Allergy immunotherapy and inhibition of Th2 immune responses: a sufficient strategy?

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Th2 immune responses mediated by the secretion of IL-4, IL-5 and IL-13 are key in the pathogenesis of atopic disorders, including allergen-induced asthma, rhinoconjunctivitis and anaphylaxis. Although such responses are downregulated to some degree by conventional specific immunotherapy, this approach is only partially effective and has a substantial risk of adverse effects. Many strategies for immunotherapeutic prophylaxis and for treatment of atopic diseases have been devised on the basis of mouse allergy and autoimmune models, including the downregulation of Th2 responses by the induction of regulatory T cell activity, Th2 to Th1 immune deviation, Th1 crossregulation of Th2 immune responses, anergy and immunosuppressive cytokines. The blockade of events that are not allergen-specific, such as T cell costimulation and downstream events dependent on IgE, cytokines and chemokines, has also been pursued. With the exception of monoclonal antibody therapy for the blockade of IgE effector function, the application of most of these strategies to humans is at an early stage. Whether the inhibition of Th2 responses without concurrent downregulation of Th1 responses will be sufficient for allergic immunotherapy, particularly for atopic dermatitis and asthma, is an important but unresolved issue.

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Abbreviations

APC	antigen presenting cell
APL	altered peptide ligand
CpG-ODNs	CpG-containing oligodeoxynucleotides
IL	interleukin
ISS	immunostimulatory sequences
sIL-4Rα	soluble form of the IL-4 receptor α chain
SIT	specific immunotherapy
TGF-β	transforming growth factor-β
vCCI	viral CC chemokine inhibitor

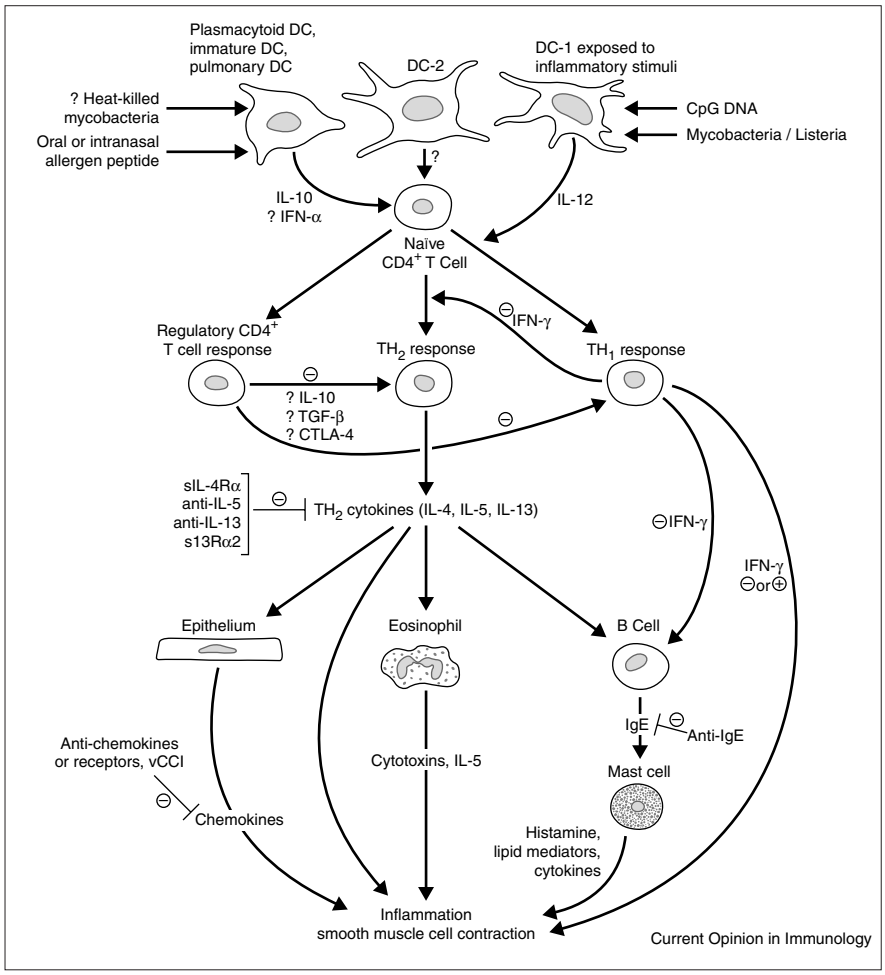
Introduction

Atopic disorders have a complex and chronic pathogenesis that provides many potential cellular and molecular targets for therapeutic intervention, but may also include redundant pathways mediating disease (Figure 1). Atopic disease is dependent on the production of the cytokines IL-4, IL-5 and IL-13 by allergen-specific Th2 cells that are generated from naïve precursors. This generation is transcriptionally regulated in a positive manner by GATA-3 [1], a DNA-binding transcription factor that preferentially binds to GATA sequences, and is inhibited by T-bet (T-box expressed in T cells; [2•]), a member of the T-box transcription factor family. IL-4 and IL-13 mediate B cell isotype switching and production of IgE. The binding of allergen-specific IgE to mast cells results in allergic sensitization [3]. Experimental allergen challenge following sensitization results in stereotypical early and late phase events: the early phase is transient, occurs within minutes after challenge, and includes vasodilatation, oedema and smooth muscle cell contraction due to mast cells releasing histamine and lipid derivatives. Mast cells also produce cytokines and chemokines that contribute to a late phase response that begins hours later and is heralded by the onset of local inflammation with Th2 cells, eosinophils, monocytes and basophils, often with recurrence of early phase events, such as smooth muscle contraction. Late phase Th2 cells may be activated by dendritic cells presenting allergen [4,5•] to release cytokines that promote and maintain chronic allergic disease (Figure 1). These effects include IL-5-mediated increases in eosinophil production and survival, increases in endothelial adhesion molecules and chemokine production mediated by IL-4 and IL-13, and the promotion of smooth muscle hyperreactivity and Goblet cell mucus secretion by IL-13 [6]. This review will initially examine the mechanisms of antigen-specific immunotherapy for atopic disorders in humans. Immunotherapy in the broader sense involving costimulatory blockade, adjuvants, cytokines, inhibitors of cytokines, chemokines or their receptors, will then be briefly covered, as these studies provide insights into targets that may be useful for future immunotherapeutic approaches.

Conventional specific immunotherapy and its modification

Conventional specific immunotherapy (SIT) for atopic disease was first described in 1911, and typically involves repeated subcutaneous injection of increasing amounts of allergen extract mixtures. Although SIT can treat anaphylaxis and allergic rhinitis, particularly for certain allergens, it remains controversial for the treatment of asthma. Unfortunately, SIT itself can induce anaphylaxis as well as new IgE responses for allergens contained in the extract [7]. The use of purified recombinant protein allergens rather than allergy extracts for SIT may permit the allergen dose to be safely increased and alternative and less invasive routes to be used, such as sublingual administration [8]. The potential therapeutic benefit of using these alternative routes is suggested by studies in rodents, which have found that administration of whole adjuvant-free protein allergens intranasally or orally may decrease subsequent experimentally-induced allergic sensitization and disease [9,10]. Such protection may be mediated by

Figure 1



Immune pathways of allergic disease and potential targets for immunotherapy. Naïve CD4+ T cells exposed to allergenic peptides by dendritic cells may differentiate into either Th1 or Th2 effector cells or regulatory T cells, depending on the dendritic cell type and the local microenvironment. Th2 cytokines promote allergic disease by multiple mechanisms, such as enhanced IgE expression, production of eosinophils, the induction of chemokines by epithelial cells and other cell types, as well as direct effects on smooth muscle cells. IFN-γ production by Th1 cells can inhibit Th2 differentiation; it may also enhance established allergic disease, particularly inflammatory mechanisms. By contrast, regulatory CD4+ T cells may suppress both Th1 and Th2 immune responses, although the mechanisms involved remain controversial. Antigen-specific immunotherapy targets early events involving CD4+ T cell activation and effector generation and function. Non-antigen-specific therapies may target either early events (e.g. mycobacterial products for induction of Th1 or regulatory CD4+ T cell differentiation) or later events (e.g. blockade of cytokines, chemokines or IgE with their respective high-affinity receptors).

the uptake of allergen by dendritic cell populations, such as those of the lung, that favor the induction of T cells with immunosuppressive regulatory activity rather than Th2 responses [11••]. Whether analogous approaches in humans will be useful for SIT is currently under investigation.

There are several approaches by which allergens may be modified to improve the safety of SIT. Allergenic proteins produced by cDNA technology can be truncated [12] or mutated, for example, by the replacement of intramolecular disulfide bonds or other key residues. These alterations may abrogate sites of IgE recognition and further reduce the risk of anaphylaxis. By using overlapping allergen fragments, T cell epitopes can be preserved with certainty.

The mechanism by which conventional SIT ameliorates allergic disease provides clues as to clinically useful targets for newer immunotherapies. Successful SIT has been associated with decreased *ex vivo* T cell allergen-specific proliferation, a reduced ratio of IL-4, IL-5 or IL-13 production relative to the production of IFN-γ, increased production of IL-10, and an elevated ratio of allergen-specific IgG4 to IgE in serum [13–15]. SIT also decreases the expression of IL-4 and IL-5 mRNA and the numbers of mast cells, basophils and eosinophils, and increases the frequency of IL-10-producing cells, at sites of allergic disease in humans in *vivo* [16–19]. The cellular sources of IL-10 following SIT appear initially to involve T cells, possibly a immunosuppressive regulatory cell subset (see below), followed by subsequent production by B cells and monocytes [13].

Increases in IgG4 and decreases in IgE often occur months after a therapeutic response is observed with SIT [13], suggesting these alterations are a late marker for successful SIT rather than IgG4 serving as a central mechanism to block the effects of IgE. These alterations in immunoglobulins by SIT may be due to increased production of IL-10, which directly acts on B cells to promote production of IgG4 relative to IgE. Children with early exposure to allergens who do not develop allergic disease also frequently develop a high ratio of allergen-specific IgG4 to IgE [20••], and it will be of particular interest to determine if this is mediated by IL-10.

Immunotherapy using peptides

Short allergen peptides, either native sequences or altered peptide ligands (APLs), with amino acid substitutions, are

attractive for SIT as they are unlikely to contain epitopes for IgE binding or to induce anaphylaxis. As for whole protein allergens, the administration of adjuvant-free peptides by various routes, such as intranasally, can suppress subsequent allergic sensitization in rodents. These reduced responses are not limited to the peptide, but also apply to the whole protein from which the peptide is derived [21,22] as well as to immunologically distinct co-immunized proteins [23]. Together, these findings suggest that reduced responses are due to the induction of regulatory T cells rather than by peptide-specific clonal deletion, anergy, or immune deviation. The effect of peptide administration on Th1 responses may vary, depending on whether it is administered before or after allergen sensitization, whereas either approach results in decreased Th2 responses.

Intradermal immunotherapy using short peptides has also provided direct evidence in humans that $CD4^+$ T cells mediate late phase allergic reactions, for example, bronchoconstriction, in the absence of an early phase response [24]. Alterations in lung inflammation, cytokine expression, or levels of lipid mediators were not detected, so the *in vivo* site where the allergenic peptide activates $CD4^+$ T cells remains unclear, as does the mechanism of bronchoconstriction [25]. This peptide-induced late phase reaction is associated with subsequent and persistent attenuation of late-phase bronchoconstriction, and with decreased T cell proliferation and Th2 cytokine production to either peptide or whole protein [26•], suggesting a regulatory cell mechanism.

Although peptide therapy is theoretically attractive as a means to avoid IgE-mediated early phase reactions, it is important to note that serum IgE in allergic individuals may often bind to relatively short linear epitopes of protein allergens [27], indicating the potential for foreign peptide administration to induce IgE-dependent disease. Such induction may account for apparent early phase type responses in patients receiving APL immunotherapy for multiple sclerosis [28]. The ability of peptide immunotherapy to induce atopic disease is also supported by the observation that anaphylaxis can be induced in mice by the administration of peptides and adjuvant [29].

Rodent studies suggest that, under highly controlled experimental conditions, allergic disease can be inhibited by APL administration. Whether this is due to Th2 to Th1 immune deviation or the induction of regulatory T cells remains unclear [30]. Unlike murine T cells, human T cells express class II MHC and can be used for autopresentation of peptides. Under such conditions, APLs induced anergy of human allergen-specific $CD4^+$ T cells *in vitro*, with anergy associated in some instances with increased production of IL-10 and IFN-γ, but not transforming growth factor-β (TGF-β; [31]). The applicability of this approach to anergy induction *in vivo*, in which APCs might also bind the APL, is unclear.

A potential barrier to peptide-based immunotherapy for allergy is the apparent complexity of the allergen-specific T cell response in terms of epitope usage in humans. An individual's allergen-specific T cell repertoire may contain multiple specificities, probably reflecting the recognition of multiple epitopes contained within the allergenic protein [32]. Moreover, the whole antigen and peptide-specific responses in terms of cytokine profile, for example IFN-γ versus IL-5 or IL-10, may not clearly segregate with whether the individual has an immediate versus a delayed-type hypersensitivity reaction to the allergen [32]. In cases where peptides are not 'promiscuous' in their binding to MHC molecules, peptide-based immunotherapy might also need to be tailored to be MHC allele-specific. Of particular concern is the possibility that the effects of peptides on T cell activation *in vitro* versus *in vivo* may differ, as was observed for the treatment of multiple sclerosis with an APL [33].

Renaissance of the regulatory T cells and emergence of immunosuppressive cytokines

Regulatory T cell populations that are generated intrathymically, such as the $CD4^+CD25^+$ subset, are important in the maintenance of peripheral tolerance to self-antigens or innocuous antigens of commensal bacteria [34]. Their physiological role in regulating the response to environmental allergens remains poorly understood. One study found that $CD4^+CD25^+$ T cells directly isolated from DO11.10 mice, in which $CD4^+$ T cells predominantly express a transgenic T cell receptor for an ovalbumin-derived peptide, increased rather than reduced ovalbumin-induced allergic disease. This was attributed to an indirect effect of decreasing Th1 responses [35]. $CD4^+CD25^+$ T cells with regulatory-like features can also be generated by oral or intranasal administration of whole protein to mice [11••,36], an approach that may mimic the consequence of SIT or high-dose allergen exposure in humans.

The induction of $CD4^+CD25^+$ regulatory T cells may be due to naïve $CD4^+$ T cells interacting with dendritic cell populations that are either immature [37–39] or that are mature but have not been exposed to inflammatory stimuli, and which produce IL-10 rather than IL-12 [11••]. Priming of murine or human $CD4^+$ T cells in *vitro* with IL-10 [38] or with certain pharmacological immunosuppressants [40•] results in an IL-10-producing $CD4^+$ T cell population, termed Tr1 cells. These Tr1 cells have regulatory activity and inhibit allergen-specific Th2-dependent responses by a mechanism that requires IL-10 secretion [41••]; however, the contexts in which Tr1-like $CD4^+$ T cells are generated *in vivo* remains unclear.

The mechanisms utilized by regulatory T cell populations generated *in vivo* for suppression of T cell-mediated immune responses remains controversial, although immunosuppressive cytokines, such as IL-10 and/or TGF-β have been implicated in some contexts. It has been proposed that the suppression of allergic disease following successful SIT or high levels of allergen exposure in humans is mediated by the production of IL-10 by regulatory T cells [13]. Although IL-10 can potently inhibit multiple aspects of APC and

T cell function [42•] and transiently inactivate chemokine receptors [43], it may also promote airway hyperreactivity [44–46] and even eosinophilia [47] in allergic models.

TGF-β either secreted by or associated with the cell membrane of regulatory T cells is also an attractive candidate to inhibit allergic responses [34]. For example, blockade of TGF-β/SMAD7 signaling in mice enhanced allergen-induced asthma [48], whereas the adoptive transfer of TGF-β-expressing allergen-specific $CD4^+$ T cell clones was protective [49]. TGF-β may be beneficial by inhibiting expression of GATA-3 and subsequent Th2 development [50], but may also contribute to some of the sequelae of atopic disease, such as airway fibrosis in asthma. Thus, for both IL-10 and TGF-β, it may be critical that these cytokines are expressed in a particular temporal and/or cellular context to achieve optimal immunotherapy and minimize adverse effects.

Th1 reponses – always protective and desirable?

A continuing and important point of controversy is whether the default response of nonatopics to allergen exposure is a Th1 response [51•] or induction of tolerance [52•]. If the former were typically the case, then Th2 to Th1 immune deviation would be expected to be generally useful for allergy immunotherapy. Many rodent studies have shown that increased *in vivo* expression of Th1 pathway cytokines, including IL-12, IL-18 and IFN-γ, achieved by various means [6,53] inhibits atopic disease. However, IFN-γ production by Th1 cells may also augment Th2-dependent pathology in murine models of atopic dermatitis [54] and asthma [55]. The co-administration of IL-12 with allergen by the intranasal route results in increased rather than decreased allergen-induced pulmonary inflammation [56]. Pulmonary infection with influenza, a respiratory virus that triggers a robust Th1 response, also interferes with the induction of tolerance to allergen by intranasal administration [57].

Some observations in humans also suggest a role for Th1-mediated immune responses in contributing to atopic disease. For example, human asthma may be characterized by increased expression of both Th2 and Th1 cytokines by T cells [58], and the airway of human asthmatics is characterized by constitutively increased activation of STAT1 [59] and expression of IL-12 p40 [60] compared to tissue from non-atopic individuals. Thus, although the central requirement for Th2 cytokines in the pathogenesis of allergic disease remains clear, Th1 and Th2 responses are not necessarily mutually exclusive or antagonistic in the context of chronic inflammatory responses to allergens. It is possible that such interactions might account, at least partially, for the disappointing results from administration of IL-12 to asthma patients who experienced a high frequency of serious side effects but no significant decrease in the late phase response to allergen challenge [61].

Adjuvants – bacteria and CpG-containing DNA

Although administration of live or heat-killed Mycobacteria [62] or other bacteria, such as Listeria, results in a robust Th1 response and can prevent or attenuate allergen-induced asthma in mice, results in humans with this approach have been mixed. Vaccination with heat-killed *Mycobacterium vaccae* may decrease the severity of atopic dermatitis in humans at least transiently [63], but was ineffective in reducing disease parameters in asthmatics [64]. Vaccination during early infancy with Bacille Calmette-Guerin, an attenuated strain of *Mycobacterium bovis*, also did not appear to reduce the subsequent risk of atopic sensitization or allergic disease [65]. Although Mycobacterial infection and product administration has been assumed to inhibit Th2 immune responses by inducing strong Th1 responses, a study using heat-killed *M. vaccae* suggests that regulatory $CD4^+$ T cells may also play role [66••].

Robust Th1 responses and increases in dendritic cell maturity can also be achieved in mice by the administration of DNA with certain immunostimulatory sequences (ISS) containing unmethylated cytosine residues adjacent to guanine residues (CpG motif; [67]). These responses are dependent on the interaction of CpG-containing DNA with Toll-like receptor 9 [68]. Oligodeoxynucleotides that incorporate these ISS (known as CpG-ODNs) are highly effective at preventing or ameliorating allergen-induced pulmonary disease and Th2 responses in mice [67,69]. In general, the direct conjugation of CpG-ODNs to allergenic proteins or peptides was more effective than their co-administration [70], possibly because of enhanced interaction with dendritic cells via the CpG moiety [71]. CpG-ODNs appear to inhibit Th2 responses and IgE production by B cells by an IFN-γ-dependent, NK cell-independent mechanism [66,72]. The evaluation of CpG-ODNs with ISS optimized for immunostimulation of primate and human leukocytes for treatment of allergic disease in humans is in progress.

Co-stimulatory blockade

Cytokine production by memory/effector T cells, particularly those of the Th2 subset, is highly dependent on costimulation via the ICOS/B7h (ICOS-ligand) pathway [73]. Blockade [74•] or genetic disruption [75] of this costimulatory pathway markedly reduced allergen-induced asthma in mice. Thus, blockade of ICOS/B7h interactions may be a promising approach for the treatment of allergic disease in humans.

Anti-IgE therapy

A humanized monoclonal antibody that blocks the interaction of human IgE with the high-affinity FcεR1 receptor expressed by mast cells and basophils has clear therapeutic benefit for asthma [76] and allergic rhinitis [77]. This unequivocally demonstrates the importance of IgE and the upstream cytokines required for its expression, IL-4 and IL-13, in the pathogenesis of asthma and other atopic disorders. This therapy has been well tolerated without reports of anti-antibody formation, but is expensive and a search for small molecules with similar blocking properties is underway.

Cytokine and chemokine targeted antagonist therapy

Evidence from rodent models and from human studies that correlated Th2 cytokine expression with allergic disease severity [3] suggested that Th2 cytokine blockade would be an effective treatment for asthma. However, only limited benefit was observed for asthma patients treated with inhaled soluble forms of IL-4 receptor α chain (sIL-4Rα) [78], a component of biologically active receptors for IL-4 and IL-13. IL-13 appears to be an important contributor to allergic pathogenesis independent of the effects of IL-4. Therefore, the finding that IL-13 induced airway hyperreactivity and inflammation in mice lacking the IL-4Rα chain suggests the existence of an alternative functional IL-13 receptor [79•], and also provides an explanation for the disappointing results with sIL-4Rα treatment. Complete IL-13 antagonism might be achieved by administration of a soluble form of the IL-13Rα2 chain [6], which serves as a high-affinity decoy receptor. Humanized anti-IL-5 monoclonal antibody treatment for asthma has also been disappointing therapeutically despite effective eosinophil depletion. Additional trials will be necessary to determine if more prolonged and complete Th2 cytokine antagonism is beneficial for asthma.

Chemokines, particularly those of the CC subfamily, are important in the pathogenesis of both inflammatory and non-inflammatory aspects of allergic disease. Several selective chemokine or chemokine receptor inhibitors are under development, although potential redundancy within the chemokine network is a possible pitfall. This obstacle might be overcome by utilizing proteins or small molecule derivatives with a broad ability to inhibit chemokines. One such protein is viral CC chemokine inhibitor (vCCI), a poxvirus-derived protein that was highly effective in inhibiting allergen-induced asthma in mice [80•] and is a potent and generic competitive antagonist of rodent and human CC chemokines.

Conclusions

New approaches for specific allergen immunotherapy, such as alternative routes of administration and the use of recombinant proteins or peptides, are promising but are only at an early stage for human therapy. The mechanisms by which these various forms of specific allergen immunotherapy act remain controversial, but there is growing evidence supporting a role for regulatory T cells and/or the immunosuppressive cytokines that they might produce. It remains unclear whether enhancing Th1 responses is always a desirable goal of immunotherapy, particularly in the case of atopic disorders in which there is established chronic inflammation, such as asthma and atopic dermatitis. Recent human trials of altered peptide ligands for the treatment of autoimmune disease indicate that it may be difficult to predict the *in vivo* consequences of these agents on T cell immunity from *in vitro* studies, and that peptide vaccination can induce early phase immune reactions. These difficulties with the application of antigen-specific immunotherapy contrast with the recent success of humanized monoclonal anti-IgE therapy, suggesting that various approaches for atopic therapy that are not antigen-specific continue to have great potential.

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