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Review Article

Antiallergic oligopeptides: therapeutic potential of peptides in type I allergy

Bijoy Kundu* *and Sanjay K. Khare*

*Division of Biopolymers, Central Drug Research Institute, Lucknow 226001, India. *Correspondence*

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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 viral respiratory infections. In the United States alone, there are 5000 deaths each year and the rate continues to increase (1).

Bronchial asthma is a chronic disease with a remitting and exacerbating course that cannot be cured at present. Both hereditary and environmental factors such as allergies, viral infections and irritants are considered to be responsible for the onset of asthma and the inflammatory exacerbations. The disease may first appear in early childhood along with viral infections, and individuals so afflicted can suffer recurrent episodes throughout their life or may outgrow the disease. On the other hand, there is adult-onset asthma; these people show no symptoms in their childhood or as young adults but suddenly develop them later in life.

Classification of asthma

Clinically, asthma can be classified in several ways and its management varies according to the classification. Allergic (extrinsic) asthma is experienced by adults and, most commonly, by children, who are allergic to house dust, pollens, foods, drugs and usually have elevated levels of circulating immunoglobulin E (IgE). Nonallergic (intrinsic) asthma is more likely to occur in adulthood and is generally severe and steroid-dependent. Episodes of intrinsic asthma may be triggered by a variety of stimuli, *e.g.*, emotional states, exposure to cold air or inert dusts. Both intrinsic and extrinsic asthmatics can be prone to exercise-induced attacks. Individuals who experience a combination of extrinsic and intrinsic asthmatic reactions have mixed asthma. Status asthmaticus refers to an acute life-threatening asthmatic attack which is generally resistant to normal treatment and often demands hospitalization.

Role of cytokines in asthma

For many years, mast cells 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 preformed and rapidly synthesized substances 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 mast cell activation, allergen can also 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 the 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 irreversible 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 mRNA for IL-2, IL-3, IL-4 and IL-5 have been found to be significantly increased. Further, higher levels of IL-4 and IL-5 were observed in asthmatics than in nonatopic controls (2). In fact, 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 a switch factor and is enhanced by IL-5 and IL-13, whereas IFN-γ, IL-8 and IL-12 antagonize IgE production. This is evident from studies in IL-4 knockout mice which have demonstrated that IgE production *in vivo* is almost entirely dependent on IL-4 co-signal at the time of antigen presentation (3). Another cytokine that is important in the regulation of IgE synthesis is IFN-γ, which acts as an inhibitor of allergic responses through its capacity to inhibit the effect of IL-4 on B-cells.

The generation, differentiation and recruitment of eosinophils, the second component of allergic diseases and the most critical determinant for the development of inflammation, is governed by IL-5 (2). For a long time it has been known that eosinophils accumulate in high numbers in the lungs of asthmatics and their presence and numbers correlate with the degree of lung dysfunction.

Furthermore, the conversion 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 (4). In addition, another cytokine that favors the progression of Th2 responses indirectly by suppressing IFN-γ 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 conversion is more crucial than the type of inhaled allergen in determining the nature of the airway immune response.

Attempts to understand the role of cytokines as well as the mechanism as to why the immune response becomes shifted towards Th2 response has provided novel therapeutic approaches for asthma. For example, whereas IFN-α, IFN-γ and IL-12 represent potential therapeutic targets for redirecting Th2 conversion and suppressing Th2 function, blocking activation and preventing the synthesis of IL-4, IL-13, IL-10 and IL-5 are important therapeutic approaches that may inhibit the effects of established Th2 cells in human asthma.

Management of asthma

In recent years, important advances have been made in the development of improved symptomatic and palliative therapy for asthma, including novel leukotriene antagonists, phosphodiesterase inhibitors, long-acting bronchodilators, corticosteroids and other mediator antagonists. However, these agents are known to simply provide symptomatic relief of asthma without controlling the inflammation. In addition, these drugs are associated with adverse effects and asthmatic patients have to understand how to manage drug therapy and their side effects. Unfortunately, despite the fact that much is known about the disease and its care, asthma is still seriously undertreated. One recent survey of 94 adult asthmatics showed that approximately 75% were not receiving the required therapy and were not using medications properly (1). Indeed, in recent years delineation of immunological mechanisms that result in allergic sensitization has contributed significantly to the development of specific strategies to cure allergy. Thus, modern treatment of asthma is directed towards lessening inflammation, thereby reducing airway reactivity and asthma severity. Novel therapeutic approaches based on new mechanisms of action are being developed with more emphasis on the prevention and cure of the disease, including immunosuppressants, neuropeptide antagonists, IL-1 inhibitors, blockers inhibiting binding of IgE to mast cells, *etc*. Most of these compounds are comprised of peptides which form new prototypes and can therapeutically interfere on various levels of immunological dysfunction.

The purpose of this review is to briefly describe the various peptide-based experimental compounds that are being developed for the treatment of type I allergy.

Neuropeptides in the respiratory tract

It is well known that the functions of the respiratory tract are controlled by specialized endocrine cells and 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 specialized myocardial cells present in pulmonary veins. In the 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 neural activity and the antidromic secretion of neuropeptides. In humans, the upper and lower airway

Table I: Neuropeptides and their effects in the respiratory tract.

Distribution	Neuropeptide	Role in Pulmonary Diseases
Neural	Substance P	Stimulates release of mediators from mast cells
Neural	Neurokinin A	Bronchoconstriction, inflammation, mucus edema
Neural	Neurokinin B	Bronchoconstriction, inflammation
Neural	Calcitonin gene related peptide	Production of mucus
Endocrine	Calcitonin	NCE
Neural	Vasoactive intestinal peptide	Vasodilation
Neural	Neuropeptide Y	NCE
Endocrine	Bombesin	NCE
Myoendocrine	Atrial natriuretic peptide	Bronchodilator
Neural	Peptide histidine methionine	Antiinflammatory
Neural	Peptide histidine isoleucine	Inhibits mast cell degranulation and mucus production
Neural	Galanin	NCE
Endocrine	Gastrin releasing peptide	Lung fibrosis
Endocrine	Enkephalin	NCE
Endocrine	Cholecystokinin	NCE
Epithelial	Endothelins*	Bronchoconstriction

NCE = Not clearly established. *Also produced by airways endocrine and endothelial cells.

functions are regulated both by cholinergic and adrenergic pathways as well as by nonadrenergic and noncholinergic pathways (NANC). The neurotransmitters for the NANC nervous system are considered to be the neuropeptides (5, 6).

Although strict anatomical demarcations in the distribution of regulatory peptides in precise anatomical structures of the lung cannot be made, a broad classification of the general distribution of the regulatory peptides along with their effects are shown in Table I. The role of some of these peptides in lung function is not yet clearly established.

Among the regulatory peptides of the respiratory tract, bradykinin, vasoactive intestinal peptide (VIP), calcitonin gene related peptide and neurokinin have been implicated in the pathogenesis of asthma. These peptides induce bronchoconstriction, vasodilation with airway edema, microvascular leakage and increased mucus secretion with coughing.

Thus, in the plethora of active peptides produced and released from specific structures of the respiratory tract, including innervation, airway epithelium and the endothelium, documentation regarding the alteration of these active peptides in diseases such as asthma is beginning to appear. Asthma is a complex disease which is poorly understood, but the potent actions of the neuropeptides modulating airway tone and participating in tissue repair are likely to be altered in asthma and 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.

Bradykinin antagonists

Bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), a nonapeptide released during the inflammatory response, is known to play an important role in the allergic pathophysiology of the airways, contributing to bronchoconstriction and edema formation. Raised levels of kinin-generating enzymes and kinins are found in the airways during allergic response (7). 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 bradykinin is one of the most potent bronchoconstrictor agents which stimulates the release of inflammatory mediators such as platelet activating factor (PAF), peptidoleukotrienes, leukotriene B_4, as well as various prostaglandins in many tissues including those of the airways.

Bradykinin receptors, therefore, may play an important role in the management of asthma. Receptors for bradykinin, B_1 and B_2, have been classified according to the relative potencies of various agonists and antagonists. Studies have shown that generally the bradykinin-induced responses in most of the inhaled smooth muscle and other tissues are mediated via B_2 receptors. Attempts to inhibit B_2 receptor-mediated effects by bradykinin led to the identification of [D-Phe7]-bradykinin as one of the first B_2 receptor antagonists (7). Since then, numerous peptide B_2 receptor antagonists have been synthesized and two of the most potent ones are NPC-567 and NPC-16371 (8) (Fig. 1). 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.

Vasoactive intestinal peptide and its analogs

Vasoactive intestinal peptide (VIP), a 28 amino acid linear peptide, was first discovered, isolated and purified from porcine intestinal extracts. Later it was recognized

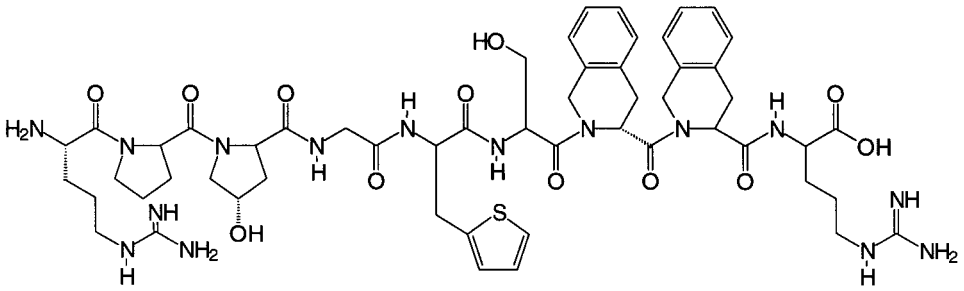


Fig. 1. Structure of NPC-16371.

as a neuropeptide widely distributed in the central and peripheral nervous system, with neurotransmitter properties and a broad spectrum of biological actions. The regulatory effect of VIP is well demonstrated in the normal lung, where it is present in high concentrations and mediates NANC 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 inflammation. It downregulates T-lymphocyte proliferation, possibly through its effect on the expression of various cytokines (9). 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-lymphocytes 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: (i) the combined administration of VIP with one or more selective peptidase inhibitors (Patent No. JP 05238950) or (ii) 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 that have similar biological activity but are relatively protease-resistant. One such compound is helodermin, which was originally isolated from the lizard *Gilamonster heloderma* but is also present in mammalian tissue and exhibits strong homology to VIP. Helodermin is a 35 residue peptide and was equipotent to VIP as a relaxant of guinea pig tracheal smooth muscle. The relaxant action was found to be 4-10 times more sustained than VIP (10). Its C-terminal extension may account for its decreased susceptibility to enzymatic degradation and its long-lasting tracheal relaxation.

In addition to helodermin, several analogs of VIP were synthesized in order 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. 2) which exhibited exceptionally high potency, metabolic stability and a long duration of action (11).

Ro-25-11553 exhibited bronchodilator activity through activation of VIP receptors in a number of *in vitro* pharmacological assays. This activity was also extended *in vivo*, where it was shown to be active by intratracheal instillation and aerosol administration against a number of different spasmogens. It was also found to suppress a number of features associated with pulmonary anaphylaxis and asthma, including IL-2 and IL-4 production (12, 13), edema formation and granulocyte accumulation in guinea pig lung. These antiinflammatory activities, together with its bronchodilatory effects, makes Ro-25-1553 a potential therapeutic agent for the treatment of bronchial asthma.

Neurokinin antagonists

The mammalian tachykinins are a family of neuropeptides which include substance P (SP), neurokinin A (NKA) and neurokinin B (NKB). They are characterized by a common C-terminal sequence, Phe-X-Gly-Leu-Met-NH_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 *in vivo* and *in vitro*, mucus secretion, plasma extravasation and neural excitation, could be considered to mimic the symptoms of asthma (14). Thus, if tachykinins were to play an important role in the pathophysiological process of asthma, selective antagonists would be of considerable clinical importance.

The neurokinin receptors can be broadly classified into three subtypes NK-1, NK-2 and NK-3, which have

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_2

Fig. 2. Structure of Ro-25-1553.

Table II: Some potent and widely studied NK-2 antagonists.

Code No.	Structure	Ref.
-	Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2	15
GR-83074	BOC-Arg-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2	15
GR-94800	PhCO-Ala-Ala-D-Trp-Phe-D-Pro-Pro-Nle-NH_2	15
L-659877	[cyclo(Gln-Trp-Phe-Gly-Leu-Met)]	16
L-659874	Ac-Leu-Met-Gln-Trp-Phe-Gly-NH_2	16
MDL-29913	[cyclo(Gln-Trp-Phe-Gly-Leu-CH_2NCH_3Leu)]	17
MEN-10207	Asp-Tyr-D-Trp-Val-D-Trp-D-Trp-Arg-NH_2	18

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 mediate the non-cholinergic constriction produced by endogenous tachykinins (14). 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 analogs has in many instances led to highly specific agonists or antagonists for each of these receptor subtypes. In the literature, more than 50 linear or cyclic peptides with potent and highly selective neurokinin antagonism for the three tachykinin receptors have been reported which may have therapeutic potential for a variety of diseases. Some of the most potent and widely studied NK antagonists are shown in Table II.

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-D-Trp-Phe-NH_2 as a lead peptide (SP antagonist) and hypothesized that the essential domain that binds to the receptor might be comprised of only few amino acid residues. They synthesized a number of unprotected and fully protected tripeptide amides and tested them in receptor binding assays. The protected tripeptide Ac-Thr-D-Trp(CHO)-Phe-NMeBzl (FR-113680) exhibited potent binding affinity to the receptor and was stable against enzymatic degradation (19). The IC_{50} value was found to be 5.8 nM, in comparison to 600 nM for the octapeptide. 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 synthesized 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 (20) (Fig. 3).

Hagiwara *et al.* reported another novel neurokinin antagonist, a cycloheptapeptide (21) lactone named WS-9326A (Fig. 4), which was initially isolated from a soil sample from Suwa, Japan. The microorganism producing this compound was identified as *Streptomyces vio-laceoniger*. Hydrogenation of two of the double bonds of WS-9326A afforded FK-224 (Fig. 4), an antagonist of both SP and NKA (21). Recently, Aramori *et al.* (22) compared the receptor binding properties and potencies of FK-224 and FK-888 using human receptor subtypes. The results indicate that FK-224 has dual actions to NK-1 and NK-2 while FK-888 is highly selective to NK-1. Similarly, the *in vivo* activity of these two compounds was evaluated in the experimental model for asthma (23). 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 respective ED_{50}s of 0.011 and 0.019 mg/kg. The bronchial contractile response to allergen in the presence or absence of FK-224 *in vitro* was also examined, and it was observed that the compound significantly inhibited ovalbumin-induced

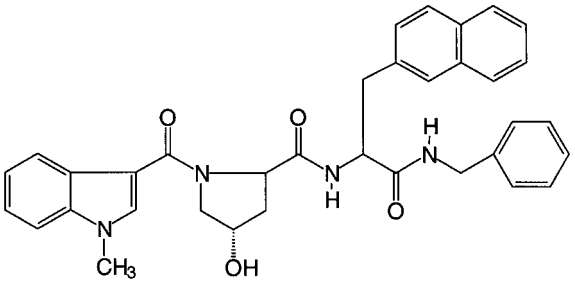
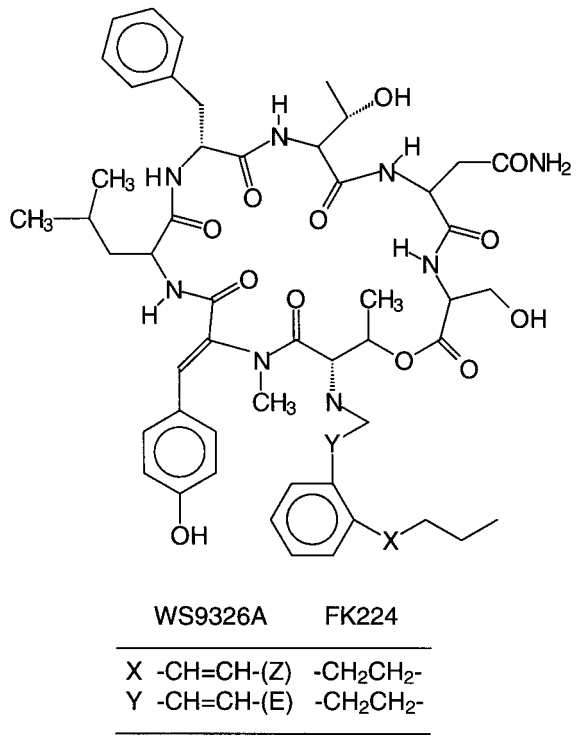


Fig. 3. Structure of FK-888.



	WS9326A	FK224
X	-CH=CH-(Z)	$-CH_2CH_2-$
Y	-CH=CH-(E)	$-CH_2CH_2-$

Fig. 4. Structure of WS-9326A and FK-224.

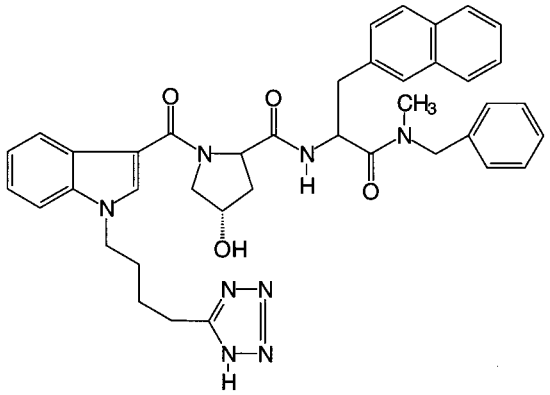


Fig. 5. Structure of S-18523.

contraction. Both FK-224 and FK-888 are undergoing clinical trials and may provide new therapies for the treatment of asthma.

Recently a water-soluble dipeptide NK-1 receptor-selective antagonist S-18523 (Fig. 5) was reported (24). The potassium salt of the dipeptide derivative was found to antagonize bronchoconstriction provoked by exogenous 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.

Inhibitors of IgE-Fc epsilon-RI interaction

Immunoglobulin E (IgE) plays an important role in mediating immediate hypersensitization such as asthma, hay fever, food and drug allergies. In recent years IgE has attracted the attention of many investigators because of the association of high levels of serum IgE with asthma. There is 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 receptor-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, Fc epsilonRI, is a critical step in the development of an allergic reaction, it has been proposed that binding to and blocking of the Fc epsilonRI by certain peptides would inhibit release of the chemical mediators. Such an IgE receptor-binding peptide, termed "isotype-specific" method of regulation, would be an ideal antiallergic agent for treating type I immediate hypersensitivity.

Several strategies have been utilized to delineate the binding region in the IgE to human Fc epsilonRI. Studies with proteolytic peptide fragments of human IgE (25), chimeric immunoglobulin molecules (26, 27), recombinant fragments (28, 29) and, most recently, with site-directed mutagenesis (30) have suggested that the binding site lies in the Fc epsilon region, particularly in the CH3 and CH4 domains. However, the exact binding site remains unidentified.

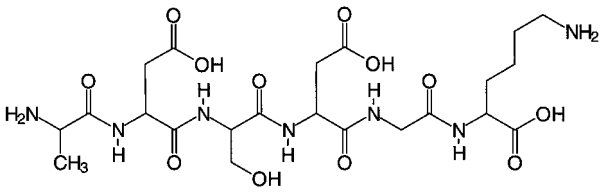


Fig. 6. Structure of Hamburger's pentapeptide.

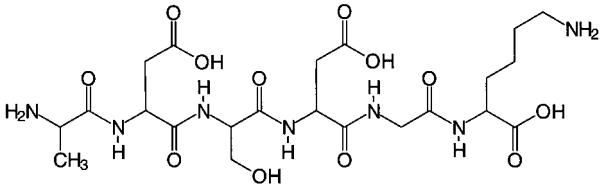


Fig. 7. Structure of Noguchi's hexapeptide.

In an attempt to identify the binding site, Stanworth *et al.* in 1968 reported for the first time that the IgE fragment, crystalline fragment (IgE-Fc), bound to the IgE receptor and was involved in allergic reactions (31). This fragment (Fig. 6) was later identified by Hamburger as a pentapeptide (320-324) which blocked the Prausnitz-Kustner action (32). Abbott Laboratories synthesized this pentapeptide by solution phase on a large scale and initiated clinical trials for the treatment of allergic rhinitis (33).

Noguchi *et al.* reported a new class of oligopeptides related to Hamburgers pentapeptide which blocked allergic response by interfering with the binding of IgE to the receptor mast cells (34). One of the hexapeptides (Fig. 7) exhibited a very high order of biological activity by inhibiting the production of IgE antibody and by preventing the contraction of rabbit aorta.

Nio *et al.* carried out the synthesis of 112 peptide fragments spanning the CH3-CH4 domain in human IgE in order to identify the exact binding site. The peptides were assayed for their capacity to inhibit passive cutaneous anaphylaxis (PCA) *in vitro*. The results suggested that an octapeptide (Fig. 8) corresponding to 345-352 in the human IgE molecule may be an IgE binding site (35, 36). It exhibited significant inhibition of PCA, probably by occupying the Fc epsilon receptor sites on the cells.

Stanworth reported (37) a new antiallergic tripeptide (Fig. 9) based on the molecular modeling 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 a trigger signal to the mast cells to release histamine, as a consequence of the cross-linking of Fc epsilonRI-bound IgE antibody by specific antigen (allergen). The tripeptide (WO 9510532) was found to be considerably more active than the established antiallergic drug Nedocromil in the inhibition of allergen-induced histamine release from sensitized mast cells *in vitro*.

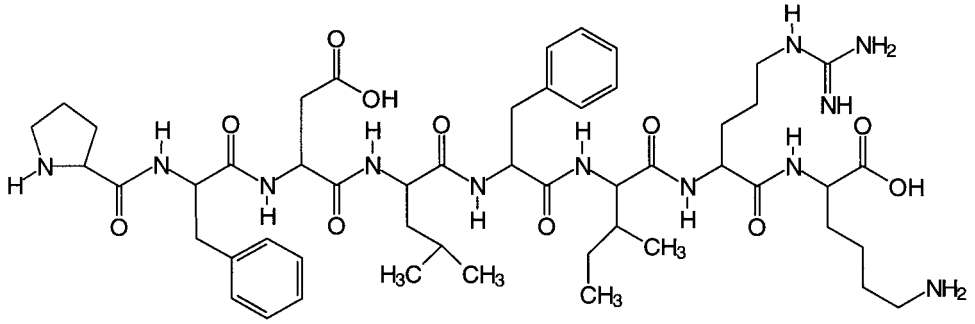


Fig. 8. Structure of octapeptide 345-352 in the human IgE.

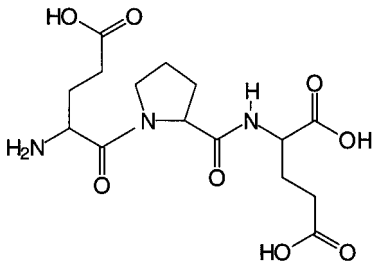


Fig. 9. Structure of WO 9510532.

Recently, McDonnell *et al.* (38, 39), using a structure-based drug design approach, described a peptide derived from the Fc epsilonRI α-chain 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 domains 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 cyclized by means of N-terminal L-Cys and C-terminal D-Cys. This type of cyclic structure (Fig. 10) was expected to adopt a native-like conformation. Several other cyclic peptides with retro-enantiomer version, with reverse sequence and with scrambled sequence were also synthesized. Cyclo(L-262) (Fig. 10) and cyclo(rD-262) exhibited significant inhibition in both the binding assay and in the mast cell stabilization assay. This interesting class of cyclic peptides provides a good lead for the development of therapeutically useful anti-asthmatic agents.

In our laboratory, we were interested in optimizing the biological activity of the hexapeptide reported by Noguchi *et al.* (34). First, structure-activity relationship studies were carried out in order to suppress asparmide formation associated with Asp-Gly and Asp-Ser in the hexapeptide sequence. A number of analogs were synthesized and one of them, CDRI-94/335 (Fig. 11) with Gly at position 2 instead of Asp exhibited high activity by both intraperitoneal and oral routes in rats (40).

Orally, 94/335 exhibited significant inhibition of PCA with an ED_{50} of 0.6 mg/kg, which was close to 0.9 mg/kg for Noguchis hexapeptide. However, in the mast cell stabilizing assay, it exhibited much better protection of mast cells, with an ED_{50} of 1 mg/kg, compared to 3 mg/kg for the lead peptide. Intraperitoneally, CDRI-94/335 was found to be at least 50 times more potent per dose than disodium cromoglycate, a standard antiallergic drug used clinically. Thus, our studies provide the first experimental evidence for the existence of antiallergic activity in small peptides by the oral route and open a new avenue for further exploration.

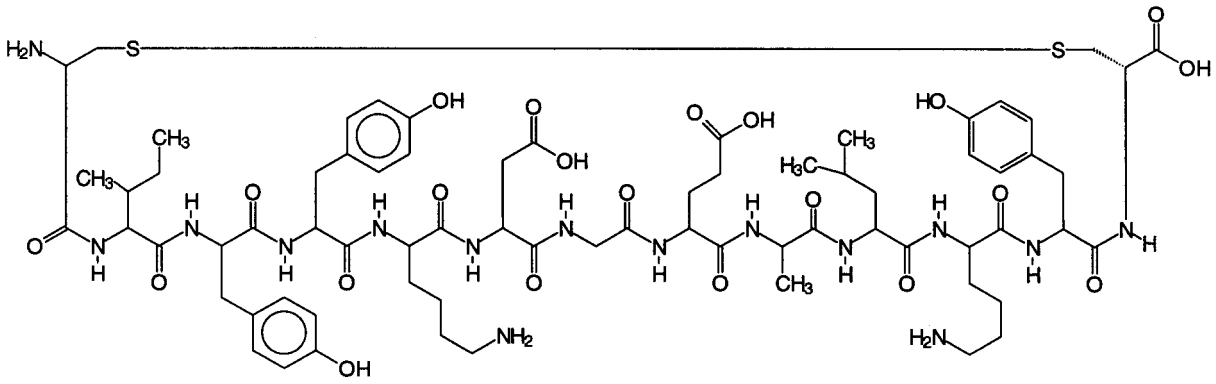


Fig. 10. Structure of cyclo(L-262).

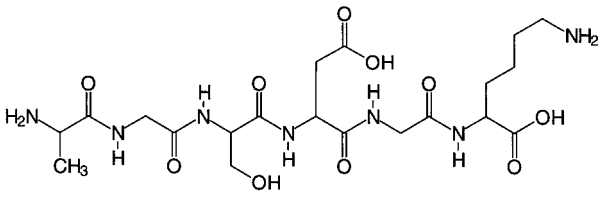


Fig. 11. Structure of CDRI-94/335.

In addition, the following patents on IgE peptides have been claimed in the literature as inhibitors of allergy: WO 9601643, JP 06336496, JP 04187091 and JP 04187088.

Muramyl dipeptides

Oral administration of muramyl dipeptide (MDP) has been shown to induce certain biological response, including the downregulation of amnestic and antigen-specific IgE responses, which are not observed following parenteral administration (41). This led to the identification of a novel analog of MDP, *N*-acetyl muramyl-L-threoninyl-D-isoglutaminyl-*sn*-glyceryl-dipalmitoyl (SDZ-280636) which exhibited significant suppression of polyclonally induced serum IgE levels in anti-IgD treated mice (42). The liposome derivative of MDP (Fig. 12) was found to selectively inhibit IgE response when administered orally without affecting the levels of other immunoglobulins. The antiallergic activity exhibited by SDZ-280636 was attributed to its suppressive effect on Th2 activity in gut-associated lymphoid tissue, which is known to be the site of the first appearance of IgE response (43). Recently, SDZ-280636 was reported to selectively suppress IL-4, but not IL-13, mRNA expression in gut-associated lymphoid tissue and mesenteric lymph nodes but not in the spleen (44). These properties make the compound a potential candidate for the treatment of type I immediate hypersensitivity, and in fact is presently being investigated as a lead compound for development as an antiallergic drug.

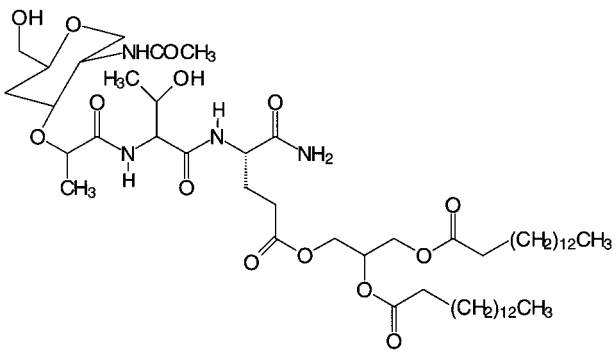


Fig. 12. Structure of SDZ-280636.

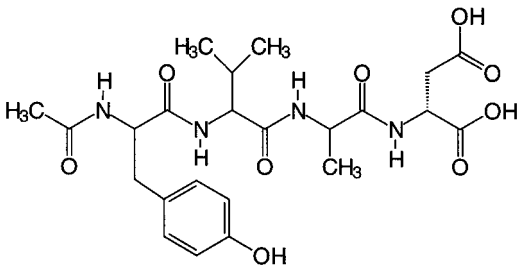


Fig. 13. Structure of IL-1 inhibitor L-709049.

Interleukin-1 inhibitors

IL-1, with a very diverse biology, has been shown to play an important role in the proliferation of Th2 cells (45) and in the recruitment of eosinophils at the sites of allergic inflammation (46) 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.* (47) 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. 13), as a novel antiallergic agent.

Ciclosporin

Ciclosporin and FK-506 are fungal metabolites widely known for their immunosuppressant 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* (48), thereby indicating their therapeutic potential for the treatment of allergic disorders. Novel ciclosporin analogs (Fig. 14) prepared by Sandoz have been claimed to be of potential use for topical application in asthma therapy.

Immunotherapy

It is well known that desensitization, or immunotherapy, with a variety of crude allergens prevents allergic symptoms in many patients, and was a mainstay of asthma treatment for many years. However, the routine use of extensive allergen testing, together with its temporary and variable effects, was a major limiting factor for its wide acceptance. While studying the mechanism of action of desensitization, it was observed that T-cell activity was suppressed (49, 50), thereby emphasizing the central role

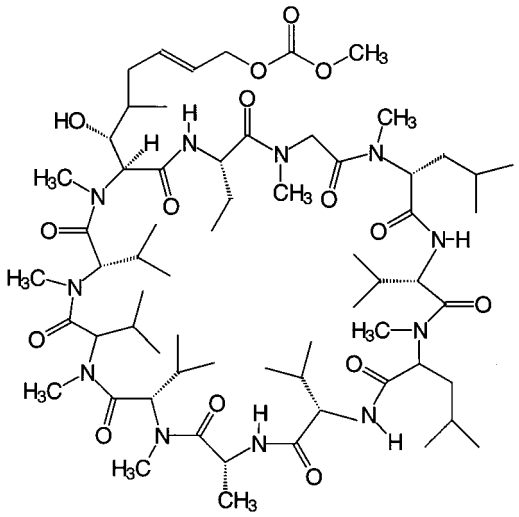


Fig. 14. Structure of ciclosporin analog.

of T-cells in allergic sensitization and IgE regulation. The allergen-specific T-cells are responsible for the enhanced production of IL-4 and IL-13, which in turn, trigger IgE synthesis followed by development of allergic diseases. Therefore, inhibiting the production of IL-4 and IL-13 or neutralizing their activities are two therapeutic approaches which can be used for inhibiting IgE production. In the first case, an IL-4 mutant protein with Tyr at position 124 instead of Asp was identified (51, 52) and found to act as a powerful antagonist of both IL-4 and IL-13 activity, including induction of IgE synthesis by these cytokines *in vitro*. Using the second approach, allergen-derived peptides were used to render Th2 cell clones nonresponsive, which in turn failed to provide the necessary B-cell help for IgE synthesis by inhibiting the production of IL-4 and IL-13. These findings led to the conclusion that allergen-derived peptides may represent an important and effective means for successful immunotherapy.

Peptides obtained by urea denaturation or enzymatic digestion of allergens have been evaluated in animal models and humans. However, in recent years short allergen-derived peptides representing minimal T-cell activating epitope have been synthesized and evaluated for use as immunotherapy, *e.g.*, immunodominant peptides from Fe1D1 cat allergen (53), DerP1 house dust mite allergen (54), Ambal ragweed allergen (55), *etc.* Clinical trials with a cat allergy vaccine based on a short peptide fragment that mimics the action of allergen are in progress.

Peptide 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 (56). 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 synthesized had the desired antagonist properties. Alternatively, it was decided to generate an 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 (57). 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 humans are under way.

Miscellaneous

In addition to the peptides already discussed, patents on several structurally diverse peptides with antiallergic activity have been claimed in the literature. These peptides were identified after investigating the antiallergic/antiasthmatic potential of immunomodulatory peptides, RGD peptides, endothelin antagonists and MSH-alpha peptides. Their structure and patent numbers are shown in Table III.

Conclusions

As outlined in this brief review, the role of structurally diverse peptides for the treatment of type I allergy is beginning to develop. The most interesting aspect of these peptides is that they can therapeutically interfere with the molecular mechanism of Th2-mediated immunity linked to the clinical expression of asthma. This is in contrast to the conventionally used drugs which are known to simply provide symptomatic relief. Furthermore, the powerful influence of oligopeptides on the immune system is already well known and some of them are being successfully used clinically as immunomodulatory agents. Therefore, small peptides comprised of 5-10 amino acid residues which can interact with the airway immune response may be of significant interest as lead molecules for realizing the long-awaited goal of preventive/curative therapy for asthma. With this in mind, detailed studies with clinically established immunomodulatory peptides may lead to better therapeutic modalities against allergic diseases. New targets for the prevention of T-cell eosinophil inflammation are likely to be identified in the future. These and other insights will play a major role in

Table III: Patents on antiallergic oligopeptides.

Patent No.	Structure of peptide[#]	Company/Institute
WO 9217191	EW and IW	Cytoven International
WO 9220360	Aerosolized substance P and antigen	Northwestern University
EP 526192	Desamino RRPYIL	Tsumura and Co.
WO 9321211	Ac-c[$(CH_2)_{17}CH_3$]E	Laboratories Menarini
JP 06172287	H_2N-CH(COOH)$CH_2CH_2CONH(CH_2)_nCOOH$	Nippon Zoki Pharm.
JP 06239887	Ac-GPCRAFAfPYGGCK	Taisho Pharma Co. Ltd.
WO 9420531	KRGCGTKFLSYKSC	Teijin Ltd.
WO 9508564	(DL-Lip)-H-HomofR	Europeen de Biologie France
WO 9513826	Peptides containing RGD sequence	Meiji Milk products Ltd.
RU 2000119	Hexapeptide	Izobreteniya, Russia

[#]Single letter code has been used for amino acids, D-amino acid has been represented by small letters.

the search for an ideal orally active drug exhibiting bronchodilatory, antiinflammatory and antihistaminic activities.

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References

1. *Focus on asthma.* Med Sci Bull 1997, 237: 12-5.

2. Howrath, P.H., Bradding, P., Quint, D., Redington, A.E., Holgate, T. *Cytokines and airway inflammation.* Ann NY Acad Sci 1994, 725: 69-82.

3. Kopf, M., Gros, G.L. Bachmann, M., Lamers, M.C., Bluethmann, H., Kohler, G. *Disruption of the murine IL-4 gene blocks the Th2 cytokine responses.* Nature 1993, 362: 245-50.

4. Jucker, J.F., Anderson, G.P. *Emerging drug targets in the molecular pathogenesis of asthma.* Prog Drug Res 1996, 47: 165-210.

5. Casale, T.B. *Neuropeptides and lung.* J Allergy Clin Immunol 1991, 88: 1-14.

6. Springall, D.R., Polak, J.M. *Localisation of peptides in the lung.* Ann NY Acad Sci 1991, 629: 288-304.

7. Farmer, S.G., Burch, R.M. *Airway bradykinin receptors.* Ann NY Acad Sci 1991, 629: 237-49.

8. Steranka, L.R., Farmer, S.G., Burch, R.M. *Antagonists of bradykinin receptors.* FASEB J 1989, 3: 2019-25.

9. Gozes, I., Brenneman, D.E. *VIP: Molecular biology and neurobiological function.* Mol Neurobiol 1989, 3: 201-36.

10. Jager, E., Bauer, S., Joyce, M.W., Foda, H.D., Berisha, H.I., Said, S.I. *Structure-activity studies on VIP IV: The synthetic agonist helodermin fragment (1-28) amide is a potent VIP agonist with prolonged duration of tracheal relaxant activity.* Ann NY Acad Sci 1996, 805: 499-504.

11. O'Donnell, M., Garippa, R.J., Rinaldi, N. et al. *Ro 25-1553: A novel long acting vasoactive intestinal peptide agonist. Part I: In vitro and in vivo bronchodilator studies.* J Pharmacol Exp Ther 1994, 270: 1282-8.

12. O'Donnell, M., Garippa, R.J., Rinaldi, N. et al. *Ro 25-1553: A novel, long acting vasoactive intestinal peptide agonist. Part II: Effect on in vitro and in vivo models of pulmonary anaphylaxis.* J Pharmacol Exp Ther 1994, 270: 1289-94.

13. Tang, H., Welton, A., Ganea, D. *Neuropeptide regulation of cytokine expression: Effects of VIP and Ro 25-1553.* J Interferon Cytokine Res 1995, 15: 993-1003.

14. Verleden, G.M. *Neural mechanisms and axon reflexes in asthma: Where are we.* Biochem Pharmacol 1996, 51: 1247-57.

15. McElory, A.B., Clegg, S.P., Deal, M.J. *Highly potent and selective heptapeptide antagonists of the neurokinin NK-2 receptor.* J Med Chem 1992, 35: 2582-91.

16. Mcknight, A.T., Maguire, J.J., Williams, B.J., Foster, A.C., Tridgett, R., Iverson, L.L. *Pharmacological specificity of novel, synthetic, cyclopeptide antagonists at tachykinin receptors.* Br J Pharmacol 1991, 104: 355-360.

17. Maggi, C.A., Patacchini, R., Astolto, M., Rovero, P., Giuliani, S., Giachetti, A. *NK-2 receptor agonists and antagonists.* Ann NY Acad Sci 199**?**, 632: 184-91.

18. Rovero, P., Pestellini, V., Maggi, C.A. et al. *A highly selective antagonist containing D-tryptophan.* Eur J Pharmacol 1990, 175: 113-5.

19. Hagiwara, D., Miyake, H., Morimoto, H., Murai, M., Fuji, T., Matsuo, M. *Studies on neurokinin antagonist 2.* J Med Chem 1992, 35: 3184-91.

20. Hagiwara, D., Miyake, H., Murano, K. et al. *Studies on neurokinin antagonist 4.* J Med Chem 1994, 7: 2090-9.

21. Hashimoto, M., Hayashi, K., Murai, M. et al. *WS9326A, a novel tachykinin antagonist isolated from Streptomyces violacensniger no. 9326 II: Biological and pharmacological properties of WS9326A and tetrahydro-WS9326A (FK224).* J Antibiot 1992, 45: 1064-70.

22. Aramori, I., Morikawa, N., Zenkoh, J. et al. *Subtypes and species selectivity of a tachykinin receptor antagonist, FK 888, for cloned rat and human tachykinin receptor.* Eur J Pharmacol 1994, 269: 277-81.

23. Murai, M., Maeda, Y., Hagiwara, D. et al. *Effects of an NK-1 receptor antagonist, FK888 on constriction and plasma extravasation induced in guinea pig by neurokinin and capsaicin.* Eur J Pharmacol 1993, 236: 7-13.

24. Bonet, J., Kucharczyk, N., Robineau, P. et al. *A water soluble stable dipeptide NK-1 receptor selective neurokinin receptor antagonist with potent in vivo pharmacology effects: S18523.* Eur J Pharmacol 1996, 310: 37-46.

25. Weetall, M., Shapes, B., Holowka, D., Baird, B. *Mapping the site of interaction between murine IgE and its high affinity receptor with chimeric immunoglobulin.* J Immunol 1990, 145: 3849-54.

26. Nissim, A., Jouvin, M-H., Esshar, Z. *Mapping of the high affinity Fc epsilon receptor binding site to the third constant region domain of IgE.* EMBO J 1991, 10: 101-7.

27. Presta, L., Shields, R., O'Connell, L. et al. *The binding site on human immunoglobulin E for its high affinity receptor.* J Biol Chem 1994, 269: 26368-73.

28. Geha, R.S., Helm, B., Gould, H. *Inhibition of the Prausnitz-Kustner reaction by an immunoglobulin epsilon-chain fragment synthesised in E. coli.* Nature 1985, 314: 577-8.

29. Coleman, J.W., Helm, B., Stanworth, D.R., Gould, H.J. *Inhibition of mast cell sensitization in vitro by a human immunoglobulin epsilon chain fragment synthesised in E. coli.* Eur J Immunol 1985, 15: 966-9.

30. Liu, F-T., Albradt, K.A., Bry, C.G., Ishizaka, T. *Expression of a biologically active fragment of human IgE epsilon chain in E. coli.* Proc Natl Acad Sci USA 1984, 81: 5369-73.

31. Stanworth, D.R., Humphrey, J.H., Bennich, H., Johansson, S.G.O. *Inhibition of Prausnitz-Kustner reaction by proteolytic cleavage fragments of a human myeloma protein of immunoglobulin class E.* Lancet 1968, 2: 17-8.

32. Hamburger, R.N. *Peptide inhibition of the Prausnitz-Kustner reaction.* Science 1975, 189: 389-90.

33. Tolle, J.C., Heinrichsmeyer, D.N., Miller, R.B., Lundell, O., Gifford, W.E., Funk, K.W. *A large scale synthesis of IgE pentapeptide.* Proc 22nd Eur Peptide Symp 1992.

34. Noguchi, K., Matsuo, K., Ohta, N. *Synthesis of peptides related to immunoglobulin E and the examination of their pharmacological activity.* Chem Pharm Bull 1990, 38: 2463-6.

35. Nio, N., Seguro, K., Ariyoshi, Y., Katsumi, I., Nakamura, H. *Inhibition of passive cutaneous anaphylaxis by synthetic human immunoglobulin E peptide fragments.* FEBS Lett 1992, 314: 229-31.

36. Nio, N., Seguro, K., Ariyoshi, Y. et al. *Inhibition of passive sensitization of human peripheral basophils by synthetic human immunoglobulin E peptide fragments.* FEBS Lett 1993, 319: 225-8.

37. Stanworth, D.R. *Novel tripeptide treatments of IgE allergies.* In: Innovation and Perspectives in Solid Phase Synthesis and Combinatorial Libraries. Eptons, R. (Ed.). Mayflower Scientific: Birmingham 1996, 173.

38. McDonnell, J.M., Beavil, A.J., Mackay, G.A. et al. *Structure based design and characterisation of peptides that inhibit IgE binding to its high affinity receptor.* Nat Struct Biol 1996, 3: 419-26.

39. McDonnell, J.M., Beavil, A.J., Mackay, G.A. *Structure based design of peptides that inhibit IgE binding to its high affinity receptor Fc epsilonRI.* Biochem Soc Trans 1997, 25: 387-92.

40. Kundu, B., Shukla, M., Khare, S.K. et al. *Antiallergic activity of some novel oligopeptides related to immunoglobulin E.* Biorg Med Chem Lett 1997, 7: 1191-4.

41. Auci, D.L., Chice, S.M., Dukor, P., Durkin, H.G. *Control of IgE responses II. Isotype-specific suppression of peak hapten of IgE antibody forming cell responses in BPO-KLH sensitized mice after oral administration of muramyl dipeptide or murabutide.* Immunopharmacology 1993, 26: 157-69.

42. Bahr, G.M., Darcissac, E., Bevec, D., Dukor, P., Chedid, L. *Immunopharmacological activities and clinical development of muramyl peptides with particular emphasis on murabutide.* Int J Immunopharmacol 1995, 17: 117-31.

43. Auci, D.L., Chice, S.M., Dukor, P., Durkin, H.G. *Origin and fate of IgE-bearing lymphocytes. II. Gut-associated lymphoid tissue as sites of first appearance of IgE-bearing B lymphocytes and hapten-specific IgE antibody-forming cells in mice immunized with benzylpenicillinoyl-keyhole limpet hemocyanin by various routes: Relation to asialo GM_1 ganglioside+ cells and IgE/CD23 immune complexes.* J Immunol 1992, 149: 2241-8.

44. Kricek, F., Zunic, M., Ruf, C., De Jong, G., Dukor, P., Bahr, G.M. *Suppression of in vivo IgE and tissue IL-4 mRNA induction by SDZ 280.636, a synthetic muramyl dipeptide derivative.* Immunopharmacology 1997, 36: 27-39.

45. Weaver, C.T., Hawry Lowicz, C.M., Unanue, E.R. *T-helper cell subsets require the expression of distinct costimulatory signals by antigen presenting cells.* Proc Natl Acad Sci USA 1988, 85: 8181-5.

46. Sanz, M.J., Weg, V.B., Bolanowski, M.A., Nourshargh, S. *IL-1 is a potent inducer of eosinophil accumulation of response by a platelet activating factor antagonist and an antihuman IL-8 antibody.* J Immunol 1995, 154: 1364-73.

47. Watson, M.L., Smith, A.D., Bourne, R.C. et al. *Cytokines contribute to airway dysfunction in antigen challenged guinea pigs.* Am J Respir Cell Mol Biol 1993, 8: 365-9.

48. Mori, A., Suko, M., Nishizaki, Y. et al. *Regulation of IL-5 production by peripheral blood mononuclear cells from atopic patients with FK-506 and cyclosporin.* Int Arch Allergy Immunol 1994, 104: 32-5.

49. Wierenga, E.A., Snoek, M., Bos, J.D., Hansen, H.M., Kapsenberg, M.L. *Comparison of diversity and function of house dust mite specific T lymphocyte clones from atopic and nonatopic donors.* Eur J Immunol 1990, 20: 1519-24.

50. Renz, H. *The central role of T cells in allergic sensitization and IgE regulation.* Exp Dermatol 1995, 4: 173-82.

51. de Vries, J.E., Yessel, H. *Modulation of human IgE response.* Eur Respir J 1996, 22(Suppl.): 585-625.

52. de Vries, J.E. *Novel fundamental approaches to intervening in IgE mediated allergic diseases.* J Invest Dermatol 1994, 102: 141-4.

53. Briner, T.J., Kuo, M-C., Keating, K.M., Roger, B.L., Greenstein, J.L. *Peripheral T cell tolerance induced in naive and primed mice by subcutaneous injection of peptide from the major cat allergen Fe1D1.* Proc Natl Acad Sci USA 1993, 90: 7608-12.

54. Hoyne, G.F., O'Hehir, R.E., Wraith, D.C., Thomas, W.R., Lamb, J.R. *Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice.* J Exp Med 1993, 178: 1783-8.

55. Smith, J.J., Olson, J.R., Klapper, D.G. *Monoclonal antibodies to denatured ragweed pollen allergen AmbaI: Characterisation, specificity for the denatured allergen and utilisation for the isolation of immunogenic peptides of AmbaI.* Mol Immunol 1988, 25: 355-65.

56. Stanworth, D.R., Coleman, J.W., Khan, Z. *Essential structural requirements for triggering of mast cells by a synthetic peptide comprising of a sequence in the Fc-epsilon4 domain of human IgE.* Mol Immunol 1984, 21: 243-7.

57. Stanworth, D.R., Jones, V.M., Lewin, I.V., Nayyar, S. *Allergy treatment with a peptide vaccine.* Lancet 1990, 336: 1279-81.