

New Methods of Prevention and Treatment of Allergic Diseases

David El-Qutob López*

FREMAP, MATEPSS no. 61, Plaza Legion Española 8, Valencia 46010, Spain

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Abstract: Over the past decades, the increasing number of patients with allergic conditions has posed a heavy burden on health care systems worldwide. This article will review recent treatments and patented methods for preventing allergies and related inflammatory and immunologic diseases. New drugs are commonly directed to known mechanisms, but there are many other pathways on which drugs can exert their action. New drug development is expected in the future as a consequence of discoveries in the pathophysiology and mechanisms of these diseases. Pharmaceuticals which would prevent the development of atopic diseases could allow us treating patients with genetic or environmental risk factors according to their conditions. Currently, a good and effective set of treatments is available for these diseases. However, the search for new treatment modalities to improve the currently available is especially important for those patients unresponsive to current therapy. In this review we summarize anti-cytokines therapies, Toll-like receptor (TLR)-mediated treatments, anti-immunoglobulin molecules, new immunomodulatory treatments and new antihistamines. The use of probiotics remains a matter of discussion and debate, since available studies have had contradictory results. In the present article we discuss current treatments for atopic diseases such as extrinsic asthma, atopic dermatitis and allergic rhinitis and relevant patents.

Keywords: Allergy, asthma, conjunctivitis, immunomodulation, prevention, rhinitis, treatment.

1. INTRODUCTION

Over the past decades the increasing prevalence and incidence of allergic conditions among affluent societies have posed a heavy burden on health care systems. The estimated prevalence of allergic rhinitis among 13- to 14-year-old participants of the International Study of Asthma and Allergies in Childhood (ISAAC) in the United States is 12% to 22%. Furthermore, the estimated prevalence of atopic sensitization among young adults in the United States is greater than 40% using the European Community Respiratory Health Survey (ECRHS) tools.

Prevalence rates for children and adults in countries around the world differ widely. The ECRHS assessed geographic variations in asthma in 140,000 adults from 22 countries. A six fold variation in the prevalence of current asthma was found among the different participating countries. A high prevalence (>7%) of asthma was described in Australia, New Zealand, United States, Ireland, and the United Kingdom. Asthma prevalences of <4% were found in Iceland, parts of Spain, Germany, Italy, Algeria and India. The ISAAC study provides the most extensive information for asthma in children around the world. There seems to be agreement between the different estimates, and the prevalence in some countries is similar to the one mentioned in the ECRHS study [1].

1.1. Respiratory Allergy

Rhinitis and asthma pose an important health problem with a wide range of etiologies. Allergic rhinitis [2] is

defined as a clinical symptomatic nasal inflammatory reaction induced by IgE-mediated allergen after exposure of the membranes of the nasal surface. It involves the following symptomatology: itch, nasal discharge, sneezing, and nasal stuffiness. Asthma is [3] a chronic inflammatory disorder of the airways with participation of various types of cells. It leads to recurrent episodes of wheezing, breathlessness, chest tightness and cough, usually accompanied by variable airflow obstruction, usually reversible with medication, as well spontaneously, and bronchial hyperresponsiveness against different stimuli. Allergic and infective causes are most common and have a substantial socioeconomic impact. Allergic rhinitis and asthma significantly impair quality of life and affect both school and work performance.

Allergic rhinitis is underdiagnosed. A proper diagnostic of asthma and rhinitis is basic for the implementation of an appropriate treatment plan. Treatment of allergic rhinitis and asthma has four cornerstones: education of the patient, allergen avoidance measures, symptomatic treatment and allergen immunotherapy. Studies on the effectiveness of avoidance measures and reduction of allergens in the home environment, as well as the different meta-analysis conducted on these studies, have shown that, while sanitation and avoidance measures are effective in reducing levels of allergens, it is not accompanied by a substantial reduction in symptoms. In addition to avoidance and environmental control measures, patients should start an individualized pharmacological treatment. The treatment should be consistent with the severity of symptoms.

The ARIA guide has recently published a new classification based on duration and presence of symptoms. Today, allergic rhinitis can be treated with oral and topical antihistamines, topical and oral corticosteroids, topical cromones,

*Address correspondence to this author at the FREMAP, MATEPSS no. 61, Plaza Legion Española 8, Valencia 46010, Spain; Tel: +34 96 339 32 90; Fax +34 96 362 02 11; Email: elqutob@comv.es

topical and oral vasoconstrictors, anticholinergics and leukotriene modifiers. Of these, the most used are second and third generation antihistamines, both oral and topical, and topical nasal corticosteroids [4].

Asthma management is based on the use of medication to reverse the bronchial obstruction. The patient's active collaboration is needed [5]. The goal of asthma treatment is the long-term control of the disease, including nocturnal symptoms and exercise-induced asthma, preventing exacerbations and achieving the best possible lung function with none, or minimal, side effects. At this time, multiple drugs are available. They can be divided into preventive medication, chronically used for control the disease, and rescue medication, used for the treatment of acute clinical symptoms Table 1.

In recent years sufficient scientific evidence is available, including the publication of numerous well-controlled studies, which demonstrate the efficacy and safety of allergen immunotherapy in patients allergic to pollen, house dust mites, mould, latex and epithelia. The World Health Organization (WHO) published a position statement, which states that immunotherapy is the only etiologic treatment of allergic diseases that can alter the natural course of the disease.

1.2. Atopic Dermatitis

The worldwide prevalence of atopic dermatitis (AD) has been estimated between 2 and 20% [6]. Eighty % of the patients with AD disease experience the first signs and symptoms of the disease before the age of 5. However, those patients with mild AD begin in adolescence.

A significant proportion of children with AD are at risk of developing allergic rhinitis and/or asthma. Acute AD is characterized by intensely pruritic, erythematous papules associated with excoriated, scaling papules. Chronic AD is characterized by thickened skin with accentuated markings (lichenification) and fibrotic papules [7]. The diagnosis is based on the presence of the following major and associated clinical features see Table 2.

Topical corticosteroids remain the first line medical treatment of AD. Other therapeutic options are tacrolimus ointment at 0.03% and 0.1%, and pimecrolimus cream at

1%, both approved for use in children younger than 2 years of age. Systemic steroids could be necessary to treat a serious outbreak of AD. Ultraviolet phototherapy has also shown efficacy in the treatment of adults and children with severe AD.

Clinicians should provide patients, and their family, various skin care recommendations. Identification and elimination of exacerbating factors should be the first step.

2. NEW PATENTS FOR PREVENTING ALLERGIES

2.1. Method for Preventing or Treating the Development of Atopic Diseases

The present invention is directed to a novel method for preventing, or treating the development of respiratory allergies. The described method comprises prenatal and/or postnatal administration of a therapeutically effective amount of *Lactobacillus* GG (LGG) [8]. *Lactobacillus* GG (LGG) is a probiotic strain isolated from healthy human intestinal flora. It was disclosed in US5032399 to Gorbach *et al.* LGG is resistant to most antibiotics, stable in the presence of acid and bile, and attaches avidly to mucosal cells of the human intestinal tract. It survives for 1-3 days in most individuals and up to 7 days in 30% of subjects. In addition to its colonization ability, LGG also beneficially affects mucosal immune responses

Several studies have focused on the pre- and postnatal administration of probiotics to prevent certain allergies in infants and children. In one of them, the study concluded that the supplementation does not protect from the development of atopic diseases except atopic eczema. The treatment is not equally effective to prevent other allergic conditions. In 2001, another study concluded that probiotics can prevent atopic eczema, but does not affect total and specific IgE levels and the prevalence of positive skin test reactions. The present invention produces a reduction or prevention in the release of pro-inflammatory cytokines (IFN- γ , MCP-1, IL-6 and IL-10) and serum IgE antibodies as well as an increase in the production of serum IgA.

There are several ways in which LGG can be effective. In a prenatal way, it can be administered to the mother in the form of a supplement. If during the first year of life the baby

Table 1. Medication for Asthma

Preventive Medication	Rescue Medication
1.Inhaled corticosteroids (ICS)	1.Short-acting- β -agonist (SABA)
2.Long-acting- β -agonist (LABA)	2.Anticholinergics
3.Leukotriene modifiers	3.Parenteral corticosteroids
4.Cromones	4.Inhaled corticosteroids (ICS)+ Long-acting- β -agonist (LABA)
5.Theophyllines	
6.Oral corticosteroids	
7.Others: omalizumab, cyclosporine, ketotifen	

Table 2. Clinical Characteristics of Atopic Dermatitis

MAJOR FEATURES	MINOR FEATURES
Pruritus	Xerosis
Facial and extensor involvement in infants and children	Cutaneous infections
Flexural lichenification in adults	Nonspecific dermatitis of the hands or feet
Chronic or relapsing dermatitis	Ichthyosis, palmar hyperlinearity, keratosis pilaris
Personal or family history of atopic disease	Pityriasis alba
	Nipple eczema
	White dermatographism and delayed blanch response
	Anterior subcapsular cataracts
	Elevated serum IgE levels
	Positive immediate-type allergy skin tests

is breast-fed, the postnatal administration of LGG can be via the mother's milk. The mother can continue LGG supplementation during the time that she breast-feeds, thereby transferring an effective amount of LGG to her baby via breast milk. However, if the baby is formula-fed, or if the mother supplements breast-feeding with formula-feeding, the LGG can be supplemented into infantile formula, which is then given to the child. This also represents an alternative method for postnatal administration of LGG.

Several studies have been completed to determine the effect of prenatal administration, the presence of LGG in the faeces of treated mice, the effect of LGG on the production of serum allergen-specific antibodies, serum IgA antibodies, the production of pro-inflammatory cytokines of spleen cells and the suppression of airway inflammation in allergic subjects. Studies comparing neonates versus control have also been conducted.

A decrease in the severity of atopic dermatitis has been found in a double-probiotic therapy study of *Lactobacillus rhamosus* GG and *Bifidobacter lactis* given in conjunction with a milk-free diet over 2 months to young children with cow's milk allergy [9].

Children with IgE-mediated atopic dermatitis induced by cow's milk allergy have a reduced Interferon- γ response that can be markedly increased by treatment with *L. Rhamosus* GG. This provides a strengthened Th1 cytokine response that could potentially reduce the Th2-mediated allergic reactions in these individuals [10]. Kalliomaki *et al.* [11] found that the incidence of atopic dermatitis in the probiotic group was less compared with the placebo group. However, when the objective markers of atopy were evaluated, there were no significant differences between the two groups. Nermes *et al.* [12] published a study concluding that specific probiotics may enhance gut barrier function and aid in the development of immune responses. In this study, 39 infants with AD were randomized to receive casein formula supplemented with, or without LGG. The results were inconclusive regarding the effects of this treatment on AD. Further studies have presented less-promising results. Helin *et al.* in 2002 treated

birch (*Betula verrucosa*) pollen allergy with *L. rhamosus* given 2-5 months before the pollen season, 1 month in the pollen season and for 2 months after the season. The probiotic group used more medication over the birch-pollen season compared with the placebo group ($p = 0.06$). However, in 2008 Kukkonen *et al.* studied the long-term safety and impact on infection rates of postnatal probiotic and probiotic treatment. They concluded that feeding symbiotics to newborn infants was safe and seemed to increase resistance to respiratory infections during the first 2 years of life. However, the same authors established that early intervention with probiotics and pre-biotics did not affect airway inflammation 5 years later [13]. Volkmar *et al.* published a double-blind, placebo-controlled prospective trial with 105 pregnant women from families with an atopic background, who were randomly assigned to receive *Lactobacillus* GG or placebo. The study suggested that supplementation with *Lactobacillus* GG during pregnancy and early infancy did not reduce the incidence of atopic dermatitis, nor altered the severity of atopic dermatitis in affected children. It was associated with an increased rate of recurrent episodes of wheezing bronchitis. Therefore, *Lactobacillus* GG cannot be generally recommended for primary prevention. Recently, a study has shown that *Lactobacillus* GG treatment during pregnancy was not sufficient for preventing eczema. The authors suggested the possibility of using postnatal treatment or the use of an alternative bacterial strain [14]. A more recent study published showed that treatment with a probiotic mixture can redirect Th2-responses to Th1, thus protecting against anaphylactic reactions in a murine model of food allergy [15].

In conclusion, none of the studies has shown any clear preventive effect on sensitization or any advantage in allergic diseases other than atopic dermatitis. There are important differences between the published studies, which include host factors, such as genetic differences in microbial responses and allergic predisposition. Variable results in different studies may also be due to exogenous factors such as preexisting intestinal microbial flora, individual organisms chosen for inclusion in the probiotic treatment, diet and treatment of the host with antibiotics.

Investigators will continue to conduct and publish studies on the subject, hoping to identify which individuals improve with probiotic treatment. More studies are needed to widely recommend the use of probiotics to prevent, or treat allergic conditions.

2.2. Prevention of Allergic Sensitization: Use of Enterovirus Vaccines

This invention relates to the prevention and treatment of allergic sensitization and diseases by treatment with an enterovirus vaccine. The enterovirus vaccine does not contain an exogenous nucleic acid sequence that is integrated into the viral genome and that encodes an allergen that induces said allergic sensitization [16]. The objective of this treatment is to decrease the risk of allergic sensitization; especially IgE mediated sensitization, and its progression to clinical illness. Allergic diseases such as asthma, eczema and allergic rhinitis and conjunctivitis may be prevented, or treated, with a vaccine comprising enteroviruses as an immunoactive element. Enteroviruses act inducing immunoregulatory pathways, or down-regulating Th2-type immune responses. Such enteroviruses can be identified by analyzing their effect on regulatory T-cells and/or Th1/Th2 balance and/or production of immunoregulatory cytokines, such as IL-10, either during *in vitro* stimulating of peripheral blood leucocytes with different enteroviruses, or *in vivo* in children with enteroviruses infection. The treatment is preferably administered to a child within 5 years after birth, and more preferably within 3 years, 2 and most preferably 1 year after birth, with boosters given later in life to prevent allergic sensitization. Individuals who have already been sensitized, regardless of symptoms of a disease or not, may be given the pharmaceutical composition at any age. This approach was studied in two cohorts of 266 school-children each; one from the Karelian Republic and the other from Finland. The children represented the respective mainstream populations and were not selected according to possible allergies, or other diseases. To obtain these cohorts, children were initially analysed for the presence of specific IgE to cat, birch pollen and egg albumin and antibodies to enteroviruses and hepatitis A. The prevalence of specific IgEs was lower among Russian than among the Finnish children, in contrast to what happened with the presence of antibodies to enteroviruses and hepatitis A. In Russian Karelia, allergic sensitization was scarcer in children who had enterovirus antibodies while no such effect was seen in terms of hepatitis A antibodies. Enterovirus antibodies were frequent in the Finnish children, but in contrast to those in Russian Karelia, they showed no association with allergen-specific IgE responses. The protective effect of enterovirus antibodies seen in Russian children and not in Finnish children could be explained because Finnish children are infected at an older age.

In this study, the presence of antibodies to enteroviruses with protective effect against the appearance of allergic disease it is not considered that sensitization is not the same as allergic disease and the presence of specific IgE does not necessarily implies the development of allergic disease, in this case, allergy to cat, birch pollen and / or egg albumin. It could be interesting to know the percentage of children with allergen-specific-IgE that developed allergic disease.

2.3. Zwitterionic Immunomodulators for the Treatment of Asthma and Allergy: Use of Zwitterionic Polysaccharides

The invention describes methods, uses and compositions involving immunomodulatory zwitterionic polymers for the induction of T regulatory cells and the treatment of asthma and allergy [17]. Zwitterionic polysaccharides activate CD4+ T cells *in vitro* by stimulating T cell proliferation and production of IL-2, IFN- γ and IL-10. The polymer includes repeating units of a charge motif characteristic of *bacterioides fragilis* polysaccharide A (PSA). Fig. (1) shows the effect of zwitterionic polysaccharide CP1 in the production of IL-10 compared with non-zwitterionic control polysaccharide PG by ICOS (inducible co-stimulator) Treg cells obtained from wildtype (WT) and ICO $^{-/-}$ mice. In Fig. (2) there is the effect in IL-13 of zwitterionic polysaccharide CP1 compared with control saline.

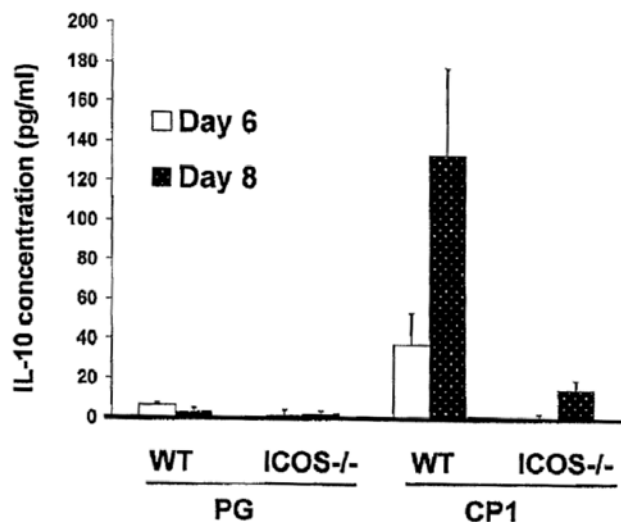


Fig. (1).

This treatment has been used in multiple sclerosis by the same mechanism, which involves the production of IL-10[18]. Oral administration with purified PSA protected mice against encephalomyelitis (EAE) prophylactic and therapeutically inducing the conversion of naïve CD4+ T cells into IL-10-producing FoxP3+ Treg cells. Protection against EAE was completely abrogated in IL-10-deficient mice. Human studies are needed for assessing the effectiveness of this invention in patients with asthma.

3. ANTI-CYTOKINES DIRECTED THERAPY

3.1. Use of an Inhibitor of TNF α Plus an Antihistamine to Treat Allergic Rhinitis and Allergic Conjunctivitis

This is a potential new method for treating allergic conjunctivitis or allergic rhinitis in human subjects, comprising topical administration to the eye, or nose of a composition comprising a effective amount of an H₁ antagonist and a pharmaceutically effective amount of an anti-TNF α compound. Tumor necrosis factor α (TNF α) is a cytokine that has been shown to play a central role in immune and inflamma-

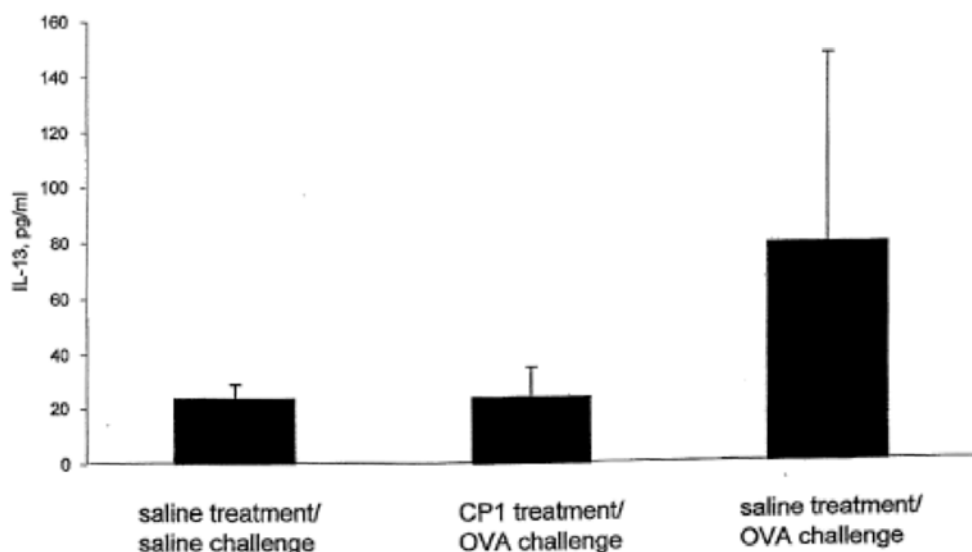


Fig. (2).

tory responses, including allergic rhinitis and conjunctivitis [19]. TNF α is a soluble homotrimer of 17 kD protein subunits, derived from mononuclear cells and macrophages, along with other cell types (neutrophils, activated lymphocytes, NK cells, endothelial cells, mast cells, smooth muscle cells). Modulation of TNF α has been proposed as a therapeutic strategy for allergic conjunctivitis and other conditions associated with activation of TNF α . It is believed that the combination of H1 antagonist and anti- TNF α will provide immediate relief from acute allergy effect such as sneezing, oedema, nasal itching and rhinorrhea because of the H₁ antagonist and protection from allergic inflammation and congestion of the anti- TNF α compound. The combination product of the present invention is devoided of the risk of steroid-induced side effects.

The anti-TNF α can be an inhibitor of the synthesis of TNF α (such as a PDE4 inhibitor, a JAK3 inhibitor, or a p38 kinase inhibitor), a TNF α antagonist such as an agent that inhibits the binding of TNF α to TNF α receptor, an antibody, or a TNF α sink. The administration is topical to the eye or nose. Topical administration to the eye includes topical compositions dropped or placed on the eye or placed underneath the eye lids. Topical administration to the nose includes delivering compositions by drops or spray into the nostrils and nasal passages.

The effect of TNF α blockage in asthma is known for many years. In a model of *Dermatophagoides farinae*-induced pulmonary eosinophilic inflammation in mice, intranasal administration of a TNF α monoclonal antibody to mice 1 h before and 24 h after challenge significantly reduced the number of eosinophils, neutrophils and lymphocytes in bronchoalveolar lavage fluid. Anti- TNF α treatment was also shown to reduce Th2 cell-mediated tissue inflammation in some strains of mice [20].

In 2006, etanercept, a TNF-receptor blocker, was given to 10 patients with severe asthma. Compared to the control group of 10 patients, the medication caused an improvement in FEV1 and quality of life in patients with asthma. In a

study of 38 patients with moderate asthma receiving inhaled corticosteroid treatment, an anti-TNF α antibody (infliximab) significantly reduced asthma exacerbation. In both studies anti-TNF α was well-tolerated. In another study, an anti-TNF α antibody (golimumab) was evaluated in 309 severe asthmatic patients. There were no measurable changes in pulmonary function, symptoms or exacerbation rates in patients receiving active treatment. In this larger study, a number of serious adverse events occurred in patients receiving golimumab, including infections and malignancies. The occurrence of these serious adverse events prompted early discontinuation of the study [21]. However, Mo *et al.* [22] published a study showing that TNF α reduces allergic responses in an allergic rhinitis mouse model. In the study, the investigators used infliximab to evaluate its effects on allergic rhinitis and to elucidate the underlying mechanisms regulating its anti-allergic effects in a mouse model. Infliximab decreased the allergic symptom score, and this anti-allergic effect could be associated with not only suppression of Th2 cytokines, but also decreased infiltration of eosinophils in the nasal mucosa. Currently there are no studies to determine the effectiveness of anti-TNF α in combination with antihistamines in patients with rhinitis or asthma but the appearance of adverse effects with the use of anti-TNF α in previous studies with asthmatic patients do not recommend it either in combination with antihistamines or alone. The large number of type cells involved in raising TNF α levels makes it a difficult target.

3.2. Interleukin-17F Antibodies and other IL-17F Signaling Antagonists

The present invention is directed to disorders in which IL-17F signalling is involved. IL-17A and IL-17F share 50% identity; both cytokines are induced by IL-23, co-expressed by T cells and considered potential targets for T cell-mediated autoimmune diseases [23]. However, expression of IL-17F by activated monocytes, activated basophils and mast cells has also been shown. IL-17F is correlated with neutrophilia and various autoimmune diseases (rheumatoid arthri-

tis, multiple sclerosis, systemic lupus erythematosus, inflammatory bowel disease, asthma and others). IL-21 and IL-23 induce IL-17A and IL-17F upon TCR/CD28 costimulation. Therefore, IL-17A and IL-17F may mediate proinflammatory effects attributed to IL-21 signaling. There are IL-17A and IL-17F homo- and heterodimers suggesting different biological functions for each one. The binding of IL-17A to its receptor (IL-17R) expressed in all tissues, results in the induction of proinflammatory cytokines through activation of NF- κ B. There are four additional receptors, sharing partial homology to IL-17R: 1) IL-17RH1 (also called IL-17RB), 2) IL-17-receptor like protein (also called IL-17RL or IL-17RC), 3) IL-17RD (also called SEF or IL-17RLM) and 4) IL-17RE. This invention presents IL-17F antagonists such as IL-17F inhibitory polynucleotides, IL-17R inhibitory polynucleotides, IL-17RC inhibitory polynucleotides, soluble polypeptides comprising IL-17R or IL-17F-binding fragments thereof, soluble polypeptides comprising IL-17RC or IL-17F-binding fragments thereof, inhibitory anti-IL-17F antibodies, inhibitory anti-IL-17R antibodies, inhibitory anti-IL-17RC antibodies, and antagonists small molecules. Antibodies inhibit the binding of IL-17F to IL-17R in the presence of increasing concentrations of one of the six anti-IL-17F antibodies Fig. (3). Anti-human IL-17F antibodies inhibit the binding of IL-17DF to IL-17RC in the presence of increasing concentrations of six anti-IL-17F antibodies Fig. (4). The invention may be administered by oral, parenteral, subcutaneous, intradermal, transdermal, transmucosal and rectal administration.

Th17 cells *via* IL-17 promote neutrophils recruitment for the clearance of bacteria and fungi. Th17 cells are mainly involved in those forms of asthma, where neutrophils more than eosinophils contribute to the inflammation[24]. Clearly these forms of asthma respond poorly to treatment with corticosteroids, the most common therapy for asthma. In those patients with asthma₇ characterized by being non-atopic, non-IgE mediated, and not eosinophilic asthma, airway neutrophilia correlates with the degree of severity. IL-17 has an important role in remodelling the lung of asthmatics patients.

Blockade of IL-17A may decrease the severity of airway hyperresponsiveness (AHR) but only in the presence of Th2 cytokines. The expression of IL-17RH1 (also called IL-17RB) has been confirmed to be upregulated in seasonal allergic rhinitis compared to healthy patients. Thus it seems interesting to use antagonists of IL-17 in cases of predominantly neutrophilic asthma, where other standard treatments, such as corticosteroids, are not very effective. However, the involvement of IL-17 in Th2 profile asthma has seen suggested. We expect that future studies in humans will assess the efficacy of this treatment in different types of asthma.

3.3. Heterocyclic Janus Kinase 3(JAK3) Inhibitors

The present invention describes a compound having an excellent JAK3 inhibition activity and being useful as an active ingredient of an agent for treating and/or preventing allergic diseases and other immune diseases [25]. JAK3 is a tyrosine kinase that functions in signal transduction and interacts with members of the STAT (signal transduction and activators of transcription) family. JAK3 is predominantly expressed in immune cells and transduces a signal in response to its activation *via* tyrosine phosphorylation by non-covalent association with the common γ chain of interleukin receptors. In the case of allergic diseases and IL-4, JAK3 acts through STAT6.

The efficacy of JAK3 inhibition was expressed as an IC₅₀ value (the half maximal (50%) inhibitory concentration (IC) of a substance). Enzyme activity (Ex) was measured as absorbance at 450nm. Table 3 shows the results obtained.

A JAK3 selective inhibitor, tofacitinib (CP-690,550) may improve autoimmune diseases such as rheumatoid arthritis by suppressing the differentiation of pathogenic Th1 and Th17 cells as well as innate immune cells signaling through inhibiting STAT1-dependent genes [26]. In patients with lymphocytic leukemia, IL-4 induced resistance to the drugs fludarabine and chlorambucil and to the novel p53-elevating agent nutlin 3 which was reserved by PF-956950, a JAK3-selective inhibitor [27]. Another JAK3-selective inhibitor,

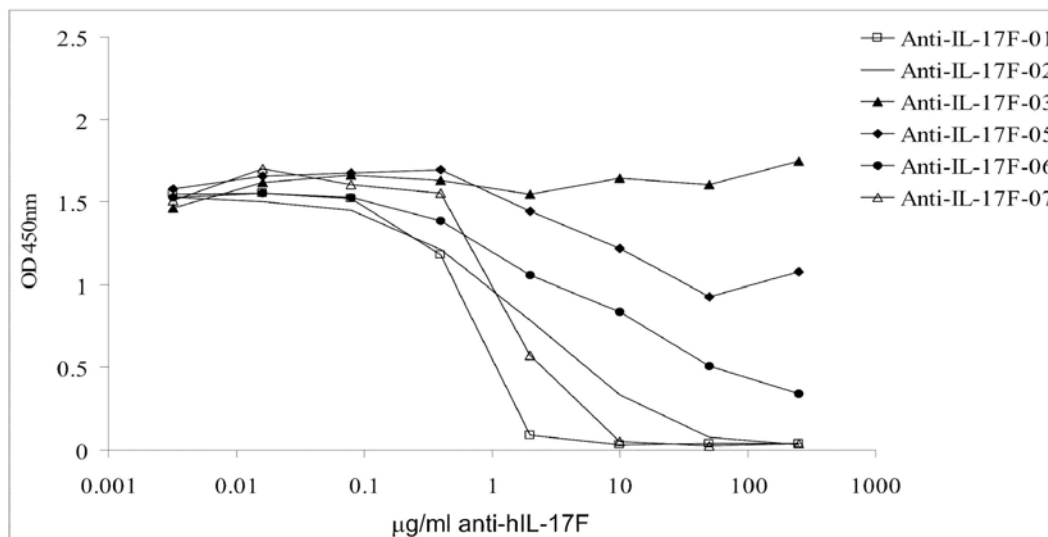


Fig. (3).

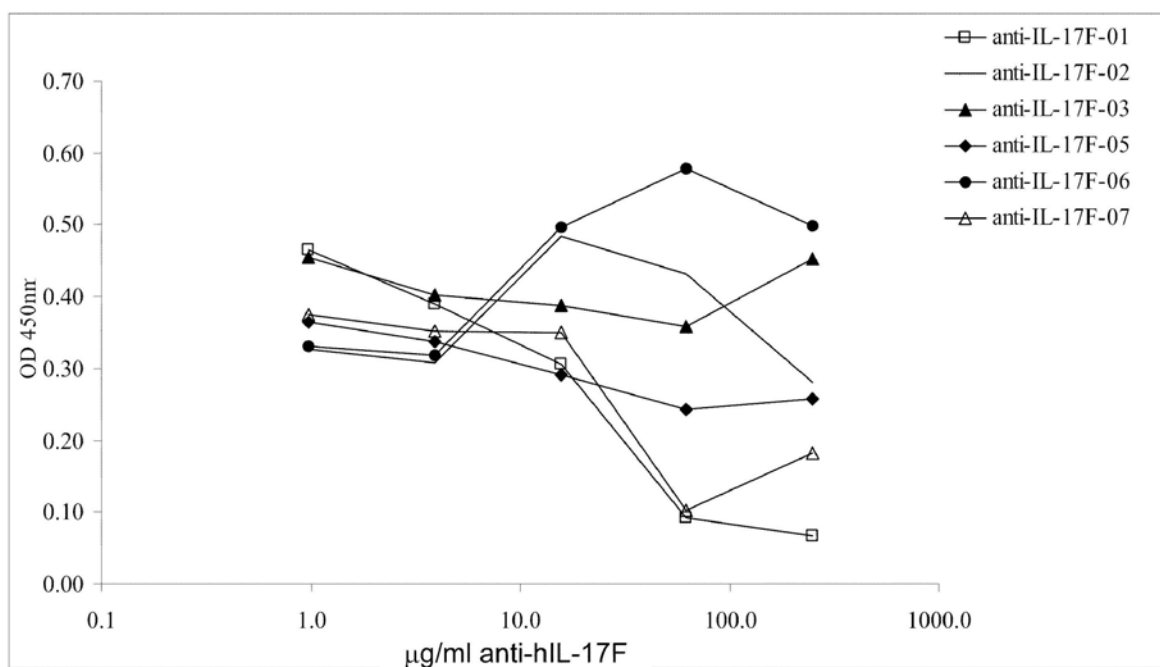


Fig. (4).

Table 3. JAK3 Inhibitory Activity of the Compound of the Present Invention

Ex	IC ₅₀ (nM)	Ex	IC ₅₀ (nM)
29	2.3	379	1.2
32	1.9	384	0.28
33	2.1	405	0.65
34	0.17	426	2.3
121	1.1	434	0.86
122	0.43	484	0.31
133	4.4	498	1.3
143	0.69	543	0.33

kaempferol, could be used in controlling cell responses to IL-4[28]. In 1999, Malaviya *et al.* demonstrated that selective inhibition of JAK3 with WHI-P131 in mast cells prevented mast cell degranulation and development of cutaneous, as well as systemic fatal anaphylaxis in mice at nontoxic dose levels[29]. The same authors showed that a potent inhibitor of JAK3, WHI-P97, could inhibit the enzymatic activity of 5-LO by translocating it in the nuclear membrane. In ovalbumin-sensitized mice, treatment with WHI-P97 prevented development of AHR to methacholine and inhibited the eosinophil recruitment [30]. Inhibitors of tyrosine kinases have been shown to block growth factor-induced hyperplasia of airway-resident cells, antigen-induced inflammatory cell activation, cytokine synthesis and chemokine-induced chemotaxis of inflammatory cells, *in vitro* [31-32]. This invention may be useful in many immune types of diseases. There are not human studies that clearly state the benefit of the JAK-3 inhibitors for allergic diseases. The development of

tyrosine kinases inhibitors can be a very attractive strategy for the treatment of asthma.

3.4. IL-13

3.4.1. Novel Anti-IL-13 Antibodies

The present invention relates to anti-IL-13 antibodies that bind specifically and with high affinity IL-13 and are useful in the treatment of IL-13 mediated allergic diseases such as asthma, allergic rhinitis, atopic dermatitis and allergic conjunctivitis [33]. These antibodies may down-regulate the activity of IL-13, reduce bronchial hyperresponsiveness and/or reduce eosinophilia in the lungs of the subject. This composition may be administered by several routes including intravenous, intraperitoneal, inhalation, intramuscular, subcutaneous and oral routes.

3.4.2. Human Antibody Molecules for IL-13

Preferred embodiments of the present invention employ antibodies which neutralise IL-13 activity employing the antibody VH and/or VL domain of the antibody molecule termed BAK502G9 and other antibody molecules of the BAK502G9 lineage and of the BAK 278D6 lineage [34] Fig. (5). This treatment may be given orally, by injection, by inhalation or topically. A typical antibody dose will be in the range 100µg to 1g for systemic applications, and 1µg to 1mg for topical applications. A composition may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

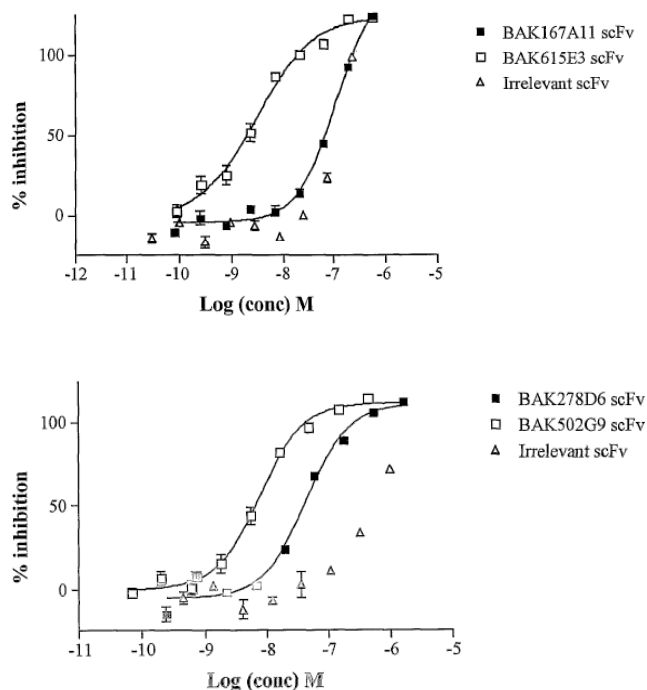


Fig. (5).

In 1998 Wills-Karp [35] published that IL-13 is necessary and sufficient for the expression of allergic asthma and induces the pathophysiological features of asthma in an IgE and eosinophil independent manner. Thus, IL-13 is critical for allergen-induced asthma. However, although IL-13 can induce IgE secretion from activated human B cells, deletion of IL-13 from mice does not markedly affect either Th2 cell development, or antigen-specific IgE, unlike IL-4 that is involved in these functions. Currently, there are several trials assessing the effectiveness of IL-13 inhibition. A pharmaceutical company has studied the effects of a single dose of QAX576 (an IL-13 monoclonal antibody) compared with placebo on allergic rhinitis[36], the safety, pharmacokinetics and pharmacodynamics of single escalating doses [37], the safety and efficacy of QAX576 in adults with moderate persistent allergic asthma [38] and, finally, its use in patients with eosinophilic esophagitis[39]. Hodges *et al.* compared AIR645, an IL-4/IL-13 dual cytokine signaling inhibitor, in healthy subjects and subjects with controlled asthma[40-41] and other trial evaluating the effects of AIR645 on allergen-

induced airway responses in subjects with mild atopic asthma [41]. Aerovant™, is a recombinant human protein that is a potent inhibitor of IL-4 and IL-13 activities. Several clinical trials have been conducted, including a phase 1 safety and pharmacokinetics study of AER 001 administered as a dry powder in asthmatic subjects [42], the evaluation of the effect of AER 001 administered by nebulization on antigen challenge in atopic asthmatics [43], a phase IIa study of the effect of subcutaneous AER 001 on antigen challenge in atopic asthmatic [44], and a study of the treatment-sparing effects of Aerovant™ AER 001 inhalation powder in asthma patients, aerotrial [45]. There are not published results from all these trials yet.

4. TOLL-LIKE RECEPTORS TREATMENT

4.1. Pyrimidine Derivatives for the Treatment of Asthma, COPD, Allergic Rhinitis, Allergic Conjunctivitis, Atopic Dermatitis, Cancer, Hepatitis B, Hepatitis C, HIV, Bacterial Infections and Dermatoses

The present invention relates to pyrimidine derivatives, processes for their preparation, pharmaceutical compositions and their use in therapy [46]. The immune system comprises innate and acquired immunity. Innate immunity can recognize pathogen-associated molecular patterns conserved through TLR expressed on the cell surface of immune cells. Recognition of invading pathogens triggers cytokine production and upregulation of cells belonging to the acquired immune system. Thus, innate immunity is closely linked to acquired immunity and can influence the development and regulation of an acquired response. Eleven TLRs have been described to date in humans and mice.

This patent application describes a class of pyrimidine derivatives having immunomodulating properties that act *via* TLR7, which are useful in the treatment of viral or allergic diseases and cancer. These compounds, and their pharmaceutically acceptable salts, act as modulators of toll-like receptor (especially TLR7) activity, and thus can be used in the treatment of many diseases, being the following linked with hypersensitivity or atopy:

1. Respiratory tract: asthma, hyper-responsiveness, sarcoidosis, hypersensitivity pneumonitis, lung fibrosis, pulmonary vasculitis, rhinitis and nasal polyposis.
2. Skin: atopic dermatitis, contact dermatitis or other eczematous dermatoses and delayed-type hypersensitivity reactions.
3. Eyes: conjunctivitis

The administered dose will vary with the compound employed, the mode of administration, the treatment desired and the disorder to treat. The pharmaceutical compositions may be administered topically or systemically, or by parenteral or rectal ways. The invention includes combination therapies wherein a compound of the invention or a pharmaceutical composition of formulation comprising a compound of the invention is administered concurrently or sequentially or as a combined preparation with another therapeutic agent or agents, for the treatment of one or more of the conditions listed.

The use of pyrimidine derivatives as antiallergic treatment has been suggested for years. This patent also proposes its use in other diseases where possible mechanisms are mediated by TLR. However, have been no studies demonstrating a beneficial effect. Therefore, it is not advisable to use this product as antiallergic treatment at this moment.

4.2. Toll Like Receptor Modulators

The invention relates to TLR9 antagonist compounds and their therapeutic or prophylactic use. These immune regulatory oligonucleotides have unique sequences that suppress, without completely ablating, TLR-mediated signalling in response to a TLR ligand or TLR signalling agonist, or bacterial unmethylated CpG (**Cytosine** Phosphate Guanine) DNA. This invention consists of the administration of an oligonucleotide sequence, partial inhibitor of TLR9-mediated immune response, avoiding the damage that involves the release of proinflammatory molecules and appearance of IL-12. TLR9 antagonist may be used for the treatment for various diseases, such as allergic asthma and respiratory inflammatory disease. It can be administered parenterally, transmucosally, orally, sublingually, transdermally, topical, inhaled, intranasally, *via* aerosol, intraocularly, intratracheally, intrarectally, vaginally, by gene therapy or in eye drops or mouthwash [47]. Fig. (6) shows the effect of several oligonucleotides in the production of IL-12.

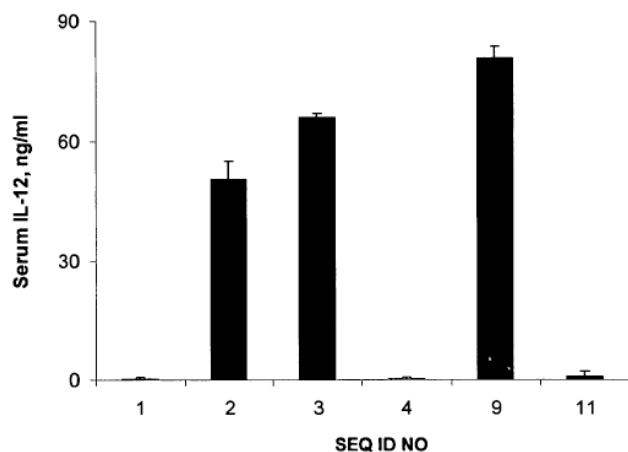


Fig. (6).

IL-12 is a cytokine that activates Th1 cells and stimulates the production and cytotoxicity of cytotoxic T cells and NK cells. It also stimulates the production of IFN- γ , promotes the activation of cellular immunity by activating Th1 and has a synergistic effect with tumor necrosis factor- α in the induction of interferon- γ . Therefore, it promotes the immune system to release of Th1 cytokines preventing the appearance and development of allergic diseases.

There are several ways in which TLR can modulate the allergic inflammatory response. The hygiene hypothesis holds that exposure to specific infections, and, perhaps, to endotoxin, diverts the immune response towards a Th1 phenotype. Although the hypothesis is certainly attractive, it has generated considerable debate. TLRs are implicitly involved in critical pathways in the maturation of the adult immune

system. In fact, the polymorphism of CD14, the coreceptor involved in transmitting signals from the interaction with LPS *via* TLR4 correlates with levels of IgE [48]. Several functional polymorphisms in human TLR4 appear to be involved in the reactivity of the airway. One goal of future research will be to establish whether such polymorphisms in human TLRs correlate with the risk of developing atopy and atopic disease. Once the allergic sensitization has been established, the endotoxin in the inhaled dust may be as important asthmatic trigger. TLR4 enhances monocytes production of TNF α resulting in a proinflammatory state. In this context, TLR signaling may worsen rather than prevent allergic inflammation. Similarly, atopic eczema may be worsened by a bacterial infection, a phenomenon that could be related, in part, by TLR signaling. In addition, TLRs are involved in responses to endogenous proteins associated with cell damage and inflammation and oxidative stress. Perhaps one of the most interesting aspects of TLR biology in relation to allergic reactivity is the possibility of exploiting the strong adjuvant properties of TLRs in modulating allergy. The use of CpG sequences of bacterial DNA associated with antigens in specific immunotherapy has been proposed. CpG sequences trigger the production of IL-12 and a subsequent T lymphocytes release of IFN- γ on antigen presenting cells (monocytes and dendritic cells). These events usually result in a Th1 phenotype, a phenomenon that could be used to modify the altered immune response of patients with allergy. Direct conjugation of CpG to allergen reduces the CpG dose required to induce immune deviation. This has been shown to be effective in altering the Th2 responses in a variety of animal species. Moreover, the possibility of reducing the amount of CpG may translate into less risk of developing side effects, such as autoimmune diseases, or the exacerbation of inflammatory reactions. The therapeutic application of TLR9 has been explored extensively in recent years, and many studies are being conducted to assess the safety and efficacy of TLR9 agonists in various diseases, including atopic and infectious diseases and cancer. Studies in murine models have shown that the development of atopic airway disease can be prevented by treating with CpG oligonucleotides (ODNs). Various clinical trials are currently ongoing to determine the efficacy of CpG ODNs as a therapeutic tool for atopic diseases [49].

5. ANTI-IGE AND OTHER ANTI-IG TREATMENTS

5.1. Anti-IgE

5.1.1 Fusion Molecules and Treatment of IgE-Mediated Allergic Diseases

The invention concerns bifunctional fusion molecules for the treatment of IgE-mediated allergic conditions and Fc ϵ RI-mediated autoimmune conditions [50]. The invention allows treating both acute and late phase allergic reactions either through inhalation, ingestion, and cutaneous or parenteral administration. It could be indicated in asthma, allergic rhinitis, atopic dermatitis, food allergy, chronic urticaria, angioedema and anaphylaxis due to Hymenoptera stings and penicillin allergy. IgE receptors (Fc ϵ Rs) are cell surface receptors binding to the constant region of immunoglobulins. There are two types: high and low-affinity receptors. The first one appears in mast cells, basophils and Langerhans cells and has

a tetrameric structure composed of an α -chain, a β -chain and two disulfide-linked γ -chains. The α -chain of the receptor interacts with the distal portion of the third constant domain of the IgE heavy chain. This invention concerns a first polypeptide sequence capable of specifically binding to a native inhibitory receptor comprising an immune receptor tyrosine-based inhibitory motif (ITIM), expressed on mast cells, basophils and/or B cells, functionally connected to a second polypeptide sequence capable of specific binding, directly or indirectly to a native IgE receptor (Fc ϵ R). Therefore, all IgE-mediated reactions are blocked by inhibiting the binding of this immunoglobulin with the Fc ϵ R1 receptor. This treatment is only indicated for type I allergic reactions (IgE-mediated). It could inhibit reactions to major environmental and occupational allergens, can be used for allergy vaccination to induce desensitization to specific allergens, and can also have a prophylactic effect by preventing allergic sensitization to environmental and occupational allergens. The route of administration could be oral, transdermal, inhaled or by injection. The determination of the dose depends on the age and size of the patient and the disease or disorder to be treated. It may be administered in combination with other known treatments for allergic disease including corticosteroids, β -agonists, theophylline, leukotriene inhibitors, immunotherapy and others.

5.1.2. Anti IgE Antibodies

The present invention relates to novel human antibodies specifically directed against human immunoglobulin E for treating allergic asthma, allergic rhinitis and food allergies [51]. This anti-IgE has an IC₅₀ of 0.5 μ g/ml, or less, as measured by their ability to reduce IgE binding in a cell binding assay see Fig. (7). The anti-IgE does not have agonist activity with isolated human blood basophils.

A previous anti-IgE treatment, omalizumab, has been successfully applied in patients with poorly controlled severe

asthma following standard treatments [52]. It is considered effective but inefficient treatment given the high cost involved. The same may be applied to these inventions due to the high costs involved. Given its mechanism of action, it may be an effective treatment, but efficiency will have to be assessed.

5.2. Methods for Treating IgE-Mediated Disorders: Activation Surface-Bound IgD Molecules on Basophils

The invention relates to methods of treating IgE mediated disorders such as allergy and asthma based on activating surface-bound IgD molecules on basophils [53]. The administration of an anti-IgD monoclonal or polyclonal antibody causes cross-linking of IgD pre-bound to the surface of basophils or targets an IgD receptor in basophils. The authors claim that this method treats IgE-mediated disorders such as asthma, atopic dermatitis, allergic rhinitis, allergic conjunctivitis, eczema, urticaria, food allergy and seasonal allergy. This agent may be administered by injections, inhalations, ingestions or implantations. This anti-IgD has a greater effect on the release of histamine than anti-IgE Fig. (8).

B lymphocytes with IgD-membrane receptors are detected between 10 and 12 weeks of gestation, and increase after birth. There is a significant increase of IgD levels observed in children at first signs of asthma. The following normalization of these levels after 18 months may represent a non specific response, or an attempt of the organism to block asthma, favouring therefore immunologic tolerance [54]. Basophils contain granules with histamine, heparin and others. The role of IgD is unknown but is thought to be involved in the process of B lymphocytes maturation, acting as a B cell antigen receptor. Normal individuals produce IgM or IgG in response to antigen, but atopic individuals produce large amounts of IgE in response to allergens that are safe for the healthy subjects. Basophils have the high affinity

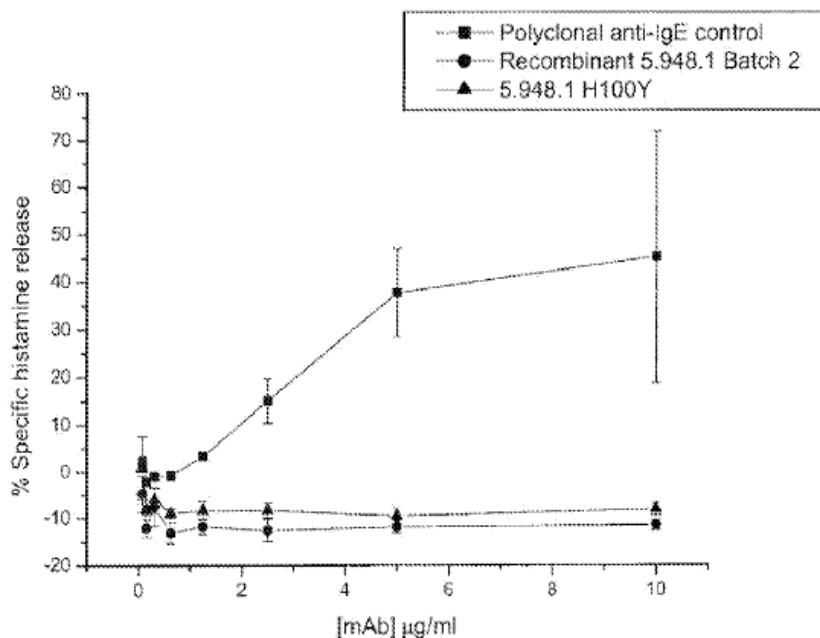


Fig. (7).

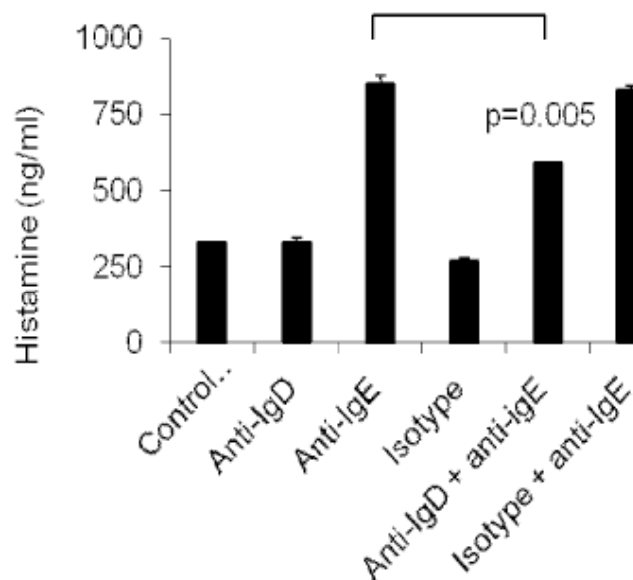


Fig. (8).

receptor (FcεRI) on their surface and the binding of IgE to this receptor is the stimulus that produces cytokines such as IL-4 and IL-13. There is not a clear role for the IgD in the type I mechanism of hypersensitivity (IgE-mediated) but serum levels of polyclonal IgD are increased in allergic disorders, although with a considerable overlap between healthy and allergic subjects. Levan-Petit showed how IL-4 and IL-10 independently induced IgD synthesis [55]. These two cytokines also induce synthesis and/or switch recombination toward IgG1 and IgG3, whereas only IL-4 induces IgE switching and synthesis. However, IFN-γ has not inhibitory effect in IgD synthesis.

In patients with asthma, basophil degranulation by a high anti-IgD concentration was reported [56] and IgD against birch and timothy pollen allergens was increased in sera of atopic individuals with IgE antibodies to birch pollen allergens compared to sera of atopics without IgE against this allergen. The allergen specificity of IgD antibodies was confirmed with an allergen inhibition test. However, no significant correlation was found between total serum IgD and IgE.

Although the basophils have high affinity receptors FcεRI, their role in IgE-mediated reactions is lower than mast cells, which are more important in the allergic reaction. Therefore, if we act by inhibiting the action of basophil we will not inhibit the mast cells. In addition, lung mast cells have a less significant role in IgE-mediated asthma than other cells such as eosinophils.

5.3. Monoclonal Antibody Reactive with CD63 when Expressed at the Surface of Degranulated Mast Cells

The present invention relates to monoclonal antibodies that specifically bind to a restricted epitope on CD63, named CD63var of degranulated mast cells and not to quiescent or other cells such as monocytes, macrophages and T cells which express CD63 in their surface [57]. This condition prevents cross-reactions with the other cell types, not caus-

ing life-threatening undesirable events. Two structurally different isoforms of the human CD63 marker exist. One isoform is on the surface of vesicles and the other on cell surfaces. Once activation of mast cell begins, CD63var is transferred to the surface cell of degranulated cells. This marker of activated human mast cells can be useful for diagnosing and treating allergic diseases mediated by degranulation of mast cells and other CD63var expressing granulocytes, such as basophils. The antibodies of the invention prevent recurrent mast cell degranulation by killing or depleting degranulated mast cells, or other CD63var expressing granulocytes. This could be a good treatment of diseases in which mast cell degranulation plays a key role. Currently, there are not clinical trials to proof this concept.

6. SPECIFIC IMMUNOTHERAPY

6.1. Novel Manner of Administering Allergen in Allergen Specific Immunotherapy

The present invention relates to a novel manner of administering the allergy vaccine in allergen specific immunotherapy directly delivering the dose slowly to subcutaneous tissue by infusion pump [58]. This allows for a more rapid up-dosing phase with fewer doses to reach maintenance phase and fewer adverse events. The administration of vaccine doses at the initiation phase has more risk of producing adverse events. The reduction of the number of doses at this phase should diminish the risk of local and systemic reactions. A fast up-dosing phase implies an increased risk of systemic reactions. This invention reduces the number of doses in the up-dosing phase of subcutaneous immunotherapy and reduces the number of patient's visit to a medical facility. The dosing time depends on the volume of the dose. The duration of administration can be maintained preferably within the range of 10 to 180 minutes.

Infusion pumps have been widely used for subcutaneous administration of different drugs. This system has been used

for years for insulin administration, and was well tolerated in diabetic patients. This implies that this new system will allow greater tolerance in the administration of immunotherapy. However, the use of a pump for the administration of immunotherapy implies an expensive treatment and more time in the clinical facility under observation. It is necessary to compare the number of adverse events with conventional administration and the new administration presented.

6.2. Vaccines Containing Allergen Peptides The present invention relates to compositions comprising peptides for preventing or treating allergy to house dust mites (HDM). The authors have used peptide fragments of allergens from group 1 (Der p 1 and Der f 1), 2 (Der p 2 and Der f 2) and 7 (Der p 7 and Der f 7) of *Dermatophagoides* (*pteronysinus* and *farinae*) to induce the production of Th1 cytokine pattern rather than Th2 in patients allergic to HDM [59]. These peptides retain their specific T cell binding capacity, losing B cell binding capacity, thus preventing the degranulation of mast cell mediators, and others. The compounds contain between 4 and 13 polypeptides. These peptide combinations target multiple different MHC molecules. If the peptide/MHC complexes are present on the antigen presenting cell surface in sufficient numbers, they may then activate T cells through T cell receptor (TCR). According to the authors, this compound can be used in sensitized patients, and in individuals at risk of being sensitized, or suspected of being sensitized. Currently, the indication for receiving immunotherapy is an IgE-mediated disease and, therefore, it is necessary to show that there is a cutaneous sensitization and/or presence of serum specific-IgE in the patient. Allergenic polypeptides from other sources apart from HDM can be combined. Once tolerated, other polypeptide can be administered to the patient. Polypeptides should be administered separately. This separation in time would prevent that the second polypeptide suffers the enzymatic action of the mite polypeptides leading to potential protein degradation.

Two clinical trials based on immunotherapy with peptides, one with cat (Fel d 1 peptides) [60] and another with one of peanut (Ara h 2 peptides) [61], show the safety of one dose of these vaccines and the release of Th1 cytokines (IFN- γ , IL-10 and IL-13). Currently, the effectiveness and efficiency of this immunotherapy has not been demonstrated. This immunotherapy could open the door to a new way of desensitization which could be safer and allergen specific.

7. CORTICOSTEROIDS & IMMUNOMODULATORY AGENTS

7.1. Novel Compounds

This invention provides compounds for the treatment of inflammatory diseases, allergy and other diseases with a non-steroidal glucocorticoid binding molecule [62]. This compound exerts its action through a transrepression mechanism and not transactivation. This compound works directly on the NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells) and AP1 instead of exerting direct action on DNA, and leading to inhibition of their pro-transcriptional activity. This mechanism of action results in greater anti-inflammatory effect with less adverse effects of the compounds. NF- κ B protein is a related eukaryotic transcrip-

tion factor that is involved in the control of a large number of normal cellular and processes in organism, such as immune and inflammatory responses, developmental processes, cellular growth, and apoptosis. In addition, these transcription factors are persistently active in a number of disease states, including cancer, arthritis, chronic inflammation, asthma, neurodegenerative diseases, and heart disease. Salts and solvates of the compounds, shown in Fig. (9), are organic and inorganic acids and bases. The compounds of the invention may have the ability to crystallise in more than one form, known as polymorphism. The invention could be administered by oral, nasal, inhaled, sublingual, parenteral, topical rectal or other topical administration.

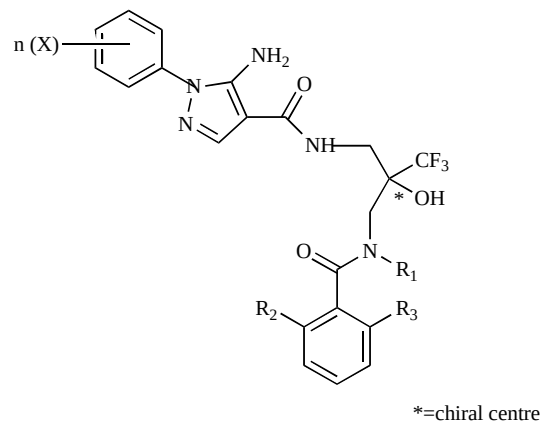


Fig. (9).

As with other glucocorticoids, a great anti-inflammatory effect in allergic diseases such as asthma is expected, with a lower rate of adverse effects. This treatment could be effective to treat inflammatory diseases.

7.2. Compositions and Methods that Modulate G-Protein Signalling for the Treatment of Inflammatory Disorders Such as Asthma and Allergic Conjunctivitis

Asthma is a pathophysiologic condition associated with a prototypical allergic response to inhaled allergens consisting of both neuromechanical and inflammatory components. β -adrenergic receptors (β ARs) are G-protein-coupled receptors (GPCRs) that activate intracellular G proteins upon binding catecholamine agonist ligands, such as adrenaline and noradrenaline [63]. Synthetic ligands have been developed that either activate or inhibit β ARs for the treatment of asthma, hypertension or cardiac dysfunction. These ligands are classified as full agonists, partial agonists or antagonists, depending on whether the cellular response is similar to that of the native ligand, reduced or inhibited, respectively [64-65]. G protein signaling mediates responses throughout the immune, nervous, and muscular systems that might contribute to the pathogenesis of allergic processes and asthma [66]. The role played by phosphorylation of the β 2-adrenergic receptor (β 2AR) by GPCR kinases (GRKs) and cAMP-dependent protein kinase A (pKA) in mediating homologous β 2AR desensitization has been demonstrated following

exposure of airway smooth muscles (ASM) cells to $\beta 2AR$ agonists. Furthermore, the role played by pKA activation in mediating heterologous $\beta 2AR$ desensitization has been demonstrated following more prolonged exposure of ASM to other receptor-coupled or non-receptor-coupled cAMP-elevating agents. The cAMP-elevating agents detected are regulated by cAMP phosphodiesterase (PDE) activity. Among the PDE superfamily, isoforms PDE4 account for most of the cAMP hydrolyzing activity in smooth muscle cells and PDE4 activity has been importantly implicated in regulating ASM contractility. This invention infers that signalling the $G\beta\gamma$ subunit of G_i protein serves to mediate upregulated PDE4 activity in $\beta 2AR$ -desensitized airway tissues in the associated pro-asthmatic state. The compositions of the invention can be directly delivered to the lung, preferably in aerosolized form and may be combined with one or more conventional agents employed in the treatment of asthma. Pretreatment with the $G\beta\gamma$ inhibitory peptide prevents the induction of altered ASM tissue constrictor and relaxation responsiveness accompanying heterologous $\beta 2AR$ desensitization. Compared with controls, the ASM tissues exposed to PGE_2 showed a significant increase in muscle contraction in response to acetylcholine while there is less relaxation in response to isoproterenol Fig. (10). Pretreatment with the $G\beta\gamma$ inhibitory peptide prevents the induction of altered relaxation responsiveness in ASM tissues passively sensitized with allergic asthmatic serum Fig. (11).

There are other mechanisms independent of G proteins for signaling of GPCRs. There are seven transmembrane-receptor of GPCRs. Common GPCR gene variants that encode receptor proteins with a distinct sequence may alter drug efficacy without always resulting in a pathologic phenotype. GPCR genetic loci have numerous variants, such as DNA insertions or deletions and single-nucleotide polymorphisms that alter GPCR expression and function, thereby contributing to interindividual differences in disease susceptibility/progression and drug responses. Thus, this invention works on one of the mechanisms that regulate the appearance of symptoms of asthma but there are many others which are not affected by this treatment. *In vitro* studies suggest that it can be effective: However, *in vivo* studies are needed to corroborate these data.

7.3. New Macrolides and their Use

The invention relates to macrolide compounds with anti-inflammatory activity through inhibition of PDE4 which makes them useful for the treatment and/or prevention of allergic diseases such as asthma and atopic dermatitis, and other non atopic diseases. Increased levels of cAMP are known to suppress cellular responses in inflammatory and immune cells by relaxing smooth muscle cells and reducing inflammation [67]. AMPc is regulated by PDE, mainly PDE4. PDE4 converts cAMP in AMP. Then, the inhibition of PDE4 deviates the route increasing the levels of cAMP by inhibiting its hydrolysis. There are some PDE inhibitors currently marketed for asthma treatment (roflumilast and cilomilast), but the number is increasing (ASP3258, GSK256066). Macrolides (calcineurin inhibitors) for have been used for many years (tacrolimus and pimecrolimus) for atopic dermatitis.

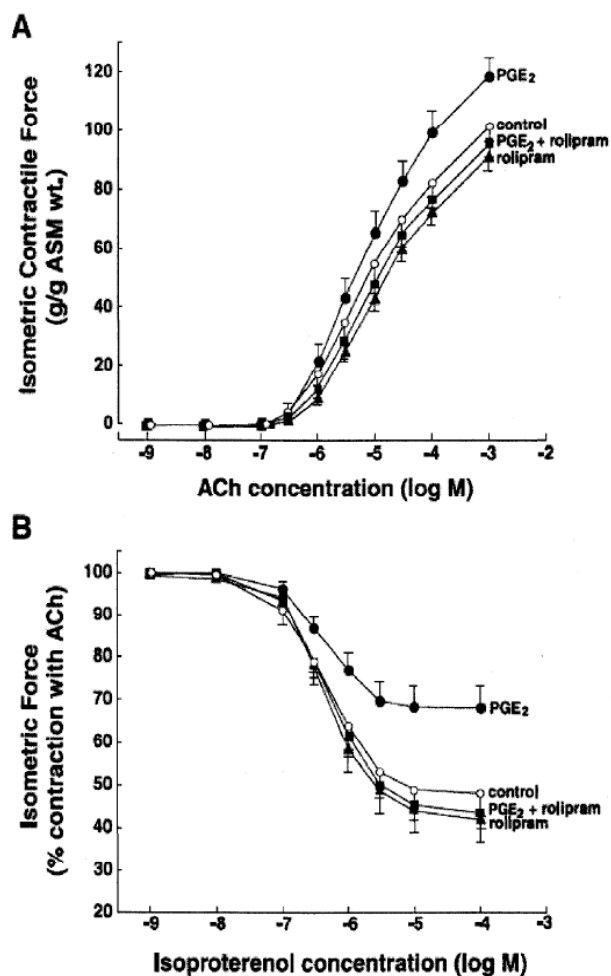


Fig. (10).

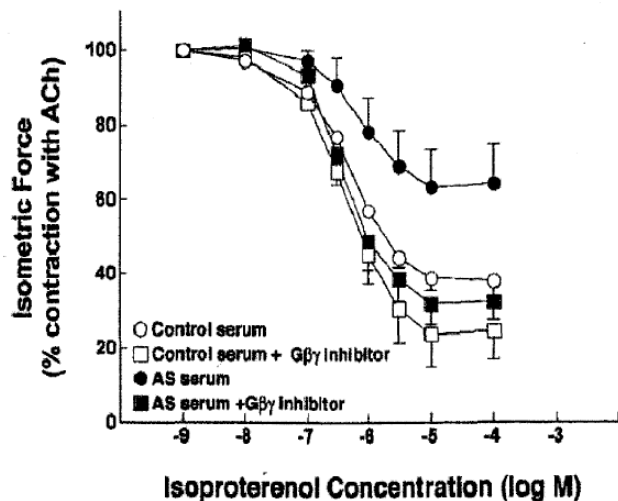


Fig. (11).

The anti-inflammatory effect of PDE4 inhibitors in distinct phases of AD is well established. In addition to blocking the abnormal PDE activity found in AD patients, PDE4

inhibitors may interfere with the most relevant features of AD immunopathogenesis: acute phase, chronic phase and bacterial superantigen-mediated effects. Future studies will determine the effectiveness of macrolides in the inhibition of PDE4 in asthma and atopic dermatitis [68].

7.4. Inhibitors of Phosphodiesterase Type-IV

The present invention relates to catechol derivatives that can be useful in asthma, allergic rhinitis, allergic conjunctivitis and atopic dermatitis and other non atopic diseases. Inhibitors of PDE such as rolipram or Ro-1724 are known as cAMP-enhancers. This invention may be administered with other therapeutic agents such as corticosteroids, β_2 -agonists, muscarinic receptor antagonists, anticholinergics, PAF antagonists, EGFR kinase inhibitors, p38 MAP kinase inhibitors, additional PDE4 inhibitors, kinase inhibitors, dopamine receptor antagonists, antihistamines, antitussives, leukotriene antagonists, 5-lipoxygenase inhibitors, chemokine inhibitors, or combinations thereof. The administration may be by inhalation, intranasal route, rectally, parenterally, intracisternally, intratracheally, intravaginally, intraperitoneally, or topically. In a PDE4 enzyme assay, the concentration of the AMPc in the sample directly correlates with the degree of PDE4 or PDE7 enzyme inhibition [69].

It has been shown [70] that combined PDE4 and PDE3 inhibition (PDE4 inhibitor Rolipram with PDE3 inhibitor motapizone) reversed glucocorticoid resistance under oxidative stress conditions and prevents alveolar macrophage activation.

8. ANTIHISTAMINES

8.1. Oxypiperadine Derivatives and Methods of Use Thereof

This invention relates to use the oxypiperidine derivatives for treating or preventing allergy, an allergic airway response and other diseases involving H_3 receptor [71]. H_3 receptors belong to the sympathetic system, where they modulate neurotransmission (acetylcholine, noradrenaline, dopamine, serotonin and substance P), not only in the central nervous system but also in the peripheral system. H_3 receptors were also localized on the epithelium and nerves in human nasal mucosa. Specifically, H_3 receptor activation by histamine attenuates norepinephrine outflow to resistance and capacitance vessels, causing vasodilatation. The presented compound could be solid or liquid. Solid form preparations include powders, tablets, dispersible granules, capsules, cachets and suppositories. Liquid form preparations include solutions, suspensions and emulsions. A study by Yokota *et al.* [72] was conducted to assess the participation of histamine H_3 receptors in nasal symptoms using Sch 50971, a potent and selective agonist of the H_3 receptor in mice. The activation of H_3 receptors with Sch 50971 or Imetit decreased antigen-induced nasal signs resulting from the inhibition of substance P release from sensory nerve endings. Oral administration of Sch 50971 and Imetit, specific H_3 receptor agonists resulted in an inhibition of nasal symptoms induced by an antigen similar to an H_1 -receptor antagonist, cetirizine. Furthermore, simultaneous use of H_3 -receptor agonists, Sch 50971 or Imetit, and an H_1 -receptor antagonist,

cetirizine, caused a significant inhibitory effect on nasal symptoms at doses that showed no effect when used separately. The number of eosinophils in the nasal mucosa of mice sensitized with antigen was significantly decreased by cetirizine. However, Sch 50971 and Imetit had no effect on eosinophil infiltration. Ling *et al.* established that H_3 receptor has not effect on eosinophil chemotaxis, cell-shape, and upregulation of adhesion molecules *in vitro*. In opinion of the authors, these results clearly indicate that H_3 receptors are involved in the mechanisms of nasal allergy, and the stimulation of H_3 receptors may be useful as a novel therapeutic approach in nasal allergy. In 2010, Sun *et al.* [73] published a study concluding that Imetit and the combination of Imetit with Loratadine can effectively relieve the symptoms of allergic rhinitis in guinea pigs by reducing substance P and the expression of Substance P-receptor mRNA, and presenting a synergism. In recent years there have been many drugs patents for use on the H_3 receptor for use in obesity. It is not clear the role of the H_3 receptor in atopic diseases, in which receptor H_1 and to a lesser extent receptor H_2 are involved. More studies in humans are needed to establish the efficacy of these drugs in allergic diseases.

9. SKIN ALLERGY

9.1. Probiotic Lactobacillus Rhamnosus Strain and Oral and Topical Uses Thereof

Probiotics can be used to prevent allergic diseases of the skin as postulated by this invention [74]. *Lactobacillus rhamnosus* is used for the treatment of inflammatory and/or allergic skin affection, or disease. The composition for local use can be as a cream, emulsion, ointment, gel, lather, liniment, powder, aqueous solution or suspension, oily solution or suspension, or biphasic solution or suspension, and mixtures thereof. Authors said that the anti-inflammatory effect of this compound is based on the elevation of IL-6 and IL-7 in increasing protein expression of peroxisome proliferator-activated receptors- γ (PPAR- γ) and the reduced levels of cyclooxygenase-2 (COX-2). The hygiene hypothesis links atopic dermatitis to a change in the intestinal colonization pattern during infancy. The manipulation of this flora could prevent, or treat atopic dermatitis. The results of the studies conducted are controversial. There is not enough scientific evidence to use this therapy for atopic dermatitis [75].

9.2. IL-31 Monoclonal Antibodies and Methods of Use

This invention is for use in atopic dermatitis, contact dermatitis, drug-induced allergic reactions and other skin diseases. The cutaneous lymphocyte antigen (CLA) is a skin receptor for T cells and it appears in several diseases [76]. The over-expression of IL-31 by CD4+ CD45RO+ results in dermatitis-like symptoms. IL-31 signals through a heterodimeric receptor composed of IL-31RA (IL-31 receptor A) and OSMR (oncostatin M receptor) that is expressed on both T cells and keratinocytes. IL-31 mRNA appears only in CLA+ T cell population in both atopic dermatitis patients and healthy individuals while IL-31RA augmented expression has been detected in skin biopsies of atopic dermatitis compared to normal individuals. Antagonists of IL-31 include soluble IL-31RA receptors and neutralizing anti-IL-31 antibodies. The immunogenicity of the invention may be in-

creased through the use of an adjuvant such as aluminium hydroxide.

In paediatric patients with atopic dermatitis, high serum levels of IL-31 correlates with severity scoring [77]. In another study, the authors found a correlation of IL-31 serum levels with severity of intrinsic and extrinsic type of atopic dermatitis [78]. IL-31 expressed in human primary keratinocytes has a link with TLR-2 ligands (Pam3Cys) and IFN- γ but it is not stimulated by IL-4 [79] pointing out to an interaction between Th1 cells and keratinocytes in the skin, enhancing CCL2 (also called MCP-1) via the phosphorylation of STAT3. CCL2 is a cytokine that recruits monocytes, Th1 cells, and dendritic cells to sites of inflammation. Therefore, it is a contributing factor, with a dysregulated production of IL-31, to the development of atopic dermatitis. IFN- γ also induces upregulation of IL-31 in human dermal microvascular endothelial cells [80]. However Neis *et al.* [81] published a correlation between IL-31 with IL-4 and IL-13 but not with IFN- γ after studying patients with atopic dermatitis and psoriasis. These inconsistencies could be explained by the fact that monocytes also express IL-31RA and OSMR when activated by IFN- γ and LPS [82]. In addition, another study showed that eosinophils constitutively expressed active receptor complex for IL-31. The co-culture of eosinophils and keratinocytes with IL-31 showed that only eosinophils are stimulated to secrete proinflammatory cytokines and also delayed their apoptosis [83]. Therefore, although it is not entirely clear which are the most important cells in the pathogenesis of atopic dermatitis, we can assume with confidence the important role of IL-31.

In a murine model (NC/Nga mice) treated with IL-31 antibodies, Grimstad *et al.* [84] observed that IL-31 does not play a key role in the pathogenesis of atopic dermatitis but did have an effect on the scratching behaviour. The authors suggested that IL-31 exerts its pruritogenic effect through sensory neurons. Venereau *et al.* generated a potent IL-31 antagonist consisting of the distal domains of OSMR associated through a linker to the IL-31RA cytokine binding domain. It showed potent neutralizing activities on various IL-31 responsive models [85]. Currently, there is a lack of clinical trials on humans with this type of treatment but it could be a good treatment options, especially in severe atopic dermatitis.

10. ANTIMUCUS THERAPY

10.1. Protein Modulators

The present invention relates to modulators of a neuregulin (NRG1 β 1) to inhibit goblet cell hyperplasia which allows the treatment of those diseases characterized by overproduction of mucus as cystic fibrosis, COPD and asthma [86]. Mucus is produced in the large airways by goblet cells and submucosal glands. In the small airways it is synthesized by goblet cells. This mucus has been documented to be the major contributing cause of fatal asthma. The mucins MUC5AC and MUC5B are major components of airway mucus secretions in respiratory diseases. These mucins and goblet cells are increased in asthmatics. Neuregulins are signalling proteins that mediate multiple cell-cell interactions via the receptor tyrosine kinases of the

ERB family. NRG1 β 1 binds to Erb3 but with higher affinity for Erb2/Erb3 heterodimer. Fig. (12) shows the effects of ErbB receptor neutralising antibodies on NRG1 β 1-induced goblet cell formation.

This invention is a consequence of previous published data about regulation of the production of mucine. Treatment of human bronchial epithelial cells with inhibitors of several kinases indicated that these kinases were involved in NRG1 β 1-induced MUC5AC and MUC5B expression. NRG1 β 1 protein was found increased in the airways of antigen-challenged mice, together with increases in MUC5AC and MUC5B [87]. Mucus secretion is linked to IL-13 and EGFR (epidermal growth factor receptor) [88]. The EGFR is a member of the ErbB family of receptors. This invention could be part of the treatment of severe asthmatic patients. Currently, there are not studies on humans.

CURRENT & FUTURE DEVELOPMENTS

In the last years, significant advances have been made in the understanding of complex diseases such as allergic and autoimmune diseases. As part of the increasing knowledge in the mechanisms involved in these inflammatory diseases, new drugs and treatment modalities have been designed. The exact mechanisms behind the favourable effects of probiotics on allergy are not entirely known. Several mechanisms have been observed *in vitro* and *in vivo* animal studies. At this moment, the probiotics pathway of action is unknown and for the future, the elucidation of these mechanisms will be an important target [89].

The development of novel biophysical techniques for monitoring the changes in the conformation of GPCRs is encouraging and has considerable promise for future studies. The discovery of a multitude of intracellular protein interactions with GPCRs points to almost limitless potential for modulation of their expression, ligand selectivity and signaling to produce an array of cellular and physiological phenotypes.

In the past years, studies about treatment of food allergy with immunotherapy using food extracts are emerging, but so far the side effects are common and dangerous. In the future the use of recombinant allergens or peptides of food allergens may improve the safety profile of this treatment. The avoidance of food is the only effective therapeutic measure currently known.

In the next years, research into the underlying pathophysiology of allergic diseases will continue to reveal new targets for immunomodulation of the IgE-mediated response. Anti-IgE has demonstrated significant efficacy in asthma; additional uses are being studied. Various anti-cytokines agents are being studied in clinical trials

Toll-like receptors can influence allergic disease by changing the Th1/Th2 cytokine balance. Understanding the roles of TLR pathways and the relationships between them and non-TLR innate immune pathways in the pathogenesis of diseases such as asthma, highlights potential treatment targets for this atopic disease. More clinical trials are needed to confirm the effectiveness of TLR-modulators as a therapeutic tool for atopic diseases.

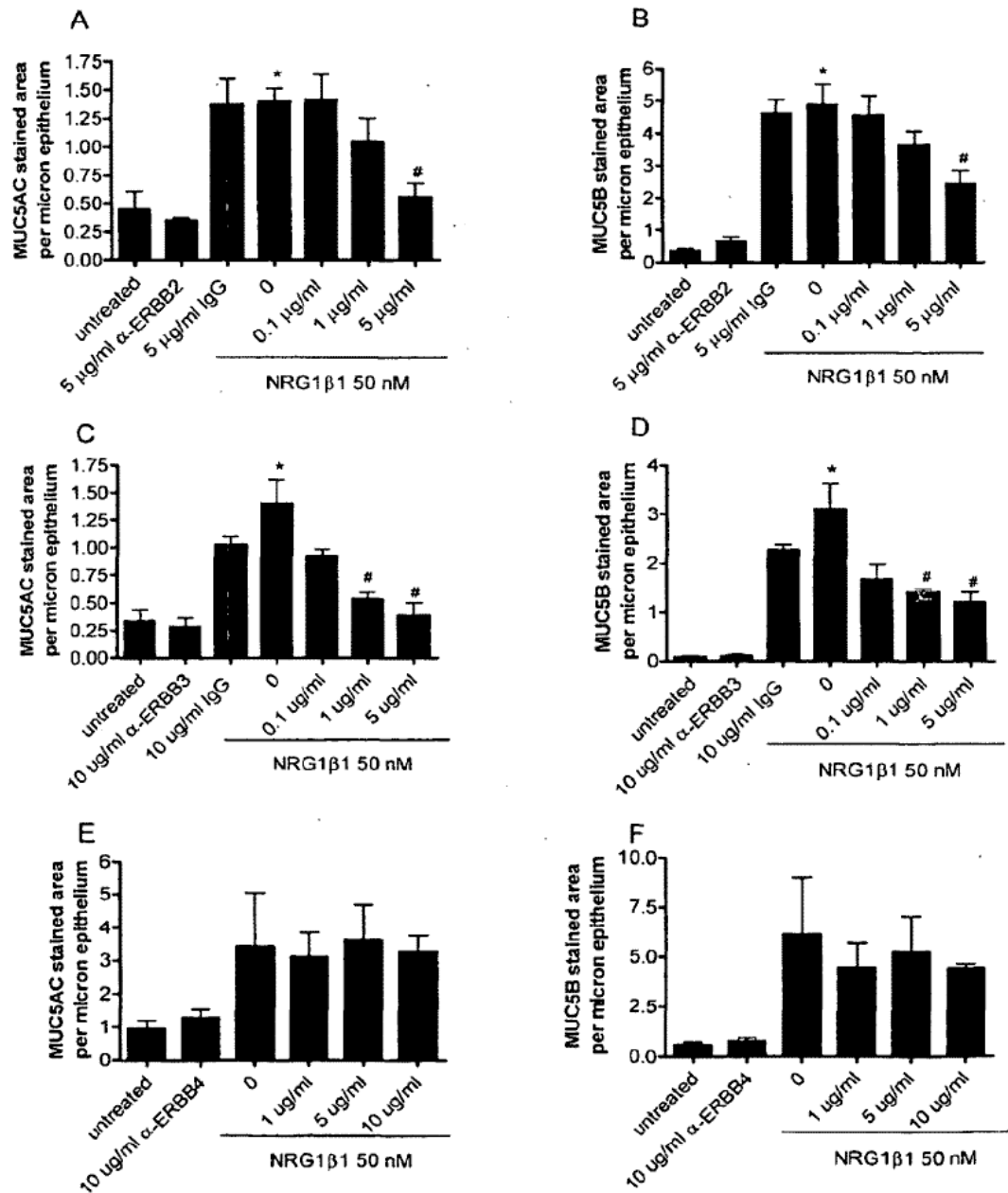


Fig. (12).

It will be very difficult, or perhaps impossible, to block the numerous pathophysiological mechanisms known in asthma using a single drug. A combination of drugs and immunotherapy will probably be the most effective way to treat this disease.

CONFLICT OF INTEREST

There is not any conflict of interest.

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