Atopic disorders: a vaccine around the corner?

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The incidence and severity of atopic disorders, in particular asthma, is steadily increasing at an alarming rate. Furthermore, no primary prevention measure exists to date. However, recent results obtained from numerous animal studies suggest that primary prevention in humans might be possible in the near future. The most promising approaches include the induction of systemic or local allergen-dependent or -independent T helper 1 (Th1) immune responses, through the use of killed bacteria (or components derived from them), CpG oligodeoxynucleotides or plasmid DNA, and the induction of allergen-specific T-cell tolerance. Here, we review the data showing that animals can be protected from developing allergic Th2 responses by vaccination. Possible future use in humans and potential side-effects of the described vaccination strategies are discussed also.

Exposure to common environmental antigens (Ags) can lead to atopic disorders, such as allergic asthma, hay fever, eczema and allergic rhinitis, which affect up to 20% of the population in developed countries. Although it is still not understood why exposure to allergen causes atopic disorders in some individuals but not others, it is clear that it involves both environmental and genetic factors.

The initial event responsible for the development of allergic diseases is the generation of allergen-specific $CD4^+$ T helper 2 (Th2) cells. The current view is that under the influence of interleukin-6 (IL-6) and IL-4, naive T cells activated by Ag-presenting cells (APCs) differentiate into Th2 effector cells[1]. IL-6, most probably secreted by the APCs, induces the initial production of IL-4 by the naive $CD4^+$ T cell. IL-4 is necessary to drive the development of Th2 cells through signaling mediated by signal transducer and activator of transcription 6 (STAT6), and the activation of specific, downstream transcription factors, such as c-Maf, GATA3, nuclear factor c of activated T cells (NFATc) and NFAT-interacting protein 45 (NIP45)[2,3]. Once generated, effector Th2 cells produce IL-4, IL-5 and IL-13 when they encounter APCs presenting peptides from processed allergen. These cytokines induce the production of allergen-specific IgE by B cells, development and recruitment of eosinophils, production of mucus and contraction of airway smooth muscle (reviewed in Refs 4,5). These events are the cause of almost all of the clinical symptoms associated with allergic diseases. Furthermore, the degranulation of eosinophils and mast cells by IgE-mediated cross-linking of receptors is the major factor leading to chronic allergic inflammation (for example, severe asthma). Moreover, recent studies indicate that chemokines, such as eotaxin, regulated on activation, normal T-cell expressed and secreted (RANTES), monocyte chemoattractant protein 1 (MCP-1) and MCP-5, are also involved crucially in the etiology of allergic disorders (reviewed in Ref. 6). Importantly, although Th2 cells are the major culprits responsible for the development of allergic diseases, Th1 cells do contribute to chronic disease (reviewed in Refs 7,8).

Although the immunological processes leading to the development of allergic diseases are relatively well understood, thus far, no effective prevention measure exists. Furthermore, the incidence, severity and mortality rate of atopic diseases has increased steadily, despite the widespread use of bronchodilators, corticosteroids and more-advanced drugs. 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 against the development of allergic disorders, in particular asthma. These findings give rise to the hope that this might also be possible in humans. Based on the observation that the presence of interferon γ (IFN-γ), IL-12 and IL-18 inhibits the development of allergen-specific Th2 cells both *in vitro* and *in vivo* (reviewed in Refs 2–4,9,10), prospective human vaccines will aim most probably to induce strong Th1 responses, leading to the induction of expression of these cytokines. This approach is supported by the finding that the administration of allergen-specific Th1 cells into mice inhibited Th2-cell-induced asthma[11,12]. However, other reports failed to confirm this result[13,14].

Two approaches seem most promising. First, using vaccines that induce strong Th1 responses in atopy-prone neonates or young children might protect the children from developing allergen-specific Th2-type cells after exposure to allergen. The basis for this approach is the suggestion that the neonatal immune response of nonatopics is biased towards a Th2 response, which shifts towards a Th1 response during the first few years of life[15,16]. By contrast, children who become atopic do not seem to lose the tendency to develop Th2 responses. For this reason, they develop allergen-specific Th2 cells after being exposed to allergen. The finding that the lack of suppression of Th2 responses in atopic children was linked to the inability of these children to produce sufficient amounts of IFN-γ as neonates supports this view[17]. Furthermore, it has been suggested that the increase in the incidence of atopic disorders is linked to a

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decrease in the prevalence of infections that induce Th1 responses early in life. It is believed that these early infections, by inducing the production of IL-12, IL-18 and IFN-γ, switch the normally Th2-biased responsiveness in atopics towards Th1 responses, thus protecting them from atopic disease. This theory is referred to as the 'hygiene hypothesis'. Several epidemiological studies showing an inverse relationship between the development of atopy and the incidence of infection support this view[18–21]. Second, an alternative approach is based on the hypothesis that nonatopic individuals are protected from the development of allergic diseases because they have developed allergen-specific Th1 responses. The secretion of IFN-γ by Th1 cells during an encounter with allergen is believed to be sufficient to suppress the development of allergen-specific Th2 cells and thereby, the development of allergies.

Live or killed bacteria to protect against atopy

Experiments in animals have provided clear evidence that the application of live or dead bacteria can inhibit the development of allergic disorders. For example, treatment of mice with killed *Mycobacterium vaccae*, *Listeria monocytogenes* or *Lactobacillus plantarum* suppressed the production of allergen-specific IgE in mice[22–24]. Furthermore, the application of live *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) intravenously or directly into the lungs of mice suppressed allergen-induced airway eosinophilia and the development of airway hyperresponsiveness (AHR)[25,26]. Also, the application of heat-killed BCG to the lungs of mice inhibited the development of allergen-induced airway eosinophilia up to two months after treatment (K.J. Erb, unpublished). Moreover, lower rates of atopy among children living in Guinea-Bissau were attributed to vaccination with BCG early in life[27]. This suggests that a simple and widely used BCG vaccination, applied to protect against tuberculosis, could reduce the risk of developing atopic disorders. However, other studies found no protective effect of BCG vaccination on the development of atopy[28,29]. The reason for this discrepancy is not clear, but might be due to different lifestyles and other environmental factors influencing the development of atopy in the children analyzed in two of the three studies (e.g. rural Africa compared with Sweden[27,28]).

Is it possible to use bacteria or their products to protect humans from the development of atopic disorders? As mentioned previously, two potential uses are conceivable. It might be possible to use attenuated bacteria or their products as adjuvants in conjunction with allergens to vaccinate children. The goal of these vaccinations would be to induce the production of IL-12 and IFN-γ at the site of allergen-specific Th-cell priming, leading to long-lasting Th1 memory responses against the allergen. It is hoped that upon future encounter with the allergen, allergen-specific Th1 cells would secrete IFN-γ, leading to the suppression of Th2-cell development. A second approach would be to apply attenuated bacteria or their products to young children (for example into the lungs), inducing strong Th1 immune responses. It is hoped that this early, and possibly first, strong immune response could then bias all further immune responses towards a Th1 type and away from a Th2 type.

However, both approaches rely on the induction of strong Th1 immune responses. For this reason, relatively large amounts of bacteria might have to be used, resulting in severe immunopathology. Furthermore, treating young children with live attenuated bacteria might lead to bacterial disease. For these reasons, the use of live bacteria might have to be discarded with respect to human use. By contrast, the use of killed bacteria, or defined bacterial components or products seems more promising. Interestingly, this might include the use of endotoxin, derived from the cell wall of Gram-negative bacteria, because it has been shown that lipopolysaccharide (LPS) abolished inflammatory bronchoconstriction of the airways after antigenic challenge of the airways[30,31]. Furthermore, epidemiological studies suggest that exposure early in life to endotoxin helps to inhibit the development of atopy[32]. The rationale behind the inhibitory effect of endotoxin on the development of allergic responses is that endotoxin induces the production of IL-12 by monocytes and dendritic cells (DCs). However, it has been reported also that allergic airway inflammation and production of IgE were enhanced when LPS was given after sensitization with allergen[33]. Furthermore, any potential use of endotoxin in humans needs to be carefully scrutinized, because the application of endotoxin can directly induce tumor necrosis factor α (TNF-α)-mediated toxic shock.

The results described suggest clearly that dead bacteria or bacterial products might prove very valuable as components of anti-atopic vaccines in humans. However, it appears that one of the major obstacles might be to develop an immunization scheme whereby sufficient amounts of dead bacteria (or bacterial products), alone or with an allergen, can be administered to suppress atopic disorders without causing serious side-effects associated with the inflammatory response initiated by the bacterial components.

Vaccines based on CpG-ODNs to protect against allergic disease

CpG oligodeoxynucleotides (CpG-ODNs) form part of the noncoding DNA sequences of bacterial DNA. Viral and vertebrate DNA also contains CpG motifs, although to a lesser extent. In addition, the CpG motifs of vertebrates are methylated and, therefore, nonmitogenic for lymphocytes (reviewed in Refs 34,35). CpG-ODNs have multiple stimulatory effects on lymphocytes, including DCs, macrophages, B cells, natural killer (NK) cells and T cells. DCs and

macrophages are stimulated directly by CpG-ODNs, resulting in the up-regulation of expression of costimulatory molecules (e.g. MHC class II, CD80 and CD86) and the production of IL-1, IL-6, IL-12, IL-18, TNF-α and IFN-α (Refs 34,35). The result of CpG-ODN-mediated activation of lymphocytes is the development of strongly Th1-biased immune responses, owing to IL-12 and IL-18 produced by APCs and IFN-γ secreted by NK cells. For this reason, several studies have addressed the question of whether CpG-ODNs can be used as a vaccine to protect against the development of atopic disorders. It was reported that the intranasal or intraperitoneal administration of CpG-ODNs reduced allergen-induced airway eosinophilia and AHR (Ref. 36). Furthermore, the production of eosinophils in the bone marrow and blood was also suppressed by the application of CpG-ODNs. Interestingly, spleen cells from CpG-DNA-treated mice secreted less IL-5 and more IFN-γ after *in vitro* stimulation with ovalbumin (OVA) than controls. These results suggest that CpG-DNA strongly suppressed the development of allergen-induced asthma in mice. It appears that the basis for this inhibition is an immune-diversion from an allergen-specific Th2 to Th1 response. Two other studies by Kline *et al.* and Sur *et al.* also showed clearly that intraperitoneal or intranasal application of CpG-ODNs inhibited the development of allergic Th2 responses in mice[37,38].

One important factor governing the use of CpG-DNA in humans is the question of how long the inhibitory effect on Th2-cell development lasts. Broide *et al.*[36] and Kline *et al.*[37] reported only short-term effects. However, Sur *et al.*[38] reported a relatively long-lasting effect; mice immunized with ragweed showed reduced lung inflammation and bronchial AHR up to six weeks after treatment with CpG-ODNs. Importantly, the inhibitory effect was associated with ragweed-Ag-specific Th1 memory responses in the spleen and lung. Furthermore, an increase in the number of B cells producing allergen-specific IgG2a and a decrease in the number producing allergen-specific IgE and IgG1 was also observed in the CpG-ODN-treated animals in comparison with the controls. Mice deficient in IFN-γ did not switch from a Th2 response to a ragweed-specific Th1 response. This finding identifies IFN-γ as one of the major players in CpG-ODN-mediated suppression of allergic Th2 responses. The current view is that the application of CpG-DNA induces the production of IFN-α, -β, and -γ, IL-12 and IL-18, thereby, leading to the development of Ag-specific Th1 cells and interfering with the development of allergen-specific Th2 cells. The generation of allergen-specific Th1 cells is believed also to be an important prerequisite of CpG-ODN-mediated inhibition of allergic responses upon subsequent encounters with allergen. However, there is also some evidence to suggest that IFN-γ and IL-12 might not be essential for CpG-ODN-mediated inhibition of allergic responses[39].

Can CpG-ODNs be used in humans to inhibit the development of asthma? *In vitro* experiments have shown clearly that human cells react to CpG-DNA in a similar manner to lymphocytes from rodents[40]. This suggests that the treatment of humans with CpG-ODNs could be very effective in inhibiting the development of asthma. The same approaches might be used as described for dead or live bacteria. CpG-ODNs could be applied systemically or locally to young children to bias all further immune responses in the lung towards Th1 and away from Th2 responses. Alternatively, CpG-DNA could be given together with the allergen to establish long-lasting allergen-specific Th1 memory responses. The results obtained from animal models suggest that it is probable that these approaches might also be successful in humans to reduce the development of atopic disorders. However, treatments using CpG-ODNs rely on both innate and adaptive pro-inflammatory Th1 immune responses to inhibit Th2 responses. For this reason, harmful side-effects of the treatment need to be ruled out. Besides the potential problem of inducing strong inflammatory responses at the site of exposure to allergen, the use of CpG-DNA could also have other serious side-effects. It has been reported that the application of CpG-ODNs can cause septic shock in mice[41]. A further potential problem might be the development of autoimmune disease after the application of CpG-DNA. Residual autoreactive T cells might become sufficiently activated to cause disease after encountering APCs that have been unspecifically activated by CpG-DNA. Supporting this view is the finding that autoimmune myocarditis[42], transient inflammatory arthritis[43] and experimental autoimmune encephalomyelitis[44] were exacerbated by the application of CpG-ODNs. However, it is totally unclear if this can also occur in healthy rodents or, more importantly, humans. Results reported by Shirota *et al.*[45] support the use of CpG-ODNs as a vaccine against atopic disorders. They found that significantly less CpG-DNA was required to inhibit allergen-induced eosinophilia in mice when the allergen was directly conjugated to the CpG-ODNs than when the allergen was mixed with CpG-ODNs.

Vaccines based on plasmid DNA to protect against allergic responses

DNA vaccination is based on the injection of plasmid (p)DNA encoding a defined Ag, leading to a strong and long-lived cellular and humoral immune response[46–48]. After the intradermal or intramuscular injection of the pDNA, transfected cells synthesize Ag continuously, providing long-lasting stimulation of the immune system, without the need for booster immunizations. A further advantage of DNA vaccines over most conventional vaccines is that they lead to the processing of Ag and loading of peptide onto both MHC class I and MHC class II molecules, thereby

activating both $CD4^+$ Th cells and $CD8^+$ cytotoxic T lymphocytes (CTLs). DNA vaccines are considered to be two-component systems (reviewed in Ref. 49). One component is the Ag-encoding transcription unit together with the eukaryotic promoter and polyadenylation terminator sequences responsible for protein synthesis. The second component is the CpG motifs contained in the plasmid backbone, which lead to very strong Th1-biased responses towards relatively small amounts of Ag.

DNA vaccination has been used successfully in several different animal species, including primates, conferring complete or partial protection against the development of infectious diseases, including hepatitis B virus, HIV-1, influenza virus or *M. tuberculosis* (reviewed in Ref. 50). In addition, there is also evidence to suggest that DNA vaccinations could be used to protect against the development of atopic disorders. It was reported that the injection of pDNA containing the cDNA encoding the house dust mite allergen Der p5 inhibited the production of Der-p5-specific IgE, AHR and the release of histamine in the airways after challenge with allergen in rats[51]. Also, injection of pDNA encoding the latex allergen Hev b5 decreased the Ag-specific IgE response after the allergy had already been established[52]. Moreover, the application of a DNA vaccine encoding OVA resulted in a shift towards an OVA-specific Th1-biased immune response, leading to the inhibition of airway eosinophilia after immunization with OVA in mice[53]. The efficacy of the DNA vaccine could be enhanced by fusing the cDNA encoding OVA with the cDNA encoding IL-18 (Ref. 54). Injection of this fusion construct, in contrast to the pDNA encoding OVA only, protected mice from allergen-induced AHR and reversed pre-existing AHR. This effect was dependent on IFN-γ and $CD8^+$ T cells. Interestingly, the fusion construct was also more efficient in inhibiting allergen-specific Th2 responses than a mixture of OVA-encoding pDNA and IL-18-encoding pDNA. Mucosal gene vaccination was also successful in inhibiting the development of asthma in a murine model, when the plasmid only contained the cDNA encoding IFN-γ, and not OVA (Ref. 55). This shows that both allergen-specific and -unspecific DNA vaccination can protect animals from developing allergic Th2 responses.

These results have led to the suggestion that DNA vaccination might also be used in humans to protect against the development of atopic disorders. However, initial trials in humans for the treatment of malaria and infections with HIV-1 (reviewed in Ref. 56) produced very low Ag-specific immune responses, in comparison to the very promising results obtained in rodents. Therefore, there appears to be a need to optimize the DNA vaccines for human use. One approach is to modify or enhance the number of CpG motifs contained in the pDNA, because optimal triggering of Th1 responses in rodents and humans is achieved by slightly different motifs[35]. However, it must be ensured that a DNA vaccine triggering optimal Th1 responses does not lead to pathological inflammatory responses or toxic shock, which have been reported to occur after the application of CpG-ODNs (Ref. 41). A further approach is the incorporation of cDNAs encoding cytokines, chemokines or costimulatory molecules associated with the suppression of Th2 responses or enhancement of Th1 responses[57–59]. Furthermore, it was shown that DNA vaccination followed by infection with a recombinant vaccinia virus (expressing the same malarial Ag as the pDNA), in contrast to pDNA or vaccinia virus given alone, induced complete protection from an infection with *Plasmodium berghei*[60]. This suggests that combining a DNA vaccine with other vaccination strategies might lead to stronger Th1 immune responses against an encoded allergen, thus protecting humans from the development of atopy, whereas each individual approach might prove fruitless. All data currently available suggest that DNA vaccination is efficient and safe in protecting animals, not only against infectious disease but also, allergic disorders. However, it seems that the DNA vaccines tested for protection against allergic disorders in animals need to be further optimized for human use. The incorporation of cDNAs encoding cytokines, chemokines or costimulatory molecules might facilitate their development.

The application of recombinant cytokines to protect against atopy

The vaccination strategies discussed are aimed at inducing the production of IL-12, IL-18 and IFN-γ *in vivo*. Therefore, it might be possible to use these cytokines directly as components of anti-atopic vaccines. Supporting this view, is the finding that nebulized IFN-γ inhibited allergen-induced AHR, cutaneous anaphylaxis and secondary production of OVA-specific IgE in an animal model of asthma[61,62]. Gavett *et al.* reported a similar effect when IL-12 was administered to the lungs at the time of antigenic challenge of the airways[63]. However, this result could not be confirmed by another study in which IL-12 had to be given together with IL-18 to produce the effect described by Gavett *et al.*[64] Interestingly, when given alone, IL-18 enhanced Ag-induced recruitment of eosinophils into the airways and exacerbated airway inflammation[65,66]. Taken together, there is some evidence to suggest that the use of IFN-γ, IL-12, or IL-12 and IL-18 as components of vaccines could help to reduce the risk of developing atopy in humans. However, owing to the problems generally associated with cytokine therapy and the reported morbidity and mortality associated with the use of IL-12 in clinical trials[67], the other approaches to a vaccine protecting against allergic disorders described in this review seem more promising.

The use of DCs as components of vaccines to inhibit allergic diseases

Recent studies have shown that distinct subsets of DCs exist in both mice and humans and these can direct immune responses differentially towards Th1 or Th2 types (Refs 68–70). Furthermore, all prospective vaccines discussed so far involve the stimulation of DCs that secrete IL-12, and drive allergen-dependent or -independent Th1 responses. It might be possible to bypass the induction of these DCs *in vivo*, by directly differentiating these cells *in vitro* from progenitors present in the blood of patients[71,72], pulsing the DCs with allergen and re-administering the cells back into the patient. At least in theory, these cells should induce allergen-specific Th1 cells upon encountering naive T cells. Supporting this view are experiments in which the vaccination of animals with DCs pulsed with Ag provided very efficient protection against infectious diseases (reviewed in Ref. 73) or cancer (reviewed in Refs 74,75). Initial clinical trials using DCs have already started in patients with cancer. However, it remains to be elucidated, both in animals and humans, whether it will be possible to generate DCs *in vitro*, which predominantly induce allergen-specific Th1 responses *in vivo*, leading to the inhibition of allergic disease.

Anti-atopy vaccines aimed at inducing CD4+ T regulatory cells

Recent studies indicate that CD4+ T regulatory (Tr) cells play a key role in the maintenance of T-cell tolerance against self- and foreign-Ags (Ref. 76). This effect is achieved by the production of the immunosuppressive and anti-inflammatory cytokines transforming growth factor β (TGF-β), IL-10 and IL-4 in an Ag-specific context, resulting in the suppression of Th effector functions. Several different subtypes of Tr cells have been described, according to which cytokines they produce. For example, the secretion of TGF-β, IL-10 and/or IL-4 characterizes Tr1 cells[77]. By contrast, Th3 cells produce TGF-β only[76]. Animal studies have shown that Tr cells are involved in the inhibition of diseases mediated by both Th1 cells (autoimmune) and Th2 cells (allergic)[78–80]. Furthermore, it was reported that the introduction of *in-vitro*-generated allergen-specific Tr cells into mice protected the animals from the development of asthma[81–83]. A human vaccine aimed at inducing allergen-specific memory Tr cells seems desirable, because this approach would eliminate the potential problems associated with the induction of pro-inflammatory Th1 responses. However, it is not well established how a vaccine might be designed to induce allergen-specific Tr cells selectively, and not Th1 or Th2 cells. Animal experiments point to oral vaccination with Ag as a potential candidate for the induction of Tr cells[78,79] (see later). Furthermore, the use of immature DCs pulsed with allergen might induce Tr cells selectively (reviewed in Ref. 84). It remains to be determined if it will be possible to induce allergen-specific Tr cells in humans to protect against atopic disorders.

Potential alternative approaches leading to an anti-allergy vaccine

Allergen-specific immunotherapy has been used extensively over the past years to treat atopic patients. The most-promising results are observed in patients suffering from rhinitis or with allergies to bee and wasp venom[85,86]. Successful treatment is achieved by multiple, subcutaneous injections of the respective allergen. The immunological mechanisms leading to a reduction in allergic effector functions are unclear. However, effective treatment is associated with an increase in the level of allergen-specific IgG. Furthermore, there is also evidence to suggest that a shift from Th2 to Th1 responses occurs, without causing potential side-effects associated with a Th1 response[87,88]. It is conceivable that allergen-specific desensitization might be used also in patients at risk from developing atopy as a preventive vaccination strategy. However, it remains to be seen whether this approach will help to reduce the risk of becoming allergic after treatment or whether the application of large amounts of allergen might actually facilitate the development of atopy and, possibly, anaphylaxis.

The oral, inhaled or sublingual administration of specific allergens (mucosal vaccination) might be another possibility to prevent the development of allergic diseases. The aim of this method is to induce Ag-specific T-cell tolerance, as a result of deletion and/or anergy of Ag-responsive cells or active suppression by Tr cells (see previous section). Although the exact mechanism by which mucosal vaccines induce tolerance against allergens remains unclear, it appears that the duration of exposure to allergen and the dose of Ag are the decisive factors responsible for the suppression of allergic responses. The application of high doses of allergen seems to be associated with anergy and/or deletion of allergen-specific Th2 cells. By contrast, the application of low doses of allergen appears to result in active suppression by Tr cells[89]. In support of the view that mucosal vaccines might be used in humans to protect against the development of atopic disorders are reports showing that the mucosal administration of allergen inhibits the development of allergic diseases in animals[90,91]. Furthermore, atopic patients treated orally, sublingually or by inhalation with mite or pollen Ags showed decreased allergic inflammation[92–94] and reduced allergic symptoms[95]. Clinical trials of mucosal vaccines have begun and it will be of great interest to see if they can be used successfully to inhibit the development of atopy.

A further prospective therapeutic approach, which might also be used as an allergy-prevention measure, is the application of allergen-derived peptides that bind to MHC class II molecules. The idea behind this approach is to supply the peptides in a manner that

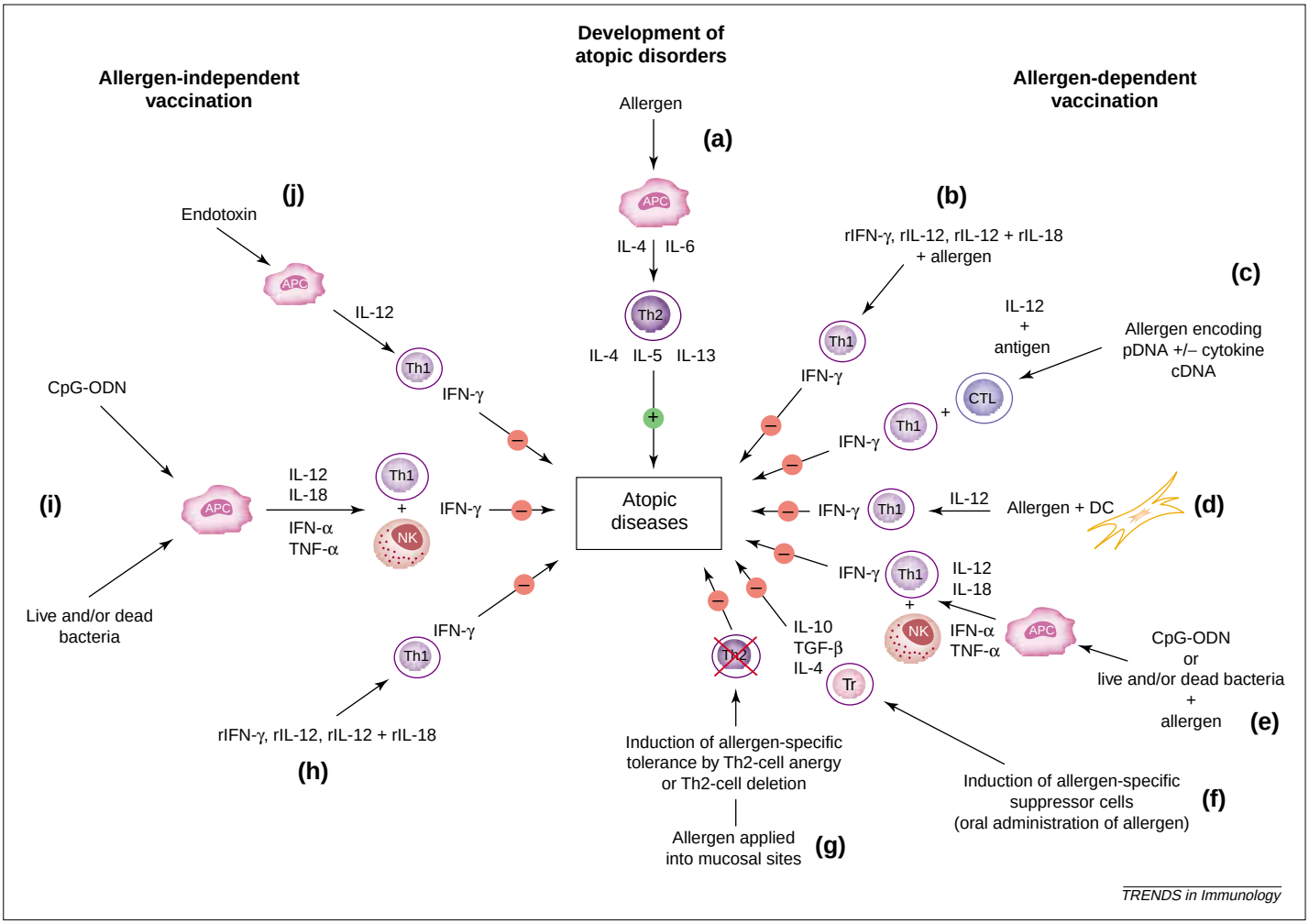

Fig. 1. Model of prospective vaccination strategies to inhibit the development of atopic disorders in humans. (a) Genetic and environmental factors determine whether allergen-specific T helper 2 (Th2) cells develop. The secretion of interleukin-4 (IL-4), IL-5 and IL-13 by Th2 cells leads to allergic disease. (b) The administration of allergen together with the recombinant cytokines interferon γ (rIFN-γ), rIL-12 or rIL-18, alone or in combination, induces the development of allergen-specific Th1 cells, leading to a long-lasting inhibition of Th2-cell development. Further approaches also possibly inducing the development of allergen-specific Th1 cells include (c) vaccinating with plasmid (p)DNA; (d) pulsing dendritic cells (DCs) with allergen and applying them back into humans; or (e) administering allergen together with CpG oligodeoxynucleotides (CpG-ODNs) or live and/or dead bacteria. (f) Administering allergen orally or by other as yet undefined routes might lead to the development of allergen-specific T regulatory (Tr) cells. These cells inhibit all further allergen-specific Th2 responses by producing transforming growth factor β (TGF-β), IL-10 and/or IL-4 at the site of naive T-cell priming. (g) Mucosal vaccines (allergen applied orally, sublingually or by inhalation) might inhibit allergen-specific Th2 responses by inducing Th2-cell tolerance or Th2-cell deletion. Vaccinations independent of the use of allergen are also conceivable. Treating young children with reagents that cause local or systemic Th1 responses might bias all further responses against allergens away from a Th2 type towards a Th1 type, resulting in protection against developing atopy later in life. This could be achieved by applying (h) the major recombinant cytokines mediating Th1-cell effector functions (rIFN-γ, rIL-12 or rIL-18, alone or in combination); (i) CpG-ODNs or live and/or dead bacteria; or (j) endotoxin, in amounts guaranteed not to cause any complications associated with the production of tumor necrosis factor α (TNF-α). Abbreviations: APC, antigen-presenting cell; CTL, cytotoxic T lymphocyte; NK, natural killer cell.

induces T-cell tolerance, thereby inhibiting the development of atopy-causing allergen-specific Th2 cells (vaccination) or suppressing the expansion of pre-existing allergen-specific Th2 cells (therapy). This approach has been used successfully in both mice and humans injected subcutaneously with peptides derived from allergen[86,96,97]. In addition, altered peptide ligands might be used to inhibit the development of atopy. It has been shown that mice vaccinated with analogs of peptides that normally induce Th2 responses were protected from developing allergic symptoms[98,99]. The inhibition of allergic responses was associated with the development of allergen-specific Th1 responses. Treating humans with altered peptide ligands derived from the most-relevant allergens might also lead to the development of long-term allergen-specific Th1 memory responses, with all the potential benefits, but also, possible negative side-effects, associated with allergen-specific Th1 responses.

Conclusions

The incidence and severity of atopic diseases are increasing and they continue to be a major health hazard in the developed nations of the world. Despite intensive research, no effective prevention measure exists to date. However, recent animal experiments have shown clearly that it is possible to vaccinate animals to protect them from developing allergic Th2 responses. This suggests that this might be possible also for humans. Based on the experiments performed in animals, three approaches seem the most promising.

First, atopy-prone children could be vaccinated with reagents that induce the production of IFN-γ and

IL-12, leading to systemic or local, allergen-independent Th1 immune responses. It is hoped that this will protect children from developing allergen-specific Th2-type cells during the immediate post-neonatal time period, when children are believed to be most susceptible to the induction of atopic disease. Second, vaccines could be used that combine reagents that induce strong Th1 responses with defined allergens. The goal is to induce selectively long-lived allergen-specific memory Th1 cells. It is hoped that upon re-encountering allergen, these cells will secrete IFN-γ, thereby suppressing the development of allergen-specific Th2 cells. Both approaches might prove successful, because adjuvants that induce Th1 responses are readily available and have been shown to inhibit the development of allergic responses in animals. The most promising candidates are killed bacteria (or components derived from them), CpG-ODNs or pDNA. However, all approaches that induce Th1 responses have the potential side-effect of Th1-cell-mediated inflammation, potentially causing serious tissue damage. Supporting this view are reports showing that allergen-specific Th1 cells adoptively transferred into naive mice caused inflammation after the application of the respective allergen[13,14]. The third and, perhaps, safest approach might be the induction of deletion and/or anergy of allergen-specific Th2 cells or the induction of allergen-specific Tr cells by mucosal vaccines.

The data currently available clearly give rise to the hope that it might be possible to vaccinate humans against atopy in the near future. However, the safety of a prospective vaccine needs to be established clearly because, generally, atopic disorders are not life threatening (with the important exception of severe asthma) and the most-promising targets for an efficient vaccination are young children. Furthermore, owing to the complex nature of atopic disorders, vaccinations might not be expected to inhibit totally the development of an allergic disorder but might, hopefully, at least result in a significant reduction of its severity. Furthermore, the vaccination strategies discussed in this article might help also to reduce allergic symptoms in patients already suffering from atopic diseases. Supporting this view is the recent report by Arkwright and David, showing that the intradermal application of a suspension of killed *M. vaccae* led to the improvement of atopic dermatitis in children[100]. Figure 1 shows a summary of the prospective vaccination strategies possibly leading to the inhibition of atopic disorders in humans.

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The mucosal immune system: primary target for HIV infection and AIDS

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Despite intensive research, several questions remain regarding the pathogenesis of infection with HIV-1. Recently, it has been shown that simian immunodeficiency virus (SIV) selectively targets and destroys specific subsets of CD4+ T cells that are abundant in mucosal tissues but rare in peripheral lymphoid tissues. This finding could be highly relevant in explaining a major paradox in the infection and elimination of CD4+ T cells during HIV infection: the progressive decline in the number of CD4+ T cells in the blood, despite the paucity of HIV-infected cells in this tissue. This article discusses the hypothesis that infection with HIV and SIV, and the resulting disease, is governed by the state of cellular activation and the expression of chemokine receptors by specific subsets of CD4+ T cells residing in mucosal lymphoid tissues, rather than those found in the peripheral blood or lymph nodes.

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The discovery that specific chemokine receptors act as co-receptors for both human and simian immunodeficiency viruses (HIV and SIV) has shed light upon the mechanisms underlying viral entry and tropism[1–6]. It is now known that, in addition to the CD4 molecule, most strains of HIV and SIV use the CC-chemokine receptor CCR5 for viral attachment and entry into host cells[1,6–9]. Furthermore, most, if not all, of the strains responsible for the transmission of HIV use CCR5, whereas viral strains that emerge later in the disease process might use the CXC-chemokine receptor CXCR4 instead of, or in addition to, CCR5 (Refs 2,10,11). It has even been hypothesized that progression from the asymptomatic stage of infection with HIV to the development of full-blown AIDS (and depletion of CD4+ T cells in peripheral blood) might be triggered by a switch in the viral usage of chemokine receptors from CCR5 to CXCR4 (Refs 10,11). If correct, this could, at least partially, explain the mechanism behind the apparent delay in disease progression, as well as the profound drop in the number of peripheral CD4+ T cells observed in the later stages of infection with HIV. However, more-recent studies have demonstrated that CCR5-utilizing strains predominate in cells from chronically infected patients[12]. Moreover, studies in the SIV rhesus macaque model indicate that such a switch in the expression of chemokine receptors is not necessary for peripheral CD4+ T-cell depletion and the development of AIDS in macaques. Recent studies indicate that all pathogenic SIV strains tested to date use CCR5 primarily[1,9]. Furthermore, CCR5 tropism persists in SIV-infected macaques sacrificed in advanced stages of SIV infection or AIDS (Ref. 13). Therefore, we hypothesize that it is the state of cellular activation and CD4+ T-cell turnover within mucosal tissues that determines the progression of disease and pathogenesis of AIDS, rather than a switch in the use of chemokine receptors.

The current controversy over CD4+ T-cell turnover in HIV infection

Early in the HIV epidemic, there seemed to be little controversy over the theory that HIV preferentially infected and then destroyed CD4+ T cells as a result of lytic viral replication. This assumption was supported by the profound loss of CD4+ T cells in the blood of patients with AIDS, as well as the discovery that the CD4 molecule is the major receptor for the attachment and entry of HIV into cells[14]. However, this relatively simple explanation was complicated by data showing that the number of infected cells in the

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