is often related to the severity of the disease and is inversely related to the degree of stability. Furthermore, analysis of exhaled breath and exhaled condensate has allowed direct assessment of H$_2$O$_2$ and nitric oxide (NO) and measurement of several indirect byproducts of oxidation in those samples (Kharitonov et al., 2001, 2002). The latter are footprints of oxidation on different substrates such as proteins (nitrotyrosine), lipids (isoprostanes and ethane), and DNA (hydroxydeoxyguanosine) (Kharitonov et al., 2001, 2002). There is a lot of interest in the effect of these new non-invasive markers on oxidative stress and in possible clinical application (Kharitonov et al., 2001; 2002; Corradi et al., 2003).

The expression of nitrotyrosine is increased in bronchial, bronchiolar epithelial cells, smooth muscle cells, eosinophils of airways and lung parenchyma of patients with stable asthma (Saleh et al., 1998; Kaminsky et al., 1999; Dweik et al., 2001). Excessive production of 3-bromotyrosine, another marker of oxidative stress, has been reported in the airways of patients with severe asthma (MacPherson et al., 2001). A significant increase in 3-bromotyrosine has also been observed in sputum proteins of asthmatic subjects. This finding is of particular interest as the level of 3-bromotyrosine is strongly related to levels of EPO (Wu et al., 2000; Aldridge et al., 2002) However, whether peroxidases simply reflect granulocytic inflammation or whether they actively contribute to tissue damage in asthma remains to be determined. Taken together, these data indicate that oxidative stress is enhanced in asthma, and such redox imbalance is not confined within the lungs.

**Antioxidant defences in asthma:** Mammals have evolved complex antioxidant strategies (enzymatic/nonenzymatic) to utilize oxygen and to minimize the noxious effects of reactive oxygen species. Although nonenzymatic antioxidants are the first line of defense against different ROS, but several enzymatic antioxidants are needed to work in concert with non-enzymatic antioxidants to form a tightly regulated antioxidant network.

Antioxidants within cells, cell membranes, and extracellular fluids can be upregulated and mobilized to neutralize excessive and inappropriate ROS formation.
(Halliwell and Gutteridge, 1996). The extent of oxidative stress will depend in part on the antioxidant defenses available within the respiratory tract. Any deficiency in this compartment will compound the ROS-mediated airway responses and tissue injury. There is evidence for an oxidant–antioxidant imbalance in asthmatic airways is that shows decreased total antioxidant capacity as well as individual antioxidants in plasma and BAL fluid of patients with asthma (De Raeve et al., 1997; Comhair et al., 2000; Kanazawa et al., 2002; Nadeem et al., 2003). Deficiencies in individual nonenzymatic antioxidants have been reported in asthmatics. Low levels of vitamin C and urate in the respiratory tract have been observed in adults with mild asthma, together with increased amounts of oxidized glutathione (GSSG) in airways (Kelly et al., 1999). Vitamin C level was reported decreased in plasma/serum, whole blood and BAL fluid by several workers in asthma (Aderele et al., 1985; Kelly et al., 1999; Kalayci et al., 2000; Vural and Uzan, 2000; Shanmugasundaram et al., 2001). Vitamin E level was also decreased in BAL fluid, bronchial wash, red cells and plasma (Mohan and Das, 1997). Disturbed reduced glutathione (GSH) status is reported in asthma, with total and oxidized GSH being elevated in BAL fluid and reduced GSH and total GSH being elevated in red cells (Smith et al., 1993; Kelly et al., 1999; Vural and Uzan, 2000; Nadeem et al., 2003). Wood et al. (2008) have shown recently that plasma alpha-tocopherol was low and sputum supernatant levels of total, reduced and oxidized GSH were elevated in asthma than controls. Sackes et al. (2008) have reported decreased levels of most of the nonenzymatic antioxidants including vitamin C, vitamin E, reduced GSH, lycopene and carotenoids in asthmatic children.

**Antioxidant therapy:** The clinical ineffectiveness of antioxidant therapy is partly due to oxidative stress, which alone is not the only factor involved with the lung diseases. Novel antioxidative drugs, among which is thioredoxin (TRX), are now under investigation for lung disorders. TRX is an endogenous redox-regulating protein and is known to have effects such as antiapoptosis and antiinflammation. It is reported to be effective in wide variety of animal models for bronchial asthma and chronic obstructory pulmonary disorder (COPD) (Tomoaki et al., 2003; Ichiki et al., 2005). Current antioxidant therapy is based on either supplementation of an antioxidant itself or an enhancement of antioxidant defenses by antioxidant inducers. However,
to restore proper oxidant/antioxidant balance disturbed by lung disease, an enormous amount of antioxidant is required, and that is likely to cause drug toxicity. Regulation of redox-sensitive pathways could be an alternative therapeutic approach. As NF-kB and AP-1 are well known pathways leading to pro-inflammation, whereas Keap1/Nrf2 signaling enhances antioxidant production. Abnormal regulation of these pathways has been detected in most lung diseases, including bronchial asthma and COPD, hence it might be a potential target for therapeutics (Saravanan et al., 2008; Hisatoshi et al., 2008). Redox regulation however, seems promising as therapeutics for lung diseases. Conventional antioxidants would still be effective with better patient selection and combination therapy. The identification of disease-specific signaling mechanism could be helpful for development of novel molecular targeted therapy.

ENZYME ALLERGENS

Over the years, many allergens have been purified from diverse sources such as pollen, fungi, insects, mites, etc employing different chromatographic techniques. For example, the major allergen Bet v 1 from birch pollen was purified from natural sources by employing ammonium sulfate precipitation, hydrophobic interaction chromatography and size exclusion chromatography (Bollen et al., 2007). Alt a Bd29K (Alt I allergen group), a major allergen of A. alternata was purified by gel-permeation and ion-exchange HPLC (Deards and Montague, 1991). Epi p 1, a major glycoprotein allergen of Epicoccum purpurascens was purified using Concanavalin A Sepharose and Sephadex G-75 chromatography followed by electro-elution (Bisht et al., 2004). PLA2 (Phospholipase A2), the major bee-venom allergen, was purified by gel filtration, inactivated by denaturing, and carboxymethylating its cysteine residues (Dhillon et al., 1992). Natural B. germanica GST (Bla g 5) was purified from whole body cockroach extract by glutathione affinity chromatography and size exclusion (Arruda et al., 1997). Der p 3, a major allergen from Dermatophagoides pteronyssinus was purified from the spent growth medium by Benzamidine-Sepharose 6B affinity and gelfiltration chromatography (Stewart et al., 1992).

Key determinants for allergenicity involve dose and route of allergen exposure, genetic predisposition of the individual towards developing a Th2
response to specific allergens and to some extent the intrinsic property of allergen as it may be involved in the mechanism causing sensitization (Pomes, 2002). Many of the characterized allergens from different sources have demonstrated biological functions such as enzyme activity (protease, enolase, chitinase, amylase, superoxide dismutase, glutathione S transferase, phospholipase, arginine kinase), structural proteins (tropomyosin, troponin C, profilin), Ca-binding proteins, protease inhibitors, pathogenesis-related proteins, etc (Arruda et al., 1995; van Ree et al., 1995; Dhillon, et al., 1992; Midoro-Horiuti et al., 2001; Ayuso et al., 2002; Martínez et al., 2002; Pomes, 2002; Bisht et al., 2004; Butteroni et al., 2005; Hindley et al., 2006; O'Neil et al., 2006; Shankar et al., 2006; Sharma, et al., 2006; Sookrung et al., 2006). This has led to the suggestion that in addition to intrinsic properties, including the integrity of the allergenic protein, resistance to degradation, binding capacity, and the degree of sequence similarity to host proteins, biologic function may be a key determinant for allergenicity (Pomes, 2002; Sehgal et al., 2005). Based on biologic function, allergens can be divided into several groups namely: enzymes, enzyme inhibitors, and binding, structural, and regulatory proteins (Smith et al., 2000). Enzyme (Protease) as allergen was first reported in workers of detergent industry by Flindt (1969). Many clinically important airborne allergens are hydrolytic enzymes, include proteases, carbohydrases, and ribonucleases from different sources. Among these, enzymes of class hydrolases and oxidoreductases are more common. Nonhydrolytic enzyme allergens include glutathione-S-transferase, amylase, plant pectin lyase, enolase, alcohol dehydrogenase, aldolase etc. Among these, few are recognized as major allergens (Thompson, 1998; Stewart, 2003). Enzyme allergens explored in most of the studies are protease, transferase, enolase, dehydrogenase and chitinase.

**Proteases:**

Among the allergens with different biological functions, proteases are the most common allergens isolated from fungi, mites, insects, parasites and pollen. The significance of protease activity in allergy was first highlighted when it was discovered that the major allergen from the house dust mite (D. pteronyssinus, Der p 1), was a cysteine protease (Tovey et al., 1981). Der p 1 is the most immunodominant dust mite allergen involved in the induction of IgE mediated
Chapter 1

Review of literature

hypersensitivity and is considered the most common cause of allergen-linked asthma worldwide (Tovey et al., 1981; Gough et al., 1999). Cysteine protease allergens are primarily reported from different mites such as *D. pteronyssinus* (Der p 1), *D. farinae* (Der f 1), *Blomia tropicalis* (Blo t 1) (Thomas et al., 2002). Aspartic protease allergens have been identified in fungi namely *A. fumigatus* (Asp f 10) and german cockroach *B. germanica* (Bla g 2) (Arruda et al., 1995; Shen et al., 2007). Metalloprotease as an allergen has been reported from *A. fumigatus* (Asp f 5) (Shen et al., 2007). Serine proteases from house dust mites and fungal extracts are important inhalant allergens. *D. pteronyssinus*, a major source of dust mite allergens contains serine protease allergens, viz. Der p 3 (trypsin), Der p 6 (chymotrypsin) and Der p 9 (Thomas et al., 2002). Several *Aspergillus* and *Penicillium* species contain both alkaline (e.g. Asp f 13 in *A. fumigatus*, Asp o 13 in *A. oryzae*, Pen ch 13 in *P. chrysogenum*, Pen c 13 in *P. citrinum*) and vacuolar serine protease (Asf p 18 in *A. fumigatus*, Asp n 18 in *A. niger*, Pen ch 18 in *P. chrysogenum*, Pen o 18 in *P. oxalicum*) as major allergen(s) (Shen et al., 1996, 1998, 2001a, 2001b, 2003, 2007). Recently, serine proteases from *C. lunata* and *E. purpurascens* were identified as major allergens (Bisht et al., 2004; Gupta et al., 2004). Thus, the serine proteases may be considered as a major pan-fungal allergen group of airborne fungal species (Shen et al., 2007).

The enzymatic activity of proteases has been implicated in mediating allergenic affect. The environmental levels of serine proteases have been linked to asthma disease (Montealegre et al., 2004). There is mounting evidence that proteinases are involved in the pathogenesis of asthma. Mast cell tryptase, a serine proteases increases in bronchioalveolar lavage (BAL) fluid of patients with asthma after allergen challenge (Schwartz, 1990). Furthermore, many aeroallergens associated with asthma, such as dust mite allergens and various fungal allergens including Cur l 1 are proteinases (Hewitt et al., 1998; Berger et al., 1999; Shen et al., 1999; Kauffman et al., 2003; Gupta et al., 2004). These proteases act via protease activated receptors (PARs), a family of G-protein coupled receptors triggering intracellular downstream signaling (Coughlin, 2000; Macfarlane et al., 2001). Protease activity of allergens can cleave complement factor C5 to its active components, which is reported to have a key role in airway hyper-responsiveness (Nagata and Glovsky, 1987; Karp et al., 2000). The primary risk factor for the
development of allergic sensitization is the delivery of allergen across the mucosal epithelium. Paracellular channels of the epithelial layer are normally sealed by tight junctions. The intrinsic proteolytic activity of the allergen leads to degradation of tight junctions in airway epithelium, thus increasing the accessibility of the sentinel dendritic antigen-presenting cells residing beneath the epithelial barrier. This suggests a major role of the enzyme allergens in decreasing the effectiveness of the epithelial barrier (Wan et al., 1999; Figure 1.13). This digestive action may facilitate the passage of allergens across the mucosa and enhance the accessibility to antigen presenting cells beneath the epithelial barriers. Airway epithelial cells do not simply act as physical barriers but also function in the regulation of immune responses through the production of cytokines and chemokines like IL-6, IL-8, RANTES (CCL5), eotaxin, TSLP, GM-CSF and increased expression of adhesion molecules via interactions with immune systems (King et al., 1998; Diamond et al., 2000). These mediators trigger the accumulation of inflammatory cells such as eosinophils and neutrophils to perpetuate the chronic allergic inflammation of the airways.

Der p 1 influences peripheral blood T cells to promote a pro-allergic Th2 type response by cleaving CD25, the 55 kDa α- subunit of the IL-12 receptor (Schulz et al., 1998). Since this receptor is pivotal for the propagation of Th1 cells, removal of CD25 by Der p 1 leads to diminished proliferation and reduced secretion of IFN-γ in response to mitogenic stimulation. There is a consequential polarization of T cells towards a Th2 phenotype, with increased secretion of IL-4 and IL-13 (cytokines responsible for inducing IgE synthesis). In addition, Der p 1 cleaves CD 23, which is involved in the negative feedback regulatory cycle of IgE synthesis from the surface of cultured human B cells and thus potentially disrupts this mechanism to cause excessive IgE synthesis and subsequent development of the allergic phenotype (Schulz et al., 1999).

The synthesis of mediators of allergic inflammation from mast cells follows the antigen mediated aggregation of IgE bound to high affinity receptor (FceRI) on the cell surface. However, the activation of mast cells by allergens can also occur via a non-IgE mediated pathway, and proteases from mites, fungi and insects are among those molecules that can generate allergic inflammation (Reed and Kita, 2004). For example, when the serine protease (Der f 1) from the D. farinae is administered into
Figure 1.13: Effect of proteases on epithelium: Endogenous and exogenous proteases can disrupt epithelium. However, this is normally counteracted by protease inhibitors. An imbalance of proteases and their inhibitors can result in epithelial disruption, trans-epidermal water loss in the skin and entry of allergens and infection beneath the epithelium. Source: Smith and Harper, 2006.
the airways of mice it induces the degranulation of mast cells and upregulation and secretion of a number of pro-allergic cytokines including IL-4, IL-6, IL-9 and IL-13, without IgE aggregation (Yu and Chen, 2003). It has been suggested that the mast cell activation is initiated by cleavage of cell surface PARs (Reed and Kita, 2004).

PARs are G protein coupled transmembrane receptors that mediate cellular responses and are activated by cleavage of an N-terminal domain (Kheradmand et al., 2002; Yu and Chen, 2003). Four PARs have been identified. PAR-1 is expressed on neutrophils, platelets, endothelial cells and fibroblasts, while PAR-2 is found on T cells, neutrophils, epithelial cells, endothelial cells, smooth muscle cells and neurons. PAR-1,3 and 4 are activated by thrombin, while PAR-2 is activated by trypsin (Yu and Chen, 2003). Many allergenic proteases have similar specificities to the mammalian serine proteases thrombin and trypsin, and therefore allergenic proteases may function by activating PARs. This activation leads to G-protein signaling cascades generating transcriptional responses through ERK (extracellular signal related kinases), mitogen activated kinases, NF-kB and production of chemokines cytokines etc that enhance IgE production and airway hyperresponsiveness (Kheradmand et al., 2002; Yu and Chen, 2003).

Airway-derived epithelial cells have been shown to increase the release of proinflammatory cytokines, such as IL-6 and IL-8, in response to proteases present in HDM-, pollen- and fungal extracts (King et al., 1998; Tomee et al., 1998; Borger et al., 1999; Kauffman et al., 2000). The release of cytokines may be mediated by protease activated receptors (PAR) present on these cells (D’Andrea et al., 1998; Cocks et al., 1999). Definitive proof for a PAR-mediated mechanism of these observations is hampered by the lack of specific PAR antagonists, but the use of human PAR expressed mouse fibroblast may elucidate whether a PAR is involved in the protease-dependent cytokine production (Kauffman et al., 2000).

In addition to being a physical barrier between the airway and the immune system, epithelial cells plays vital roles in both innate and adaptive immune responses (Hammad et al., 2008). Epithelial cells produce chemokines and cytokines that recruit and enhance survival of dendritic cells (DCs) and interact directly with DCs through membrane-associated chemokines (Reibman et al., 2003; Sha et al.,
Epithelial cells also express cell surface molecules that regulate recruitment, differentiation, proliferation, and function of T cells and B cells (Kato et al., 2006; Schleimer et al., 2007). In particular, newly discovered epithelial-derived cytokines, such as thymic stromal lymphopoietin (TSLP), IL-33, and B cell-activating factor of the TNF family, may play key roles in shaping the functional differentiation and activation of T cells and B cells in the mucosal organs (Kato et al., 2007). Thymic stromal lymphopoietin (TSLP) is produced by epithelial cells and triggers dendritic cell-mediated Th2-type inflammation (Liu et al., 2007). In humans, exposure to ubiquitous airborne fungi, such as *Alternaria*, is implicated in the development and exacerbation of asthma. When BEAS-2B cells or normal human bronchial epithelial cells were exposed to *Alternaria* extract, TSLP was potently induced. The TSLP-inducing activity of *Alternaria* was partially blocked by treating the extract with a cysteine protease inhibitor, E-64, or by infecting BEAS-2B cells with small interfering RNA for PAR-2. Protease-induced TSLP production by BEAS-2B cells was enhanced synergistically by IL-4 and inhibited by IFN-γ (Kouzaki et al., 2009). These findings demonstrate that TSLP expression is induced in airway epithelial cells by exposure to allergen-derived proteases and that PAR-2 is involved in the process. By promoting TSLP production in the airways, proteases associated with airborne allergens may facilitate the development and/or exacerbation of Th2-type airway inflammation, particularly in allergic individuals. TSLP expression is induced in airway epithelial cells by exposure to allergen-derived proteases and that PAR-2 is involved in the process. By promoting TSLP production in the airways, proteases associated with airborne allergens may facilitate the development and/or exacerbation of Th2-type airway inflammation, particularly in allergic individuals (Kouzaki et al., 2009).

There is mounting evidence that proteases are involved in the pathogenesis of asthma and serine protease inhibitors may be studied as a therapeutic target for airway inflammation (Reed and Kita, 2004; Smith and Harper, 2006). To clarify the involvement of serine proteases in the development of allergic airway inflammation, Ishizaki et al (2008) investigated the effect of nafamostat mesilate (FUT), a serine protease inhibitor, in a murine model of allergic asthma (Ishizaki et al., 2008). Non antigenic synthetic serine protease inhibitors like nafamostat mesilate and gabexate mesilate (FOY) have therapeutic effect in allergen-induced airway inflammation as a
result not only of the inhibitory action in the early phase of mast cells activation but also of the immunoregulatory function in the late inflammatory phase of decreasing pulmonary eosinophilia, and attenuating Th2 as well as other inflammatory cytokines production in asthma (Chen et al., 2006; Ishizaki et al., 2008). Such properties of protease inhibitors like FUT and FOY might be useful in combination, or as an alternative treatment with current anti-asthma medications for patients with asthma.

**Enzyme allergens from fungi:**

Fungi have unique capability of surviving on variety of substrates and are thus equipped with several enzymes. Some of the enzymes of molds have been implicated in IgE mediated reactions (Table 1.4). Among these, enzymes of class hydrolases and oxidoreductases are more common. Serine proteases, enolases, alcohol dehydrogenases, malate dehydrogenases, phosphoglycerate kinase, Glutathione-S-transferases etc. are the allergenic enzymes reported from fungi.

Fungi, owing to the presence of proteases, are well equipped for causing airway inflammation and remodeling process. A recent study reported strong allergic inflammatory response in a murine model upon sensitization to protease allergens released by viable *Penicillium chrysogenum* conidia (Schwab et al., 2004) where they determined the concentration of protease extract that would induce allergic response in mice. Kheradmand et al. employed specific inhibitors of proteases (in case of allergen extract) as well as purified fungal protease for induction of allergic lung inflammation in murine model and demonstrated that protease content influences the disease induction and severity (Kheradmand et al., 2002). Furthermore it showed that proteases can overcome airway tolerance and induce pulmonary disease. They categorized fungal proteases as type II allergens i.e. which have intrinsic ability to bypass induction of airway tolerance and thus can induce inflammation without an additional adjuvant. In contrast, allergens like Ovalbumin (OVA) were categorized as type I i.e. which do not have such intrinsic capability. However, the study used protease in combination with a non-enzymatic allergen (OVA) and focused on evaluation of the adjuvant properties of the protease
Studies have established the role of proteases in fungal extracts in mediating allergic inflammation (Schwab et al., 2004; Shin et al., 2006; Tai et al., 2006). However, the role of biochemical activity of these proteases in the pathology of allergic inflammation remains to be elucidated.

Table 1.4: Enzyme allergens of fungi

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mold(s)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enolase</td>
<td><em>Cladosporium herbarum</em>, <em>Alternaria alternata</em>, <em>Aspergillus fumigatus</em>, <em>Penicillium citrinum</em>, <em>Curvularia lunata</em></td>
<td>Sharma et al., 2006</td>
</tr>
<tr>
<td>Aldehyde dehydrogenase</td>
<td><em>Alternaria alternata</em>, <em>Cladosporium herbarum</em></td>
<td>Achatz et al., 1995</td>
</tr>
<tr>
<td>Phosphoglycerate kinase</td>
<td><em>Candida albicans</em>, <em>Saccharomyces cerevisae</em></td>
<td>Singh et al., 2005</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td><em>Aspergillus fumigatus</em></td>
<td>Singh et al., 2005</td>
</tr>
<tr>
<td>Protease</td>
<td><em>Penicillium citrinum</em>, <em>Aspergillus fumigatus</em>, <em>Curvularia lunata</em>, <em>Epicoccum purpurascens</em></td>
<td>Shen et al., 1997; Chow et al., 2000; Gupta et al., 2004; Bisht et al., 2004</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td><em>Curvularia lunata</em></td>
<td>Sharma et al., 2007</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td><em>Malassezia furfur</em></td>
<td>Singh et al., 2005</td>
</tr>
<tr>
<td>Glutathione-s-transferase</td>
<td><em>Alternaria alternata</em></td>
<td>Shankar et al., 2006</td>
</tr>
</tbody>
</table>

In the last decade, many enzymes have been reported as important allergen, 3D-structure and mechanism of action for some of them was predicted. However, number of enzyme allergens need to be elucidated for mechanism of action in the target tissue. Also they are requiring to be developed in bulk amounts in recombinant form for in vitro (sera / blood) and in vivo studies in animal model. The enzyme like glutathione-S-transferase (GST) has antioxidant property but the
allergenic nature restricts its use for therapeutic purpose. The hypoallergenic variant of this enzyme may be of therapeutic value in airway inflammatory disorders.

Keeping above facts in mind, the present study was aimed to achieve the following objectives:
1. To study antioxidant activity of enzyme allergen GST and mutated GST (mGST) in mouse model of airway disease.
2. To evaluate the therapeutic potential of enzymatic and non-enzymatic antioxidants in airway inflammation.
3. To investigate proinflammatory effect of alkaline serine protease (Cur l 1) in mouse model of airway inflammation.
4. Cloning, expression and characterization of a serine protease allergen of *Curvularia lunata*. 