Novel vaccines protecting against the development of allergic disorders: a double-edged sword?

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The severity and incidence of allergic disorders is steadily increasing despite the widespread use of steroids and other drugs. Recent results obtained in animals suggest that it may be possible to develop novel anti-allergy vaccines for human use, thereby stopping this alarming worldwide increase in allergic diseases. The most promising approaches are the induction of allergen-specific T helper 1 or allergen-specific T regulatory responses. However, both approaches potentially harbour negative side effects that need to be ruled out before vaccinating young children – the best candidates for the primary prevention of allergic disorders.

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Abbreviations

AHR	airway hyperresponsiveness
APC	antigen presenting cell
BAL	bronchoalveolar lavage fluid
BCG	*Mycobacterium bovis* bacillus Calmette-Guerin
CpG-ODN	CpG oligodeoxynucleotide
DC	dendritic cell
IFN-γ	interferon-γ
IL	interleukin
i.d.	intradermal
i.n.	intranasal
i.v.	intravenous
LPS	lipopolysaccharide
MPL	monophosphoryl lipid
NK	natural killer
OVA	ovalbumin
(p)DNA	plasmid DNA
PIT	peptide-based immunotherapy
SIT	specific immunotherapy
Th	T helper
Tr	T regulatory

Introduction

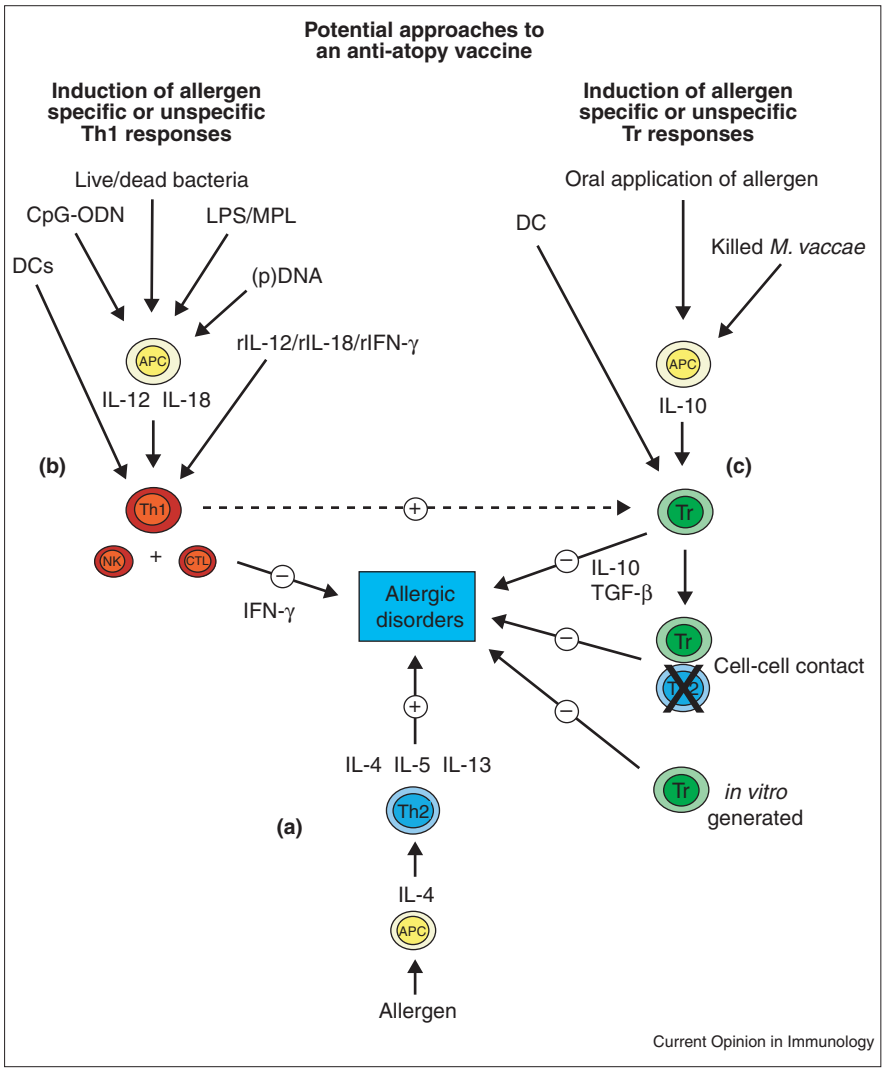
Exposure to allergens, followed by the production of IL-4 and the generation of allergen-specific $CD4^+$ T helper (Th)2 cells are together the most important prerequisites for the development of atopic disorders [1]. When generated, effector Th2 cells are recruited into the sites of allergen exposure by the guidance of specific chemokines. After coming into contact with allergen-derived peptides, which are bound to MHC class II expressed by APCs, they secrete IL-4, IL-5 and IL-13 (Figure 1a). These cytokines induce the production of allergen-specific IgE by B cells, the development and recruitment of eosinophils, smooth muscle contraction and mucus production. Degranulation of eosinophils and mast cells (via IgE cross-linking) in turn are the two major factors leading to most clinical manifestations of allergic diseases.

Although a relatively large amount is known about the immunological processes leading to the development of allergic disorders, thus far no effective prevention measure exists. Furthermore, over the past decade the incidence, severity and mortality rate (due to severe asthma) of allergic disorders has steadily increased, despite the widespread use of steroids and other drugs. For these reasons it is very important to develop novel therapeutic approaches and effective prevention measures. Recent animal experiments using live bacteria, bacterial components, CpG oligonucleotides (CpG-ODNs), plasmid DNA ([p]DNA) and other approaches suggest that it will be possible to develop novel vaccines for protecting humans from allergic disorders. Currently, two approaches seem to have the greatest potential. Firstly, vaccines inducing allergen-specific or unspecific Th1 responses during early childhood in atopy-prone children may prove successful in inhibiting Th2 responses (Figure 1b; [2•]). Secondly, recent publications have shown that in addition to Th1 cells, T regulatory (Tr) cells are able to inhibit the development of allergic Th2 responses (Figure 1c). This finding has led to the suggestion that future anti-allergy vaccines should aim at inducing Tr and not Th1 responses, as the generation of Tr cells harbours fewer potential negative side effects than the generation of Th1 cells. This review will focus on the prospective use of anti-allergy vaccines that induce Th1 or Tr responses in humans, pointing out the potentially negative side effects of both approaches.

Vaccines associated with the induction of Th1 responses

Numerous publications have clearly shown that Th1 responses induce the production of IFN-γ, IL-12 and IL-18, and are associated with the inhibition of Th2 cell development and effector functions both *in vivo* and *in vitro* [2•,3]. Furthermore, it appears that nonatopic-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. These findings led to the 'hygiene hypothesis', suggesting that infections early in life that induce Th1 responses help protect atopy-prone children against the development of allergic disorders later in life by biasing immune responses in these children away from Th2 and towards Th1. Epidemiological and experimental studies demonstrating an inverse correlation between the development of allergic disorders and the incidence of infection support this view [3]. 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

Figure 1



Prospective vaccination strategies possibly leading to an anti-atopy vaccine in humans. **(a)** Allergens are taken up by APC. Environmental and genetic factors then determine if naïve T cells develop into allergen-specific Th2 cells under the influence of IL-4. These Th2 cells then secrete IL-4, IL-5 and IL-13, leading to the development of allergic disorders. There are two major approaches towards developing an anti-allergy vaccine that seem the most promising: the induction of allergen-specific Th1 or Tr responses. **(b)** Allergen-specific Th1 immune responses protect from the development of atopic disorders by inducing the production of IFN-γ, which inhibits the development of Th2 cells. The application of DCs pulsed with allergen or the application of the cytokines rIL-12/rIL-18/rIFN-γ can directly induce the development of allergen-specific Th1 responses. By contrast, the application of live/dead bacteria, (p)DNA, CpG-ODN, or LPD/MPL induce the generation of Th1 cells by inducing the expression of IL-12 and IL-18 by DCs, leading to Th1 cell development. **(c)** Tr cells can inhibit the development of Th2 responses either by the secretion of IL-10 , or by directly anergizing Th2 cells through an cell-contact dependent and allergen dependent or independent mechanism. Allergen-specific Tr cells could be induced by oral administration of allergen or killed *M. vaccae* by inducing the secretion of IL-10 by APCs. The application of DCs pulsed with antigen may directly induce the development of Tr cells. Tr cells may also be generated from PBMC from patients *in vitro* and re-administered back into the patients. Furthermore, vaccines aimed at inducing Th1 responses may also induce Tr cells which may contribute to the anti-allergy effects of Th1 responses.

development of allergic Th2 responses. For this reason, two separate approaches to developing an anti-allergy vaccine utilising Th1 responses can be envisioned. Young children could be vaccinated with reagents that induce strong Th1 responses, leading to a general decrease in the tendency to develop allergic Th2 responses, thereby resulting in the development of allergen-specific Th1 cells after the exposure to allergen. Alternatively, vaccines could be used that 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 successful inhibition of Th2 cell induced asthma in mice by the administration of *in vitro* generated allergen-specific Th1 cells supports this view [4,5••].

Application of live/killed bacteria or bacterial products

Mycobacterial infections induce profound Th1 responses. The administration of mycobacteria or their products may therefore be used as a vaccine or as components of vaccines aimed at inhibiting Th2 responses. In support of this view are our findings that the application of live or heat-killed *Mycobacterium bovis* bacillus Calmette–Guerin (BCG) into the lung of mice strongly suppressed the development of airway eosinophilia with the effect lasting up to two months after application [6•,7]. The inhibitory effect was clearly dependent upon IFN-γ signalling and, therefore, Th1 responses. Furthermore, the application of BCG intravenously (i.v.) into mice or subcutaneously (s.c.) into guinea pigs led to the generation of allergen-specific Th1 responses and the inhibition of both allergen-induced airway hyperresponsiveness (AHR) and allergen-induced airway eosinophilia [8,9]. In addition to BCG, *Mycobacterium vaccae* was also shown to mediate protection against the development of allergic Th2 responses. Hopfenspirger *et al.* [10•] reported that intranasal (i.n.) administration of *M. vaccae* and BCG could also reduce established allergen-induced Th2 responses, with BCG being more effective

that *M. vaccae*. Importantly, the authors found that only the treatment with BCG but not *M. vaccae* lead to increased IFN-γ levels in the bronchoalveolar lavage fluid (BAL) of the mice. Furthermore, Wang and Rook [11] reported that *M. vaccae*-induced reduction of established allergen-specific IgE responses were not associated with increased Th1 responses. Taken together these findings suggest that BCG-induced, but not *M. vaccae*-induced, suppression of allergic responses is mediated by Th1 cells. It has been suggested that *M. vaccae* mediates the suppression of Th2 responses by the induction of Tr cells ([12••]; see below). However, Janssen *et al.* [13] recently reported 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. In this system, recombinant *M. vaccae* was at least as efficient as recombinant BCG in reducing Th2 responses.

In addition to mycobacteria, the application of other bacteria has also been shown to suppress the development of allergic responses. For example, the application of killed *Listeria monocytogenes* or *Lactobacillus plantarum* suppressed the induction of allergen-specific IgE in mice [14,15]. The *Listeria*-induced suppression was associated with increased Th1 responses. Importantly, Hansen *et al.* [16] could show that the application of heat-killed *Listeria* was also able to reduce established allergen-specific Th2 responses in mice. This effect was dependent upon $CD8^+$ T cells and IL-12, and was associated with increased IL-18 mRNA expression.

A further adjuvant of an anti-allergy vaccine could be endotoxin derived from gram negative bacteria. Animal studies have shown that allergen-induced AHR was suppressed by prior i.n. administration of lipopolysaccharide (LPS; [17,18]). Although the mechanism underlying this effect is unclear, it has been speculated that LPS activates macrophages and dendritic cells (DCs) leading to IL-12 secretion and, therefore, the production of IFN-γ by n atural killer (NK) and T cells. Recently, however, Tulic *et al.* [19] reported that the LPS-mediated inhibition of the late-phase response to allergen was associated with increased IL-10 production, suggesting that Tr responses might also be involved in mediating the suppressive effect. These results suggest that applying LPS to humans might also help to reduce the development of allergic disorders. Supporting this view are the results of epidemiological studies, suggesting that children that grow up on farms develop less allergies in their life time because they have been continuously exposed to LPS (derived from animal manure) early in life [20,21]. However, animal experiments also suggest that LPS treatment may also exacerbate allergic responses. It was reported that enhanced allergic airway inflammation and IgE production was observed when LPS was given after the sensitisation with allergen [22]. Furthermore, it was also shown that inhalation of endotoxin later in life, contrary to exposure early in life, causes chronic bronchitis and is associated with AHR and the exacerbation of pre-existing asthma [23]. The potential problems of using LPS, in particular toxic shock, may be overcome more easily by using a detoxified derivative of LPS, for example, monophosphoryl lipid (MPL)-A derived from *Salmonella minnesota* R595. In mice it could be demonstrated that immunisation with allergen (ovalbumin [OVA] or ragweed pollen extract) in combination with MPL (and the depot adjuvant L-tyrosine) resulted in increased allergen-specific Th1 responses and reduction of Th2 responses [24]. MPL is currently being tested as a component of anti-allergy vaccines designed to help protect against grass pollen or dust mite allergies [25,26].

CpG-ODN

CpG-ODNs are part of the noncoding sequences of microbial DNA. CpG motifs bind directly to toll-like receptor 9 [27]. The application of CpG-ODN to humans or animals leads to strongly Th1-biased responses *in vivo* by directly inducing the secretion of IL-12 and IL-18 by macrophages and DCs, and the secretion of IFN-γ by NK cells [28]. Animal studies have shown that the application of CpG-ODN (applied via intraperitonal [i.p.] injection or i.n. given without allergen) led to the suppression of allergen-induced asthma in mice [29–31]. The inhibitory effect of CpG-ODN was clearly associated with a shift from allergen-specific Th2 responses towards allergen-specific Th1 responses, characterised by increased IFN-γ and IgG2a levels, suggesting that the induction of Th1 responses was responsible for the inhibitory effect. Supporting this view is the finding that CpG-ODN-mediated suppression of allergic Th2 responses was not observed in IFN-γ deficient mice [31]. However, IFN-γ and IL-12 independent suppressive effects of CpG-ODN on Th2 responses have also been reported [32]. In contrast to the above described results, Shirota *et al.* [33] showed that the inhibition of allergen-induced AHR and airway eosinophilia was only observed when CpG-ODN was given in combination with allergen, and not alone. This suggests that allergen-specific immune responses were responsible for the inhibition. Importantly, it was also reported that allergen directly conjugated with CpG-ODN can be used 100-fold less concentrated than a mixture of CpG-ODN and allergen to achieve similar suppressive effects on allergen-induced airway eosinophilia and Th2 cell differentiation *in vivo* [34•]. The enhancement of the immunomodulatory potential of CpG-DNA as a strong Th1 response-inducing adjuvant by conjunction of CpG-motifs with the relevant allergen was also reported by Tighe *et al.* [35]. Furthermore, Santeliz *et al.* [36] demonstrated that i.d. applications of Amb a 1 (a major antigen of rag weed pollen) conjugated to CpG also resulted in the reduction of already established allergen-induced eosinophilia and AHR in mice that were sensitised and challenged with ragweed pollen extract. This effect correlated with increased IFN-γ secretion of the spleen cells after antigen stimulation and the shift in the antibody profile towards IgG2a secretion. Shirota *et al.* [37] recently showed that antigen–CpG-ODN conjugates cause increased

antigen uptake and DC activation. Antigen and CpG are co-incorporated by the same DCs, which results in increased CD40 and CD86 expression, and high levels of IL-12 expression, promoting the development of IFN-γ secreting Th1 cells. This finding may explain why much less CpG-ODN conjugate can be used in comparison to CpG-ODN alone or in combination with allergen to induce the suppression of allergic disorders. The first human clinical trials using CpG-ODN conjugated to ragweed antigen have been initiated and the first reports indicate that the treatment is well tolerated [38].

DNA vaccination

Another possible method of inducing strong and long-lasting Th1 responses is by DNA vaccination [39]. This relatively new immunisation method is based on i.d. or intramuscular (i.m.) injections of (p)DNA encoding a defined antigen. After cellular uptake the transfected cells produce antigen continuously for the duration of their survival. This leads to a persistent long-lasting Th1 memory response. The reason why DNA vaccination leads to strong Th1-biased responses is believed to be because of the CpG motifs in the plasmid backbone, which act as an intrinsic adjuvant.

Protective effects of gene vaccination on the development of allergic Th2 responses have been demonstrated in several animal models. 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 [40,41]. In another animal model using the latex allergen Hev b 5, it could be shown that DNA immunisation is also efficient in reducing established allergen-specific IgE responses [42]. Recently another group succeeded in reversing established AHR using an improved DNA vaccination strategy [43•]. 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. Although all three approaches protected immunised mice from the induction of AHR (correlating with increased IFN-γ production and decreased OVA-specific IgE production) only the OVA–IL-18 fusion (p)DNA was able to reverse established AHR (correlating with reduced allergen-specific IL-4 and increased allergen-specific IFN-γ production). The protective effect was dependent on IFN-γ and $CD8^+$ cells. Allergen-independent DNA vaccination has also been shown to be effective. In an OVA model of asthma, pulmonary Th2 responses were successfully inhibited by mucosal DNA vaccination using cDNA encoding IFN-γ [44]. Furthermore, the application of (p)DNAs encoding for IFN-γ and IL-12, either 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 immunised with Kentucky blue grass antigen [45]. The strongest inhibitory effects were achieved when using a combination of (p)DNA–IL-12 and (p)DNA–IFN-γ. Interestingly, Hertz *et al.* [46••] used (p)DNA encoding murine IL-5 and modified to contain a foreign Th epitope to protect mice from developing allergen-induced AHR. The Th epitope encoded together with IL-5 bypassed immunological tolerance to IL-5, leading to the production of anti-IL-5 antibodies and thereby neutralising the effects of IL-5 after allergen challenge.

Additional approaches

The application of bacteria, CpG-ODN, or (p)DNA are the best characterised ways of inducing Th1 responses, leading to the inhibition of allergen-specific Th2 responses. Most published reports indicate that IL-12, IL-18 and IFN-γ are responsible for the inhibitory effect. For this reason it may be feasible to bypass the vaccination strategies discussed above by directly applying recombinant IL-12, IL-18 or IFN-γ either alone or in combination, with or without allergen, to suppress Th2 responses. In support of this view is the finding that the administration of nebulized IFN-γ inhibited allergen-induced AHR, cutaneous reactivity and secondary OVA-specific IgE production in mice [47,48]. However, treatment with IL-12 led to somewhat conflicting data. Whereas one group observed inhibitory effects comparable to those observed when using IFN-γ [49] another group needed to co-administer IL-18 to have this effect [50]. On the other hand, application of IL-18 alone exacerbated allergic asthma by enhancing allergen-induced recruitment of eosinophils into the airways and airway inflammation [51,52].

The administration of allergen-pulsed DCs may be another strategy to induce allergen-specific Th1 responses. DCs can easily be obtained from progenitors present in the blood of patients and adjuvants inducing the secretion of IL-12 by DCs are readily available (for example, CpG-ODN; [53]). Furthermore, animals vaccinated with DCs pulsed with antigen *in vitro* showed very efficient protection against infectious diseases and cancer [54,55]. The first clinical trials in humans, based on DC vaccination, have already yielded positive results in patients with cancer. However, it remains to be seen both in animals and humans, if DCs matured to secrete IL-12 and pulsed with allergen (or peptides) induce allergen-specific Th1 responses leading to the inhibition of Th2 responses. Furthermore, it needs to be ruled out that DCs from atopic-prone humans do not induce Th2 responses.

A further vaccination method also possibly associated with Th1 responses is specific immunotherapy (SIT). It is efficiently and widely used in people who are allergic to bee or wasp venom or who suffer from rhinitis. By repeated injections (usually s.c.) increasing amounts of allergen are applied. The mechanism underlying successful desensitisation is not entirely clear, but it has been suggested to be caused by a downmodulation of IgE and upregulation of IgG in the serum through the action of Th1 cells. However, recent results indicate that Tr cells possibly

mediate this effect (see below). SIT harbours the risk of IgE-mediated anaphylaxis and cannot be used in all patients. Using allergen-derived peptides that contain short T cell but no B cell epitopes, or B cell epitopes known not to bind to IgE (peptide-based immunotherapy [PIT]) may be more effective, as IgE-mediated mast cell degranulation can be ruled out [56]. Currently, it is not clear if these peptides selectively induce Th1 or Tr responses or both. A further problem with both SIT and PIT is that they may directly lead to increased Th2 responses in some atopic individuals. The use of altered peptide ligands may overcome this potential problem, as it was reported that mice vaccinated with peptide analogues of peptides, which normally induced Th2 responses, developed peptide-specific Th1 responses [57,58]. Currently, SIT and PIT are only used for therapeutic purposes and not for primary prevention as it is not clear how atopy predisposed but not yet allergic patients will react to the applied allergen or peptides.

Vaccines associated with CD4$^+$ Tr cells

Tr cells

The immune system can rapidly react to invading pathogenic organisms normally leading to protective immunity without causing severe immune pathology or autoimmune disease. Although it is not entirely clear how this selective immune suppression is achieved, recent evidence suggests that CD4$^+$ T cells in particular are responsible for this effect by shutting down or dampening T cell responses. Other cell types, including CD8$^+$, γδTCR$^+$ or NK T cells have also been implied to play a role in transient immune suppression [59,60]. CD4$^+$ T cells responsible for immune suppression have been termed regulatory T cells (Tr). Currently four major Tr cell populations have been described. CD4$^+$CD25$^+$ Tr cells naturally occur both in humans and rodents and suppress T cell responses in a contact-dependent manner. However, not all cells expressing CD4 and CD25 are Tr cells, as activated effector T cells also express CD25, making the identification of CD4$^+$CD25$^+$ Tr cells difficult. A further Tr cell type has been named Tr1 cells. They can be generated *in vitro* by stimulating murine or human CD4$^+$ T cells in the presence of IL-10 [61]. Tr1 cells can inhibit both Th1 and Th2 responses by secreting large amounts of IL-10 and lower amounts of TGF-β. Tr cells induced after oral administration of antigen and secreting TGF-β have been named Th3 cells [59,60]. In addition, T cell clones, which are themselves anergic but can suppress other T cell responses by influencing APC function, are also considered to be Tr cells [59,60]. Currently the relationship between the different Tr populations remains unclear with respect to their development, activation and the mechanisms by which exact immune suppression is achieved. However, numerous animal experiments have clearly shown that Tr cells can suppress both Th1 and Th2 responses *in vivo* and thereby actively suppress the development of autoimmunity and allergic responses. For a detailed overview on Tr cells we would like to refer the reader to reviews on this specific topic [59,60].

Tr cells and allergic disorders

Animal experiments have provided the first evidence that Tr cells can inhibit allergen-mediated Th2 responses. Hansen *et al.* [62] and Cottrez *et al.* [63] reported that the application of *in vitro* engineered allergen-specific Tr cell lines (secreting TGF-β or IL-10) protected mice from developing allergen-induced Th2 responses. Furthermore, inhibition of experimental tracheal eosinophilia was also due to the induction of CD4$^+$ T cells secreting TGF-β [64]. However, Suto *et al.* [65] recently reported that CD4$^+$CD25$^+$ Tr cells may also contribute to allergic inflammation by selectively downmodulating Th1 and not Th2 responses in Rag-2$^{-/-}$ mice reconstituted with T cells from OVA TCR transgenic mice (most CD4$^+$ T cells in these animals express a transgenic-derived TCR, which recognises a peptide from OVA bound to MHC class II). Taken together, these reports indicate that Tr cells can inhibit the development of allergen-induced Th2 responses in most experimental animal models that have so far been used to address this question.

In humans thus far there is only circumstantial evidence to suggest that Tr cells play a major role in the inhibition of allergic disorders. It has been reported that IL-10 levels in the BAL of asthmatic patients is lower than in healthy controls, and that T cells from children suffering from asthma also produce less IL-10 mRNA than T cells from control children [66,67]. These findings indicate that increased IL-10 production may be associated with decreased allergic responses. Supporting this view are two reports, the first one indicates that a polymorphism in the human IL-10 promoter region is associated with increased total serum IgE levels [68] and the second report indicates that suppression of allergen-mediated mast cell degranulation in humans infected with schistosomes was associated with increased amounts of IL-10 present in the serum of the patients [69••]. As Tr cells are a major source of IL-10 it has been speculated that Tr cells secreting IL-10 are involved in the suppression of allergic Th2 responses in humans. Supporting this hypothesis are the findings from Müller *et al.* [70], Akdis *et al.* [71] and Pierkes *et al.* [72]. They report that successful immunotherapy against bee or wasp venom in hypersensitive patients was associated with the induction of anergic allergen-specific T cells that secreted IL-10. However, other reports clearly failed to confirm the hypothesis that increased IL-10 levels are associated with less allergic disease [73,74]. In addition, animal models also yielded somewhat contradictory results with some reports suggesting that IL-10 is involved in the downmodulation of allergen-induced Th2 responses and others indicating either no effect or that IL-10 is needed for the development of Th2 responses [75–77].

Another anti-inflammatory cytokine associated with Tr cells is TGF-β. Interestingly, to date there is no evidence suggesting that the presence of TGF-β is associated with the suppression of allergic Th2 responses in humans. The opposite may be true though, as some reports imply a role

for TGF-β in the pathogenesis of asthma in humans [78,79]. However, a recent report indicates that the increased allergic inflammation observed after blocking cytotoxic T lymphocyte-associated antigen 4 (CTLA-4, a negative regulator of T cell function) in mice is clearly associated with decreased TGF-β levels in the BAL of these animals [80].

In conclusion, it is not currently clear if Tr cells, or the anti-inflammatory cytokines IL-10 or TGF-β play a major role in the inhibition of allergic Th2 responses in healthy humans. Further studies are needed to answer this very important question.

Inducing Tr cells *in vivo*

Although it is not clear if the lack of allergic responses in healthy humans is due to the presence of allergen-specific or unspecific Tr cells, the findings obtained in animal models imply that a vaccine which selectively induces the development of Tr cells may also protect humans from the development of allergic Th2 responses. A prerequisite for such a vaccine is the knowledge of how to induce Tr cells *in vivo*. Thus far, animal experiments indicate two potential vaccination strategies for human use — the oral application of antigen and the use of DCs pulsed with antigen — both of which can lead to the selective induction of allergen-specific Tr cells [81]. In particular, the application of specific DC populations may be very effective, as a recent report suggests that pulmonary DCs secreting IL-10 mediate the suppression of allergen-induced Th2 responses in the lung of mice by inducing Tr cells [82••]. Recently, Zuany-Amorim *et al.* [12••] reported a novel vaccination strategy of inducing allergen-specific Tr cells in mice. They show that the s.c. application of a killed *M. vaccae* suspension leads to a reduction in OVA-induced airway eosinophilia and lung hyper-reactivity. Importantly, they show that this effect was dependent upon the presence of $CD4^+CD45RB^{lo}$ T cells (known to contain Tr cells), IL-10 and TGF-β. Furthermore, the effect seems to be allergen-specific, as only the transfer of $CD4^+CD45RB^{lo}$ T cells from the spleen of mice treated with OVA and *M. vaccae* protected mice from airway eosinophilia, and not the transfer of $CD4^+CD45RB^{lo}$ cells from *M. vaccae*-only treated mice or mice treated with *M. vaccae* and immunised with a different Th2 response-inducing antigen (cockroach extract antigen). This approach seems to be the most promising for human use as it appears to be both safe (using killed *M. vaccae*, which even when alive is relatively avirulent) and easy to use. Supporting this view is the report from Arkwright and David [83•] showing that the intradermal application of a *M. vaccae* suspension improved atopic dermatitis in children. However, a similar approach did not result in the improvement of asthma [84]. Applying killed *M. vaccae* before the onset of asthma may yield more promising results. The recently published report from Barrat *et al.* [85••] suggests that it may also be possible to directly generate allergen-specific Tr cells *in vitro* from peripheral blood monocytes (PBMC) of allergic patients by stimulating $CD4^+$ T cells in the presence of allergen and immunosuppressive drugs. Re-administering allergen-specific Tr cells back into the patient may then also help reduce allergic Th2 responses.

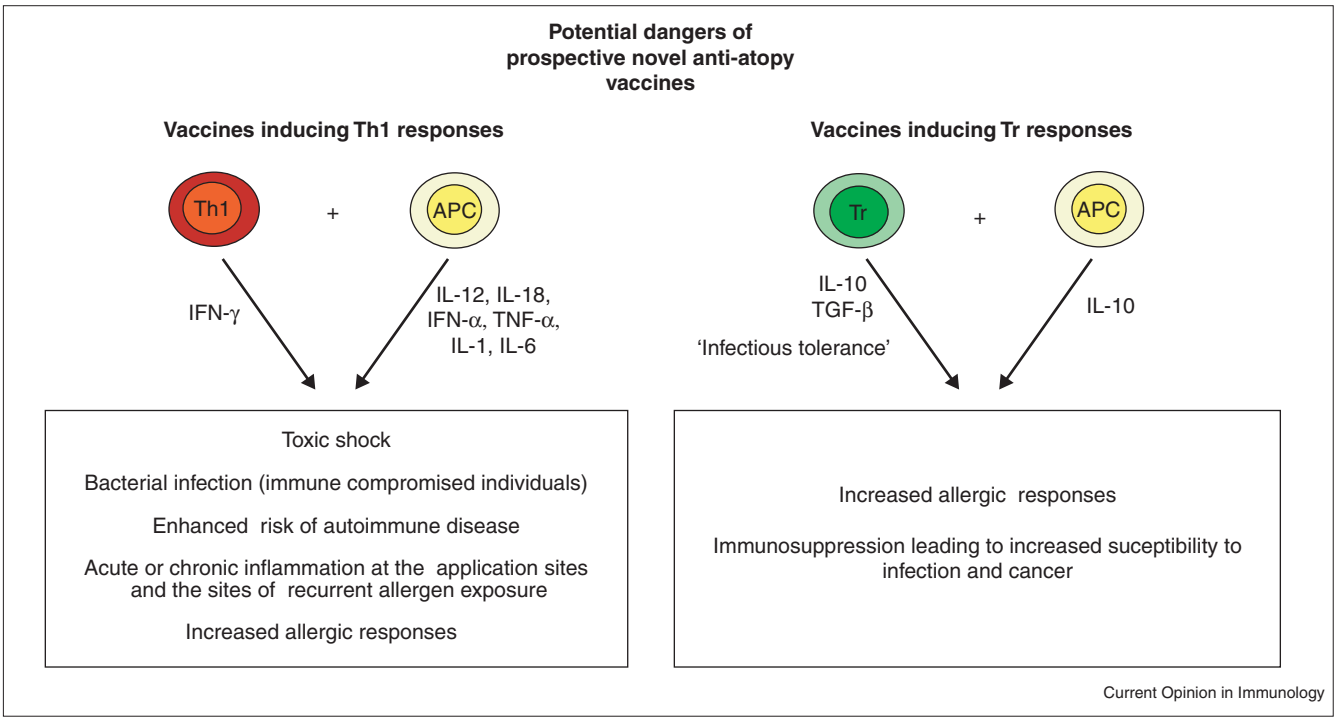
It remains to be elucidated if it will be possible to selectively induce allergen-specific Tr cells leading to the inhibition of allergic disorders in humans. Furthermore, it may not be necessary to induce allergen-specific Tr cells. Generally inducing more immune regulation (by 'unspecific' vaccines, leading to the generation of more Tr cells) during early infancy may also help atopy-prone children not to develop allergen-specific Th2 responses in their lifetime.

Potential side effects of prospective anti-allergy vaccines

The most promising approaches that might lead to a human vaccine for protecting against the development of allergic disorders are the induction of allergen-specific or unspecific Th1 or Tr responses. The question is, which approach is the most effective and the safest? Safety may be the most important prerequisite for an anti-allergy vaccine, as allergies are not normally life threatening (in contrast to cancer, or some infectious or autoimmune diseases) and may have to be applied at an very early age to be effective. Should vaccines that directly induce allergen-specific Th1 responses be used? Most animal experiments show that allergen-specific Th1 responses seem to be effective in inhibiting allergen-specific Th2 responses. However, some reports also indicate that allergen-specific Th1 cells fail to counterbalance Th2 cell-mediated allergic inflammation in mice [86–88]. In addition, OVA-specific Th1 cells caused lung neutrophilia and lung inflammation. These results clearly show that allergen-specific Th1 cells are capable of causing inflammatory responses and tissue destruction in mice and suggest that this may also occur in humans. Therefore the 'worst case scenario' would be that any vaccine that induces allergen-specific Th1 responses might lead to acute or chronic Th1-mediated inflammatory diseases at the sites of reoccurring antigen exposure in humans (for example, chronic obstructive pulmonary disease [COPD] in the lung). This may occur independently of the vaccine used to induce the Th1 response. However, it is important to keep in mind that these conclusions are based on experiments that transfer relatively large amounts of monoclonal TCR transgenic Th1 cells and then challenge with allergen within a short time interval after i.v. application of the T cells. It is not clear what happens in these animals when they are continuously rechallenged with allergen during the following weeks and months. Furthermore, it is not clear if allergen-specific Th1 cells, directly generated in the vaccinated animals, also lead to Th1-mediated immunopathology after frequent exposure to allergen.

Further dangers associated with vaccines that induce Th1 responses may be toxic shock, bacterial disease and increased susceptibility to autoimmune disorders (Figure 2).

Figure 2



Potential dangers of vaccines designed to inhibit the generation of allergen-induced Th2 responses. The greatest danger of vaccines that induce Th1 responses is the development of acute and/or chronic inflammation mediated by Th1 cells secreting IFN-γ and APCs secreting the pro-inflammatory cytokines IL-12, IL-18, IFN-γ, TNF-α, IL-1 and IL-6. The secretion of large quantities of these pro-inflammatory cytokines may also lead to toxic shock (in particular following the application of LPS or CpG-ODN) or the breaking of immunological tolerance leading to increased autoimmunity. Induction of strongly increased numbers of Tr cells may lead to increased immunosuppression. This may result in increased susceptibly towards infection or cancer. Vaccines designed to induce allergen-specific Th1 or Tr responses may unexpectedly lead to or exacerbate allergic Th2 responses in some genetically or environmentally predisposed individuals.

For example, one should take great care when utilising the anti-allergy effects of LPS or CpG-ODN in humans, as both reagents can cause toxic shock [89]. Furthermore, the use of live bacteria, which induces very strong Th1 responses, although effective, may have to be totally discarded for human use. The reason is that treating young children with live bacteria may directly lead to bacterial disease and strong inflammatory responses (such as those seen when BCG is directly given into the lung of mice) or later in life during acquired or induced immunosuppression (for example, during treatment for autoimmune disease or cancer, or after being infected with HIV). In addition, it needs to be totally ruled out that vaccines that induce strong Th1 responses do not favour the generation of autoimmune disease, as it was reported that the application of CpG-ODN exacerbated autoimmunity in different animal models [2•]. The production of large amounts of pro-inflammatory cytokines (induced by the vaccine) might lead to the breaking of immunological tolerance needed to inhibit the development of autoimmune disorders.

As outlined above, vaccines that induce Th1 responses may have numerous harmful side effects. For this reason, vaccines that induce allergen-specific Tr cells seem to be a much better alternative as they do not cause inflammatory responses. However, research into Tr cell biology has just begun and important questions in respect to their function, activation, and so on have not been answered in detail, so they may also have negative side effects (Figure 2). For example, it has been shown that the depletion of $CD25^+$ Tr cells leads to enhanced protection against syngenic tumours [90]. Will increased numbers of Tr cells lead to 'too much' immune regulation, thereby increasing the risk of developing cancer? On the basis of current knowledge, this appears unlikely. Furthermore, Segal *et al.* [91] recently reported that IL-10-producing $CD4^+$ T cells mediated tumour rejection. However, some reports indicate that immune suppression during chronic helminth infections, believed to be associated with Tr cells, leads to decreased Th1 responses and possibly results in increased susceptibility towards infection [92,93]. At least in theory it is conceivable that strongly increased amounts of Tr cells could shut down or dampen the T cell responses needed for the efficient clearance of bacteria, viruses or tumours by anergizing the T cells. The finding that T cells anergized by Tr cells can themselves again anergize other T cells (known as 'infectious tolerance') supports this view.

A further prerequisite for an prospective anti-allergy vaccine is that the vaccine does not lead to the development or the exacerbation of allergic Th2 responses. Results obtained in animal experiments using inbred animals of a defined immunological status and genetic background cannot be transferred to humans without caution. This is especially true for allergic diseases where environmental and genetic factors play a major role in determining if allergic Th2 responses develop or not.

Conclusions

Allergic disorders continue to be a major health hazard in the developed countries of the world and, to date, no effective preventative measure exists. Experiments in animals have clearly shown that they could be protected from developing allergic Th2 responses by using different vaccination approaches. These results suggest that it may now also be possible to develop an anti-allergy vaccine for human use. On the basis of published results, the most promising approaches seem to be the induction of allergen-specific Th1 or Tr cells. Young children could be treated with vaccines (with or without allergen) that induce the production of IL-12, IL-18 and IFN-γ, leading to allergen-specific Th1 immune responses. It is hoped that this will protect children from developing allergen-specific Th2 type responses later in life. This could be achieved by immunising children with or without allergens in conjunction with live/dead bacteria, LPS/MPL, CpG-ODN alone or conjugated with allergen or (p)DNA. However, allergen-specific Th1 cells may cause chronic inflammatory responses upon repeated encounters with allergen. Supporting this view are the reports that allergen-specific Th1 cells adaptively transferred into naïve mice induced the recruitment of macrophages and neutrophils into the lung after allergen airway challenge [86–88]. However, it is not currently clear if allergen-specific Th1 responses lead to chronic inflammatory diseases after recurrent exposure to allergen. Animal experiments that answer this important question need to be performed before discarding the use of vaccines that selectively induce Th1 responses. Live bacteria may never be used in humans because they may have to be applied to mucosal surfaces (for example nose, gut or the lung) to be effective. This view is supported by reports indicating that children vaccinated s.c. with BCG were not protected from developing allergic disorders [94–96]. By contrast, mice treated i.v. or i.n. with BCG were protected from allergic Th2 responses [7,8]. The induction of Tr cells seems to be the best approach for a novel anti-allergy vaccine, as Tr cells can inhibit the development of allergic Th2 responses without causing inflammation. However, it needs to be ruled out that vaccines which induce the generation of Tr cells lead to disease-promoting immunosuppression.

Taken together, recent publications suggest that it may be possible to develop a novel anti-allergy vaccine for human use in the near future. However, it remains to be elucidated which approach is the safest and most effective. The safety issue cannot be overemphasised, as it is important to point out that allergic disorders are not usually life threatening. Furthermore, a well-balanced vaccine that induces both Th1 and Tr responses may be most desirable. Th1 responses may very efficiently inhibit the development of Th2 cells via IFN-γ, leading to a life-long protective Th1 memory response. Allergen-specific Tr cells may in turn dampen the anti-allergic Th1 response, ensuring a well-balanced protective but nonpathological Th1 response. In this context it will be of great importance to define the immunological mechanisms (enhanced/reduced Th1 and/or Tr responses) responsible for the inhibition/induction of allergic Th2 responses in allergen exposed healthy/atopic humans, respectively, to design the most effective and safest vaccines.

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