Future and experimental therapeutic strategies for allergic rhinitis and asthma

Giovanni Passalacqua MD

Allergy & Respiratory Diseases, Dept of Internal Medicine, Padiglione Maragliano, L.go R. Benzi 10, 16132 Genoa, Italy
Tel.: +39 10 353 8908
Fax: +39 10 353 8904
passalacqua@unige.it

The rapid progress in our understanding of basic immunology has recently enabled the identifcation of many possible therapeutic targets for the treatment of respiratory allergy and allergic inflammation. As a result, new approaches have been proposed and tested in rhinitis and asthma, including new drugs and mediator antagonists, monoclonal antibodies and immunosuppressant agents. Only a small number of these approaches have become a reality, however, all of them have provided us with the opportunity to improve our knowledge in the field. We will review herein some of the experimental approaches used in humans in relation to the pathogenic aspects of allergic inflammation.

Keywords: allergic inflammation, asthma, IgE, mediators, monoclonal antibodies, rhinitis, therapy

Future Drugs Ltd

The overall view of respiratory allergy has deeply changed and evolved over the last 10 years, mainly due to the rapid development of basic science which facilitated in a more and more detailed manner, the elucidaton of the pathogenic aspects of immune inflammation. It has become progressively clear that allergy is much more than the classic allergen–immunoglobulin (Ig)E–mast cell interaction. Numerous factors contribute to the development of the atopic status and to the subsequent appearance of the clinical disease. Moreover, after the allergen–IgE interaction has taken place, a complex inflammatory network is established, sometimes leading to chronic inflammation and tissue remodelling (**Figure 1**). In particular, many of the components of the allergic inflammatory reaction (e.g., adhesion molecules, cytokines, chemokines and receptors) and their roles have been identified and described. These advances have opened new research pathways since many specific targets for therapy were also identified. In parallel, the immunological and biochemical techniques have made it possible to synthesize new molecules, engineered antibodies, and in some cases, therapeutic gene constructs. In many cases it has become possible to test the effect of treatments designed to interfere with single components of the immune reaction.

Currently, experimental or exploratory therapies (those that differ from more traditional approaches) are still at the laboratory stage. Nevertheless, some of the newer approaches have rapidly become reality and are being introduced into clinical practice.

This paper reviews the experimental approaches, focusing mainly on those already being applied to human diseases. In order to explain the rationale for those approaches, a brief overview of the immune inflammation of allergy will be provided.

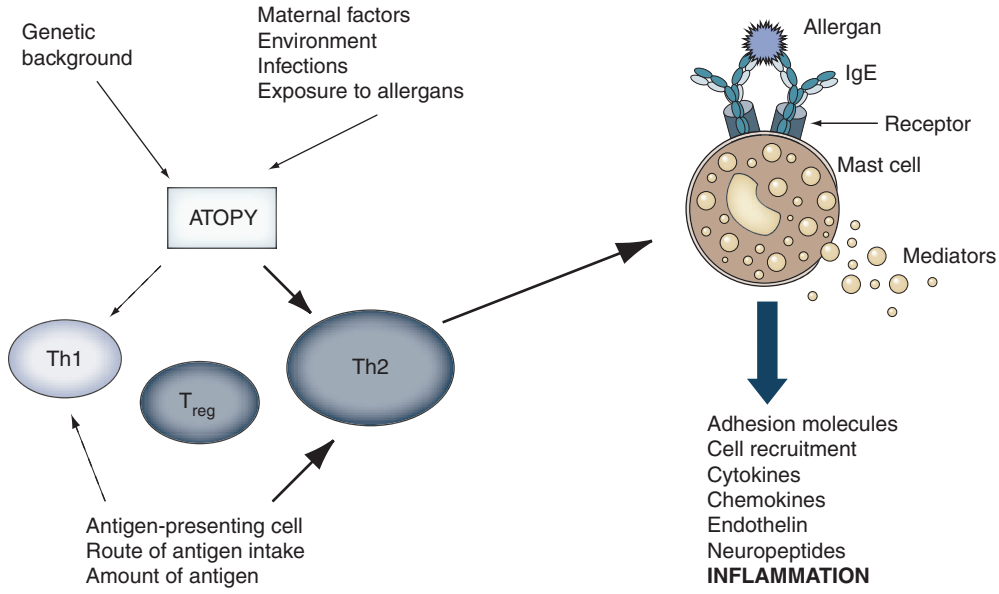
Immunology of the allergic reaction

Before the IgE reaction

Allergic response is characterized by both an overproduction of specific IgE (atopy) and the activation of mast cells in response to the allergen. The processes leading to the develoment of the IgE response are complex and thus far, not completely elucidated. There is of course a genetic background, in fact, atopy is at least in part an inherited condition and a family history of the disease is the strongest risk factor for developing atopy. Although it is unlikely that a 'gene of atopy' or a 'gene of asthma' can be discovered, genetic studies remain an attractive approach and many candidate loci of 'susceptibility' have been described [1]. The genetic background interacts with numerous factors: maternal feeding, maternal smoking, early exposure to allergens, infections in early infancy, environment and lifestyle. The importance of each of these factors for the early development of atopy have been repeatedly described [2–4], but the exact interaction and the relative weight of each of them still remains unclear.

It has been ascertained that the atopic status is characterized by a T-helper Type (Th)2 cell skewed phenotype, whereas the response from nonatopic individuals is commonly dominated by the Th1 phenotype. These two subsets can be distinguished based on their cytokine production pattern [5]. Th2 lymphocytes typically secrete IL-4, IL-5 and IL-13, whereas Th1 cells produce mainly interleukin (IL)-2 and interferon (IFN)γ. IL-4 (in cooperation with IL-13)

Figure 1. Factors influencing the development of the atopic phenotype, IgE-mediated reactions and components of inflammation.



Ig: Immunoglobulin; Th: T-helper cell.

induces ε class-switching and thus the production of IgE by B-cells [6]. IL-5 is an eosinophil-committed cytokine in that it enhances maturation, recruitment and activation of eosinophils and prolongs their survival [7]. IL-13 is also involved in the activation and differentiation of fibroblasts which intervene in many processes of inflammation in asthma [8]. The skew towards the Th2 phenotype is also affected by microenvironmental factors such as antigen presentation, the amount and affinity of antigen and the human leukocyte antigen (HLA) genotype of the responder, the type of antigen-presenting cell (APC) and the cytokine pattern.

Another subset of Th lymphocytes has recently been described: the so-called $CD4^+CD25^+$ regulatory T-cell or T_{reg} [9]. These cells produce a relevant amount of IL-10 that inhibits T-cell activation, IgE production, eosinophil recruitment and other aspects of allergic inflammation [10]. It has been proposed that a defect in the T_{reg} function is primarily responsible for the Th2/Th1 imbalance.

The IgE-mediated reaction – early phase

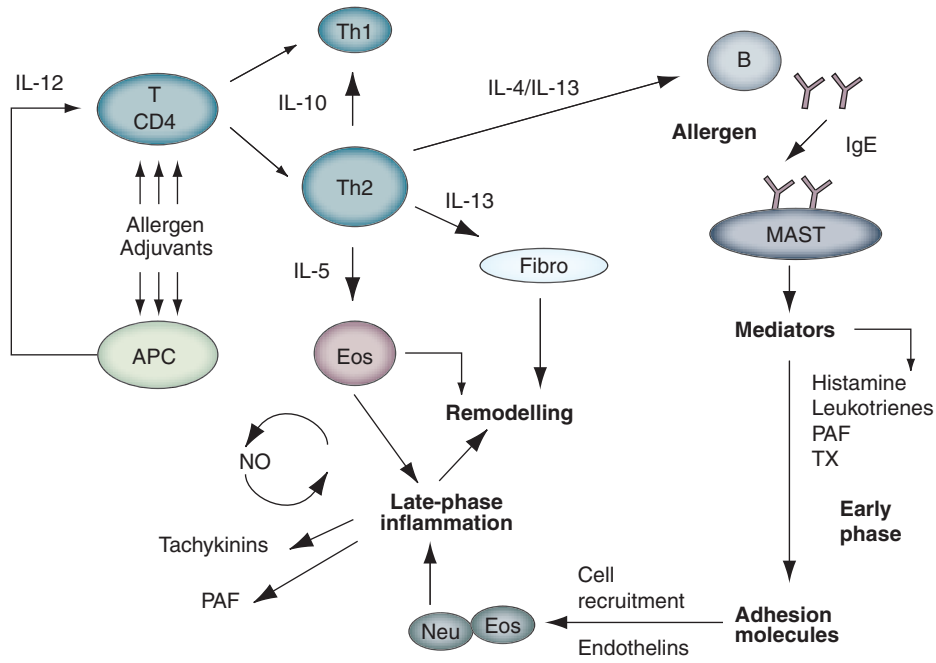
The allergen-specific IgE are bound to mast cells and basophils via the high-affinity IgE receptor (FcεRI), and at a lesser extent, by the low-affinity receptor (FcεRII, CD23). Mast cells are predominantly localized in the epithelium of the upper and lower airways in the gut and the skin – the most important target organs for allergic reactions.

When the allergen reaches the target organ it crosslinks specific IgE bound to high-affinity IgE receptors on mast cells and induces the prompt (within minutes) release of vasoactive and inflammatory mediators (so-called early phase) [11]. Histamine is predominant in this phase, since it is stored in large amounts in mast cells. Histamine, via the H1 receptor, is responsible for vasodilatation, increased permeability and bronchoconstriction. The activation of mast cells initiates the synthesis of other mediators and cytokines [12]. Leukotrienes are a good example of the proinflammatory mediators synthesized during the allergic reaction. Their synthesis (from membrane phospholipids through arachidonic acid metabolism) by mast cells begins when the cells activate in the early phase and continues for hours. Leukotrienes have a potent bronchoconstrictor–vasodilator effect and a chemotactic action on many inflammatory cells, including neutrophils and eosinophils [13].

Beyond IgE & mast cells: inflammation & remodeling

The early phase is followed by a complex network of inflammatory phenomena, in which T-lymphocytes, cytokines and adhesion molecules are involved (**Figure 2**) [14]. The adhesion machinery, in

Figure 2. Overview of the allergic reaction with the most relevant therapeutic targets.



Overview of the allergic reaction with the most relevant therapeutic targets.
Eos: Eosinophil; Fibro: Fibroblast; IL: Interleukin; LT: Leukotrienes; Neu: Neutrophil; NO: Nitric oxide; TX: Thromboxanes.

particular, seems to be crucial for the recruitment of inflammatory cells at the target organ. During the early phase, specific adhesion molecules are newly expressed or upregulated on the surface of endothelium (selectins) and epithelium (integrins). The adhesion molecules favor the rolling over, extravasation and migration towards epithelium of inflammatory cells [15]. If the allergenic trigger persists, such as happens in natural exposure, the inflammation becomes chronic and may provoke architectural and functional changes in the target organ (e.g. remodeling and bronchial hyperreactivity). Interestingly, a weak inflammatory infiltration is present in the mucosae even in the absence of symptoms, when a subthreshold exposure to the allergen persists. This is known as minimal persistent inflammation (MPI) [16]. A broad spectrum of inflammatory mediators contribute to clinical symptoms of respiratory allergy, involving for instance: leukotrienes, nitric oxide (NO), tachykinins, platelet-activating factor (PAF) and tromboxanes.

Another important aspect of respiratory allergy is the remodelling phenomenon that is quite characteristic of bronchial asthma and poorly represented in rhinitis [17]. The remodeling is characterized by sub-basement membrane thickening, smooth muscle hypertrophy, epithelial disruption and vascular proliferation [18]. It was previously believed that remodeling was a direct consequence of the chronic inflammatory processes that take place in allergic asthma. Nevertheless, it has recently been demonstrated in asthmatic children that remodelling is at least in part, independent of the inflammation [19] and is a background characteristic of allergic asthma.

Future therapeutic approaches for rhinitis & asthma – general aspects

As described, the allergic reaction is extremely complex. Despite this, we know in detail many of its components and their role in the inflammatory network. This has lead to several interesting and promising approaches to the treatment of rhinitis and asthma. There are currently several different strategies. The most obvious and attractive of which is to improve the pharmacologic characteristics of existing drugs or, alternatively, to develop new drugs acting on specific mediators and receptors such as antileukotrienes and phosphodiesterase (PDE) inhibitors, or to use already existing drugs in

combination. Another possible approach is to selectively block the molecules (e.g., adhesion molecules, cytokines and receptors) with monoclonal antibodies (mAbs). This is now possible thanks to advanced molecular and genetic techniques that facilitate the building of humanized (chimeric) antibodies with a given specificity. This strategy is expensive, but it provides the unique opportunity to assess the clinical role of each therapeutic target in diseases. A third approach involves the administration of the allergen in various and modified forms. This belongs to the large field of so-called specific immunotherapy or allergy vaccinations (including DNA vaccination, peptides, recombinant allergens and adjuvanted immunotherapy) that will not be reviewed in detail herein. Finally, there is currently an increasing interest in strategies involving modification of the immune response specifically acting on the Th1/Th2 balance, possibly using probiotics and bacterial products. This latter approach is not specifically targeted to respiratory allergy but rather to the general development of an 'atopic phenotype'.

An overall and arbitrary summary of the experimental therapeutic approaches investigated for human use, is reported in **Figure 3**.

Drug therapy

Improved & modified inhaled steroids

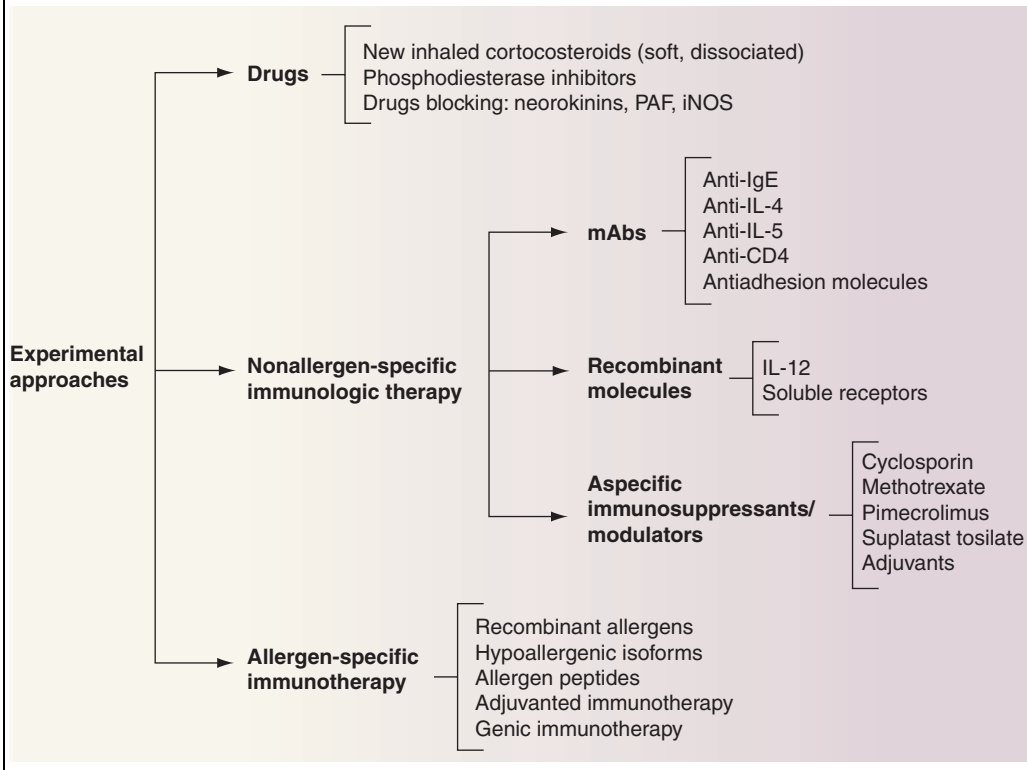
Inhaled corticosteroids (ICSs) are currently considered to be the cornerstone in the long-term management of bronchial asthma. They are the most potent anti-inflammatory agents and exert their activity on almost all of the components of allergic inflammation. The available ICSs (beclometasone, budesonide, fluticasone, flunisolide, mometasone and triamcinolone) are highly effective and overall safe. Nevertheless, some concerns still remains regarding the possible endocrine and metabolic effects in long-term treatments and particularly in infancy. Therefore, efforts have been made to improve the pharmacologic characteristics of ICSs – especially in terms of safety. One of the first attempts was to synthesize molecules that are immediately inactivated in the lung – so-called soft steroids. Many of these molecules appeared to be poorly effective and were therefore rapidly abandoned. Presently, only two molecules etiprednol and loteprednol, are undergoing clinical studies [20]. Another interesting approach was that of the dissociated steroids. These molecules only possess the transrepressive and not the transactivating activity. This results in steroidal drugs with no metabolic effect (mediated by transactivation) and retained anti-inflammatory effect (mediated by transrepression). There are some studies in animal models with the drugs RU44858 and ZK216348 [21,22], demonstrating that these molecules are capable of reducing experimental inflammation without inducing metabolic effects (osteopenia, dyslipidemia or hyperglicaemia). No data in humans and clinical disease is thus far available.

A third approach is to build a steroid that is activated only when it reaches the respiratory epithelium. In this case, the fraction of ICS that is invariantly swallowed or directly absorbed in the mouth can exert no metabolic action. This approach resulted in a new molecule, ciclesonide (Alvesco®, Altana AG), a special type of soft steroid, which has proven to be safe and effective and which is expected to be soon commercialized. Ciclesonide was tested in both asthma and rhinitis [23–25]. In a clinical study in 209 asthmatics it was shown that ciclesonide 200 µg once daily improved morning and evening peak expiratory flow and reduced asthma exacerbations [23]. In 24 subjects with allergic rhinitis, a significant symptomatic improvement with intranasal ciclesonide appeared from the second day of treatment upon nasal challenge [25]. The measurement of plasma cortisol (index of the metabolic effect of steroids *in vivo*) have shown that no impairment of the pituitary adrenal axis occurs with ciclesonide 200, 800 and 3200 µg daily [23,26] .

Selective PDE inhibitors

PDEs are a group of isoenzymes involved in the metabolism of cyclic AMP. Blockage of PDE results in bronchodilation due to intracellular accumulation of cAMP, but also in inhibition of the production of several pro-inflammatory mediators [27]. Theophyllines, which have numerous side effects, are a good example of nonspecific PDE inhibitors. Pharmacological research has recently produced some PDE inhibitors that are quite specific for the isoform four and that maintain the bronchodilator action without relevant adverse events. The first member of this class of drugs, cilomilast (Ariflo®, GlaxoSmithKline) proved effective in the treatment of chronic obstructive pulmonary disease (COPD) [28], however, theophylline-like side effects were frequent. For this reason, cilomilast did not gain US Food and Drug Administration (FDA)

Figure 3. A tentative classification of the new and experimental approaches for respiratory allergy.



Ig: Immunoglobulin; IL: Interleukin; iNOS: Inducible nitric oxide synthase; mAb: Monoclonal antibody; PAF: Platelet-activating factor.

marketing approval. The newest compound roflumilast (Daxas®, Altana AG) displayed a good bronchodilator effect and seemed to be devoid of the main side effects (nausea, stomachache, headache and tachycardia), thus making it a good candidate for clinical use. Numerous clinical trials in chronic bronchitis and asthma are presently ongoing and roflumilast is awaiting marketing approval in several countries [29]. A third molecule of this class, piclamilast, is now undergoing Phase I studies.

Traditional drugs & their combinations in allergic rhinitis

Allergic rhinitis shares the same pathogenic mechanisms as asthma and the same mediators are also involved, including leukotrienes. This is the reason why leukotriene receptor antagonists that are widely used in asthma have been recently proposed in the treatment of rhinitis. Indeed, cysteinyl-leukotriene receptor antagonists (LTRAs) proved more effective than placebo in controlling symptoms of both seasonal and perennial rhinitis, however, systematic reviews of the available trials have clearly shown that LTRAs are not more effective than antihistamines. Thus far, it seems that LTRAs alone do not offer significant advantages on established therapies for the treatment of allergic rhinitis.

Since respiratory allergy is sustained by numerous mediators, it was reasonably hypothesized that blocking different mediators at the same time would increase the clinical efficacy of treatment. Therefore, various combinations of LTRAs plus antihistamines (cetirizine plus montelukast and loratadine plus montelukast) have been tested in rhinitis, confirming that the combination is more effective than each drug alone [32,33]. Nevertheless, a recent randomized controlled six-way study has evidenced that nasal corticosteroids still remain the most effective drugs in allergic rhinitis and that adding an antihistamine or a LTRA provides only a marginal benefit [34].

Nevertheless, it is now well established that asthma and rhinitis often coexist and represent the expression of the same respiratory disease [35]. In this case, the association of an antihistamine plus a LTRA is equally effective

to the association of nasal and ICSs [36]. In addition, it has been demonstrated that an effective treatment for allergic rhinitis with an antihistamine may result in a beneficial effect on concomitant asthma symptoms [37]. This aspect has lead to a partial reassessment of the role of antihistamines in asthma [38].

Other mediator antagonists

The effector mediators (e.g., histamine or leukotrienes) are highly redundant and their actions often overlap. Nevertheless, some of these mediators appeared to be of particular relevance and therefore attempts to antagonize them have been made. Tachykinins belong to the neurokinin family. They are released by nerve ends and exert a powerful bronchoconstrictor action via the neurokinin (NK)1 and 2 receptors. A selective NK2 receptor antagonist proved ineffective in clinical asthma [39], whereas in a recent controlled study an oral NK1/2 antagonist was able to protect asthmatic adults against neurokinin A bronchial challenge [40]. PAF is another mediator involved in asthma, where it is responsible for vascular leakage. Several studies have nonetheless demonstrated that PAF antagonists are clinically ineffective and only a marginal benefit on gas exchange parameters can be achieved [41]. It is for this reason that this approach has been virtually abandoned for the treatment of asthma. NO is considered a relevant inflammatory mediator in asthma since asthmatic subjects exhale increased amounts of this compound. NO is formed by epithelial cells via an inducible NO synthase (iNOS). One single study in humans has tested the effect of an oral iNOS inhibitor, SC51, demonstrating that the drug almost abrogates the production of NO in the airways [42]. Thus far there is no data on the clinical effects of such an antagonism, however the approach is an attractive one due also to the safety of the compound.

Monoclonal antibodies & recombinant molecules

Anti-IgE mAbs

Due to their role in allergy, IgE was considered the ideal target for a mAb-based approach. In fact, IgE triggers the early phase of the allergic reaction and begins the cascade of inflammatory events. Thus, selectively blocking IgE was immediately regarded as an attractive approach. Modern techniques have allowed for the production of several mAbs directed to IgE [43]. Omalizumab (commercialized as Xolair®, Novartis) is the first to be used and studied. This chimeric humanized antibody binds the Fcε3 domain of human circulating IgE, preventing them from binding to mast cell receptors. Once administered (subcutaneously or intravenously) at an appropriate dose, omalizumab reduces the circulating free IgE level by 95% within 24 hours [44]. The IgE–anti-IgE immune complexes are small, inert and do not activate complement. A conspicuous number of Phase II and III studies have been performed in both rhinitis and asthma, demonstrating a good clinical efficacy and virtually no relevant side effects [45]. A recent Cochrane review of omalizumab included eight randomized control trials (2037 patients with asthma of varying severity) [46]. Omalizumab significantly reduced inhaled steroid consumption by an average of -114 µg/day, (95% confident interval [CI] -150 to -78.13). There were significant increases in the number of participants who completely withdrew or reduced steroids by greater than 50%: odds ratio (OR) 2.50; 95% CI 2.00–3.13. Also, there was a significant overall decrease in asthma exacerbation (OR 0.49; 95% CI 0.37–0.60). The therapy proved to be well tolerated. Moreover, after 1 year of omalizumab therapy, children with asthma did not have significantly more adverse events compared with the placebo group and no children developed immune complex disease [47]. While conducting the trials, it was noticed that the clinical efficacy was greater in more severe asthma and that there was an important reduction in exacerbations, emergency visits and near-fatal attacks [48,49]. Since the mAb is very expensive, it is reasonable to state that it is cost effective only in severe, uncontrolled and steroid-dependent asthma.

Very recently, anti-IgE was successfully employed in severe peanut [50] and latex allergy [51]. Therefore, other possible uses of anti-IgE could be that of protecting children with severe food allergy from inadvertent ingestion of the culprit food, or in healthcare workers allergic to latex. Finally, omalizumab was shown to be capable of greatly enhancing the effect of traditional specific immunotherapy in respiratory allergy [52].

Anti-IL-5 mAbs

IL-5, at variance with other pleiotropic cytokines, is quite specific for eosinophils. It stimulates the maturation of bone marrow precursors, promotes chemotaxis and migration, and enhances the activation and granule secretion of eosinophils. It also prolongs the survival

of these cells at the site of allergic inflammation [7]. Thus, IL-5 appeared an ideal candidate for specific antagonism. A humanized anti-IL5 (Mepolizumab) was produced and rapidly developed [53]. The first data in animals *in vivo* were encouraging, since in a monkey model of asthma, a single administration of mepolizumab resulted in a long-lasting clinical benefit [54]. Anti-IL5 was then used in a Phase II study in asthmatic patients [55]. It induced, as expected, a significant decrease in sputum and blood eosinophils but had only a marginal effect on symptoms and no effect on bronchial reactivity. This fact raised some concerns over the real role of eosinophils in allergic diseases [56]. Indeed, a pilot study with another anti IL-5 antibody (SCH55700) provided more encouraging results, but the clinical effects were again low [57]. Therefore, this therapeutic approach still remains open for future developments.

Anti-IL-4 mAb & recombinant IL-4 receptor

Since IL-4 is deeply involved in the isotypic switch of B lymphocyte to IgE production, it was considered a good target for mAbs [6]. An anti-IL-4 mAb (Pascolizumab) was synthesized and tested in various animal models (reviewed in [58]) with promising results. Nevertheless, it has to be remembered that IgE synthesis is regulated not only by IL-4, but also other factors [59] and in fact an early study demonstrated that selective blockage of IL-4 had only a marginal effect on IgE levels [60].

On the contrary, the aerosolized administration of a soluble recombinant IL-4 receptor (rIL-4R, Nuvance™, Immunex Corp.) in a single dose of 1500 µg was capable of preventing asthma worsening in steroid-dependent asthmatic adults after ICS withdrawal [61]. It is thought that the rIL-4R works by blocking the locally acting IL-4, thus impeding its binding to specific receptors on effector cells.

Anti-CD4 mAb

It is well known that Th cells ($CD4^+$) play a central role in orchestrating allergic inflammatory phenomena via cytokine production and molecular signaling [62]. It is expected that selective blockade of these cells would result in a reduction of many inflammatory events. This is also the rationale for the use of nonspecific immunosuppressant agents. A humanized anti-CD4 antibody (keliximab) was synthesized in the last few years [63] and rapidly tested in human asthma in a Phase II study [64]. The results were encouraging in clinical terms, although side effects were common. Thus, this approach did not seemed to be cost effective or safe enough to explore further and no human studies appeared in the literature after 1998.

Antiadhesion molecule mAbs

Adhesion machinery is responsible for selective migration and extravasation of inflammatory cells at the site of the allergic reaction. Therefore blocking those molecules would result in a decreased accumulation of inflammatory cells. The efalizumab (Raptiva®, Genentech Inc.) antibody selectively blocks the CD11a adhesion molecule, an integrin that mediates the migration of eosinophils and neutrophils to inflamed tissues. Efalizumab has been already used for the treatment of psoriasis [65]. So far, there is one single clinical study of efalizumab in asthmatic subjects [66]. After 8 weeks of treatment, a significant symptomatic improvement over placebo was observed together with an increase in pulmonary function. Nevertheless, the symptomatic effect was clinically irrelevant as well as the increase in forced expiratory volume, that was on average less than 10 ml. The marginal clinical effect is probably due to the fact that the adhesion system is highly redundant and blocking one single molecule does not result in a quantitative relevant method of cell migration.

Recombinant IL-12

IL-12 is secreted by APCs, especially dendritic cells, in response to antigens and it is a potent Th1 phenotype-inducer [67]. Subcutaneous administration of recombinant human IL-12 has been in clinical trials in mild allergic asthmatic patients [68]. Eosinophil numbers in blood and sputum were significantly reduced compared with placebo, however, differences in histamine-induced bronchoconstriction and the late asthmatic reaction to inhaled allergen were not significant. Conversely, conjugation of IL-12 to allergen has been shown to downregulate allergen-specific Th2 responses in animals [69]. However, this approach is at a very early stage and far from being applicable in humans.

Other immunological strategies

The availability of mAbs makes it possible to antagonize virtually all components of the allergic reaction. Nevertheless, only a few molecules seem to be of primary relevance and worth such an expensive attempt. Indeed, several possible targets have been identified, including chemokines,

IL-9, IL-18 and surface receptors. To date, there is one single Phase I study in asthmatic subjects employing a mAb specific for the low-affinity IgE receptor (CD23) [70]. In this study, single intravenous doses of the mAb demonstrated an ability to significantly reduce the total IgE levels without significant side effects.

IL-9 is another important Th2 cytokine, involved in the synthesis of IgE and activation of inflammatory cells. Anti-IL-9 mAbs have been tested with good results in animal models [71], but no data in humans is available. Similarly, specific antagonism to chemokines that are potent chemo -attractants for inflammatory cells has been proposed as a future strategy for allergy treatment, however, no clinical result is thus far available.

Nonspecific immunosuppressants & immunomodulators

The immune-mediated inflammation in asthma has been well recognized for decades, therefore, many immunosuppressant therapies have been proposed – especially for refractory and severe asthma. Those therapies include cyclosporine, methotrexate, intravenous Igs, rapamycin and others. However, a number of studies have shown that immunosuppressant agents may provide some benefit in the more severe forms of asthma and act as steroid-sparing agents, the safety profile and the risk–benefit of which are clearly unfavorable and strongly limit their use [72–74].

Recently, new immunosuppressant agents with a good safety profile have been introduced in clinical practice. Pimecrolimus (Elidel®, Novartis) is currently marketed as a topical treatment for atopic dermatitis. Pimecrolimus inhibits cytokine synthesis and release from Th cells, with a mechanisms that is similar to cyclosporine and tacrolimus [75]. A multicenter Phase II clinical trial of oral pimecrolimus for moderate-to-severe asthma is presently ongoing. In addition, IFNs are potent immunomodulators and typical Th1 cytokines. Despite this, there are few studies of IFNs in asthma and allergy. A recent article reported encouraging results obtained in severe asthma with low doses of IFN-α.

Suplatast tosilate (IPD®, Taiho Pharmaceutical Co. Ltd) is a new molecule, currently marketed in Japan that selectively inhibits the synthesis of IL-4 and IL-5 (Th2-type cytokines), as testified by the reduction of bronchial eosinophilic inflammation in allergic asthma [77]. There are a small number of controlled randomized studies of suplatast in clinical asthma confirming its clinical efficacy in improving symptoms, reducing the need for corticosteroids and bronchodilators and improving pulmonary function [78,79]. Of note, positive clinical results have also been recently reported in allergic rhinitis [80]. Therefore, suplatast tosilate seems to represent a real advance in the field of immunosuppressant agents with a good perspective for routine clinical use.

Adjuvants (usually represented by killed bacteria or microbial components) have been used since the dawn of immunology with the purpose of enhancing the immunogenicity of antigens. It is now clear that some adjuvants preferably potentiate the Th1 response and have therefore also been proposed in allergic diseases in order to restore the Th1/2 unbalance in favor of the Th1 response. Indeed there are few studies utilizing adjuvants alone in allergic disease, and the results were controversial [81,82]. Moreover, in one study, a high rate of adverse events was described [82]. Therefore, the use of adjuvants alone seems not to be a currently viable approach, whereas the association of adjuvants with immunotherapy is of course more promising.

Brief outline on the advances in allergen-specific immunotherapy

Allergen-specific immunotherapy (subcutaneous injection of increasing doses of allergen extract) is a unique immunological treatment and the only allergen-oriented therapy. At variance with other strategies, it modifies the natural history of the disease, can provide a long-lasting effect after discontinuation and redirects the Th1/2 balance. Since the mid-80's, due to the progress in basic science and immunology techniques, there was a dramatic change which involved the introduction in clinical practice of local routes of administration [83] and the adjuvanted allergens [84]. Moreover, new experimental approaches were established in order to maintain the immunogenicity of the vaccine without the capacity to bind allergen-specific IgE (improved safety). One of these approaches is the possible use of recombinant/engineered allergens, although no human studies are currently available. Another strategy is the use of allergen fragments or peptides. In this case, favorable results in humans with cat allergen peptides were recently published [85]. The most attractive and modern field of research in allergen-immunotherapy is the genetic approach, that includes the use of allergens conjugated with DNA immunostimulatory sequences. These short sequences acts as potent adjuvants and redirect the immune response toward the Th1 phenotype.

The first exploratory study performed in humans with DNA-conjugated ragweed allergen provided positive results and disclosed new horizons in the field of specific immunotherapy [86]. The genetic approach also includes the administration of cDNA plasmids encoding for a given allergen. However, reviewing the strategies for immunotherapy is not the purpose of the present article. Interesting and updated reviews on immunotherapy were recently published by Nelson [87] and Norman [88].

Expert opinion

Recent and progressive developments in our knowledge of the immunology of allergic inflammation have disclosed new therapeutic horizons. The complex interaction between genetic background and environmental factors has begun to be elucidated and an important field of research in this sense is that of the primary prevention, intended as the possible modalities to impede the establishment of the atopic phenotype. Some of these strategies claim to act in very early infancy or even during pregnancy. In addition, interest in allergen-specific immunotherapy has progressively increased, leading to the development of new modes of administration, modified allergen and genetic interventions. Indeed, the greatest efforts have been made in the field of drugs and immunological interventions with the aim of modulating or downregulating the inflammatory events once established. Some of these approaches, including new drugs and mAbs, have reached an advanced stage of development and some are ready for marketing or are already marketed. In parallel, the possible role in therapy of traditional associated drugs was investigated in various situations.

All of these experimental approaches require, in most cases, a relevant economic effort, often resulting in disappointing clinical results. Nevertheless, these negative aspects are largely counter-balanced by the opportunities that clinical research with experimental therapies offers for clarifying mechanisms, identifying new targets and better defining the role of traditional treatments.

Highlights

- Exploratory treatments involve: modified traditional drugs, antimediators, monoclonal antibodies (mAbs), immunosuppressants and engineered immunotherapy.
- Among modified steroids, Ciclesonide (Alvesco®, Altana AG) is the most advanced in research and most promising for clinical use.
- Anti-immunoglobulin E mAbs are effective in severe uncontrolled asthma.
- mAbs directed against single cytokines proved poorly effective in clinical disease.
- Allergen-specific immunotherapy is rapidly developing with adjuvants and DNA-based techniques.

Outlook

The more and more detailed knowledge of the allergic reaction has allowed the identification of new and specific therapeutic targets. New and modified drugs have been studied and proposed, some of them clinically effective. On the other hand, blocking single mediators by mAbs is not a good strategy due to the redundancy of mediators themselves.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Howard TD, Meyers DA, Bleecker ER.
• Mapping susceptibility genes for allergic diseases. *Chest* 123(Suppl. 3), S363–S368 (2003).
2. Peebles JR. Viral infections, atopy, and asthma: is there a causal relationship? *J. Allergy Clin. Immunol.* 113(Suppl. 1), S15–S18 (2004).
3. Van Bever HP. Early events in atopy. *Eur. J. Pediatr.* 161(10), 542–546. Epub 2002 Aug 15 (2002).
4. Bousquet J, Jacot W, Yssel H, Vignola AM, Humbert M. Epigenetic inheritance of fetal genes in allergic asthma. *Allergy* 59, 138–147 (2004).
•• **A comprehensive review of the data concerning the epigenetic hypothesis for the development of asthma since fetal life.**
5. Romagnani S. Immunologic influences on allergy and the TH1/TH2 balance. *J. Allergy Clin. Immunol.* 113, 395–400 (2004).
6. Wills Karp M, Gavett SH, Schofiels B, Finkelman F. Role of interleukin 4 in the development of allergic airway inflammation and airways hyperresponsiveness. *Adv. Exp. Med. Biol.* 409, 343–347 (1996).
7. Lalani T, Simmons RK, Ahmed AR. Biology of IL-5 in health and disease. *Ann. Allergy Asthma Immunol.* 82, 317–332 (1999).
8. Wyin TA. IL-13 effector functions. *Ann.*
• *Rev. Immunol.* 21, 425–456. Epub 2001 Dec 19 (2003).
9. Shevach EM. Certified professionals:
• $CD4^{(+)}CD25^{(+)}$ suppressor T-cells. *J. Exp. Med.* 193(11), F41–F46 (2001).
10. Koulis A, Robinson DS. The anti-inflammatory effects of interleukin-10 in allergic disease. *Clin. Exp. Allergy* 30, 747–750 (2000).
11. Reischl IG, Coward WR, Church MK. Molecular consequences of human mast cell activation following immunoglobulin E-high-affinity immunoglobulin E receptor (IgE-FcεRI) interaction. *Biochem. Pharmacol.* 58, 1841–1850 (1999).
12. Boyce JA. Mast cells: beyond IgE. *J. Allergy Clin. Immunol.* 111, 24–32 (2003).
13. Nagata M. The roles of cysteinyl leukotrienes in eosinophilic inflammation of asthmatic airways. *Int. Arch. Allergy Immunol.* 131(Suppl. 1), 7–10 (2003).
14. Kay AB Allergy and allergic diseases. Second
• of two parts. *N. Engl. J. Med.* 344, 109–113 (2001).
15. Bochner BS, Schleimer P. Mast cells,
• basophils, and eosinophils: distinct but overlapping pathways for recruitment. *Immunol. Rev.* 179, 5–15 (2001).

16. Storms WW. Minimal persistent inflammation, an emerging concept in the nature and treatment of allergic rhinitis: the possible role of leukotrienes. *Ann. Allergy Asthma Immunol.* 91, 131–140 (2003).
•• **An updated review on the concept of minimal persistent inflammation in allergy, and its possible pharmacologic modulation.**
17. Bousquet J, Jacot W, Vignola AM, Van Cauwenberge P. Allergic rhinitis: a disease remodeling the upper airways? *J. Allergy Clin. Immunol.* 113, 43–49 (2004).
•• **Deals with the characteristics of remodelling and addresses the difference and similarities of the phenomenon in rhinitis and asthma.**
18. Chiappara G, Gagliardo R, Siena A *et al*. Airway remodelling in the pathogenesis of asthma. *Curr. Opin. Allergy Clin. Immunol.* 1, 85–93 (2001).
19. Payne DN, Rogers AV, Adelroth E *et al.*
• Early thickening of the reticular basement membrane in children with difficult asthma. *Am. J. Respir. Crit. Care Med.* 167, 78–82 (2003).
20. Belvisi M, Hele D. Soft steroids: a new approach to the treatment of inflammatory airways diseases. *Pulm. Pharmacol. Ther.* 16, 321–325 (2003).
21. Belvisi MG, Wicks SL, Battram CH *et al*. Therapeutic benefit of a dissociated glucocorticoid and the relevance of in vitro separation of transrepression from transactivation activity. *J. Immunol.* 166, 1975–1982 (2001).
22. Schacke H, Schottelius A, Docke WD *et al.* Dissociation of transactivation from transrepression by a selective glucocorticoid receptor agonist leads to separation of therapeutic effects from side effects. *Proc. Natl Acad. Sci. USA* 101(1), 227–232 (2004).
23. Postma DS, Sevette C, Martinat Y, Schlosser N, Aumann J, Kafe H. Treatment of asthma by the inhaled corticosteroid ciclesonide given either in the morning or evening. *Eur. Respir. J.* 17, 1083–1088 (2001).
•• **First clinical trial in humans demonstrating the efficacy of the new steroid prodrug ciclesonide in asthma.**
24. Larsen BB, Nielsen LP, Engelstatter R, Steinijans V, Dahl R. Effect of ciclesonide on allergen challenge in subjects with bronchial asthma. *Allergy* 58, 207–212 (2003).
25. Schmidt BM, Timmer W, Georgens AC. The new topical steroid ciclesonide is effective in the treatment of allergic rhinitis. *J. Clin. Pharmacol.* 39, 1062–1069 (1999).
26. Weinbrenner A, Huneke D, Zschiesche M
• *et al.* Circadian rhythm of serum cortisol after repeated inhalation of the new topical steroid ciclesonide. *J. Clin. Endocrinol. Metab.* 87, 2160–2163 (2002).
27. Sturton G, Fitzgerald M Phosphodiesterase 4 inhibitors for the treatment of COPD. *Chest* 121(Suppl. 5), S192–S196 (2002).
28. Compton CH, Gubb J, Nieman R *et al.* Cilomilast, a selective phosphodiesterase-4 inhibitor for treatment of patients with chronic obstructive pulmonary disease: a randomised, dose-ranging study. *Lancet* 358, 265–270 (2001).
29. Roflumilast: APTA 2217, B9302–107, BY 217, BYK 20869. *Drugs* 5(3), 176–181 (2004).
30. Nathan RA. Do leukotriene receptor antagonists have a place in pharmacotherapy of allergic rhinitis? *Ann. Allergy Asthma Immunol*. 90, 466–468 (2003).
31. Wilson AM, O'Byrne PM, Parameswaran K. Leukotriene receptor antagonists for allergic rhinitis: a systematic review and meta-analysis. *Am. J. Med*. 116, 338–344 (2004).
•• **Comprehensive meta-analysis of all studies with leukotriene receptor antagonists in rhinitis, with a critical evaluation of the magnitude of their effect.**
32. Wilson AM, Orr LC, Sims EJ, Lipworth BJ. Effects of monotherapy with intra-nasal corticosteroid or combined oral histamine and leukotriene receptor antagonists in seasonal allergic rhinitis. *Clin. Exp. Allergy* 31, 61–68 (2001).
33. Nayak AS, Philip G, Lu S, Malice MP, Reiss TF. Montelukast Fall Rhinitis Investigator Group. Efficacy and tolerability of montelukast alone or in combination with loratadine in seasonal allergic rhinitis: a multicenter, randomized, double-blind, placebo-controlled trial performed in the fall. *Ann. Allergy Asthma Immunol.* 88(6), 592–600 (2002).
34. Di Lorenzo G, Pacor ML, Pellitteri ME *et al*. Randomized placebo-controlled trial comparing fluticasone aqueous nasal spray in monotherapy, fluticasone plus cetirizine, fluticasone plus monteleukast and cetirizine plus monteleukast for seasonal allergic rhinitis. *Clin. Exp. Allergy* 34, 259–267 (2004).
•• **Very interesting and well conducted controlled trial evaluating the relative efficacy of munerous combinations of drug therapies for allergic rhinitis.**
35. Passalacqua G, Ciprandi G, Pasquali M, Guerra L, Canonica GW. An update on the asthma-rhinitis link. *Curr. Opin. Allergy Clin. Immunol.* 4, 177–183 (2004).
36. Wilson AM, Orr LC, Sims EJ, Dempsey OJ, Lipworth BJ. Antiasthmatic effects of mediator blockade versus topical corticosteroids in allergic rhinitis and asthma. *Am. J. Respir. Crit. Care Med.* 162, 1297–1301 (2000).
37. Baena-Cagnani CE, Berger WE, DuBuske LM *et al*. Comparative effects of desloratadine versus montelukast on asthma symptoms and use of β 2-agonists in patients with seasonal allergic rhinitis and asthma. *Int. Arch. Allergy Immunol.* 130, 307–313 (2003).
38. Nelson HS. Prospects for antihistamines in
• the treatment of asthma. *J. Allergy Clin. Immunol.* 112(Suppl. 4), S96–S100 (2003).
39. Kraan J, Vink-Klooster H, Postma DS. The NK-2 receptor antagonist SR 48968C does not improve adenosine hyperresponsiveness and airway obstruction in allergic asthma. *Clin. Exp. Allergy* 31, 274–278 (2001).
40. Joos GF, Vincken W, Louis R *et al*. Dual tachykinin NK1/NK2 antagonist DNK333 inhibits neurokinin A-induced bronchoconstriction in asthma patients. *Eur. Respir. J.* 23, 76–81 (2004).
41. Evans DJ, Barnes PJ, Cluzel M, O'Connor BJ. Effects of a potent platelet-activating factor antagonist, SR27417A, on allergen-induced asthmatic responses. *Am. J. Respir. Crit. Care Med.* 156, 11–16 (1997).
42. Hansel TT, Kharitonov SA, Donnelly LE *et al*. A selective inhibitor of inducible nitric oxide synthase inhibits exhaled breath nitric oxide in healthy volunteers and asthmatics. *FASEB J*. 17, 1298–1300 (2003).
43. Chang TW, Davis FM, Sun NC *et al.* Monoclonal antibodies specific for human IgE producing cells: a potential therapeutic for IgE mediated diseases. *Biotechnology* 8, 122 (1990).
44. Corne J, Djukanovitch R Thomas L *et al*. The effect of inravenous administration of chimeric anti IgE antibody on serum IgE levels in atopic subjects. Efficacy, safety and pharmacokinetics. *J. Clin. Invest.* 99, 879–887 (1997).
45. D'Amato G. Therapy of allergic bronchial asthma with omalizumab – an anti-IgE monoclonal antibody. *Expert Opin. Biol. Ther.* 3, 371–376 (2003).
46. Walker S, Monteil M, Phelan K, Lasserson T, Walters E Anti-IgE for chronic asthma in adults and children. *Cochrane Database Syst. Rev. 3*, CD003559 (2004).
•• **Most recent and updated review on the clinical effects of omalizumab in asthma.**
47. Berger W, Gupta N, McAlary M, Fowler-Taylor A. Evaluation of long-term safety of the anti-IgE antibody, omalizumab, in children with allergic asthma. *Ann. Allergy Asthma Immunol.* 91(2), 182–188 (2003).

•• **Addresses the crucial issue of the long-term safety of anti-IgE therapy in children.**

48. Holgate S, Bousquet J, Wenzel S, Fox H, Liu J, Castellsague J. Efficacy of omalizumab, an anti-immunoglobulin E antibody, in patients with allergic asthma at high risk of serious asthma-related morbidity and mortality. *Curr. Med. Res. Opin.* 17, 233–240 (2001).
49. Corren JA, Casale T. Omalizumab, a recombinant humanized anti-IgE antibody, reduces asthma-related emergency room visits and hospitalizations in patients with allergic asthma. *J. Allergy Clin. Immunol.* 111, 87–90 (2003).
50. Leung DY, Sampson A, Yunginger Y *et al.* Effect of anti-IgE therapy in patients with peanut allergy. *N. Engl. J. Med.* 348, 986–993 Epub 2003 March 10 (2003).
51. Leynadier F, Doudou O, Gaouar H *et al*. Effect of omalizumab in health care workers with occupational latex allergy. *J. Allergy Clin. Immunol.* 113, 360–361 (2004).
52. Kuehr J, Brauburger J, Zielen S *et al.* Efficacy of combination treatment with anti-IgE plus specific immunotherapy in polysensitized children and adolescents with seasonal allergic rhinitis. *J. Allergy Clin. Immunol.* 109, 274–280 (2002).
53. Danzig M, Cuss F. Inhibition of interleukin 5 with a monoclonal antibody attenuates allergic inflammation. *Allergy* 52, 787–794 (1997).
54. Mauser P, Pitman AM, Fernandez X *et al.* Effects of an antibody to IL-5 in a monkey model of asthma. *Am. J. Respir. Crit. Care Med.* 152, 467–469 (1995).
55. Leckie MJ, ten Bincke A, Khan J *et al.* Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2144–2148 (2000).

•• **Fundamental clinical study evaluating the effects of anti-IL-5 in asthma showing that the reduction of eosinophils does not result in clinical improvement, thus questioning the real pathogenic role of eosinophils.**

56. Flood-Page PT, Robinson DS. Eosinophil's
• role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am. J. Respir. Crit. Care Med.* 167, 199–204 Epub 2002 Oct 17 (2003).
57. Kips JC, O Connor J, Langley SJ *et al.* Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am. J. Respir. Crit. Care Med.* 167, 1655–1659 Epub 2003 Mar 20 (2003).
58. Hart TK. Preclinical efficacy and safety of pascolizumab (SB 240683): a humanized anti-interleukin-4 antibody with therapeutic potential in asthma. *Clin. Exp. Immunol.* 130, 93–100 (2002).
59. Van der Pouw Kraan CT, Aalberse RC, Aarden LA. IgE production in atopic patients is not related to Il-4 production. *Clin. Exp. Immunol.* 97, 254–259 (1994).
60. Zhang X, Polla B, Hauser C, Zubler RH. T cells from atopic individuals produce IgE inducing acctivity which is incompletely blocked by anti IL-4 antibody. *Eur. J. Immunol.* 22, 829–833 (1992).
61. Borish LC, Nelson HS, Corren J *et al.*
• Efficacy of soluble IL-4 receptor for the treatment of adults with asthma. *J. Allergy Clin. Immunol.* 107, 963–970 (2001).
62. Kon OM, Kay AB. T cells and chronic asthma. *Int. Arch. Allergy Immunol.* 118, 133–135 (1999).
63. Burgelski PJ, Herzyk DJ, Rehma S *et al.* Preclinical development of keliximab, a primatized anti CD4 monoclonal antibody in human CD4 transgenic mice: characterizationof the model and safety studies. *Hum. Exp. Toxicol.* 19, 230–242 (2000).
64. Kon OM, Sihra BS, Compton CH, Leonard TB, Kay AB, Barnes N. Randomised dose-ranging placebo controlled study of chimeric antibody to CD4 (keliximab) in chronic severe asthma. *Lancet* 352, 1109–1113 (1998).
65. Jullien D, Prinz JC, Langley RG *et al*. T-cell modulation for the treatment of chronic plaque psoriasis with efalizumab (Raptiva): mechanisms of action. *Dermatology* 208, 297–306 (2004).
66. Gauvreau GM, Becker AB, Boulet LP *et al.* The effects of an anti-CD11a mAb, efalizumab, on allergen-induced airway responses and airway inflammation in subjects with atopic asthma. *J. Allergy Clin. Immunol.* 112, 331–338 (2003).

•• **First clinical randomized trial in humans assessing the efficacy and safety of the antiadhesion molecule strategy for therapy.**

67. Leonard P, Sur S. Interleukin-12: potential role in asthma therapy. *BioDrugs* 17(1), 1–7 (2003).
68. Bryan SA, O'Connor BJ, Matti S *et al.* Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2149–2153 (2000).

•• **Another important clinical trial, confirming that a reduction in blood and sputum eosinophils has a weak clinical outcome in asthma.**

69. Kim TS, DeKruyff RH, Rupper R, Maecker HT, Levy S, Umetsu DT. An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type 1-dominated immune response and inhibiting antigen-specific IgE production. *J. Immunol.* 158(9), 4137–4144 (1997).
70. Rosenwasser LJ, Busse WW, Lizambri RG,
• Olejnik TA, Totoritis MC. Allergic asthma and an anti-CD23 mAb (IDEC-152): results of a phase I, single-dose, dose-escalating clinical trial. *J. Allergy Clin. Immunol.* 112, 563–570 (2003).
71. Zhou Y, McLane M, Levitt RC. Th2 cytokines and asthma. Interleukin-9 as a therapeutic target for asthma. *Respir. Res.* 2, 80–84 (2001).
72. Kay AB, Barnes NC. Nonsteroidal anti inflammatory therapy for asthma. *Lancet* 351, 672 (1998).
73. Spector S. Alternative treatments in the
• patient with intractable asthma. *Curr. Opin. Pulm. Med.* 3, 23–29 (1997).
74. Hill JM, Tattersfield AE. Corticosteroid sparing agents in asthma. *Thorax* 50, 577–582 (1995).
75. Schops RE. Pimecrolimus. Novartis. *Curr. Opin. Investig. Drugs* 3, 720–724 (2002).
76. Simon HU, Seelbach H, Ehmann R, Schmitz M. Clinical and immunological effects of low-dose IFN-α treatment in patients with corticosteroid-resistant asthma. *Allergy* 58, 1250–1255 (2003).
77. Sano Y, Suzuki N, Yamada H *et al.* Effects of suplatast tosilate on allergic eosinophilic airway inflammation in patients with mild asthma. *J. Allergy Clin. Immunol.* 111, 958–966 (2003).
78. Tamaoki J, Kondo M, Sakai N *et al*. Effect of suplatast tosilate, a Th2 cytokine inhibitor, on steroid-dependent asthma: a double-blind randomised study. Tokyo Joshi-Idai Asthma Research Group. *Lancet* 356, 273–278 (2000).

•• **Provided for the first time evidence of the clinical efficacy of the Th2-suppressant, suplatast.**

79. Sano T, Nakamura Y, Yanagawa H *et al.* Add-on effects of suplatast tosilate in bronchial asthma patients treated with inhaled corticosteroids. *Lung* 181, 227–235 (2003).
80. Furukido K, Takeno S, Ueda T, Hirakawa K, Yajin K. Suppression of the Th2 pathway by suplatast tosilate in patients with perennial nasal allergies. *Am. J. Rhinol.* 16, 329–336 (2002).

81. Awkwright PD. Intradermal administration of a killed Mycobacterium vaccae suspension (SRL 172) is associated with improvement in atopic dermatitis in children with moderate-to-severe disease. *J. Allergy Clin. Immunol.* 107, 531–534 (2001).
82. Shirtcliffe PM, Easthope SE, Weatherall M, Beasley R. Effect of repeated intradermal injections of heat-inactivated Mycobacterium bovis bacillus Calmette–Guérin in adult asthma. *Clin. Exp. Allergy* 34(2), 207–212 (2004).
83. Passalacqua G, Guerra L, Pasquali M, Lombardi C, Canonica GW. Efficacy and safety of sublingual immunotherapy. *Ann. Allergy Asthma Immunol.* 93, 3–12 (2004).
84. Drachenber KJ, Wheeler A, Stubner P, Horak F. A well tolerated grass pollen specific allergy vaccine containing a novel adjuvant MPL reduces allergy symptoms after only four preseasonal injections. *Allergy* 56, 498–505 (2001).
85. Oldfield WL, Larchè M, Kay AB. A double blind placebo controlled study of short-peptides derived from Fel d 1 in cat allergic subjects. *Lancet* 360, 47–53 (2002).
86. Simons FE, Shikishima Y, Van Nest G, Eiden JJ, HayGlass KT. Selective immune redirection in humans with ragweed allergy by injecting Amb a 1 linked to immunostimulatory DNA. *J. Allergy Clin. Immunol.* 113, 1144–1151 (2004).
•• **The first study conducted in humans with a DNA-based specific immunotherapy.**
87. Nelson H. Advances in upper airways
• diseases and allergen immunotherapy. *J. Allergy Clin. Immunol.* 113, 635–642 (2004).
88. Norman PS. Immunotherapy 1999–2004. *J. Allergy Clin. Immunol.* 113, 1013–1023 (2004).
•• **General overview on immunotherapy for asthma and rhinitis and its mechanism of action, with an expanded section on the novel approaches.**

Affiliation

• *Giovanni Passalacqua MD,*
Allergy & Respiratory Diseases,
Dept of Internal Medicine,
Padiglione Maragliano,
L.go R. Benzi 10,
16132 Genoa, Italy
Tel.: +39 10 353 8908
Fax: +39 10 353 8904
passalacqua@unige.it