ANTI-IGE AND OTHER NEW IMMUNOMODULATION-BASED THERAPIES FOR ALLERGIC ASTHMA

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ABSTRACT

Understanding of the cellular and molecular mechanisms in asthma has lead to the recognition of a number of potential therapeutic targets, a few of which have been evaluated in clinical studies. Parenteral administrations of both anti-IL-5 and IL-12 inhibit eosinophil recruitment to the airways, but display a lack of clinical efficacy. Interrupting the IL-4 pathway thus far has also shown disappointing results in clinical studies. Omalizumab is the first anti-IgE monoclonal antibody developed for the treatment of moderate to severe asthmatics to receive FDA approval. In a number of clinical trials treatment with omalizumab was associated with moderate improvements in a number of relevant endpoints, including the rate of occurrence of disease exacerbations. Newer DNA-based therapeutic strategies including DNA vaccination and the antisense oligonucleotides show promise but thus far have only been tested in animal models.

KEYWORDS

Anti-IgE, asthma, immunomodulation

Allergic asthma is now considered to be a complex syndrome rather than a single disease entity. Its major clinical characteristics include a variable degree of airway obstruction and bronchial hyper-responsiveness (BHR): the capacity to react with an (increase in) airway obstruction in response to a variety of nonspecific stimuli, such as cold air and exercise. In addition, the majority of patients have elevated serum levels of IgE, as well as specific IgE antibodies to common environmental allergens, such as house dust mite and animal dander.

In clinical studies substantial inflammation has been found in bronchial biopsy specimens from patients with asthma, even in those with mild disease. Current therapy therefore emphasises suppression of this airway inflammation by the regular use of inhaled corticosteroids (ICS), which provides reasonable efficacy for patients with mild asthma. In this group, as well as in patients with more severe disease, there is an obvious need to make existing therapies readily available and to educate patients as to how and why the medication must be taken on a regular basis. However, even when given appropriately, existing therapies do not completely solve the clinical problems of subjects with moderate and severe asthma. These patients still experience significant residual symptoms and are sometimes subject to frequent exacerbations of their disease associated with consumption of healthcare resources and poor quality of life, despite the use of higher doses of inhaled corticosteroids as well as adjunctive therapy, such as long-acting β2-agonists and antileukotrienes. The prevalence of asthma in Western Europe has doubled in the last decade, leading to an estimated prevalence in the adult population of 10 to 15%. It is estimated that about 5% of these patients have poorly controlled asthma despite the use of maximally recommended doses of inhaled therapy and an additional number of patients can only be managed well at the cost of the use of high doses of ICS. The latter is relevant in view of concerns about systemic side effects such as cataract, osteoporosis and skin atrophy as a consequence of the long-term use of higher doses of inhaled corticosteroids. So there are a substantial number of patients who may benefit from novel therapies designed to target specific mechanisms underlying airway inflammation in asthma.
The chronic inflammatory reaction in the airway walls of asthmatics is dominated by eosinophilic and neutrophilic granulocytes and T helper lymphocytes as well as mast cells. Dendritic cells appear to be the key cells for antigen presentation in asthmatic airways. Following antigen stimulation, naïve T helper precursor T cells acquire a restricted capacity for cytokine production. Those producing predominantly interleukin (IL)-4, IL-13 and IL-5 have been termed Th2 cells and those producing predominantly interferon-γ T cells (figure 1). Microbes are probably the chief stimuli of “protective” T cells to produce IL-12, which in turn stimulates T helper lymphocytes. IL-4 is required for the production of Th2 cells and together with IL-13 regulates IgE production. IL-5 is the major Th2 cytokine involved in the accumulation of eosinophils in allergic inflammation (figure 2). The inflammatory process is accompanied by, and probably the driving force behind, structural changes in the airway wall, generally referred to as airway remodelling.

![Diagram showing factors involved in the balance between lymphocytes with a predominantly Th1- or Th2-like profile of cytokine excretion.](image)

**Figure 1**
*Factors involved in the balance between lymphocytes with a predominantly Th1- or Th2-like profile of cytokine excretion.*

Within this concept immunostimulatory sequence oligodeoxynucleotide (ISS-ODN)-based therapies and CpG-DNA provide a signal which leads to the increased production of IL-12 by antigen presenting cells (APC), which in its turn shifts the Th1/Th2 balance in a Th1-like direction. Under the influence of IL-4 naive Th0 cells differentiate in a Th2-like direction. Interleukin 2 and interferon-γ are important effectors cytokines of Th lymphocytes; IL-4, IL-13, IL-5 and IL-10 of Th2 lymphocytes. Treg = regulatory T cells.

These structural changes are the result of the interaction of inflammatory mediators and resident cells as well as of plain tissue injury. Structural cells in the airways and the matrix respond to the inflammation in an apparently coordinated fashion, which can be viewed as an attempt to repair the damage in an effort to keep the airway intact. The net result is an increase in airway wall thickness, to which all the tissue elements can contribute, which leads to a reduction in airway luminal diameter. Remodelling and inflammation result in, or at least contribute to, airway hyper-responsiveness which together with the reduced diameter of the airways cause the (periodic) breathlessness and wheezing that are so characteristic of clinical asthma. There is at least doubt as to whether ICS are able to prevent or reverse the process of airway wall remodelling. The dominant mechanism through which IgE determines the expression of atopy is through its binding to high-affinity receptors (FcεRI) expressed on the surface of tissue mast cells and basophils. During an immediate hypersensitivity reaction cross linkage of IgE with allergen results in the release of an array of preformed and newly generated mediators of inflammation, which are responsible for the early asthmatic response (EAR) (figure 2). During late-phase allergic reactions (LAR) eosinophils and neutrophils accumulate, followed by CD4+ T cells and basophils. The view that microbial stimuli may skew the Th1/Th2 balance forms the basis of the so-called hygiene hypothesis. According to present insights, the development of asthma is the result of a complex interaction of a genetic predisposition and environmental factors. Asthma is strongly associated with atopy and both have shown a remarkable increase in prevalence during the last 30 to 40 years. According to the hygiene hypothesis, this rise is related to the adoption of a Western lifestyle in which the human immune system is deprived of microbial encounters. This would then lead to a lack of stimulation in a Th1-like direction, which results in a shift of the Th1/Th2 balance in a Th2 dominated direction. A subpopulation of suppressive T cells, termed regulatory T cells, recently (re)gained attention since these appear to play a key role in the maintenance of immunological balance. In animal models and *in vitro*, regulatory T cells prevent the development of autoimmunity by inducing tolerance to (Th1) self antigens, but also appear to be able to suppress allergen-induced activation of Th2 cells. It seems that part of the suppressive activity is mediated via direct cell-cell interactions and part via cytokines such as IL-10 and TGF-β, but much is still to be elucidated about the precise mode(s) of action of these regulatory T cells.

The understanding of the cellular and molecular mechanisms of the allergen-induced and Th2 lymphocyte driven inflammatory process in asthmatic airways has lead to the recognition of a number of potential therapeutic targets, a few of which have been evaluated in clinical studies.
INTERLEUKIN 12

IL-12 is considered to be a key cytokine involved in regulating the balance between Th1 and Th2 cells (figure 1). Furthermore, IL-12 inhibits airway hyper-responsiveness and airway eosinophilia after antigen challenge in several animal models for allergen sensitisation.4-7 In patients with mild asthma weekly infusions of human recombinant IL-12 in escalating doses over a four-week period caused a progressive fall in peripheral blood eosinophil numbers, a reduction in the rise in circulating eosinophils after allergen challenge and a concomitant reduction in eosinophils in induced sputum.8 However, this was not accompanied by a suppression of the late asthmatic airway reaction nor an increase in bronchial hyper-reactivity after allergen challenge. Furthermore, due to the IL-12 infusions most of the patients suffered from malaise and one had an episode of cardiac arrhythmia. These side effects in combination with lack of clinical efficacy suggest that IL-12 is not a suitable therapy for patients with established asthma. In a mouse model administration of an IL-12-allergen fusion protein remarkably resulted in the development of a specific Th1 response to the allergen, with increased production of allergen-specific IgG2, instead of a Th2 response with IgE formation.9 This indicates the possibility of (local) administration of IL-12 as an adjuvant in conjunction with specific allergens to provide a more effective form of immunotherapy, which may even be preventive or curative when given early in the course of atopic disease, in early childhood for instance.

ANTI-IL-5

Eosinophilic infiltration is a characteristic feature of asthma, and based upon its biological properties this cell has assumed a position as the principal inflammatory cell in asthma. The eosinophil is a rich source of inflammatory proteins, such as major basic protein, which can damage...
airway epithelium and increase bronchial hyper-reactivity. IL-5 came forward as a key target in the Th2 cascade in mouse models for asthma using allergen sensitisation and challenge that were extrapolated to the delayed allergen response in humans. In a monkey model with ascariis-induced asthma a monoclonal antibody to IL-5 almost completely abolished eosinophilia and airway hyper-reactivity. An initial study evaluated the effect of a high-affinity humanised monoclonal anti-IL-5 antibody on the airway responses to allergen challenge in patients with mild asthma. Although the usual increase in blood and sputum eosinophils to allergen challenge was nearly abolished by anti-IL-5 treatment, it did not affect either the EAR or the expected post-allergen-challenge increase in BHR. In a follow-up study, anti-IL-5 treatment nearly ablated circulating and bronchial lavage fluid eosinophils, but in contrast in mucosal biopsies the reduction of eosinophils was only about 50%, and the deposition of major basic protein was unaffected. The different susceptibility for anti-IL-5 treatment of eosinophils in different biological compartments may explain why airway functional outcomes appear to be resistant to this therapy. The biology of eosinophil recruitment and activation in the airways appears to be more complex than traditionally viewed, which may explain the absence of significant clinical efficacy of anti-IL-5, also in patients with more severe asthma. There is evidence to support in situ eosinophilopoiesis in asthmatic airways. However, the number of the eosinophil progenitor cells is hardly if at all decreased by anti-IL-5 therapy. Furthermore, the role of IL-5 may be limited to the recruitment of mature eosinophils from the bone marrow, since airway eosinophils express reduced numbers of surface IL-5 receptors relative to their circulating counterparts; the decrease in IL-5 receptors is associated with a loss in capacity to degranulate to IL-5.

Eosinophil recruitment to the airways and the local biological activity almost certainly requires a coordinated effort of IL-5 and other cytokines and chemokines such as eotaxin. As stated earlier, asthma is probably a complex syndrome rather than a single disease. Therefore, anti-IL-5 therapy may only benefit those patients in whom eosinophilic inflammation contributes significantly to the signs and symptoms of disease. It may be important to design studies that allow identification of such patients. It is noteworthy that variability in the response to medication is characteristic in asthma. In a study in mild to moderate asthmatics, one third of patients did not respond to therapy with the leukotriene receptor antagonist montelukast. Furthermore, it may be worthwhile to look for treatment regimens that lead to more profound reductions of numbers of eosinophils and their biological activity in the airways. Since corticosteroids reduce eosinophil numbers in the airways by apoptosis, such reduction may be achieved by the administration of high doses of systemic corticosteroids followed by anti-IL-5 as a kind of maintenance treatment to prevent repopulation of the airways with eosinophils. Support for such an approach can be found in a recent study showing significant reductions in airway eosinophils by high-dose systemic corticosteroids in severe asthmatics.

**INTERRUPTING THE IL-4 PATHWAY**

In view of its role in Th2 cell development, neutralisation of IL-4 is likely to be most effective at the time of initial allergen encounter. However the contribution of IL-4 in the effector phase of immunity and the theoretical role in promoting ongoing Th2 cell commitment during established inflammation provide a rational basis for interrupting IL-4 signalling in asthma. Treatment with soluble recombinant IL-4 receptor (to compete with the endogenous ligand) showed encouraging results in an initial placebo-controlled trial in atopic asthma. A single nebulised dose of soluble IL-4 receptor prevented the fall in lung function induced by the withdrawal of inhaled corticosteroids in patients with moderately severe asthma and sustained asthma control was obtained by weekly nebulisations over a 12-week period. However, the results of an as yet unpublished large-scale trial indicate that the agent has no clinical efficacy in asthma. This negative result for IL-4 blockade does not preclude that other approaches aiming at the inhibition of the IL-4 pathway, such as interrupting downstream IL-4 receptor signalling by targeting transcription factors such as Stat6 and GATA-3 may be more successful.

**ANTI-IGE**

Preclinical studies have convincingly shown that anti-IgE antibodies are able to abrogate IgE-mediated responses in allergic disease. Omalizumab is a humanised recombinant monoclonal anti-IgE antibody that selectively binds to the Ce3 domain of free IgE at the FcεI receptor binding site. It is very important that these antibodies bind to the FcεI receptor binding part of IgE, to prevent cross-linking of mast cell bound IgE and subsequent anaphylactic reactions. Omalizumab appears to be safe and well-tolerated during long-term administration to adults, adolescents and children with asthma. In patients with allergic asthma, omalizumab results in a rapid decline in free serum IgE levels in a dose-dependent manner. In addition, FcεI receptor expression on basophils dramatically decreases. In clinical trials of a six-month duration, treatment with subcutaneously administered omalizumab...
resulted in reductions in serum IgE levels of 90% or more. Short-term treatment of asthmatic patients with omalizumab inhibited the EAR and LAR after allergen challenge, as well as the accompanying increases in airway eosinophilia and BHR. These observations suggest anti-inflammatory effects of maintenance therapy with omalizumab.

In a number of well-designed clinical studies, the efficacy of omalizumab as add-on with ICS has been evaluated in patients with moderate to severe allergic asthma. In these studies patients typically received omalizumab subcutaneously every two or four weeks. The results showed improvements in symptom scores, reduced use of rescue \( \beta_2 \)-agonists and improvements in lung function. Furthermore, significant reductions in exacerbation rates were observed in adults and adolescents (Table 1).

<table>
<thead>
<tr>
<th>STUDY</th>
<th>AGE GROUP</th>
<th>ASTHMA SEVERITY</th>
<th>REDUCTION IN ICS DOSE VS PLACEBO</th>
<th>REDUCTION IN ORAL CS DOSE VS PLACEBO</th>
<th>REDUCTION IN EXACERBATIONS VS PLACEBO*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milgrom28</td>
<td>12-18?</td>
<td>Moderate-severe</td>
<td>33%</td>
<td>NS, 85%</td>
<td></td>
</tr>
<tr>
<td>Milgrom27</td>
<td>11-50</td>
<td>Moderate-severe</td>
<td>60%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soler29</td>
<td>12-75</td>
<td>Moderate-severe</td>
<td>33%</td>
<td>58%, 52%</td>
<td></td>
</tr>
<tr>
<td>Busse30</td>
<td>12-75</td>
<td>Severe</td>
<td>25%</td>
<td>48%, 41%</td>
<td></td>
</tr>
</tbody>
</table>

*Reduction during stable corticosteroid phase and corticosteroid reduction phase, respectively. ICS = (inhaled) corticosteroids.

The therapeutic goal of allergen immunotherapy is the induction of protective immunity to an allergen to which a clinical hypersensitivity pre-exists, by inducing a change in the allergen-specific adaptive immune response. Desensitisation can be achieved by traditional protein-based immunotherapy. However, allergen immunotherapy requires multiple injections, takes at least several months to achieve a therapeutic effect, is generally less effective than pharmaceutical therapeutics for the treatment of allergic airway symptoms, and has associated risks, such as potentially life-threatening anaphylaxis. Although it is has been shown that effective therapy is associated with an increase in the level of specific IgG and a shift from Th2 to Th2 responses, precise information on what immunological mechanisms lead to a reduction in allergic effector functions is lacking. Promising results have been achieved in patients suffering from allergic rhinitis or allergies to bee and wasp venom but there is a need for more effective strategies to prevent and reverse the Th2-biased immune deviation that drives the pathogenesis of allergic airway conditions. In this respect regulatory T cells are an emerging target for immunomodulation.

In a DNA vaccine (Figure 3) the gene for the antigen of interest is cloned into a bacterial plasmid that is engineered to augment the expression of the inserted gene in mammalian cells. After being injected, the plasmid enters the host cell, where it stays in the nucleus but is not integrated into the host's DNA. Using the host cell protein-synthesising 'machinery', the plasmid DNA directs the synthesis of the antigen it encodes. This approach involving the synthesis of antigen within host cells has potential advantages over immunisation with exogenous (recombinant) proteins. A protein produced by transfected cells is more likely to be folded in its native configurations, which favours the production of effectively neutralising antibodies. Furthermore, in the course of
evaluating the immunogenicity of various gene vaccination vectors, it was discovered that effective vaccination was dependent upon the presence of immunostimulatory DNA sequences - unmethylated cytidine phosphate guanosine (CpG) motifs - within the plasmid, which provide adjuvant Th1 immunity for the responses that develop towards the gene product. These unmethylated CpG motifs, which are more highly present in microbial DNA, are recognised as foreign by the innate immune system via Toll-like receptor 9.

Several ISS-ODN-based therapeutic strategies were evaluated and proved to be effective for the prevention and reversal of Th2-mediated models of allergic disease in animals (for review see Horner & Raz 2002). Vaccination with allergen mixed with ISS-ODN proved more effective than vaccination with allergen alone in the induction of Th1-biased and the reversal of Th2-biased immune responses. In their turn, allergens physically conjugated to ISS-ODN have shown improved immunogenicity and reduced allergenicity compared with allergen-ISS-ODN mixtures. In animal models, ISS-ODN conjugate vaccine induced antigen-specific Th1-biased immune responses that were maintained for at least one year.

Preliminary clinical data in patients with ragweed-sensitive allergic rhinitis show that allergen-ISS-ODN conjugate vaccination is feasible and well tolerated.

**Antisense oligonucleotides (ASONs)**

Antisense oligonucleotides (ASONs) are short oligonucleotides, modified to slow degradation, which code for peptides that match the non-coding strand of DNA. When introduced into a cell they inhibit protein synthesis by binding RNA in a sequence-specific manner and blocking translation. The advantages of the antisense technology include its high specificity, ability to be delivered locally to the lung (respirable antisense oligonucleotides (RASONs)) avoiding systemic side effects, and relative low production cost. The main disadvantages are the tedious work to find the appropriate antisense and evaluate its specificity and effectiveness, and the generation of anti-DNA antibodies.

A human antisense against the adenosine receptor has been tested in a rabbit model for allergic inflammation. Adenosine A1 receptor binding activity as well as receptor numbers were significantly (75%) and specifically reduced by administration of the antisense by direct inhalation of the nebulised material. In physiological terms the RASON reduced the BHR and the EAR in rabbits sensitised to house dust mite. Recently an antisense oligodeoxynucleotide for IL-4 was found to inhibit allergic inflammation ex vivo in nasal mucosal biopsies of ragweed allergic rhinitis patients. These data support the concept of treating allergic airway diseases by local administration of antisense oligonucleotides.

**CONCLUSIONS**

Although it may be too early to draw firm conclusions, the rather limited beneficial effects in clinical studies interrupting the IL-4 and IL-5 pathways at least challenge the Th2 paradigm in human asthma. However, even if these observations are supported by further studies, this would not be the definite proof that these cytokines are not important in the pathogenesis of asthma. Blockade of Th2-mediated or other pathways relevant in asthma will not necessarily lead to a rapid reversal of physiologically relevant abnormalities that were in fact initially the result of it. Therefore, lack of a rapid meaningful clinical effect cannot be taken as evidence that a specific cytokine is not involved in asthma pathogenesis, but rather might be taken as an indication that therapy must be started much earlier in the course of the disease.

In the majority of patients with persistent asthma the disease can be well controlled by the currently available
inhaled anti-inflammatory (ICS) and bronchodilator agents. The new recombinant DNA-based high-molecular-weight proteins such as anti-IL-5 and anti-IgE are expensive drugs that need parenteral administration at least partly under medical supervision. None of the above-mentioned treatment modalities are likely to lead to a cure of asthma. Additive value above existing therapies must be based on a better control of asthma, reduced need for the use if inhaled therapy or the induction of long-term disease remissions with infrequent (parenteral) administration.

Studies showing that omalizumab can be an effective and well-tolerated add-on therapy for moderate to severe patients with allergic asthma already on ICS have formed the basis of the recently obtained FDA approval. Procedures to allow omalizumab on the European market are ongoing, but it is to be foreseen that in this license the emphasis will be on more severe asthmatics.

REFERENCES


