Chapter 1
Review of Literature
1.1. INTRODUCTION

Immune system is needed for protection from environmental hazard and pathogens. It uses a complex array of protective mechanisms to prevent invasion and elimination of pathogen from the body. Two mainstay of immunological defense mechanism includes: Innate and Adaptive immune response. Once a pathogen comes in contact with physical barrier of body, the innate immune system is activate and provides an immediate, but non-specific response. Innate immune response includes epithelial cells, dendritic cells, natural killer cell, phagocytic leukocytes and circulating plasma proteins. If pathogens successfully evade the innate response, the vertebrate manifest a second type of immune response i.e. adaptive immune response (Cooper & Alder, 2006). It is antigen specific and retains an immunological memory in the form of B- and T-memory cells, even after the elimination of antigen. Memory cells facilitate the immune response to mount faster and stronger protection on further encounter.

The structural properties of pathogens which are different from the host cells, is the main criteria by which innate and adoptive system work. This host-pathogen differentiation is essential to eliminate the pathogen without excessive damage to host cells. Human immunity possesses many cells and effector molecules that are capable of differentially damage the pathogen. Recruitment of effector cells during pathogenic invasion at the particular site, termed as inflammation. These inflammatory cells counteract pathogens especially by producing reactive oxygen species (ROS) and digestive enzymes. But sometimes, these inflammatory mediators may have detrimental effects resulting in significant host tissue damage, morbidity or mortality (Serhan et al., 2007). Such an inappropriate immune response to an antigen is termed as hypersensitivity or Allergy.

1.2. HYPERSENSITIVITY CLASSIFICATION

Hypersensitivity may be classified into five categories (Table 1):
Table 1: Types of hypersensitivity

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type-I (anaphylactic)</th>
<th>Type-II (cytotoxic)</th>
<th>Type-III (immune complex)</th>
<th>Type-IV (delayed type)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody</td>
<td>IgE</td>
<td>IgG, IgM</td>
<td>IgG, IgM</td>
<td>None</td>
</tr>
<tr>
<td>Antigen</td>
<td>Exogenous</td>
<td>Cell surface</td>
<td>Soluble</td>
<td>Tissues &amp; organs</td>
</tr>
<tr>
<td>Response time</td>
<td>15-30 minutes</td>
<td>Minutes-hours</td>
<td>3-8 hours</td>
<td>48-72 hours</td>
</tr>
<tr>
<td>Appearance</td>
<td>Weal &amp; flare</td>
<td>Lysis and necrosis</td>
<td>Erythema and edema, necrosis</td>
<td>Erythema and induration</td>
</tr>
<tr>
<td>Histology</td>
<td>Basophils and eosinophils</td>
<td>Antibody and complement</td>
<td>Complement and neutrophils</td>
<td>Monocytes and lymphocytes</td>
</tr>
<tr>
<td>Mediator</td>
<td>Antibody</td>
<td>Antibody</td>
<td>Antibody</td>
<td>T-cells</td>
</tr>
<tr>
<td>Examples</td>
<td>Allergic asthma, Hay fever</td>
<td>Erythroblastosis fetalis</td>
<td>Farmer’s lung disease</td>
<td>Tuberculin test, Granuloma</td>
</tr>
</tbody>
</table>
1.2.1. Type I hypersensitivity or immediate type

Immediate hypersensitivity is a rapid onset of IgE- mediated immune response that occurs in susceptible individuals called atopic, upon re-exposure to certain previously exposed antigens. However on late phases the reaction is amplified and modified by eosinophils, neutrophils, helper T cells and platelets (Fadal, 1993; Mulder & Justinich, 2010). The antigens which instigate such a reaction are called allergens. The manifestations of these reactions can range in severity from minor inconvenience, mild hay fever to anaphylaxis or death. After antigen exposure immune reaction starts in 30 minutes, although sometimes it may delayed to 10 - 12 hours.

1.2.2. Type II hypersensitivity

Type II hypersensitivity is also called as IgG-Mediated Cytotoxicity. Normally endogenous antigens are involved, but sometimes chemicals which can attach to cell surface may instigate this reaction. Antibodies IgM or IgG are directed against antigens on the surface of cells. These antibodies may trigger the compliment system which creates pores in the membrane of a target cell thereby causing cellular destruction. This process is called as antibody dependent cell-mediated cytotoxicity. In this process, cytotoxic cells bind to the Fc region of antibodies on target cells and promote killing of the cells. Type II hypersensitivity includes drug-induced hemolytic anemia, granulocytopenia etc.

1.2.3. Type III hypersensitivity

Type III hypersensitivity involves antigen-antibody immune complexes and hence known as immune complex hypersensitivity. Generally antigen-antibody complex activate components of compliment system (Issekutz et al., 1990) which activate mast cell degranulation and increases vascular permeability. Compliment factor C3a, C5a, and C5b67 infiltrate neutrophil at the site of immune-complex deposition. Much of the tissue damage occurs from release of lytic enzymes by neutrophils in attempt to phagocytose immune complexes. The appearance of symptom generally takes upto 10 hours after antigen exposure (Shmagel & Chereshnev, 2009). The reaction may be general (serum sickness) or affect organs such as skin (e.g., Arthus reaction, systemic lupus erythematosus), joints (e.g., rheumatoid arthritis), kidneys (e.g., lupus nephritis).
1.2.4. Type IV hypersensitivity or delayed type hypersensitivity

Type IV hypersensitivity has several phases; sensitization phase includes the first exposure of antigen and activation of helper and cytotoxic T cells. Subsequent antigen exposure induces the effector phase response. A DTH response generally peaks at 48–72 h after antigen re-exposure. A range of cytokines are released by Th1 cells in the effector phase, which recruit and activate inflammatory cells such as macrophages (Black, 1999). Th1 cells, cytotoxic T cells and macrophages are the major participants of DTH. The macrophages’ activation is important in host defense against parasites and bacteria which are dwelling inside the cells, which cannot be accessed by antibodies. The phagocytic activity and lytic enzymes from macrophages lead to nonspecific destruction of cells. Generally, the pathogen is easily eliminated with slight tissue damage. However, prolonged DTH response can itself become destructive to the host. Type IV hypersensitivity refers to pathogenesis of autoimmune and infectious diseases like tuberculosis, leprosy, blastomycosis, leishmaniasis, contact dermatitis etc.

1.2.5. Type V hypersensitivity

Type V hypersensitivity is also called as stimulatory hypersensitivity. In this immune response, antibodies IgG or IgM bind to the cell surface receptors instead of binding to cell surface components unlike Type II. Bound antibodies either prevent the binding of ligand with the receptor or mimic the effects of the ligand thus impairing cell signaling. Graves’ disease, Myasthenia gravis etc. are clinical examples for type V hypersensitivity reactions.

1.3. ALLERGY

Clemens von Pirquet introduced the term ‘allergy’ for “an altered capacity of the body to react to a foreign substance”. However this definition was an extremely comprehensive as it encompasses all type of immunological reactions. In other words, allergy can be defined more specifically as “disease following a response by the immune system to an otherwise innocuous antigen”. Allergy is a type of immune responses that are otherwise called hypersensitivity reactions. On the basis of time elapsed between the exposure to the antigen and the appearance of clinical symptoms, hypersensitivity can be classified into
immediate or delayed type. Coombs and Gell (1963) classified hypersensitivity reactions into four types. Allergy is often corresponded with immediate-type hypersensitivity reactions mediated by specific IgE (will be used in this sense here) against antigens called as allergens. 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.

1.4. HISTORY

The report on allergy was made as early as 2641 BC, when King Pharaoh Menes experienced anaphylactic reaction on being stung by a wasp (Vernersson et al., 2002). Modern era of allergy started in 1800s with the description of hay fever by Dr. John Bostock. First Skin prick test was performed by Charles Blakely in 1869 to investigate his own hay fever and found grass pollen as causative agent. Clemens von Pirquet, introduced the term ‘allergy’ in 1906 (Zenner, 1987). The term ‘anaphylaxis’ was coined by Portier and Richet describing the fatal manifestations by Jellyfish stung (Ring et al., 2004). Prausnitz and Kustner (1922) showed the transfer of the type I hypersensitivity by serum. In 1967, Ishizaka and Ishizaka characterized a serum component responsible for type I hypersensitivity as ‘IgE antibodies’ (Zenner, 1987).

In 1911, Dale and Laidlaw discovered histamine, a principle mediator of allergy. The histamine induced bronchoconstriction in asthma was first described by Herxheimer in 1949 and mast cells were discovered as the source of histamine by Riley and West in 1953. While in asthma therapy Hench and Kendall got Nobel Prize in 1950 to introduced cortisone into clinical medicine as anti-inflammatory drug for the treatment of arthritis. Soon after arthritis corticosteroids were also introduced for the treatment of allergic disease.

1.5. PREVALENCE OF ALLERGIC DISEASES

In last 50 years, the prevalence of allergic diseases has increased rapidly and it has become a major public-health concern (Devereux, 2006d). Statistics suggest allergic rhinitis affects 10% - 30% of adults and the majority of patients with asthma (60 - 80%) have rhinitis, whereas Peters et al. estimated 20 - 40% of patients with rhinitis may have
asthma (Peters et al., 2006). It has been estimated that allergy and asthma together affect in excess of 700 million people world over (Bateman & Jithoo, 2007).

Asthma has multifunction etiology, among them allergic asthma is predominant and causes most serious problem in individuals. According to Global Initiative for Asthma (GINA) report approximately 300 million people of all ages and all ethnic backgrounds, suffer from asthma worldwide (Masoli et al., 2004). Data suggests that asthma prevalence has increased globally by 50% every ten years (Braman, 2006). The prevalence of asthma is higher in western countries and also increasing with the adoption of Western lifestyle and become urbanized. Global prevalence of asthma ranges from 1-18% in different countries. Asthma causes approximately 239,000 deaths (1 in every 250 deaths worldwide) per year (0.4% of all deaths due to disease) and results in approximately 15 million disability-adjusted life year annually.

Limited data is available for the epidemiology of allergy and asthma from the developing nations, including India. Survey carried out in India has shown that 20-30% of populations suffer from allergic rhinitis (Chhabra et al., 1998). However a multi-center study in India revealed that overall prevalence of asthma is 2.38% (Aggarwal et al., 2006). They also found that the prevalence of “recurrent coryza” is 3.45%, “recurrent skin rashes” is 2.1%, and “recurrent eye itching” is 2.78% in Indian adults. The ISAAC phase I survey done in 14 centers in India revealed that nasal symptoms alone are present in 12.5% children in the 6-7 yrs. age group whereas 18.6 % in the 13-14 yrs age group. Same study suggests that prevalence of Hay fever is 5.5% and 8% respectively, while allergic rhinoconjunctivitis affects 3.3% and 5.6%, respectively, among aforesaid age group (Beasley et al. 1998).

1.6. MACHENISM AND PHASE OF ALLERGY

Allergy reaction includes two phases: 1) Sensitization and 2) Effector phase.

1.6.1. Sensitization phase

Sensitization phase comprises of an initial exposure of allergen through different routes like airway, eye, lung, skin etc. Allergen sensitization is a complex immune process
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involves interplay of dendritic cells, epithelial cells, T cells and B cells. Epithelial barrier is molecular sieve of body and generally allergen first comes in contact with the epithelial tissue and dendritic cells. Allergen activates the epithelial cells and induces an array of cytokine production. Protease induces production of TNF-α, Chemokine ligand 20 (CCL20), GM-CSF, IL-8, Eotaxin, TSLP, IL-25, IL-33 etc. from epithelial cells. Some cytokine induces ingestion of immune cells including neutrophil, eosinophil, macrophage and naïve dendritic cells to the site of allergen exposure. Many of these cytokine facilitate dendritic cells to skew the immune response toward Th2. Dendritic cells migrate to the local draining lymph nodes where these cells come in contact with T and B cells. In presence of Th2 cytokine, class switching occurs in B cells for IgE secretion. IgE binds with basophils and mast cells through the Fc receptor. These immune cells (mainly mast cells) resides beneath the epithelial surface provide immune surveillance for prevention of pathogen evasion.

Allergic sensitization may be affected by many factors like host genotype, biochemical property of allergen, abundance in the environment and presence of adjuvants (Cookson, 2004; Vercelli, 2008; Galli et al., 2008). These agents include certain ligands of Toll-like receptors, including endotoxin (Herrick & Bottomly, 2003), chitin (Dickey, 2007), adjuvant (Kool et al., 2008) which is found in many organisms and environmental pollutants (Saxon & az-Sanchez, 2005). Studies have shown that proteases are important player in allergic sensitization (Arizmendi et al., 2011). Proteases can diminish epithelial barrier function (Wan et al., 1999) and cleave surface molecule like CD23, CD25, CD40 and DC-SIGN to employ Th2 immune response (Hammad & Lambrecht, 2008).

1.6.2. Effector Phase

The allergic reaction may be classified into three temporal phases (Galli, Tsai & Piliponsky, 2008). Early-phase reactions include the response that start within seconds to minutes of allergen exposure whereas, late-phase reactions appears after many hours. Persistent inflammation at sites of repeated allergen exposure is included under chronic allergic inflammation (Figure 1.1).
Figure 1.1: Mechanism and phases of allergy: First exposure of allergen leads to IgE class switching and memory cell production. IgE binds to the mast and basophil cells. Re-exposure of allergen crosslink IgE and degranulate mast cells to release mediators in early phase (immediate phase) reaction. These mediators recruit inflammatory cell which leads to inflammation (late phase reaction) (Larche, et al., 2006).
1.6.2.1. Early-phase reaction
In sensitized individuals, mast cells and basophils possess IgE on surface bound to high-affinity IgE receptors (FcεRI). Exposure of allergen leads to IgE crosslinking and subsequently mast cell degranulation to releases cytoplasmic granules (Kraft & Kinet, 2007; Rivera & Gilfillan, 2006). Mast cells release mediators like amines (such as histamine, serotonin) (Marshall, 2004; Galli et al., 2005), proteoglycans (such as heparin and chondroitin sulphate) (Stevens & Adachi, 2007), serine proteases (such as tryptase, chymase and carboxypeptidases) (Pejler et al., 2007), various other enzymes, cytokines and growth factors (TNF-α and vascular endothelial growth factor A) (Robinson, 2004). Mast cells activation also releases lipid-derived mediators like prostaglandins (particularly prostaglandin D2), leukotriene B4 and cysteinyl leukotrienes (Boyce, 2007). These mediators cause vasodilation, and increase in vascular permeability with edema and acute functional changes in affected organs (such as bronchoconstriction, airway mucus secretion, urticaria, vomiting and diarrhea). Some of the cytokines and chemokines also promote activation and recruitment of leukocytes, leading to the late-phase reactions (Kraft & Kinet, 2007).

1.6.2.2. Late-phase reaction
Late-phase reaction develops after 2–6 h and peaks at 6–9 h of allergen exposure. It may not arise in all affected individual and may not be clearly distinguished from early phase response (Kay, 2001). Late-phase reactions are assumed to be coordinating in part by some of the long term consequences of mediators released during early phase reactions i.e. chemokines, to infiltrate and activate innate immune cells (i.e. TNF-α, IL-8, LTB4, CCL2 and IL-5) and to affect many aspects of the biology of dendritic cells, T cells and B cells (i.e. IL-10, TNF-α, TGF-β and histamine) (Galli et al., 2005; Galli et al., 2008).

Late phase reaction is also regarded as T cell-mediated effector phase. Allergen-specific tissue-resident memory T cells rapidly produce higher amounts of cytokines, like IL-4, IL-5 and IL-13 on re-challenge with the cognate allergen (Islam & Luster, 2012). IgE on surface of antigen presenting cells facilitates antigen uptake and presentation to activate T-cells. However IgE secretion has been observed in case of allergic rhinitis and asthma but not in case of dermatitis (Larche et al., 2006).
Eosinophils are the major inflammatory cells infiltrate in lungs of asthmatic individuals (Larche et al., 2006). IL-5 guides the recruitment growth and activation of the eosinophils. Eosinophils poses Fc receptors for IgE and IgG isotypes, which bind to allergen coated with antibodies. Binding of antibody-coated antigen activates eosinophils, leads to the degranulation and release of inflammatory mediators like major basic protein, leukotrienes, platelet-activation factor, eosinophil-derived neurotoxin and eosinophil cationic protein. The mediators derived from eosinophil may play protective role in parasitic infections but in case of allergy, these participate in extensive tissue damage. Neutrophils are major participant of late-phase reactions and infiltrated due to chemotactic factor released from mast cells and epithelial cells (IL-8). On activation neutrophil release granule contents possesses lytic enzymes, platelet-activating factor and leukotriene. Eosinophils and neutrophils are major player of late phase reaction causes extensive host tissue damage.

1.6.2.3. Chronic allergic inflammation

The late phase allergic response may convert into a chronic inflammatory response by prolonged or repetitive allergens exposure, which in turn promotes eosinophilia and further IgE secretion. This phase is characterized not only by the presence of large numbers of innate and adaptive immune cells but also by substantial changes in the number, phenotype and function of structural cells, alterations in the extracellular matrix and tissue remodeling.

1.7. SOURCE OF ALLERGEN

Common source of allergen includes house dust mites, molds, insects, pollens, foods, animals etc.

1.7.1. House dust mites (HDM)

HDM are the common sources of airborne allergens Worldwide and it affects 15-20% of the population from industrialized countries (Zock et al., 2006). Exposure of HDM to sensitized individuals may lead to development of inflammatory diseases like allergic asthma, perennial rhinitis and atopic dermatitis (Arshad, 2010). Dermatophagoides
pteronyssinus and Dermatophagoides farinae are common HDM species isolated from dust samples and both co-exist throughout the world (Thomas et al., 2010). D. pteronyssinus and D. farinae derived allergen shows sequence homologies and similar biological activities. HDM also include allergens from the siboney and the microceras species. Some allergens derived from members of other genus such as Blomia or Euroglyphus are also included in HDM. To date, more than twenty different IgE binding components have been identified in D. farinae and D. pteronyssinus sensitized patients (Bessot & Pauli, 2011). In a study in Assam (India) house dust sample from allergic patient’s house were analyzed for the presence of HDM, in which Dermatophagoides was found to be dominant followed by Blomia, Acarus, Cheyletus whereas least number of Caloglyphus was recorded (Sharma et al., 2011)

Although the biological function of HDM allergen repertoire remains to be fully elucidated, some of these are characterized as proteases, lipid binding proteins and non-enzymatic components (Thomas et al., 2002). Group 1, 3, 6 and 9 of HDM allergen possess protease activity (Thomas et al., 1991). Der p 2, has structural similarity with MD2 and can activate Toll-like receptor-4 (TLR-4) signaling complex, like MD2 (Trompette et al., 2009). On the basis of structure and sequence homologies, groups 2, 5, 7, 13, 14 and 21 were described as lipid binding proteins yet their role in the mite is still unknown (Thomas et al., 2010). Non proteolytic enzyme include group 4, 8, 12, 15, 18 and 20. Allergen groups 4, 8 and 20 are amylase, glutathione-S-transferase and arginine kinase respectively whereas groups 12, 15 and 18 display homologies with chitinases (Lake et al., 1991; Hales et al., 2007; O’Neil et al., 2006). HDM allergen groups 10 and 11 include the tropomyosin and paramyosin respectively (Jeong et al., 2006; Tsai et al., 2005). Groups 16 and 17 HDM allergens have been identified as gelsolin-like and EF- and Ca2+-binding proteins (Kawamoto et al., 2002; Kawamoto et al., 2002).

1.7.2. Molds

Fungal spores are universal atmospheric components indoors and outdoors and associated with the lower airway allergic reaction, more frequently than do pollen allergies (Lehrer et al., 1983). Actual prevalence of fungal allergy is not known
however skin test result suggest that at least 3 to 10% of adults and children worldwide are affected by fungal allergy (Beaumont et al., 1985; Gergen et al., 1987). More than 80 genera of the major fungal groups have been documented. Commonly *Aspergillus, Alternaria, Cladosporium, Curvularia, Epicoccum, Penicillium, Rhizopus, Candida, Helminthosporium, Phoma, Acrothecium, Scopularia* and *Saccharomyces* are important sources of fungal allergens (Kurup et al., 2002; Mezzari et al., 2003; Bisht et al., 2000). Among these Deuteromycetes (Einarsson & Aukrust, 1992) are dominant (Einarsson & Aukrust, 1992; Bush & Portnoy, 2001). They spent saprophytic life on the dead and decaying organic matter of plant or animal origin and reproduce asexually. Sharma et al., (2011) have reported that dominance of *Aspergillus* followed by *Cladosporium* species in air sample of home of allergic children’s in Delhi (India). *Alternaria* and *Penicillium* species were also obtained in higher quantity from these houses.

Biochemical property of fungal allergen reflects a huge diversity including enzymes, structural proteins and host interacting molecules. Serine proteases, enolases, alcohol dehydrogenases, malate dehydrogenases, phosphoglycerate kinase, glutathionese transferases etc. enzymes were reported from fungal allergen (Shankar et al., 2006; Sharma et al., 2006; Sharma et al., 2008; Achatz et al., 1995). Allergen with proteolytic activity have been also identified and characterized from *Aspergillus, Penicillium, Curvularia* and *Epicoccum* (Bisht et al., 2004; Chow et al., 2000; Gupta et al., 2004; Shen et al., 1997). Ribosomal proteins from different species of fungi were reported as allergen. Glycoproteins implicated in host pathogen interaction from *Penicillium marneffei, A. fumigatus, Beauveria bassiana, C. albicans* and *Volveriella volvacea* species were also identified as immunomodulatory proteins (Calderone, 1993; Hamilton et al., 1998; Peczynska-Czoch et al., 1992; She et al., 1998).

1.7.3. Insects: bee, cockroach, mosquito

Allergy to insects has been known for a longtime as early as 2641 B.C (IM-PINTO et al., 1956). Cockroach, hornets, bumblebees, ants, mosquitoes, flies or kissing bugs are common source of insect allergy (Bircher, 2005). Generally clinical manifestations of insect allergy start from biting/stings of bees, wasps and ants. Besides, injectants are also important source of aeroallergen. Materials such as saliva, venom, digestive tract
enzymes are likely to be the allergens (Palm et al., 2012). A study on rural population showed that sensitization was maximum to mayfly, followed by the housefly, caddis fly, moth and ant (Smith et al., 2005). According to Rosenstreich et al., (1997) among the indoor allergens, sensitization to cockroach allergens was found to be strongly associated with asthma morbidity among inner-city children with asthma. A high prevalence of cockroach hypersensitivity was observed in case of atopic (20-55%) and asthmatic (49-60%) populations (Wu et al., 2003). Cockroach allergen Per a 1, 3 and 7 from *P. americana* and Bla g 2, 4, 5 and 6 from *B. germanica* have been identified as major allergen (Arruda et al., 2001). Insect allergen includes a variety of molecules like serine protease, phospholipase, troponin C, hyaluronidase and tropomyosin. Phospholipase A2, hyaluronidase and acid phosphatase are the three most potent honeybee venom allergens (Grunwald et al., 2006). A study at Lucknow (India) has shown that patients of nasobronchial allergy demonstrated highest positive skin prick test with insect allergen (21.2%) which includes cricket, cockroach, grasshopper and locust (Prasad et al., 2009).

### 1.7.4. Pollen allergens

From approximately 200,000 known plant species, about 50 are included in the official allergen list of the IUIS (Mothes et al., 2004). Atmospheric content of pollen allergen vary according to climate, geography and vegetation.

In USA, frequently encountered source of allergen are *Artemisia, Ambrosia, Xanthium, Quercus* (Anderson et al., 1978). *Acer, Artemisia, Betula, Quercus, Rumex* etc. are important pollen source in Canada. In New York hypersensitivity to oak, birch, and maple tree pollens are frequent (Lin et al., 2002) whereas eastern red cedar or white cedar sensitization is common (Deane, 2005). In Cincinnati abundance of ragweed, oak, maple and Pinaceae pollen has shown correlation with asthma hospitalization (Zhong et al., 2006). In Canada ragweed is common source of aeroallergen.

Birch (Betula) is considered as most important tree pollen in north central and eastern Europe implicated in allergy, followed by Olive (Olea europaea) and cypress (Cupressus) in the Mediterranean regions. Betulaceae family comprises Betula along with genera Alnus (alder). Hazel, hornbeam and hopbeam of Corylaceae family sheds
their pollen in December to April and act as primer for birch allergic sensitization. In the central Alpine Regions *Alnus viridis* pollen are abundant. *Olea europaea* pollen of Oleaceae family is most important causes of respiratory allergy in Mediterranean region (D’Amato et al., 2007). The airborne grass pollen in Europe belongs to tall meadow grasses such as meadow foxtail, timothy, orchard and cultivated rye grass (Griffith et al., 1991). Urticaceae family includes genus *Parietaria* in which species *P. judaica* and *P. officinalis* are leading allergen contributor in Europe.

In South Africa important source of pollen are Acacia, *Cupressus*, *Morus*, *Cannabis*, *Prosopis*, *Celtis*, *Pinus*, Cynodon, Compositae, Graminae, Asteraceae and Fabaceae (Singh & Mathur, 2012).

Japanese cedar and pine are most important aerospora in Japan (Ishizaki et al., 1987). In China *Artemisia*, Casuarinaceae, Graminae and Pinaceae pollens are abundant (Chen et al., 1988) 1988). West Asia atmosphere is comprises of Amaranthaceae, Cyperaceae, Gramineae, Plantaginaceae, Poaceae, *Acer*, *Cupressus*, *Morus*, *Populus*, *Pinus* etc (Singh & Mathur, 2012).

In India (Delhi) *Prosopis juliflora* has been identified as major cause of pollinosis whereas other pollens are also clinically important (Shivpuri et al., 1979). In an aeropalynology study in West Bengal positive correlation between atmospheric pollen count and skin prick tests has been shown for Poaceae. *Amaranthus Argemone*, *Ricinus communis*, *Morus*, *Quercus*, *Cedrus* etc are clinically important pollen. In South India *Ageratum*, *Albizia lebbeck*, *Artemisia scoparia*, *Ricinus* and *Salvadora* have been reported as clinically important aeroallergens (Singh & Mathur, 2012).

Pollen allergens include a wide variety of molecules i.e enzymes like enolase (Phl p 22), protease (Cyn d CP), endoxylanalase (Cyn D EXY), structural and functional proteins like polcalcin (Phl p 7), cytochrome C (Lol p 10) and pathogenesis-related proteins (Phl p 24). Group I allergens of grasses are the most widespread allergenic component (Esch & Klapper, 1989; Griffith, et al., 1991; Perez et al., 1990). These allergens are glycoproteins and profusely released upon hydration (Cosgrove et al., 1997). Beside these grass pollen includes calcium binding protein (Cyn d 7, Phl p 7), profilin (Cyn d 12, Phl p 12, Zea m 12) cytochrome C (Lol p 10, Poa p 10),
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ribonuclease (Phl p 5), trypsin inhibitor (Lol p 11), Polygalacturonase (Phl p 13) etc. The major allergens from *Parietaria* are glycoproteins which show high cross-reactivity with other grass allergen (Colombo et al., 2003). Pollen extract of *P. judaica* possess aminopeptidase activity which is able to disrupt epithelium barrier and enhance the antigen delivery to dendritic cells (Cortes et al., 2006). Among Compositae family ragweed (*Ambrosia*) and mugwort (Monteseirin et al., 2003) are well known responsible factor for pollenosis (D’Amato et al., 2007).

1.7.5. Food allergens

The prevalence of food allergy has increased notably as reported by Sampson (1999). Food allergies cases were mostly reported in infants, affecting up to 6% of children (Sampson, 2003). Food allergen generally includes ligand binding proteins and protein molecules interacting with cell membrane or other lipids such as paravalbumin, lipocalin, blactoglobulin. Parvalbumins a ligand binding protein from white muscle of many fishes, assumed to be vital for the muscle relaxation through binding with intracellular calcium (Pauls et al., 1996). Apart from parvalbumins, caseins (Cow’s milk allergens) also binds calcium but in a different manner. Lipocalins and nonspecific lipid-transfer proteins (LTPs), which possess a lipid-binding pocket, have been shown to increase stability when the pocket is occupied. Thus the thermostability of blactoglobulin increases on lipid binding (Creamer, 1995), as nsLTP of wheat (Douliez et al., 2001). Bet v 1-homologous proteins might be involved in the transport of steroid ligands came from the discovery of a high structural similarity between Bet v 1 and the steroid-binding domain of the human and also have homology with sweet cherry allergen Pru av 1, interacte with phytosteroids (Tsujishita & Hurley, 2000).

Many plant food allergens are also able to associate with cell membranes and other types of lipid structures formed in foods. One commonly observed mode of action whereby proteins can protect plants against microbial pathogens is destabilization of bacterial or fungal membranes resulting in leakage of cytoplasmic content. Proteins acting in this way include thionins, thaumatin-like proteins (TLPs), 2 types of prolamin superfamily members (2S albumins and nsLTPs) and some defensins have shown allergic potential (Selitrennikoff, 2001). TLPs have been reported as PR-5proteins
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(Breiteneder, 2004). Many TLPs have been reported to inhibit the growth of fungi, including the apple allergen Mal d 2 (Krebitz et al., 2003) and flax seeds 2S albumins is a seed storage proteins, other functions ascribed to them, including antifungal activity resulting from membrane permeabilization in mustard (Onaderra et al., 1994), radish (Terras et al., 1992) and rape (Brassica napus) (Terras, et al., 1992; Terras et al., 1993).

Egg, milk, cereals and legumes are commonly studied food allergen in India. In a study in India 4.5 % of asthma and rhinitis patient have shown positive skin prick test to food allergens such as rice, black gram, fruits etc. (Kumar et al., 2010). Among legumes kidney bean, peanut and chick pea are the common cause of food allergy in India (Kasera et al., 2011).

1.8. ALLERGEN SOURCE DERIVED PROTEASES

Allergens are biochemically active molecules possess a wide variety of function in their host organism. However their roles in allergic diseases are being explored. Protease activity is common in allergens which augment allergic effect.

1.8.1. Pollens

Pollen grains from various plants possess proteases activity. Gunawan et al.(2008) reported presence of serine protease activity in pollen of Japanese cedar (Cryptomeria japonica), Japanese cypress (Chamaecyparis obtusa) and Rocky Mountain juniper (Juniperus scopulorum) belong to the Cupressaceae, Taxodiaceae family. A protease from Japanese cedar pollen has been purified (Noguchi et al., 2002; Nagata et al., 2005) and characterized as aspartic protease by Ibrahim et al. (2010) using a proteomic approach. Among grasses pollen, two serine proteases have been identified from short ragweed (Ambrosia artemisiifolia) (Bagarozzi, Jr. et al., 1996; Bagarozzi, Jr. et al., 1998). Bermuda grass (Cynodon dactylon), Kentucky blue grass (Poa pratensis) and Rye grass (Lolium perenne) pollen also have serine proteases (Raftery et al., 2003). Cysteine protease activity has also been detected in pollen of Kentucky and Rye grass, which belongs to group 1 allergen (Grobe et al., 1999; Grobe et al., 2002). Bermuda grass pollen Cyn d 1 has been identified as a major group 1 allergen and characterized as cysteine proteases using proteomic approach (Rafteryet al., 2003).
1.8.2. House dust mites (HDMs)

Protease activity of HDMs play important role in allergic exacerbation. HDM *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, produce at least four groups of protease allergen. Major group 1 allergen is considered as a papain like cysteine proteinase whereas groups 3, 6, 9 are trypsin-like, chymotrypsin-like and collagenolytic serine proteases respectively (Bennett & Thomas, 1996; King et al., 1996; Schulz et al., 1995). Some group 1 allergen preparations of mites, exhibit cysteine protease activity along with serine protease activity (Hewitt et al., 1995; Hewitt et al., 1997). However, recombinant group 1 allergen protein has shown only cysteine protease activity, indicating that the detected serine protease activity is due to contamination (Takai et al., 2005). Besides whole body extract, cysteine protease activity also exist in fecal extract of mite (Ino et al., 1989), even, serine protease is abundant in the fecal extract than body extract of mite (Stewart et al., 1994). Protease allergens were detected in the gut of mite as well as in the feces indicating the possible role in digestion (Thomas et al., 1991).

1.8.3. Cockroaches

American cockroach (*Periplaneta americana*) and German cockroach (*Blattella germanica*) are common indoor source of allergen having proteolytic activity in their extract (Hivrale et al., 2005; Kondo et al., 2004; Sudha et al., 2007). Per a 10, a trypsin-like serine protease allergen has been identified from American cockroaches extract (Sudha et al., 2008; Sudha et al., 2009). Bla g 2 is an allergen from German cockroaches, has been purified and characterized as an inactive aspartic protease (Pomes et al., 2002; Wunschmann et al., 2005). German cockroach extract rich in proteases foster inflammatory response on airway epithelial cells. These proinflammatory effects could abolished by serine protease inhibitors (Bhat et al., 2003), suggesting the involvement of proteases. The presences of protease in fecal remnants (frass) from cockroaches indicate the role in digestive system in insects (Page et al., 2007).
1.8.4. Fungi

A large number of mold species possess proteases allergen. Serine proteases from different airborne fungal species have been identified such as Aspergillus, Curvularia, Cladosporium, Penicillium and Rhodotorula (Schwab et al., 2003; Schwab et al., 2004; Shen et al., 2007a; Su et al., 1999). Protease allergen of different fungal species shows cross-reactivity with each other (Chou et al., 2008; Poll et al., 2009a; Shen et al., 2007b; Bowyer et al., 2006). Group 1 allergens derived from Epicoccum purpurascens Epi p 1 and Curvularia lunata Cur l 1 induced airway allergic responses in murine model in protease dependent manner (Kukreja et al., 2008; Tripathi et al., 2009). Group 13 allergen from different species of Aspergillus and Penicillium has been characterized as alkaline serine protease, whereas group 18 has been identified as vacuolar serine protease (Shen et al., 1999b). Cla h 9 a minor allergen from Cladosporium herbarum possesses vacuolar serine protease activity and has demonstrated cross-reactivity with vacuolar serine proteases with Aspergillus fumigatus and Penicillium species (Poll et al., 2009b). Cur l 4 from Curvularia lunata has been identified as a vacuolar serine protease. Protease activity of fungal allergen plays important role for the exacerbation of allergic response (Kauffman et al., 2006; Kouzaki et al., 2009; Namvar et al., 2014). Besides serine protease, Asp f 5 and Asp f 10 from A. fumigatus have been characterized as metalloprotease and aspartate protease respectively.

1.9. PROTEASE IN ALLERGY

Allergens from different sources possess protease activity. Studies suggest that protease play a pivotal role in allergic exacerbation. Blocking of protease activity of Aspergillus fumigatus (Kheradmand et al., 2002), German cockroach frass (Page et al., 2008), Per a 10 (from American cockroach) (Sudha et al., 2009), Epi p1 (from the fungus Epicoccum purpurascens) (Kukreja et al., 2008) and Cur 11 (from Curvularia lunata) (Tripathi et al., 2009) showed reduced airway inflammation and hyperresponsiveness in animal models. Moreover, administration ovalbumin (tolerogenic antigen) with active proteases from A. fumigatus resulted in allergic sensitization (Kheradmand et al., 2002) indicating that proteases found in ambient air may facilitate allergic response. Proteases aggravate allergic response through multiple ways (Figure 1.2).
Figure 1.2: Role of protease allergen in allergy: Protease allergen degrades epithelial barrier and increases the permeability. Protease allergens participate in infiltration and activation of inflammatory cells. Proteases from allergens are also involved in neurogenic inflammation. (Reed & Kita, 2004).
1.9.1. Epithelial barrier disruption

One of the mechanisms by which protease facilitate allergic response is increment of epithelial permeability by the cleaving of tight junctions as well as lowering ZO-1 and occludin content (Wan et al., 1999). *In vitro* and *in vivo* studies on HDM revealed that its proteases cause epithelial barrier dysfunction and increase permeability (Wan et al., 1999; Wan et al., 2001). The role of cockroach allergen in disruption of epithelial barrier is not demonstrated till now. However, cockroach allergen could decrease the electrical resistance across bovine aortic endothelial cells monolayers. Neutralizing antibodies of vascular endothelial growth factor has inhibited the effect of cockroach allergen in BAEC electrical resistance indicating that reduced resistances VEGF mediated process (Antony et al., 2002). Topical application of cockroach allergens and HDM also decreases barrier recovery rate in a PAR-2-specific manner as observed in hairless mice or humans (Jeong et al., 2008).

Protease allergen from fungal sources affects the epithelial barrier permeability. *Aspergillus fumigatus* derived proteases promote human epithelial cell detachment (Robinson et al., 1990). Pen ch 13 from *Penicillium chrysogenum* disrupts the epithelial barrier by proteolytically cleaving the tight junction protein occludin and induced proinflammatory cytokines in epithelial cells (Tai et al., 2006). Pen ch 13 also decreases cell surface expression of CD44 from epithelial cell line and the primary culture of bronchial epithelial cells (Tai et al., 2010), which contribute in repair of epithelial damage (Teder et al., 2002). CD44 is an adhesion molecule spanning the cell membrane; act as a major receptor for extracellular matrix component hyaluronan. CD44 removes extracellular matrix from sites of injury. Persistent inflammation has been observed in case of impaired clearance of extracellular matrix (Jiang et al., 2007).

Substrate preference profiles of serine proteases from different pollen sources has been evaluated by dipeptide ester substrates which further confirmed the role of protease in airway injury and allergy (Widmer et al., 2000). Proteases from pollens of Easter lily, Giant ragweed, Kentucky blue grass and White birch assisted allergen
delivery across epithelia by cleaving occludin, claudin-1 and ZO-1 in MDCK and Calu-3 cells. This effect of protease allergen could be partially blocked by serine and cysteine protease inhibitors (Runswick et al., 2007). Conclusively, the protease allergen increases epithelial permeability to facilitate the allergen to interact with immune cells.

1.9.2. Epithelial cells activation and cytokine production

Protease allergen activates the airway epithelium via PAR-2 receptor and induces the release of interleukin IL-6, IL-8 and granulocyte monocyte colony-stimulating factor (GM-CSF) (Bhat, et al., 2003; King et al., 1998; Wanet al., 1999). IL-6 and IL-8 participate in the recruitment of inflammatory cells like neutrophils. GM-CSF is an important factor for recruitment, activation, maturation and prolonged survival of DCs at the mucosal surface. Protease allergen also stimulates epithelial cells to secrete IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) and facilitate Th2 polarization.

Cockroach allergen induces IL-6 and IL-8 secretion from human airway epithelial cells (Bhat et al., 2003; Page et al., 2003) via extracellular signal regulated kinase (ERK) (Page, Strunk & Hershenson, 2003) and nuclear factor for IL-6 (NF-IL-6) (Page et al., 2005) in PAR-2 dependent manner. Recently, chemokine ligand 20 (CCL20), a strong chemoattractant for lymphocytes dendritic cells and GM-CSF release has been confirmed in primary mouse tracheal epithelial cells in PAR-2 dependent manner (Day et al., 2012).

Fungal protease allergens from Alternaria alternata, Aspergillus fumigates and Cladosporium herbarum leads to desquamation and morphological changes in epithelial cells along with induction of pro-inflammatory cytokines (Borger et al., 1999; Kauffman et al., 2000). The protease activity of Pen ch 13 promote PGE2, IL-8, TGF-β1 and COX-2 induction from epithelial cell line as well as from primary cultures of human bronchial epithelial cells (Tai et al., 2006). Pen c 13, has induced the expression of IL-8 in human airway epithelial cells through protease dependent process (Chiu et al., 2007). Conclusively protease allergens derived from various sources activate epithelial cell to promote inflammation and Th2 immune response.
1.9.3. Recruitment, Development and Maturation of DCs and Th2 polarization

Dendritic cells are regarded as the transient cells to decide the type of immune response either Th1 or Th2. Cysteine protease from HDM cleaves CD40 from the surface of on human monocyte-derived DCs and consequently reduces IL-12 production which is important for Th1 cell differentiation (Ghaemmaghami et al., 2002). Th2 inducing response in allogeneic naive T cells has also been observed on exposure of proteolytically active allergen (Lamhamedi-Cherradi et al., 2008).

GC frass exposure to naïve mice increases TNFα, KC, CCL20 and GM-CSF in BALF within a single hour (Day et al., 2010; Day et al., 2012). This study has also confirmed that induction of cytokines by GC frass was a protease mediated response. Early release of TNFα from DCs is a protease induced response which modify the cytokine expression in epithelial cells (Lutfi et al., 2012). Same study has indicated that TNFα activate airway epithelial cells to increased production of crucial cytokines such as CCL20 and GM-CSF along with the recruitment and activation of subepithelial DCs. Exovesicles containing TNFα secrete from DCs on LPS stimulation, activate epithelial cells to release chemokines (Obregon et al., 2009). Thus, both the DC and epithelium may play roles to set the allergic response.

The turnover of the DCs in airway epithelium is very high. The percentage of mDC in the lung increases following exposure of GC frass by PAR-2 dependent process (Day et al., 2012). Besides recruitment, development of DCs is also regulated by protease. Fields et al., (2003) has shown that PAR-2 knockout mice failed to develop DCs; however, the addition of TNFα or crosslinking of CD40 restore the development. The presence of protease inhibitor hinders DC development which can also be restored by TNFα. Recently, a study has shown that GC frass increases the activation marker CD80 and CD86 on pulmonary mDCs (Lewkowich et al., 2011). Allergen uptake by DCs is also an important process for allergic response. PAR-2 agonist enhances the uptake of allergen by DCs and also increases the percentage of allergen containing DCs in draining lymph node and the spleen (Ebeling et al., 2007). In contrast, Lewkowich et al., (2011) has observed that AlexaFluor labeled GC frass uptake by mDCs, is independent of PAR-2. Gao et al. (2010) has investigated that cockroach antigen elevate
proinflammatory cytokines IL-13 and TNFα from pDC and T cell co-culture but no IL-12p70 or IFNα.

HDM protease cleaves DC-SIGN and DC-SIGNR, which are closely related C-type lectin transmembrane receptor from DCs surface. Binding of DC-SIGN and DC-SIGNR involved in DC trafficking and skewing of the immune response in favor of Th 1 (Furmonaviciene et al., 2007). Allergen-derived proteases can also set Th2 immune response, by cleavingsurface molecules like CD25 and CD23. Der p 1 cleaves the alpha chain of the CD25 (Bodas et al., 2006) on human T cells (Schulz et al., 1998). As IL-2 stimulation is involved in maintenance of regulatory T cells in the periphery, cleavage of CD25 might also alter the regulatory function. The overall effect may favor a Th2 response.

1.9.4. Enhanced IgE

Der p 1 cleaves the low-affinity receptor for IgE; CD23, from the surface of B cell (Schulz et al., 1995). As CD23 receptor signaling negatively regulates the IgE secretion, cleavage of CD23 by protease allergen, disrupt the inhibitory signal and enhance IgE secretion. Kikuchi et al. (2006) have confirmed that tertiary architecture of the allergen protein itself is not sufficient, but presence of proteolytic activity enhances IgE and IgG level in naïve mice. Immunization of mice with protease allergen Der p 1 leads to significant increase in serum IgE (del-Patient et al., 2000) as compared to animals immunized with inactive Der p 1 (Gough et al., 1999).

1.9.5. Impair innate defense mechanism

Protease activity of allergens may impair innate defense mechanism of lung by inactivating surfactant proteins such as SP-D and SP-A. SP-D and SP-A are calcium-dependent carbohydrate-binding proteins with innate immune functions like bacterial agglutination and modulation of leukocyte functions (Deb et al., 2007). These provide protection against *A. fumigatus* induced allergic inflammation in mice, through binding to glucan moieties of inhaled allergens and facilitating the clearance (Brandt et al., 2008; Madan et al., 2001). Der p 1 and Der f 1 can inactivate SP-A and SP-D and lower innate immune defense of lung (Debet et al., 2007). Moreover, α1-antitrypsin a naturally
occurring protease inhibitor protects respiratory tract against proteases. Cleavage of α1-antitrypsin by Der p 1 leads to enhanced inflammation (Kalsheker et al., 1996).

1.9.6. PAR-2 activation

Protease-activated receptor 2 (PAR-2) is a family of proteolytically activated G-protein-coupled receptors. Evidence suggested that PAR-2 play an important role in protease induced allergic response. It is expressed by many structural and immune cells, such as epithelial cells (Asokananthan et al., 2002) fibroblasts, (Akers et al., 2000) macrophages (Colognato et al., 2003) and mast cells (D’Andrea et al., 2000). More importantly, patients with asthma have higher expression of PAR2 on respiratory epithelial cells (Knight et al., 2001). Allergen extract from HDM and cockroach along with purified protease allergen Der p 1, Der p 3 and Der p 9 are able to activate PAR-2 (Hong et al., 2004). HDM extract (Asokananthan et al., 2002), German cockroach (Page et al., 2003) and the mold allergen Pen c 13 (Chiu et al, 2007) has been reported to induce inflammatory cytokine release by airway epithelia through PAR2.

PAR-2 agonists potentiate broncho-constriction in human (Roche et al., 2003; Schmidlin et al., 2001) whereas lack of PAR-2 expression is associated with lower inflammation and reduced airway resistance in mice (Schmidlin et al., 2002). PAR-2 is involved in increased IgE level, airway hyperresponsiveness, elevated proinflammatory cytokine (IL-6, IL-8, GM-CSF and eotaxin) and matrix metalloproteinase-9 (MMP-9) release, (Ebeling et al., 2005; Reed & Kita, 2004). MMP-9 can further disrupt the tight junction integrity along with remodeling of the airway (Vermeer et al., 2009). Additionally, some studies also suggest that IL-6 and IL-8 release from epithelial cells by Der p 1 can also occur via a mechanism independent of Ca$^{2+}$ mobilization and PAR-2 activation (Adam et al., 2006; Kauffman et al., 2006).

1.9.7. Regulation of allergic airway inflammation

Cockroach extract is type II allergen does not requires addition of adjuvant for Th2 response. Intratrachial administration of GC frass (devoid of serine protease activity) has shown decreased AHR and mucin production (Page et al., 2007). Arizmendi et al.(2011) has shown that heat-inactivated CE sensitization to mice induced significantly
less AHR, eosinophilia and serum IgG1 when compared to active CE. A purified proteolytically active Per a 10 allergen from *P. americana* reveal that its sensitization to mice induces AHR, cellular infiltration in lung (Sudha et al., 2009). Protease activity of allergen is also able to mediate sensitization to bystander antigens. HDM extract can demonstrate adjuvant effect, as its exposure develops a micro-environment for the allergic sensitization by otherwise weak antigens (Fattouh et al., 2005; Gough et al., 2001).

Protease from fungal extracts also influences the severity of allergic airway disease. Proteolytic allergen facilitates the presentation of other components to immune cells for example alkaline serine protease allergen derived from *A. fumigatus* (Asp f 13) has synergistic effects on the Asp f 2-induced allergic immune response in mice (Kurup et al., 2007).

1.10. INTRINSIC PROTEASES

Endogenous proteases have important role in allergic reaction. Large amounts of different proteases are released from immune cells like mast cells and neutrophils. Mast cell tryptase and chymase along with neutrophil elastase and matrix metalloprotease is major participant in allergic disease. The roles of these enzymes are discussed below:

1.10.1. Mast cell tryptase

During effect or phase of allergic reaction IgE crosslinking leads to mast cell degranulation. Mast cells release tryptase along with histamine, heparin and cytokines. Tryptase potentiates allergic reaction by multiple ways. Tryptase further triggers mast cell degranulation by positive feedback (Molinari et al., 1996). It also facilitates leukocyte recruitment and promotes inflammation by activating epithelial cell to release chemokine. β-tryptase induces expression of adhesion molecule and IL-8 release, which facilitates neutrophil accumulation in airway (Cairns & Walls, 1996). Inflammatory role of tryptase has been demonstrated by directly placing human tryptase βI into mouse airway (Huang et al., 2001) and the peritoneal cavity (mouse MCP-6) (Huang et al., 1998). Recombinant human γ-tryptase induced IL-13 cytokine and bronchial irritability when introduced in airway (Wong et al., 2002). Besides, tryptase induces keratinocyte
growth and pruritus mediated by PAR-2 on human keratinocytes (Steinhoff et al., 1999). Indeed, tryptase and PAR-2 both are involved in itching (Steinhoff et al., 2003; Ui et al., 2006; Dugas-Breit et al., 2005) and the interaction suggest a mechanism for neurogenic inflammation in skin, gut etc..

*In vitro* and *ex vivo* studies suggest that tryptase promote bronchoconstriction by cleaving peptides, having bronchodilating properties such as vasoactive intestinal peptide and peptide histidine-methionine (Tam et al., 1990). Patrick Berger and colleagues have been reported that tryptase induced accumulation of mast cells in vicinity of smooth muscle in airway sub-epithelium (Berger et al., 1999). Further, mast cell mediated infiltration of smooth muscle bundle (as in mast cell myositis) consistently and perhaps uniquely observed in asthmatic bronchi (Brightling et al., 2002).

Asthma-associated airway remodeling includes goblet cell metaplasia, increased airway smooth muscle mass and reticular basement membrane thickening/ sub epithelial fibrosis. Ruoss and Hartmann have reported mast cell tryptase as a growth factor by demonstrating upregulated DNA synthesis and proliferation in fibroblasts (Hartmann et al., 1992; Ruoss et al., 1991). These studies have revealed tryptase as a strong mitogen and also have synergistic effect with PDGF and FGF. Furthermore tryptase can also stimulates fibroblast chemotaxis and collagen synthesis (Cairns & Walls, 1997; Gruber et al., 1997) and regulate tissue remodeling in allergic disease. The detailed mechanism of direct tryptase-mediated airway remodeling and mitogenesis remain to be elucidated.

A study have shown inflammatory yet protective role of tryptase. Human βI tryptase, when placed into mouse provides protection against *Klebsiella pneumonia* (Huang et al., 2001) by increasing neutrophil influx that kills bacteria. Thus, positive contributions of tryptase are inseparable from the inflammation. On the other hand, tryptase-induced inflammation can lead to sever problem as in inflammatory bowel disease, e.g. ulcerative colitis (Tremaine et al., 2002).

1.10.2. Chymase

Chymase is regarded as biomarker of anaphylactic mast cell degranulation. In parasitized mice, worm antigen challenge induces chymase secretion which translocates
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to gut lumen after intestinal anaphylaxis (Miller et al., 1983; Scudamore et al., 1995). In mouse model of HDM induced allergic disease (Yu & Chen, 2003), intratracheal challenge with Der f increases serum chymase MCP-1. Studies have also shown that cathepsin G and mast cell chymase stimulated fluid secretion from cultured airway serous gland cells (Sommerhoff et al., 1989; Sommerhoff et al., 1990). Purified dog chymase, which is highly similar to the human enzyme (Caughey et al., 1990; Caughey et al., 2000), augments the size of skin wheal (Rubinstein et al., 1990). Although chymase by itself does not trigger wheal formation it can lead to augmentation in the size of wheal on co-injection with histamine into the skin. The effect of chymase activity in wheal formation has also been confirmed by using inactivated chymase in skin (Rubinstein et al., 1990). Thus it seems that chymase can increase the severity of allergic inflammation by acting in combination with other inflammatory products of mast cells.

Although chymase have shorter active life due to circulating inhibitors such as serpins and α2-macroglobulin, it plays a pivotal role in allergic diseases. Chymase (MCP-4) deficient mice have thicker ears and more connective tissue in comparison to wild-type animal, suggesting that MCP-4 require balancing the ECM production and clearance, favoring tissue remodeling. Chymase contribute to matrix clearance directly by cleaving proteins such as fibronectin and non-helical collagen (Tchougounova et al., 2005) and also by activating matrix metalloproteases (MMPs) to degrade matrix proteins. Chymase are major activator of MMP-9 in vivo as demonstrated by Gunnar Pejler and colleagues that MCP-4-deficient mice possess inactive MMP-9 (pro-MMP-9) in tissue extract, compared to wild type mice (Tchougounova et al., 2005). Chymase is more effective in activating MMP-9 than tryptase, however tryptase can indirectly activates MMP-9 by activating MMP-3 (Gruber et al., 1989). Tryptase, mastin, chymase and MMP-9 itself are strong gelatinase (Coussens et al., 1999; Fajardo & Pejler, 2003; Raymond et al., 2005) and may have in vivo importance to hydrolyze denatured collagens.

Chymase has protective role as elucidated in mice lacking MCP-1 (chymase). Mice lacking MCP-1 shows difficulty in expelling the intestinal nematode, Trichinella spiralis (Knight et al., 2000). Because of lack of mouse chymase MCP-1 counterpart in human, it is difficult to speculate the similar protective role in human.
1.10.3. Neutrophil elastase

Simpson et al. (2006) reported that among adults with stable asthma and adults are under inhaled corticosteroid treatment; ~40% have eosinophilic asthma whereas 25% have neutrophilic asthma. Neutrophilic infiltration in airways is a feature of acute severe asthma (Ordonez et al., 2000). Neutrophils are predominate in fatal attacks of short duration asthma (Simpson et al., 2006), and also in the early stages of asthma, (Ramirez-Velazquez et al., 2013). Azurophilic granules of neutrophils contain set of serine proteases namely cathepsin G, proteinase 3 and neutrophil elastase, which are implicated in immunological reaction particularly for antimicrobial defense by degrading engulfed microorganisms inside the phagolysosomes of neutrophils (Reeves et al., 2002; Segal, 2005). Proteinase 3 and neutrophil elastase are closely related enzymes, with overlapping and potentially redundant substrate specificity.

Neutrophil elastase is implicated in tissue injury and alters airway function (Suzuki et al., 1996). Reports say that neutrophil elastase activity is closely associated with asthma exacerbation (Baines et al., 2011; Nyenhuis et al., 2010). Elastase promotes extravasation of neutrophil by facilitating the adhesion to endothelial layer of blood vessels (Heutinck et al., 2010). Study on guinea pigs has suggested that reduction of neutrophil recruitment or inhibition of neutrophil elastase activity prevent goblet cell degranulation (Agusti et al., 1998; Nadel et al., 1999). In allergic sheep the elastase inhibitor ONO-5046, could effectively attenuated airway resistance but did not affect the number of eosinophils and lymphocytes in BALF (Fujimoto et al., 1995). However the role of neutrophil elastase in allergic diseases remains to be evaluated.

1.10.4. Matrixmetalloprotease

The Extracellular Matrix (ECM) serves mainly as an inert scaffold influencing the cellular function such as migration, differentiation and proliferation (Akiyama et al., 1989; Anwar et al., 1993; Erle & Pytela, 1992; Spurzem, 1996; Spurzem, 1996). Asthmatic airways possess higher deposition of collagen I, III, V, fibronectin, hyaluronan, laminin, tenascin and versican (Laitinen et al., 1997; Roberts & Burke, 1998) whereas lower deposition of collagen IV and elastin (Bousquet et al., 1992). The exact causes of altered ECM deposition are not known; however, the microvascular leakage is one of the main reasons (Shiels et al., 1996; Johnson et al., 2000). Activation of MMPs could also be a reason for tissue remodeling in case of allergic diseases.
MMPs release in the inactive pro-form for example MMP-9/gelatinase B is release from mast cells (and perhaps all cells) as pro-MMP-9. Pro-MMP-9 remain bound to an inhibitor, TIMP-1, chymase removes TIMP-1 from pro-form during the activation process (Frank et al., 2001). Other proteases, such as tissue- or urokinase-type plasminogen activator, trypsin or by membrane-anchored MMP can also remove TIMP-1 from pro-form to activate MMPs. In the BALF of asthmatic, MMP-1, -2 and -9 are predominant and involved in tissue remodeling (Ohno et al., 1997). MMPs can be secreted from epithelial cells and different inflammatory cells. When asthmatic and non-asthmatic tissues are compared conflicting results obtained (Mautino et al., 1999; Ohno et al., 1997). Rajah et al. (1996) observed that level of MMP-1 significantly elevate in the smooth muscle cells of bronchi and trachea.

Increased number of eosinophil in the airways is characteristics of asthma. In asthmatic patients enhanced levels of MMP-9 mRNA in eosinophils have been reported in mucosal biopsy. Increased expression of TIMP-1 in alveolar macrophages has been observed in asthmatic indicating the correlation with eosinophils (Ohno et al., 1997).

Human airway smooth muscle cells exposed to asthmatic serum produce increased amounts of chondroitin sulphate (a glycosaminoglycan related to hyaluronan), perlecan, laminin and fibronectin (Black, 1999). Johnson & Knox (1999) have demonstrated an antiproliferative effect of protease inhibitors of autocrine derived MMP-2 on the ASM cells. The imbalance in the production of MMP-2 and MMP-9 between asthmatic and non-asthmatic ASM cells may be one of the mechanisms involved in this increased growth of airway smooth muscle. The effect of ECM/MMP/TIMP to increase smooth muscle mass has been observed in asthma is an important area of research.

1.11. TREATMENT STRATEGY FOR ALLERGIC DISEASES

Multiple strategies are being used for the management of allergic diseases. In pharmacotherapy inhaled corticosteroids and β2-adrenoceptor agonists are the mainstay treatment of asthma (Figure 1.3). In the case of rhinitis, α-adrenoceptor agonists and non-sedating H1-antihistamines and topical corticosteroids are well-established therapies. For most allergic diseases, a combination of symptom relieving and control therapies are the basis of management. Therapies for allergy management are summarized below:
Figure 1.3: Stepwise approach for managing asthma as recommended by the Expert Panel of the “National Asthma Education and Prevention Program” (Levine & Wenzel, 2010).
1.11.1. Allergen avoidance

Allergen exposure in early life may influence the extent of allergic sensitization as found in a study on HDM (Illi et al., 2006). However, early life allergen exposure not always leads to sensitization rather provides protection against sensitization (Turcanu et al., 2003). Allergen exposure to atopic results in allergic exacerbation and therefore, allergen avoidance reduces chances of allergic reaction.

1.11.2. Corticosteroids

Corticosteroids are most successful anti-inflammatory agent being used in allergic inflammatory diseases. Corticosteroids diffuse into cytoplasm and interact with cytoplasmic glucocorticoid receptors to activate the receptor. Activated receptor translocate to the nucleus and increases the transcription and expression of anti-inflammatory genes. Corticosteroid modulated the expression of target genes by several different mechanisms, including gene transrepression and transactivation (Barnes & Adcock, 2003). These lower the expression of allergen induced cytokines, chemokines and adhesion molecules (Barnes et al., 1998), which are regulated by transcription factors such as nuclear factor-κB (NF-κB) and activator protein 1 (AP1) (Barnes & Adcock, 1998). These inhaled corticosteroids are highly effective in suppression of NF-κB and AP1 and consequently reduce inflammation (Barnes & Adcock, 2003). However, corticosteroids are found less effective to reduce virus mediated allergic response and to minimize the allergic symptom in smokers (Harrison et al., 2004; Chaudhuri et al., 2003).

1.11.3. β2-adrenoceptor agonists

β2-adrenoceptor agonist is established pharmacotherapy for asthma includes short-SABAs and LABAs. Agonists bind to the β2-adrenoceptor and transduce a signal to activate adenylate cyclase. Adenylate cyclase increases cyclic adenosine 3’5’-monophosphate level, to activate protein kinase A, which subsequently leads to smooth-muscle relaxation and bronchodilation through the phosphorylation of myosin light-
chain kinase and by opening Ca\(^{2+}\)-dependent K\(^+\) channels. Generally used inhaled LABAs supplement (Palmqvist et al., 1997) may have bronchodilation effect at least for 12 h (Palmqvist et al., 1997). LABA monotherapy is not recommended as it may enhance the inflammatory response. LABAs used in combination with corticosteroids may increase the efficacy of therapy (Usmani et al., 2005), however this is controversial (Mcivor et al., 1998). Turbutaline and salbutamol are common inhaled SABAs and are the most effective bronchodilators used for the instant relief in asthma.

1.11.4. Phosphodiesterase inhibitors (PDE)

Theophylline a xanthine has dual activity in treatment of asthma. It acts as a cAMP phosphodiesterase (PDE) inhibitor and also as an adenosine-receptor antagonist. It has marked bronchodilator effect but has lower therapeutic index and also associated with cardiac and central nervous dysfunction at required therapeutic dose. Theophylline also has some anti-inflammatory effect, but the evidence is rather weak. First and second generation phosphodiesterase inhibitors like cilomilast, ciclamilast, roflumilast, and rolipram have shown therapeutic effect in vivo and in vitro (Bundschuh et al., 2001; Mata et al., 2005). Phosphodiesterase are also effective to prevent airway remodeling. A selective PDE-4 inhibitor non-xanthine drug roflumilast is under phase 3 clinical trial (Boswell-Smith et al., 2006). Besides, MK-0359 has also shown great promises in allergic airway disease and is under clinical trial (Lu et al., 2009).

1.11.5. Mediator antagonists and synthesis inhibitors

H1-antihistamines are first specific agent used to control allergic exacerbation. Chlorpheniramine an early antihistamine product is effective in controlling allergic symptoms, but has problematic sedative and anti-cholinergic side-effects. Second generation of antihistamines such as cetirizine, levocetirizine, loratadine and desloratadine are more selective and has limited side effects due to decrease capacity to cross the blood–brain barrier (Del et al., 2006). CysLTs are important mediator of allergic reaction and increased level of CysLTC4 and CysLTE4 have been reported in
case of asthma and rhinitis. The CysLTs interact with CysLT receptor 1 (CysLTR1) and induces airway smooth muscle contraction and also have effects on mucous glands, microvessels, eosinophils and nerves. Oral leukotriene modifiers CysLTR1 antagonists (pranlukast, montelukast and zafirlukast) are being used in allergic diseases. Corticosteroids are not effective to reduce biosynthesis or action of CysLTs (Gyllfors et al., 2006). Leukotriene inhibitors also provide symptomatic relief in allergic rhinoconjunctivitis (frequently coexists with asthma) (Nayak & Langdon, 2007), but not in atopic dermatitis (Friedmann et al., 2007). Other modifiers like 5-lipoxygenase inhibitor; zileuton is also being evaluated for the therapeutic effect (Kemp, 2003).

1.11.6. Drugs for refractory disease

Conventional treatments are not adequate to control asthma in some patients most possible reason is lack of adherence to treatment. However, refractory asthma sometimes responds to immunomodulators such as low-dose methotrexate, azathioprine or cyclosporine with some side-effects. By contrast, calcineurin inhibitors such as tacrolimus and pimecrolimus (locally applied) and oral cyclosporine A inhibit T-cell responses and are effective treatments for atopic dermatitis (Hijnen et al., 2007). In more severe forms of the disease higher level of tumor-necrosis factor (TNF) and other associated cytokines has been reported in asthmatic airways (Howarth et al., 2005), (Waserman et al., 2000). Due to presence of these cytokines in severe forms of the disease, corticosteroids have limited effects in reduction of allergic reaction (Truyen et al., 2006). Studies targeting TNF have also been performed to combat the severe case of allergy using soluble TNF-receptor fusion protein etanercept (Howarth et al., 2005; Berry et al., 2006) and TNF-specific monoclonal antibodies. Kinase inhibitor such SB 220025 (Duan et al., 2005) and TPCA-1 (Birrell et al., 2006) (p38 mitogen-activated protein kinase and IκB kinase (IKK) respectively) are effective in lowering pro-inflammatory cytokines levels such as TNF and IL-1 and hence seems to be new attractive therapeutic approaches for refractory asthma.
1.11.7. Mast cell stabilizer

Sodium cromoglicate was initially characterized as mast cell stabilizer. Sodium cromoglicate inhibit the flux of chloride ions in mast cells, epithelial cells and neurons to increase their threshold for their activation (Alton & Norris, 1996). It has anti-inflammatory effect as prevent activation and infiltration of immune cells and promote Annexin 1 (anti-inflammatory) release (Yazid et al., 2009). Nedocromil sodium is another mast cell stabilizer is also being used in allergic airway diseases.

1.11.8. Allergen-specific immunotherapy

Allergen-specific immunotherapy (SIT) also termed as hypo-sensitization is an effective mean to modify immune response. SIT increases level of allergen-specific IgA and IgG4 antibodies and decreases IgE. IgG4 is blocking antibody reduces allergic inflammation (Aalberse & Schuurman, 2002). SIT also directs the immune response toward Treg and induces IL-10 and TGFβ for immunological tolerance to attenuates allergen-specific TH2- responses. IL-10 suppresses mast-cell, eosinophil and T-cell response (Wu et al., 2007). TGFβ maintains a diverse and self-tolerant Treg cells repertoire (Wan & Flavell, 2007). SIT also reduces the recruitment of mast cells, basophils and eosinophils and consequently the allergic reaction. Although SIT has therapeutic effect in allergic diseases such as asthma, rhinitis and venom hypersensitivity it has risk of serious adverse effect and may be life threatening (Fujita et al., 2012). For SIT appropriate selection of patient is also important. It may have adverse effect in patient those are already under beta agonist treatment (Moote & Kim, 2011). To combat with high risk of anaphylaxis hypo allergic recombinant allergen and chemically modified allergen are also being developed. CpG oligonucleotides conjugate to allergen are also important measure to improve the therapeutic effect as seen in case of ragweed allergy (Creticos et al., 2004).

Numbers of therapeutic agent are being used in different allergic diseases. However studies are being performed for further advancement in current therapies, to reduce side effect and potential adverse effect. Besides, new therapeutic agents are also being developed and evaluated for therapeutic potential (Figure 1.4).
Figure 1.4: Potential targets for development of new therapy of airway allergic diseases (Adcock et al., 2008).
1.12. NEWER TREATMENT FOR ALLERGIC DISEASES

1.12.1. Anti- IgE as a therapeutic target

Anti IgE are molecules that targets IgE and reduced its function. Different strategies to block IgE mediated responses are being studied. The Cε3 region of Fc fragment of IgE binds selectively to α-chain of the tetrameric FcεR1 (α1β1γ2). Antibodies specific for the C3 domain of IgE that block IgE binding to its receptor on surface of mast cells and reduces allergen mediated response (Corne et al., 1997). A humanized IgE-specific; non-anaphylactic IgG1 Omalizumab has been proven effective for asthma treatment (Holgate et al., 2005). Omalizumab can form trimer or hexamer with IgG/IgE without compliment activation. Reduction of free IgE with Omalizumab also contribute to lower FcER1 receptor in effector cells and contribute to treatment efficacy (MacGlashan, 2004). Omalizumab is effective in lowering of symptoms in multiple diseases such as allergic aspergilosis, rhinitis, urtiacaria, angioedema, eosinophilic gastroenteritis etc. On omalizumab administration level of IgE decreases rapidly but optimal clinical significance observed only after a long term treatment. Anti-IgE may influence allergen processing by airway DCs. IgE blocking reduces the allergic inflammatory cascade such as eosinophil and basophil recruitment (Schroeder et al., 2010). Raising concerns about induction of malignancy by omalizumab therapy have recently been largely dispelled (Long et al., 2014). However, omalizumab treatment does not shown reduction in methacholine and histamine induced airways responsiveness (van Rensen et al., 2009). Omalizumab has also role in replenishment of allergen induced airway remodeling (Hoshino & Ohtawa, 2012). Omalizumab treatment is also effective in allergic rhinoconjunctivitis, but therapy has to start long before the pollen season (Bez et al., 2004; Plewako et al., 2002). Recently, a new form of anti-IgE, QGE031 (Novartis) which is much more potent than omalizumab has been successfully assessed for the safety in treatment of IgE-driven diseases (Arm et al., 2014). Lumiliximab, a specific antibody for FcεRII receptor, decreases circulating IgE levels in Phase I clinical trial (Poole et al., 2005).

1.12.2. Cytokine-based immunotherapy

TH2 cytokines have sentinel role in allergic inflammation. Blocking monoclonal antibodies, fusion proteins targeting cytokines and the receptors are important
therapeutic strategies. Besides, inhibitors of the TH2-cell transcription factors STAT6 and GATA3 are also therapeutic target (Wong et al., 2002).

IL-4 is involved in class switching in B-cells to IgE synthesis. Preclinical studies on IL-4 blocking molecule have shown clinical benefit in allergic diseases. Altrakincept is a non-immunogenic recombinant human IL-4Rα (extracellular portion) administered to asthma patient for 12 weeks allowed withdrawal of corticosteroids treatment (Borish et al., 1999; Borish et al., 2001). However, in Phase III clinical trial Altrakincept therapy did not showed satisfactory outcomes, most probably due to low bioavailability. Further, humanized IL-4 and IL-4Rα blocking antibodies are under phase II clinical trial (Hart et al., 2002). Studies on mouse have revealed that secondary IgE reaction is not a Th2 mediated response and hence there is doubt over therapeutic efficacy (Linhart et al., 2007).

IL-13 participates in IgE synthesis, mucus secretion, cellular infiltration (eosinophil, T cells and monocyte) (Wynn, 2003). IL-13 binds with subunit IL-13Rα1 receptor (low affinity) and subunit complex of IL-4Rα1 & IL-13Rα1 (high affinity). IL-4 binding stabilizes IL-13 binding with high affinity receptor whereas IL-13Rα2 non-signaling receptor subunits inhibit IL-13 binding with high affinity complex (Andrews et al., 2006). IL-13Rα2 has shown therapeutic potential in reduction of AHR and mucus plugging in allergen immunized mice (Grunig et al., 1998). Monoclonal IL-13 specific antibodies have also shown therapeutic effect in animal model. A specific monoclonal IL-13 antibody CAT-354 is being evaluated in phase II study. Pitrakinra a mutant IL-4 protein inhibit binding of IL-4 and IL-13 to high affinity receptor is being therapeutic effect in asthmatic (Wenzel et al., 2007).

IL-5 is important player for eosinophil recruitment and activation. Humanized specific monoclonal antibodies to IL-5, Sch-55,700 andSB-240,563 (mepolizumab) have demonstrated therapeutic benefit in a small double blind trail. Although, these reduced the eosinophil in circulation and sputum, have no satisfactory effect on AHR and late response (Leckie et al., 2000). The IL-5 targeting may have less clinical benefit as airway eosinophils lacking IL-5R.
IL-10 is a Treg cytokine and has regulatory effect in Th2 cytokine mediated response. IL-10 knockout mice have show increased inflammatory response on allergen challenge (Grunig et al., 1997). Administration of IL-10 to healthy volunteer demonstrated reduction in circulatory Th1 and CD8 T cells. IL-10 also reduces endotoxin induced IL-1β and TNF and T cell proliferation as well (Chernoff et al., 1995). IL-10 is being studied for clinical advancement in inflammatory diseases but remain to be evaluated in allergic asthma.

1.12.3. Inhibitors of mast cells

K (Ca) 3.1 channel promotes mast-cell chemotaxis and activation. TRAM-34 (1- [(2-chlorophenyl) diphenylmethyl]-1H-pyrazole) is a K (Ca) 3 inhibitor might be a new approach to reduce allergic response (Cruse et al., 2006; Mark et al., 2004). Mast-cell activation can also be reduced by blocking SYK that propagates FccRI signaling (Matsubara et al., 2006). Intranasal administration of 2, 4-diaminopyrimidine (R112) a SYK inhibitor (Rossi et al., 2006) attenuate allergen induced nasal obstruction and rhinorrhea. R112 also inhibit prostaglandin D2 production (Guyer et al., 2006). Mast cell development relay on interaction of tyrosine-kinase receptor KIT with stem-cell factor. It is also required for survival, proliferation, homing, adhesion of mast cell and also for optimal IgE mediated mast cell degranulation and cytokine production (Okayama & Kawakami, 2006). Drug targeting SCF or KIT signaling molecules may be therapeutic approach for allergic disease (Reber et al., 2006).

1.12.4. Rho Kinase inhibitor

It is a GTP binding protein cause calcium sensitization to smooth muscle of airways. Rho kinase is involved in muscle contraction along with cell attachment, proliferation and migration. Trans-4-(1-Aminoethyl)-N-(4-Pyridyl)cyclohexanecarboxamide dihydrochloride (Y-27632) is a rho kinase inhibitor reduces not only the airway hyperresponsiveness but also the eosinophil infiltration when administered intranasally to ovalbumin immunized mice (Henry et al., 2005). Rho kinase inhibitor has promise to reduce allergic airway response are also in developmental phase.
1.12.5. Statins

Statins are HMG-CoA reductase inhibitor. Statins comprises a group of drugs used to lower cholesterol. Statins have shown therapeutic advancement in airway allergic diseases. Statin prevents IgE, cellular infiltration and inflammatory cytokine secretion by regulating MAP kinase, GTPase protein such as Ras, Rho and Nf-kB (Kim et al., 2006). Simvastatin a lipophilic statin has shown preventive effect in mouse model of airway inflammatory diseases (Kim et al., 2007). However the studies on statins are in infancy.

1.12.6. PI3K inhibitors

Activation of MAPK such as PI3K is important for cellular infiltration, proliferation, migration and proinflammatory cytokine induction. Phosphorylation of MAPK kinases is one of the reasons for glucocorticoid insensitivity. MAPK activation reduced the ability of glucocorticoid receptor to translocate into the nucleus (Ito et al., 2000) and subsequent activation of transcription factor. Additionally, PI3K phosphorylation reduced histone deacetylase 2 activity (To et al., 2010). Oxidative stress also increases PI3K and responsible for a glucocorticoid resistance. Intratracheal administration of Wortmannin and LY294002 PI3K inhibitors significantly reduces airway response, cellular infiltration along with Th2 cytokine in animal model (Lee et al., 2006). PI3k inhibitors also have potential to lower mucus secretion and smooth muscle contraction and migration (Duan et al., 2005; Gosens et al., 2004). PI3k inhibitors are emerging molecules for therapeutic intervenes in allergic inflammatory disease. Kinase inhibitors of P38 MAPK, ERK, JUN etc. are also promising therapeutic agent in allergic diseases.

1.12.7. P38 kinase inhibitor

MAP p38 kinase involved in expression of proinflammatory mediators such as IL-8, TNF-α and MMPs. MAP p38 kinase is also implicated in tissue remodeling. MAPK p38 inhibitors like SB2439063 treatment have shown reduction in inflammatory mediator release (Adcock et al., 2008). MAP p38 kinase inhibitors also increases sensitivity of glucocorticoids in allergic diseases particularly in case of oxidative stress (Bhavsar et al., 2008). However safety issues remain to resolve in use of P38 MAP kinase inhibitors (Kotlyarov et al., 1999).
1.12.8. Transcription factor blocker

IKK2 or Kb selective inhibitors to target Nf-kB are currently in development phase. However long term use may have immunosuppressive role and hence need further advancement in this field (Roshak et al., 2002). GATA3 is another target blocking of which prevent allergic inflammation in asthma. Other transcription factors are involved in expression of pro-inflammatory cytokines in allergic diseases such as NF-AT and STAT6 are also important target to develop new therapeutic agent (Adcock et al., 2008).

1.12.9. CpG-Oligonucleotides

CPG- oligonucleotide treatment reduces Th1 to Th2 ratio and hence diverts the immune response from IgE to IgG (Sur et al., 1999). Oligonucleotide reduced airway resistance and eosinophilic asthma in mice (Santeliz et al., 2002). The clinical trial for such oligonucleotide is currently underway.

1.12.10. Antisense oligonucleotide

An antisense oligonucleotide targeting adenosine A1 receptor have shown promising potential in Rabbit model of allergic asthma (Nyce & Metzger, 1997). Clinical trial of same is also completed (Sandrasagra et al., 2002). Double standard oligonucleotide blocking transcription factor are also emerging area of research in allergic diseases. TPI ASM8 a oligonucleotide targeting CCR3 and beta chain of IL-3/IL-5/GM-CSF receptor is currently under clinical trials (Guimond et al., 2008).

1.12.11. Peptide immunotherapy

Peptide immunotherapy has several advantages over extract based immunotherapy. Unlike allergen extract, peptides have defined composition, presence of allergen component only and lack of other allergic component. Moreover short peptide inhibit cross linking due to lack in structural motif, while maintaining immunogenicity (Sharma et al., 2011) and reduces chance of systemic allergic reaction and anaphylactic reaction. The mechanism in peptide immunotherapy involved the activation of Treg cells followed by skewing of the response away from Th2 type, leading to T cell anergy.
Initial studies on mice with T-cell peptide derived from Fel d 1 and Bet v 1 showed tolerogenic effect (Bauer et al., 1997; Briner et al., 1993). Gefter and colleagues developed peptide derived from Fel d 1 (Wallner & Gefter, 1994) as well as ragweed allergen were clinically evaluated in collaboration with Norman et al., (1996). Among different regime of therapy, subcutaneous treatment of peptides significantly lowered allergic airway symptoms against cat allergen and ragweed allergen. However, these peptides were longer and need to be administered in higher amount showed late onset of adverse effects. Further advancement includes the use of synthetic peptide immunoregulatory epitopes (SPIREs) which are comparatively shorter and to be used in lower doses. Such a peptide for cat allergen i.e. Cat-SPIRE is under phase III clinical trial (Creticos, 2014). Recently, ragweed SPIRE and HDM SPIRE have shown therapeutic benefit in allergic diseases (Hafner et al. 2012; Larche et al. 2013).

T cell peptide derived from Der p 2 has shown reduced allergic response against whole protein in mice (Hoyne et al., 1993). T cell peptide and its derivative of fungal allergen Cur l 3 have demonstrated skewing of immune response from Th2 to Treg in mice model and have promise for immunotherapy in allergic diseases (Sharma et al., 2011). However further studies are still required for the clinical uses of peptides.

1.12.12. Antioxidant treatment

Low levels of antioxidant are associated with increased severity of allergic diseases (Rubin et al., 2004), and hence antioxidants supplement exert therapeutic promises in allergic diseases. Presence of oxidant also lowers the corticosteroid sensitivity which can be minimized by antioxidant supplement. Antioxidants may be either enzymatic or non-enzymatic.

Non-enzymatic antioxidant such as ascorbate, bilirubin, glutathione, lipoic acid, curcumin, resveratrol, tocopherol, N-acetylcysteine, nacystelyn and urate can have therapeutic advantage in allergic airway diseases. An endogenous protein thioredoxin have shown anti-inflammatory response in animal model of asthma and COPD (Ichiki et al., 2005). Despite of its lower bioavaibility, oral application of curcumin has shown efficicacy as add-on therapy in asthmatics (Abidi et al., 2014). Recently, a study has
demonstrated the reduced allergic parameters in mice on vitamin C and selenium administration and has additive therapeutic potential in combination with anti-inflammatory agent (Bansal et al., 2014). S-nitrosoglutathione is as a nitric oxide (NO) reservoir. Decreased level of S-nitrosoglutathione is found in asthmatic due to increases level of catabolic enzyme S-nitrosoglutathione reductase. S-nitrosoglutathione reductase has shown therapeutic potential in ovalbumin immunized mice (Blonder et al., 2014). Redox sensitive pathway mediators such as NF-κB, AP-1 may also be alternative target to lower allergic response. Keap1/Nrf2 signaling is also important to enhance antioxidant level that can provide therapeutic effect.

Apart from non-enzymatic antioxidant enzymatic oxidant also have therapeutic importance in allergic diseases. Tripathi et al. (Tripathi et al., 2008) have shown that mutated glutathione S-transferases from *A. aternata* reduced Th2 response in ovalbumin immunized mice. They also demonstrated that administration of mutated glutathione S-transferases with non-enzymatic antioxidant GSH further enhanced the therapeutic potential in allergic mice (Tripathi et al., 2010). The reduced level of antioxidant enzymes superoxide dismutases (SOD) and catalase was reported in asthma (Comhair & Erzurum, 2010). Mimetics of SOD have shown potential therapeutic effect in animal model of allergic diseases (Masini et al., 2005).

The therapeutic effect of antioxidant and vitamins are still controversial in allergic diseases, as some studies have shown no clinical benefits on antioxidant supplement in (Fogarty et al., 2003).

### 1.13. PROTEASE INHIBITOR THERAPY

Enzyme inhibitors are considered as potential therapeutic agent in many diseases. Angiotensin-converting enzyme inhibitors are already in common usage for the treatment of hypertension and congestive heart failure. The approval of aspartyl protease inhibitors for the treatment of HIV infection has provided a new turn in the pharmaceutical industry. Multiple protease inhibitor programs have been started for the development of novel protease inhibitors that target further viral proteolytic enzymes, such as the NS3 protease of the hepatitis C virus and 3C protease of common cold virus. Disturbance of local balance of protease and antiprotease is a common feature of
inflammatory airway disease which may lead to chronic remodeling and lung degeneration. Increased proteolytic activity in the vicinity may also disturb the signaling and regulatory molecules and consequently the pulmonary function. Certain protease inhibitors are being used as standard therapy for inflammatory disease emphysema (hereditary) from many years. Recent studies suggested broader role for protease inhibitors in the treatment of airway inflammatory disease.

Serine protease inhibitors which are proposed for the therapeutic potential in allergic inflammatory disease include the inhibitors of mast cells tryptase and chymase, neutrophil elastase, cathepsin G and Proteinase 3 and plasminogen.

1.13.1. Synthetic protease inhibitor

1.13.1.1. Bis (5-amidino-2-benzimidazolyl) methane (BABIM)

BABIM is a synthetic non peptidic serine protease inhibitor having high specificity for tryptase and inhibit tryptase by zinc dependent mechanism. BABIM has demonstrated a marked reduction in glomerular necrosis in immune complex-mediated glomerulonephritis (Jennette et al., 1987). Clark et al. (1995) have shown that aerosol administration of BABIM and APC 366 (tryptase inhibitor) effectively reduced late phase airway resistance in allergic sheep. In a study aimed to determine contribution of mast cell tryptase in airway contraction, Gailit et al. (2001) have observed that the contraction of collagen gels containing fibroblasts and mast cells was reduced by BABIM.

1.13.1.2. N-(1-hydroxy-2-napthoyl)-L-arginyl-L-prolinamide hydrochloride (APC366)

APC366 is a peptidic serine protease inhibitor with high potential and specificity to inhibit tryptase enzyme. Molinari et al. (1995) have demonstrated that APC366 reduces the immediate cutaneous response in allergic sheep. Tryptase induces histamine release from mast cells which increases the airway resistance. APC366 treatment to sheep either 30 min before or after tryptase challenge, reduces airway resistance and hyperresponsiveness (Molinari et al., 1996). Clark et al. (1995) confirm the effect of same inhibitor in attenuation of allergen induced airway response and eosinophilia in sheep. In an in vitro study Barrios et al. (1998) have shown that tryptase induces
hyperresponse in isolated bronchi of guinea pig that is reduced by APC366 and SLPI (an endogenous protease inhibitor). APC366 suppress IgE dependent histamine release from synovial mast cells (He et al., 2001), may be due to mast cell-stabilizing effect of tryptase inhibitor by non-PAR2-mediated action of tryptase (He et al., 2004). APC366 reduces the acute airway resistance and airway response induced by ascaris antigen in pigs (Sylvín et al., 2002). In a study it has been observed that short-term repeated administration of APC 366 significantly reduces the magnitude of antigen-induced late allergic response (Krishna et al., 2001). APC366 reduces hepatic fibrosis scores and collagen content in hepatic fibrosis rat model indicating the potential of APC366 to reduce airway remodeling in inflammatory diseases. In phase 2 clinical trials APC366 has indicated efficacy in mild and moderate asthma.

Both, APC-366 and BABIM are mast cell tryptase inhibitor blocks tryptase activity by different mechanism. Preclinical studies showed both have therapeutic potential but only APC 366 was selected for clinical studies. APC-366 effectively reduce the late phase airway response but had no significant effect in reduction of early phase response. APC366 was also not effective in reduction of histamine induced bronchial hyperresponsiveness (Krishna et al., 2001). In phase II clinical trial bronchospasm was reported in treated patient, which preclude further clinical study on APC366 (Rice et al., 1998).

1.13.1.3. Nafamostae mesilate FUT-175 (6 amidino-2-naphthyl-4-guanidino benzoate-dimethanesulfonate)

Nafamostae mesilate is a synthetic protease inhibitor. It has broad specificity and can blocks the enzyme activities of various proteases, such as C1r, C1 esterase, thrombin, kallikrein, plasmin and trypsin. Nafamostae mesilate strongly inhibits the immunological reaction such as complement-mediated hemolysis, passive cutaneous anaphylaxis and delayed hypersensitivity (Hitomi & Fujii, 1982). Nafamostae mesilate exhibits anti-inflammatory effect in zymosan-induced increased vascular permeability and granuloma-pouch. Same inhibitor is also effectively prevents Arthus reaction and scald paw edema. Nafamostae mesilate also reduce complement mediated acetic acid-induced writhing (Issekutz, Roland & Patrick, 1990). Nafamostat mesilate inhibits
nuclear factor (NF)-κB activity in lung tissues along with AHR and goblet cell hyperplasia, eosinophil and neutrophil infiltration, Th2 cytokines in HDM as well as ovalbumin induced mouse model. Nafamostat mesilate exert therapeutic effect in allergen-induced airway inflammation, due to their inhibitory action in the early phase of mast cells activation. Late phase of allergic inflammation can also prevented by immunoregulatory function of inhibitor. Such properties might be a potential therapeutic approach for asthma (Chen et al., 2006) (Ishizaki et al., 2008). Besides allergic airway disease, nafamostat mesilate has also therapeutic potential in dermatitis and (Tsujii et al., 2009) chronic urticaria (Takahagi et al., 2010).

Positive skin reaction and allergic immune response was observed against nafamostat mesilate (Higuchi et al., 2000; Okada et al., 1998; Yamazato et al., 2002), which may limit the therapeutic use of same.

1.13.1.4. MOL 6131
MOL 6131 is a synthetic reversible specific tryptase inhibitor. Oh et al.(2002) have found that MOL6131 reduces total cells and eosinophils infiltration, goblet cell hyperplasia, Th2 cytokines, mucus secretion and peribronchial edema in allergen induced mouse model of airway disease. However MOL6131 was not effective in reduction of methacholine induced airway hyper-reactivity in mice.

1.13.1.5. NX21909
NX21909 is an inhibitor of elastase and the first oligonucleotide (aptamer) introduced to attenuate lung injury and neutrophil influx in animal model of acute lung inflammatory disease (Bless et al., 1997).

1.13.1.6. ICI 200,355
ICI 200,355 is a specific neutrophil elastase inhibitor. Neutrophil elastase appeared in the perfusate after allergen exposure and was positively correlated with lysozyme secretion. ICI 200,355 has attenuated allergic late-phase response in dog by blocking neutrophil elastase (Tabachnik et al., 1992). Allergen instillation into airway of immunized guinea pigs causes early recruitment of neutrophils and goblet cell
degranulation. Pretreatment of ICI 200,355 to guinea pig abolish mucous plugging, neutrophil recruitment (Agusti et a., 1998). Nadel et al. (1999) observed similar effect and suggested ICI 200,355 as potential therapy for serious pathophysiological abnormality in asthma.

1.13.1.7. Sivelestat (ONO-5046)

Sivelestat is a neutrophil elastase inhibitor. ONO-5046 treatment significantly reduces both early and late phase airway resistance, neutrophil recruitment and LTB4 in BALF of mice (Fujimoto et al., 1995). A study also suggests that beside airway resistance and inflammation ONO-5046 treatment suppress worsening of diseases by regulating neutrophil activity (Furuno et al., 1997). Neutrophil accumulation into the airway and the subsequent release of neutrophil elastase play a role in the airway microvascular leakage which is almost completely abolish on ONO-5046 treatment (Takeda et al., 1997). ONO-5046 treatment to mice significantly attenuates airway response, mucus secretion and inflammatory cell accumulation following ovalbumin challenge. Allergen induced IL-4, IL-5, IL-13 and eotaxin in early phase has been significantly suppressed by the elastase inhibitor. Early phase allergic airway response in mice is predominantly mediated by neutrophils. ONO-5046 treatment during late phase (Wada et al., 2010) significantly reduces the levels of IL-13 and TGF-β1 in the BAL fluid (Koga et al., 2013).

Among elastase inhibitor only sivelestat was approved for clinical trial for airway inflammatory disease (Lucas et al., 2013). But it was discontinued for further clinical trial due to adverse effect such as endocannabinoid (Maryanoff & Costanzo, 2008).

1.13.1.8. JNJ-10311795 (RWJ-355871)

JNJ-10311795 is a potent dual inhibitor of neutrophil cathepsin G and mast cell chymase. JNJ-10311795 exhibited anti-inflammatory activity in rats for glycogen induced peritonitis and LPS induced airway inflammation. JNJ-10311795 reduces glycogen induced neutrophil influx, inflammatory mediators IL-1α, IL-1β, TNFα and MCP-1 in LPS induced airway nitric oxide levels. These findings demonstrate that inhibiting
both cathepsin G and chymase with JNJ-10311795 is an exciting opportunity in the treatment of airway inflammatory diseases (de et al., 2005). Intravenous administration of RWJ-355871 to ovalbumin immunized rats prevents increase in paw volume. Aerosol pretreatment of same inhibitor to allergic sheep reduces early and late phase allergic airway response. In tobacco-smoke-exposed mice, RWJ-355871 administration significantly reduces neutrophil infiltration. The anti-inflammatory effects of RWJ-355871 in animal model indicate potential therapeutic ability for treating airway inflammatory diseases (Maryanoff et al., 2010).

1.13.2. Natural protease inhibitors

1.13.2.1. Secretory leukocyte protease inhibitor (SLPI)

SLPI constitutively expressed in mucosal tissues and immune cells and found in large quantities in bronchial and mucosal fluids. SLPI is a natural inhibitor of trypsin, mast cell tryptase and neutrophil elastase and play important roles in lung homeostasis (Seto et al., 2009).

Administration of aerosolized SLPI prior to antigen prevents antigen induced AHR, eosinophils and neutrophils infiltration as well as decreases mucus production in sheep (Wright et al., 1999). Tryptase-inhibiting activity is responsible factor for SLPI mediated in vivo suppression of lung vascular permeability and neutrophil recruitment (Mulligan et al., 2000). Forteza et al. (2001) have observed the same using SLPI and alpha (1)-PI to block tryptase mediated bronchoconstriction in sheep and found only SLPI is effective. SLPI and alpha (1)-antitrypsin inhibit IgE-dependent histamine release from lung, tonsil and skin mast cells which may be another mechanism of these inhibitor to attenuate allergic response (He et al., 2004). Fath et al. (1998) have observed that heparin, in combination with SLPI is more effective in reduction of early and late phase bronchoconstriction. Besides, topical application of SLPI has also reduced conjunctival recruitment and degranulation of eosinophils and inhibited the development of allergic conjunctivitis in guinea pigs. Mite extract upregulate IL-6 and IL-8 gene expression in the human conjunctival epithelial cell line which is inhibited effectively by SLPI and alpha1-antitrypsin (Seto, et al., 2009).
SLPI is an endogenous protease inhibitor. Lee et al., (1993) has demonstrated that the concentration of SLPI increased significantly in atopic patients. In a recent study, it has been shown that the cleaved portion of SLPI increases in subjects with allergic rhinitis and asthma compared to healthy controls (Belkowski et al., 2009). Overexpression of SLPI prevents the development of AHR and prevented ovalbumin mediated IgE, while ablation of the SLPI gene led to more severe responses to allergen (Marino et al., 2011). Further, studies are being performed for the further evaluation of therapeutic effect of SLPI in allergic diseases.

1.13.2.2. Elafin

Elafin is a natural protease inhibitor also known as peptidase inhibitor 3 (PI3) or skin-derived antiprotease. It expresses in mucosa of healthy individual. Tremblay et al. (1996) demonstrated that elafin is a constituent of BAL fluid from normal subjects and is found in enhanced concentrations in farmer’s lung and in farmers with lymphocytic alveolitis. This suggests that elafin may play a role in lung homeostasis and inflammation. The mucosal expression of elafin diminishes in patients with inflammatory bowel disease. This defect is associated with increased elastolytic activity (elastase-like proteolysis) in colon tissue. Food grade bacteria expressing elafin protects against inflammation and restore colon homeostasis in animal models (Motta et al., 2012).

1.13.2.3. Lactoferrin (LF)

LF is a cell secreted glycoprotein mediator, which has affinity with iron and can be regarded as and link between innate and adaptive immunity. It is secreted by epithelial and granulocyte and provides first line of defense against bacteria (Sanchez et al., 1992). Intranasal administration of LF in BALB/c mice prior or after ovalbumin challenge reduces airway inflammation, number of eosinophils and goblet cells, expression of Th2, Th17 and Treg cells in the nasal cavity (Wang et al., 2013). Although this property of LF is not directly correlated to protease inhibition, LF alters the expression of adhesion molecule in eosinophil that is involved in infiltration (Curran & Bertics, 2012). LF also inhibits eotaxin-stimulated eosinophil migration with no effects on eosinophil viability (Bournazou et al., 2010). Enzymatically active tryptase is
heparin stabilized tetramer, LF a cationic protein released from activated neutrophils, binds tightly to heparin and hence selectively prevent the activation of tryptase. In an allergic sheep model of asthma; aerosolized LF abolishes both late-phase bronchoconstriction and airway hyperresponsiveness (Elrod et al., 1997). However, some studies reported that LF induces adhesion of leukocyte to endothelial and exert proinflammatory effect (Brock, 1995). LF also activate eosinophil activation and degranulation which may limit therapeutic uses in allergic disease (Travis et al., 1999)

1.13.2.4. Prolastinor alpha1 antitrypsin (A1AT)

A1AT is a naturally occurring protease inhibitor also called as alpha 1 proteinase inhibitor. A1AT found in serum and the main function of A1AT is to maintain the physiological balance of protease anti-protease. The development of late phase allergic airway response in sheep is associated with increased tissue kallikrein activity (Koblinski et al., 2000) and decreased A1AT in bronchoalveolar fluid. The aerosol alpha A1AT given to allergic sheep effectively reduced airway hyperresponsiveness. The anti-allergic effect of A1AT has also been seen against aerosol challenge with high-molecular-weight kininogen (HMWK), a substrate of tissue kallikrein (Forteza et al., 1996). A1AT inhibits neutrophil elastase, mast cell tryptase and cathepsin G and other serine proteases. The A1AT therapy improve the quality of life of asthma patient (Blanco et al., 2008).

Previous studies have shown that serine protease inhibitors have therapeutic potential in allergic airway diseases. Protease inhibitors such as BABIM, APC-366, nafamostae mesilate, MOL 6131, sivelestat, SLPI etc. have reduced allergic parameters in animal models. However, only few of them were selected for clinical trial. In phase II clinical APC-366 has demonstrated increased risk of bronchospasm in patient and shown no efficacy to lower histamine induced bronchial hyperresponsiveness. MOL 6131 also has no effect in methacholine induced bronchial hyperresponsiveness reduction in mice. Nafamostae mesilate reduces bronchial hyperresponsiveness and allergic airway response in mice; however previous study reported nafamostae mesilate itself has some allergic response. LF an endogenous protease inhibitor has shown reduction in bronchial hyperresponsiveness and airway resistance but studies also
indicated proinflammatory effect of the same. Even though protease inhibitors have therapeutic effect in allergic airway disease very few of them are being studied hence there is a need of exploration and evaluation of the new inhibitors in allergic diseases.

AEBSF is an irreversible serine protease inhibitor with broad specificity (Trypsin, chymotrypsin, plasmin, thrombin and kallikreins) with high affinity. It is a less toxic (LD50 of 76mg/kg), soluble in water (200mg/ml) compound which has excellent stability in acidic pH as in inflammatory condition. Owing to these properties, AEBSF may be a suitable molecule to be studied for therapy in allergic diseases. The present study is therefore aimed to explore the therapeutic effects of AEBSF in allergic airway disease.
OBJECTIVES

Following are the objectives to be achieved under the present study:

1. To study the prophylactic and therapeutic effect of AEBSF in ovalbumin induced airway inflammatory disease.

2. To evaluate the prophylactic and therapeutic effect of AEBSF in cockroach allergen induced airway inflammatory disease.

3. To study the effect of protease inhibitor in combination with kinase inhibitor in airway inflammatory disease.