

Inflammation versus Immunoregulation: What Is the Key to the Development of an Effective Antiallergy Vaccine?

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Key Words

Th1 cells · Th2 cells · T regulatory cells · Asthma ·
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Abstract

The incidence and severity of allergic disorders is currently increasing worldwide. The reason for this development is unclear. However, epidemiological studies and experimental data suggest that this increase may be due to a decline in infectious diseases which induce T helper type 1 (Th1) and/or T regulatory (Tr) responses. Based on these observations, antiallergy vaccines are currently being tested in animal studies aimed at inducing Th1 or Tr responses. This review focuses on novel approaches potentially leading to a vaccine protecting humans from the development of allergic disorders.

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Introduction

The CD4+ T helper (Th) cell is the most important cell type controlling adoptive immune responses. Th cells can generally be divided into two major subsets (Th1 and Th2) based on the types of cytokines produced. Th1 cells secrete predominantly IFN- γ and IL-2, whereas Th2 cells produce IL-4, -5, -9, -10 and -13. The major cytokines

governing the generation of Th1 versus Th2 immune responses are IFN- γ , IL-4 and IL-12 [1]. Interaction of IL-12 with its receptor activates Stat4 and induces Th1 cell development. Type 1 responses are commonly induced during infections with intracellular pathogens such as bacteria, protozoa or viruses. They are characterized by the production of a number of cytokines, including IL-2, TNF- α , IFN- γ , IL-12 and IL-18, and increased IgG2a antibody titers.

Type 2 responses predominate during helminth infections and allergic disorders including atopy. The current view is that under the influence of IL-4, naive T cells activated by antigen-presenting cells (APC) differentiate into Th2 effector cells. IL-6, most likely secreted by the APC, induces the initial IL-4 production by the naive CD4+ T cell necessary to drive Th2 cell development via Stat6-mediated signaling [2]. Effector Th2 cells produce IL-4, IL-5 and IL-13. These cytokines induce the production of allergen-specific IgE by B cells, the development and recruitment of eosinophils, mucus production and airway smooth muscle contraction. Degranulation of eosinophils and mast cells (via IgE cross-linking) are two major factors leading to chronic asthma [3, 4]. Moreover, recent studies also indicate that chemokines such as eotaxin, RANTES, monocyte chemoattractant protein (MCP)-1 and MCP-5 and neurotrophins such as NGF and BDNF may also be critically involved in the etiology of asthma [5]. Importantly, although Th2 cells are responsible for the develop-

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ment of allergic diseases, Th1 cells contribute to chronic disease.

A third CD4⁺ Th population has been discovered recently. These cells are called regulatory T (Tr) cells [6, 7]. Some secrete anti-inflammatory and immunosuppressive cytokines (TGF- β and/or IL-10) after antigen-specific activation leading to the suppression (and thus regulation) of both Th1 and Th2 cell-mediated responses. Furthermore, other Tr subpopulations (CD4⁺CD25⁺ cells) inhibit Th1 and Th2 cell proliferation in an antigen-specific and possibly more importantly also in an antigen-unspecific manner through cell-cell interaction. How Tr cells inhibit the proliferation of T cells remains unclear but appears to be associated with the suppression of autocrine IL-2 production by the Th1 or Th2 cells needed for cell proliferation. Although it is not completely understood how Tr cells develop, IL-10 has been implicated as a differentiation factor for Tr cells.

The incidence and severity of allergic disorders are increasing at an alarming rate. For these reasons, it is important to develop novel and effective prevention measures. Recent studies have shown that it is possible to vaccinate animals to protect them from the development of allergic disorders, in particular asthma, by inducing proinflammatory reactions leading to the production of IFN- γ , IL-12 and IL-18, which inhibit the development of allergen-specific Th2 cells. Recent publications have shown that besides Th1 cells, Tr cells are also able to inhibit the development of allergic Th2 responses. This finding has led to the suggestion that future antiallergy vaccines should aim at inducing Tr and not Th1 responses, since presumably the generation of Tr cells harbors fewer potential negative side effects than the generation of Th1 cells. This review will address the question of which approach seems to be more suitable for the design of novel antiallergy vaccines.

Inhibition of Allergen-Specific Th2 Responses by the Induction of Th1 Responses

Th1 responses inducing the production of IFN- γ , IL-12 and IL-18 are associated with the inhibition of Th2 cell development and effector functions both in vivo and in vitro [8, 9]. Furthermore, it appears that non-atopic-prone children show increased Th1 responses in early childhood and lose the neonatal bias of the immune system towards Th2 responses more rapidly than atopic-prone children. Epidemiological and experimental studies demonstrating an inverse correlation between the devel-

opment of allergic disorders and the incidence of infection support this view [9]. Currently, it is not clear if allergen-specific Th1 cells or the general downmodulation of Th2 responses by Th1 responses are responsible for the inhibitory effect on the development of allergic Th2 responses. For this reason, two separate approaches to an antiallergy vaccine using Th1 responses may be successful. Young children could be vaccinated with reagents inducing strong Th1 responses, leading to a general decrease in the tendency to develop allergic Th2 responses and resulting in the development of allergen-specific Th1 cells after the exposure to allergen. Alternatively, vaccines could be used which directly induce the development of allergen-specific Th1 cells. The secretion of IFN- γ by Th1 cells during the encounter with allergen is then believed to be sufficient to suppress allergen-specific Th2 cell development or effector functions later in life.

The Use of CpG-Oligodeoxynucleotides

CpG-oligodeoxynucleotides (ODN) are part of the noncoding sequences of microbial DNA and directly induce strongly Th1-biased immune responses in vitro and in vivo by inducing the secretion of IL-12 and IL-18 by macrophages and dendritic cells (DC) and IFN- γ by NK cells [10]. Animal studies have shown that the application of CpG-ODN leads to the suppression of allergen-induced asthma in mice [11–13]. This effect was clearly associated with a shift from allergen-specific Th2 towards allergen-specific Th1 responses characterized by increased IFN- γ and IgG2a levels, suggesting that the induction of Th1 responses was responsible for the inhibitory effect. The finding that CpG-ODN-mediated suppression of allergic Th2 responses was not observed in IFN- γ -deficient mice supports this view [13]. Furthermore, it has also been reported that the inhibition of allergen-induced airway hyperresponsiveness (AHR) and airway eosinophilia was only observed when CpG-ODN were given in combination with allergen, and not alone [14], suggesting that allergen-specific immune responses were responsible for the inhibition. It was also shown that allergen directly conjugated to CpG-ODN can be used at a 100-fold lower concentration than a mixture of CpG-ODN and allergen to achieve similar suppressive effects on allergen-induced airway eosinophilia and Th2 cell differentiation in vivo [15]. Tighe et al. [16] reported a similar finding. Furthermore, recently, Santeliz et al. [17] found that intradermal applications of Amb a 1 (a major antigen of ragweed pollen) conjugated to CpG also resulted in the reduction of already established allergen-induced eosinophilia and allergen-induced AHR in mice. This effect correlated with

increased IFN- γ secretion by the spleen cells after antigen stimulation and a shift in the antibody profile towards IgG2a. It was recently found that antigen-CpG-ODN conjugates cause increased antigen uptake and DC activation. Antigen and CpG are taken up by the same DC, which results in increased CD40 and CD86 expression and high levels of IL-12 expression, promoting the development of IFN- γ -secreting Th1 cells [18]. This may explain why much less CpG-ODN conjugate can be used than CpG-ODN alone or in combination with allergen to induce the suppression of Th2 responses.

Using Bacteria or Bacterial Products to Induce Th1 Responses

Because mycobacteria induce profound Th1 responses, they or their products may therefore be used as vaccines or components of vaccines aimed at inhibiting Th2 responses. The findings that the application of live or heat-killed *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) into the lung of mice strongly suppressed the development of airway eosinophilia, with the effect lasting up to 2 months after the application of the mycobacteria, support this view [19, 20]. The inhibitory effect was clearly dependent upon IFN- γ . Furthermore, the application of BCG in guinea pigs led to the generation of allergen-specific Th1 responses and the inhibition of AHR and allergen-induced airway eosinophilia [21, 22]. *Mycobacterium vaccae* also induces protection against the development of allergic Th2 responses. It was reported that intranasal administration of *M. vaccae* and BCG could also reduce established allergen-induced Th2 responses, with BCG being more effective than *M. vaccae* [23]. Treatment with BCG but not *M. vaccae* led to increased IFN- γ levels in the bronchoalveolar lavage (BAL) fluid of the mice. Furthermore, Wang and Rook [24] reported that *M. vaccae*-induced reduction of established allergen-specific IgE responses was also not associated with increased Th1 responses. Taken together, these findings suggest that BCG- but not *M. vaccae*-induced suppression of allergic responses is mediated by Th1 cells. It has been suggested that *M. vaccae* mediates suppression of Th2 responses by the induction of Tr cells [25] (see below). However, recently it was found that the application of recombinant *M. vaccae* expressing the house dust mite antigen Der p I into mice resulted in a reduction of Th2 responses correlating with increased Der p I-specific Th1 responses [26].

Other bacteria besides mycobacteria have also been shown to suppress the development of allergic responses. For example, the application of killed *Listeria monocytogenes* or *Lactobacillus plantarum* suppressed the induc-

tion of allergen-specific IgE in mice [27, 28]. The *Listeria*-induced suppression was associated with increased Th1 responses.

A further adjuvant of a vaccine protecting against allergic disorders could be gram-negative bacteria-derived lipopolysaccharide (LPS). Animal studies have shown that allergen-induced AHR was suppressed by the prior intranasal administration of LPS [29, 30]. These results suggest that applying LPS to humans may also help to reduce the development of allergic disorders. Supporting this view are the results of epidemiological studies indicating that children growing up on farms develop less allergies in their lifetime because they have been continuously exposed to LPS [31, 32]. The potential problems with using LPS, in particular toxic shock, may be overcome more easily by using a detoxified derivative of LPS, for example monophosphoryl lipid A. It has been demonstrated that immunization of mice with allergen [ovalbumin (OVA) or ragweed pollen extract] in combination with monophosphoryl lipid A resulted in allergen-specific Th1 responses and reduction of Th2 responses [33].

Efficient DNA Vaccination Is Associated with the Induction of Th1 Responses

DNA vaccination induces strong and long-lasting Th1 responses [34]. This immunization method is based on intradermal or intramuscular injections of plasmid (p)DNA encoding a defined antigen. After uptake, the transfected cells produce antigen continuously. This leads to a long-lasting Th1 memory response. The reason why DNA vaccination induces Th1-biased responses is due to the CpG motifs in the plasmid backbone, which act as an intrinsic adjuvant (see above).

Several studies have shown protective effects of gene vaccination on the development of allergic Th2 responses. After injection of (p)DNA coding for the house dust mite allergen Der p 5 or OVA, prevention of allergic Th2 responses was observed in rats and mice, respectively, correlating in both cases with increased allergen-specific Th1 responses [35, 36]. Using the latex allergen Hev b 5, it could be shown that DNA immunization is also efficient in reducing established allergen-specific Th2 responses [37]. Another group succeeded in reversing established AHR using an improved DNA vaccination strategy [38]. The injected DNA plasmid contained the cDNA of OVA fused to the cDNA of IL-18 and resulted in enhanced effectiveness compared to OVA-(p)DNA alone or when using a mixture of OVA-(p)DNA and IL-18-(p)DNA. The protective effect was dependent on IFN- γ and CD8+ cells. In an OVA model of asthma, pulmonary Th2 responses

were successfully inhibited by mucosal DNA vaccination using cDNA encoding IFN- γ [39]. Furthermore, the application of (p)DNAs encoding for IFN- γ and IL-12 alone or in combination resulted in the reduction of total serum IgE levels, increased grass allergen-specific IgG2a serum levels, reduced pulmonary inflammation and reduced AHR in mice immunized with Kentucky blue grass antigen [40]. The strongest inhibitory effects were achieved when using a combination of (p)DNA-IL-12 and (p)DNA-IFN- γ .

Inhibition of Asthma by Tr Cells

Normally, the result of immune responses directed against invading pathogens is protective immunity. To avoid the development of autoimmune diseases or severe immune pathology at the same time and site, T cell responses are selectively suppressed by shutting them down or dampening them. Recent evidence suggests that in particular CD4⁺ T cells – termed regulatory T (Tr) cells – are responsible for this effect [6, 7]. These immunosuppressive cells differ from Th1 and Th2 cells by their distinct cytokine profile and their anti-inflammatory properties. To date, four major Tr cell populations have been described, but the relationship between the different Tr populations remains unclear in respect to their development, activation and the exact mechanisms used to suppress immune responses.

One type of Tr cells are the Tr1 cells, which are characterized by secretion of high levels of IL-10 and low to moderate levels of TGF- β . They can inhibit both Th1 and Th2 responses through the production of IL-10. Repeated in vitro stimulation of murine or human CD4⁺ T cells in the presence of IL-10 results in the generation of Tr1 cells [41]. In contrast, the differentiation factors for another Tr cell population (Th3 cells) are IL-4 and TGF- β . Th3 cells are induced after oral administration of antigen. These cells secrete primarily TGF- β and seem to suppress immune responses by a mechanism dependent on this cytokine [6, 7]. In contrast to Tr1 or Th3 cells, a third subset, called CD4⁺CD25⁺ Tr cells, do not mediate suppression by cytokine production but through cell-cell contact. Another group of Tr cells is characterized by an anergic state. Their suppressive function on T cell responses is mediated by their influence on APC [6, 7].

The Role of Tr Cells during Allergic Responses

The inhibitory effect of Tr cells on allergic Th2 reactions was first shown in animal experiments. Haneda et

al. [42] reported that experimental tracheal eosinophilia was ameliorated by CD4⁺ T cells secreting TGF- β [42]. Furthermore, it has been shown that the development of allergen-induced Th2 responses could be prevented by application of in vitro engineered allergen-specific Tr cell lines (secreting TGF- β or IL-10) [43, 44]. On the other hand, CD4⁺CD25⁺ Tr cells may also contribute to allergic inflammation by downmodulating Th1 responses. This effect became evident in experiments using Rag-2^{-/-} mice transferred with T cells from OVA TCR transgenic mice [45]. Nevertheless, in most animal experiments used to address this question, Tr cells inhibited at least partly the development of allergen-induced Th2 responses.

In humans thus far, conclusive evidence is not available showing that Tr cells suppress allergic reactions. However, there are findings indicating that decreased allergic responses are associated with increased IL-10 production. One report shows that the T cells from healthy children produce more IL-10 mRNA than the T cells from asthmatic children [46]. Another study reveals lower IL-10 levels in the BAL of asthmatic patients compared with healthy controls [47]. In unison with these findings is a report indicating that there is an association between a polymorphism in the human IL-10 promoter region and increased total serum IgE levels [48]. Furthermore, van den Biggelaar et al. [49] reported that decreased atopy in children infected with schistosomes is associated with increased IL-10 serum levels in these children. It has been speculated that the suppression of allergic Th2 responses is caused by the anti-inflammatory cytokine IL-10 secreted by Tr cells. Although this is only circumstantial evidence pointing at a role for Tr cells in the suppression of allergic reactions in humans, findings from Müller et al. [50], Akdis et al. [51] and Pierkes et al. [52] support this view. Their studies showed that successful bee or wasp venom immunotherapy was associated with increased IL-10 levels. However, other reports showed no association between decreased atopy and increased amounts of IL-10 [53, 54]. In animal models, there are also conflicting results concerning the role of IL-10 in the downmodulation of allergen-induced Th2 responses [55–57].

In addition to IL-10, Tr cells also secrete TGF- β , another anti-inflammatory cytokine. At present, the role of TGF- β in the inhibition of allergic Th2 responses in healthy humans is not clear. There is some evidence suggesting that TGF- β plays a role in inhibiting the development of asthma in humans [58, 59]. However, recently it was demonstrated in a mouse model that a decrease in TGF- β in the BAL was associated with an increase in eosinophilic airway inflammation after blocking CTLA-4

[60]. Taking these results together, it is not clear if Tr cells play an important role in the inhibition of atopic disorders in humans.

Induction of Tr Cells in vivo

Considering the results obtained from animal models (see above), the development of vaccines designed to induce allergen-specific or allergen-unspecific Tr responses seems to be a very attractive vaccination strategy protecting against allergic disorders in humans. As shown in animal experiments, oral application of antigen or the use of DC pulsed with antigen seem to be very promising methods to selectively induce allergen-specific Tr cells [61]. Furthermore, Akbari et al. [62] suggested that specific DC populations (pulmonary DC) induced the development of Tr cells in mice by IL-10 secretion, resulting in the suppression of allergen-induced Th2 responses in the lung.

A novel approach to induce allergen-specific Tr cells in vivo was recently published by Zuany-Amorim et al. [25]. In a mouse model of asthma, OVA-induced airway eosinophilia and lung hyperreactivity were reduced by subcutaneous application of a suspension of killed *M. vaccae*. The presence of IL-10, TGF- β and a T cell subpopulation (CD4+CD45RBlo) which is known to contain Tr cells was essential for this effect. Furthermore, it is very likely that the induced effect is allergen specific, as shown by transfer experiments. Protection from airway eosinophilia was mediated only by transferred CD4+CD45RBlo T cells from the spleen of mice treated with OVA (specific antigen) and *M. vaccae*. The transfer of CD4+CD45RBlo T cells from mice immunized with cockroach extract antigen (another Th2 response-inducing antigen) and treated with *M. vaccae* or from mice only treated with *M. vaccae* showed no effect. These results are in unison with the finding that atopic dermatitis in children was improved by the intradermal application of an *M. vaccae* suspension [63]. In contrast, Shirlcliffe et al. [64] could not confirm the improvement of asthma using a similar approach.

Conclusion

Experiments have clearly shown that animals can be protected from developing allergic Th2 responses by using different vaccination approaches. It may now also be possible to develop a vaccine for human use. The two most promising approaches are the induction of proinflammatory Th1 responses or immunoregulatory responses me-

diated by Tr cells. Which is the safest and most efficient strategy? Although very efficient in suppressing Th2 responses, allergen-specific Th1 cells may cause chronic inflammatory responses. The finding that allergen-specific Th1 cells adoptively transferred into naive mice induced the recruitment of macrophages and neutrophils into the lung after airway allergen challenge supports this view [65–67]. Currently, it is not clear if allergen-specific Th1 responses lead to chronic inflammatory diseases after recurrent exposure to allergen in humans. However, the potential dangers associated with Th1 responses may be too great a risk to take to immunize healthy children. A vaccine based on the induction of Tr cells may therefore be the best approach to a novel antiallergy vaccine since Tr cells can inhibit the development of allergic Th2 responses without causing inflammation. However, enhanced immunosuppression needs to be ruled out. Furthermore, Th1 responses are easily induced and monitored, while Tr responses are not. It remains to be elucidated which approach is the safest and most effective. A well-balanced vaccine inducing both Th1 and Tr responses may be the best approach. Th1 responses inhibit the development of Th2, and allergen-specific Tr cells in turn dampen the antiallergic Th1 response, ensuring non-pathological Th1 responses.

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