PART - 1

DESIGN AND SYNTHESIS OF SOME NOVEL HEXAPEPTIDE ANALOGUES RELATED TO IgE Fc FRAGMENT (330-334) AS ANTI-ALLERGIC/ANTI-ASTHMATIC AGENTS
CHAPTER - 1

ASTHMA : AN OVERVIEW
1. INTRODUCTION

Asthma is a complex disorder characterized as chronic inflammatory disease of the airways combined with nonspecific bronchial hypersensitivity to a variety of stimuli and manifests as episodes of coughing, wheezing, chest tightness and shortness of breath. The airway obstruction results from both contraction of airways smooth muscle and excessive bronchial edema. Edema, characteristic of inflammatory states, is accompanied by the formation of a viscous mucus which can completely block the small airways. The atopic diseases - allergic rhinitis and asthma are among the most widely spread afflictions of industrialized countries. Epidemiological studies suggest that the prevalence severity, cost of care and mortality are rising at a time when mortality from other treatable conditions is falling. Asthma prevalence has almost doubled in western countries in the last 20 years and this can be attributed to the worldwide increase in environmental pollutants and allergens and, as a result, greater human exposure to vital respiratory infections. In the United states alone, there are 5000 deaths each year and the rate continues to increase.

Bronchial asthma is an obstructive lung disorder that can not be cured at present and are usually associated with bronchospasm, mucosal oedema and mucous secretion. During an asthma attack, the tissue of the airway inner wall is inflamed and mucous is thick and sticky. Continuing production of mediators and messenger substance of inflammation such as histamine and leukotriene keeps the process going.

Many different factors have been suggested as possible causes for the increased asthma encumbrance in urban populations. These include over crowded living environments, perennial indoor allergens, dysfunctional family situations, deterioration of dwelling facilities along with exposure to pollutants, and inaccessibility to health care. Other factors may be influenced by patient-physician interaction and include poor patient understanding of the disease process, lack of adherence to prescribed medical regimens, and inability to deliver medications properly (particularly metered dose inhalers). In addition, the possibility exists that as a result socioeconomic factors, physician care of inner-city asthmatic patients may
be inadequate in terms of state of the art medical therapy, patient education and therapeutic steps necessary to minimize morbidity during exacerbation.

Some times asthma is even provoked by cold air and exercise, regarding this a mechanism\textsuperscript{12} has been suggested that cold air itself or rapid, deeper breathing during exercise cools all the airways. In response, increased amount of blood move into the capillaries surrounding the airways to rewarm the airways tissue. This large amounts of blood in the abnormally plastic capillary beds of asthmatics causes collection of fluid in tissues thereby constricting the airway.

Since asthma has been found to affect people of all ages and from all walks of life, two major classification has been made: (i) allergic and (ii) non allergic. Allergic atopic asthma has been called extrinsic, because the cause of an episode comes from outside the system whereas non allergic nematopic asthma has been called intrinsic because it just "happens". Recent studies\textsuperscript{13} suggest that the evidence of atopy shows up better at some ages than others. The true incidence may be 100% and all asthma may be of the allergic type. The original cause of asthma, then may be the immune system "learning" to respond to antigens with immunoglobulin E (IgE). There seems to be a time in earliest childhood when the immune system learns preferentially to mount IgE response to antigens in particular organs and tissue, with the result that the person develops allergic dermatitis, hay fever or asthma.

Both intrinsic as well as extrinsic asthmatics are known to be prone to exercise induced asthmatic attacks. Those individuals who experience a combination of extrinsic and intrinsic asthmatic reactions have mixed asthma. Status asthmatics refers to an acute life threatening attack which is generally resistant to normal treatment and often demands hospitalisation of the patient.

2. **CELLS AND MEDIATORS INVOLVED IN ASTHMA:**

Asthma is a disease of unknown etiology and involves many types of inflammatory cells and mediators, that have multiple effects on the various target cells in the airways. Important advances have been made in the understanding of biochemical events involved in signal transduction in inflammatory cells, in mediator
synthesis and release, and in the contraction and relaxation of airway smooth muscle. This may lead to the development of more effective and specific therapies for the treatment of asthma.

2.1 **Inflammatory Cells:**

2.1.1 **Mast cells:**

These are present in the loose connective tissues of all organs\(^{14}\), airway epithelium, near blood vessels, in the submucosa, adjacent to submucous glands, scattered throughout the muscle bundles, in the intra-alveolar septa and in the bronchial lumen. They are assumed to play a central role in the pathogenesis of asthma. Mast cells when activated through its high affinity immunoglobulin E (IgE) receptor, releases a diverse array of biologically active molecules, including cytokines, leukotrienes, prostaglandins, amines, proteoglycans and the protease. The presence of activated mast cells in the bronchial airways of individuals with asthma suggests that this hematopoietic effector cells play an important role in the pathophysiology of asthma. In allergic individuals, inhalation of specific allergens leads to mast cell degranulation. Mediators released from these cells loosen the intra-epithelial cell tight junctions allowing entrance of more allergen to deeper mast cells\(^{15}\). The primary and secondary mediators induce further increased vascular permeability, leading to the entrance of plasma proteins as well as platelets. The other immediate responses induced by mediator release are smooth muscle constriction and mucus secretion. Thus mast cells get activated by either inhaled allergen via an IgE dependent mechanism by physical factors such as hyper- or hypo-osmolar solutions. They possess high-affinity receptors (FceRI) which bind IgE and the F (ab\(_2\)) fragment functions as the recognition site for specific antigenic binding. The physiologic consequences of exposing IgE- sensitised mast cell to antigen against which the IgE molecule is directed result from the secretion of mast cell granules from which are derived the mediators of anaphylaxis.
2.1.2 Platelets:

Though platelets have classically been considered for their well recognised role in haemostasis and thrombosis, it is now several years since the first report appeared in the literature that platelets can possess low affinity (FcεRI) for IgE receptors\textsuperscript{16} and that the activation of platelets via an immunoglobulin E (IgE) dependent mechanism was central to mounting a successful allergic response against parasite infections\textsuperscript{17}. These observations suggest that platelets and platelets activating factor (PAF) may be involved in the pathogenesis of asthma\textsuperscript{18}.

In addition to this other substances believed to play a role in the inflammatory process have been demonstrated to activate platelets without necessarily causing platelets aggregation. Other forms of platelets activation, which may be independent of classical aggregation and secretion are supported by the observations, that existing antiallergic agents such as Cetirizine\textsuperscript{19} and disodium cromoglycate (DSCG)\textsuperscript{20} have been observed to inhibit IgE activation of platelets. Whilst these drugs are ineffective against classical platelets aggregation. Platelets have been shown to release a variety of agents such as platelet factors 4 (PF\textsubscript{4}) (a chemoattractant for neutrophils, monocytes\textsuperscript{21} and eosinophils\textsuperscript{22}), various lipooxygenase metabolites of arachidonic acid metabolism (e.g. 12-HETE, a chemoattractant for neutrophils\textsuperscript{23}), cationic proteins (Which can induce vascular permeability and cause neutrophil accumulation)\textsuperscript{24} as well as a range of mediators capable of contracting airway and/or vascular smooth muscle.

2.1.3 Basophils:

Like mast cells basophils have also high affinity receptors for IgE antibody and contain histamine and other markedly proinflammatory mediators. Basophils, which may be thought of as the circulating form of the mast cell, infiltrates tissues and is responsible for mediators release in the late phase response to antigen which constitute a type of inflammation which is now felt to be characteristic of chronic allergic diseases such as asthma. Early investigators of basophils viewed them as surrogates
for tissue-fixed mast cells, which were much more difficult to isolate and study. The latter is known to exist in the respiratory mucosa and are assumed to be responsible for disease process such as rhinitis and asthma. Basophils were found to be elevated in peripheral blood and nasal secretions of subjects with allergic rhinitis, and in the peripheral blood and bronchial secretions of atopic subjects with asthma.

It is no longer tenable to consider the basophil a surrogate for the mast cell. The basophil is a far more excitable cell, releasing mediators not only in response to IgE related stimuli but also to other putatively relevant stimuli such as complement products (C3a, C5a), formylated bacterial products, cytokines including IL-1, IL-3, IL-8, GM-CSF, histamine-releasing factors and platelet activating factor. Of all the stimuli mentioned above which activate the basophil, only anti-IgE is the effective secretogogues for all mast cell studied to date.

### 2.1.4 Eosinophils:

As efforts to establish the pathogenesis of asthma have begun to focus upon mechanism of bronchial inflammation, the eosinophil has emerged as an important cell in the process, e.g. increased number of eosinophils are found in the circulation of asthma patients, usually in relationship to the severity of asthma. Eosinophils are also a prominent cell in sputum and lavage fluid from patients with asthma. Furthermore, bronchial biopsy and histological specimens from patients dying of asthma demonstrate an increase in tissue eosinophils. Finally corticosteroid treatment of acute asthma reduces sputum and blood eosinophils as well as airway obstruction. These clinical observations imply an association between eosionophils and asthma; recent evidence extends this hypothesis and suggest that eosinophils may have a role in the pathogenesis of asthma.

Eosinophil mediators which may contribute to asthma may be either granule associated or membrane-derived. Membrane-derived mediators include eicosanoids, whose generation follows activation of 5- and 15-lipoxygenase pathway and platelet activating factor (PAF) which is the predominant sulfidopeptide leukotriene generated
and a potent bronchial smooth muscle contractor. Eosinophils granules contain several basic protein which can directly contribute to asthma and bronchial hyperresponsiveness\textsuperscript{40}. These granule protein's include major basic protein (MBP), eosinophil cationic protein (ECP), eosinophil derived neutrotoxin (EDN) and eosinoperoxidase (EPO). Of these granule proteins MBP has been extensively studied and shown to be a likely participant in asthma.

2.1.5 Neutrophils:

The neutrophil is the predominant circulating white blood cell (WBC) that has an essential role in local host defence mechanism. Complex mechanism exist to detect sites of infection or injury and trigger the local extravascular release of chemical signals that stimulate the emigration of neutrophils from blood microvessels. The survival of rare individuals with genetic defects in the mechanism involved in this process is severely compromised and these patients suffer from serious recurrent infections. Asthma appears to result from an inappropriate overreaction of the inflammatory response. Thus, neutrophils may contribute to tissue dysfunction in the lung.

In some types of reactions waning of neutrophil number coincides with increased number of monocytes, T-lymphocytes, basophils and eosinophils. These cells persist in the tissues for much longer than neutrophils. Because of the chronic nature of asthma, leukocytes that persist in the lung tissue, especially eosinophils, macrophages and lymphocytes, have become more strongly associated with asthma symptoms. Because of the chronic nature of asthma the importance of the neutrophil is contentious and much more attention is being directed towards the cells of chronic inflammation such as eosinophils, macrophages and lymphocytes. This may be well justified: however, neutrophils have been shown to be important in certain animal models of asthma and these cells do appear in man during the critical phase between immediate bronchial smooth muscle reaction and late reactions leading to hyperresponsiveness. Thus neutrophils may be involved in this link by facilitating the
accumulation of other leukocyte type. Alternatively, neutrophils may cooperate with eosinophils in producing spasmogens.

2.1.6 Airway epithelial cells:

Under healthy physiological conditions the respiratory epithelium lines the airways system and forms a continuous barrier to the diffusion of airborne particles that may be inhaled and passed to the lower regions of the respiratory tract. In patients with asthma bronchial hyperresponsiveness to various spasmogens is associated frequently with a damaged respiratory epithelium. The first evidence to suggest a direct correlation between the functional integrity of the epithelium and airways hyperreactivity came from experiments on canine isolated bronchi.

The airway epithelial cells may release lipid mediators (cyclo-oxygenase and lipoxygenase product) but may also secrete a bronchodilator substance termed epithelium-derived relaxant factor (EpDRF). If epithelial cells are shed or damaged in asthma the loss of EpDRF may increase bronchoconstrictor response although its effect is likely to be relatively small. Epithelial cells also express various enzymes that degrade inflammatory mediators, thus acting as a metabolic barrier under normal conditions.

The influence of the epithelium on the tone of airway smooth muscle varies throughout the respiratory system. e.g. epithelium-dependent relaxations to isoproterenol becomes more pronounced with the narrowing of the bronchi whereas an inverse relationship is observed in response to arachidonic acid.

In asthma the epithelium may be damaged or dysfunctional thereby leading to the development of bronchial hyperresponsiveness. Thus, it is clear that epithelium has an important role to play in the modulation of airway smooth muscle tone and could be an important site for amplification of the immune response and be a target for drug therapy.
2.1.7 T-Cells:

Although T cells are known to play a central role in initiating and maintaining pulmonary inflammation and airway obstruction in asthma, little consideration has been given to their role in establishing baseline airway responsiveness. Several factors contribute to airway responsiveness including genetic predisposition, geometric factors related to airway caliber, autonomic neural pathways and bronchial inflammation.

Recently De Sanctis et al\textsuperscript{52} reported that in the absence of a defined antigen challenge or gross tissue inflammation, airway responsiveness is also regulated by T-cells. A number of additional critical issues emerge from the De Sanctis study. Passive transfer of T-Cells in the absence of antigen challenge has generally been insufficient to transfer hyporesponsiveness\textsuperscript{53}. Environmental factors may be contributing significantly to such effects. Although the experimental animals were housed in a protected environment, they were not maintained under germ free conditions. Low level exposure to antigen may simply have unmasked an interstrain difference in the ability of the T-cell to be activated by such stimuli, resulting in the release of certain cytokines or other factors which regulate airway responsiveness\textsuperscript{54}.

Although other cell types (mast cell, basophils, eosinophils) may contribute to the production of cytokines such as interleukin (IL-4, IL-5, IL-3), it appears that T-cells are essential for developing an allergic inflammatory response. T-cells deficient animals fail to develop eosinophilic infiltration of the airways or altered airway function following sensitization and challenge to allergen. The role for T-cells in the development of altered airway function is presumably through the elaboration of cytokines and not a direct contact effect of T-cells on the airways.

However, to what extent these finding can be extrapolated to the human condition is not yet clear? The control of the biological trails of airway hyperresponsiveness and asthma by genes and their environmental interactions are obviously very complex. Multiple cell types and numerous cellular control
mechanisms are involved. In mice at least three genetic loci have been linked to the control of airway hyperresponsiveness. In humans there is also evidence of heritability of airway hyperresponsiveness, and it appears that alleles at multiple single loci are involved in the complex etiology of asthma. The pivotal role of T-lymphocytes, which was previously defined in response to allergen challenge, now appear to extend into establishing native or baseline levels of airway responsiveness in the absence of identified environmental influence.

3. **ROLE OF CYTOKINES:**

Mast cells, for many years have been known to play a central role in the pathogenesis of asthma. The traditional understanding has been that these cells are activated as a result of the interaction of allergen with IgE coated mast cells. This in turn, releases a series of performed and rapidly synthesised substance that mediate the onset of vasodilation, vascular leakage, smooth muscle contraction and irritant nerve receptor stimulation. Recent studies, however, have established that in addition to the mast cell activation, allergen can interact with and activate T-lymphocytes and mononuclear phagocytic cells, leading to the secretion of cytokines and other inflammatory substances. In fact, the interaction of allergen with immune system is a complex cascade which is capable of producing the chronic inflammatory changes characteristic of allergic disorder. The interaction of allergen with the immune system also promotes the secondary release of inflammatory neuropeptides. Thus, the known spectrum of mediator released after allergen exposure has vastly expanded. These include numerous chemotactic and activating peptides, leukotrienes, platelet activating factor, several proteases, neuropeptides and most importantly the cytokines. These mediators in turn, initiate infiltration of bronchial mucosa and epithelium with activated eosinophils, neutrophils, monocytes, basophils macrophages, T-lymphocytes, and induced mast cell proliferation with further mast cell degranulation. Eventually, subepithelial fibrosis occurs, with irreverible obstruction.
Several cytokines have been found to be associated with allergic diseases and may contribute to the characteristic inflammatory state. In the bronchoalveolar lavage fluid obtained from asthmatics and patients with allergic rhinitis, the number of cells expressing m-RNA for IL-2, IL-3, IL-4 and IL-5 have been found to be significantly increased. Further, in the pathogenesis of asthmatics, higher levels of IL-4 and IL-5 were observed, than nonatopic controls.56 These cytokines which are released from T-cells, partially control the two major components of allergic responses: IgE production and eosinophilia. Production of IgE by B lymphocytes depends primarily on IL-4 which functions as switch factor and is enhanced by IL-5 and IL-13 whereas gamma-IFN, IL-8 and IL-12 antagonises the IgE-production. This is evident from the studies in IL-4 knockout mice which have demonstrated that IgE production in vivo is almost entirely dependent on IL-4 cosignal at the time of antigen presentation.57 Another cytokine that is important in the regulation of IgE synthesis is gamma-IFN. It acts as an inhibitor of allergic responses through its capacity to inhibit the effect of IL-4 on B-cells.

On the other hand generation, differentiation and recruitment of eosinophils, the second component of allergic disease and which is the most critical determinant for the development of inflammation, is governed by IL-5.56 For a long time it has been known that eosinophilia accumulate in high numbers in the lung of asthmatics and their presence and numbers correlate with the degree of lung dysfunction.

Further the commitment of TH⁰ cells to TH² phenotype has been also found to be controlled by IL-4 and this is evident by the extensive documentation demonstrating the presence of TH² cells or TH² pattern cytokines in human allergy and asthma.58 In addition to this another cytokine that favours the progression of TH² responses indirectly by suppressing gamma-IFN production is IL-10, derived from TH² cells. These findings clearly suggest that the presence of soluble cytokine(s) signal at the time of TH⁰ committment is more crucial than the type of inhaled allergen in determining the nature of the airway immune response.
Thus, attempts to understand the role of cytokines as well as the mechanism as to why immune response becomes shifted towards TH\(^2\) response has provided novel therapeutic approaches for asthma, e.g. whereas on one hand alpha-IFN, gamma-IFN and IL-12 represent potential therapeutic target for redirecting TH\(^2\) commitment and suppressing TH\(^2\) function, on the other hand, blocking the activation or preventing the synthesis of IL-4, IL-13, IL-10 or IL-5 are an important therapeutic approaches that may inhibit the effects of established TH2 cells in human asthma.

4. FACTORS AND RECEPTORS INVOLVED IN ASTHMA

4.1 Nuclear Factor- KB:

The inflammatory response in asthma is similar to the defence mechanism mounted against worms and parasites, which is beneficial and self-limiting in the case of these infectious diseases, but in the case of asthma persists due to continued exposure to allergens. The inflammatory response in asthma is due to the increased expression of genes that encode inflammatory protein, such as cytokines, chemokines, enzymes, receptors and adhesion molecules. These inducible genes are regulated by transcription factors which are activated extracellularly and bind to the promoter region of 'inflammatory' genes to increase their rate of transcription. Although many transcription factors are involved in the regulation of these inflammatory genes, one nuclear factor, KB (NF-KB), appear to be of particular importance\(^{59}\).

NF-KB, first identified as a factors that regulates k-Light chain expression in murine B-lymphocytes, is present in most cell types and plays a key role in immune and inflammatory responses\(^{60,61}\). It is made up of two submits which belong to the Rel family. Many stimuli activate NF-KB, including proinflammatory cytokines, oxidants, viruses and activators of protein kinase C (PKC). Reactive oxygen intermediates may play an important role in mediating the activation of NF-KB, and oxidants such as pyrroline dithiocarbamate and N-acetyl cysteine, may inhibit the activation of NF-KB in response to a variety of stimuli\(^{62}\).
Many of the stimuli that increase inflammation in asthmatic airways result in the activation of NF-KB. These include pro-inflammatory cytokines that may be released from macrophages and other inflammatory cells on exposure to allergens. Recent studies suggest that increased activation of NF-KB in the airways of asthmatic patients, with predominant localization by immunocytochemistry to epithelial cells and macrophages. Many of the inflammatory proteins that are expressed in asthmatic airways are regulated at least in part, by NF-KB. These include: (a) the pro-inflammatory cytokines IL-1β and TNF-α that may amplify airway inflammation particularly in several disease (b) granulocyte macrophage colony stimulating factor (GM-CSF) that prolong eosinophil survival and (c) chemokines, MCP-3 (monocyte-chemotaetic protein-3) and eotaxin, that attract eosinophils into the airways. Adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) that are involved in eosinophil recruitment into the airway from the circulation are also regulated via NF-KB. Thus NF-KB may lead to the co-ordinated expression of inflammatory gene in asthmatic airways and may result in the amplification and perpetuation of the inflammatory process.

Oxidants activate NF-KB in epithelial and other cells and may play in asthmatic inflammation. A plausible explanation for the world-wide increase in asthma is reduction in the dietary intake of antioxidants such as vitamin C and E. Asthma may be more common in the areas with a low dietary intake of selenium as this is an essential co-factor for glutathione peroxidase one of the most important antioxidant enzymes in the lung.

NF-KB is a compelling target for the development of new anti-inflammatory drugs for asthma. Antioxidants have the ability to block activation of NF-KB in response to a wide variety of stimuli and drugs, but do not block all of the effects of NF-KB inhibitors may also be useful in other chronic inflammatory disease where current anti-inflammatory therapy is often difficult.
4.2 Adenosine Receptors:

Over the past decade, widely recognized growth in the incidence of asthma has increased the need for new therapies. The aim of any asthma therapy is to control the inflammation and hypersensitivity of the airways that result from the condition. The purine adenosine has been implicated in both the development of bronchial hypersensitivity and the control of inflammation. So adenosine receptors have been suggested as possible therapeutic targets.

Recently it was confirmed that adenosine is involved in an animal model of allergic asthma by using antisense oligodeoxyneucleotides (ODNs) to reduce the number of adenosine $A_1$, receptors in the lung. The observed decrease in the constriction of airways confirms that the adenosine $A_1$, receptor is a potential therapeutic target for allergic asthma.

Two interesting changes that occur in the human lung with the onset of asthma are an increase in the mucosal concentration of adenosine and the appearance of the adenosine $A_1$ receptor. The $A_1$ receptor is one of the four known cell-surface adenosine receptor and it is very difficult to detect in normal lung tissue. But in the asthmatic lung its presence is associated with the ability of adenosine to induce bronchoconstriction - an effect that is not seen in normal lungs. So adenosine and the $A_1$ receptors seem to be central players in the developments of airways hypersensitivity and the inflammatory response to allergens.

These findings suggest that adenosine is an important mediator of both airway obstruction and inflammation and that some portion of these effects are mediated through the pulmonary adenosine $A_1$ receptor in the asthmatic lung. They further indicate that the lung may have great potential as a target for antisense ODN-based disease intervention in asthma and related lung pathologies.
5. THERAPEUTIC APPROACHES TO ASTHMA:

Several new drugs are now under development for the treatment of asthma including those representing an improvement over existing classes of effective drugs, those based on rational drug design and finally those arising from chance observations.

5.1 Bronchodilator:

Patients with asthma suffer intermittent episodes of wheezing so the use of a rapid acting inhaled bronchodilator is a valuable treatment strategy. The frequency of 'as needed' use serves as a rough guideline to the degree of asthma control and the need for supplemental preventive medication. The main bronchodilators used in the critical care setting are summarised below-

(a) B<sub>2</sub> adrenoceptor agonists:

The autonomic nervous system is known to be responsible for regulating airway tone through the release of neurotransmitters that activates specific autonomic receptors. It is comprises the cholinergic (parasympathetic) system and the adrenergic (sympathetic) system. The adrenergic system controls branchdilation via cyclic adenosine monophosphate (cAMP), and is further subdivided into α- and β-components. α- Receptor activation is associated with vasoconstriction and the inhibition of norepinephrine release. β- Adrenoreceptors have been divided into β<sub>1</sub>, which are responsible for both chrontropic and inotropic effect on the heart, and β<sub>2</sub>, which mediate bronchodilation. Most bronchodilators in current use produce their effects by interchanging with β<sub>2</sub>-adrenoceptors. They are found on airway smooth muscle and in lungs, blood vessels and other tissues through out the body. In the airway, stimulation of, since the introduction of Salbutamol in 1968, inhaled β<sub>2</sub>-adrenoceptor agonists remains the treatment of choice in acute asthma. There are atleast 12 β<sub>2</sub> agonists in clinical use (Table 1) and they can be classified under two catagories.
Table 1. $\beta_2$-agonist as antiasthmatic agents.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>YEAR</th>
<th>COMPOUND</th>
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<tbody>
<tr>
<td>1.</td>
<td>1968</td>
<td>Salbutamol sulfate</td>
</tr>
<tr>
<td>2.</td>
<td>1970</td>
<td>Terbutaline sulfate</td>
</tr>
<tr>
<td>3.</td>
<td>1971</td>
<td>Fenoterol HBr</td>
</tr>
<tr>
<td>4.</td>
<td>1974</td>
<td>Rimiterol HCl</td>
</tr>
<tr>
<td>5.</td>
<td>1977</td>
<td>Reproterol HCl</td>
</tr>
<tr>
<td>6.</td>
<td>1980</td>
<td>Procaterol HCl</td>
</tr>
<tr>
<td>7.</td>
<td>1981</td>
<td>Tulobuterol HCl</td>
</tr>
<tr>
<td>8.</td>
<td>1983</td>
<td>Pirbuterol HCl</td>
</tr>
<tr>
<td>9.</td>
<td>1986</td>
<td>Bitolterol mesylate</td>
</tr>
<tr>
<td>10.</td>
<td>1986</td>
<td>Formeterol fumarate</td>
</tr>
<tr>
<td>11.</td>
<td>1990</td>
<td>Bambuterol</td>
</tr>
<tr>
<td>12.</td>
<td>1990</td>
<td>Salmeterol xinafoate</td>
</tr>
</tbody>
</table>

(a₁) **short-acting $\beta_2$-agonists:**

Inhalation of these compounds results in rapid bronchodilation that begins within a few minutes. The rapid onset of action of this class of drugs e.g. terbutaline makes their useful not only as a rescue medication for acute asthma symptoms but also as an effective preventive measure against broncho-constricting stimuli such as exercise, cold air or other inhaled irritants. An increased demand for short acting bronchodilator therapy is likely to be a sign of deterioration of asthma control and may need the use of anti-inflammatory agents.

(a₂) **Long-acting $\beta_2$-agonists:**

Long-acting $\beta_2$-agonists, such as salmeterol demonstrate prolonged receptor occupancy, which is thought to produce persistent functional antagonism at $\beta_2$-receptors in bronchial smooth muscle and other locations. This leads to prolonged
bronchodilation that helps decrease symptoms of asthma and improve airflow for up to 12 hours. Although its duration of action may be longer, the onset of action is lower than short-acting \( \beta_2 \)-agonists, making it inappropriate for use as a rescue medication. The position of long-acting inhaled \( \beta_2 \)-agonists in clinical practice has yet to be fully established. If a long-acting \( \beta_2 \)-agonist is used concomittantly with inhaled corticosteroids in patients with moderate to severe asthma whose asthma is poorly controlled despite anti-inflammatory therapy, long-acting \( \beta_2 \)-agonists should not be used as replacement for oral or inhaled corticosteroids.

(b) Anticholinergics:

Since the introduction of inhaled quaternary anticholinergic bronchodilators, their clinical usefulness have been explored in a variety of settings. They have emerged as the inhaled bronchodilators of first choice in the management of asthma. It appears to be more effective when combined with adrenergic bronchodilators. Ipratropium bromide, the most widely available quaternary anticholinergic, is the most potent bronchodilator than the other class of inhaled bronchodilators such as \( \beta_2 \)-adrenergic drugs. The gradual but sustained bronchodilator effect of ipratropium bromide contributes to its perceived value as a maintenance therapy in the management of asthma.

Recently, studies were conducted with ipratropium bromide in its metered-dose inhaler format and on comparison with \( \beta_2 \)-selective agonists, it was found that ipratropium bromide is not the quick-releif bronchodilator of first choice for young asthmatics. However ipratropium bromide produces additional bronchodilatory effect when given with a \( \beta_2 \)-agonists and this may allow reduction of corticosteroid dosages. This supplemental bronchodilator benefit in moderate-to-severe asthma is sustained during chronic therapy with no evidence of tachyphylaxis. Recently a group of derivatives related to ipratropium bromide have been developed, which include the long-acting anticholinergic tiotropium bromide (fig. 1, Boehringer Ingelheim), thereby offering the possibility of twice- or even once-a-day application.
During the last decade, increasing interest in the therapeutic potential of muscarinic antagonists has been observed as a result of the discovery that different subtypes of muscarinic receptors exist throughout the respiratory system. Novel selective antimuscarinic drugs with high affinity for these receptors may provide improved cholinergic therapy for asthmatics. Quinuclidine esters developed by Pfizer (fig. 2) shown below have selectivity for pulmonary muscarinic receptors and are more effective bronchodilators than ipatropium bromide.

Thus although anticholinergics act as a safe and effective bronchodilators, neither the β₂-agonists nor ipatropium bromide and its derivatives are able to reduce bronchial hyper-responsiveness. Therefore these agents should not be considered as prophylactic therapy for chronic asthma.

(c) Phosphodiesterase Inhibitors:

Cyclic 3',5'-adenosine monophosphate (cAMP) modulates a variety of cellular and physiological functions in mammals, such as cell division, endocrine function and the immune response. The level of cAMP is controlled, by phosphodiesterases that
enzymatically degrade cAMP. The phosphodiesterase IV (PDE IV), a cyclic nucleotide phosphodiesterase isoenzyme, is formed in several cells and is in the predominant form in human leukocytes. PDE IV is responsible for the breakdown of cAMP in airway smooth muscle and in the inflammatory cells. Inhibition of this enzyme has proved not only to produce airway smooth muscle relaxation, but also to suppress degranulation of mast cells, basophils and neutrophils, along with inhibiting the activation of monocytes and neutrophils. PDE IV inhibitors are therefore expected to offer a new approach for the treatment of bronchial asthma with advantages over currently available agents.

Rhone-Poulene Rorer has developed RP-73401 (fig. 3) as one of the most potent and selective PDE IV inhibitor with bronchodilating and anti-inflammatory activities as well. In view of its exceptional potency in preclinical test it has been selected for clinical investigations.

![Fig. 3: Phosphodiesterase (PDE) IV (Rhone-Poulene Rorer)](image)

(d) Methyl Xanthines:

Since its introduction more than 50 years ago, theophylline has remained the most widely prescribed bronchodilator as maintenance therapy for asthmatic patients. Its mechanism of action is although unknown, it is hypothesised that theophylline may be acting as a non selective phosphodiesterase inhibitor. Theophylline (fig. 4) relaxes bronchial smooth muscle, besides inhibiting intracellular release of calcium, late response to allergens and the release of chemical mediators from mast cells.
Several theophylline formulations for once daily administration have been launched during the last few years, including those of Pierre Fabre (1984), Elan (1984), and Byk Gulden (1990). The most interesting xanthine derivative doxofylline (fig. 5) (Ansimar, ABC) was launched in 1987 and Malesci’s isbufylline is in clinical trials.

However, the major drawbacks associated with theophyllines are toxicity, such as irritability, dehydration, severe vomiting, hematemesis, stupor, and convulsions. Report are also available that nausea and vomiting are the most frequent and earliest side effects with chronic oral therapy. According to Piafsky and Ogilive patient may not tolerate oral theophylline even when plasma levels are less than optimal and a local irritant effect in the gastrointestinal tract may be predominant in these patients.

(c) Potassium Channel Openers:

Potassium channel openers are a structurally heterogenous group of compounds that relax smooth muscle by activating potassium channels in the plasmalemma. Many different types of potassium channels have been characterized. Activation of potassium channels has been proposed as a target for the design of drugs for asthma.
The potassium channel openers Cromakalim and its active (-)-isomer Levromakalim (smithkline Beecham) have been evaluated in asthma. But both compounds failed to meet the criteria to be developed as antiasthmatics.

The search for airway selective and long-acting compounds continues to be pursued. BRL-55834 (Smithkline Beecham) is a novel potassium channel opener that shows significant tissue selective relative to the first generation compounds. Benzopyran derivatives have been also claimed by chugai and syntex to be potassium channel activators that are potentially useful as bronchodilators, other potassium channel openers that have a potential as antiasthmatic agents are SR-47063 (fig: 6, Elf Sanofi) and YM-934 (Yamanouchi) which are now undergoing clinical trials.

![Fig. 6 : SR 47063 (Elf Sanofi)]

5.2. Anti-inflammatory Drugs:

Asthma being a chronic and dynamic inflammatory disease of the airways, anti-inflammatory drugs were considered to be of significted interest in the managemant of asthma. Although the autopsy evidence of inflammation has long been recognised in patients who have died as a result of an asthma attack, recent studies have shown the presence of inflammation even in mild and subclinical disease. Electron micrographs of bronchial mucosa from patients with mild asthma show epithelial destruction at all levels of the airways.

Recruitment and activation of inflammatory cells at the sites of allergen exposure can cause release of both preformed and newly formed mediators that can damage airway epithelium and enhance the bronchial response to various stimuli. Certain resident tissue cells, such as epithelial cells, mast cells and fibroblasts, probably also participate in the inflammatory process. All these inflammatory cells
produce a wide range of inflammatory mediators including histamine leukotrienes, PAF and cytokines. It is quite likely that asthma may be due to the complex interplay of different inflammatory cells and mediators. Therefore, anti-inflammatory drugs represents the first line treatment approach in asthmatic patients. Certain anti-inflammatory drugs have been discussed below.

5.2.1 Corticosteroids/Glucocorticoids:

Inhaled corticosteroids have a potent anti-inflammatory effect on the airways, and their mechanism of action in asthma is of considerable interest. Inhaled corticosteroids reduce the infiltration of mast cells, macrophages, T-lymphocytes and eosinophils in the bronchial mucosa.

The regular use of inhaled corticosteroids for 6 to 8 weeks reduces airway hyperreactivity and improves pulmonary function in asthma patients. These favourable effects are not seen in patients receiving long term treatment with β2-agonist. The clinical efficacy of inhaled corticosteroids is well established in moderate to severe asthma, both in adult and pediatric patient. A substantial proportion of patients receiving long term therapy with oral corticosteroids are able to stop or reduce the dose of oral prednisone after the addition of even low doses of inhaled corticosteroids.

Recent studies strongly support the early use of inhaled corticosteroids in asthma patients, since these agents ameliorate abnormalities of the bronchial mucosa and reduce airway inflammation. Some studies suggest that the early use of inhaled corticosteroids decreases the deterioration of lung function and may prevent the development of irreversible airway obstruction. Nevertheless, the cessation of inhaled corticosteroids after 1 to 2 years of therapy is often followed by exacerbation of the disease. Systematic corticosteroids (betamethasone, dexamethasone, methyl prednisolone, prednisone & triamcinolone) are the most efficacious compounds available for the management of asthma. One of the major advances in the corticosteroids therapy is the introduction of beclomethasone dipropionate and
budesonide. Both are steroids of high topical potency that were introduced in 1972 and 1981, respectively.\textsuperscript{112}

The most common adverse effects of inhaled corticosteroids therapy are dysphonia and oropharyngeal candidiasis. There is no evidence that even long term use of inhaled corticosteroids causes clinically relevant suppression of the hypothalamic pituitary-adrenal axis.\textsuperscript{113,114}

\subsection*{5.2.2 Antiallergic Drugs:}

Drugs in this category were previously named as mast cell stabilizers because their primary mode of action was believed to be inhibition of the release of mast cell inflammatory mediators. It has now been demonstrated that these agents affect several other inflammatory cells and sensory nerves as well. There are good reasons for implicating mast cells in the pathogenesis of asthma. First mast cells are abundant in the airways of humans\textsuperscript{115,116} and contain high affinity IgE receptors. Further these IgE antigen-antibody interactions lead to the release of multiple performed and newly formed mediators. Secondly mast cells degranulations have been demonstrated in the airways of asthmatic subjects,\textsuperscript{117-119} suggesting that mast cells are stimulated to secrete in asthmatics.

Because of this strong circumstantial evidence implicating the participation of mast cells in asthma, investigators have sought that mast cell mediators might explain the various manifestations of asthma such as (a) smooth muscle contraction and particularly the increased responsiveness of the airway smooth; (b) increased vascular permeability, leading to edema of the airway walls; and (c) abnormal mucus secretion. Although various mediators have been identified in mast cells, most investigators believe that these mediators do not explain adequately the major manifestations of asthma. Therefore, the search continues for other key drug and their mechanism.

Since its introduction in 1967, disodium cromoglycate (DSCG) has remained an important drug in the prophylactic therapy of bronchial asthma. It appears to have a specific action on allergic inflammation, and yet its molecular mechanism of action
remains a mystery. A great deal of synthetic work has been directed towards the
discovery of more potent and orally active antiallergic drugs. For 20 years DSCG
remained the only agent of this type in the market, until the introduction of nedocromil
sodium (fig.7) in 1986 an anti-inflammatory and antiallergy agent which, like DSCG,
must be administered by insufflation. Several orally active antiallergy agents have
been launched more recently including amtexanox\textsuperscript{120}, repirinast\textsuperscript{121}, tazar solvent\textsuperscript{122}. and
pepirolast potassium\textsuperscript{123}. Other antiallergy agents under development include Cl-959,
batebulast hydrochloride and TYB-2285\textsuperscript{124}.

![Fig. 7: Nedocromil Sodium](image)

Even though DSCG is being used for 25 years, clinically it is an enigma
because it is effective in some patients and yet in other, apparently similar patients it
affords little protection. Further repeated administration of DSCG has been found to
exhibit tachyphlaxis.

5.2.3 Peptidoleukotriene antagonists:

In 1960 it was observed that stimulated lung tissue could produce a material
that slowly induced sustained contractions of guinea pig smooth muscle, called slow
reacting substance of anaphylaxis (SRS-A). This SRS-A is produced by human lungs
upon immunological challenge by antigens. During the last 20 years this crude
substance has been extensively studied and has proved to play an important role in the
pathogenesis of bronchial asthma. In 1979 new metabolites derived from arachidonic
acid via the 5-lipoxygenase pathway were shown to have the chemical and biological
properties of SRS-A and to exert pharmacological effects on the respiratory systems.
These metabolites were named leukotrienes because their structure contain conjugated triene system and they are isolated from leukocytes.

Leukotrienes are generally classified in two subclasses: the peptidoleukotrienes (leukotrienes C4, D4 and E4) and the hydroxy leukotrienes (leukotriene B4). Peptidoleukotriene are implicated in the biological response associated with the slow-reacting substance of anaphylaxis. The pharmacological activities of peptidoleukotrienes include smooth muscle contractions, increased vascular permeability and enhanced mucus production. During the past ten years, a major goal has been the discovery and development of novel, selective antagonists of peptidoleukotrienes as potential therapeutic agents for combatting the asthmatic condition. Hydroxy leukotriene B₄ (LTB₄) on the contrary, is believed to be an important mediator of inflammation. It exerts its biological effects through stimulation of leukocytes and leukocyte functions. Specific LTB₄-receptor antagonists could find application in the certain inflammatory conditions such as asthma. CGS-25019C, is a leukotriene B₄ antagonists is under clinical development for the inhibition of acute inflammatory reaction associated with bronchial asthma.

A series of 1,2,4,5-substituted phenols and series of 1,2,4,5-substituted hydroxy acetophenone synthesised by Eli Lilly. have been found to possess LTB₄ receptor antagonists activity¹²⁵. Out of these the o-phenyl phenol leukotriene B4 antagonist LY-280748¹²⁶ (Fig. 8) displayed good oral efficacy in a guinea pig model of broncho-constriction, and has been selected for the further development in the treatment of human inflammatory diseases.

![Fig. 8 : LY-290748 (Eli-Lilly)](image-url)
In recent years Leukotriene D₄ receptor antagonists have been developed as a new approach to antiasthma drug therapy. A variety of them are in clinical evaluation for the treatment of asthma, as is apparent from the growing number of new compounds appearing in the patent literature. Ibudilast was launched in Japan for the treatment of asthma in 1989 and it represents the first leukotriene antagonists to enter the international market for use in treating bronchial asthma.

Bayer's Bay-X-7195\textsuperscript{127} is a new potent, orally active leukotriene D₄ antagonist that was obtained through systematic structural modification of LTD₄. Good activity and tolerability in animal studies led to the initiation of clinical trials of this compound for the treatment of asthma. It remains to be seen whether it will be clinically acceptable or not.

### 5.2.4 PAF antagonists:

Platelet activating factor (PAF) is an endogenous phospholipid mediator\textsuperscript{128-130} and is chemically identified as 1-0-alkyl-2-0-acetyl-sn-glycero-3-phosphocholine. It is considered to be implicated as a mediator of pathophysiological reactions in several human diseases particularly in allergic inflammation. It is released from a variety of inflammatory cells which are relevant to asthmatic inflammation, such as basophils, neutrophils, platelets, macrophages, endothelial cells and IgE-sensitized bone marrow mast cells.

PAF antagonists make up a group of structurally diverse compounds that inhibit the biological effects of PAF and have been proposed as agents with great therapeutic potential for asthma. More than 15 PAF antagonists are undergoing clinical trials for the treatment of asthma, however, none of the compounds have yet shown any promising results.

Recently, a dual PAF antagonists, UR 12592 (fig. 9) which also acts as an antihistamine is being developed as an antiallergic agent.
Another potent antagonist of PAF and histamine has been also reported by Schering-plough\cite{131} (fig. 10) as a potential anti-inflammatory and antiallergy agent. Clinical trials with both the products are in progress.

5.2.5 Thromboxane A₂ antagonists:

Thromboxane A₂ (TxA₂), the major cyclogenase product in platelets, is one of the most potent vasoconstricting and platelet aggregating agents known. The potent biological activity of TxA₂ has been implicated in a variety of renal and respiratory diseases.

Two TxA₂ antagonists Bay-u-3405\cite{132} (fig. 11) and AA-2414\cite{133} (fig. 12) are in clinical trials for treating bronchial asthma.
Recently Kyowa Hakko have synthesized dibenz [b,e] oxepin derivative KF-15766 and evaluated for its antiallergy activity. This novel compound represents the first antiallergy agent to combine TxA₂ and histamine H₁ antagonism in the same molecule, and further boasts an exceptional margin of safety. It has been selected as a lead compound for further study and modification.

Terumo\(^{134}\) has claimed that phenoxyacetic acid and its derivative have potent TxA₂ and leukotriene-antagonist activities and may have potential for the treatment of allergic and ischemic conditions.

5.2.6 Thromboxane synthase inhibitors:

Inhibition of the biological effects of TxA₂ can also be attained through inhibition of thromboxane synthesis. Recently, ozagrel hydrochloride was launched in Japan for the oral treatment of allergy and asthma. It appears to inhibit TxA₂ production through the selective inhibition of thromboxane synthase therapy inhibiting airway hyperresponsiveness and bronchoconstriction. The thromboxane synthase inhibitors S-1452, CS-518 and Y-20811 are in clinical trials as antiasthmatic agents.

Phthalazinone derivative represent a new class of antiasthmatic agents, possessing dual thromboxane A₂ synthase-inhibitory and bronchodilator activities. The compounds from this series are now under development and its pharmacological and toxicological activities are being further investigated\(^{135}\) KK-505 is another important compound of this series recently described in the patent literature.
A series of phthalazinone derivative with imidazolyalkyl group were also evaluated as antiasthmatic agents. (fig. 13). They were of special interest due to its unexpectedly high in vivo activity and absence of significant in vitro effects. Compounds of this series possesses dual thromboxane A$_2$ synthase inhibitory and bronchodilatory activities$^{136}$

![Phthalazinone](image)

**Fig. 13: Phthalazinone**

5.2.7 Leukotriene biosynthesis inhibitors:

Conversion of arachidonic acid by 5-lipoxygenase produces straight chain hydroperoxide acid (5-HPETE) which is subsequently metabolized to a series of highly potent leukotrienes (LTA$_4$, LTB$_4$, LTD$_4$, LTE$_4$). These oxygenated eicosanoids are powerful mediators of a broad range of physiological responses, and have been implicated in human inflammatory and allergic reactions such as asthma, allergic rhinitis and inflammatory bowel disease. Intervening in the 5-lipoxygenase pathway is of therapeutic use in the treatment of asthma.

Several 5-lipoxygenase inhibitors are being developed for the treatment of bronchial asthma, including zileuton, A-78773, AD-3264, MK-591 and E-6080. A-79175, an N-hydroxyurea compound, is a second generation lipoxygenase inhibitor that has entered into phase-I clinical trials due to its good activity and long lasting effects in monkeys after oral administration.$^{137}$ Recently a compound BI-RM-270 (fig. 14)$^{138}$ developed by Boehringer Ingelheins has been found to act as a potent and enantio-selective leukotriene biosynthesis inhibitor. This compound acts at the level of arachidonic acid release rather than through direct inhibition of 5-lipoxygenase and is being developed for the treatment of asthma.
CGS-23885\textsuperscript{139} is a member of a new series of orally active 5-lipoxygenase inhibitor based on the chromene template. It displays good potency and a long duration of anti-inflammatory activity.

5.2.8 Leukotriene A\textsubscript{4} hydrolase inhibitors:

Recently Rhone-poulenc\textsuperscript{140} has claimed phenyl alkonic acid with leukotriene A\textsubscript{4} hydrolase-inhibitory activity to be potentially useful for the treatment of diseases in which excess or unnecessary LTB\textsubscript{4} production occurs i.e. chronic inflammatory disease such as skin disease and inflammatory conditions such as asthma, allergic etc. An exemplified compound is shown below-

5.2.9 Phospholipase inhibitors:

Since the enzyme phospholipase A\textsubscript{2} plays a key role in the generation of all lipid mediators, it has been selected as a target for inhibitory drugs. Phospholipase D and phospholipase C may also be target enzymes in asthma. Substituted naphthofuran derivatives have been reported by DuPont Merck to have phospholipase C-inhibitory activity and to be potentially useful as anti-inflammatory and anti-allergic agents.
Recently American Home products (fig. 16) have reported that phospholipase A₂ inhibitors is useful in the treatment of immunoinflammatory conditions such as allergic, asthma, anaphylaxis and inflammation.

![Fig. 16: WO 9322305 (American Home Products)](attachment:image)

5.3 Anti-histamine:

The allergic reaction to a specific antigen is characterised by a series of complex immunological process consisting of an early specific immune response and a late inflammatory reaction. The release of active substances such as histamine from cytoplasmic granules of mast cells and basophils in response to antigen challenge is responsible for many of the symptoms observed in the early phase of the allergic reaction. Histamine is widely distributed throughout the body mainly in the lungs, skin and intestinal tract. Although mast cells and basophils contain the most histamine, it is also found in the central nervous system, gastric mucosa, epidermal cells and fast growing tissues.

There are three known types of histamine receptors (H₁, H₂, H₃) through which histamine induces a wide variety of responses. The interaction of histamine with H₁ receptors produces a series of biological effects, importantly contraction of bronchial smooth muscle, increased vascular permeability, stimulation of peripheral nerve endings to produce reflux broncho constriction and cough. In the late 1970's clinical investigation of the H₁-antihistamines like Clemastime and Chlorpheniramine revealed that these agent had a bronchodilating effect and provided some protection against exercise induced asthma. But the side effects, induced at H₁ receptors in the central nervous system which produced sedation and also at cholinergic receptors limited the maximal doses that could be given. Therefore, these drugs are not useful as antiasthmatic agents.
Astemizole (fig. 17) and terfenadine (fig. 18) are the two newer potent selective H₁-antagonist. They have neither the sedative nor the anticholinergic problems associated with earlier agents.

![Chemical structure of Astemizole and Terfenadine]

Astemizole seems to delay the onset of exercise-induced asthma but does not affect the severity of the bronchoconstriction. Terfenadine effectively blocks the histamine induced bronchospasm when taken orally. It causes modest bronchodilation and provides limited protection from either exercise-induced asthma or challenge using nebulized water.

Ebastine (fig. 19) is a new second generation agent that has shown antihistamine activity in preclinical studies and clinical efficacy in providing relief from symptoms in patients with allergic disorders. Importantly, it was virtually without affect in a complete set of regulatory toxicity studies. Its efficacy in asthmatic patients are being carried out.
5.4 Diuretics:

Inhalation administration of the diuretic compound furosemide (fig. 20)\textsuperscript{146} has been shown to provide protection against bronchoconstriction induced by various stimuli, inducing allergen exposure in sensitized individuals.

![Furosemide](image)

Furosemide is not a direct bronchodilator, but has been reported to improve the effects of B\textsubscript{2}-adrenergic agonists such as salbutamol, reducing edema in the airway wall. Amiloride, a compound launched as a diuretic in 1967, is under clinical development for the inhalation treatment of asthma. It is possible that new derivatives with less diuretic potency may in the future be developed for the treatment of asthma.

5.5 Anti-asthmatic Drugs from Natural Origin:

Some naturally occurring alkaloids also take part in reducing the symptoms of asthma, e.g. ephedrine is an alkaloid derived from *Ephedra equisitina* and *Ephedra vulgaris*. It can also be synthesised and used mainly in combination with other
agents.\textsuperscript{147,148} Atropine is another naturally occurring alkaloid isolated from plant origin and block transmission at parasympathetic neuroeffector junctions inhibits vagally induced smooth muscle contraction and secretory activity. Theobromine, theophylline and caffeine are also naturally occurring alkaloids useful in the treatment of asthma and have been discussed earlier.

The powdered fruits of pepper Cubeb (tailed pepper)\textsuperscript{148} are also employed in the treatment of asthma and as an expectorant and stimulant to the bronchial mucous membrane. Some Ayurvedic drugs also have been helpful in controlling asthma from time to time and isolated from natural origin in crude state.

5.6 New shots for allergy:

5.6.1 Naked DNA

Downregulation of immune responses to inert nonpathogenic antigen is central to the maintenance of immunologic homeostasis at the mucosal surfaces in the respiratory and gastrointestinal tracts, and failure of the underlying control mechanisms has been suggested as a key etiologic factor in allergic disease.\textsuperscript{149} An important component of this process is the selective suppression of T helper type 2 (Th2)-dependent IgE response to inhaled or ingested antigens, which is mediated by antigen-specific CD8\textsuperscript{+} T cells.\textsuperscript{150} It is therefore possible to generate antigen specific regulatory T cells to modulated the IgE antibody response.

Previous method of immunotherapy has used injection of pollen extracts as a treatment for allergic rhinitis, however, the routine use of an extensive battery of allergens for testing remained the major drawback. Recently Hsu et al\textsuperscript{151} have used a new therapeutic approach based on allergen immunization for allergic diseases they found that intramuscular injection of rats with a plasmid DNA encoding a house dust mite allergen into the muscle resulted in its long term expression and the induction of
specific immune responses. This approach resulted in the prevention of IgE synthesis, histamine release in bronchoalveolar fluids, and airway hyperresponsive-ness in rats challenged with aerosolized allergen. Further more, this suppression was found to be persistent and could be transferred to naive rats by CD8$^+$ T cells from gene immunised rats. These findings$^{152}$ suggest that allergen-gene immunisation is effective in modulating allergic responses, and may provide a novel therapeutic approach for allergic diseases.

5.6.2 Interleukin-1 Inhibitors

IL-1, with a very diverse biology, has been shown to play an important role in the proliferation of Th2 cells$^{153}$ and in the recruitment of eosinophils at the sites of allergic inflammation$^{154}$. Consequently, antagonism to the naturally occurring IL-1 may have a therapeutic potential in chronic asthma. Experimental evidence in this direction was provided by Watson et al$^{155}$ with the development of a pure recombinant form of IL-1 receptor antagonist (rhIL-1RA), which upon administration to guinea pigs was found to substantially reduce the intensity of inhaled allergen-provoked eosinophilic airway inflammation and hyperreactivity. Thus, any agent targeted at IL-1 may find therapeutic application in chronic asthma rather than in the early stages of asthma. Intensive research in the area of IL-1 inhibition led to the discovery of a tetrapeptide, L-709049 (fig. 21) as a novel antiallergic agent.

![IL-1 Synthetic inhibitor](image)

Fig. 21: IL-1 Synthetic inhibitor
5.7 Peptides as Antiallergic/Antiasthmatic agents

5.7.1 Neuropeptides in respiratory tract:

Since long it has been known that the function of the respiratory tract are controlled by specialised endocrine cells and the innervation, together known as the ‘diffused neuroendocrine system’. Production of bioactive regulatory peptides and other chemicals by the components of the diffused neuroendocrine system is well established, but such production is increasingly demonstrated by other tissues, such as endothelium and specialised myocardial cells present in pulmonary veins. In respiratory tract, neuropeptides are also present at the nerve endings within the airway and in inflammatory cells. Stimulation of exposed vagal nerve endings in the airway epithelium by inflammatory stimuli can result in a reflux increase in efferent nerual activity and the antidromic secretion of neuropeptides. In humans, the upper and lower airway functions are regulated both by cholinergic and adrenergic pathways as well as by nonadrenergic and noncholinergic pathway (NANC). The neurotrans-mitters for the NANC nervous system are considered to be the neuropeptides.\textsuperscript{156,157}

Although strict anatomical demarcations in the distribution of regulatory peptides in precise anatomical structure of the lung can not be made, a broad classification of the general distribution of the regulatory peptides along with their effects are shown in the table 2. The role of some of the peptide in the lung function is not yet clearly established.
Table 2 Neuropeptides and their effects in respiratory tracts

<table>
<thead>
<tr>
<th>Distribution</th>
<th>Neuropeptides</th>
<th>Role in Pulmonary disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neural</td>
<td>Substance P</td>
<td>Stimulates release of mediators from mast cells</td>
</tr>
<tr>
<td>Neural</td>
<td>Neurokinin A</td>
<td>Bronchoconstriction, inflammation, mucus edema</td>
</tr>
<tr>
<td>Neural</td>
<td>Neurokinin B</td>
<td>Bronchoconstriction, inflammation</td>
</tr>
<tr>
<td>Neural</td>
<td>Calcitonin gene related peptide</td>
<td>Production of mucus</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Calcitonin</td>
<td>n.c.e.</td>
</tr>
<tr>
<td>Neural</td>
<td>Vasoactive intestinal peptide</td>
<td>Vasodilation</td>
</tr>
<tr>
<td>Neural</td>
<td>Neuropeptide Y</td>
<td>n.c.e.</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Bombesin</td>
<td>n.c.e.</td>
</tr>
<tr>
<td>Myoendocrine</td>
<td>Atrial natriuretic peptide</td>
<td>Bronchodilator</td>
</tr>
<tr>
<td>Neural</td>
<td>Peptide histidine methionine</td>
<td>Antiinflammatory</td>
</tr>
<tr>
<td>Neural</td>
<td>Peptide histidine isoleucine</td>
<td>Inhibits mast cell degranulation and mucus production</td>
</tr>
<tr>
<td>Neural</td>
<td>Galanin</td>
<td>n.c.e.</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Gastrin releasing peptide</td>
<td>Lung fibrosis</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Enkephalin</td>
<td>n.c.e.</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Cholecystokinin</td>
<td>n.c.e.</td>
</tr>
<tr>
<td>Endocrine</td>
<td>Endothelins*</td>
<td>Bronchoconstriction</td>
</tr>
</tbody>
</table>

n.c.e. = Not clearly established, * Also produced by endocrine and endothelial cells of airways.

Out of all the regulatory peptides of the respiratory tract, Bradykinin, Vasoactiveintestinal peptides, CGRP and Neurokinin have been well implicated in the pathogenesis of asthma for quite sometime. These peptides induce bronchoconstriction, vasodilation with airway edema, microvascular leakage and increased mucus secretion with coughing. Some of these peptides degranulates mast cells and possess chemotactic activities and were found to increase both number and length in asthmatic airways.
Thus, in the plethora of active peptides produced and released from specific structure of the respiratory tract including the innervation, airway epithelium and the endothelium, documentation regarding the alteration of these active peptides in disease such as asthma is beginning to appear. Asthma is poorly understood and complex disease or disease family but the potent actions of the neuropeptides modulating airway tone and participating in tissue repair are likely to be altered in asthma and in other hyperreactive conditions. Therefore, these active peptides in the hyperreactive respiratory tract provide new therapeutic approaches for the development of clinically efficacious antiallergic/antiasthmatic agents.

5.7.2 Bradykinin Antagonists:

Bradykinin (BK, Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), a nonapeptide released during the inflammatory response, is known to play a role in allergic pathophysiology of the airways, contributing to bronchoconstriction and edema formation. Raised levels of kinin generating enzymes and kinin are found in the airways during allergic response\textsuperscript{158}. Thus, there is substantial evidence that these peptides contribute to the inflammatory response associated with symptoms of allergy, arthritis, viral rhinitis and asthma. In asthmatic patients, inhaled BK is one of the most potent bronchoconstrictor agents which stimulates the release of inflammatory mediators such as platelet activating factor (PAF), peptidoleukotrienes, leukotriene B4, as well as various prostaglandins in many tissue including those of the airways.

Bradykinin receptors, therefore, may play an important role in the management of asthma. Receptors for bradykinin, B\textsubscript{1} and B\textsubscript{2}, have been classified according to the relative potencies of various agonists and antagonists. Studies have shown that generally the BK-induced response in most of the inhaled smooth muscle and other tissues are mediated via B\textsubscript{2} receptors. Attempts to inhibit B\textsubscript{2} receptor mediated effects by BK led to the identification of [D-Phe\textsubscript{7}]-BK as one of the first B\textsubscript{2} receptor antagonist\textsuperscript{158}. Since then, numerous peptide B\textsubscript{2} receptor antagonist have been synthesised and two of the most potent ones\textsuperscript{159} are NPC567 and NPC16371 (Fig. 22)
These two compounds were found to inhibit the onset of antigen-induced airway hyperresponsiveness to acetylcholine in sensitized guinea pigs, when administered chronically with inhaled ovalbumin.

**5.7.3 Vasoactive Intestinal Peptide (VIP) and its analogues:**

Vasoactive intestinal peptide (VIP), a 28 amino acid linear peptide was first discovered, isolated and purified from porcine intestinal extracts. Later it was recognised as a neuropeptide widely distributed in the central and peripheral nervous system, with neurotransmitter properties and a broad spectrum of biological actions. The regulatory influence of VIP is well exemplified in the normal lung, where it is present in high concentrations and mediates nonadrenergic, noncholinergic airway relaxation. Altered VIP production or its metabolism in the lung plays a major role in the pathogenesis of asthma and other lung diseases.

VIP exerts distinct and potent antiinflammatory actions on both cellular and chemical mediators of inflammations. It down regulates T-lymphocyte proliferation, possibly through its effect on the expression of various cytokines\textsuperscript{160}. Because asthma is a disease of chronic inflammation of the airways and not merely a state of airway constriction, these antiinflammatory properties of VIP enhance its potential usefulness as an antiasthmatic. Briefly, VIP inhibits T-lymphocyte, and alveolar macrophage function and counteracts the bronchoconstriction of all known bronchoconstrictors.

In the asthmatic lung it has been proposed that the normal inhibitory effects of nerve containing VIP may be absent. If this is the case, then replacement therapy with
inhaled VIP may restore the status quo. However, clinical trials with inhaled VIP have been disappointing either due to the low order of potency or due to degradation of VIP by airway proteases. These findings suggest two possible alternatives for enhancing its therapeutic effectiveness: (a) the combined administration of VIP with one or more selective peptidase inhibitors (Patent No. J P05: 238,950) or (b) to search for a VIP like peptide that may have similar bronchial relaxant activity but is more resistant to inactivation by airway mucosal protease. The latter alternative led to the identification of a number of naturally occurring peptides as well as synthetic analogues of VIP that have similar biological activity but are relatively protease resistant. One such naturally occurring compound identified as Helodermin, originally isolated from the lizard, *Gilamonster Heloderma* But also present in mammalian tissue and exhibiting strong homology to VIP. Helodermin is a 35 residue peptide and was equipotent to VIP as relaxant of guinea pig tracheal smooth muscle. The relaxant action was found to be 4 to 10 times more sustained than VIP\textsuperscript{161}. Its C-terminal extension may account for its decreased susceptibility to enzymatic degradation and its long lasting tracheal relaxation.

In addition to this, several analogues of VIP were also synthesised with the view to enhance its potency, stability and duration of action using SAR and enzyme degradation studies. These studies led to the identification of peptide Ro-25-1553 (Fig. 23) which exhibited exceptionally high potency, metabolic stability and a long duration of action\textsuperscript{162}.

\begin{center}
\textbf{Ac His-Ser-Asp-Ala-Val-Phe-Thr-Glu-Asn-Tyr-Thr-Lys-Leu-Arg-Lys-Gln-Nle-Ala-Ala-
Lys-Lys-Tyr-Leu-Asn-Asp-Leu-Lys-Lys-Gly-Gly-Thr-NH\textsubscript{2}}
\end{center}

\textbf{Fig. 23: RO-25-1553}

It exhibited bronchodilator activity through activation of VIP receptors in a number of in vitro pharmacological assay and this activity was also found to be extended in vivo where it was shown to be active by intratracheal instillation and
aerosol administration against a number of different spasmogens. Beside this it was also found to suppress number of features associated with pulmonary anaphylaxis and asthma, including II-2 and II-4 production\textsuperscript{163,164}, edema formation and granulocyte accumulation in guinea pigs lung. These antiinflammatory activity together with bronchodialatory effect of Ro-25-1553 makes it a potential therapeutic agent for the treatment of bronchial asthma.

5.7.4 Neurokinin Antagonists:

The mammalian tachykinins are a family of neuropeptides which include substance P(SP), Neurokinin A (NKA) and Neurokinin B (NKB). They are characterised by a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH\textsubscript{2} where X is Phe or Val. These peptides are widely distributed in the central nervous system and in peripheral tissues and exhibit extensive and potent biological effects on airways. These actions which include bronchoconstriction \textit{in vivo} and \textit{in vitro} mucus secretion, plasma extravasation and neural excitation could be considered to mimic the symptoms of asthma\textsuperscript{165}. Thus if tachykinins were to play an important role in the pathophysiological process of asthma, selective antagonists would be of considerable clinical importance.

The receptors of neurokinins have been extensively studied in pharmacologi-cal as well as genetic aspects. They are classified into three subtypes NK-1, NK-2 and NK-3, which have high affinity to SP, NKA and NKB respectively. Functional and receptor binding studies in different laboratories have provided evidence for NK-1 and NK-2 receptors in guinea pig airways and that these receptors mediated the noncholinergic constriction produced by endogeneous tachykinins\textsuperscript{165}. The wide range of biological activity of SP has been attributed to its lack of specific selectivity for the three different types of receptors, which in turn is related to the conformational flexibility of the peptide. Introduction of restriction to the conformation flexibility of SP and its analogues has in many instances led to highly specific agonists or antaginists for each of these receptors subtypes. A neurokinin antagonist would be expected to have clinical potential for variety of diseases such as treating pain,
psychosis, inflammation, rheumatoid arthritis and respiratory diseases such as asthma and bronchitis.

Extensive studies with the design and synthesis of analgues of SP, NKA and NKB have been carried out with the view to develop highly selective NK antagonists. In the literature around more than fifty linear or cyclic peptides with potent and highly selectively NK antagonist for the three tachykinins receptors have been reported which may have therapeutic potential for variety of disease. Some of them have been summarised in table 3 which may have therapeutic potential for the treatment of asthma.

**Table 3: Some of the potent and widely studied NK₂ antagonist**

<table>
<thead>
<tr>
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<tbody>
<tr>
<td>-</td>
<td>Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH₂</td>
<td>166</td>
</tr>
<tr>
<td>GR 83074</td>
<td>BOC-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH₂</td>
<td>166</td>
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<tr>
<td>GR 94800</td>
<td>PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH₂</td>
<td>166</td>
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<tr>
<td>L 659877</td>
<td>[cyclo(Gln-Trp-Phe-Gly-Leu-Met)]</td>
<td>167</td>
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<tr>
<td>L659874</td>
<td>Ac-Leu-Met-Gln-Trp-Phe-Gly-NH₂</td>
<td>167</td>
</tr>
<tr>
<td>MDL 29913</td>
<td>[cyclo(Gln-Trp-Phe-Gly-Leu-CH₂NCH₃Leu)]</td>
<td>168</td>
</tr>
<tr>
<td>MEN 10207</td>
<td>Asp-Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-NH₂</td>
<td>169</td>
</tr>
</tbody>
</table>

Further in an attempt to discover a low molecular weight SP antagonist, Hagiwara et al selected octapeptide D-Pro-Gln-Gln-D-Trp-Phe-D-Trp-Phe-NH₂ as a lead peptide (SP antagonist) and hypothesised that the essential domain that bind to the receptor might be comprised of few amino acid residues. They synthesised a number of unprotected and fully protected tripeptide amide and tested them in receptor binding assay. One of the protected tripeptide Ac-Thr-D-Trp(CHO)-Phe-NMeBzl exhibited potent binding affinity to the receptor and was stable against enzymatic degradation. The IC₅₀ value was found to be 5.8 nM, in comparison to IC₅₀ value of
600 nM for the octapeptide. Beside this, the tripeptide was also shown to be a specific and potent SP antagonist in in vivo models. However, this compound lacked solubility in water, and had a poor oral absorption. In order to overcome this problem, a variety of branched tripeptides were designed and synthesised which can mimic the spatial orientation of the essential features of Ac-Thr-D-Trp(CHO)-Phe-NMeBzl. Subsequently, these studies culminated in the discovery of the most potent compound FK 888\textsuperscript{171} (fig. 24).

![Fig. 24 : FK-888](image)

The same group reported another novel neurokinin antagonist, a cycloheptapeptide\textsuperscript{172} lactone named WS 9326A (fig. 25) which was initially isolated from a soil sample of Suwa city Japan. The microorganism producing this compound was identified as Streptomyces Violaceoniger. Hydrogenation of two of the double bonds of WS 9326A afforded FK 224 (fig. 25), and antagonists of both SP and NKA\textsuperscript{172}. Recently Aramori et al\textsuperscript{173} compared the receptor subtypes. The result indicates that FK224 has dual actions to NK-1 as well as NK-2 but FK 888 is highly selective to NK-1. Similarly the in vivo activity of these two compounds was also evaluated in the experimental model for asthma\textsuperscript{174}.
FK 224 suppressed the edema induced by SP and capsaicin with ED$_{50}$ values of 0.14 and 0.30 mg/kg, respectively, whereas FK 888 exhibited more potent activity with ED$_{50}$ values of 0.011 and 0.019 mg/kg. Further, bronchial contactile response to allergen in the presence or absence of the tachykinin antagonist FK 224 in vitro was examined and it was observed that the compound significantly inhibited ovalbumin induced contaction. Both FK 224 and FK 888 are undergoing clinical trials and may provide new therapeutic approach for the treatment of asthma.

Recently a water soluble dipeptide NK-1 receptor selective antagonist S 18523 (fig. 26) was reported$^{75}$. The potassium salt of the dipeptide derivative was found to antagonise bronchoconstriction provoked by exogeneous SP in the guinea pig when administered by aerosol.
Thus, with the introduction of very potent tachykinin antagonists, it may be possible to find a solution to the enigma associated with the treatment of asthma.

5.7.5 Cyclosporins (CS):

Cyclosporin and FK 506 are fungal metabolites, widely known for their immuno-
suppressant activity. They are used during organ transplantation for the treatment of autoimmune diseases and dermatological disorders. Recently, they were found to completely suppress IL-5 induction *in vitro*\(^{176}\), thereby suggesting their therapeutic potential for the treatment of allergic disorders. Novel CS analogues prepared by Sandoz have been claimed to be potential use for topical application in asthma therapy (fig. 27).

### 5.7.6 Muramyl dipeptides:

Oral administration of muramy dipeptides (MDP) has been shown to induce certain biological response, including the downregulation of anmnestic and antigen specific IgE responses which are not observed following parenteral administra-
tion\(^{177}\). This led to the identification of a novel analogue of MDP, N-acetyl muramyl-threoninyl-D-isoglutaminyl-sn-glyceryl-dipalmitoyl (SDZ 280.636) which exhibited significant suppression of polyclonally induced serum IgE levels in anti IgD treated mice\(^{178}\). The liposomised MDP derivative (fig. 28) was found to selectively inhibit IgE response when administered by oral route without affecting the levels of other immunoglobulins. The antiallergic activity exhibited by SDZ 280.636 was attributed to the suppressive effect on TH\(^2\) activity in gut-associated lymphoid tissue (GALT) which is known to be the sites of the first appearence of IgE response\(^{179}\). Recently, it has been reported to selectively suppress IL-4 and not IL-13 mRNA expression only in gut associated lymphoid tissue and mesentric lymph nodes but not in the spleen\(^{180}\). These properties make this molecule a potential candidate for the treatment of type I immediate hypersensitivity and is therefore now being investigated as lead compound for the development of antiallergic drug.
5.7.7 IgE-Related Peptide:

(a) Antiallergy Peptide:

Immunoglobulin E (IgE) plays an important role in mediating immediate hypersensitivity such as asthma, hayfever, food and drug allergies. In recent years IgE has attracted the attention of many investigators, because of association of high levels of serum IgE with asthma. There exist a direct correlation between IgE titers and the distribution of mast cells and basophils which bear high affinity receptors for IgE (Fc epsilonRI). Bridging the receptors bound IgE by a specific multivalent antigen triggers secretion of chemical mediators such as histamine, slow reacting substance of anaphylaxis and platelet activating factor, which are responsible for many of the symptoms of allergic diseases. Since the interaction between IgE and its high affinity receptor, FceRI, is a critical step in the development of an allergic reaction, it has been proposed that binding to and blocking of the FceRI by certain peptide would inhibit release of the chemical mediators. Such an IgE receptor-binding peptide will become an ideal antiallergic agent for treating type I immediate hypersensitivity, termed as an 'isotype-specific' method of regulation.
Several strategies have been utilised to delineate the binding region in the IgE to the human FcεRI. Studies with proteolytic peptide fragments of human IgE\textsuperscript{181}, chimearic immunoglobulin molecules\textsuperscript{182,183}, recombinant fragments\textsuperscript{184,185} and most recently with site directed mutagenesis\textsuperscript{186} have suggested that binding site lies in the Fc epsilon region, particularly in the CH3 and CH4 domains. However, the exact binding sites remain unidentified.

In an attempt to delineate the binding sites, Stanworth et al in 1968 for the first time reported that the IgE fragment crystalline (IgE-Fc) fragment bound to the IgE receptor and that the IgE Fc fragment was involved in an allergic reaction\textsuperscript{187}. This fragment (fig. 29) was later identified by Hamburger as a pentapeptide (320-324) which blocked the Prausnitz-Kustner action\textsuperscript{188}. Recently, Abbott laboratories have synthesised this pentapeptide by solution phase on large scale and have initiated clinical trials for the treatment of allergic rhinitis\textsuperscript{189}.

![Fig. 29: Hamburger Pentapeptide](image)

Fig. 29: Hamburger Pentapeptide

Noguchii et al in 1990 reported a new class of oligopeptides related to Hamburger’s pentapeptide which blocked allergic response by interfering with the binding of IgE to the receptor mast cells\textsuperscript{190}. One of the hexapeptide (fig. 30) exhibited very high order of biological activity by inhibiting the production of IgE antibody and by preventing the contraction of rabbit aorta.
Fig. 30: Noguchii's hexapeptide

Nio et al. in 1992 and 1993 reported synthesis of 112 peptide fragments spanning the CH3-CH4 domain in human IgE with the view to identify the exact binding site. The peptides were assayed for their capacity to inhibit passive cutaneous anaphylaxis (PCA) \textit{in vitro}. The results suggested that an octapeptide (fig. 31) corresponding to 345 to 352 in the human IgE molecule may be an IgE binding site\textsuperscript{191,192}. It exhibited significant inhibition of PCA probably by occupying the Fc receptor site on the cells.

Fig. 31: Octapeptide 345-352 in the human IgE

Stanworth in 1996 reported\textsuperscript{193} a new antiallergic tripeptide (fig. 32) based on the molecular modelling studies of a decapeptide Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe present in the CH4 domain of IgE. This decapeptide was responsible for providing trigger signal to the mast cells to release histamine, as a consequence of the cross linking of FcεRI bound IgE antibody by specific antigen (allergen). The tripeptide (WO 9510532) was found to be considerably more active than the
established antiallergy drug Neodromil in the inhibition of allergen induced histamine release from sensitized mast cells *in vitro*.

![Chemical structure](image)

**Fig. 32 : WO 9510532**

Recently, McDonnell et al.\textsuperscript{194,195} have elegantly reported an IgE related peptide, using structure based drug design approach, for inhibiting the interaction between IgE and its high affinity receptor. In the first instance, the potential contact residues were identified on the basis of exhaustive mutagenesis studies. This was followed by the generation of a model for the two extracellular immunoglobulin-like domain of the alpha helix chain. Based on this model they designed a conformationally constrained peptide that would mimic the region which makes substantial contact with IgE-Fc. An undecapeptide corresponding to this region in the proposed model was selected and cyclised by means of N-terminal L-Cys and C-terminal D-Cys. This sort of cyclic structure (fig. 33) was expected to adopt a native like conformation. Besides this several other cyclic peptides with retro-enantiomer version, with reverse sequence and with scrambled sequence were also synthesised. Cyclo(L-262) and cyclo(rD-262) exhibited significant inhibition in both the binding assay as well as in the mast cell stabilisation assay. These interesting class of cyclic peptides provides a good lead for the development of therapeutically useful antiasthmatic agent.
In our laboratory, we have been interested in optimising the biological activity of the hexapeptide reported by Noguchii et al.\textsuperscript{190} In the first instance structure activity relationship studies were carried out with the view to suppress aspartimide formation associated with Asp-Gly and Asp-Ser in the hexapeptide sequence. A variety of analogues were synthesised in order to established the role of individual amino acids in the expression of biological activity. Out of which two analogues CDRI-94/335 (fig. 34) with Gly at position 2 instead of Asp and 95/220 with Gly at position 2,3 instead of Asp, Ser exhibited high order of activity both by i.p. as well as \textit{p.o.} route in rats.\textsuperscript{196}

By oral route, 94/335 exhibited significant inhibition of PCA with ED\textsubscript{50} value of 0.6 mg/kg which was close to 0.9 mg/kg for the Noguchi’s hexapeptide. However, in mast cell stabilising assay, it exhibited much better protection of mast cells and was determined to have ED\textsubscript{50} value of 1 mg/kg, compared to 3 mg/kg for the lead peptide.
By i.p. route, 94/335, was found to be at least 50 times more potent in terms of dose per dose of disodium cromoglycate (DSCG), a standard antiallergy drug used clinically. Thus, our studies provide first experimental evidence for the existence of antiallergy activity in small peptides by oral route and opens a new avenue for further exploration.

In addition, following patents on IgE peptides have been also claimed in the literature as inhibitors of allergy: WO 9601643, JP 04187091 and JP 04187088.

(b) Antiallergy Vaccine:

One of the most interesting approaches in the treatment of IgE allergies has been the pioneering work carried out by Stanworth et al who developed a novel form of peptide vaccine derived from mechanism-based leads. Many years of investigation into the role of IgE antibody and SAR studies on model histamine releasing peptide led to the identification of a decapeptide, Lys-Thr-Lys-Gly-Ser-Gly-Phe-Phe-Val-Phe as an effector site within the CH4 domain of IgE for providing a trigger signal to the (nonsensitized) mast cells to release histamine. The triggering phenomenon by the putative decapeptide within the Fc region of IgE was identical to the regular allergen-IgE antibody trigger situation. Based on these findings, it was proposed that this decapeptide might behave like a hormone and it might be possible to design an antagonist by chemical modification of the peptide. However, none of the analogs synthesised had the desired antagonist properties. Alternatively, it was decided to generate antibody against this decapeptide to abrogate the trigger signal. Thus, the human epsilon-chain decapeptide was linked to KLH as a protein carrier and an antibody was raised in rabbits. The results were very encouraging as it was shown to inhibit rat IgE antibody-mediated PCA reactions in rats, when administered at the time of allergen challenge. Clinical trials with this novel vaccine on human are under way.
5.7.8 Inter Cellular Adhesion Molecule-1:

A major characteristic of asthma is the extreme (10 to 1000 times normal) sensitivity of the bronchi to inhaled agents\textsuperscript{199,200}. The severity of this "airway hyperresponsiveness" correlated with the intensity of asthmatic symptoms\textsuperscript{200-202} and therapy\textsuperscript{200,203} required. Although the underlying pathogenic mechanism is not known, many studies suggest that eosinophil infiltration and desquamation of the bronchial epithelium are involved\textsuperscript{204-206}. Since eosionphil derived mediators damage airway epithelial cells \textit{in vitro} these two events may be linked\textsuperscript{207}.

Adhesion of leucocytes to microvascular endothelium is essential for their migration into inflammed tissue. Infected tissue in severely affected patients contain little or no neutrophils. A ligand for some\textsuperscript{208,209} of these adhesion receptor is inter cellular adhesion molecule-1 (ICAM-1). ICAM-1 was shown to be upregulated on endothelium and skin epithelium both \textit{in vitro} and \textit{in vivo} 4 to 24 hrs after an inflammatory stimulus\textsuperscript{209-211}. In addition monoclonal antibodies (MAbs) to ICAM-1 attenuate neutrophil adhesion to endothelium and inhibit neutrophil trans endothelial migration \textit{in vitro}\textsuperscript{209-211} and \textit{in vivo}\textsuperscript{214}. Thus antagonism of ICAM-1 may provide a therapeutic approach to reducing airway inflammation hyperresponsiveness and asthma symptoms.

5.7.9 Other antiallergic peptides:

In addition to the peptides already discussed, patents on several structurally diverse peptides have been claimed in the literature with antiallergic activity. The peptides have been identified after investigating the antiallergy/antiasthmatic potential of immunomodulatory peptides, RGD peptides, endothelin antagonists and alpha MSH. Their structure and patent numbers have been shown in table 4.
Table 4: Patents on antiallergic oligopeptides

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<tr>
<th>Patent No.</th>
<th>Structure of peptide&lt;sup&gt;a&lt;/sup&gt;</th>
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<tr>
<td>WO 9217191</td>
<td>EW and IW</td>
<td>Cytoven International</td>
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<tr>
<td>WO 9220360</td>
<td>Aerosolised Substance P and Antigen</td>
<td>Northwestern University</td>
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<tr>
<td>EP 526192</td>
<td>Desamino RRYPYL</td>
<td>Tsumura and Co.</td>
</tr>
<tr>
<td>WO 9321211</td>
<td>Ac-c[(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;17&lt;/sub&gt;CH&lt;sub&gt;3&lt;/sub&gt;]E</td>
<td>Laboratories Menarini</td>
</tr>
<tr>
<td>JP 06172287</td>
<td>H&lt;sub&gt;2&lt;/sub&gt;NCH(COOHCH&lt;sub&gt;2&lt;/sub&gt;CH&lt;sub&gt;2&lt;/sub&gt;CONH(CH&lt;sub&gt;3&lt;/sub&gt;)&lt;sub&gt;2&lt;/sub&gt;COOH</td>
<td>Nippon Zoki Pharm</td>
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<sup>a</sup> Single letter code has been used for amino acids, D-amino acid has been represented by small letters.

6. CONCLUSION:

Asthma is a chronic inflammatory disorder characterised by an airway hyper-reactivity to a variety of stimuli and manifests as episodes of coughing, wheezing, chest tightness and shortness of breath. More than 100 million people worldwide have asthma, making it a serious global health problem. In Western countries asthma affects up to 5% of the adult population and perhaps up to 10% of children. Epidemiological studies from several countries suggest that the prevalence of wheezing and self-reported asthma has increased during the past 2 to 3 decades.
The studies carried out so far for treating asthma indicates that conventional therapy directed at the symptomatic relief of bronchospasm is ineffective. On the contrary, drugs that regulates the influx of inflammatory cells into tissue by regulating cellular adhesion molecule or blocking particular chemotactic factors offer potential therapeutic approach. Agents which are involved in blocking the IgE-Fc receptor are currently undergoing trials in animals and soon may undergo trials in human beings. IgE based vaccine is a new concept in this direction in an attempt to prevent binding of IgE to the mast cells. It is thus evident that coming years, will atleast to their suitability investigate targets for prevention of allergic and other mast cell mediated diseases, instead of looking for symptomatic releifs.

DSCG, though the useful drugs for the treatment of mild to moderate asthma which is relatively free from potentially dangerous unwanted effect associated with other antiasthmatic agents, yet the drugs has proved to be rather an enigma. Hence the search for an ideal orally active antiasthmatic drug exhibiting bronchodilatory, anti-inflammatory, anti-histaminic and antiallergic properties continues. An effort in this regard could be to tackle the problem at its very foundation stone, i.e prevention of mast cells to IgE-Fc fragment which triggers the release of various mediators.