2. REVIEW OF LITERATURE

Bronchial asthma is a common disease of children and adults. Although the clinical manifestations of asthma have been known since antiquity, it is a disease that still defies precise definition.

Asthma is a chronic inflammatory disease of the airways characterized by fibrosis of the airways, hyperplasia and hypertrophy of smooth muscle cells and mucous secreting cells due to infiltration of activated eosinophils and activation of resident mast cells and lymphocytes. These chronic inflammatory changes are mediated by secretion of cytokines from inflammatory cells. Cytokines play an integral role in the co-ordination and persistence of inflammatory processes in chronic inflammation of the airways.

Since then the word Asthma was not only used to describe any disorder with episodic shortness of breath but also dyspnea. Now the terms bronchial and cardiac asthma etc have been used to delineate the etiologies of dyspnea. Asthma can be defined as a lung disease characterized by

- Airway obstruction that is reversible either by treatment or spontaneously
- Airway inflammation
- Increased airway responsiveness to a variety of stimuli

This disease has its root in infancy, and both genetic factors such as atopy and environmental factors such as viruses, allergens and occupational exposures contribute to its inception and evolution.

The natural history of Asthma is not well defined. Most of the studies are retrospective and either clinical or hospital based. Between 30 and 70% of children with Asthma will markedly improve or become symptom free by early adulthood; chronic disease persists in about 30% of patients. Although asthmatic patients who develop the disease in childhood are more likely to have remissions, ongoing airway obstruction may persist undetected in asthmatic children who are clinically well if objective measures are not made. Approximately 60% of patients who are symptom free as adults continue to exhibit bronchial hyperactivity to inhale histamine challenges. In general, subjects with less frequent attacks and normal pulmonary functions on initial assessment have higher remission rates. Most deaths from Asthma occur outside of the hospital and death is rare after hospitalization. The most common cause of death from Asthma is inadequate assessment of severity of airway obstruction by the patient or physician and
inadequate therapy. Thus the key to prevent death from Asthma is education, which includes education of patients as well as the clinicians caring for them.

2.1. BRONCHIAL HYPERREACTIVITY

Hyperreactivity of the airways to physical, chemical and pharmacological stimuli is the hallmark of asthma. Bronchial hyperreactivity also occur in some patients with chronic bronchitis and allergic rhinitis. Normal healthy subjects may also develop an increased bronchial reactivity after viral respiratory infections. The degree of reactivity is quantitatively greater in asthmatic patients than in other groups who demonstrate hyperreactivity. The degree of bronchial hyperreactivity with asthmatics correlates with the clinical course of their disease and medication requirement necessary to control symptoms.

Much of the necessary research on the pathogenesis of asthma has focussed on explaining airway hyperreactivity. Increased bronchial responsiveness seen in asthma is at least in part due to an inflammatory response within the airway. The intact lungs of patients at autopsy are hyperinflated because of air plugging. The histological examination is characterized by three findings:-

- Marked hypertrophy and hyperplasia of airway smooth muscle.
- Increased airway wall thickness caused by an exudative inflammatory reaction and edema.
- Mucous gland hypertrophy and mucus hypersecretion

2.2. HISTOLOGICAL CHANGES IN THE LINING OF AIRWAYS

Histological studies performed on patients with mild intermittent to moderate chronic asthma have shown marked inflammatory changes within the airway along with extensive epithelial damage. Similar but more severe changes have also been seen in patients who have died from acute asthma attacks. The correlation between the degree of epithelial denudation and airway reactivity suggests that patients with most reactive airways have the least amount of normal bronchial epithelium. Subepithelium fibrosis has also been described in bronchi of patients with mild asthma.
2.3. IMMUNOHISTOPATHOLOGY OF ASTHMA

Evidence that inflammation was a component of asthma was initially derived from findings at autopsy in patients with fatal asthma. Their airways showed infiltration by neutrophils and eosinophils, degranulated mast cells, sub-basement-membrane thickening, loss of epithelial-cell integrity, and occlusion of the bronchial lumen by mucus. Hyperplasia and hypertrophy of bronchial smooth muscle and hyperplasia of goblet cells were also present. More recent studies have found substantial inflammation in bronchial-biopsy specimens from patients with asthma. These inflammatory changes can occur throughout the central and peripheral airways and often vary with the severity of the disease although not observed uniformly, denudation of the airway epithelium, deposition of collagen beneath the basement membrane, mast-cell degranulation, and infiltration of the airway by lymphocytes and eosinophils have been found in patients with mild-to-moderate asthma (Figure R1). Many of the cells in the airway appear to be activated, implying that by releasing preformed or newly synthesized mediators, they have a direct role in asthma.

Further evidence of an inflammatory response in asthma is the presence of cytokines that mediate inflammation and chemotactic chemokines in bronchoalveolar lavage fluid or pulmonary secretions. Since these cytokines and chemokines are elaborated by resident and inflammatory cells in airways and have many effects on these cells, a variety of autocrine, paracrine, and endocrine networks could participate in asthma (Table R1). Some cytokines initiate inflammatory responses by activating transcription factors, which are proteins that bind to the promoter region of genes.

Transcription factors involved in asthmatic inflammation include nuclear factorκ B, activator protein-1, nuclear factor of activated T cells, cyclic AMP responseelement binding protein, and various members of the family of signal transduction-activated transcription (STAT) factors. These transcription factors act on genes that encode inflammatory cytokines, chemokines, adhesion molecules, and other proteins that induce and perpetuate inflammation. Corticosteroids modulate immunoinflammatory responses in asthma by inhibiting these transcription factors. The ability of cytokines to induce the expression of adhesion molecules such as intercellular adhesion molecule, vascular-cell adhesion molecule, and endothelial-leukocyte adhesion molecule provides a mechanism for the adhesion of inflammatory cells to the endothelium and the migration of these cells from the circulation into the lamina propria, the epithelium, and in many cases, the airway lumen itself.
Figure R1: Specimen of Bronchial Mucosa from a Subject without Asthma (Panel A) and a Patient with Mild Asthma (Panel B) (Hematoxylin and Eosin, x 40). In the subject without asthma, the epithelium is intact; there is no thickening of the sub-basement membrane, and there is no cellular infiltrate. In contrast, in the patient with mild asthma, there is evidence of goblet-cell hyperplasia in the epithelial-cell lining. The sub-basement membrane is thickened, with collagen deposition in the submucosal area, and there is a cellular infiltrate.

Table R1: CYTOKINES THAT MAY HAVE A ROLE IN THE PATHOGENESIS OF ASTHMA
<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Primary Sources</th>
<th>Primary Targets</th>
<th>Effects or Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic fibroblast growth factor</td>
<td>Endothelial cells</td>
<td>Fibroblasts, matrix</td>
<td>Production of fibroblasts, matrix formation</td>
</tr>
<tr>
<td>Granulocyte colony stimulating factor</td>
<td>Macrophages, epithelial cells</td>
<td>Neutrophil precursors, monocytes, macrophages</td>
<td>Maturation and differentiation of target cells</td>
</tr>
<tr>
<td>Granulocyte-macrophage colony-stimulating factor</td>
<td>Monocytes, macrophages; activated macrophages and T cells</td>
<td>Eosinophils, neutrophils, macrophages</td>
<td>Proliferation, differentiation, activation, and prolonged survival of target cells; enhanced cytokine production; degradation of eosinophils</td>
</tr>
<tr>
<td>Interferon-α</td>
<td>Monocytes, macrophages</td>
<td>Virus-infected cells</td>
<td>Inhibition of viral replication</td>
</tr>
<tr>
<td>Interferon-β</td>
<td>Monocytes, macrophages</td>
<td>Virus-infected cells</td>
<td>Inhibition of viral replication</td>
</tr>
<tr>
<td>Interferon-γ</td>
<td>Monocytes, macrophages</td>
<td>Macrophages; activated macrophages; leading to the expression of Fcγ receptors, MHC class I and II molecules, nitric oxide synthase, interleukin-1, tumor necrosis factor</td>
<td>Shift in cytokine profile from Th2 type to Th1 type; increased expression of interleukin-2 receptors; increased cytokoty of CD8+ T cells; activation of natural killer cells; production of cytokines; cellular cytotoxicity; production of cytokines; differentiation of B cells; proliferation of B cells; production of immunoglobulin</td>
</tr>
<tr>
<td>Interleukin-1</td>
<td>Monocytes, macrophages</td>
<td>CD4+ T cells</td>
<td>Inhibition of proliferation</td>
</tr>
<tr>
<td>Interleukin-2</td>
<td>CD4+ T cells</td>
<td>T cells</td>
<td>Proliferation and differentiation of target cells</td>
</tr>
<tr>
<td>Interleukin-3</td>
<td>T cells</td>
<td>CD8+ T cells</td>
<td>Growth and activation of B cells; production of MHC class II molecules, interleukin-6, tumor necrosis factor, CD8+; class switching to IgE; enhancement of IgE, IgG1, and IgG4 and inhibition of IgM, IgG2, and IgG3 production</td>
</tr>
<tr>
<td>Interleukin-4</td>
<td>CD4+ Th2 cells</td>
<td>Hematopoietic stem cells</td>
<td>Maturation of B cells into plasma cells; class switching to IgG1 and IgA; inhibition of lipopolysaccharide; production of interleukin-1 and tumor necrosis factor α</td>
</tr>
<tr>
<td>Interleukin-5</td>
<td>CD4+ T cells, CD8+ T cells</td>
<td>Eosinophils</td>
<td>Proliferation, chemotaxis, adhesion, activation, enhanced survival, and degranulation of eosinophils</td>
</tr>
<tr>
<td>Interleukin-6</td>
<td>Monocytes, macrophages</td>
<td>B cells</td>
<td>Proliferation of activated T cells</td>
</tr>
<tr>
<td>Interleukin-7</td>
<td>Bone marrow stromal cells</td>
<td>Pre-B cells</td>
<td>Directed migration of neutrophils to endothelium but inhibition of adhesion of these cells</td>
</tr>
<tr>
<td>Interleukin-8</td>
<td>Macrophages</td>
<td>Neutrophils</td>
<td>Direct migration of neutrophils to endothelium but inhibition of adhesion of these cells</td>
</tr>
<tr>
<td>Interleukin-9</td>
<td>CD4+ T cells (especially Th2)</td>
<td>B cells</td>
<td>Enhancement of response to interleukin-4</td>
</tr>
<tr>
<td>Interleukin-10</td>
<td>CD4+ Th1 cells, Th2 cells, Th2 cells, CD4+ T cells</td>
<td>Monocytes, Macrophages</td>
<td>Inhibition of the expression of MHC class II molecules and adhesion molecules; inhibition of interferon-γ and tumor necrosis factor production, resulting in switching of T-cell differentiation from Th1 to Th2; inhibition of interleukin-4 and interferon-γ by Th2 cells</td>
</tr>
<tr>
<td>Interleukin-11</td>
<td>Bone marrow stromal cells</td>
<td>B cells and plasma cells</td>
<td>Similar to those of interleukin-6</td>
</tr>
<tr>
<td>Interleukin-12</td>
<td>Monocytes, macrophages</td>
<td>Natural killer cells</td>
<td>Production and proliferation of interleukin-2; production of interleukin-γ and tumor necrosis factor α</td>
</tr>
<tr>
<td>Interleukin-13</td>
<td>CD4+ Th2 cells</td>
<td>Th0 cells</td>
<td>Production of interferon-γ and tumor necrosis factor α</td>
</tr>
<tr>
<td>Interleukin-14</td>
<td>Activated T cells</td>
<td>B cells</td>
<td>Similar to those of interleukin-4</td>
</tr>
<tr>
<td>Interleukin-15</td>
<td>Monocytes, macrophages</td>
<td>T cells, natural killer cells</td>
<td>Proliferation and increased cytoxicity of target cells; expression of intercellular adhesion molecule 3</td>
</tr>
<tr>
<td>Interleukin-16</td>
<td>CD8+ T cells</td>
<td>CD4+ T cells</td>
<td>Chemotaxis, growth factor</td>
</tr>
<tr>
<td>Interleukin-17</td>
<td>CD8+ memory cells</td>
<td>CD4+ T cells</td>
<td>Proliferation and activation of autocrine factors</td>
</tr>
<tr>
<td>Interleukin-18</td>
<td>Macrophages</td>
<td>Activated B cells</td>
<td>Similar to those of interleukin-12, inhibition of IgE production by increasing interferon-γ</td>
</tr>
<tr>
<td>Macrophage colony-stimulating factor</td>
<td>Monocytes, fibroblasts, epithelial cells</td>
<td>Eosinophils, neutrophils, macrophages</td>
<td>Differentiation of monocytes</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>Alpha granules of platelets, monocytes, macrophages</td>
<td>Fibroblasts and smooth muscle cells</td>
<td>Proliferation of target cells; chemotaxant for fibroblasts; active in wound healing, angiogenesis, and tissue remodeling</td>
</tr>
<tr>
<td>Stem-cell factor (also called c-kit ligand)</td>
<td>Bone marrow stroma, fibroblasts</td>
<td>Mast cells</td>
<td>Chemotaxis; along with interleukin-3, stimulation of growth; induction of histamine release</td>
</tr>
</tbody>
</table>

*Th1 denotes type 1 helper T, MHC major histocompatibility complex, Th2 type 2 helper T, and Th0 precursor of Th1 and Th2.
2.4. INFLAMMATORY MEDIATORS

Asthma is a highly complex disease (Figure R2) that involves many inflammatory cells, mediators and inflammatory proteins, and therefore treatments that target a single cell or mediator are unlikely to be effective.

Figure R2: The pathophysiology of asthma. Several inflammatory cells are recruited and/or activated in the airways, releasing a variety of inflammatory mediators that have acute effects on the airway (such as bronchoconstriction, plasma leakage, vasodilatation, mucus secretion, sensory nerve activation and cholinergic reflex-induced bronchoconstriction), together with structural changes (remodelling) that include subepithelial fibrosis, increased numbers of blood vessels and mucus-secreting cells, and increased thickness of airway smooth muscle as a result of hyperplasia and hypertrophy.

The inflammatory reaction appears to be the key mechanism to explain the pathological changes seen in asthma. In addition inflammation of the airways and the release of mediators of inflammation appear to be necessary for the development of bronchial hyperactivity.\textsuperscript{13}
Inflammation can also account for mucus hyper-secretion. Therefore recent research on the pathogenesis of bronchial hyperactivity has focused on inflammation and the mediators of inflammatory process. Inflammatory process involves large number of mediators like:

- **Inflammatory cells**
- Mast cells
- Eosinophils
- Alveolar macrophages
- T-lymphocytes
- Neutrophils
- Preformed mediators
- Membrane derived lipid mediators
- Prostaglandins
- Thromboxanes
- Leukotrienes
- Platelet activation factor and Adhesion molecule

**Inflammatory cells**

Numerous types of leukocytes are present in the circulation, lung tissues and lumen of airways. The involvement of mast cells, eosinophils, neutrophils, alveolar macrophages, and lymphocytes within the airways and surrounding tissues are important in asthma. The contribution of these cell types to asthma is important in terms of pathogenesis and for understanding how therapy may alter cellular presence and function.

**Mast cells**

Mast cells degranulation is important in the initiation of immediate responses following exposures to allergens. Mast cells are found throughout the walls of respiratory tract, and increased number of these cells has been described in the airways of asthmatics with an allergic component. Once binding of allergen to cell bound IgE occurs, mediators such as neutrophil chemotactic factors, Leukotrienes C4, D4, and E4, prostaglandin, platelet activation factor, and others are released from mast cells.

There are at least two subpopulation of mast cells; mast cell with tryptase and mast cell with both tryptase and chymase activity. Although the role of these enzymes is not fully defined,
inhibitors of tryptase have been shown to modulate the response of the airway to allergen.\textsuperscript{52} Mast cells also contain proteglycans with diverse biological properties or functions, ranging from being supporting structures for various proteins to exerting effects on the differentiation and proliferation of cells, the adhesion and motility of cells and tissue morphogenesis. Mast cells produce various cytokines including IL-1, IL-2, IL-3, IL-4, IL-5, GM-CSF, Interferon-\(\gamma\), and TNF-\(\alpha\).\textsuperscript{53} The potential for the extra cellular release of these cytokines raises the possibility that mast cells contribute to both acute and chronic allergic inflammation.\textsuperscript{54}

**Eosinophils**

Eosinophils have long been linked to asthma, primarily due to association between asthma and peripheral blood eosinophils. The degree of bronchial activity has been related to number of eosinophils in peripheral blood and bronchoalveolar lavage fluid (BAL fluid). Major basic proteins (MBP), a constituent of granules present in the eosinophils, is responsible for damage to airway epithelium and has been found in high quantities in the sputum of patients with asthma.\textsuperscript{15}

Eosinophilopoiesis begins in the bone marrow and is regulated by IL-3, IL-5 and GM-CSF; IL-5 induces terminal differentiation of immature eosinophils.\textsuperscript{55} The mature eosinophil has dense intracellular granules that are source of inflammatory proteins, including major basic protein, eosinophil-derived neurotoxin, peroxidase and cationic protein. Major basic protein, in particular, can directly damage airway epithelium, intensify bronchial responsiveness, and cause degranulation of basophils and mast cells. These effects increase the severity of asthma.\textsuperscript{56}

A number of cytokines regulate the function of eosinophils and other cells in asthma. IL-5 stimulates the release of eosinophils into the circulation and prolongs their survival. Challenge of the airway with allergens increase the local concentration of IL-5, which correlates directly with the degree of eosinophilia.\textsuperscript{57} In mice lacking the gene for IL-5, eosinophilia does not occur after challenge by the allergen. Direct administration of IL-5 to the airway in human causes mucosal eosinophilia and an increase in the bronchial responsiveness.\textsuperscript{58} (Figure R3)

To participate in the allergic inflammatory response, the eosinophil must migrate from the circulation to the airway.\textsuperscript{59, 60} The first step in this process is the phenomenon of cell rolling, which is mediated by P-selection on the surface of eosinophils.\textsuperscript{59, 60}
Inhaled antigen activates mast cells and Th2 cells in the airway. They in turn induce the production of mediators of inflammation (such as histamine and leukotrienes) and cytokines including interleukin-4 and interleukin-5. Interleukin-5 travels to the bone marrow and causes terminal differentiation of eosinophils. Circulating eosinophils enters the area of allergic inflammation and begin migrating to the lung by rolling, through interactions with selectins, and eventually adhering to endothelium through the binding of integrins to members of the immunoglobulin superfamily of adhesion proteins: vascular-cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1). As the eosinophils enter the matrix of the airway through the influence of various chemokines and cytokines, their survival is prolonged by interleukin-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF). On activation, eosinophil releases inflammatory mediators such as leukotrienes and granule proteins to injure airway tissues. In addition, eosinophils can generate granulocyte-macrophage colony-
stimulating factor to prolong and potentiate their survival and contribution to persistent airway inflammation. MCP-1 denotes monocyte chemotactic protein, and MIP-1α macrophage inflammatory protein.

**Alveolar macrophages**

The role of alveolar macrophages in the pathogenesis of asthma is becoming clearer. The primary function of these cells in the normal airway is to act as “Scavengers”, engulfing and digesting bacteria and other foreign materials. They are found in large and small airways, ideally located for affecting the asthmatic response. A number of mediators produced and released by macrophages have been identified and their roles in initiating and amplifying inflammation in allergic asthma have been determined.

**T-lymphocytes**

Much emphasis has been placed on the role of T-Lymphocytes in the pathogenesis of asthma, specifically the regulation of inflammation. Several studies have demonstrated increased number of T-Lymphocytes in BAL fluid and airway biopsy specimens of asthmatics even in mild and diagnosed cases. In addition, the presence of T-lymphocytes has been correlated to bronchial hyper responsiveness. The Th2 subset of T-Lymphocytes has received particular attention due to the profile of cytokines produced and released by these cells, which include IL-4, IL-5, IL-6 and IL-10. Conversely Th1 cells secrete IL-2, IFN-γ and TNF-α, with both Th1 and Th2 like cells in the pathogenesis of allergic inflammation includes their requirement for the importance of Th2 like cells expressing mRNA for IL-4 and IL-5 in the airways of atopic asthmatics.

**Neutrophils**

The role of neutrophils is the pathogenesis of asthma remains somewhat unclear, because they would normally be present in the airways and usually do not infiltrate tissue showing chronic allergic inflammation despite the potential to participate in late phase inflammatory reactions. However, the importance of this cell was highlighted by a recent finding of high numbers of neutrophils present in the airways of patient who dies from sudden onset fatal asthma. This suggests that neutrophils may play a vital role in the disease process, with perhaps a lesser role in the chronic inflammation of asthma. Neutrophils can also be a source of a variety of mediators like platelet activation factors (PAF), prostaglandins and Leukotrienes contribute to bronchial hyper responsiveness and airway inflammation.
Performed mediators

Histamine, associated with asthma for many years is capable of inducing smooth muscle constriction, bronchospasm and is thought to play a role in mucosal edema and mucus secretion. Lung mast cells are an important source of histamine. The release of histamine can be stimulated by exposure of airway to a variety of factors including physical stimuli and exposures to relevant allergens.\textsuperscript{64, 14}

Membrane derived lipid mediators

Chemical substances known as phospholipids are found in rich supplies in the membranes of most cells involved with inflammation. Several classes of important mediators, including arachidonic acid and its metabolites, prostaglandins, Leukotrienes and platelet activation factor are derived from this membrane phospholipids.\textsuperscript{64, 14}

Prostaglandins

Once arachidonic acid is released it can be broken down by the enzyme cyclooxygenase (COX) to form the prostaglandins. A further breakdown product, prostaglandin D\textsubscript{2} can produce sustained effects on the airway function or inflammation; however, its role in asthma remains to be determined. Similarly, prostaglandin F\textsubscript{2} is a potent bronchoconstrictor in patients with asthma and can enhance the effects of histamine. It is not clear whether this substance has any other pathophysiological effects and its specific origin from within the lung is also unknown. Another cyclooxygenase (COX) product, prostacyclin is known to produce in the lung. It is unclear whether prostaglandin I\textsubscript{2} is important as a bronchoconstricting agent in humans; it may contribute to inflammation and edema due to its effects as a vasodilator.\textsuperscript{64, 13}

Thromboxanes

The cyclooxygenase (COX) products known as Thromboxanes have received increasing attention. Of these, Thromboxane A\textsubscript{2} is the best understood. Thromboxane A\textsubscript{2} is produced by alveolar macrophages, fibroblasts, epithelial cells, neutrophils and platelet within the lung\textsuperscript{14}. Indirect evidence from animal model suggests that Thromboxane A\textsubscript{2} may have several properties, including bronchoconstriction, involvement in the late asthmatic response, and involvement in the development of airways inflammation and hyperactivity. Potent and specific Thromboxane synthetase inhibitors will be crucial tools for understanding the role of Thromboxane in asthma.
Leukotrienes

The lipoxygenase pathway of arachidonic acid breakdown is responsible for production of the class of compounds called Leukotrienes C₄, D₄ and E₄ constitutes the slow reacting substance of anaphylaxis (SRS-A). These Leukotrienes are liberated during inflammatory processes in the lung and have significant effect on airway smooth muscle, mucociliary function, microvascular permeability, and airways edema. In theory, potent Leukotriene antagonists should be able to prevent or reverse some of the pathologic features of asthma. Specific leukotriene antagonists and 5-lipoxygenase inhibitors are undergoing clinical trials at this time. Zileuton, a 5-lipoxygenase inhibitor recently gained approval from FDA advisory panel for the treatment of asthma.

Platelet activation factor

Thought to be produced by macrophages, eosinophils, and neutrophils within the lung, platelet-activating factor (PAF) is involved in the mediation of many of the important steps in the development of the asthmatic response. These steps include immediate bronchoconstriction and sustained induction of airway hyperactivity, edema formation, and cellular changes associated with generalized inflammatory responses, including chemotaxis of eosinophils. PAF is the only mediator known to produce a sustained increase in bronchial reactivity. As selective and potent PAF-receptor antagonists are developed and clinical trials are completed, the relative importance of PAF as a mediator in asthma will be more completely understood.

Adhesion molecules

An important step in the inflammatory process is the adhesion of the various cells to each other and the tissue matrix to facilitate infiltration and migration of these cells to the site of inflammation. To promote this, cell membranes express a number of glycoproteins, or adhesion molecules. Adhesion molecules have additional functions involved in the inflammatory process aside from promoting cell adhesion, including activation of cells and cell-to-cell communication and promoting cellular migration and infiltration. The many adhesion molecules are divided into families on the basis of their chemical structures. These families are the integrins, cadherins, immunoglobulin supergene family, selectins, vascular adressins, and carbohydrate ligands. Those thought to be important in inflammation include integrins, immunoglobulin supergene family, selectins, and carbohydrate ligands. Adhesion molecules are found on a variety of cells, such as neutrophils, monocytes, lymphocytes, basophils, eosinophils, granulocytes, platelets,
endothelial cells, epithelial cells, and can be expressed or activated by the many inflammatory mediators present in asthma. Thus complex interactions occur whereby mediators effect expression of adhesion molecules. In addition to these interactions, a major role of adhesion molecules is in the requirement of leukocytes from the vascular lumen to tissues. The initial step involved in this leukocyte-endothelial cell adhesion cascade is transient and reversible binding of the adhesion molecule to specific ligands on endothelial cells results in slowing or rolling of the circulating leukocyte along the surface of vasculature. Activation of the leukocyte or endothelial cell follows in response to a mediator or the initial adhesion event. Finally, firm adhesion, or anchoring, of the leukocyte to the endothelial cell surface allows for diapepdesis between endothelial cells and migration of the leukocyte into the extra cellular matrix.

2.5. TYPES OF ASTHMA

Child-onset asthma

When asthma does begin in childhood, it often does so in a child who is likely, for genetic reasons, to become sensitized to common "allergens" in the environment (atopic person). When these children are exposed to house-dust mites, animal proteins, fungi, or other potential allergens, they produce a type of antibody that is intended to engulf and destroy the foreign materials. This has the effect of making the airway cells sensitive to particular materials. Further exposure can lead rapidly to an asthmatic response. This condition of atopy is present in at least one-third and as many as half of the general population. When an infant or young child wheezes during viral infections, the presence of allergy (in the child itself or a close relative) is a clue that asthma may well continue throughout childhood.

Adult-onset asthma

Adult-onset asthma develops after age 20. It is less common than asthma in children, and it affects more women than men. Allergenic materials may also play a role when adults become asthmatic. Asthma can actually start at any age and in a wide variety of situations. Although less common than asthma in children, adult-onset asthma can also be triggered by allergies. Between 30 percent and 50 percent of all adult cases are associated with allergies, but often allergic exposures don't seem to be the most important, driving factors. This nonallergic adult-onset asthma is sometimes called "intrinsic." Many adults who are not allergic do have such conditions as sinusitis or nasal polyps, or they may be sensitive to aspirin and related drugs.
Another major source of adult asthma is exposure at work to animal products, certain forms of plastic, wood dust, or metals.

**Exercise-induced asthma**

Shortness of breath and/or wheezing occurring after strenuous exercise is called exercise-induced asthma. Although this phenomenon happens in up to 80% of people with recognized asthma, it frequently takes place as an isolated event without any other symptoms of asthma at any other time. This complicates any diagnosis of asthma as an underlying cause because frequently this form of asthma is confused with poor physical conditioning or possible heart problems. Nevertheless, asthma should always be suspected as a possible cause of exercise-induced wheezing or shortness of breath, especially when the person is otherwise healthy. Exercise-induced asthma involves symptoms that occur about 5-20 minutes after beginning an exercise that involves breathing through the mouth. Sports and games that require continuous activity or that are played in cold weather (for example, long-distance running, hockey, soccer, and cross-country skiing) are the most likely to trigger an asthma attack. Other physical exertions that can trigger an attack include laughing, crying, and hyperventilating.

**Cough-variant asthma**

Coughing can occur alone, without the other symptoms of asthma that are usually present and recognized by the physician or patient. Cough variant asthma causes great difficulty for the physician to accurately diagnose the true underlying cause of the cough as being asthma because it can be easily confused with other conditions, such as chronic bronchitis and post nasal drip due to hay fever or sinus disease. Coughing can occur day or night. Nighttime coughing is most disruptive, interfering with sleep.

**Occupational asthma**

Occupational asthma occurs in response to a trigger in the workplace. Triggers include contaminants in the air, such as smoke, chemicals, vapors (gases), fumes, dust, or other particles; respiratory infections, such as colds and flu (viruses); allergens in the air, such as molds, animal dander, and pollen; extremes of temperature or humidity; and emotional excitement or stress. In occupational asthma, the trigger is a substance or condition in the workplace that causes asthma symptoms. Most of these substances and conditions are very
common and are not normally considered hazardous. Although these substances and conditions can be encountered in almost any workplace, occupational asthma is most common in workers in the following industries and jobs. In most people with occupational asthma, the symptoms appear a short time after beginning work and subside after leaving work.

**Nocturnal asthma**

Nocturnal asthma occurs between midnight and 8 am. It is triggered by allergens in the home such as dust and pet dander or is caused by sinus conditions. Nocturnal or nighttime asthma may occur without any daytime symptoms recognized by the patient. This is called "nocturnal asthma." The patient may have wheezing or short breath when lying down or may not notice these symptoms until awoken by them in the middle of the night, usually between 2 and 4 am. Nocturnal asthma may occur only once in a while or frequently during the week. Nighttime symptoms may also be a common problem in people who have daytime asthma as well, but then its true nature is more readily recognized. When there are no daytime symptoms to suggest asthma is an underlying cause of the nighttime cough, this type of asthma will be more difficult to recognize and usually delay proper therapy. The cause (or causes) of this phenomenon is unknown, although many possibilities are under investigation.
2.6 CAUSES AND SYMPTOMS

In most cases, asthma is caused by inhaling an allergen that sets off the chain of biochemical and tissue changes leading to airway inflammation, bronchoconstriction, and wheezing. Because avoiding (or at least minimizing) exposure is the most effective way of treating asthma, it is vital to identify which allergen or irritant is causing symptoms in a particular patient. Once asthma is present, symptoms can be set off or made worse if the patient also has rhinitis (inflammation of the lining of the nose) or sinusitis. When, for some reason, stomach acid passes back up the esophagus (acid reflux), this can also make asthma worse. A viral infection of the respiratory tract can also inflame an asthmatic reaction. Aspirin and a type of drug called beta-blockers, often used to treat high blood pressure, can also worsen the symptoms of asthma. The most important inhaled allergens giving rise to attacks of asthma are:

- animal dander
- mites in house dust
- fungi (molds) that grow indoors
- cockroach allergens
- pollen
- occupational exposure to chemicals, fumes, or particles of industrial materials in the air

Inhaling tobacco smoke, either by smoking or being near people, who are smoking, can irritate the airways and trigger an asthmatic attack. Air pollutants can have a similar effect. In addition, there are three important factors that regularly produce attacks in certain asthmatic patients, and they may sometimes be the sole cause of symptoms. They are:

- inhaling cold air (cold-induced asthma)
- exercise-induced asthma (in certain children, asthma is caused simply by exercising)
- stress or a high level of anxiety

Wheezing is often very obvious, but mild asthmatic attacks may be confirmed when the physician listens to the patient's chest with a stethoscope. Besides wheezing and being short of breath, the patient may cough and may report a feeling of "tightness" in the chest. Children may have itching on their back or neck at the start of an attack. Wheezing is often loudest when the patient breathes out, in an attempt to expel used air through the narrowed airways. Some
asthmatics are free of symptoms most of the time but may occasionally be short of breath for a brief time. Others spend much of their days (and nights) coughing and wheezing, until properly treated. Crying or even laughing may bring on an attack. Severe episodes are often seen when the patient gets a viral respiratory tract infection or is exposed to a heavy load of an allergen or irritant. Asthmatic attacks may last only a few minutes or can go on for hours or even days (a condition called status asthmaticus).

Being short of breath may cause a patient to become very anxious, sit upright, lean forward, and use the muscles of the neck and chest wall to help breathe. The patient may be able to say only a few words at a time before stopping to take a breath. Confusion and a bluish tint to the skin are clues that the oxygen supply is much too low, and that emergency treatment is needed. In a severe attack that lasts for some time, some of the air sacs in the lung may rupture so that air collects within the chest. This makes it even harder to breathe in enough air. Almost always, even patients with the most severe attacks will recover completely.

2.7. DIAGNOSIS

Apart from listening to the patient's chest, the examiner should look for maximum chest expansion while taking in air. Hunched shoulders and contracting neck muscles are other signs of narrowed airways. Nasal polyps or increased amounts of nasal secretions are often noted in asthmatic patients. Skin changes, like atopic dermatitis or eczema, are a tipoff that the patient has allergic problems.

Inquiring about a family history of asthma or allergies can be a valuable indicator of asthma. The diagnosis may be strongly suggested when typical symptoms and signs are present. A test called spirometry measures how rapidly air is exhaled and how much is retained in the lungs. Repeating the test after the patient inhales a drug that widens the air passages (a bronchodilator) will show whether the airway narrowing is reversible, which is a very typical finding in asthma. Often patients use a related instrument, called a peak flow meter, to keep track of asthma severity when at home.

Often, it is difficult to determine what is triggering asthma attacks. Allergy skin testing may be used, although an allergic skin response does not always mean that the allergen being tested is causing the asthma. Also, the body's immune system produces antibody to fight off the allergen, and the amount of antibody can be measured by a blood test. This will show how sensitive the
patient is to a particular allergen. If the diagnosis is still in doubt, the patient can inhale a suspect allergen while using a spirometer to detect airway narrowing. Spirometry can also be repeated after a bout of exercise if exercise-induced asthma is a possibility. A chest x ray will help rule out other disorders.

2.8. MANAGING ASTHMATIC ATTACKS

A severe asthma attack should be treated as quickly as possible. It is most important for a patient suffering an acute attack to be given extra oxygen. Rarely, it may be necessary to use a mechanical ventilator to help the patient breathe. A beta-receptor agonist is inhaled repeatedly or continuously. If the patient does not respond promptly and completely, a steroid is given. A course of steroid therapy, given after the attack is over, will make a recurrence less likely.

2.9. MAINTAINING CONTROL

Long-term asthma treatment is based on inhaling a beta-receptor agonist using a special inhaler that meters the dose. Patients must be instructed in proper use of an inhaler to be sure that it will deliver the right amount of drug. Once asthma has been controlled for several weeks or months, it is worth trying to cut down on drug treatment, but this must be done gradually. The last drug added should be the first to be reduced. Patients should be seen every one to six months, depending on the frequency of attacks.

Starting treatment at home, rather than in hospital, makes for minimal delay and helps the patient to gain a sense of control over the disease. All patients should be taught how to monitor their symptoms so that they will know when an attack is starting, and those with moderate or severe asthma should know how to use a flow meter. They should also have a written "action plan" to follow if symptoms suddenly become worse, including how to adjust their medication and when to seek medical help. If more intense treatment is necessary, it should be continued for several days. Over-the-counter "remedies" should be avoided. When deciding whether a patient should be hospitalized, the past history of acute attacks, severity of symptoms, current medication, and whether good support is available at home all must be taken into account.

Referral to an asthma specialist should be considered if:

- there has been a life-threatening asthma attack or severe, persistent asthma
• treatment for three to six months has not met its goals
• some other condition, such as nasal polyps or chronic lung disease, is complicating asthma
• special tests, such as allergy skin testing or an allergen challenge, are needed
• intensive steroid therapy has been necessary

2.10. TREATMENT

Patients should be periodically examined and have their lung function measured by spirometry to make sure that treatment goals are being met. These goals are to prevent troublesome symptoms, to maintain lung function as close to normal as possible, and to allow patients to pursue their normal activities including those requiring exertion. The best drug therapy is that which controls asthmatic symptoms while causing few or no side-effects.
2.11. DRUG THERAPY OF ASTHMA

Despite considerable effort by the pharmaceutical industry, it has proved very difficult to develop new classes of therapeutic agents for asthma. This is partly because existing drugs are effective and safe, and partly because animal models of asthma are poor and do not seem to be predictive of clinical efficacy.

The drug therapy of asthma currently involves the use of following agents:

**BRONCHODILATORS**

Non Selective PDE Inhibitors

PDE-4 Selective Inhibitors.

β₂ - AGONISTS

Anticholinergic

**NEW BRONCHODILATORS**

Vasoactive Intestine Peptide

Prostaglandin E₂

K⁺ Channel openers

**ANTIINFLAMATORY DRUGS**

Glucocorticosteroids

Mast Cell Stabilizers

Antileukotriene Drugs

**MISCELLANEOUS AGENTS**

Antihistamines

Leukotriene Modifiers
2.11.1. BRONCHODILATORS

Non Selective PDE Inhibitors

Methyl xanthines have been used for asthma therapy for 50 years. Theophylline (1), a primary methylxanthine of interest, is more potent than Caffeine (25), dyphylline, and enprophylline as bronchodilator. Like the $\beta_2$-agonists, the methylxanthines are functional antagonists; however their clinical potency is limited by their low therapeutic index. Methyl xanthines are ineffective by aerosol and therefore must be taken systemically. Theophylline as a sustained release is preferred oral preparation, whereas its complex with ethylenediamine also known as aminophylline is the preferred injectable product.
The mechanism by which theophylline produces bronchodilation is unknown but may involve inhibition of phosphodiesterases. Theophylline is a competitive antagonist of bronchoconstrictor adenosine, this property is not shared by enprofylline, a more potent bronchodilator. Inhibition of PDEs results in increase in cyclic AMP and cyclic GMP concentrations. The PDE isozymes currently thought to be important in asthma are PDE III, predominant in airway smooth muscle, and PDE IV, important in inflammatory cell regulation such as mast cells, eosinophils, and T-lymphocytes. PDE inhibition is consistent with various nonbronchodilator activities that may be relevant to asthma including decreased eosinophil basic protein release, decreased T-lymphocyte proliferation, decreased plasma exudation.

Theophylline is a nonselective PDE inhibitor although this property indicate a potential for theophylline to provide anti-inflammatory activity in asthma, todate clinical trials have been bone this out. Theophylline produces linear increases in bronchodilation with logarithmic increments in serum drug concentration. Theophylline inhibits pulmonary edema by decreasing vascular permeability, enhances mucociliary clearance, and strengthens contraction of a fatigued diaphragm. In vitro theophylline inhibits the release of histamine in sensitized lung fragments but has provided protection against early asthmatic response to allergen.

In the 1970s and 1980s, theophylline was the primary drug for the treatment of both acute and chronic asthma. However, the availability of safer effective inhaled β2-agonists and anti-inflammatory agents coupled with a better understanding of pathogenesis of asthma and bronchial hyperreactivity, has curtailed the use of theophylline. Comparative studies between sustained release theophylline and oral sustained release β2-agonists have not shown any advantage for theophylline. Disadvantages to chronic theophylline therapy include theophylline’s lack of effect on underlying bronchial hyperreactivity and death at serum concentration two folds greater than optimal therapeutic concentrations. Due to its high risk/benefit ratio, theophylline should be considered as second or third line drug therapy.
SAR of Methylxanthines

Methylxanthines have been examined for their ability to inhibit cyclic nucleotide phosphodiesterases and to antagonize receptor mediated actions of adenosine.

- Both activities are reduced in derivatives that lack substitutions at position 1 or contain substituents at position 7, as compared with corresponding 1,3-dialkylxanthine. e.g. The order of potency for the methylxanthines is Theophylline > Caffeine > Theobromine.

- Enhancement of both activities is obtained with larger nonpolar substituents at positions 1 and 3.

- Addition of aromatic, cyclohexyl and/or cyclopentyl groups at position 8 usually markedly increases the affinity for adenosine receptors but reduces the inhibition of cyclic nucleotide phosphodiesterases.

- Theophylline (1) and caffeine (26) both are weak Bronsted bases with pKas 0.8 and 0.6 respectively. This is due to imino group at position 9. In theophylline, a proton can be donated from position 7 (i.e. it can act as a Bronsted acid) but Caffeine cannot donate a proton from position 7 (i.e. it can not act as a Bronsted acid).

- Caffeine have electrophilic sites at positions 1, 3 and 7. Theophylline has electrophilic sites at 1 and 3 positions, in addition it is having a Bronsted acid site at position 7 due to which it acts as a proton donor in most pharmaceutical system.

PDE-4 Selective Inhibitors

PDE 4 selective inhibitors have the ability to reduce the bronchospasm induced by histamine, leukotriene D4 (LTD4), carbachol and methacholine. Selective PDE 4 may address not only
asthmatic bronchoconstriction but also underlying bronchial inflammation. Thus the profile of selective PDE 4 inhibitors seems to fulfil the requirements for a unique therapy for asthma.

From a structural point of view, selective PDE 4 inhibitors can be divided into four classes.

- Catechol ethers: structural analogs of rolipram. (2)
- Heterocyclics and analogues: structural analogs of nitraquazone. (26)
- Xanthines and related compounds: structural analogues of theophylline. (1)
- Miscellaneous selective PDE 4 inhibitors.

![Catechol Ethers](2)

![Rolipram](26)

**Catechol Ethers**

Rolipram (2) is the prototype of this class which was originally developed as an antidepressant and is now the most studied of all selective PDE 4 inhibitors. SAR studies of a series of rolipram analogues as PDE inhibitors have been published.\(^69,70\) Rolipram has been found to bind to a high affinity site on PDE 4, distinct from catalytic site.\(^70,71\) While high affinity rolipram binding site do not appear to be present in all tissues that contain PDE 4, this activity is coexpressed with human recombinant PDE 4 activity.\(^18,70\) The bronchodilatory effect of several PDE 4 inhibitors correlate better with displacement of rolipram binding than with PDE inhibition.\(^69\) In addition to having desirable inhibitory effects on inflammatory, anaphylaxis and smooth muscle contraction, selective PDE 4 inhibitors also produce undesirable effects including nausea and vomiting. Emetic potency of PDE 4 inhibitors is correlated with an affinity for rolipram binding site in brain rather than potency of inhibiting PDE 4 enzyme activity.
Heterocyclics and analogues

This class of PDE 4 inhibitors is exemplified by nitraquazone (26). The archetypical quinazolindione moiety of (26) has been extensively manipulated to afford a variety of structure derived compounds. 3’-NO₂ group of 12 has been replaced by a different nonprotic, electron-withdrawing functionalities like -Cl, -Br, -COOCH₃ to study SAR.¹⁷ Corresponding acid and n-methyl amide produces a substantial and complete loss in potency respectively.¹⁷,¹⁸ Pyridopyrimidinedione analogues have also been prepared. Although benzene-pyridine isosteric replacement led to decrease in potency in case of (27) as compared to (28) but introduction of bulkier groups at N₃ afforded (29-31) with substantially increased potency. The corresponding 4-pyridyl derivative (32) has proved to be four times potent inhibitor of PDE 4 with respect to prototype.⁷²

![Chemical Structure](image)

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<th>X</th>
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<tr>
<td>(32)</td>
<td>N</td>
<td>4-pyridyl</td>
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Compounds of the type (3) containing pyridopyridazinone nucleus are nanomolar selective inhibitors of PDE 4.⁷³,⁷⁴ The concomitant increase in their side effects limited their development. Attempts were made to reduce these adverse effects and compounds were synthesized by replacing pyridine with a series of heterocyclic systems such as pyrrole, pyrazole, 1, 2-dihydropyridine and thiophene.⁷⁵,⁷⁶ These compounds showed significantly better balance between PDE 4 inhibition and emetic side effects. Further simplification led to quinoline derivatives RS 14203 (33) which is one of the most potent PDE 4 inhibitor.
Xanthine and related compounds

Theophylline represents this class of selective PDE 4 inhibitors. Xanthines have long been known to cause a variety of physiological effects. The CNS stimulatory properties of caffeine have been utilized for centuries. Tachycardia and bronchoconstriction are other responses elicited by xanthines. Inhibition of phosphodiesterase in heart, brain and lungs may be the mechanism by which xanthines exert their effects.\(^77\) Theophylline preparations have found continuos use as bronchodilators in the treatment of asthma for almost a century. It is a weak non selective phosphodiesterase inhibitor, but it is believed that PDE inhibitory activity may contribute to both its bronchodilatory and anti-inflammatory activities.\(^78\) Although theophylline is useful in the treatment of asthma, the value of theophylline is limited to a narrow therapeutic index due to wide range of gastrointestinal, CNS and cardiovascular side effects.

Attempts to develop an "improved theophylline" has emerged recently,\(^79\) using xanthine skeleton novel compounds are designed which are selective PDE 4 inhibitor and also retain the therapeutic efficacy of theophylline. Xanthine nucleus present in theophylline is also present in the PDE substrate and will bind to and block highly conserved enzyme catalytic site. Miyamoto \emph{et al.} studied SAR of this class and reported an interesting series of heterocyclic fused xanthines. A series of xanthines with varied subtituents at the 1, 3 and 8 positions have been prepared in order to understand the SAR for alkyl xanthines as inhibitor of phosphodiesterase.\(^80\)
A number of 7-alkylated derivatives of theophylline such as dyphylline (34), doxyphylline (35), and thioanalogues of doxyphylline (36), proxiphylline (37), bamiphylline (38), acephylline (39) and etophylline (40) have been synthesized and studied. These derivatives are generally less active and exhibit low or moderate PDE 4 inhibitory activity than theophylline but are stable in solution and in vivo.

Arofylline (4) is in phase III clinical trials for oral asthma therapy. It is a weak but selective inhibitor of PDE 4 as compared to theophylline. It is 25-30 folds less emetic than rolipram. Cipamphylline (5) was the prototype of a series of potent PDE 4 inhibitors reported and have been synthesized by Smithkline Beecham.
8-Substituted piperazine derivatives (41) have been reported by Ragnier et al.\textsuperscript{87} having a combination of antiallergic and antihistaminic properties. Among these, exhibited inhibitory action on mast cell degranulation and phosphodiesterase enzyme.\textsuperscript{81,87}

One of the xanthine analogues, Ibudiblast (42), marketed as an orally acting antiasthmatic drug in Japan\textsuperscript{88} has been found to be a nonspecific and moderately potent PDE 4 inhibitor (43) is an effective bronchodilator in human bronchus precontracted with different spasmogens. The presence of bromine on position 6 and an alkylamino group on position 8 plays a critical role in the activity.\textsuperscript{89}
Miscellaneous selective PDE 4 inhibitors

PDE 4 inhibitors based on benzopyran (44) nucleus have been reported. Compound (45) having an indazole nucleus represents PDE 4 inhibitor synthesized by Pfizer labs.  

Correlation between PDE 4-mediated side-effects and affinity for the rolipram binding site

The ubiquity of PDE 4 enzymes in different cell types and tissues not only promises anti-inflammatory effects but also raises the possibility of widespread pharmacodynamic actions of systemically administered PDE 4 inhibitors. Oral administration of rolipram (2) to rats (10 - 100 mg/kg for up to 14 days) produces decreased locomotor activity, unusual behaviour, salivation, abdominal distension and a lowered pain threshold. Necropsy showed stomach and intestinal inflammatory changes, with mesenteric vessels being particularly affected.  

Higher doses were associated with widespread tissue damage involving multiple organs. Studies in higher species, including man, have identified nausea, emesis and gastro-intestinal discomfort as major adverse reactions. In addition to inhibiting the PDE 4 catalytic activity (IC₅₀ 0.31 μM), rolipram (2) binds to a high affinity binding site at the enzyme (Kiₐ 0.0017 μM). There is currently a debate as to whether the high affinity binding site is a distinct allosteric site or whether the enzyme can exist in two conformationally different forms, one representing a 'high-affinity'
Irrespective of which model is correct, structure-activity data with PDE 4 inhibitors indicate different relationships depending on whether [3H]-rolipram binding or catalytic activity is measured. Interestingly, behavioural CNS changes and gastric acid secretion seem to correlate with the rank order of potency for inhibition of [3H]-rolipram binding to the enzyme. On the other hand, inhibition of TNF-α release from human blood monocytes and guinea-pig mast cells seems to correlate with inhibition of cAMP catalytic activity. CDP-840 (46) and SB207499 (47) are less potent at inhibiting ‘rolipram binding’, and it will be interesting to see whether this is accompanied by an improved tolerance in human subjects. It might be possible to design more specific PDE 4 inhibitors which have fewer side-effects by synthesising compounds that are selective for the ‘low-affinity’ conformer. Working toward this hypothesis, Duplantier and coworkers have synthesised a biarylcarboxylic acid (48) which is 4 times more active as a PDE 4 inhibitor than as an inhibitor of rolipram binding to mouse brain homogenate. After oral dosing, this compound potently inhibited antigen-induced bronchospasm in sensitised guinea-pigs, but had little emetic potential in ferrets in vivo (being present in high concentrations in plasma). These data support the hypothesis that certain side-effects, such as emesis, are directly related to inhibition of rolipram binding to PDE 4 enzyme although these data do not necessarily demonstrate whether emesis is triggered from the primary area or from the gastrointestinal tract. It remains to be seen whether the same relationship holds true in humans.

**Effects of PDE inhibitors in patients with asthma**

Despite the large amount of preclinical data, little is still known about the effects of PDE inhibitors in patients with asthma or other airway diseases. Of the selective inhibitors, those that affect the PDEs 3, 4 and 5 have attracted the greatest interest for their potential as anti-asthma drugs. PDE 3 inhibitors are effective bronchodilators in a number of species although they have profound cardiovascular effects at the same doses. The PDE 3 inhibitor enoximone (MDL 17043, 49) has been shown to improve lung function in patients with chronic obstructive lung disease. In a placebo-controlled, double-blind, cross-over trial, zaprinast (PDE 5 inhibitor, 50) reduced exercise-induced but not histamine-induced bronchoconstriction in asthmatics, suggesting that it was acting indirectly, possibly on mast cells. A relatively weak PDE 4 inhibitor, tibenelast has been investigated in subjects with asthma. At a single oral dose a slight, but non-significant improvement in FEV1 was observed in asthmatics. This effect may not be representative for novel, potent and more selective PDE 4 inhibitors which may exert qualitatively different effects. The PDE 3/4 inhibitor AH 21-132 (51) and zardaverine (52) relax
bronchial smooth muscle in vitro and act as bronchodilators in vivo.\textsuperscript{104,105} DeBoer and co-workers\textsuperscript{106} showed that combined PDE 3/4 inhibitors produce a more marked relaxation of human bronchi in vitro than selective inhibitors alone, but whether this is the case also in human subjects remains to be demonstrated. AH 21-132 (51) has been shown to reverse methacholine-induced bronchospasm in healthy subjects and to induce a small, transient bronchodilation when administered intravenously.\textsuperscript{105} However, when zardaverine (52) was administered by a metered dose inhaler (1.5, 3 and 6 mg) to patients with chronic airflow obstruction, there was no improvement in lung function even though salbutamol clearly produced bronchodilator response in these patients.\textsuperscript{107} Tolafentrine (53), a combined PDE 3/4 inhibitor, has been administered by nebulisation to subjects with mild asthma. At a dose of 0.5 mg, tolafentrine had no significant effect on airway tone or airway hyper-responsiveness to inhaled AMP.\textsuperscript{108} Recently, clinical data with the selective PDE 4 inhibitor CDP 840 (46) were presented. A single oral dose of either 15 or 30 mg was administered p.o. to patients with asthma.\textsuperscript{109} The investigators found, however, no effect on bronchial tone when FEV1 was used as a measurement. When dosed repeatedly for up to 9 days (15 mg b.i.d.), there was no reduction in the airway hyper-responsiveness to inhaled histamine.\textsuperscript{110} The effect on the early (EAR) and late (LAR) asthmatic reactions was studied although it was not possible to define whether enough compound was administered to effectively control the bronchoconstriction of the asthmatic patients after antigen challenge. With the same dosing regimen (15 mg b.i.d. for 9 days) there was a statistically significant reduction of the LAR, but not EAR.\textsuperscript{111} These data suggest that PDE 4 inhibitors may have anti-inflammatory actions but are without direct bronchodilator effects. Reportedly, CDP 840 (46) has been administered p.o. to patients with asthma in a Phase II clinical study. According to a company bulletin, CDP 840 (46) did not improve asthma symptoms sufficiently in this study to warrant further development of the compound as an oral anti-asthmatic, although no details were given. Thus, despite the wealth of preclinical data demonstrating bronchodilator and anti-inflammatory effects, surprisingly little encouraging clinical data has been forthcoming. The limited effects of CDP 840 (46) (which is a nM potent PDE 4 inhibitor) are disappointing, but can perhaps be explained by poor oral bioavailability. Compounds that have entered Phase II clinical trials all seem to have some limiting side-effects. It seems reasonable to assume that higher doses would produce a more substantial therapeutic benefit than has been reported to date. The key to achieving this will be to synthesise compounds with a markedly improved therapeutic ratio, perhaps through a greater separation between inhibition of catalytic activity and 'rolipram binding'.
Future developments

PDE 4 inhibitors, like RP 73401 (54) and CDP 840 (46), represent a new generation of potent and selective drugs which have been shown to suppress inflammatory cell function in vitro, relax airway smooth muscle in vitro and to have anti-inflammatory and antibronchoconstrictor properties in a range of acute and chronic animal models. This type of compound thus shows great promise as a novel anti-asthma agent and the high potency and selectivity over other PDE isoenzymes may produce therapeutic agents with distinct advantages over theophylline. Intense patenting activity in this area demonstrates the huge interest from pharmaceutical companies and the promise of a longawaited new therapeutic principle for asthma therapy. Selective PDE 4 or mixed PDE 3/4 inhibitors are currently in preclinical and clinical development for asthma and other chronic inflammatory and/or allergic conditions. Although preliminary clinical results with, for example, CDP 840 (46) have been disappointing, we suggest that PDE 4 subtype-selective compounds or compounds that do not interact with the 'high affinity' rolipram binding site will possess more interesting pharmacodynamic properties and should be pursued. Exploratory clinical studies have pointed towards a possible use also in other allergic conditions such as rhinitis and atopic dermatitis. Whether inflammation and tissue remodelling in chronic obstructive pulmonary disease and lung fibrosis will be sensitive to PDE 4 inhibitors remains an intriguing possibility. The potent immuno-modulatory effect, including suppression of TNF-α production, suggests that this class of drugs may also be of value in diseases with aberrant TNF-α production such as inflammatory bowel disease and rheumatoid arthritis. The proven therapeutic benefit of these compounds continues to stimulate rapid progress in many areas of PDE research and it is anticipated that the results from clinical studies will further our understanding of physiological and pathological processes and contribute to the development of novel and more efficacious medicines.
β₂-Agonists

Asthma mortality is increasing worldwide despite the availability and widespread use of more powerful and longer acting agonists selective for β₂ receptor subtype.\(^{112,113,114}\) β₂-agonists are the only agents shown to be immediately effective in the setting of acute, severe asthma. In clinical studies, regular use of β₂-agonists led to worse control of asthma. Prophylactic use of β₂-agonists in the setting of exercise induced asthma is highly effective and safe.\(^{21}\)

For inhalation treatment of bronchospasm there seems to be little basis for choice among the selective such as Bitolterol (55), Albuterol (56), Terbutaline, Pirbuterol. Albuterol and terbutaline are available in nebuliser solutions in addition to metered dose inhalers.\(^{115}\)
Isoetharine (57) and Metaproterenol (59) are somewhat shorter acting and less selective for $\beta_2$ adrenergic receptors, while Isoproterenol (58) activates $\beta_1$ and $\beta_2$ receptors equally and has a still shorter duration of action. Inhalations of a $\beta_2$ selective agonist usually produce excellent bronchodilation or short term protection against a challenge without appreciable cardiac or other systemic effects, especially in younger asthmatic patients. Salmeterol xinafoate, a long acting $\beta_2$ selective agonist has been approved for use. Although its slow onset of action led it not suitable for treatment of acute bronchospasm but the place for Salmeterol in the regular treatment of asthma remains to determined.

Orally administrated adrenergic agonists for bronchodilation has not gained wide acceptance, largely because of the greater risk of producing side effects, especially tremulousness, muscle cramps, cardiac tachyarrhythmia's and metabolic disorders. Oral $\beta_2$-agonists are used in two situations. First, in young children (<5 years old) who cannot manipulate metered dose inhalers yet have occasional wheezing with viral upper respiratory infections. Secondly, in some patients with severe asthma exacerbations, any aerosol can be irritating and cause a worsening of
cough and bronchospasm. Even stimulation of $\beta_2$ receptors either orally or by inhalation, does not reduce bronchial hyper responsiveness.\textsuperscript{118}

\[
\begin{align*}
\text{R}_1 & \quad \text{R}_2 & \quad \text{R}_3 \\
(57) & \text{Isoetharine} & -\text{CH}(\text{CH}_3)_2 & -\text{C}_3\text{H}_53,4-(\text{OH})_2\text{phenyl} \\
(58) & \text{Isoproterenol} & -\text{CH}(\text{CH}_3)_2 & -\text{H} & 3,4-(\text{OH})_2\text{phenyl} \\
(59) & \text{Metaproterenol} & -\text{CH}(\text{CH}_3)_2 & -\text{H} & 3,5-(\text{OH})_2\text{phenyl}
\end{align*}
\]

SAR Of $\beta_2$ Agonists

\[
\begin{align*}
\text{General formula}
\end{align*}
\]

- Only one of the aromatic hydroxyl groups is necessary (usually at para position but sometimes at meta position of the aromatic ring).

- An $\alpha$- methyl group is preffered for vascular effects. e.g. Isoxsuprine.

\[
\begin{align*}
\text{Only one of the aromatic hydroxyl groups is necessary (usually at para position but sometimes at meta position of the aromatic ring).}
\end{align*}
\]

- If the $\alpha$-methyl group is removed then more selectivity for $\beta_2$ receptor is obtained e.g. Salbutamol (9), Terbutaline (61)
Anticholinergics

Stramonium herbal treatment have been used for asthma for centuries however their systemic effects, particularly involving the central nervous system, limited their usefulness. The introduction of quaternary ammonium derivatives such as ipratropium bromide (10), has renewed interest in these compounds. Anticholinergics bronchidilators are competitive inhibitors of muscarinic receptors. Unlike $\beta_2$-agonists and theophylline, they are not functional antagonists; they only produce bronchodilation in cholinergic-mediated bronchoconstriction. A number of triggers and mediators of asthma (e.g. histamine, prostaglandins, sulphur dioxide, exercise, and allergens) produce bronchoconstriction in part through vagal reflex.

Anticholinergics attenuate but do not block allergen or exercises induced asthma in a dose dependent manner and have no effect on the late asthmatic response.

NEW BRONCHODILATORS

Although several novel classes of BRONCHODILATOR have now been explored, it is difficult to find a drug class of comparable efficacy and safety to the $\beta_2$-adrenoceptor agonists which also
counteract all known bronchoconstrictor mechanisms. Several new β2-adrenoceptor agonists with a long duration of action that will be suitable for once-daily administration are now being tested, and are likely to become the bronchodilators of choice in the future when used in combination inhalers with a long-acting corticosteroid. Several novel bronchodilators have been developed on the basis of knowledge of the mechanism of action of β2-adrenoceptor agonists (Figure R4).

Figure R4: Molecular mechanisms of action of bronchodilators. Activation of β2 adrenoceptors, vasoactive intestinal peptide (VIP) and prostaglandin E2 (PGE2) receptors results in activation of adenylyl cyclase (AC) via a stimulatory G-protein (Gs) and an increase in cAMP concentration. This activates protein kinase A (PKA), which then phosphorylates several target proteins, resulting in the opening of calcium-activated potassium channels (KCa) or maxi-K channels, decreased phosphoinositide (PI) hydrolysis, increased Na+/K+ ATPase and decreased myosin light chain kinase (MLCK) activity, which leads to relaxation of airway smooth muscle. In addition, β2-adrenoceptors can be coupled directly via Gs to KCa. cAMP is broken down by phosphodiesterases (PDE), which are inhibited by theophylline and selective PDE3 inhibitors, and which could therefore be potential asthma therapies.

Vasoactive intestinal peptide

Vasoactive intestinal peptide (VIP) is a potent relaxant of constricted human airways in vitro, but its degradation in airway epithelium means that it is ineffective in asthmatic patients. A
more stable cyclic analogue of VIP (Ro-25-1553) has a more prolonged effect in vitro and in vivo and is effective in asthmatic patients by inhalation.120

**Prostaglandin E2**

Although prostaglandin E2 (PGE2) relaxes airways in vitro and is involved in the refractory response of the airways to exercise,121 it is not effective as a bronchodilator in vivo, and can even lead to constriction and coughing in asthmatics through stimulation of sensory nerves in airways. PGE agonists that are selective for receptor subtypes could avoid the problem of coughing and might be worthy of further exploration as bronchodilator/anti-inflammatory drugs.122 Atrial natriuretic peptide. Intravenous infusion of atrial natriuretic peptide (ANP) produces a significant bronchodilator response and protects against bronchoconstriction induced by inhaled bronchoconstrictors such as methacholine.123 Although ANP itself is susceptible to enzymatic breakdown, it is possible that non-peptide agonists of ANP receptors could be developed in the future. The related peptide urodilatin (ularitide) has a longer duration of action than ANP, is less susceptible to degradation and is as potent as salbutamol when intravenously infused in asthmatic subjects.124

**K⁺ channel openers**

Drugs that selectively open an ATPdependent K+ channel (K+ channel openers (KCOs)), such as levcromakalim, are effective bronchodilators of human airways in vitro, but are ineffective in vivo at maximally tolerated oral doses125. KCOs might also be effective as inhibitors of sensory nerve activation, and therefore could be useful in inhibiting cough and AIRWAY HYPERRESPONSIVENESS (AHR)126. Several KCOs have been studied in Phase I/II trials, but their development for asthma was halted because of dose-limiting vasodilator side effects (headaches and postural hypotension).
2.11.2. ANTIINFLAMMATORY DRUGS

**Glucocorticosteroids**

Glucocorticoids can alter the level of hyperreactivity, this evidence has reestablished the utility of glucocorticoids in the therapy of asthma.\textsuperscript{12,13,23} The mechanism of action useful in asthma include:-

- Increasing the number of $\beta_2$ receptor and improving the receptor responsiveness to $\beta_2$ agonist stimulation.
- Reducing mucus production and hypersecretion.
- Inhibiting the inflammatory response at all levels.\textsuperscript{23}

Glucocorticoids constrict the microvasculature inhibiting fluid and protein influx, and inhibiting migration of neutrophils and eosinophils into tissues as well as inhibiting their function. Glucocorticoids inhibit the production of prostaglandins and Leukotrienes by inhibiting phospholipase release of arachidonic acid from membrane phospholipids.\textsuperscript{23}

Cortisol and its synthetic derivatives such as prednisolone (11), methylprednisolone (62), triamcinolone (63), dexamethasone and beclomethasone (12), all have beneficial effects in the treatment of asthma related to prevention and suppression of airway inflammation.\textsuperscript{14,23,24} The major cellular and biochemical activity of the Glucocorticoids includes decreasing synthesis and release of several proinflammatory cytokines such as IL-1, GM-CSF, IL-3, IL-4, IL-5, IL-6 and IL-8 reducing inflammatory cell activation, recruitment, and infiltration; and decreasing vascular permeability.\textsuperscript{127} Supressing the ongoing airway inflammation results in prevention or inhibition of mucus secretion, decreased edema of airway mucosa, and perhaps decreased airway epithelial denudation, leading to a reduction in airway reactivity.\textsuperscript{23}
Glucocorticoids in asthma are used in two forms:

- Systemic Glucocorticoids
- Inhaled Glucocorticoids

**Systemic Glucocorticoids**

Acute severe asthma, status asthmaticus, is treated with high dose systemic glucocorticoids combined with frequent administration of inhaled \( \beta_2 \)-agonists. Glucocorticoids such as methylprednisolone, hydrocortisone are administrated by the parental route and prednisolone (11), methylprednisolone (62) are given orally which provide a rapid onset of action and a systemic effect.\(^{128}\) Glucocorticoids are also recommended for the treatment of impeding episodes of severe asthma unresponsive to bronchodilator therapy.\(^{12,16}\) The effects of Glucocorticoids in asthma are dose and duration dependent, this is true as well as for the adverse effects of systemic steroids. Because short term high dose steroids do not produce serious toxicity, the ideal use is to administer the glucocorticoids for a short course or "burst" and then maintain the patient on bronchodilators, inhaled corticosteroids and Cromolyn with long periods between systemic glucocorticoids treatment.\(^{128}\)

**Inhaled Glucocorticoids**

The inhaled glucocorticoids are becoming popular as the first line drug therapy for chronic therapy in asthma. This is because the contribution of inflammation to the pathogenesis of asthma is becoming better understood and the inhaled glucocorticoids allow the application of potent topical anti-inflammatory agents to the relevant site of action within the airways.\(^{23}\) The glucocorticoids currently available for inhaled use are beclomethasone, triamcinolone and flunisolide. Fluticasone and budesonide are undergoing clinical trials for asthma.

The "ideal" glucocorticoid for inhaled use should have a high degree of topical potency, minimal systemic absorption of active drug, and minimal local or systemic side effects. None of the available inhaled glucocorticoids are considered ideal and ongoing investigations into topical/systemic potency ratios will reveal important and much needed information. The bronchoselectivity of inhaled glucocorticoids is primarily a result of metabolism to less active substances following absorption from the lung. Beclomethasone, triamcinolone and flunisolide appear to have relatively similar topical/systemic potency ratios, whereas budesonide and fluticasone have improved bronchoselectivity.\(^{127}\)
SAR of Glucocorticoids

- Prednisolone (11), which is a 1, 2-dehydro derivative of fludrocortisone is 4 times potent as an anti-inflammatory agent.
- The introduction of 6 α-methyl group in prednisolone leads to methylprednisolone (62) in which anti-inflammatory activity is increased.
- Dexamethasone combines all the 1, 2-dehydro, 9α, 16α-methyl modifications and its anti-inflammatory activity is 25 times as that of prednisolone.
- Beclomethasone (12) is the 16-methyl analogue of cortisol. The presence of methyl group eliminates the sodium retaining effect and increases its anti-inflammatory effect.
- Triamcinolone (63), having a 16-hydroxyl group further eliminates the sodium retaining effect, but the anti-inflammatory property is slightly modified.
- 1, 2-dehydro and 6α-fluoro increases the anti-inflammatory and glucocorticoid activity.
- The introduction of 6α-fluoro, 16β-methyl and 16α-hydroxy substituents decrease or eliminate sodium retaining activity but only slightly modify the anti-inflammatory potency.

Mast Cell Stabilisers

**Chromolyn sodium and Nedocromil sodium**

Cromolyn sodium (64) has been available for the prophylactic uses in asthma for almost 20 years, while Nedocromil sodium (65), a pyranoquinoline dicarboxylic acid that is pharmacologically similar, has just recently been released for the treatment of asthma. The exact mechanism of action of these agents is still unknown. Initially it was thought that all of Cromolyn’s activity was a result of mast cell stabilization. As such it inhibits the Early Asthmatic Response (EAR) to allergen challenge as well as Eosinophil infiltration assay (EIA).\(^\text{129}\)
Unlike β₂-agonists and experimental mast cell membrane stabilizer, cromolyn and nedocromil also inhibit the Late Asthmatic Response (LAR) and prevent the subsequent increased bronchial hyperresponsiveness. Long-term prophylaxis with cromolyn prevent the usual increase in bronchial hyperreactivity associated with specific pollen season and may produce a modest decreased in baseline bronchial hyperreactivity. Both these inhibit invitro activation of human neutrophils, macrophages, and eosinophils.²⁵,¹³⁰ Each agent also inhibits neural mediated bronchoconstriction through C-fiber sensory nerve stimulation in the airways.¹³¹ Neither drug has a bronchodilator effect.

Both these are effective only by inhalation and are available as Metered Dose Inhalers (MDIs), while cromolyn also comes as a nebulizer solution and spinhaler. They are not bioavailable orally but the portion of the doses that reaches the lung is completely absorbed²⁵,¹³⁰ cromolyn and nedocromil are indicated for the prophylaxis of chronic mild to moderate asthma in both children and adults regardless of etiology. They are particularly effective for the allergic asthmatics on a seasonal basis or just prior to an acute exposure.¹³⁰ Cromolyn is the second drug of choice for the prevention of EIA and may be used in conjunction with a β₂-agonist in more severe cases not completely responding to either agent alone.¹³² In those patients with a history of LAR following exercise, cromolyn would be the first choice. The efficacy of each drug is directly related to its deposition in the lung so when therapy, it is important that the airways are patent. A short course of systemic glucocorticoids and around-the-clock inhaled β₂-agonist may initially be required in patients with significant obstruction.²³,¹³²

**Antileukotriene Drugs**

These drugs belong to four classes.

- Drugs that inhibit the synthesis of leukotrienes C₄ (LTC₄), leukotrieneD₄ (LTD₄), and LeukotrieneE₄ (LTE₄)
- Drugs that inhibit the synthesis of leukotrienes B4 (LTB₄)
- Cysteinyl receptor antagonists.
- LTB₄ receptor antagonists.

The fact that no significant clinical difference have been observed between these four classes of drugs, the term antileukotriene drugs are used. The 5-lipoxygenase inhibitors block the activity of 5-lipoxygenase. The 5-lipoxygenase activating protein inhibitors displace arachidonic acid from its binding sites on the FLAP molecule and prevent this substrate from being presented to 5-lipoxygenase. Exercise induced asthma is partially inhibited by antileukotriene drugs. Inhaled antigen causes an EAR with peak effect after 15 minutes and recovery after next hour or so. The EAR is followed by LAR after about 6-8 hrs in 50% of patients. LAR is accompanied by swelling of airway wall and infiltration by inflammatory cells, together with increased airway response to methacholine and histamine. Zafirlukast (66), 20 mg given orally two hrs before exercise, had variable efficacy ranging from complete to little or no protection in individuals and 40 mg given two hours before antigen challenge attenuates the early response by 80% and LAR by 50%. It partially reduces the increase in airway reactivity 6 hours after the challenge. 

Allergen induced hyperreactivity may be an important mechanism in the symptoms and progression of disease. The ability of drugs to prevent this effect would be an important component of long term drug therapy. In asthma the target site for the delivery of drug is the lungs which reduces the risk of systemic toxicity. 

In contrast montelukast and zafirlukast have been developed as oral formulations because of patient’s compliance for this type of medication. In chronic asthma compared with placebo, day time asthma scores, night wakenings and use of β agonists, have improved with zafirlukast.
Eosinophil count in blood and sputum was decreased with montelukast 10 mg daily as compared with placebo in Patients who usually only used β agonists for their mild asthma. 13-Week comparative trial between zafirlukast and sodium cromoglycate shows reduced symptom scores and improved lung function compared with placebo.\(^{133}\)

Inhaled corticosteroids when compared with zafirlukast and montelukast shows equal potency with beclomethasone. Asthmatic patients which cannot tolerate aspirin because it can induce bronchospasm, noso-ocular and gastrointestinal reactions. Such patients having an abnormally High leukotriene production, measured by urinary LTE\(_4\), concentration using anti-leukotriene drugs diminish the bronchoconstrictor response.\(^{133}\) Zileuton (68), a selective inhibitor of 5-lipoxygenase inhibit the formation of all 5-lipoxygenase products. Which means that inhibiting the formation of cys-LTs along with the formation of leukotriene B\(_4\), a potent eicosanoid that depend on LTA\(_4\) synthesis.\(^{134}\)

![Zileuton](image)

(68)

Antileukotriene drugs may have a role in asthma, which can be controlled with modest doses of inhaled corticosteroid.
2.11.3 MISCELLANEOUS AGENTS

Antihistamines

Although classical antagonists of the H1 receptor are of little clinical value in asthma\textsuperscript{23}, the recent discovery of H4 receptors expressed on mast cells, T cells and eosinophils has raised the possibility that H4 receptor antagonists could be beneficial in asthma\textsuperscript{24}. The selective H4 receptor antagonist JNJ 7777120 potently inhibits mast-cell activation and chemotaxis, and might therefore be of potential benefit in reducing asthma symptoms and exacerbations\textsuperscript{25}.

Antihistamines have had a controversial role in the asthma therapy. Earlier studies determining the role of histamine release in bronchoconstriction suggested a potential benefit of antihistamine therapy\textsuperscript{25}. However, studies in chronic and asthma did not support the initial enthusiasm. Antihistamines through their anticholinergic effect could be harmful in asthma, but they are not contraindicated. Some of the newer agents like terfenadine (13), cetirizine (14), loratadine (69) have demonstrated their efficacy in asthma by anti-inflammatory and azelastine (70) by their bronchodilatory properties\textsuperscript{25}.

\begin{center}
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Methotrexate

These oral drugs are also beneficial for those who comply poorly with inhaler device. Initial trials of this agent in the treatment of severe steroid dependent asthma allowed the reduction of systemic steroid dose in some patients. Although its primary mechanism of action is not understood, methotrexate may act as an anti-inflammatory agent.
Leukotriene modifiers

- Drugs that inhibit the synthesis of leukotrienes C₄ (LTC₄), Leukotriene D₄ (LTD₄), and LeukotrieneE₄ (LTE₄)
- Drugs that inhibit the synthesis of leukotrienes B₄ (LTB₄)
- Cysteinyl receptor antagonists
- LTB₄ receptor antagonists

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Antileukotriene drugs may have a role in asthma, which can be controlled with modest doses of inhaled corticosteroid.

Leukotriene modifiers, including antileukotrienes (for example, montelukast, pranlukast and zafirlukast) and 5-lipoxygenase (5-LO) inhibitors (zileuton), were, 5 years ago, the first new class of antiasthma treatment to be introduced in 30 years.\textsuperscript{135}

Although antileukotrienes have had some clinical success in asthma, they are considerably less effective and more expensive than inhaled corticosteroids.\textsuperscript{122} Current antileukotrienes are potent and selective competitive antagonists of the leukotriene CysLT1 receptor, which mediates bronchoconstriction, plasma exudation and mucus secretion; however, a second receptor, termed CysLT2, might also be an important target for asthma, because it mediates some responses to CysLTs, such as airway smooth-muscle-cell proliferation.\textsuperscript{121} Zileuton is a relatively weak 5-LO inhibitor, and has a short duration of action. However, in terms of clinical efficacy it is similar to the more potent antileukotrienes which perhaps indicates that more potent 5-LO inhibitors might be more effective clinically. 5-LO inhibitors block the generation of CysLTs, but also of the leukotriene LTB4 receptor, which might have a role in more severe asthma. However, LTB4 receptor antagonists (BLT1 antagonists) do not inhibit allergen-induced responses in asthmatic patients.\textsuperscript{136} The development of 5-LO inhibitors has been limited by liver toxicity, and although inhibitors of 5-LO-activating protein (FLAP) seem to be less toxic, they lacked efficacy in clinical studies.\textsuperscript{137}

5-Lipoxygenase (5-LO), present in cells of the myeloid lineage (e.g., polymorphonuclear leukocytes (PMNs), eosinophils, macrophages, etc.), is the first enzyme in the metabolism of arachidonic acid to leukotriene A4 (LTA4).\textsuperscript{138,139} which can be further metabolized to the peptidoleukotrienes (LTC4 and LTD4), potent vasoactive and spasmogenic mediators capable of inducing increased vascular permeability and bronchoconstriction, as well as leukotriene B4 (LTB4) and 5-hydroxyeicosatetraenoic acid (HETE), which function as chemotactic and chemokinetic mediators playing a central role in inflammatory cell infiltration. Current evidence suggests that several inflammatory diseases are associated with the presence and actions of leukotrienes. First, bronchoalveolar lavages and sputum from patients with asthma are replete with lipid-derived mediators including peptidoleukotrienes and LTB4.\textsuperscript{138} Elevated levels of these mediators have been associated with the key features of the disease including, increased vascular permeability, airway obstruction, mucus hypersecretion, and leukocytic infiltration of
pulmonary tissue. The improvement of acute airway function in asthmatic patients upon administration of either a peptidoleukotriene antagonist or a 5-LO inhibitor is strong evidence supporting the importance of peptidoleukotrienes in this disease.

Thus, compounds that restrict LT synthesis by inhibition of 5-LO will have therapeutic utility in such pathological conditions. Encouraging preliminary clinical results for some of these pathological conditions have been reported for zileuton (68), the most clinically studied 5-LO inhibitor. Recognition that the reduction of multiple lipid mediators via the inhibition of 5-LO theoretically offers a greater therapeutic benefit than a selective leukotriene antagonist has made the goal of developing effective 5-LO inhibitors an important therapeutic target. Within the field of 5-lipoxygenase inhibition, multiple strategies have been developed, and compounds derived from these efforts are currently being tested in the clinic.

Inhibitors of 5-LO can be separated into four distinct classes based on their putative mechanisms. Three of these classes of inhibitors interact with the 5-LO enzyme via a redox, a nonredox, or an iron ligand mechanism, while the fourth class blocks the association of the enzyme to the cellular membranes (FLAP inhibitors). Representative inhibitors of these four classes are shown below BW755C (72) (redox), ICID2138 (73) (nonredox), zileuton (68) (iron ligand), and MK886 (74) (FLAP inhibitor)).
Prostaglandin antagonists

Prostaglandin D2 (PGD2) (75), the major cyclooxygenase metabolite produced by mast cells in response to IgE-dependent stimuli,\(^{151}\) has a variety of inflammatory effects.\(^{152}\) Thus, PGD2 is considered to be an important mediator in various allergic diseases such as allergic rhinitis, atopic asthma, allergic conjunctivitis, and atopic dermatitis.\(^{153}\) Despite much speculation about the roles of PGD2, studies on its antagonists have only yielded BWA868C,\(^{155}\) which is used as a tool for pharmacological examination of the PGD2 receptor. There are also few reports on the efficacy of PGD2 receptor antagonists in animal allergic models or against human allergic diseases.\(^{156}\) We focused on the possible therapeutic value of selective PGD2 receptor antagonists in the treatment of various allergic disorders. Screening of prostaglandin derivatives revealed that (5Z)-7-[3-(biphenyl-4-sulfonylamino)bicyclo[2.2.1]hept-2-yl]hept-5-enoic acid, previously reported to be a thromboxane (TX) A2 receptor antagonist,\(^{156}\) exhibited fairly strong binding to the PGD2 receptor. With this seed compound in hand, we initiated structure-activity relationship (SAR) studies of R and \(\sigma\) side chains of the compounds. On the basis of our previous study on TXA2 receptor antagonists, \(\sigma\) side chain modification seemed to be the most important for enhancing the biological activities against the PGD2 receptor. These modifications pointed to the need for aromatic moieties linked with a proper spacer to be conjugated or the existence of a rigid structure. Since these studies revealed that compounds with the bicyclo[2.2.1]heptane ring system, which has fused aromatic rings, were effective for inhibiting PGD2 receptor binding, we synthesized and investigated various types of compounds to obtain a drug candidate. In an earlier paper, we reported that novel prostaglandin D2 (PGD2) receptor antagonists having the bicyclo[2.2.1]heptane ring system as a prostaglandin skeleton were synthesized as a potential new class of antiallergic agents. These compounds exhibited selective antagonism of the PGD2 receptor in radioligand binding and cAMP formation assays with IC50 values below 50 nM and exhibited much less antagonism of TXA2 and PGI2 receptors. Furthermore, they suppressed various allergic inflammatory responses such as those in rhinitis, conjunctivitis, and asthma models. Clearly, PGD2 plays an important role in the pathogenesis of allergic diseases,\(^{151}\) and support for this comes from a study of DP knockout mice recently reported by Narumiya et al.\(^{152}\)

We have been trying to develop PGD2 receptor antagonists having other prostaglandin skeletons in order to enhance the biological activities. As described in an earlier paper,\(^{1a}\) the bicyclo[2.2.1]heptane ring system was derived from a TXA2 receptor antagonist.\(^{153}\) At that time, we obtained two different types of prostaglandin (PG) skeletons, both of which exhibited strong
TXA2 antagonism. One (S-1452) is the bicyclo[2.2.1]heptanes ring system, and the other (S-6877) is the 6,6-dimethylbicyclo[3.1.1]heptane ring system\textsuperscript{154}. In the case of the bicyclo[2.2.1]heptane ring system, compounds having the enantiomer skeleton of S-1452, which is a strong TXA2 antagonist, exhibited strong PGD2 inhibitory activities. This suggested that 6,6-dimethylbicyclo[3.1.1]heptane ring derivatives having the enantiomer structure of S-6877, which also displays strong TXA2 antagonism, would have PGD2 inhibitory activity. However, the enantiomer skeleton of S-6877 was unsuitable for an SAR study and as a drug candidate because of its synthetic difficulty. We have therefore tried to investigate types of the 6,6-dimethylbicyclo[3.1.1]heptanes ring system that are regio- and stereoisomeric to S-6877 to find a new seed compound for PGD2 antagonists. Examination of the three types of regio- and stereoisomers having the sulfonamide moiety showed that compound with a \(1R,2R,3S,5S\)-6,6-dimethylbicyclo[3.1.1]heptane ring skeleton exhibited PGD2 antagonist activity. Several sulfonamides with this stereo structure were synthesized as done in the SAR study on the bicyclo[2.2.1]heptane ring system, but no better results were obtained. However, evaluation of compounds having the amide moieties revealed that the simple compound having the benzoyl group and its analogues of the \(\sigma\)-chain showed strong activity especially in the in vivo assay. No improvement of the antagonistic activity was noted for the other regio- and stereoisomers having the amide moiety, despite the conversion of the sulfonamide. We therefore initiated further SAR studies of the \(1R,2R,3S,5S\)-6,6-dimethylbicyclo[3.1.1]heptanes(76) ring system having the amide moiety. The results revealed that the compound with the benzothiophene-3-carbonyl moiety as the \(\sigma\)-chain exhibited fairly strong antagonistic activity against the PGD2 receptor. In this paper, we describe the synthesis and development of a new class of PGD2 receptor antagonists with the 6,6-dimethylbicyclo[3.1.1]heptane ring system having the benzothiophene-3-carbonyl moiety. Ramatroban (77), a thromboxane A2 receptor (TP) antagonist with clinical efficacy in asthma and allergic rhinitis, was recently shown to also antagonize the prostaglandin D2 receptor CRTH2. Here we report that minor structural changes to ramatroban result in a compound with complete lack of activity on TP but sub-nanomolar potency toward CRTH2. This is the first selective CRTH2 antagonist described to date, and should prove highly valuable in further elucidating the biological significance of CRTH2.

Deletion of prostaglandin D2 (PGD2) receptors in mice significantly inhibits inflammatory responses to allergen and AHR, which indicates that PGD2 might be important in asthma.\textsuperscript{157} PGD2 activates the chemoattractant receptor of TH2 cells (CRTH2), which is expressed on TH2 cells, eosinophils and basophils, and which mediates the chemotaxis of these cell types —
thereby providing a possible link between mast-cell activation and allergic inflammation. However, blocking the production of PGD2 with cyclooxygenase inhibitors has not been beneficial in asthma, antagonists could be beneficial in asthma. The selective H4 receptor antagonist JNJ 7777120 potently inhibits mast-cell activation and chemotaxis, and might therefore be of potential benefit in reducing asthma symptoms and exacerbations.

![Chemical structures](75-77)

**Endothelin antagonists**

Endothelin-1 (ET1) induces airway smooth-muscle-cell proliferation and promotes a pro-fibrotic phenotype, and might therefore have a role in chronic inflammation and airway remodelling in asthma. Several potent antagonists of endothelin receptors have been developed. But because both ETA and ETB receptors might be involved in bronchoconstriction and structural changes in asthma, the development of non-selective antagonists would be preferable. However, it would be difficult to detect the effect of a drug on slow remodeling processes in the absence of validated biomarkers.

**Nitric oxide inhibitors**
The concentration of nitric oxide (NO) in the exhaled air of asthma patients is higher than that of normal subjects, probably as a result of increased inducible NO synthase (iNOS) expression in airway epithelial cells and infiltrating inflammatory cells. An inhibitor of iNOS might therefore be useful in the treatment of asthma, particularly for restoring steroid responsiveness in patients with severe disease. Several potent and long-lasting iNOS inhibitors are now in development. For example, the prodrug L-N6-(1-iminoethyl)lysine-5-tetrazole amide (SC-51), which is rapidly converted \textit{in vivo} to the active metabolite L-N6-(1-iminoethyl)lysine (L-NIL), markedly reduces the levels of exhaled NO in asthmatic patients for several days after oral administration.

**Adenosine antagonists**

Adenosine is an endogenous nonselective agonist that activates all four subtypes of adenosine receptors (AdoRs): A1, A2A, A2B, and A3.1 Adenosine has been implicated to play a role in inflammatory airway diseases such as asthma. High adenosine levels are observed in the bronchoalveolar lavage (BAL) fluid and in exhaled breath condensate of asthmatics compared to those of normal controls. It is believed that the activation of the A2B AdoR on human lung mast cells leads to mast cell degranulation, releasing inflammatory cytokines (IL-4, IL-8, and IL-13). It has also been shown that the A2B AdoR subtype is the predominant AdoR expressed in bronchial smooth muscle cells (BSMC), and its activation increases the expression and release of interleukin-6 (IL-6) and monocytic chemotactic peptide-1 (MCP-1). The presence and functional coupling of human A2B AdoRs in different peripheral blood cells that play a role in immune and inflammatory process in which A2B AdoRs are thought to be involved have been recently characterized. Therefore, we choose to explore the potential of selective, high-affinity A2B adenosine receptor (AdoR) antagonists in the treatment of asthma. Prior to a description of our approach to obtain high-affinity A2B AdoR antagonists, we described relevant background information on human AdoR antagonists that influenced our design. Theophylline (I), 1,3-dimethyl xanthine, is a PDE IV inhibitor and a nonselective AdoR antagonist that has a $K_i$ of 9 $\mu$M for the A2B adenosine receptor (AdoR). Theophylline is currently approved for use in the treatment of asthma in both iv rescue therapy for acute asthma attacks and chronic oral treatment. Theophylline has a low therapeutic index due to both CNS and cardiac side effects. We hypothesize that a more selective A2B AdoR antagonist devoid of PDE IV activity may have an enhanced therapeutic index. Replacing the methyl groups of (I) with propyl groups as increases the A2B AdoR affinity ($K_i$) 610 nM without any enhancement in selectivity. Enprofylline (78), a 3-propyl xanthine derivative, has moderate
affinity for the A2B AdoR (Ki) 4.7 nM) and has moderate selectivity against the other AdoR subtypes also. Suzuki and co-workers have shown that substitution at the 8-position of xanthine with cycloalkyl groups increases the A1 AdoR affinity. For example, 8-cyclopentyl-1,3-dipropylxanthine, (79) (DPCPX), is a known A1 antagonist that also exhibits considerable affinity for the A2B AdoR (Ki) 56 nM. Several research groups have synthesized 8-phenyl substituted xanthines that have high A2B AdoR affinity and selectivity against the other AdoR subtypes. Jacobson and co-workers demonstrated that the introduction of a parasubstituted phenyl derivative at the 8-position of the xanthine core increases the A2B AdoR affinity and selectivity against the other AdoRs as illustrated by (80). As A2B AdoR antagonists, our main focus still remains on the xanthine class. Even though several A2B antagonists are known in the literature with high affinity, there are very few A2B AdoR antagonists known with good affinity and selectivity. Herein, we report the exploration of the 8-pyrazolyl xanthine derivatives represented by (82) as a new class of adenosine receptor antagonists with the goal of achieving high affinity for the A2B AdoR and selectivity over the other AdoRs. To our knowledge, there were no examples of 8-pyrazolyl xanthines evaluated as A2B AdoR antagonists in the literature prior to our exploration. Recently, Baraldi and co-workers have reported the 8-(5-pyrazolyl)-xanthines represented by structure (81) that typically contain the amide functionality found in the MRS-1754 (80) class of compounds. Our approach to the discovery of a selective, high-affinity A2B AdoR antagonist through the preparation of 8-(4-pyrazolyl)-xanthines was guided by a systematic optimization of the SAR.

Adenosine seems to activate mast cells via adenosine A2B receptors; antagonists of this receptor might therefore be of value in asthma, although it has been difficult to identify compounds that selectively target this receptor. Conversely, adenosine itself has an inhibitory effect on granulocytes, including eosinophils, and this action is mediated via adenosine A2A receptors, several selective A2A agonists are currently in development, such as CGS 21680, which inhibits allergic inflammation in rats. ATP also enhances the release of mediators from sensitized human mast cells via the P2Y2 receptor that is expressed on eosinophils, indicating that P2Y2 antagonists might also be beneficial in the treatment of asthma.
Tryptase inhibitors

The mast cell is proposed to play a central role in modulation of the inflammatory response and tryptase, a major mast cell secretory protease, is implicated as a key mediator of mast cell related allergic and inflammatory pathologies, including asthma. First generation tryptase inhibitor APC-366 provide support for the involvement of tryptase in asthma pathology and possibly other inflammatory diseases. As an extension of our efforts to identify novel tryptase inhibitors for development as antiinflammatory drugs, a series of analogues of the potent tryptase inhibitor APC-1390 (83) were synthesized. A major focus was to vary the terminal nitrogen base as a range of amino-, amidino-, and guanidino- derivatives while retaining an optimal core scaffold. Herein, we highlight the structure activity relationships (SARs) and structural requirements for potent tryptase inhibitory activity in a series of C2-symmetrical and asymmetric dibasic analogues of APC-1390 as well as the potent antiinflammatory activity of
lead analogue APC-2059 (84) in a sheep model of allergic asthma. Chemistry It is therefore believed that inhibition of tryptase may offer a therapeutic benefit for the treatment of asthma. Our preceding report4 described the identification of BMS-262084 (85) as a potent inhibitor of tryptase (IC50=4 nM) with moderate to good selectivity against related serine proteases but not for trypsin. In ovalbumin-sensitized guinea pig models, intratracheally dosed BMS-262084 demonstrated efficacy in preventing allergen-induced bronchoconstriction and in protecting against inflammatory cell infiltration into the lung. It was recognized that a significant portion of any inhaled drug would be swallowed; consequently, good selectivity against the gastric protease trypsin was considered highly desirable. This Letter, reported on their efforts to improve upon BMS-262084 by exploring the effects of conformationally constrained guanidino groups at C-3 and new substituents at N-1 of the azetidinone nucleus. These investigations led to the discovery of BMS-363131 (86) as a potent inhibitor of tryptase having excellent selectivity versus other serine proteases including trypsin and having improved hydrolytic stability (Figure R5).
Mast-cell tryptase increases the responsiveness of airway smooth muscle to constrictors, increases plasma exudation, potentiates eosinophil recruitment and stimulates fibroblast and airway smooth-muscle proliferation.\textsuperscript{191} Some of these effects are mediated by activation of the protease-activated receptor PAR2, which is widely expressed in the airways of asthmatic patients.\textsuperscript{192} The tryptase inhibitor APC366 is effective in a sheep model of allergen-induced asthma,\textsuperscript{193} but is only poorly effective in asthmatic Patients.\textsuperscript{194} Selective tryptase inhibitors with greater potency and selectivity are currently in development,\textsuperscript{195} for example, BMS-363131 has
nanomolar potency and is 3,000-fold more selective for tryptase compared with other serine proteases.\textsuperscript{196} There are several possible approaches to inhibiting specific pro-inflammatory cytokines, which are summarized in (Figure R5) Conversely, some cytokines that suppress the allergic inflammatory process might themselves have therapeutic potential in asthma.\textsuperscript{197,198}
Interleukin inhibitors

Interleukin-5 (IL-5) is essential in orchestrating the eosinophilic inflammation of asthma (Figure R6). The eosinophilic response to allergen in IL-5 gene knockout mice, and subsequent AHR, are markedly suppressed, and yet animals have a normal survival. Blockage of IL-5 has also been achieved using antibodies, and inhibits eosinophilic inflammation and AHR in primate models of asthma. Humanized monoclonal antibodies to IL-5 have been developed, and a single intravenous infusion of one of these antibodies (mepolizumab; GlaxoSmithKline) markedly reduces blood eosinophils for more than 3 months and prevents eosinophil recruitment to the airways after allergen challenge in patients with mild asthma. However, this treatment has no significant effect on the early or late response to allergen challenge or on baseline AHR, which indicates that eosinophils might not be of crucial importance for these responses in humans. Indeed, a clinical study of an anti-IL-5 antibody in patients with moderate to severe asthma that was not controlled by inhaled corticosteroids confirmed a reduction in circulating eosinophils, but no significant improvement in either asthma symptoms or lung function. In both of these studies it would be expected that high doses of corticosteroids would improve these functional parameters. These surprising results raise doubts about the supposedly crucial role of eosinophils in asthma and indicate that other strategies aimed at inhibiting eosinophilic inflammation might not be effective. However, although mepolizumab reduces circulating eosinophils by more than 95%, it is less effective at reducing eosinophils in bronchial biopsies (~50%), which might explain its lack of clinical efficacy. Nevertheless, this indicates that blocking IL-5 itself is unlikely to be useful as an anti-asthma strategy. Non-peptidic antagonists of the IL-5 receptor would be an alternative strategy, and would have the potential advantage of allowing oral administration. Molecular modelling of the IL-5 receptor α-chain and large-scale, high-throughput screening was used to discover YM-90709, a relatively selective inhibitor of IL-5 receptors. However, the lack of clinical benefit of anti-IL-5 antibodies makes this a less
an attractive approach. As well as its involvement in eosinophil recruitment to the airways, IL-4 plays a unique function in promoting differentiation of TH2 cells, acting at a proximal and crucial point in the allergic response. IL-4-blocking antibodies inhibit allergen-induced AHR, goblet-cell metaplasia and pulmonary eosinophilia in a murine model of asthma. A single nebulized dose of soluble humanized IL-4 receptor (sIL-4r) prevents the fall in lung function induced by withdrawal of inhaled corticosteroids in patients with moderately severe asthma, and weekly nebulization improves asthma control. Subsequent studies in patients with milder asthma proved disappointing, however, and this treatment has now been withdrawn. Recently, a heterodimeric soluble receptor containing each component of the IL-4 receptor (termed cytokine trap) has been shown to have a much higher affinity for IL-4, and might therefore be more useful. Another approach is to use a mutated form of IL-4 (BAY 36-1677) that binds to and blocks the IL-4 receptor and IL-13 receptor α1, thereby blocking both IL-4 and IL-13 actions. However, this treatment has a short duration of action. IL-4 and the closely related cytokine IL-13 signal through a shared surface receptor, IL-4Rα, which activates the transcription factor STAT6. Deletion of the gene encoding STAT6 has a similar effect to IL-4 gene knockout. This has led to a search for inhibitors of STAT6, and although peptide inhibitors that interfere with the interaction between STAT6 and Janus-activated kinases linked to IL-4Rα have been discovered, it will be difficult to deliver these intracellularly, and therefore small-molecule inhibitors are being sought through screening efforts. There is increasing evidence that IL-13 causes features in animal models that mimic asthma, including AHR, mucus hypersecretion and airway fibrosis, independently of eosinophilic inflammation. It potently induces the secretion of eotaxin from airway epithelial cells and transforms airway epithelium into a secretory phenotype. Knocking out the gene encoding IL-13 in mice, but not IL-4, prevents the development of AHR after allergen challenge, despite a vigorous eosinophilic response, and the increase in AHR induced by IL-13 is only seen when the expression of STAT6 is lost in airway epithelial cells. IL-13 signals through IL-4Rα, but might also activate different intracellular pathways via activation of IL-13Rα1, and its broad spectrum of effects makes it an important potential target for the development of new therapies. A second specific IL-13 receptor, IL-13Rα2, exists in soluble form and has a high affinity for IL-13, thereby acting as a decoy receptor for secreted IL-13. Soluble IL-13Rα2 is effective in blocking the actions of IL-13, including IgE generation, pulmonary eosinophilia and AHR in mice. In the murine asthma model, soluble IL-13Rα2 is more effective than IL-4-blocking antibodies, which highlights the potential importance of IL-13 as a mediator of allergic inflammation. Blocking IL-13 might be more important in established asthma, in which the concentration of IL-13 is much higher than
that of IL-4. Humanized IL-13Rα2 and anti-IL-13 antibodies are now in clinical development as therapeutic approaches for asthma. IL-9 is a TH2 cytokine that enhances TH2-driven inflammation, amplifies mast-cell mediator release and IgE production,\textsuperscript{216} and enhances mucus hypersecretion.\textsuperscript{217}

IL-9 and its receptors show an increased expression in asthmatic airways,\textsuperscript{218} correspondingly, a blocking antibody to IL-9 inhibits airway inflammation and AHR in a murine model of asthma.\textsuperscript{219} Strategies to block IL-9, including the use of humanized blocking antibodies, are now in development.\textsuperscript{220} IL-1 expression is increased in asthmatic airways\textsuperscript{221} and activates many inflammatory genes that are expressed in asthma. There are no small-molecule inhibitors of IL-1, but the endogenous cytokine IL-1 receptor antagonist (IL-1ra)\textsuperscript{222} was shown to reduce AHR induced by allergen in animal models. Human recombinant IL-1ra does not seem to be effective, however.\textsuperscript{223}
Several strategies are possible for the inhibition of eosinophil inflammation in tissues, including immunomodulators (for example, CyA, tacrolimus, rapamycin, mycophenolate, brequinar and suplatast tosylate), inhibitors of proinflammatory cytokines (for example, interleukin (IL)-4 and IL-5), inhibition of crucial adhesion molecules (for example, very late antigen-4, selectins, intercellular adhesion molecule-1), blockade of chemokine receptors on eosinophils (for example, chemokine receptor-3 (CCR3)) and induction of apoptosis by corticosteroids, lidocaine and p38 mitogen-activated protein kinase (MAPK) inhibitors.

Cytokines as asthma drugs

Allergic asthma is a chronic lung disease characterized by airflow obstruction, symptoms of cough, breathlessness and chest tightness, increased airway responsiveness together with evidence of airway inflammation. Inflammatory phenomena include denudation of the airway epithelium, edema of the submucosa, smooth muscle hypertrophy, and the infiltration or
activation of inflammatory cells, in particular eosinophils and T lymphocytes. The activated T lymphocytes belong to the Th-2 cell subpopulation and display a cytokine production profile that includes interleukin (IL)-4, IL-5, monocyte chemotactic protein (MCP)-1, MCP-2, and MCP-3. IL-4 induces the synthesis of IgE antibodies, whereas IL-5 is responsible for growth, differentiation, activation, and survival of eosinophils. MCP-1, MCP-2, and MCP-3 attract or activate basophils, monocytes, or eosinophils in the airways. Thus, suppression or antagonism of proallergic cytokines may represent a viable approach for the treatment of asthma.

Through compound library screening using the phytohemagglutinin (PHA)-activated human whole blood assay, we identified a series of 1-phenyl-substituted 6-azauracil derivatives with micromolar IL-5 inhibiting efficacy. Subsequent targeted chemical synthesis resulted in the characterization of R146225 (87) as a nanomolar potent, orally active IL-5 inhibitor capable of reducing the pulmonary infiltration of eosinophils in Cryptococcus-challenged mice. However, chronic dosing of 87 to pregnant rats and rabbits induced signs of teratogenesis and efforts to develop this compound were halted. However, convinced of the intrinsic antiasthmatic properties of compound (87), we sought to eliminate the teratogenic side effect by designing 1-substituted 6-azauracil-containing antedrugs, i.e., compounds that exert their desired topical effect in the lung (target tissue) but are rapidly converted into inactive/nontoxic metabolites after entry into the systemic circulation. Synthetic efforts aimed at combining the 1-substituted 6-azauracil pharmacophore with “labile” carboxylic ester functionalities led to the synthesis of a series of triazinylphenylalkylcarboxylic acid esters with lungspecific antedrug characteristics.

Although it might not be feasible or cost-effective to administer anti-inflammatory cytokines as a longterm therapy, it might in the future be possible to develop drugs that increase the release of these endogenous cytokines or activate their receptors and specific signal-transduction pathways. IL-10 inhibits the synthesis of many inflammatoryproteins that are overexpressed in asthma. Indeed, there might be a defect in IL-10 transcription and secretion from macrophages in asthma, which indicates that IL-10 might be defective in atopic diseases. In sensitized animals, IL-10 is effective in suppressing the inflammatory response to allergen, and cells that carry the CD4 antigen engineered to secrete IL-10 suppress airway inflammation in a murine model of asthma. Specific allergen immunotherapy results in increased production of IL-10 by a subpopulation of regulatory TH cells that are thought to mediate the beneficial effects of such immunotherapy. Recombinant human IL-10 has proved to be effective in controlling Crohn’s disease and psoriasis, a disease in which similar cytokines are expressed,
and can be given as a weekly injection. In mice, drugs that elevate cyclic AMP increase IL-10 production, but this does not seem to be the case in human cells.

Interferon (IFN)-γ inhibits TH2 cells and should therefore reduce atopic inflammation by blocking the release of IL-5, which drives eosinophilia, and of IL-4 and IL-13, which induce immunoglobulin E (IgE) formation. Administration of IFN-γ by nebulization to asthmatic patients does not significantly reduce eosinophilic inflammation, possibly because of the difficulty in obtaining a high enough local concentration in the airways. Specific immunotherapy increases IFN-γ production by circulating T cells in patients and has shown clinical benefit in asthma, immunotherapy also increased the number of IFN-γ-expressing cells in nasal biopsies of patients with allergic rhinitis. IFN-γ could be useful in the treatment of patients with severe asthma who have reduced responsiveness to corticosteroids. IL-12 and IL-18 have a synergistic effect on inducing IFN-γ release and inhibiting IL-4-dependent IgE production and AHR. however, there are no reported clinical studies of IL-18 in asthma. IL-12 regulates TH1 cell development and determines the balance between TH1 and TH2 cells, partly through the release of IFN-γ from TH1 cells to suppress TH2 cells. In patients with mild asthma, weekly infusions of human recombinant IL-12 in escalating doses over 4 weeks caused a progressive fall in circulating eosinophils, and a reduction in the normal rise in circulating eosinophils after allergen challenge. However, as with anti-IL-5 therapy, there was no reduction in either early or late response to inhaled allergen challenge or any reduction in AHR. Moreover, there was evidence of toxic side effects. Taken together, these findings indicate that recombinant IL-12 is not a suitable treatment for asthma. An IL-12–allergen fusion protein administered to mice resulted in the development of a specific TH1 response to the allergen, with increased production of an allergenspecific IgG2, rather than the normal TH2 response with IgE formation. The use of local IL-12 in conjunction with specific allergens might even be curative if applied early in the course of the atopic disease.
Chemokine receptor inhibitors

Monocyte chemoattractant protein-1 (MCP-1) is a major chemoattractant for monocytes and memory T cells through binding to its specific cell-surface receptor, CC-chemokine receptor-2 (CCR2). Animal model studies of chronic inflammatory diseases have demonstrated that inhibition of binding between MCP-1 and CCR2 by an antagonist suppresses the inflammatory response. MCP-1 and its receptor CCR2 have been implicated in inflammatory disease pathologies such as uveitis, rheumatoid arthritis, multiple sclerosis, allergic rhinitis, chronic obstructive pulmonary disease (COPD), allergic asthma, and solid tumors. Monocyte migration is inhibited by MCP-1 antagonists (either antibodies or soluble, inactive fragments of MCP-1) that have been shown to inhibit the development of arthritis, asthma, and uveitis. MCP-1 and CCR2 knockout (KO) mice have demonstrated that monocyte infiltration into inflammatory lesions is significantly decreased. In addition, such knockout mice are resistant to the development of experimental allergic encephalomyelitis (EAE, a murine model of human multiple sclerosis), cockroach allergen-induced asthma, atherosclerosis, and uveitis. Rheumatoid arthritis and Crohn’s disease patients have demonstrated a reduction in symptoms during the treatment with TNF-R antagonists (e.g., monoclonal antibodies and soluble receptors) at dose levels that correlated with decreases in MCP-1 expression and the number of infiltrating macrophages. MCP-1 has been implicated in the pathogenesis of seasonal and chronic allergic rhinitis, having been found in the nasal mucosa of most patients with dust mite allergies. MCP-1 has also been found to induce histamine release from basophils in vitro. During allergic conditions, allergens and histamines have been shown to trigger (i.e., to up-regulate) the expression of MCP-1 and other chemokines in the nasal mucosa of people with allergic rhinitis, suggesting the presence of a positive feedback loop in such patients. There remains a need for
small-molecule CCR2 antagonists for preventing, treating, or ameliorating a CCR2-mediated inflammatory syndrome, disorder, or disease. Many reports regarding the discovery of CCR2 antagonists have been published to date.\textsuperscript{269-281} Recently, we disclosed a series of potent phenylpiperidine-based CCR2 antagonists. Those compounds demonstrated good selectivity over CCR1, CCR3, and 5-HT, an excellent cytochrome P450 profile, and reasonable pharmacokinetics.\textsuperscript{282} Our search for more potent CCR2 antagonists led to the discovery of a series of substituted dipiperidines exemplified by the structure. In this communication, we report the synthesis, structure-activity relationships, and anti-inflammatory activities of these compounds. Initial SAR studies on the CH2 linker between the two piperidine moieties revealed that carboxylic acid derivative (88) showed a marked improvement in binding affinity to the human CCR2 receptor relative to the unsubstituted, amide, and ester analogues.

More than 50 different CHEMOKINES are now recognized to be involved in the recruitment of inflammatory cells via the activation of more than 20 different surface receptors.\textsuperscript{283} Chemokine receptors are G-protein-coupled receptors, which makes them amenable to small-molecule inhibitors — an approach that has not yet proved feasible for classical cytokine receptors.\textsuperscript{284} Another strategy is to use antibodies, which can produce a long duration of blockade and avoid some of the toxicity issues associated with many small-molecule inhibitors. Some chemokine inhibitors seem to be selective for single chemokines, whereas others are promiscuous and mediate the effects of several related chemokines several chemokines, including eotaxin, eotaxin-2, eotaxin-3, CCR1 (formerly known as RANTES) and macrophage chemoattractant protein-4 (MCP4) activate chemokine receptor-3 (CCR3) on eosinophils.\textsuperscript{285} Accordingly, there is increased expression of eotaxin, eotaxin-2, MCP3, MCP4 and CCR3 in the airways of asthmatic patients and this is correlated with increased AHR.\textsuperscript{286} A neutralizing antibody against eotaxin reduces both eosinophil recruitment to the lung after allergen challenge and the associated AHR in mice.\textsuperscript{287} Several small-molecule inhibitors of CCR3, including UCB35625, SB-297006 and SB-328437, are effective in inhibiting eosinophil recruitment in allergen models of asthma, and are currently undergoing clinical trials in asthma.\textsuperscript{288} There is also evidence for the expression of CCR3 on TH2 cells and mast cells, and these inhibitors might therefore have a more widespread effect in asthma treatment. Blocking MCP1-mediated activation of CCR2 on monocytes and T cells using neutralizing antibodies reduced the recruitment of both T cells and eosinophils in a murine model of ovalbumin-induced airway inflammation, with a marked reduction in AHR,\textsuperscript{287} and blocked the development of AHR in response to allergen in sensitized mice. MCP1 also recruits and activates mast cells, an effect that is mediated via CCR2.\textsuperscript{289}
MCP1 is instilled into the airways in mice, a marked and prolonged reduction of AHR associated with mastcell degranulation is observed. MCP1 is therefore an attractive target for asthma therapy, and small-molecule inhibitors of CCR2 are now in clinical development. CCR4 and CCR8 are selectively expressed on TH2 cells and are activated by the chemokines monocytederived chemokine (MDC) and thymus- and activationdependent chemokine (TARC), both of which are expressed in asthmatic airways. Inhibitors of CCR4 and CCR8 might therefore inhibit the recruitment of TH2 cells and persistent eosinophilic inflammation in the airways. However, deletion of the Ccr8 gene in mice has no effect on allergic inflammation, which indicates that this receptor might not be an effective target. The small-molecule compound AMD3 inhibits allergen-induced inflammation in a murine model of asthma by inhibiting CXCR4, which is selectively expressed on TH2 cells.

Matrix metalloprotease 12 inhibitor

Asthma is a chronic pulmonary disease that is characterized by airway inflammation, lung tissue remodeling, and progressive airflow obstruction that is reversible. This respiratory condition affects more than 300 million people worldwide, and this number is expected to grow due to increased prevalence with increasing age and environmental factors. Presently, there are only symptomatic therapies, and no disease-modifying drugs are available for this disease. Chronic inflammation and the pathologic degradation of the extracellular matrix (ECMa) of the bronchial wall may represent important causes of airflow obstruction in asthma. Matrix metalloproteinases (MMPs) have been suggested to be the major proteolytic enzymes that induce this airway remodeling. Macrophage metalloelastase (MMP-12) in particular, has been demonstrated to play a significant role in allergic airway inflammation and remodeling.
MMP-12 is the primary elastolytic enzyme of alveolar macrophages. Preclinical studies support blocking MMP-12 as a valid approach for therapeutic intervention in asthma. Specifically, MMP-12 deficient mice display markedly reduced airway eosinophilia and airway hyper-responsiveness in response to allergen. These mice also have less peribronchial fibrosis accompanied by reduced levels of R-smooth muscle actin and collagen type III deposition as detected by immunohistochemistry (IHC). Furthermore, transgenic animals that overexpress IL-13 develop alveolar and lung enlargements, compliance alterations, respiratory failure, and death that are, in part, mediated by MMP-12. MMP-12 also makes a critical contribution to the accumulation of eosinophils and macrophages within the lungs of these mice and plays an important role in the IL-13-mediated induction of mRNA for MMP-2, -9, -13, and -14. Significant increases in the expression of MMP-12 following antigen challenge or IL-13 exposure have been observed in both mouse and rat models of allergen-induced asthma. IHC analyses in these studies revealed that MMP-12 was primarily expressed in airway epithelia and alveolar macrophages. These findings are consistent with in vitro data that both human bronchial epithelial cells and human airway smooth muscle cells can also express and secrete MMP-12 upon stimulation with pro-inflammatory cytokines. Moreover, as detected by IHC, significantly increased levels of MMP-12 have been noted within airway smooth muscle of large airways in human fatal asthmatic patients when compared to nonasthmatics. Collectively, these findings provide support for the potential involvement of MMP-12 in the inflammatory response and tissue remodeling in asthma and its role in contributing to the development of disease pathology. Human MMP-12 is a 54 kDa proenzyme containing 470 amino acids composed of three domains: the pro-domain (9 kDa), the catalytic domain (22 kDa), and the hemopexinlike domain (23 kDa). The pro-domain includes a highly conserved cysteine residue that coordinates with the zinc ion to maintain the enzyme’s latency. The catalytic domain (22 kDa) bears the zinc-binding motif composed of three conserved histidines coordinated with the zinc ion. This is the domain that was used in our FRET assay for compound screening. The hemopexin domain is attached to the catalytic domain by a hinge region. The functions of this domain include substrate recognition, tissue inhibitor binding, and localization of the enzyme in the extracellular matrix compartment. Although MMP-12 is considered to be the most active MMP against elastin, its substrates have been identified to include many other extracellular matrix components. Those include fibronectin, fibrillin-1, laminin, entactin, type IV collagen fragments, chondroitin sulfate, proteoglycans, and vitronectin. In addition to inflammatory respiratory diseases, MMP-12 has been considered to be a therapeutic target for other chronic inflammatory, as well as musculoskeletal,
neurological, cardiovascular, and neoplastic diseases. Support for targeting these disease areas and MMP-12’s role in their disease pathophysiology has been obtained largely with animal models, including gene knockout and transgene studies. These, in part, include in vivo studies in models of multiple sclerosis, aortic aneurysm and atherosclerosis, and rheumatoid arthritis. Herein, we report our drug discovery efforts focused on targeting MMP-12 for asthma with the identification of a potent and orally efficacious compound, MMP145.

Transcription factor inhibitors

Many transcription factors are involved in the expression of inflammatory genes in asthmatic airways and are therefore possible targets for anti-inflammatory drugs. The pro-inflammatory signalling molecule NF-κB is naturally inhibited by inhibitor of NF-κB (IκB), which is degraded after activation by specific IκB kinases (IKKs). IKK2 is an isoenzyme that is important for activation of NF-κB by inflammatory stimuli. Selective inhibitors of IKK2 or the proteasome (the multifunctional enzyme that degrades IκB), and therefore of NF-κB, are currently in development. However, one concern about long-term NF-κB inhibition is that it could result in immune suppression and impair host defences. As an alternative, there are other pathways of NF-κB activation that might be more important in inflammatory disease and more amenable to long-term modulation. Cyclosporin A, tacrolimus and pimecrolimus inhibit T-lymphocyte function by inhibiting the nuclear factor of activated T-cells (NF-AT) by blocking activation of calcineurin. This results in suppression of IL-2, IL-4, IL-5, IL-13 and granulocyte-macrophage colony stimulating factor (GM-CSF), a cytokine that is important for eosinophil survival; these drugs therefore have therapeutic potential in asthma. However, cyclosporin A is of little value for treating chronic asthma, because the dose is limited by toxicity, in particular nephrotoxicity. Inhaled formulations of cyclosporin and tacrolimus are being tested for efficacy in asthma, but it remains to be determined whether this would provide a favourable therapeutic ratio. Rapamycin (sirolimus) has a similar action to calcineurin inhibitors, but acts more distally and has a better toxicity profile because it is not nephrotoxic; it can, however, induce hyperlipidaemia. GATA-binding protein-3 (GATA3) is important in the differentiation of TH2 cells and the expression of TH2 cytokines. Blocking GATA3 with an antisense oligonucleotide or a dominant-negative mutant prevents the differentiation of TH2 cells and the development of eosinophilic inflammation in mice, but the development of a small-molecule inhibitor of GATA3 could be difficult until the specific activation pathways for this transcription factor have been identified. An alternative approach is to activate the opposing transcription factor T-bet, the expression of which is reduced in asthma.
Kinase inhibitors

There has been particular interest in the p38 mitogen-activated protein (MAP) kinase pathway, which is involved in expression of several inflammatory proteins that are relevant to asthma.\(^{333}\) p38 MAP kinase is blocked by a novel class of drugs, the cytokine suppressant anti-inflammatory drugs (CSAIDs), which include SB203580, SB239063 and RWJ67657. These drugs inhibit the synthesis of many inflammatory cytokines, chemokines and inflammatory enzymes. Interestingly, they seem to have a preferential inhibitory effect on synthesis of TH2 compared with TH1 cytokines, indicating their potential application in the treatment of atopic diseases.\(^{334}\) Furthermore, p38 MAP kinase inhibitors decrease eosinophil survival by activating apoptotic pathways\(^ {335}\) and several inhibitors of p38 MAP kinase are now in Phase II development. p38 MAP kinase is also involved in corticosteroid resistance in asthma.\(^ {336}\) Whether this new class of anti-inflammatory drugs will be safe in long-term studies remains to be established; it is likely that such a broad-spectrum anti-inflammatory drug will have some toxicity, but inhalation might be a feasible therapeutic approach. Jun N-terminal kinases (JNKs) could be involved in activation of the transcription factor AP-1, which is activated in asthmatic airways, and small-molecule inhibitors have now been developed that have anti-inflammatory effects in allergen-exposed sensitized animals.\(^ {337}\) Steroid resistance in asthma is also associated with increased activation of JNKs,\(^ {338}\) indicating that JNK inhibitors could be useful in severe asthmatic patients with reduced steroid responsiveness. Several protein tyrosine kinases have been implicated in allergic inflammation. For example, Syk (p72Syk) kinase is pivotal in signalling of the high-affinity IgE receptor (FcεRI) in mast cells. In syk-deficient mice mast-cell degranulation is inhibited, which indicates that this might be an important potential target for the development of mast-cell-stabilizing drugs.\(^ {339}\) Syk is also involved in antigen receptor signalling of B and T lymphocytes and in eosinophil survival in response to IL-5 and GM-CSF.\(^ {340}\) Aerosolized Syk antisense oligodeoxynucleotide inhibits allergen-induced inflammation in a rat model, indicating that this could be a target for asthma drug development.\(^ {341}\) Lyn is a tyrosine kinase that acts upstream of Syk, and its inhibitor kinase, PP1, inhibits inflammation and mast-cell activation\(^ {342}\). Lyn is also involved in eosinophil activation and IL-5 signalling,\(^ {343,344}\) and a Lyn-blocking peptide inhibits eosinophilic inflammation in a murine model of asthma.\(^ {344}\) However, Lyn and Syk are widely distributed in the immune system, so there are consequently concerns about the long-term safety of selective inhibitors of these kinases.
Cell-adhesion blockers

Infiltration of inflammatory cells into tissues is dependent on the adhesion of blood-borne inflammatory cells to endothelial cells before migration to the inflammatory site. This requires specific glycoprotein adhesion molecules, such as integrins and selectins, on both leukocytes and on endothelial cells, which are upregulated and show increased binding affinity in response to various inflammatory stimuli. Monoclonal antibodies that inhibit adhesion molecules can therefore prevent infiltration by inflammatory cells. A monoclonal antibody against intercellular adhesion molecule-1 (ICAM1) on endothelial cells prevents the infiltration of eosinophils into airways and the increase in bronchial reactivity after allergen exposure in sensitized primates, although this has not been found in other species. The interaction between the α4 integrin very late antigen-4 (VLA4) and vascular cell-adhesion molecule-1 (VCAM1) is important for eosinophil inflammation. Small-molecule peptide inhibitors of VLA4 have been developed that inhibit allergen-induced responses in sensitized sheep and are now in clinical trials for asthma. Moreover, natalizumab (Antegren; Elan), a monoclonal antibody against α4 integrin, a component of VLA4, has recently been shown to be effective in Crohn’s disease, indicating its anti-inflammatory efficacy in humans. Inhibitors of selectins based on the structure of sialyl-Lewisx (particularly L-selectin, which is expressed on granulocytes and T lymphocytes) inhibit the influx of inflammatory cells in response to inhaled allergen in sensitized sheep and inhibit adhesion of human eosinophils in vitro. However, there could be potential dangers associated with inhibiting immune responses by preventing T-cell trafficking, because this could lead to an increased risk of infection and neoplasia. VCAM-1 is a key regulator of leukocyte trafficking to sites of inflammation and has been implicated in numerous inflammatory diseases such as asthma, rheumatoid arthritis (RA), and atherosclerosis. While it is endogenously expressed at very low levels in healthy tissues, increased expression of VCAM-1 has been observed in inflammatory disease states in humans and in animal models. Increased plasma levels of soluble VCAM-1 have also been observed in some patient populations. Antibodies directed against VCAM-1 and inhibitors of VCAM-1 expression have shown antiinflammatory effects in animal models. Natalizumab, a specific humanized monoclonal antibody to very late antigen-4 (VLA-4, the counter receptor of VCAM1), showed efficacy in treating patients with multiple sclerosis and might also be promising for patients with Crohn’s disease. The chalcone class of compounds, with a common 1,3- diphenyl-2-propen-1-one framework, has been known for over a century. Natural chalcones occur mainly as petal pigments and have also been found in the heartwood, bark, leaf, fruit, and root of
a variety of trees and plants. Chalcone-containing plants such as *Glycyrrhiza* species have long been used as folk remedies. Naturally occurring and synthetic chalcone compounds have shown interesting biological activity as antioxidant, antiinflammatory, anticancer, or antiinfective agents. Recently we disclosed the discovery of some chalcone derivatives as inhibitors of VCAM-1 expression. Herein we report on the lead evolution from our initial discovery, subsequent SAR studies, and biological activities of a novel series of carboxylated, heteroaryl-substituted chalcones.
ANTI-ALLERGY DRUGS

Anti-allergy drugs have the potential to more selectively target the atopic disease process. There are several approaches to inhibiting allergen-induced responses (Figure R7).

**Figure R7: Strategies to inhibit the allergic response underlying asthma.** Immunoglobulin E (IgE) can be inhibited by the antibody omalizumab (a) and low-affinity IgE receptors by anti-CD23 (b). Mast cells can also be blocked by cromones and furosemide (c), probably acting on a chloride channel and by inhibitors of Syk kinase, which inhibit the signal-transduction pathways activated by IgE receptors (d). Antigen presentation can be blocked by inhibitors of costimulatory molecules (e), including B7.2, CD28, inducible co-stimulatory molecule (ICOS) and cytotoxic T-lymphocyte antigen-4 (CTLA4). TH2 cells can also be directly inhibited by interferon-γ (IFN-γ), interleukin (IL)-12 and IL-18 (f).

**Cromones**

Sodium cromoglycate (cromolyn sodium) and nedocromil sodium are the most specific antiallergy drugs so far discovered, but their effectiveness is considerably less than low doses of inhaled corticosteroids, probably because of their short duration of action. Cromones have a specific action on allergic inflammation, yet their molecular mechanism of action remains
obscure. Although it was believed that their primary mode of action involves inhibiting mast-cell mediator release, cromones also inhibit other inflammatory cells and sensory nerves, and can act on and possibly inhibit certain types of chloride channels that are expressed in mast cells and sensory nerves.\textsuperscript{364,365} The identification of the molecular mechanism of action could aid the development of more potent and long-lasting cromone-like drugs in the future. Both cromoglycate and nedocromil must be given topically, and all attempts to develop orally active drugs of this type have been unsuccessful, which perhaps indicates that topical administration is crucial to their efficacy.

**Furosemide**

The diuretic furosemide (frusemide) shares many of the actions of cromones; for example, both classes of drug inhibit indirect bronchoconstrictor challenges but not direct bronchoconstriction (histamine, methacholine) when given by inhalation.\textsuperscript{366} The mechanism of action of furosemide is thought to involve inhibition of the same chloride channel that is inhibited by cromones. Furosemide itself does not seem to be very effective when given regularly by metered-dose inhaler in asthma,\textsuperscript{367} but it is possible that more potent and long-lasting chloride-channel blockers might be developed in the future.

**Anti-IgE**

The release of mediators from mast cells in asthma is IgE-dependent, and blocking the activation of IgE using antibodies that do not result in cell activation is therefore an attractive approach to treat asthma. A humanized monoclonal antibody directed to the high-affinity IgE-receptor (FcεRI)-binding domain of human IgE (omalizumab) has beneficial effects in the treatment of patients with asthma when given by subcutaneous injection every 2–4 weeks, particularly for those with severe steroid-dependent disease.\textsuperscript{368} Omalizumab is now approved for asthma therapy in some countries, but its high cost means that it is only likely to be used in patients with very severe disease that is not controlled by low doses of oral corticosteroids. However, the success of omalizumab indicates that small-molecule inhibitors of IgE-activated signal-transduction pathways might be beneficial.

An important drug recently approved for the treatment of asthma is the injectable humanized anti-IgE antibody omalizumab (Xolair).\textsuperscript{369} The effectiveness of this antibody validates IgE as an important mediator in atopic diseases and has led to an influx of smallmolecule IgE inhibitors under development for the treatment of asthma and allergic rhinitis.\textsuperscript{370} The latter compounds
work via various mechanisms to suppress IgE responses such as inhibiting the release of the Th2 cytokine IL-4. While their oral bioavailability provides an advantage over the anti-IgE antibody, previous work with omalizumab has shown that optimal therapeutic effectiveness requires greater than 95% suppression of IgE, a level that has been unachievable in vivo by small-molecule IgE inhibitors. Our objective was to address one of the foremost challenges in the field of asthma pharmacotherapy, to suppress disease development at its foundation without imparting significant liability to the patient. Herein, we describe a group of compounds that is novel in structure and apparent mechanism. These agents appear to act via a single target shared by multiple cell types to suppress their activation to allergic stimuli, including production of IgE and Th2 cytokines and expression of CD23 and IL-4 receptor-R. The lead compound in this series (AVP-13358) is orally active in mouse models of asthma and currently in phase I clinical trials for the treatment of asthma and allergic rhinitis. Identification of these compounds started with the premise that since IgE is central to allergic manifestations, a compound that interferes with the IgE response would act on a target that is fundamentally linked to its development. This allergic cascade was experimentally reconstituted in mice by immunizing BALB/c mice with an antigen (DNP-KLH) and adjuvant (alum) followed 2 weeks later by removing the spleen and establishing cultures of lymphocytes with specific antigen, in the presence and absence of drug. Allergen sensitization in vivo initiates the cascade of events that prepares the animal to produce IgE upon subsequent challenge. The response to allergen challenge (exposure), which is pertinent to the clinical situation, is thus reconstituted in the spleen cell culture, wherein equal numbers of B and T cells act in concert to produce IgE.

In this system, B cells likely function to present the introduced antigen (DNP-KLH) to T cells, which respond by producing IL-4 (and/or IL-13) and expressing costimulatory molecules such as the T cell receptor and CD40 ligand, which in turn activate B cells/plasma cells to produce IgE. Thus, although IgE is the downstream product of this experimental system, the assay identifies drug candidates that act on numerous potential targets within either of two cell types that are involved in the generation or propagation of allergic responses. To identify lead structures that would potentially interfere with this process, an in-house universal informer library (developed from over 300 000 compounds) was screened for biological activity using a cell-based ex vivo IgE response assay. This effort resulted in the identification of compounds with the 2-phenylbenzimidazole core structure and modest activity against the IgE response.
The evidence that the human eosinophils play a central role in the pathogenesis of bronchial asthma and an increased number of eosinophils in the circulation and BAL fluids has been reported as a characteristic feature of chronic bronchial asthma. Activation of allergen specific helper (CD4+) T-lymphocytes of Th-2 subset and subsequent release of cytokines including IL-3, IL-5, and GM-CSF, with IL-5 as the most likely to be specific to eosinophil proliferation and activation. The glucocorticoid dexamethasone supported this fact by inhibiting the activation of T-lymphocytes and also shows efficacy against pulmonary infiltration by eosinophils in actively sensitized rats.

The dual cyclooxegenase (COX) and lipoxygenase inhibitor BW-755C as well as PAF antagonist BN52021 and WEB2086 have inhibitory activity which suggests that arachidonic acid metabolites produced by COX and lipoxygenase or PAF, may be involved in eosinophilia. PDE inhibitors especially PDE-IV inhibitors prevent allergen induced lung eosinophilia in rats by directly or indirectly inhibiting the activation of eosinophils and/or cell responsible for attracting them into lungs.

The phenomenon of eosinophils is not fully understood but several derivatives have been found effective in eosinophilia models. GCC-AP0341 was found very potent in sephadex induced lung eosinophilia model in rats. It blocks the allergen induced airway hyper-reactivity in guinea pigs. This was the first that an eosinophilia inhibitor alone could be a suitable for the treatment of asthma. Other potent compounds based on GCC-AP0341 were
screened to confirm that eosinophilia is necessary for chronic asthma and airway hyper-reactivity. On the basis of this screening a highly potent eosinophilia inhibitor 3h was found.

The SAR studies based on the compound GCC-AP0341 were done and the effect of various ring systems was studied. The results are shown in (Table R2).

**TABLE R2:**

<table>
<thead>
<tr>
<th>No.</th>
<th>Structure</th>
<th>Mp(°C)</th>
<th>Formula</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1" alt="Structure 1" /></td>
<td>Oil</td>
<td>C₅H₈N₄S</td>
<td>27</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2" alt="Structure 2" /></td>
<td>*</td>
<td>C₃H₆N₆S</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3" alt="Structure 3" /></td>
<td>235-237</td>
<td>C₅H₈N₄S</td>
<td>-22</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4" alt="Structure 4" /></td>
<td>202-204</td>
<td>C₅H₈N₄S</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5" alt="Structure 5" /></td>
<td>Oil</td>
<td>C₆H₈N₂S₂</td>
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The SAR study was continued by determining the effect of substituents at the 3-position of the triazole ring and a series of phenyl derivatives obtained by the introduction of different substituents on the phenyl ring. The results are shown in the table 2.

The SAR study was continued by determining the effect of substituents at the 3-position of the triazole ring and a series of phenyl derivatives obtained by the introduction of different substituents on the phenyl ring. The results are shown in the (Table R 2)
Table R3:

<table>
<thead>
<tr>
<th>No.</th>
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<th>Mp(°C)</th>
<th>Formula</th>
<th>% inhibition</th>
</tr>
</thead>
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<tr>
<td>23a</td>
<td>Ph</td>
<td>174-175</td>
<td>C₁₀H₁₁N₅S</td>
<td>54</td>
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<tr>
<td>23b</td>
<td>Ph(4-Me)</td>
<td>180-182</td>
<td>C₁₁H₁₃N₅S</td>
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<tr>
<td>23c</td>
<td>Ph(4-Cl)</td>
<td>203-206</td>
<td>C₁₀H₁₀ClN₅S</td>
<td>(88)***</td>
</tr>
<tr>
<td>23d</td>
<td>Ph(4-CN)</td>
<td>213-216(dec)</td>
<td>C₁₁H₁₀N₆S.O₃H₂O</td>
<td>87</td>
</tr>
<tr>
<td>23e</td>
<td>Ph(4-OMe)</td>
<td>188-189</td>
<td>C₁₁H₁₃N₅OS</td>
<td>70</td>
</tr>
<tr>
<td>23g</td>
<td>Ph(4-NH₂)</td>
<td>172-174</td>
<td>C₁₀H₁₂N₆S.O₃H₂O</td>
<td>*</td>
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<td>23h</td>
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<td>261-262</td>
<td>C₁₄H₁₆N₆O₃S</td>
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<tr>
<td>23i</td>
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<td>190-192</td>
<td>C₁₂H₁₅N₅S₂.O₂H₂O</td>
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<tr>
<td>23j</td>
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<tr>
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<td>Ph(4-CF₃)</td>
<td>193-195</td>
<td>C₁₁H₁₀F₃N₅S</td>
<td>(91)***</td>
</tr>
<tr>
<td>25d</td>
<td>Ph(2,4-Cl₂)</td>
<td>192-193</td>
<td>C₁₀H₁₀Cl₂N₅S</td>
<td>(91)***</td>
</tr>
<tr>
<td>25e</td>
<td>Ph(3,4-Cl₂)</td>
<td>216-218</td>
<td>C₁₀H₁₀Cl₂N₅S</td>
<td>(85)***</td>
</tr>
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* All animals died. ** SE value for 23c was determined to be ± 4.2 from six experiments (n=30) *** Value in parentheses is % inhibition at an ip dose of 3mg/kg.

Compound 3h showed highly potent activity in the sephadex induced model but less effective against antigen induced airway hyper responsiveness model as compared with GCCAP0341. Compound 3h suppress the antigen (ascaris) induced hyper responsiveness in guinea pigs less than GCCAP0341 compared at an i.p. dose of 1mg/Kg. despite having highly potent activity for eosinophilia inhibition.

The effects of 3h and GCCAP0341 were examined on IL-5 mediated human eosinophil survival and 3h shows less activity as compared to GCCAP0341.

**SYNTHESIS AND RELEASE OF IL-5**

IL-5 was first isolated from supernatants of activated murine spleen cells which were shown to induce eosinophil colony formation. The isolated soluble activity was shown to stimulate eosinophil production from murine bone marrow selectively and was termed eosinophil differentiation factor. IL-5 was isolated from this soluble activity. It is produced by T-lymphocytes and an increased expression of IL-5 mRNA has been demonstrated in CD4+ T cells in asthmatic airways using in situ hybridization. Bronchoalveolar lavage CD4+ and CD8+ T cells can also secrete IL-5. Human eosinophils can express IL-5 mRNA and release IL-5 protein in vitro.384

**EFFECTS OF IL-5**

IL-5 can influence the production, maturation, and activation of eosinophils. It acts predominantly at the later stages of eosinophil maturation and activation and can also prolong the survival of eosinophils. IL-5 appears to be the main cytokine involved in the development of eosinophilia in vivo. Administration of exogenous IL-5 causes eosinophilia in many in vivo models. IL-5 transgenic mice behave normally indicating that eosinophils need other factors for degranulation and subsequent tissue damage. Thus, intratracheal administration of another eosinophil chemotactic agent, eotaxin, leads to further eosinophil accumulation in the lungs with bronchial hyper responsiveness and this effect not observed in wild type mice. IL-5 may cause eosinophils to be released from the bone marrow while local release of another
chemoattractant may be necessary to cause tissue localization of eosinophils. On the other hand, IL-5 instilled into the airways of patients with asthma induces significant airway eosinophilia and inhaled IL-5 caused eosinophilia in induces sputum and bronchial hyper responsiveness.

**BIOLOGICAL FUNCTIONS OF IL-5**

IL-5 has been shown to have string secretagogue effects on eosinophils. These effects are likely mediated via adhesion through the β2 integrin as a prerequisite for degranulation. IL-5 can also enhance eosinophil peroxidase levels \textit{in vitro} in comparison to IL-3 and GM-CSF.

The direct linkage of IL-5 with eosinophils has been demonstrated through several laboratory experimental systems. IL-5 transgenic mice, for example, have a constant high number of eosinophils in their circulation. On the other hand mice deficient in IL-5 fail to elicit a pronounced eosinophilic response to aeroallergen challenge or parasitic infections. Although these mice can generate a basal level of functionally normal eosinophils, they appear to be insufficient to mount a pulmonary inflammatory response to aerosolized antigens and for normal host defense against parasites.

IL-5 is unusual among the T-cell produced cytokines in being a disulphide linked homodimeric glycoprotein. It is highly homologous between species as indicated by the highly sequence homology between mouse and human IL-5 and the cross reactivity of the protein across a variety of mammalian species. Mature human IL-5 monomer comprises 115 amino acids (Molecular weight of 12000 and 24000 for the dimmer). Studies with mouse IL-5 indicate that the monomer has no biologic activity and has no inhibitor activity suggesting that they do not form high – affinity interaction with the IL-5 receptor (IL-5R).

**IL-5 RECEPTOR (IL-5R)**

The human IL-5R has been identified \textit{in vitro} on eosinophils but not a neutrophils or monocytes. It consists of a heterodimer with two polypeptide chains, a low affinity binding β chain and a non-binding β chain shared with IL-3R and GM-CSF. Both chains belong to the cytokine receptor superfamily. The α subunit alone is sufficient for ligand binding affinity and is specific for IL-5, but association with β chain leads to a 2-3 folds increase in binding affinity and allows signaling to occur.
The membraneous form interacts with β subunit, leading to a substantial increase affinity for IL-5. Some IL-5R mutants have antagonistic effects and may acts as receptor antagonists. The membraneous form is selected in body fluids and interacts with IL-5 and antagonizes the action of IL-5 on target cells.\textsuperscript{397, 398, 399} The expression of IL-5R is restricted to eosinophils and their immediate precursors to eosinophils.

There are two major signaling pathways of IL-5 in eosinophils. IL-5 activates the tyrosine kinases Lyn, Syk and JAK2 and propagates signals through the Ras-MAPK and JAK-STAT pathways. For eosinophil survival Lyn, Sky and JAK2 tyrosine kinases and SHP-2 tyrosine phosphatase are important, and for eosinophil degranulation and adhesion molecule expression Raf-1 kinase is critical.\textsuperscript{400}

Binding studies of IL-3, IL-5 and GM-CSF to human eosinophils showed cross inhibition suggesting some common components in the receptors for each cytokines.\textsuperscript{401, 402} Murine eosinophils have been calculated to express approximately 50 times high affinity receptors for IL-5. Recent work has shown that each of human receptors consists of α and a β-chain. The α chains are unique to each cytokine and form a group of homologous glycoproteins within the hematopoietin receptor super family.\textsuperscript{403} The α chain forms a low affinity bond with the analogous cytokine. The β-chain is common to all three receptors. It seems likely that the cross inhibition exhibited within the group is due to limiting number of β chains.

**IL-5 ACTIVITY IN VIVO**

In contrast to studies *in vitro*, experiments *in vivo* clearly indicate the central role of IL-5 in eosinophilia and indicate that experiments *in vitro* may be misleading, possibly because the culture conditions do not accurately reproduce the microenvironment of the bone marrow.\textsuperscript{404} The administration of anti IL-5 antibody to mice injected with *Nippostrongylus brasiliensis*, *Schistosoma mansoni*, Heligmosomoides polygyrus, or *Strongyloides venezuelensis* totally blocked the development of eosinophilia. These experiments show the essential role that IL-5 performs in the control of eosinophilia in such parasite infections.\textsuperscript{405} In addition, they show that the apparent redundancy seen *in vitro*, in which both IL-3 and GM-CSF are able to induce eosinophil production, does not operate in these infections.\textsuperscript{406}
EVIDENCE FOR THE ROLE OF IL-5 IN ASTHMA

Direct evidence for the role of IL-5 in airway hyper-reactivity and eosinophilia was obtained in a placebo-controlled study of inhaled IL-5 in patients with allergic bronchial asthma. Inhaled IL-5 increased airway responsiveness, infiltration of activated eosinophils in BAL fluids and showed elevated concentrations of eosinophilic cationic protein (ECP) in induced sputum.

Studies have also shown that the presence of IL-5 in induced sputum is a good indicator of eosinophilic inflammation in atopic and non-atopic asthmatics.\textsuperscript{407} Increased IL-5 production by BAL cells has been linked to an increased physiological response to allergen challenge.\textsuperscript{408} In a controlled cross-over study to evaluate the direct effect and time course of repeated low-dose allergen challenge an airway hyper-reactivity and airway inflammation, it was found that IL-5 production and eosinophilia were linked to hyper-responsiveness.\textsuperscript{409} The levels of IL-5 induced by Japanese cedar pollen induced allergic rhinitis were also linked to the episodic seasonal rhinitis in allergic patients.\textsuperscript{410} Intriguing data has also emerged from systematic studies of patients undergoing specific immunotherapeutic regimens where immunotherapy converts Th2 responses to Th1 or Th0 responses and is associated with clinical improvements.\textsuperscript{411}

IL-5 may play an important part in eosinophil maturation, chemo attraction and activation in asthma, and may underlie bronchial hyper-responsiveness. It may also interact with other eosinophil chemoattractants and activators such as chemokines to activate and induce chemoattraction of eosinophils.\textsuperscript{412} The studies with IL-5 monoclonal antibodies clearly support a role for IL-5 in asthma. Pre-treatment with anti IL-5 monoclonal antibodies can suppress allergen induced airway eosinophils. Studies with IL-5 monoclonal antibodies clearly support that IL-5 monoclonal antibodies can suppress allergen induced airway eosinophilia. There is some debate about whether the IL-5 induced eosinophilia is the direct cause of bronchial hyper-responsiveness induced by allergen exposure. In some studies there is an effect of anti-IL-5 antibodies on bronchial hyper-responsiveness,\textsuperscript{413, 414} which such an affect is not reported in another study despite inhibition of eosinophilia.\textsuperscript{415} In IL-5 knockout mice both allergen induced eosinophilia recruitment and airway hyper-responsiveness was abolished.\textsuperscript{416} Transgenic mice over expressing IL-5 in BAL fluid and serum, lung histopathological changes reminiscent of asthma, and display baseline airway hyper-responsiveness. On the other hand, studies in mice indicate that circulating but not local lung IL-5 is required for the development of antigen induced airways eosinophilia.\textsuperscript{417} Indeed, sensitization and allergen challenge of mice leads to an increase in IL-5 producing T cells in the bone marrow. In addition to its effects in mobilizing
eosinophil from the bone marrow, there is evidence for its effect as a regulator of eosinophil homing and migration into tissues in response to local chemokine release.