Chapter 1

Review of Literature
IMMUNE SYSTEM
A system for distinguishing what is self from non-self is necessary in all living organisms. The mammalian immune system protects the individual from infectious microbes that possess diverse pathogenic mechanisms, at the same time avoiding immune responses that produce excessive damage to self-tissues. The environment contains a range of microbes that threaten the host by continuous exposure. The immune system uses a complex array of protective mechanisms to control and eliminate these organisms. All of these mechanisms rely on detecting structural features of the pathogens that mark them as distinct from host cells. This host-pathogen discrimination is essential to permit the host to eliminate the pathogen without excessive damage to its own cells.

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

HISTORY OF ALLERGY
The term ‘allergy’ conceived by von Pirquet (1905) is defined as abnormal immunological reactivity on contact with foreign particles. The foreign agents causing altered immunological reactivity are called allergens which include a broad spectrum of substances. An allergen is an antigen that the body mistakenly perceives as a threat and trigger a specific chain of events (allergic cascade) leading to hypersensitivity.
The term atopy was first used by Coca and Cooke (1923) to describe a tendency to develop immediate-hypersensitivity reactions to allergens that are commonly encountered in the general environment. Atopic individuals produce high amount of Immunoglobulin (Ig) E in response to specific allergens (Mekori, 1996). The development of suitable technology, led to the identification of IgE by Ishizaka and Ishizaka followed by Johansson and Bennich (1967). Allergic individuals experience exaggerated reaction against foreign proteins on second or subsequent exposures to which their body become sensitized in the first exposure. The term hypersensitivity refers to undesirable (damaging, discomfort-producing and sometimes fatal) reactions produced by the immune system. Hypersensitivity reaction requires a pre-sensitized (immune) state of the host. Gell and Coombs classified hypersensitivity in four groups in 1963 (Figure 1.1). Later, a fifth type of hypersensitivity was also added to this list (Rajan, 2003).

1. Type I - Anaphylactic / Immediate hypersensitivity (allergy) is triggered by the interaction of IgE and allergens on the surface of mast cells or basophils resulting in the release of pharmacologically active mediators such as histamine, serotonin, leukotrienes, prostaglandins, eosinophil chemotactic factors etc. This reaction is manifested within minutes after exposure to an allergen and is therefore referred as immediate hypersensitivity. The reaction involves two phases (Figure 1.2).
   a. Sensitization: This phase sets in upon first exposure to allergen. The antigen presenting cells (APCs) in the airway epithelium internalize, process and present the allergen to T-lymphocytes. The interaction of these T-cells with B-lymphocytes signals heavy chain switching to ε type and secretion of allergen specific-IgE. The released IgE binds to high affinity receptors on mast cells and basophils (Figure 1.2). This completes the sensitization phase (Descotes and Choquet-Kastylevsky, 2001; Goldsby et al., 2003). In this phase immune system is stimulated for IgE production, but no symptoms occur.
   b. Re-exposure: Subsequent exposure to allergen causes memory B cells to proliferate and secrete allergen specific IgE in large amount. The released IgE binds to receptors on mast cells and basophils. Interaction of multivalent allergen with receptor bound IgE causes cross-linking of receptors. This initiates intracellular signaling that leads to degranulation of cells, with the release of pro-inflammatory mediators (Figure 1.2) (Goldsby et al., 2003).
Figure 1.1: Type of hypersensitive reactions: Gell and Coombs’ classification of hypersensitive reactions. Source: Goldsby et al., 2003.

Figure 1.2: Allergic reaction: Allergen exposure activates B cells to form IgE secreting plasma cells. These IgE molecules bind to IgE-Fc receptors on mast cells and basophils. Subsequent exposure to the allergen leads to cross-linking of the bound IgE, thereby activating the mast cells/basophils to release pharmacologically active mediators. These mediators are responsible for the pathologic reactions of immediate hypersensitivity. Source: Goldsby et al., 2003.
The mediators exert their effect on different parts of system such as smooth muscles, blood vessels etc. The reaction manifests itself in two phases:

i. **Early phase response**: It occurs within 5 to 30 minutes of allergen exposure. IgE receptor cross-linking following allergen exposure signals microtubule polymerization. The polymerized microtubules allow transport of cytoplasmic granules to plasma membrane to which they fuse (Leung, 1997). Fusion of these granules empties the preformed mediators e.g. histamine, serotonin etc. Since the constricting effects of histamine on smooth muscles last only for 1-2 hours, the changes tend to subside after most of the granules are empty (Goust and Finn, 2003). Such reactions e.g. vasodilatation and mucus secretion can lead to nasal blockage in upper respiratory tract and to sneezing, edema and broncho-constriction in the lungs (Leech, 2002).

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

Upon repeated exposure to allergen, the symptoms may progress to take the form of severe disease. The severity of the symptoms is related to the amount of mediators released, which in turn is dependent on the number of sensitized immune cells (Goust and Finn, 2003). The immediate hypersensitivity manifest in various forms given below:

*Cutaneous reaction*: Mostly occurs when antigen enters by oral route. The examples are urticaria, hives etc.

*Respiratory reaction*: Usually occurs when antigen enters by inhalation and can lead to allergic rhinitis, asthma and/or both.

*Anaphylactic reaction*: It may appear as severe systemic reaction on exposure to allergen in highly sensitive cases. Reactions present as erythema, vomiting, diarrhea, respiratory distress and in severe cases as laryngeal edema and vascular collapse leading to shock or mortality (Goust and Finn, 2003).
2. **Type II - Cytolytic / Cytotoxic hypersensitivity** occurs when antigen bound to cells is recognized by antigen specific IgG or IgM. The surface associated immune complexes are recognized by complement resulting in cell lysis. Complement is usually (but not always) necessary to affect cellular damage. The target cells can also be killed non-specifically through antibody dependent cell mediated cytotoxicity. This mechanism involves binding of non-sensitized cells like monocytes, polymorphs and killer cells to target by their specific receptor. The reactions include mismatched blood transfusion, organ transplant rejection, etc.

3. **Type III - hypersensitivity (Immune complex-mediated reactions) reactions** cause tissue injury by forming immune complexes. Such reactions occur when antigen-antibody reaction forms micro-precipitates in and around small blood vessels. Such circulating immune complexes are generally cleared by phagocytic systems. When clearance capacity of phagocytic system exceeds (Goust and Finn, 2003), soluble immune complexes (aggregations of antigens and IgG and IgM antibodies) are deposited in the endothelial lining of blood vessel walls and tissues (typically the skin, kidney and joints). The deposited complexes trigger complement activation. Macrophages, neutrophils and platelets are subsequently attracted to the deposition site and contribute to the tissue damage. Typical manifestations include allergic bronchopulmonary aspergillosis (ABPA) caused by the inhalation of *Aspergillus* conidia, glomerulonephritis, rheumatoid arthritis, serum sickness, arthus reaction and systemic lupus erythematosus (Rajan, 2003).

4. **Type IV - hypersensitivity (cell-mediated)** is initiated by sensitized T-delayed hypersensitivity (TDH/T-helper type-1) cells, which react with the antigens deposited at the local site. The interaction results in release of cytokines that activate macrophages or T-cytotoxic (Tc) cells which mediate direct cell damage. The reaction takes hours to develop. It includes tuberculin hypersensitivity, graft rejection, allergic contact dermatitis, etc.

5. **Type V - Stimulatory hypersensitivity reaction** is an additional type that is sometimes (often in Britain) used as a distinction from Type II. Here, instead of binding to cell surface components, antibodies recognize and bind to the cell surface receptors, which either prevent the intended ligand binding with the receptor or mimic the effects of the ligand thus impairing cell signaling. Some clinical examples for type V reactions are Graves' disease, Myasthenia gravis, etc.
TYPE I HYPERSENSITIVITY

Immunoglobulin (Ig) E plays central roles in type I hypersensitivity. Allergic diseases affect an individual in terms of personal sufferings as well as the society in terms of quality of life, work performance and productivity (Bolin and Lindgren, 2002).

Immunoglobulin E

Immunoglobulins are glycoproteins that recognize and interact with antigens (foreign substances) and cause their neutralization and removal. IgE was first described as ‘reagin’ (serum factor) by Prausnitz and Kustner in 1921. This serum factor was later identified as IgE in 1967 (Johansson and Bennich, 1967). IgE is an antibody subclass present primarily in the skin and mucus membrane of mammals. It is also found circulating in mucosal secretions and or in serum. It is a heat labile glycoprotein of 190 kDa. The concentration of IgE (0-0.0001 g/L, constituting 0.004% of total serum immunoglobulins) in serum is lowest of the five immunoglobulin isotypes (IgA, IgM, IgG, IgD & IgE) and is age dependent. IgE has a half life of 1-5 days in peripheral blood (Prussin and Metcalfe, 2003).

IgE structure: The IgE molecule has basic Y-shaped structure, consisting of two identical light chains (23 kDa) and two identical heavy chains (50-70 kDa) folded into constant (C) and variable (V) domains (Sutton and Gould, 1993; Mekori, 1996). IgE however differs from other immunoglobulin in an additional C-chain domain (C_{H4}). Within the variable (V) region, there are complementarity-determining regions (CDRs) called hypervariable regions. The antigen-binding specificity of immunoglobulin molecules is determined by these CDRs (Sutton and Gould, 1993; Mekori, 1996). The heavy (H) and light (L) chains and the two heavy chains are held together by inter-chain disulfide bonds and by non-covalent interactions. There are intra-chain disulfide bonds within each polypeptide chains also.

IgE synthesis and regulation: B-lymphocytes differentiate into IgE producing plasma cells when B-cell receptors present on the cell surfaces recognize specific B-cell epitopes on the antigen surface (Figure 1.3; Huby et al., 2000). Furthermore, the isotype switching for the IgE-secreting B-cells requires help from T-lymphocytes (Vercelli and Geha, 1992; Corry and Kheradmand, 1999). B-lymphocytes need two signals to get mature as IgE producing plasma cell. The first signal is provided by T-helper (Th) cells by release of cytokines such as interleukin (IL)-4 and IL-13 (Vercelli, 1995). Another signal required for IgE synthesis is provided by physical interaction between CD40L expressed on the activated Th cells and the CD40
molecule expressed constitutively on B cells (Romagnani, 1995). Mast cells and basophils also express CD40L and provide cell contact signal required for IgE synthesis by human B cells (Marone et al., 1995).

IgE molecules have high affinity for mast cells and basophils (Hasegawa et al., 1999). The antibody mediates its functions by binding to IgE receptors present on the surface of its target cells (Oettgen and Geha, 2001). There are two cell surface receptors for IgE.

1. **High affinity receptor (FcεRI):** It is found principally on mast cells and basophils (Blank et al., 1989). Clustering of IgE bound to the FcεRI on mast cell surface, with multivalent allergens, results in the release of mediators that cause allergic symptoms (Kinet et al., 1990; Sutton and Gould, 1993).

2. **Low affinity receptor (FcεRII):** FcεRII (CD23) is a single chain transmembrane glycoprotein expressed on B-cells and macrophages, eosinophils, follicular dendritic cells, Langerhans cells and T-cells (Goldsby et al., 2003, Novak et al., 2003). Cross-linking of CD23 leads to the activation of B-cells, alveolar macrophage and eosinophils (Prussin and Metcalfe, 2003).

**THE ALLERGIC CASCADE**

Contact with multivalent allergen causes cross-linking of IgE that leads to FcεRI aggregation. This initiates calcium dependent (LYN/SYK/PLC/Ca2+ mediated) and calcium independent (Fyn/GAB2/PKC mediated) degranulation of mast cell vesicles (Figure 1.4; Nishida et al., 2005).
Figure 1.3: Synthesis and regulation of IgE. Source: Miescher and Vogel, 2002; Larche, 2006.
Figure 1.4: Allergic cascade and signaling events: Calcium dependent and independent pathways are triggered upon IgE receptor cross-linking. These pathways eventually cause release of mediators from effector cells. *Constructed from Goldsby et al., 2003 and Nishida et al., 2005.*
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Calcium dependent signaling: Clustering of FcεRI initiates cellular response by 3 ways:

a) It promotes phosphorylation of tyrosine in ITAMs of β and γ chains by protein tyrosine kinase (PTK) ‘Lyn’ (Cambier, 1995). This signal is for recruitment and activation of cytosolic ‘Syk’, another PTK (Oliver et al., 2000; Bruhns et al., 2005). The lyn-syk dependent signaling cascade causes membrane recruitment of cytosolic phospholipase Cγ1. This enzyme catalyses the formation of inositol-1, 4, 5-trisphosphate (IP3) and diacylglycerol (DAG) from membrane phospholipids (Figure 1.4). IP3 opens calcium channels on the surface of intracellular calcium stores. DAG activates protein kinase C (PKC) which causes microtubule assembly and granule fusion in presence of calcium ions.

b) It also activates an enzyme that converts phosphatidyl serine (PS) to phosphatidyl ethanolamine (PE). The PE is methylated by methyl transferases to form phosphatidylethanolamine (PC). Accumulation of PC on membrane surface increases membrane permeability and helps in formation of Ca2+ channels (Goldsby et al., 2003).

c) Cross-linking activates membrane adenylate kinase, leading to transient increase in cAMP which activates protein kinases. These kinases phosphorylate membrane proteins thereby changing permeability of granular membrane to water and Ca2+. This leads to swelling and fusion of granules to plasma membrane. The increase in cAMP levels is transient and is followed by drop in the levels to below baseline. Sustained rise in cytosolic Ca2+ leads to formation of arachidonic acid (AA) which is converted into leukotrienes and prostaglandins by action of 5-lipoxygenase and cyclo-oxygenase, respectively.

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

Mast Cells: The most crucial cell type in the early asthmatic reactions (EAR) are mast cells (Bloemen et al., 2007; Ryan and Brown, 2002). Mast cells are important effector cells for adaptive immune responses and are involved in the pathophysiology of asthma to secrete a wide variety of mediators after activation by allergens (Bloemen et al., 2007). They are relatively abundant in skin, thymus, lymphoid tissue, lung, nasal mucosa, conjunctiva, uterus, urinary bladder, tongue, synovia, and mesentery; around large and small blood vessels, below the skin, in subserosal and submucosal layers of the airway and gastrointestinal tract and along blood vessels in the connective tissue of all organs, except the brain. They express a variety of membrane receptors, high-affinity IgE receptor (FceRI) and FcγRIIb (CD32), cytokine receptors (IL-3R, IL-4R, IL-5R, IL-9R, IL-10R), GM-CSF, chemokine receptors and nerve growth factor receptors. It has been recently observed that the number of infiltrated mast cells into airway smooth muscle correlates with the degree of AHR in asthma (Brightling et al., 2002). There are two types of mast cell phenotypes:

(i) MC_{T} phenotype: MC_{T} cells contain neutral peptidase tryptase and have primary role in host defense. They are mainly located at mucosal surfaces and present in large numbers in areas of T lymphocyte infiltration and in allergic diseases.

(ii) MCTC phenotype: MCTC cells contain both tryptase and chymase and are primarily involved in angiogenesis and tissue remodeling. They are found predominantly in submucosal and connective tissues.

Basophils: Basophils are granulocytes (1% of peripheral blood leukocytes) with segmented nucleus sharing common features of mast cells, such as high-affinity IgE receptor (FceRI) expression, metachromatic staining, Th2 cytokine expression, and histamine release. Basophils develop from CD34+ pluripotent stem cells, differentiate and mature in the bone marrow under the influence of IL-3 (Prussin and Metcalfe, 2003). Basophils induce tissue infiltrations and play a crucial role in the initiation and maintenance of allergic diseases following FceRI cross-linking (Prussin and Metcalfe, 2003). Basophils express a variety of cytokine receptors (IL-3R, IL-5R, GM-CSFR), chemokine receptors (CCR2, CCR3), complement receptors (CD11b, CD11c, CD35, CD88), prostaglandin receptors (CRTH2), and immunoglobulin Fc receptors (FceRI, FcγRII).
Eosinophils: Eosinophils comprise less than 5% of the circulating white cell population in healthy individuals and are morphologically distinguished by their bi-lobed appearance (Kariyawasam and Robinson, 2007). They functions as APCs and directly amplify the Th2 immune response (Shi, 2004). Eosinophils get activated by number of factors, including IL-3, IL-5, GM-CSF, CC-chemokines (CCL2, CCL3, CCL5, CCL7, CCL8 (MCP-2), CCL11, CCL13), and platelet activating factor (PAF), express FceRI on their surfaces and bind to IgE (Janeway et al., 2001; Pearlman, 1999). Activated eosinophils release cytotoxic and pro-inflammatory mediator leading to mucosal damage in chronic asthma (Figure 1.5). These products include reactive oxygen species and cytotoxic granule and vesicular proteins such as major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN), as well as cytokines and chemokines together with phospholipids-derived, pharmacologically active mediators (Prussin and Metcalfe, 2006).

Eosinophils are capable of producing significant quantities of cysteinyl leukotrienes (Figure 1.5). Cysteinyl leukotrienes contract airway smooth muscle (100-1000 fold more potent bronchoconstrictors than histamine), increase vascular permeability, stimulate mucus secretion, decrease muco-ciliary clearance, stimulate eosinophil and neutrophil recruitment into the airways (Busse, 1998). They also promote some of the pathophysiological hallmarks of asthma such as AHR and cause neuronal dysfunction (Dahlen, 1998; Kariyawasam and Robinson, 2007).

During the inflammatory response, complement-derived anaphylatoxins C3a and C5a bind to specific cellular receptors and activate respiratory burst in eosinophils. C5a acts as a chemoattractant for neutrophils and eosinophils and represents a major metabolic activator for eosinophils inducing the release of granule proteins and free oxygen radicals that cause damage to the airway tissue (Elsner and Kapp, 1999). Moreover, eosinophils are a source of a number of profibrotic cytokines and fibrogenic mediators that are implicated in airway remodeling of asthma such as IL-11, IL-17, IL-17E (IL-25), transforming growth factor-alpha (TGF-α), TGFβ1, and matrix metalloproteinase (MMP)-9 (Foley et al., 2007).
Figure 1.5: Role of eosinophils in the late asthmatic reaction: Source, Filipovic and Cekic, 2001.
Neutrophils: These are polymorphonuclear lymphocytes (PMN) which play important role in pathophysiology of asthma. These are produced by hematopoiesis in the bone marrow. Neutrophils are active phagocytic cells and exhibit larger respiratory burst by generating more of reactive oxygen and nitrogen intermediates as compared to macrophages. Studies have shown increased number of PMN in bronchial biopsies in severe asthmatics (Balzar et al., 2002). Increased PMNs in BAL fluid and sputum of asthmatics are also reported (Tanizaki et al., 1993; Sampson, 2000). They can produce a wide range of products, including lipids (LTA4, LTB4, PAF, thromboxane (TX) A2), cytokines (IL-1β, IL-6, TNF-α, TGF-β, CXCL8), proteases (elastase, collagenase, matrix metalloproteinase (MMP) 9), and microbicidal products (lactoferrin, myeloperoxidase, lysozyme). Neutrophil products can cause airway narrowing, increased mucus secretion and increased airway smooth muscle (ASM) responsiveness (Gibson et al., 2001).

Macrophages: Macrophages play a dual role in allergic responses and inflammation in the airways. They secrete cytokines and chemokines, including IL-1, IFN-γ, TNF-α, IL-6, CCL2, CCL3 and CXCL8 which further helps in the recruitment and activation of other inflammatory cells (Bloemen et al., 2007).

Lymphocytes: Lymphocytes constitute 20-40% of white blood cells and 99% of the cells in the lymph that play a major role in both immunity and allergy. They are divided into two types, the B and T-lymphocytes:

B-lymphocytes are tiny antibody factories that produce antibodies to help destroy foreign substances when stimulated to do so by Th cells. B-cell display membrane bound antibody molecules which serve as receptors for antigen. Interaction between antigen and the membrane bound antibody on B-cells, as well as interactions with T-cells and macrophages, selectively induces the activation of differentiation of B-cells (Melchers and Rolink, 1999). In case of allergic response, B-cells in presence of appropriate cytokines such as IL-4 and IL-13 released by Th2 cell produce IgE antibodies which in turn cause mast cell degranulation leading to allergic symptoms.

T-lymphocytes play a fundamental role in the initiation and regulation of inflammatory response. T-lymphocytes have specific antigen receptors which recognize foreign antigens and initiate immune response responsible for attacking foreign substances (cell-mediated immunity) (Corrigan, 1997). T-cell recognize antigen only when it is bound to a self-molecule encoded by genes within the major
histocompatibility complex (MHC). T-cells express T-cell receptor, a complex of polypeptides that includes CD3; and most can be distinguished by presence of CD8 and CD4 membrane molecules. T-lymphocytes are of two types, 1) cytotoxic or killer T-cells, and 2) T helper cells (Th cells).

I) T cytotoxic (Tc) cells: They belong to CD8+ T-cells and capable of inducing the death of infected somatic or tumor cells. These cells have TCRs that recognize antigens bound to class I MHC molecules, present on all nucleated cells and proliferate and differentiate into an effector cell i.e. cytotoxic T-lymphocyte (CTL). In contrast to the TC cell, the CTLs generally do not secrete many cytokines and instead exhibit cell-killing or cytotoxic activity. The CTLs have a vital function in monitoring the cells of the body and eliminating anything that display antigen, such as virus-infected cells, tumor cells, altered self-cells and cells of a foreign tissue graft.

II) T helper (Th) cells: T helper cells express CD4 on their surfaces and are activated by recognition of an antigen-class II MHC complex on an APC. Once activated, they divide rapidly and secrete cytokines to activate B cell, T cell and other cells that regulate the immune response. Depending on the cytokine signals received, these cells differentiate into Th1, Th2, Th17 or Treg subsets, which generate different regulatory signals (Figure 1.6).

i) T-helper type 1 (Th1) cells participate in cell-mediated immunity essential for controlling pathogens such as viruses and certain bacteria (Figure 1.7). They produce cytokines that supports inflammation and activates mainly certain T-cells and macrophages (Romagnani, 2000; Troye-Blomberg, 2002). They are produced when naïve T cells interact with dendritic cells type 1 (DC-1) presenting antigen to the T cell's receptor and secreting IL-12. This IL-12 activates the Th1 cells to secrete tumor-necrosis factor-beta (TNF-β) and interferon-gamma (IFN-γ), which in turn stimulate macrophages to kill the pathogens. They engulf pathogens and recruit other leukocytes to the site producing inflammation. There is now evidence that Th1 cell responses might also be responsible for some of the pathogenic features in patients suffering from chronic forms of atopy, including epithelial apoptosis and smooth muscle cell activation (van Oosterhout and Bloksma, 2005).
Figure 1.6: Schematic overview of T-cell phenotypes in allergic disease: Th1, Th2, Th17, and Treg cells are characterized by cytokines, which mediate specific functions in different tissue cells such as dendritic cells (Dc), keratinocytes (Ker), epithelial cells (Epit), B cells, eosinophils (Eos), mast cells, fibroblasts (Fib) and neutrophils (Neut). Source: Schmidt-Weber et al., 2007.
Figure 1.7: Development of Th1 and Th2 lymphocytes: Antigens enter through the endobronchial tree, cross the epithelial surface, and interact with naive Th cells and DCs. As a result of signals from the surrounding microenvironment, they differentiate into Th1 cells, which produce IFN-\(\gamma\), IL-2, and lymphotoxin (LT), or Th2 cells, which produce IL-4, IL-5, IL-9, IL-13, and IL-10. Polarization into Th1 cells occurs via a STAT-1 and T-bet–dependent pathway under the influence of CD8\(\alpha^+\) DCs and macrophage-derived cytokines such as IFN-\(\gamma\), IL-12, and IL-18. Differentiation into Th2 cells occurs via a pathway that involves STAT-6, GATA-3, nuclear factor of activated T cells-c (NFATc), and c-maf under the influence of CD8\(\alpha^-\) DCs and IL-4, which may come from mast cells. Source, Elias et al., 2003.
ii) T-helper type 2 (Th2) lymphocytes play an important role in the initiation, progression and persistence of allergic diseases and asthma (Figure 1.7). Th2 cells are produced when DC-2 dendritic cells present antigen to the T cell receptor (TCR) and induce these Th2 cells to participate mainly in humoral immune reactions to promote antibody synthesis. Th2 secretes IL-4 (promotes IgE synthesis; positive-feedback device promoting more pre-Th cells to enter the Th2 pathway and blocks the IFN-γ receptors on pre-Th cells thus inhibiting them from entering the Th1 pathway); IL-5 (attracts and activates eosinophils); IL-10 (inhibits IL-12 production by DCs thus inhibiting pre-Th cells from entering the Th1 pathway) and IL-13 that promotes synthesis of IgE, mucus production, promotes eosinophil differentiation and limit the development of AHR (Burton et al., 2002). These cells contribute many of the pathophysiological features of asthma, including airway inflammation, mucus secretion and AHR (Cohn et al., 2004; Ying et al., 2006).

A number of transcription factors and signaling molecules have potential roles in asthma, including c-maf, nuclear factor κB (NF-κB), and signal transducer and activator of transcription 6 (STAT6) (Robinson and Lloyd, 2002). GATA3 and T-bet are the 2 transcription factors with the potential to control Th2 or Th1 respectively. GATA3 is implicated in Th2 development and is an important controller of the IL-5 gene locus (Zheng and Flavell, 1997). The level of GATA-3 expressed cells was increased in biopsy samples taken from asthmatics lungs (Nakamura et al., 1999). T-bet was described as a controller of Th1 development and is shown to direct IFN-γ production and IL-12 receptor β2 expression (Szabo et al., 2000; Larche et al., 2003). Reduced number of cells expressing T-bet was reported from asthmatics and impulsive AHR in mice deficient in T-bet. These findings suggest that asthma is associated with reduced airway Th1 cells (Finotto et al., 2002).

iii) Treg (Regulatory T) cells play crucial roles in the induction of peripheral tolerance to self and foreign antigens. These cells express both CD4 and CD25 and secrete TGF-β and IL-10 and provide important down regulating signals. There are two most relevant classes of Treg cells within CD4+ T cells: 1) type 1 regulatory T cells (Tr1), and 2) CD4+CD25+Treg cells, differing in a number of important biological features, including their specific cytokine production, surface markers, ability to differentiate in antigen-specific responses, and patterns to exert their functions, i.e., through secretion of cytokines or cell-cell contact mechanisms. Impaired regulatory T cell activity can cause autoimmune diseases and allergy (Romagnani, 2006; Ying et al., 2006).
iv) Th17 cells are a recently-identified subset of Th cells, found at the interfaces between the external environment and the internal environment, e.g., skin and lining of the GI tract protecting them against extracellular bacteria by recruiting neutrophils (Bettelli et al., 2007). On activation they enter a pathway distinct from that of Th1 and Th2 cells, secrete IL-17 and IL-21, and induce synthesis of plasma membrane receptor for IL-23. Interaction of IL-23 with the receptor drives rapid proliferation of the Th17 cells. These cells have also been implicated in Crohn's disease, ulcerative colitis, psoriasis, etc (Schmidt-Weber et al., 2007).

Natural killer (NK) cells: These cells are a form of cytotoxic lymphocytes that constitute 5-10% of lymphocytes in human peripheral blood. These are the major component of the innate immune system and play a major role in the rejection of tumors and virally infected cells. NK cells kill by releasing small cytoplasmic granules of perforin and granzyme that cause the target cell to die by apoptosis. They were named "natural killers" because of the initial notion that they do not require activation in order to kill cells that are "missing self" markers of MHC class I.

There has been a growing recognition of a cell type, the NKT cells, that has some features of both NK cells and T cells. NKT cells constitute a lymphocyte subpopulation that is abundant in the spleen, thymus, bone marrow, liver and lungs (Bendelac et al., 1997; Kronenberg and Gapin, 2002). A large majority of NKT cells express an invariant TCR consisting of the Vα14–Jα18 (previously named Jα281) α-chain (Vα24–JαQ in humans) paired with a Vβ8 or Vβ2 β-chain, and so these cells are referred to as invariant NKT (iNKT) or Vα14 NKT cells (Berzins et al., 2004; Akbari, 2006). iNKT cells play a prominent role in the pathogenesis of AHR and asthma via production of IL-4 and IL-13 cytokines (Akbari, 2006).

Antigen Presenting Cells (APCs): Antigen presenting cells such as dendritic cells (DC), macrophages and B cells have a crucial effect on the initiation and chronicity of allergic diseases and contact allergy. They critically control T-cell mediated immune responses, thus directing the outcome of the encountered allergen toward silent elimination, allergy and or tolerance. APCs internalize the antigen either by phagocytosis or endocytosis, and then display a part of that antigen on class II MHC molecule. T helper cell recognizes and interacts with the antigen-class II MHC molecule complex on APC. An additional co-stimulatory signal is then produced by the APC, leading to activation of the Th cell. Also APCs and Th2 cells play an
important role in B cell maturation and differentiation into cells producing allergen specific IgE (Goldsby et al., 2003).

Role of Dendritic cells (DCs) in the Th1/Th2 switch: As DCs are the major APCs localized at the interface with the environment, it is presumed that these cells carry the signal for the differentiation of T cells into Th1 and Th2 (Bubnoff et al., 2001). DCs are involved in both allergen presentation and the late-phase of asthmatic cascade. DC1s are responsible for inducing Th1 cells, whereas DC2s induce Th2 differentiation (Rissoan et al., 1999). Th2 cytokine, IL-4 enhances the maturation of DC1s and leads to apoptosis of pDC2s. Interferon gamma (IFN-γ) from Th1 cells protects progenitor DC2s against IL-4 and IL-10 induced killing and promotes DC2 differentiation. The critical factor for the polarizing mechanism seems to be the level of IL-12 produced by DC1s. IL-12 producing capacity can be influenced and modulated directly by the pathogen, micro-environmental factors, and by affecting DC1 maturation at different stages. DCs upregulate the expression of several co-stimulatory molecules that might be involved in T-cell activation or in the differentiation of regulatory T cells (Heijink and Van Oosterhout, 2005). DCs are not only very crucial during Th2 initiation, but also control the inflammatory Th2 effector response during ongoing asthma when IL-17 was given during allergen challenge (Hammad and Lambrecht, 2008).

PRODUCTS OF EFFECTOR CELL ACTIVATION

Cytokines: They are small, soluble proteins that activate and modulate leukocytes and structural cells during inflammatory and immune responses. Th1 and Th2 cells develop from the same naive Th cell under the influence of both environmental and genetic factors acting at the level of antigen presentation in association with a series of modulatory factors such as TCR, activation of co-stimulatory molecules, predominance of a given cytokine in the microenvironment of the responding Th cell (Romagnani, 2000). The type and the combination of cytokines secreted during the course of inflammation, determine type of hypersensitivity reaction ensued by influencing the development of Th1 and Th2 type cells. Several features specifically associated with the asthmatic state are regulated by cytokines. These include the regulation of IgE, eosinophilia, and mast cell proliferation. The important cytokine involved in allergic immune response are summarized below:
• **IL-4**: It is produced primarily by Th2 cells but NK cells, mast cells, basophils and eosinophils can also produce IL-4 (Seder et al., 1991; Ying et al., 1997). It exists as preformed peptide in cells and thus can be released rapidly. IL-4 stimulates growth and differentiation of B-cells, enhances surface expression of CD23, MHC class II and IL-4R on B cells and monocytes. It promotes synthesis of IgG1, IgE, IgA and drives initial differentiation of T cells to the Th2 phenotype and e-type switch. Other B-cell activating cytokines, such as IL-2, IL-5, IL-6, and IL-9, synergize with IL-4 to increase the secretion of IgE (Banchereau et al., 1994). Release of IL-4 within airways also increases goblet cell metaplasia, mucus hypersecretion and eosinophil recruitment (Hamid and Minshall, 2000).

• **IL-5**: IL-5 is crucial for the maturation, activation, and survival of eosinophils. It is increased in parasitic infestation and at sites of chronic allergic inflammation. IgE-dependent stimulation of human lung mast cells also induces IL-5 production (Jaffe et al., 1995).

• **IL-6**: IL-6 is secreted by Th2 cells, eosinophils and macrophages. Under the influence of IL-6, B lymphocytes differentiate into mature plasma cells and secrete immunoglobulins (Borish and Steinke, 2003).

• **IL-13**: It is produced by activated Th2 cells, mast cells and natural killer (NK) cells (Hoshino et al., 1999). Its function is similar to that of IL-4 but it is less potent and act independently of IL-4 (Punnonen et al., 1993).

• **IL-25**: It is a novel Th2 cytokine of the IL-17 family that plays a key role in allergic inflammation (Ballantyne et al., 2007; Wang et al., 2007). Recent studies suggest that over-expression of IL-25 in mouse induces eosinophilia (Tamachi et al., 2006).

• **IL-3**: It is produced by activated T-cells, eosinophils and mast cells. It acts as haemopoietic growth factor including mast cell growth factor (Shen et al., 2008).

• **IL-9**: It is important for growth and survival of mast cells (Renauld, 2007).

• **IL-10**: IL-10 inhibits production of pro-inflammatory cytokines (IL-4 and IL-5) and chemokines (eotaxin I & II), expression of HLA-DR, and some co-stimulatory molecules by Th1 macrophages, neutrophils, and eosinophils. Those with asthma have a decreased expression of IL-10 illustrating IL-10’s important role in regulating allergic responses (Yssel et al., 2001).
• **IFN-γ:** It is produced by APC’s and Th1 lymphocytes in response to either specific antigen or mitogenic stimulation. Studies show that IFN-γ inhibits the IL-4-dependent IgE response *in vitro* (Yoshimoto et al., 1997) and promotes Th1 response.

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

• **IL-8:** It is a pro-inflammatory cytokine (chemokine) present along cytoplasmic membranes and in intracellular granules and induces chemotaxis of neutrophils and T-cell lymphocytes (Cheng et al., 2007).

• **IL-18:** It has both anti-allergic and allergy-promoting effect. It induces IFN-γ production and Th1 response under the influence of IL-12 and inhibits IgE isotype switching. It can also stimulate IL-4 production and histamine release from basophils, IL-4 and IL-13 production from NK, T and NKT cells under the influence of IL-2 and induces IgE synthesis by B cells (Izakovicova, 2003).

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

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

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

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

**Chemokines:** They are a group of small molecules (8 to 12 kDa) involved in inducing chemotaxis in a variety of cells and play a role in immune homeostasis and in driving the maturation, homing, and activation of leukocytes. The chemokines, CCL2, CCL11, CCL22, and CCL17, play a role in T-cell polarization, recruitment, or both into an inflammatory lesion (Lloyd et al., 2000). CCL2 skews *in vitro* T-cell differentiation towards the Th2 phenotype (Kim et al., 2001). CCL11, CCL22, and CCL17 appear to selectively recruit Th2 lymphocytes to sites of inflammation (Sallusto et al., 1997) as Th2 lymphocytes express CCR3 and CCR4 (Zingoni et al.,
CCR8 selectively expressed on Th2 cells might play a role in their recruitment into allergen-inflamed tissues through CCL17 (Hirata et al., 2003). CCL2 and CCL5 play an important role in mast cell recruitment and activation in the lung (Trautmann et al., 2000). In humans, 7 chemokines (CCL11, CCL24 and CCL26, CCL5, CCL7, CCL13, and CCL3) and IL-5 have been implicated in the recruitment of eosinophils into various tissues (Rot et al., 1992).

**Lipid mediators:** The cysteinyl leukotrienes (LTC4, LTD4, and LTE4) can be generated by eosinophils, mast cells and alveolar macrophages and combine with specific receptors, CysLT1 and CysLT2 (Busse, 1998). The majority of actions of cysteinyl leukotrienes are generated by interaction with the CysLT1 receptor which can lead to airway smooth muscle contraction, leukocyte chemotaxis, and increases in vascular permeability.

**ALLERGIC DISEASES**

During the last few decades a rapid upsurge has been observed in incidence of allergic ailments (Bousquet et al, 2005). Studies suggest that over 20% individuals worldwide suffer with IgE-mediated diseases such as asthma, allergic rhinitis, rhino-conjunctivitis, eczema, and anaphylaxis (Linhart and Valenta, 2005). The common eye and respiratory diseases are described below:

**Allergic Conjunctivitis:** Allergic conjunctivitis is the inflammation of the conjunctiva i.e. membrane covering the white part of the eye, due to allergy. Symptoms consist of redness, edema of the conjunctiva, itching and increased lacrimation (production of tears) (Bielory and Friedlaender, 2008). If this is combined with rhinitis, the condition is termed allergic rhino-conjunctivitis. The symptoms are due to release of histamine and other active substances by mast cells, which stimulate dilation of blood vessels, irritate nerve endings and increase secretion of tears (Riedi et al., 2005).

On exposure to a particular allergen, sensitization takes place and prepares the system to launch an antigen specific response. Th2 differentiated T-cells release cytokines, which promote the production of antigen specific IgE. On subsequent exposure and interaction with IgE on mast cells histamine is released, which leads to the release of cytokines, prostaglandins, and platelet activating factor (Bielory et al., 2007). Histamine released from mast cells binds to H1 receptors on nerve endings and causes the ocular symptom of itching. Histamine also binds to H1 and H2 receptors of
the conjunctival vasculature and causes vasodilatation. Mast cell derived cytokines such as chemokines, IL-8 are involved in recruitment of neutrophils. Th2 cytokines such as IL-5 recruit eosinophils and IL-4, IL-6, and IL-13 which promote increased sensitivity (Ono and Abelson, 2005).

Allergic conjunctivitis is best treated by avoiding the allergen e.g. avoiding grasses in bloom during the "hay fever season" and with antihistamines, either topical in the form of eye drops, or systemic in the form of tablets (Buckley, 1998). In addition to antihistamines, mast cell stabilizers, dual mechanism anti-allergenic agents, or topical antihistamines are also used (Ono and Abelson, 2005). Non specific measures to ameliorate symptoms include: cold compresses and eyewashes with tear substitutes. Corticosteroids are another option, but considering the side effects of cataracts and increased intraocular pressure, corticosteroids are reserved for more severe forms of allergic conjunctivitis (Ono and Abelson, 2005; Origlieri and Bielory, 2008).

**Allergic Rhinitis:** Allergic rhinitis (AR) is clinically defined as a symptomatic disorder of upper airways induced by an IgE-mediated inflammation after allergen exposure of the membrane lining the nose (Bousquet et al., 2001). It is characterized by nasal obstruction, rhinorrhea, sneezing, watery eyes, itching of the nose and/or postnasal discharge which are reversible spontaneously or under treatment. Allergic rhinitis represents a global health problem affecting 10 to 25% of the population worldwide (International Consensus Report on Diagnosis and Management of Rhinitis, 1994; Sibbald, 1993, Wuthrich et al., 1995, Strachan et al., 1997).

About 40% of the world’s population suffers with atopic disorders and allergic rhinitis is the commonest of them. Allergic rhinitis affects 24% of the population in the United Kingdom, 20.6% in Norway and 19.6% in Germany whereas in Asia, it afflicts 10-50% (Anonymous, 1998; Wong et al., 2004). Allergic rhinitis is considered as a first step towards asthma in many cases. The nasal and bronchial mucosa present similarities and most patients with asthma also have rhinitis (Vignola et al., 1998). Epidemiological, patho-physiological and clinical studies have strongly suggested a relationship between rhinitis and asthma (Wright et al., 1994).

Previously, allergic rhinitis was subdivided, based on the time of exposure, into seasonal, perennial and occupational diseases (Strachan et al., 1997; Dykewicz et al., 1998; Van-Cauwenberge et al., 2000). But, sometimes the allergens (pollens, moulds) are present throughout the year, so it is confusing to designate as seasonal
allergic rhinitis in patients. Also the symptoms of perennial rhinitis may not be present throughout the year. Thus a revision to subdivide allergic rhinitis has been proposed on the basis of presence of symptoms while retaining the term seasonal and perennial rhinitis. It is subdivided into “intermittent” or “persistent” allergic rhinitis (Bousquet et al., 2001).

1. **Intermittent allergic rhinitis** means that the symptoms are present less than 4 days a week or for less than 4 weeks.

2. **Persistent allergic rhinitis** means that the symptoms are present more than 4 days a week and for more than 4 weeks.

Rhinitis may be caused by allergic, non-allergic, infectious, hormonal, occupational, and other factors (Dykewicz et al., 1998; Bousquet et al., 2001). Allergic rhinitis is the most common type of chronic rhinitis, but 30%-50% of patients with rhinitis have non-allergic causes (Settipane and Lieberman, 2001).

**Pathophysiology**

Allergic rhinitis is caused by aeroallergens which include proteins and glycoproteins in airborne dust mite fecal particles, cockroach residues, animal dander, molds, and pollens etc. Upon inhalation, allergen particles get deposited on nasal mucosa and diffuse into nasal tissues. The sensitization process is initiated when antigen-presenting cells (dendritic cells, especially CD1+ Langerhans-like cells, and macrophages) present allergen to CD4+ T lymphocytes (Godthelp et al., 1996). Stimulated CD4+ Th2 cells release IL-3, IL-4, IL-5, IL-13 and other cytokines that lead to a cascade of events promoting local and systemic production of IgE by plasma cells as well as chemotaxis and inflammatory cell placement, localization, proliferation, activation and prolonged survival within the airway mucosa (Bousquet et al., 2001).

**Early/immediate allergic response**

Within minutes of inhalation of allergen in sensitized individuals, they are recognized by IgE fixed on mast cells and basophils, causing degranulation and release of preformed mediators such as histamine and tryptase, and the rapid generation of mediators, including cysteinyl-leukotrienes (LTC4, LTD4, and LTE4) and prostaglandin D2 (Bousquet et al., 2001). Mediators cause plasma leakage from blood vessels and stimulate secretion of mucus from glandular and goblet cells. Histamine elicits itching, rhinorrhea, and sneezing whereas other mediators such as leukotrienes
and PGD2 produce congestion in the nasal mucosa (Naclerio, 1991; Bousquet et al., 2001).

**Late-phase response**

Mediators and cytokines released during the immediate phase set off a cascade of events over the ensuing 4-8 hours that lead to an inflammatory response called late response. Although this reaction may be clinically similar to the immediate reaction, nasal congestion is more prominent (Naclerio, 1991). Mediators and cytokines released during the early response act upon post-capillary endothelial cells to promote vascular cell adhesion molecule (VCAM) and E-selectin expression that promotes adherence of circulating leukocytes, such as eosinophils to endothelial cells. Factors with chemoattractant properties, such as IL-5 for eosinophils, promote the infiltration of the superficial lamina propria of the mucosa with many eosinophils, some neutrophils and basophils, and eventually CD4+ (Th2) lymphocytes and macrophages (Naclerio, 1991; Bousquet et al., 2001). These cells become activated and release more mediators, which in turn activate many of the pro-inflammatory reactions seen in the immediate response. The eosinophils predominate in nasal secretions whereas CD4+ (Th) lymphocytes predominate in nasal biopsy specimens (Dykewicz et al., 1998).

**Diagnosis**

Clinical history is essential for an accurate diagnosis of rhinitis, assessment of severity and response to treatment. The evaluation of a patient with rhinitis should include assessment of specific symptoms e.g., nasal congestion, pruritus, rhinorrhea, sneezing, the pattern of symptoms viz., intermittent, seasonal, perennial, identification of precipitating factors, response to medications, coexisting conditions, and a detailed environmental history including home and occupational exposures (Dykewicz et al., 1998). Generally allergic rhinitis is often associated with allergic conjunctivitis and the presence of eye pruritus and lacrimation is an indication that a patient’s has an allergic rhinitis.

Skin prick testing (SPT) and radioallergosorbent test (RAST) or Enzyme linked immunosorbant assay (ELISA) can be utilized to identify the triggers responsible for allergic rhinitis. The tests used in the diagnosis of allergic rhinitis detect the free or cell-bound IgE. The results of allergy tests provide useful information when analyzed together with information about the symptoms and clinical history of the patients. Apart from the above two test, other test are also
available which can be applied to the patients such as nasal provocation tests, nasal cytology, endoscopy etc on individual basis.

ASTHMA
Asthma is a chronic lung disease where inhalation and exhalation are obstructed by the production of excess mucus and the swelling of the airway membranes, giving rise to coughing and wheezing. It is estimated that allergy and asthma together affect in excess of 700 million people world over (Bateman and Jithoo, 2007). Since 1980, the frequency of asthma has almost doubled (Umetsu et al., 2002). As a result, asthma now affects approximately 8-10% of the population in the US, is the leading cause of hospitalization among children less than 15 years of age, and costs society billions of dollars annually. Although much progress has been made in our understanding of bronchial asthma over the past decade its pathogenesis is still unexplored (National Asthma Education and Prevention Program, 2003).

Asthma is an inflammatory disorder of the lower airways characterized by chronic eosinophilic inflammation, increased AHR and variable airway obstruction (Elias et al., 2003). Many cells and cellular elements play a role in asthma pathogenesis, in particular, mast cells, eosinophils, T-lymphocytes, macrophages, neutrophils, and epithelial cells. In susceptible individuals, this inflammation causes recurrent episodes of wheezing, breathlessness, chest tightness, and coughing. It is further distinguished by multiple phenotypes that might differ on the basis of age of onset, triggering factors and patterns of severity both during acute exacerbations and on a more chronic basis, as reflected by variably reversible loss of lung function (Aubier et al., 2005; Magnan and Vervloet, 2005). It has long been assumed that patients with asthma experience intermittent attacks and have relatively normal lung function during intervening periods. Recent studies have demonstrated that asthma can cause progressive lung impairment and in some patients, eventuate in partially reversible or irreversible airway obstruction (National Asthma Education and Prevention Program, 2007).

DISORDERED AIRWAY PATHOLOGY
Until recently, information on airway pathology in asthma has come largely from post-mortem examinations (Dunnill, 1960; Jeffery, 2000), which shows that both large and small airways often contain plugs composed of mucus, serum proteins, inflammatory cells and cellular debris. Microscopically there is usually extensive
infiltration of the airway lumen and wall with eosinophils and lymphocytes accompanied by vasodilatation, evidence of microvascular leakage and epithelial disruption. Presence of increased levels of T-cells, eosinophils and mast cells has been reported from the airway samples taken from asthmatic and control subjects (Foley et al., 2007). In asthmatics, more severe and prominent inflammation was apparent in the small airways than in large airways (Hamid, 1997). Compared to specimens from patients with mild to moderate disease, inflammatory cells in the small airways of severe asthmatic patients persisted, despite the aggressive treatment with steroids (Wenzel et al., 1997).

The relationship between the pathological changes and clinical indices has been difficult to obtain. Clinicians have long recognized an association of sputum and blood eosinophilia with asthma (Reed, 1993). Eosinophils play an important role in bronchial inflammation in asthma. These cells are in part recruited to the site of the inflammation by chemoattractants liberated by the structural cells of the mucosa, especially epithelial cells (Van Wetering et al., 2007). Eosinophils liberate proteins like the major basic proteins (MBP) and the eosinophil cationic protein (ECP) that are thought to be involved in damage of airway tissues.

Studies in patients of severe asthma, in both acute and chronic states, suggest that in addition to eosinophils and lymphocytes, neutrophils are also present and play a role in this chronic disease (Wenzel et al., 1997). There are other forms of asthma which are non-eosinophilic and are dominated by neutrophils and show increased levels of IL-8 in the airways (Gibson et al., 2001). Non-eosinophilic forms of asthma are common (Douwes et al., 2002) and are difficult to treat with even high doses of inhaled corticosteroids along with long acting β-agonist (LABA). These therapies act specifically on eosinophilic inflammation and allergic IgE pathways and offer little or no relief to neutrophilic airway inflammation, a recognized feature of severe asthma or refractory asthma (Wenzel et al., 1997).

The application of immunological and molecular biology techniques has revealed that T-lymphocytes play pivotal role in orchestrating the inflammatory response in asthma through the release of multifunctional cytokines (Robinson et al., 1992). The generation of cytokines by “structural” (constituent) cells of the airways, including fibroblasts, endothelial and epithelial cells, is increasingly considered to be important in the maintenance of the inflammatory response (Holgate et al., 2000).
Th1 and Th2 cells have distinct functions, and dysregulation of one or the other subset can cause different types of pathology. Cytokines associated with a Th2-like response appear to play a role in disease progression, particularly IL-4 and IL-5 (Romagnani, 1991; Hasday et al., 1994). IL-4 enhances secretion of IgE by B cell, mast cell growth, and endothelial cell up-regulation of adhesion molecules, especially vascular cell adhesion molecule-1 (VCAM-1) which is involved in selective recruitment of eosinophils (Coffman et al., 1986; Coffman and Carty, 1986; Schleimer et al., 1992; Robinson et al., 1993). IL-5 acts on eosinophils by enhancing differentiation, adhesion to vascular endothelial cells, chemotaxis, cytotoxic activity, and degranulation (Sanderson, 1992; Robinson et al., 1993). IL-4 and IL-5 proteins and the frequencies of cells expressing these mRNAs are increased in bronchoalveolar lavage (BAL) fluid and lung tissue samples from asthmatics but not from normal subjects (Robinson et al., 1993).

The bronchial epithelium forms a dynamic barrier that protects the mucosa from inhaled foreign agents. The airway epithelium protects the internal milieu of the lung by secreting mucus and signaling and interacting with the innate and adaptive immune systems through the secretion of cytokines and chemokines (Marini et al., 1992). Epithelial damage is one of the many pathophysiological features of asthma that has been observed, even in mild asthma, but not in other pulmonary diseases such as chronic obstructive pulmonary disease (COPD) and chronic bronchitis (Hamilton et al., 2001; Laitinen et al., 1985). It is characterized by the weakening of the ciliated cells that detach in clumps and expose the basal membrane. There is a correlation between the desquamation of the epithelium and the degree of bronchial hyperreactivity (Laitinen et al., 1985). Epithelial fragilization may occur through cleavage of desmosomes (Montefort et al., 1993). In addition to the fragility of the epithelium, goblet cell hyperplasia has been observed, which may contribute to the hypersecretion of mucus in asthma (Montefort et al., 1993). Excessive epithelial damage can arise from an enhanced susceptibility to injury, an inability to proliferate and repair properly or an inadequate response to an environmental stimulus. It is reported that cultured asthmatic bronchial epithelial cells are more sensitive to oxidative stress (Bucchieri et al., 2002). Bronchial epithelial cells from mild asthmatics are more permeable after exposure to ozone or dioxides and release more IL-8 and Granulocyte-macrophage colony-stimulating factor (GM-CSF) than cells from non-asthmatic subjects when exposed to diesel exhaust particle (Devalia et al.,
Following chronic epithelial injury in asthma, epithelial cells express high levels of transcription factors such as NF-kB, STAT-6, STAT-1 and AP-1 and overproduces a large array of cytokines which are involved in the recruitment, maturation and activation of inflammatory cells (Demoly et al., 1992; Hart et al., 1998; Sampath et al., 1999; Mullings et al., 2001; Chakir et al., 2001).

ASSOCIATION OF AIRWAY PATHOLOGY TO DISORDERED LUNG FUNCTION

Airway (bronchial) hyperresponsiveness (AHR/BHR) and acute airflow limitation are the two predominant manifestations of disordered lung function.

Airway hyperresponsiveness (AHR): The key feature of persistent asthma is the development of the state of AHR. However, the hyperresponsive state acquired is poorly understood. The chronic allergic inflammation in atopic asthma results in airway remodeling, involves thickening of the airway wall, increases sub-epithelial collagen deposition and vascularity as well as hypertrophy and hyperplasia of airway smooth muscle (ASM) (Barnes et al., 1998).

Airway hyperresponsiveness has become an important tool in the diagnosis of asthma. Measurement of AHR has been standardized for histamine and methacholine administered via aerosol inhalation by tidal breathing (Cockcroft et al., 1977) or administered in predetermined amounts via a dosimeter (Chai et al., 1975). In carefully controlled conditions, response to histamine and methacholine is similar (Juniper et al., 1978; Salome et al., 1980; Hargreave et al., 1981). Methacholine is better tolerated even at higher doses but the recovery time is slightly slower as compared to histamine (Salome et al., 1980; Nelson, 1983). Among different tests of lung function to measure changes in airway caliber following provocation, the FEV1 (forced expiratory volume in one second) has been most broadly accepted, with the position of the stimulus-response curve identifying the provocative concentration (or dose) of agonist. The provocative concentration reduces FEV1 by 20 % from baseline (PC20 or PD20) and serves as an index of responsiveness.

Airflow Limitation: The repeated episodes of airflow limitation in asthma relate to the airway inflammatory reaction.

Acute bronchoconstriction: The mechanism of acute airflow limitation varies according to the stimulus. Allergic inflammation is classified into three sequential phases. Early phase reactions are induced within minutes of allergen challenge
whereas late phase reactions occur within several hours. By contrast, chronic allergic inflammation is a persistent inflammation that occurs at sites of repeated allergen exposure (Kay, 2001; Holgate and Polosa, 2008). Early phase reactions mainly reflect the secretion of mediators by mast cells at the affected site. When cross-linking of adjacent IgE molecules by bivalent or multivalent allergen occurs aggregation of FcεRI triggers a complex intracellular signalling process that results in the secretion of biologically active product: those stored in the cytoplasmic granules, lipid-derived mediators, cytokines, chemokines, growth factors and other products that contract the smooth muscle and lead to bronchospasm (Galli et al., 2008). The release of preformed and other mediators contributes to the acute signs and symptoms associated with early-phase reactions which forms the basis of bronchoconstriction upon exposure to aeroallergens.

**Swelling of the airway wall:** Airflow limitation also results from edematous swelling of the airway wall with or without smooth muscle contraction or bronchoconstriction. This leads in the reduction of airway caliber that characteristically occurs 6 to 24 hours following allergen challenge and is referred to as the late asthmatic response (Holgate, 1993). The increase in microvascular permeability and leakage leads to the mucosal thickening and swelling of the airway outside the smooth muscle. This causes swelling of the airway wall and loss of elastic recoil pressure, both of which contribute to AHR (James et al., 1989; Hogg, 1993).

**Airway wall remodeling:** Examination of bronchial biopsies from patients has shown that inflammation and changes in the bronchial structure are common features in mild and severe asthmatic patients (Bentley et al., 1993). Airflow limitation sometimes fails to reverse with glucocorticosteroid treatment. The cellular and molecular basis of this lack of response may be associated with structural changes to the airway matrix accompanied with severe persistent airway inflammation. Airway inflammation is the most likely factor to account for varying severity of asthma and is therefore the element most responsive to controller medications such as inhaled glucocorticosteroids. However, even in the absence of symptoms and overt airflow limitation, asthma continues to exist in the form of mild airway inflammation and AHR (Jacoby et al., 2001).
PREVALENCE OF ASTHMA IN ADULT AND CHILDREN

Epidemiological studies show that asthma prevalence is highest in economically developed countries e.g. New Zealand, UK, USA and Australia (Figure 1.8). Around 300 million people in the world currently suffer with asthma which is equally common in both children and adults (Masoli et al., 2004). The increase in the prevalence of asthma has been associated with an increase in atopic sensitization and is paralleled by similar increases in other allergic disorders such as eczema and rhinitis (Farrar, 2005). As communities adopt western lifestyle, asthma is expected to increase involving 400 million people by 2025 (GINA, 2006). As per estimates, asthma accounts for about one in 250 deaths worldwide. The economic burden of asthma is considerable both in terms of direct medical costs (drugs and hospitalization) and indirect medical costs (time lost from work and premature death). The number of disability-adjusted life years (DALYs) lost due to asthma worldwide is currently estimated to be 15 million/year, and asthma accounts for around 1% of all DALYs lost, reflecting the high prevalence and severity (Humbert, 2006).

The steering committee of the International Study on Asthma and Allergies in Childhood (ISAAC) recently reported that the prevalence of atopic diseases in childhood (including asthma) has increased considerably in Western industrialized countries. Surveillance of asthma in North America, Western Europe, and Australia has demonstrated recent increases in asthma prevalence and mortality (Janson et al., 1997; Anonymous, 1998). The prevalence of asthma ranges from 7% in France and Germany to 11% in the USA and 15-18% in the United Kingdom. Approximately 20% of these patients have severe asthma, of which 20% is inadequately controlled (Peters et al., 2006). Allergic rhinitis affects 10% to 30% of adults and the majority of patients with asthma (60-80%) have rhinitis and 20-40% of patients with rhinitis can have asthma (Peters et al., 2006). Rhinitis symptoms in patients with asthma are associated with worse asthma outcomes and have a significant impact on the quality of life (Lehman and Lieberman, 2007).

The incidence of asthma in India is reported in the range of 2.3-16.6 % (Chhabra et al., 1998; Gaur et al., 2006; Aggarwal et al., 2006; Jindal, 2007), which is higher than reported (< 1 %) earlier (Viswanathan et al., 1966). Asthma prevalence was reported to be 3.5% with diagnosis, whereas it was 9-12% of symptomatic subjects without diagnosis (Chowgule et al., 1998). The reported prevalence of asthma was 2.3-3.3% in the children from Lucknow (Awasthi et al., 2004) whereas it
Figure 1.8: Global prevalence of allergy and asthma: Mapping the prevalence of allergy and asthma on world map. Red colored zones (Western industrialized nations) represent most affected population (≥10% population affected). India represents relatively lesser affected region (2.5-5.0% population affected). Source: Masoli et al., 2004
was 2.6% in rural children from Ludhiana (Singh et al., 2002). In Bangalore, prevalence of asthma was 9% in 1979 that reached to 29.5% in 1999 (Paramesh, 2002). This may be attributed to environmental pollution, urbanization and change in demography of the city. A study in Tamil Nadu on urban and rural children of 6-12 years showed 18% prevalence of wheeze (Chakravarthy et al., 2002).

RISK FACTORS FOR ASTHMA

It has long been established that many common diseases are the result of interactions between genetic susceptibility and environmental exposure. The expression of asthma is a complex, interactive process between the two major factors-host factors and environmental exposures that occur at a crucial time in the development of the immune system (Figure 1.9). These two factors play a leading role in the development, severity or limiting the resolution of allergic inflammation (Cookson, 2004; Galli et al., 2008; Vercelli, 2008).

Host factors (Genetics): Asthma has an inheritable component to its expression, but the genetics involved in the eventual development of asthma remain complex and not well understood (Holgate, 1999; Ober, 2005). This led to the recognition that allergies and asthma represent complex genetic disorders that have numerous contributing genes, each with variable degrees of involvement in any given individual (Steinke et al., 2003).

Clinical observations suggest that 70% of offspring from both asthmatic parents can suffer from asthma (Geha, 2003). Linkage studies have identified more than a dozen genomic regions linked to asthma and/or increased serum IgE levels (Wjst and Immervoll, 1998). Asthma, often defined as a single disease, in reality includes several different phenotypes, each of which is likely to reflect different pathogenic mechanisms. ADAM33 (a disintegrase and metalloprotease) has been identified as an asthma susceptibility gene in ethnically diverse populations which is significantly associated with AHR, airway remodeling and asthma (Van Eerdewegh et al., 2002; Geha, 2003).

Chromosomes 11, 12, and 13 contain several genes that could be important in the development of atopy and asthma (Holloway et al., 1999). Several genes on chromosome 5q are suggested to be important in the development or progression of inflammation associated with asthma and atopy, including genes encoding the
cytokines such as IL-3, IL-4, IL-5, IL-9, IL-12 (β-chain), IL-13, and GM-CSF (Holloway et al., 1999; Wiesch et al., 1999). Toll-like receptor-10 is a potential asthma candidate gene, as two SNPs in Toll-like receptor-10 are associated with asthma (Fabbri et al., 2005). Despite the significant progress in the knowledge about genetics of allergic disease, the biological relevance of the genes and genetic variations associated with expression of the allergic or asthmatic phenotype is still unclear (Holgate, 1999; Hakonarson and Wjst, 2001).

**Environmental factors:** Asthma prevalence rates tend to be highest in economically developed countries with a temperate climate (Janson et al., 1997; Anonymous, 1998) and low in rural subsistence and economically developing communities and may increase with adoption of a more affluent lifestyle (Weinberg, 2000; Bjorksten, 1998). Environment is an important determinant of atopic disease. Airborne allergens and viral respiratory infections are the two most important environmental factors, which play a crucial role in the development, persistence and severity of asthma. Apart from allergen exposure, air pollution, tobacco smoke and ozone have been demonstrated to play a significant role in disease initiation and exacerbation. Suspended particles of less than 2.5 mm in diameter carry cat, dog and pollen allergens on their surface to respiratory tract. Diesel exhausts particles and tobacco smoke act as an adjuvant, upon interaction with allergens, enhance IgE production and induce allergic inflammation (Salvi et al., 1999; Lee et al., 2002). Improved hygiene, antibiotics and vaccination to viral and bacterial infections have also been related to increasing allergy prevalence particularly in developed nations (Yazdanbakhsh et al., 2002). Infection with respiratory syncytial virus, para influenza virus, rhinovirus and adenovirus has been reported to induce asthma by release various inflammatory cytokines (McCunney, 2005; Myers and Maynard 2005; Trasande and Thurston, 2005). Maternal smoking during pregnancy is associated with impaired lung growth and diminished lung function and in asthmatic children parental smoking increases symptoms and the occurrence of asthma exacerbation (Kabesch, 2006).

**The hygiene hypothesis:** Exposure in early life to infectious agents and their products can potentially limit atopic immune responses through the induction of Th1 immunity (in turn suppressing Th2 immunity), or through the induction of immune regulating mechanisms involving suppressive T lymphocytes (Holt, 2000). Improved hygiene, use of antibiotics and vaccination to viral and bacterial infections early in life have
Figure 1.9: Interplay of genetic and environmental factors in the development of allergic diseases. Source: Miescher and Vogel, 2002.
been related to increasing allergy prevalence particularly in developed nations (Lee et al., 2002; Figure 1.9). Epidemiological studies that support the ‘hygiene concept’ show association between exposures to commonly found bacteria that exist in the gut of humans, mycobacteria, measles and hepatitis A in early life and less atopy (Matricardi and Ronchetti, 2001).

ALLERGENS
The term ‘allergen’ refers specifically to non-parasitic, innocuous antigens capable of stimulating type I hypersensitive responses in atopic individuals. They are usually proteins or glycoproteins capable of inducing synthesis of IgE antibodies, thereby sensitizing the predisposed individuals. Allergens arise from a variety of sources, including pollens, mites, animals, insects, moulds, foods, etc (Huby et al., 2000). Based on the route of exposure, allergens are classified into four categories i.e. inhalants (aeroallergens), ingestants (food), contactants (cosmetics) and injectants (insect bite, stings etc).

The most common offending allergens are inhalants or aeroallergens that travel easily from one place to other along with desiccated particles in the air. In developed countries, asthma is strongly but not exclusively associated with allergic sensitization to *Dermatophagoides pteronyssinus* and other environmental allergens (Tattersfield et al., 2002). The allergens originating from the natural environment are small, soluble proteins or glycoproteins of molecular weight higher than 10 kDa (Puc, 2003). Allergens belong to several protein families and have diverse biological functions: (i) indoor allergens such as house dust mite and cockroach belong to protein families of enzymes, ligand-binding proteins or lipocalins, albumins, tropomyosins, and calcium-binding proteins; (ii) pollen allergens from grasses, trees and weeds belong to families such as pathogenesis-related proteins, calcium-binding proteins, pectate lyases, expansins and trypsin inhibitors; and (iii) plant and animal food allergens are usually lipid transfer proteins, profilins, seed storage proteins and tropomyosins (Chapman et al., 2007).

Over the last decade, many allergens have been purified / characterized, and the amino acid sequences and three-dimensional structures of allergenic proteins have been spectacularly increased in the allergen database (Brusic et al., 2003; Chapman et al., 2007). The allergens are compiled on the basis of their known biochemical and immunological properties using the systematic nomenclature of the Allergen
Nomenclature Sub-Committee of the World Health Organization and International Union of Immunological Societies (King et al., 1994). The allergens from mites, animal dander, pollens, insects, and foods have been cloned, and three-dimensional structures of > 40 allergen are submitted in the Protein Database. Analyses of the protein family database suggest that the universe of allergens comprises more than 120 distinct protein families (Chapman et al., 2007).

Pollen grains (from grasses and weeds) are amongst the earliest known allergens (Weber, 2005). They are significant cause of allergic diseases afflicting more than 25% of the atopic subjects (Suphioglu, 1998; Mohapatra et al., 2005). Pollen allergens are water-soluble proteins or glycoproteins of molecular masses from 10-70 kDa. Ragweed (*Ambrosia* spp.), Plantain (*Plantago* spp.), Mugwort (*Artemisia* spp.), Sorrel (*Rumex* spp.), Birch (*Betula* spp.), Alder (*Alnus* spp.), Hazel (*Corylus* spp.), and *Poaceae* (grasses) are some of the most prevalent pollen allergens worldwide (Puc, 2003). Insect also represents an important group of allergens. Allergy to insects usually occurs due to bites or stings of insects, such as bees, yellow jackets, hornets, wasps, mosquito, fire ants etc. Insect’s stings cause systemic reaction which sometimes can be fatal in few cases (Chiu and Kelly, 2005). Apart from these, house dust mite, cockroach represents an important source of indoor allergen sensitization and the development of allergy/asthma. High prevalence of cockroach hypersensitivity in atopic (20-55%) and asthmatic (49-60%) populations has been reported (Wu and Lee, 2005; Arruda LK, 2005). The fungal spores are present in much higher concentration than pollens allergens (Horner et al., 1995). *Alternaria, Aspergillus, Cladosporium, Curvularia, Helminthosporium, Fusarium* and *Epicoccum* are predominant aeroallergens responsible for type I and III allergies (Kurup et al., 2002; Adhikari et al., 2004; Mezzari et al., 2003; Bisht et al., 2000).

**DIAGNOSIS OF ASTHMA**

**History and Measurements of Symptoms**

Clinical diagnosis of asthma is often driven by symptoms such as breathlessness, cough, wheezing and chest tightness. Seasonal variability of symptoms and a positive family history of asthma and atopic disease are also helpful in diagnosing the disease. Physical examination, pulmonary function tests (PFT) and AHR on challenge with histamine or methacholine provide the requisite information for correct diagnosis of asthma.
Physical examination: Clinical signs such as dyspnea, airflow limitation (wheeze), and hyperinflation are likely to be present if patients are examined during symptomatic days. As asthma symptoms are variable, so the physical examination of the respiratory system may be normal during asymptomatic days. But during an exacerbation of asthma, contraction of airway smooth muscle, edema, and hypersecretion tend to close the smaller airways leading to airflow limitation. So, wheezing is the most typical physical finding in asthma, but may be absent in severe asthma exacerbations. However, patients in this state usually have other physical signs reflecting severity, such as cyanosis, drowsiness, difficulty in speaking, tachycardia, hyperinflated chest, use of accessory muscles, and intercostal recession (GINA, 1995).

Measurements of Lung Function: Patients with asthma frequently have poor recognition of their symptoms, especially if their asthma is severe and venerable. Assessment of symptoms such as dyspnea and wheezing may also be inaccurate. Measurements of lung function, particularly the reversibility of lung function abnormalities, provide a direct assessment of airflow limitation (GINA, 2006).

**Spirometry:** A wide range of different methods to assess the level of airflow limitation exists, but two methods have widespread acceptance for use in patients over 5 years of age. These are the measurement of FEV1 and its accompanying forced vital capacity (FVC), and the measurement of peak expiratory flow (PEF) as per GINA guidelines.

Reversibility is determined either by an increase in FEV1 of ≥12% from baseline or by an increase of ≥10% from predicted FEV1 after inhalation of a short-acting bronchodilator. Significant reversibility is indicated by American Thoracic Society (ATS) standards as an increase in FEV1 of >200 mL and ≥12% from the baseline measure after inhalation of a short-acting bronchodilator (e.g., albuterol, 2-4 puffs of 90 mcg/puff) (ATS, 1991; ATS, 1995).

**Airway hyperresponsiveness:** Bronchoprovocation with methacholine, histamine, cold air or exercise challenge may be useful when asthma is suspected and lung function test is normal or near normal. These measurements are sensitive for a diagnosis of asthma, but have low specificity. The most widely used method of measuring AHR relies on measuring response in terms of change in FEV1, after inhalation of increasing concentrations of histamine or methacholine (Crapo et al., 2000). The agent can be delivered by breath-activated dosimeter, via a nebulizer using tidal
breathing, or via a hand held atomizer. A positive bronchoprovocation test is
diagnostic for the presence of AHR, a characteristic feature of asthma that also can be
present in other conditions e.g., allergic rhinitis, cystic fibrosis and COPD also. Thus,
although a positive test is consistent with asthma, a negative bronchoprovocation may
be more helpful to rule out asthma.

Histamine is the most commonly used agent as it is cheap, stable in solution
and rapidly metabolizes. Histamine is required in low doses to elicit response in
asthmatics. In the dose range required to elicit response in asthmatic it is very safe
drug. However, higher dose is required to assess bronchial response in normals.
Higher doses of histamine can cause headache, cough and flushing of face (Hargreave
et al., 1981). Late reactions are very rare with histamine (Salome et al., 1980).

Measurements of Allergic Status: The presence of an allergic component in asthma or
rhinitis can be identified by skin testing with allergen extracts or measurement of
specific IgE in serum. The skin prick test (SPT) is the method of choice for
diagnosing immediate type (IgE mediated) hypersensitivity. In case of strong history
but negative SPT, intradermal (ID) test should be performed to detect the suspected
sensitization with allergen.

Skin tests are done on volar aspect of forearm or lateral aspect of upper arm
and or on the patient’s back (Nelson, 1983). In SPT, a drop of glycerinated allergen
extract is placed on the skin and pierced with a needle, allowing antigen to enter
(Dreborg, 1989; Kumari et al., 2006). Intradermal test is performed by administering
0.02 ml of allergen extract intradermally into the skin, raising a bleb of about 2 mm
(Aggarwal et al., 2000; Dhyani et al., 2006). Histamine diphosphate (5 mg/ml for SPT
or 100 µg/ml for ID) is used as a positive control. SPT wheal ≥ 3 mm of glycerinated
buffer saline or ≥ to histamine is considered as positive reaction whereas phosphate
buffered saline (PBS) is used as a negative control. ID wheal equal to the size of
positive control or more is considered positive reaction. ID test is most sensitive but
can give false positive reaction. SPT is specific but may give false negative reaction at
times (Boyd, 2003; Oppenheimer and Nelson, 2006).

In vitro tests like RAST or ELISA measure specific IgE against respective
allergens (Negrini et al., 1985; Singh et al., 2000). RAST was the first serological
assay developed to detect allergen-specific IgE in the serum (Dolen, 2001). Allergen
bound to solid phase is used to detect specific IgE in serum. Bound IgE is detected
using anti-IgE antibody (125I labeled or enzyme conjugated) by RAST or ELISA. The
amount of radioactivity or the colour reaction developed by the antigen–antibody complex corresponds to bind IgE. Over the years, IgE antibody assays have improved from the first generation qualitative assays (RAST, ELISA), through the second generation semi-quantitative IgE assays (AutoCAP, Alastat, HYTech, Matrix, MagicLite), to the present state-of-the-art quantitative “third generation” autoanalyzers (ImmunoCAP System, Immulite 2000) (Hamilton and Adkinson, 2004). It is recommended to use a combination of in vivo and in vitro tests to detect sensitization against aeroallergens.

**Biomarkers of inflammation:** The measurement of biomarkers of inflammation (e.g., total and differential cell count and mediator assays) in sputum, blood, urine, fractional exhaled nitric oxide concentration and exhaled air aids to the diagnosis and assessment of asthma and is currently being evaluated in clinical trials.

Assessment of a combination of historical features and of biomarkers may allow accurate estimation of the risk of future adverse events, but it must be kept in mind that laboratory tests only indirectly estimate control of risk. In the end, only symptoms, exacerbations, and quality of life over time are the measures of asthma control.

**CLASSIFICATION OF ASTHMA**

Asthma may be classified on the basis of etiology, severity and pattern of airflow limitation (GINA, 2002). Conventional assessments of asthma severity have combined assessment of symptoms, amount of β-2-agonist used to treat symptoms and lung function. Both the level of airflow limitation and its variability enable asthma to be subdivided by severity into four steps: Intermittent, Mild Persistent, Moderate Persistent, and Severe Persistent (GINA, 2002). This type of asthma classification, based on severity, is important when decisions must be made about management at the initial assessment of a patient.

**STEP 1: Intermittent**

- Symptoms less than once a week
- Brief exacerbations
- Nocturnal symptoms not more than twice a month
  - FEV1 or PEF ≥ 80% predicted
  - PEF or FEV1 variability < 20%
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STEP 2: Mild Persistent
Symptoms more than once a week but less than once a day
Exacerbations may affect activity and sleep
Nocturnal symptoms more than twice a month
• FEV1 or PEF ≥ 80% predicted
• PEF or FEV1 variability 20-30%

STEP 3: Moderate Persistent
Symptoms daily
Exacerbations may affect activity and sleep
Nocturnal symptoms more than once a week
Daily use of inhaled short-acting β2-agonist
• FEV1 or PEF 60-80% predicted
• PEF or FEV1 variability > 30%

STEP 4: Severe Persistent
Symptoms daily
Frequent exacerbations
Frequent nocturnal asthma symptoms
Limitation of physical activities
• FEV1 or PEF ≤ 60% predicted
• PEF or FEV1 variability > 30%

MANAGEMENT OF ASTHMA AND ALLERGIC RHINITIS

Avoidance
Asthma exacerbations may be caused by a variety of risk factors including allergens, pollutants, foods, tobacco smoke and drugs. The first line of treatment for airway disease is the avoidance of risk factors. Emphasis is given on patient education regarding the disease and its management. Both pharmacotherapy as well as immunotherapy is practiced for treatment of allergic diseases.

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

Pharmacological management

Pharmacotherapy is used to prevent and control asthma/allergic rhinitis symptoms, reduce the frequency and severity of asthma exacerbations and reverse airflow obstruction. Following are the drugs being presently used for the treatment of asthma/AR (Table 1.1).

β2-Adrenergic agonists: β2-Adrenergic drugs are the most potent and rapidly acting bronchodilators in clinical use today (Barnes, 1996a; Holgate and Polosa, 2008). Their availability in multiple forms (short, intermediate and long-acting) and delivery systems (metered-dose inhalers, nebulizer solutions, oral liquids and tablets, respirable powders) give them wide clinical flexibility. In addition to relaxing airway smooth muscle, β2-agonists enhance mucociliary clearance, decrease vascular permeability, and may modulate mediator release from mast cells (Nelson, 1995). Side effects of selective β2-agonists include tremor, tachycardia, and increased anxiety (Nelson, 1995). The long-acting β-agonists (LABA) salmeterol and formoterol induce bronchodilation for at least 12 hours and are effective against moderate to severe persistent asthma (Kips and Pauwels, 2001; Holgate and Polosa, 2008). LABA should not be used as monotherapy in patients requiring daily controller medications (Mcivor et al., 1998). However, in patients receiving inhaled corticosteroids (ICS) whose asthma is suboptimally controlled, these agents produce better overall asthma control (Woolcock et al., 1996).

Inhaled short-acting β-agonists (SABAs) such as salbutamol and turbutaline are the most effective bronchodilators currently available for the rapid relief of asthma symptoms. After binding of these agonists to the β2-adrenoceptor, adenylate cyclase is stimulated by the signal-transducing Gs protein to increase production of cyclic adenosine 3′5′-monophosphate (cAMP), thereby activating protein kinase A. This mediates smooth-muscle relaxation through the phosphorylation of myosin light-chain kinase and by opening Ca2+ dependent K+ (KCa) channels, which relieves bronchoconstriction in asthma (Holgate and Polosa, 2008).

Phosphodiesterase (PDE) inhibitors: Theophylline, a methylxanthine, is a bronchodilator that may have mild anti-inflammatory effects and activity as cAMP phosphodiesterase (PDE) inhibitor and an adenosine-receptor antagonist (Holgate and
Polosa, 2008). Sustained-release theophylline preparations and aminophylline can be used as controller medications in asthma in both children and adults (Tinkelman et al., 1993; Reed et al., 1998). Serum levels of theophylline, due to liver metabolism, may be markedly affected by a number of variables including age, diet, disease states and drug interactions, all of which contribute to the complexity of using this medication (Weinberger and Hendeles, 1996). In addition, theophylline may produce a number of dose-related side effects. Gastrointestinal symptoms may be intolerable to some patients, even well within the usual therapeutic drug levels. At higher doses (10 mg/kg body weight/day or more), theophylline has the potential for significant adverse effects (Lipworth, 2005).

In addition to theophylline, an oral PDE4 inhibitor, roflumilast, has an inhibitory effect on allergen induced responses in asthma and also reduces symptoms and lung function. However its use is limited due to number of side-effects like nausea, vomiting, headaches and gastrointestinal discomfort (Lipworth, 2005).

**Cromolyn and nedocromil:** Cromolyn sodium and nedocromil sodium are two structurally different anti-inflammatory medications for the treatment of chronic asthma. They are rapidly absorbed from the lungs and are safe. Both medications have been shown to inhibit inflammatory cell activation and mediator release and reduce AHR (Hoag and McFadden, 1991; Schwartz et al., 1996). The mechanism of action of these agents may be related to their effects on airway epithelial chloride channels (Alton et al., 1996) or their effects on local neuronal reflexes. Both these drugs can be modestly effective prophylactically in the attenuation of exercise-induced bronchospasm (Kelly et al., 2000), but less than β2-agonists.

**Leukotriene antagonist:** Leukotrienes are biologically active fatty acids derived from the oxidative metabolism of arachidonic acid, an integral part of the cell membrane. The actions of leukotrienes can be prevented by inhibition of cysteinyl leukotriene synthesis [5-lipoxygenase inhibitors (zileuton)] or antagonists or leukotriene receptors (zaflurilukast and montelukast) (Horwitz et al., 1998; Drazen et al., 1999). The receptor antagonists have been shown to inhibit exercise-induced bronchospasm and to improve airway function in patients with chronic asthma. In patients with asthma, leukotriene receptor antagonists can improve airflow obstruction between 8% and 13%, reduce the need for β-agonists and reduce asthma exacerbations. In head-to-head trials with inhaled corticosteroids, the leukotriene receptor antagonists are less effective in terms of improvement in lung function and reduction in exacerbations.
Churg-Strauss syndrome has occurred in association with use of the leukotriene antagonist (Health advisory for new asthma drug, 1998).

**Humanised monoclonal antibodies:** Clinical studies of humanized monoclonal antibodies to IgE, CD4+ cells and IL-4 and IL-5 are underway. Omalizumab (a recombinant humanized monoclonal antibody); antibody against IgE binds to circulating IgE, inhibiting its interaction with FceRI receptors and decreasing the number of FceRI receptors on basophils (Prussin et al., 2003). This has been shown to give benefit in allergic rhinitis patients (Casale et al., 2006). A recent evaluation by the Cochrane group found that omalizumab led to a significant reduction in inhaled steroid consumption compared to placebo (Walker et al., 2006). Omalizumab has a low incidence of side effects but it is quite expensive and requires administration at frequent intervals (Casale et al., 2006).

IL-4 has also been targeted by a soluble form of the IL-4 receptor α chain (sIL-4R α), which binds to and inactivates IL-4. A study reported partial efficacy of IL-4 inhibition by sIL-4R α in adult asthmatics (Borish et al., 2001). Patients with mild or severe asthma treated with 2 closely related neutralizing anti-IL-5 antibodies showed no clinical improvement, despite marked suppression of blood eosinophilia (Leckie et al., 2000; Kips et al., 2003). Another cytokine that is currently targeted in asthma is the TNF-α, which seems to have a role in severe asthma. Etanercept, a soluble receptor that blocks TNF-α, has some efficacy in patients with refractory asthma who are not responsive to conventional therapies (Berry et al., 2006), but another anti-TNF-α therapy (infliximab) is less effective in less-severe asthmatics than in severe asthmatics (Erin et al., 2006).

**Glucocorticoids:** Glucocorticosteroids are the most potent anti-inflammatory agents available for the treatment of asthma (Barnes, 1996b). Their efficacy is related to many factors including the decrease in inflammatory cell function and activation, stabilization of vascular leakage, decrease in mucus production and an increase in β-adrenergic response. Glucocorticoids produce their effect on various cells by binding to intracellular glucocorticoids receptors (GR), which go on to regulate transcription of certain target genes. Steroid-bound GR form dimers that bind to DNA glucocorticoid response elements (GREs), resulting in increased transcription, an increase in mRNA, and increased synthesis of proteins (Rhen and Cidlowski, 2005).
However, in asthma, it is more likely that control of inflammation comes from repression of gene transcription.

Inhaled corticosteroids (ICS) are recommended as first-line treatment for asthma/allergic rhinitis. Several clinical studies have shown that adding a LABA to ICS leads to better symptomatic asthma control and lower exacerbation frequencies than increasing the dose of inhaled steroids (Shrewsbury et al., 2000). Because of the increased effectiveness of this add-on therapy with respect to symptomatic asthma control, it has been suggested that adding a LABA to ICS exerts an additional effect on bronchial inflammation, complementary to the effect of ICS (Taylor and Hancox, 2000).

Inhaled corticosteroids have the potential for producing systemic side effects that are dependent on the dose, potency, its bioavailability, absorption in the gut, first-pass metabolism in the liver, and the half-life of its systemically absorbed fraction (Rhen and Cidlowski, 2005). Although ICS, when used in recommended doses, have minimal adverse effects with the exception of oral candidiasis when oral hygiene is suboptimal. However, concern has been raised that the use of these agents in children may be associated with growth suppression (Allen, 2002). The dose and type of medication may be influenced by age (delivery systems, side effects), cost and the familiarity of the clinician with the nuances of the various available products. It should be emphasized that the abrupt discontinuation of ICS is an important cause of asthma exacerbations (Guilbert et al., 2006).

Intranasal corticosteroids are the most effective medication for treatment of allergic rhinitis and are particularly useful for severe allergic rhinitis (Bousquet et al., 2001). During use for long duration, it is associated with some side effects such as epistaxis. A delay in the attainment of normal height has been reported in children using intranasal beclomethasone (Beswick et al., 1985; Lipworth 1999; Juniper et al., 2005). Oral corticosteroids such as prednisone, methylprednisolone etc are used in brief courses (e.g., prednisone 30 mg/QD for 3-7 days for adults) for the treatment of very severe nasal symptoms in rhinitis patients, but it is associated with HPA axis suppression and long-term corticosteroid side effects (Bousquet et al., 2001). Leukotriene receptor antagonists help in relieving nasal symptoms of allergic rhinitis but it is quite weak as monotherapy (Meltzer et al., 2000; Bousquet et al., 2001).

In addition to the local adverse effects associated with ICS therapy, such as oral candidiasis, clinically important systemic adverse effects may arise with the use
of these agents, even at licensed doses. Such effects include adrenal suppression, osteoporosis or changes in bone mineral density, growth retardation in children, cataracts and glaucoma (CSM/MCA, 1998). It is evident that for those drugs with a high degree of first-pass metabolism (e.g. Fluticasone and budesonide), such systemic effects are caused by absorption of the drug from lung tissue, rather than the 60% or more (with a pressurised metered dose inhaler) which is swallowed and absorbed from the gastrointestinal tract (Lipworth, 1999). This has led to a concerted effort to find safer corticosteroids that have reduced oral bioavailability, are less absorbed by the lungs or inactivated in the circulation. Ciclesonide, a newly introduced steroid, is a pro-drug that becomes activated (desciclesonide) by the action of esterases in the lungs. This corticosteroid seems to have less systemic effects than currently available corticosteroids might be due to long-term retention in the lung, no oral-bioavailability and high degree of binding with circulating proteins (Barnes, 1996b; Cohen et al., 2008).

Medications should be selected in consideration of the individual patient’s symptoms, type and severity of disease. The anti-histamines are the main line treatment in allergic rhinitis but have little role in treating non-allergic rhinitis syndromes. Anti-histamines reduce sneezing, rhinorrhea, and nasal and ocular pruritus associated with allergic rhinitis, but has less effect on nasal congestion (Bousquet et al., 2001; Dykewicz, 2003). But all patients not respond and the drugs are required to be given for months to control the symptoms. The most common side effect of anti-histamines is sedation or drowsiness. In persons who experience drowsiness, the sedation effect usually lessens over time, but there could still be performance impairment. Another often encountered side effect is excessive dryness of the mouth, nose and eyes. Less common side effects include restlessness, nervousness, over excitability, insomnia, dizziness, headaches, euphoria, fainting, visual disturbances, decreased appetite, nausea, vomiting, abdominal distress, constipation, diarrhea, increased or decreased urination, high or low blood pressure, nightmares (especially in children), sore throat, unusual bleeding or bruising, chest tightness or palpitations.

Decongestants help relieve the stuffiness and pressure caused by swollen nasal tissue. They do not relieve the other symptoms of allergic rhinitis, such as runny nose, post-nasal drip and sneezing. Decongestants are available as prescription and non-prescription medications and are often given in combination with antihistamines or other medications. It is not uncommon for patients using decongestants to experience
insomnia if taking the medication in the afternoon or evening. At times, men with prostate enlargement may encounter urinary problems while on decongestants. Unlike decongestant nose sprays, a saline nose spray can be used as often as needed.

ASTHMA: A METABOLIC DISORDER

Asthma is a serious health problem worldwide. The social burden and cost to public and private health care system due to this disease are substantial. It is estimated that about 70-80% asthmatics are of atopic origin. Earlier, the increased serum levels of lysophosphatidyl choline (LPC) was observed associated with a high ratio of esterified to free-cholesterol in asthma (Agarwal and Nath, 1978). The observations at Mayo clinic have consistently shown significantly higher levels of LPC in asthmatics. This suggested increased activity of the cholesterol-esterifying enzyme-lecithin cholesterol acyltransferase (Agarwal et al., 1985). It was observed that during hibernation, myocardium shows β to α-adrenergic shift (Kunos and Szentivanyim 1968; Kunos et al., 1973) as well as a 3.5 fold increase in lysophospholipids (Aloia et al., 1974). An exposure to NO2 leads to increase airway reactivity in asthmatics, as well as increased tissue levels of lysophospholipids in guinea pig lungs (Trzeciak et al., 1977). An increase in the leucocyte phospholipase A2 activity and plasma LPC levels was reported in asthma and rhinitis (Mehta et al., 1990). Thus LPC plays an important role in the pathogenesis of asthma by producing β-adrenergic sub sensitivity, α-adrenergic over reactivity and airway hyperreactivity (Agarwal et al., 1986). Asthma was thus perceived of as a metabolic disorder (Agarwal, 1979).

Presently, steroids and disodium cromoglycate are the drugs used as an anti-inflammatory agent for treatment of bronchial asthma. Bronchodilators like β-agonists, anti-cholinergic and xanthines are used for symptomatic relief. Leukotriene modifiers, a new class of anti-asthma drugs and second-generation anti-histamines are used with some inhibitory effects on allergic responses. Controlled clinical trials have demonstrated that long term treatment with high doses of ICS may be associated with systemic effects, including skin thinning, easy bruising, adrenal suppression, and decreased bone mineral density (Lipworth, 1999). At higher doses (10 mg/kg body weight/day or more), theophylline intoxication can occur involving multiple organs of the body. Further, theophylline intoxication in children and adults can result in seizures, arrhythmias and even death. Leukotriene modifiers have been
associated with liver toxicity, and there are reports of Churg-Strauss syndrome
associated with leukotriene modifier therapy (Health advisory for new asthma drug,
1998). The side effect of some second-generation antihistamines is sedation in the
initial treatment period. Further, the action of these drugs is short lived and once the
drugs are withdrawn, symptoms come back quickly because the underlying bronchial
inflammation recurs. Therefore, new drugs are required which can control immune
inflammation for a prolong period with no or minimum side effects. Such drugs can
have a prophylactic role in asthma management.

CHOLINE

Choline is a lipotropic agent, involved in maintaining cell structure and facilitates the
movement of fats in and out of the cells (Blusztajn, 1998). It is widely distributed in
foods, principally in the form of phosphatidylcholine (PC) and also as free choline. It
is also present in foods in the form of phospholipid sphingomyelin. Choline is the
major constituent of PC, present in soybean, liver, oatmeal, cabbage and cauliflower.

Choline, a dietary component is important for the structural integrity of cell
membranes, methyl metabolism, cholinergic neuro-transmission, transmembrane
signaling, lipid and cholesterol transport and metabolism. Its deficiency results in loss
of membrane PC and induction of apoptosis in PC12 cells in vitro (Yen et al., 1999).
Choline is used as an appetizer for years as sorbilene and trichodol solution. Choline a
precursor to acetylcholine and CDP-choline elicits a variety of useful pharmacological
effects in many diseases, including stroke, dementia, Alzheimer’s and Parkinson’s
disease. Previous studies with choline showed anti-anaphylactic activity in animals
(Smith, 1961). Choline magnesium trisalicylate had been used for treatment of asthma
with aspirin hypersensitivity (Szczechlik et al., 1990). Studies with tricholine citrate in
asthmatics showed beneficial effects in improving symptoms and airway
hyperresponsiveness (Gupta and Gaur 1997; Gaur et al., 1997). However the molecule
needs further evaluation as an anti-inflammatory agent with advanced methods of
drug screening. The present study has been aimed to achieve the following objectives:
OBJECTIVES:

1. To evaluate the anti-inflammatory effect of choline in animal model.

2. To study the toxicity of choline in mouse model.

3. To evaluate the anti-oxidant activity of choline in mouse model of allergic airway inflammation.

4. To investigate anti-inflammatory activity of choline chloride in bronchial asthma patients.
<table>
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<th>DRUGS</th>
<th>EXAMPLE</th>
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| **β-adrenergic agonists**         | Epinephrine, Isoproterenol, Albuterol | • ↑ c-AMP  
• Inhibit mast cell degranulation | ➢ Do not affect eosinophil infiltration, thus bronchitis keeps progressing  
➢ Drug requirement keeps rising                                          |
| Methylxanthines                   | Theophylline              | • Blocks phosphodiesterase  
• ↑ c-AMP persistently                  | -                                                                           |
| Anti-cholinergic agents           |                          |                                         |                                                                               |
| Corticosteroids                   |                          | • Inhibition of transcription factors AP-1, NF-κB | High dose may cause systemic side effects                                    |
| Mediator antagonist               | Loratidine               | -                                       | Inhibitors of single mediator may not have major clinical benefit             |
| - Antihistamines                  | Cetrizine                |                                         |                                                                               |
| - Anti-Leukotrienes               | Montelukast              |                                         |                                                                               |
| Specific anti-allergic drugs      | Sodium cromoglycate      | • Phosphorylates cytoskeletal protein ‘moesin’ | Only prophylactic use                                                        |
| Cromones                          |                          |                                         |                                                                               |
| Inhibition of APCs & Th2 lymphocytes | Anti-CD28         | • Inhibit co-stimulatory molecules  
• Inhibits T-cells                 | -                                                                           |
|                                   | Anti-B7-2                |                                         |                                                                               |
|                                   | Cyclosporine-A           |                                         |                                                                               |
| Anti-IgE                          | Omalizumab               |                                         |                                                                               |
| **OTHER POTENTIAL DRUGS**         |                          |                                         |                                                                               |
| Cytokine modulators               | Anti-IL4, Anti-IL5, Anti-IL13, Anti-TNF |                                         |                                                                               |