

EDITORIAL

Immunotherapy — anergy, deviation or suppression?

Introduction

Clinical evidence, reviewed in several official position papers, suggests that immunotherapy is an effective modality for the treatment of allergic rhinitis and venom anaphylaxis [1,2], whilst there remains controversy regarding its usefulness in asthma [3–6]. The goal of immunotherapy is to alter the underlying cause of allergic disease, namely inappropriate immunological reactivity to innocuous environmental substances. If this is achievable then the need for medications may be reduced or eliminated. It should be borne in mind however, that whilst immunotherapy may 'cure' (permanently?) specific allergies, there has been no suggestion that it is the answer to the problem of atopy itself.

The potential for immunotherapy to alter the long-term course of atopic disease has been hinted at by the demonstration that immunotherapy, administered early in the course of allergic sensitizations, may prevent the acquisition of new sensitizations [7]. In addition, there are tantalizing data suggesting that immunotherapy for rhinitis may prevent the development of asthma, signalling a real interruption in the atopic 'march' [2,8]. There is no doubt that these observations require corroboration from larger trials; if confirmed, they signify a major advance in the understanding and treatment of atopic diseases. Holt and Sly [9] have hypothesized that clear-cut allergy with TH₂-like immunological responses to allergens precedes the onset of atopic disease. An important implication of this hypothesis is that desensitizing treatment may need to be started early, before the development of multiple sensitizations and multiorgan disease, perhaps even before the development of symptoms; certainly a provocative proposition.

Potential mechanisms of immunotherapy

The mechanism of action of immunotherapy in preventing specific allergic reactions remains enigmatic. Most information to date has been based on empirical observation, often made in retrospective studies. Innovative forms of immunotherapy based on modification of extracts, alternative routes of delivery, low-dose schedules and other forms of treatment closer to the fringes of conventional practice have been proposed and in some cases tested, but none has demonstrated overwhelming and convincing benefits. By

obtaining an understanding of underlying mechanisms it may be possible to redesign immunotherapy treatments to improve efficacy and reduce disadvantages.

There are many steps in the pathway from T cell sensitization to allergic inflammation where immunotherapy might potentially act (Fig. 1). Immunotherapy has been demonstrated to reduce both early and late phase responses to allergen [10,11] and may modify the expression of markers of the inflammatory responses, such as ECP [12,13], and PAF [14]. The influx of effector cells, such as eosinophils [15] into challenge sites may be reduced, as well as the number of mucosal mast cells present [16]. The alterations in a variety of late events suggests a fundamental change at an early stage in the pathway, most likely the underlying T cell sensitization, which then affects a large number of downstream events. The major contribution of the T cell to the allergic response has often been considered to be in providing help for B cells to make IgE. However, the putative linkage of an alteration of T cell function to the amelioration of atopic disease, in the face of the persistent elevation of IgE, reinforces the notion that T cell derived cytokines play a major ongoing role in allergic inflammation.

Humoral mechanisms

IgE is clearly a central mediator in allergic responses. Serum IgE may initially rise with immunotherapy, but the usual seasonal rise is blunted. Eventually, after several years of treatment, IgE may fall but this occurs well after symptoms have been reduced. Paradoxically despite the presence of continuing high levels of IgE, cutaneous sensitivity, as determined by intradermal weal size or skin-prick sensitivity threshold, is often reduced [15,17,18]. The early phase response in the target organs (measured after allergen challenge), known to be directly mediated by IgE, is also clearly reduced [15,18]. It has been suggested [18] that interferon-gamma (IFN γ) production may reduce the immediate response by direct effects on mast cell development or degranulation, and indeed in some studies increased IFN has been measured systemically, however, this does not account for the antigen specificity of the effect.

One of the most familiar theories for the mechanism of immunotherapy was suggested by the widely observed finding that antigen specific IgG antibodies particularly of the IgG4 subclass rise significantly during immunotherapy [19]. Specific IgG was shown to be able to block IgE responsiveness [20], suggesting that immunotherapy acted by inducing allergen specific 'blocking' antibodies which

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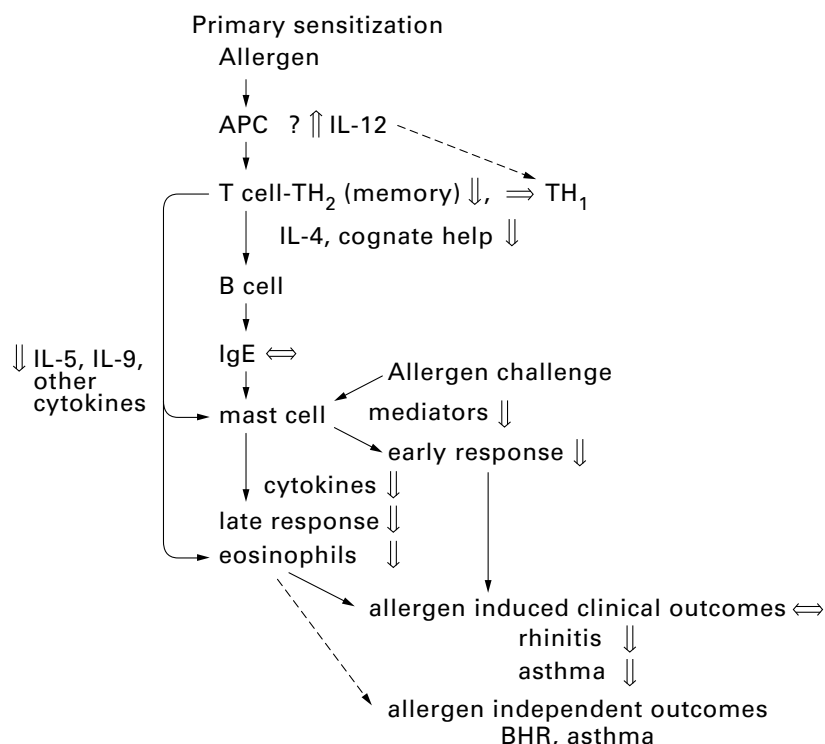


Fig. 1. Pathways of allergic sensations and responses to allergen challenge. Effects of immunotherapy: ↓, reduced by immunotherapy; ↔, no change or uncertain (in the case of IgE, changes occur late); ⇒, deviation towards TH₁; ↑, possible increase in IL-12 from APC.

compete with IgE for the binding of allergen. Whilst there is still some support for this hypothesis from *in vitro* [20,21], and *in vivo* [22,23] studies, there is little correlation between levels of IgG and other parameters of allergy in treated or untreated patients [24,25] and indeed, elevation of total IgG4 levels has been associated with treatment failure [26]. Rush desensitization protocols protect effectively and reduce skin reactivity [27] in the absence of alteration in IgG antibodies. Longer term studies of house dust mite immunotherapy also showed a lack of correlation between clinical response and IgG4 levels [28,29], and interestingly, a recent study indicated that sublingual immunotherapy could be efficacious despite the lack of any alteration in allergen-specific IgG or IgG4 [30].

T cell anergy

It has long been apparent that immunotherapy results in a reduction in antigen-specific lymphoproliferation [31–33], related to a reduction in IL-2 production [34]. Inhibition of T cell proliferation can be demonstrated to occur within weeks in rush venom protocols [18,35,36], but this was not accompanied by B cell tolerance [37]. The decreased proliferative responses were not due to deletion of antigen-reactive T cells since they could be restored by

the addition of IL-2 or IL-15 [37]. In vitro, anergy may be induced by treatment of T cells with peptides [38], or stimulation via the TCR in the absence of costimulation [39,40], but it is unclear by what mechanism T cell responses are inhibited by immunotherapy *in vivo*. It is also unlikely that failure of antigen specific T cells to proliferate is in itself the most important mechanism of the clinical benefit at least in venom allergy, since anaphylaxis is obviously not directly dependent on a T cell proliferative response.

Immune deviation

It has become evident in recent years that the TH₁/TH₂ paradigm [41] provides a useful, if not entirely comprehensive [42], framework for understanding the nature of allergic responses at a fundamental level and hence possibly for the understanding of the mechanism of immunotherapy. There is certainly evidence to suggest that lack of allergy is not simply due to an absence of reactivity to allergens at the T cell level, since T cells from non-allergic subjects readily proliferate in response to environmental allergens [43]. Allergen specific T cell precursor frequencies in allergic and non-allergic subjects were similar in one study [44]. The precursor frequency for allergen-specific cytokine-producing

T cells is also similar [45]. What is clearly different is the nature of the cytokine response. Although there is not a strict polarization into one pattern or the other, the data suggest a relatively consistent difference in the balance or ratio of TH₁ to TH₂ cytokines in atopics vs non-atopics. This has now been examined at the level of *in vitro* generated clones [46,47], allergen-specific PBMC cultures [48], and cytokine producing cells in limiting dilution analysis, where a 27-fold alteration in the ratio of allergen specific IL-4 to IFN γ producing cells is found in allergics [45]. Using cytokine mRNA and ELISA studies of allergen stimulated primary cultures, it has recently been shown in our laboratory that these patterns of relative cytokine polarization in response to allergens develop during infancy and are essentially established by the age of 5 years [49,50].

There are now a number of studies addressing the production of TH₁ and TH₂ type cytokines by allergen specific T cells in response to immunotherapy; some have been of notably high quality, with well characterized groups of matched patients studied prospectively. Most, as expected, have demonstrated a shift from the TH₂ pattern seen in allergy to the production of the TH₁ cytokine IFN γ . Studies vary considerably in the details of this shift. Some report that IFN increases while IL-4 and IL-5 diminish [17,35,36,51,52]; in some cases, IL-4 was reported to diminish, whilst IFN was not altered [53,54], whilst in others, IFN increased whilst IL-4 and IL-5 remained unchanged. These differences do not seem to be related to the nature of the allergen extract or the protocol used, and are most likely to be accounted for by methodological differences.

Although IL-2 is also considered to be predominantly a TH₁ factor, its production is in fact reduced due to the anergy-like effect of immunotherapy. It is interesting that the shift to IFN γ production can be demonstrated so readily, since TH₁ immunity seems usually to be associated with a brisk proliferative response. Indeed, tolerization of TH₁ immune responses e.g. as seen in classic neonatal tolerance, has been accounted for by a switch to TH₂ cytokines, associated with reduced proliferation [55]. Furthermore, as has been pointed out [42], allergic individuals do not suffer from a cell mediated immune response to allergen *in vivo*, and so do not demonstrate typical TH₁ immunity despite such a T cell response evident *in vitro*. For these reasons alone, it is clearly simplistic to think that immunotherapy acts simply through a shift from TH₂ to TH₁ response patterns.

Immunomodulatory cytokines

It is unclear how immunotherapy may bring about the observed changes in cytokine balance. It would not appear to be related to adjuvants, since similar effects are seen

when aqueous extracts are used. It is certainly possible that the altered T cell response is brought about by the major increases in concentration of antigen exposure between injected extracts and natural exposure, or by the route of delivery (although this argument applies only in the case of aeroallergens, not venom). Both allergen dose, and the nature of the antigen presenting cell, have been shown to affect the production of IL-4, even in those with established allergy [56].

The major immunomodulatory cytokine to be associated with TH₁ biased immune responses is IL-12 [57]. This factor is able to inhibit allergic inflammation in presensitized animals [58], suggesting the ability to divert existing TH₂ responses *in vivo*. Although IL-12 was not found to be altered in PBMC cultures [54], Hamid *et al.* demonstrated an increase in IL-12 positive macrophages at sites of allergen challenge associated with increases in IFN γ positive T cells in immunotherapy treated, but not untreated, allergic individuals [17]. Although not directly demonstrated, one could infer that this might be antigen-specific. It was suggested that the TH₁ cells accumulating locally were IL-12 driven. Yet macrophage IL-12 production is part of the innate (i.e. non-specific) immune response, therefore it is perhaps more likely that the IL-12 is IFN γ driven, due to the TH₁ type response to the allergen. Nevertheless, the induction of IL-12 by immunotherapy remains an attractive explanation for the observed T cell phenotypic shifts. In this regard, it is notable that β_2 -agonists have recently been shown to inhibit IL-12 production by monocytes and dendritic cells [59]. One is tempted to speculate that this may provide an explanation for the difficulty in demonstrating effectiveness of immunotherapy in asthma compared with allergic rhinitis; let alone an explanation for the rising incidence of atopy and asthma in our population.

A more comprehensive explanation for the mechanism of immunotherapy is hinted at in the paper by Bellingshausen *et al.* [35], in which it was shown that the apparently anergic PBMC from patients desensitized to bee venom specifically produced IL-10 in response to the antigen. IL-10 has been reported to inhibit the synthesis of many cytokines, to inhibit allergic inflammation in animal models [60], to inhibit antigen-specific T cell proliferation [61] and induce T cell anergy [62]. Indeed, neutralization of IL-10 *in vitro* restored proliferative responses to bee venom, and increased IFN γ production but did not restore IL-4 [35]. IL-10 was shown to be produced by CD4⁺ T cells; could these be the *in vivo* counterparts to the recently described IL-10 producing regulatory T cell clones, designated Tr1 [63]? Whilst TH₂ to TH₁ shifts or T cell anergy do not alone satisfactorily explain the effects of immunotherapy, the combination of a cytokine shift with IL-10 mediated immune suppression may indeed do so.

It is not yet evident whether the effect of immunotherapy

can be equated to the return of T cell responses to a pattern resembling that of 'normal' non-allergic responsiveness or if the tolerance induced is of an artificial nature. This may be relevant to the issue of whether the immune response can be durably, permanently modulated by immunotherapy or whether the response is transient and subject to later reversion to allergic type. At this point, we are reaching some important insights into both the clinical and mechanistic aspects of immunotherapy. What is clear is that more and better quality prospective studies are required in order to elucidate how effective immunotherapy can be at reversing or possibly preventing allergic sensitization and atopic disease and the mechanism/s by which this occurs. Only then will we be in a position to truly alter the course of atopic diseases.

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