

EDITORIAL

Immunological responses to immunotherapy

The concept of allergen immunotherapy, whereby allergic reactivity is treated and ameliorated by administration of initially very small quantities of the specific allergen to which a subject is sensitive, then gradually increasing the dose until the subject appears clinically desensitized, and current indications for its use, are well documented [1]. There are many published studies describing the effectiveness of specific allergen immunotherapy, particularly to common aeroallergens such as dust, pollens, animal proteins and recombinant peptides derived therefrom [2,3,4], and also to systemic allergens such as venom from various insect species (mainly hymenoptera (bees and wasps)) [5]. The treatment, however, is not without drawbacks which include the potentially fatal side-effect of induced uncontrolled anaphylaxis [6], the avoidance of which will be discussed below, albeit in an immunological context.

From a clinical viewpoint insect venom immunotherapy offers an opportunity to effectively treat potentially life-threatening allergic (anaphylactic) responses to insect stings, and from a more pragmatic immunological stance provides a useful model with a clearly defined end-point (ability of the sensitive subject to tolerate a venom concentration of greater than one natural sting). Another major advantage is that many subjects who develop severe allergic sensitivity to bee or wasp stings are not atopic, so the analysis of changes in immunological reactivity is not confounded by a variable atopic background. This article will be based around two studies examining certain immunological changes during insect venom immunotherapy published in the current edition of *Clinical and Experimental Allergy*. That of Jutel *et al.* [7] addresses the phenomenon of rapid desensitization of allergic effector cells which enables clinical immunotherapy protocols to be condensed from the more conventional period of several weeks or months to within hours (rush immunotherapy), followed by maintenance injections, without inducing anaphylaxis. The study by Tsicopoulos *et al.* [8] examines the expression of certain co-stimulatory signals which may be important in regulating T cell responses in the context of antigen presentation by antigen presenting cells (APCs). However, before discussing the findings in detail it may be prudent to describe the more established immunological responses to immunotherapy so that these new findings and the speculation that they invite can be considered in context.

Allergic reactivity is primarily mediated by the interaction of allergen with allergen-specific IgE bound to high affinity Fc ϵ receptors on the surface of mast cells and basophils. Cross-linking of the IgE/receptor complexes results in degranulation of these cells and mediator release.

These mediators include vaso-active amines (such as histamine), enzymes (tryptases and chymases) and arachidonic acid metabolites (particularly leukotriene C4 (LTC4) and prostaglandin E2 (PGE2)) amongst others, all of which have potent effector functions and can initiate the recruitment and activation of late phase inflammatory cells [9]. It might, therefore, appear that removal of specific IgE from the system would be the desired aim of immunotherapy. Experimental evidence, however, suggests this does not happen, at least not in the induction phase of treatment nor within several months on maintenance therapy, but it may fall in the longer term [10]. Many studies have shown that during effective conventional immunotherapy, levels of IgG rise significantly, particularly IgG1 and IgG4 sub-classes.

Combined with the observation that passive transfer of specific IgG could block acquired allergic (IgE) responsiveness, these observations led to the idea that IgG antibodies were directly competing for allergen and thus blocking-IgE mediated events, the so called 'blocking antibody' hypothesis [11]. Again, experimental data have shown little correlation between levels of IgG or its subclasses with IgE production or efficacy of insect venom immunotherapy [12], suggesting that this mechanism does not occur to any great extent *in vivo*. Probably the most convincing evidence to dispel this idea was the development of rush immunotherapy protocols where hyposensitization occurs over a time period where there is no possibility for *de novo* antibody synthesis. However, the production of IgE and IgG subclasses (particularly IgG4) may well be of considerable importance for other reasons and its relevance should become apparent in the discussion of potential mechanisms of longer term desensitization below.

With the realization that T cell cytokines, notably interferon- γ and IL-4, had profound effects on the regulation of IgE [13]; and with the discovery of distinct T helper cell functional phenotypic subsets in mice, by Mosmann and Coffman [14] and confirmed in humans by Wierenga *et al.* [15], there emerged the possibility that T cell mediators could themselves be responsible for the mechanisms of desensitization apparent in immunotherapy. Functional studies suggest that Th2 type cytokines (from whatever cell source) are responsible for enhancing humoral immune responses and, of considerable relevance to allergic reactions, promote immunoglobulin isotype switching from IgG to IgE (IL-4 and IL-13), enhance eosinophil survival (IL-5), enhance terminal B cell differentiation (IL-6) and inhibit Th1 responses (IL-4 and IL-10). On the other hand Th1 type cytokines up-regulate cell-mediated immune defense mechanisms, promoting phagocytosis and natural killer

cell activity (IFN- γ and IL-12) and cross-inhibit Th2 responses [16]. It therefore seemed natural to hypothesize that specific allergen immunotherapy might down-regulate Th2 responses and/or up-regulate Th1 responses.

Although the picture is not clear-cut, this is indeed what appears to happen in both rush and conventional bee venom immunotherapy in the peripheral blood compartment [17,18]. Using an *in vitro* system where allergen specific T cells were selected and then expanded, Jutel *et al.* [17] were able to demonstrate that during the course of rush immunotherapy levels of IL-4 decreased whilst those of IFN- γ increased. In a different model employing only primary stimulation *in vitro*, our group has also demonstrated similar findings in both rush and conventional immunotherapy [18,19]. Although rapid changes in cytokine production can be observed, it is probable that these reflect the compartment from which they were measured and that during immunotherapy allergen reactive T cells may be removed from the peripheral blood, either by rapid migration into inflammatory foci induced by high allergen concentrations, or by sequestration in lymphoid tissue during antigen presentation. It therefore seems likely that the mechanisms which result in rapid hyposensitization are not directly mediated by cytokine production, but that the switch in cytokine production from a predominantly Th2 to a Th1 profile may be responsible for maintenance of longer term hyposensitization. Exposure to specific allergen in the context of a Th1 memory response might theoretically be sufficient to perpetuate allergic hyposensitization.

Before addressing the mechanisms of immediate desensitization it is worth considering how the above mentioned cytokine switching occurs. There is compelling evidence from a number of sources that antigen concentration and the type of antigen presentation that takes place can directly influence cytokine production [20,21]. It has been shown that treating T cells, from a bee venom-allergic donor, with increasing amounts of phospholipase A2 (PLA-2) (a major allergen of bee venom) results in the production of IL-4 at low to intermediate doses of PLA-2 and IFN- γ at high doses [21]. Similar observations have been made by a number of different groups, employing a variety of models [20]. If this phenomenon is occurring during immunotherapy where there is a rise in cumulative dose of allergen, how is this achieved? There are three interlinking strands of evidence which might suggest an answer.

First, presentation of antigen to T lymphocytes without appropriate co-stimulatory signals results in a state of antigen-specific unresponsiveness (anergy) [22], and it has been demonstrated that necessary co-stimulatory signals between APCs and T cells are modulated by the dose of antigen to which APCs are exposed as well as by the specific type of APC [20,23]. In the interesting article by Tsicopoulos *et al.* [8] in this issue, CD28 expression on peripheral blood T cells

was examined during the course of rush immunotherapy to hymenoptera venom. They observed that CD28 levels, although raised in venom-allergic subjects (particularly on CD8+ T cells), remained unaltered during and after rush venom immunotherapy. The implication from this is that the changes in T cell responses (cf. cytokine switching from Th2 to Th1 and reduction in T cell proliferation) are not related to overall CD28 expression on T cells, suggesting allergen-specific T cell anergy may not be a major mechanism of T helper cell subset down-regulation. Their results, however, do not preclude a role for allergen-specific T cells being regulated by this mechanism, as their number in the peripheral blood compartment would be low, and reduced by immunotherapy. However, what it does strongly suggest is that CD28/B7 co-stimulation is a feature of venom allergy and, if representative, may be a major co-stimulatory signal required for the perpetuation of Th2 responses when allergenic peptides are presented in the context of MHC class II to the CD3/Ti T cell receptor complex.

Second, Barcy *et al.* [24] have recently shown that B7-CD28/CTLA-4 expression on monocytes is strongly inhibited by cross-linking Fc receptors (FcR) with a consequence that the capacity of those monocytes to function as APCs and to stimulate T cell activation is severely impaired. The mechanism of such impairment may well be related to the density of FcRs (G and E immunoglobulin isotypes) on the surface of monocytes (and other APCs) which is known to be up-regulated when immunoglobulin levels increase [25], and on the ability of antigen to cross-link these receptors via bound immunoglobulin. This mechanism of anergy at the level of the APC actually ties in quite nicely with the observed changes in circulating immunoglobulin, as mentioned earlier, and may also be of relevance to immediate desensitization linked with FcR expression on mast cells and basophils (see below). The results of Tsicopoulos *et al.* [8] of unaltered CD28 expression on T cells may not reflect the expression of B7-1 or B7-2 on APCs, nor can they shed light on whether the co-stimulatory functional capacity of B7/CD28 is compromised after putative cross-linking of FcRs on monocytes by increasing concentrations of multivalent allergen.

The third aspect, representing another mechanism by which T cell functional subsets may be regulated, is by deletion of antigen specific cells by the phenomenon of activation induced apoptosis which could relate to either T cell or APC unresponsiveness. In this situation activated cells fail to receive the necessary co-stimulatory signals and enter a genetically predetermined pathway of programmed cell death. Removal of one subset, say Th2, would then permit expansion of Th1-type cytokine expression on subsequent exposure to allergen.

If changes in immunoglobulin or cytokine production are not responsible for immediate hyposensitization to

venom challenge, then what mechanisms might underlie this phenomenon? This question has been asked by Jutel *et al.* [7] in another paper in this issue and they have produced some extremely interesting data. In order to avoid triggering anaphylactic reactions during rush (or ultra-rush) immunotherapy there has to be a rapid inhibition of the ability of the inflammatory effector cells which cause anaphylaxis to release their mediators. Various studies have shown that the release of mediators from circulating basophils may provide at least the initial trigger for systemic anaphylactic reactivity. Mast cells in the lungs and skin [26,27] may also be important producers of potent vasoactive mediators although in the skin Jutel *et al.* suggest that their reactivity does not alter during venom immunotherapy [7]. Gradual and sub-clinical depletion of preformed mediators from mast cells and basophils in response to increasing concentrations of allergen during immunotherapy has been postulated, but anecdotal evidence showing that systemic reactions can occur at any point during immunotherapy suggest this not to be a major mechanism of desensitization [18].

Jutel *et al.* [7] have measured histamine release and LTC-4 production from basophils in a whole polymorph preparation to investigate changes in basophil responsiveness during venom immunotherapy. They have shown that total leucocyte histamine was reduced after ultra rush immunotherapy. Although no differences were observed in per cent of total histamine after stimulation with allergen (bee venom or PLA) or with anti-IgE, anti-IgE receptor or with C5a, between the start and end of immunotherapy, the actual amounts of allergen specific release were reduced. Similarly, changes in LTC-4 were only significantly reduced in samples stimulated with specific allergen. In a previous report by Eberlein-Konig *et al.* [28], where patients undergoing bee venom immunotherapy were challenged with a bee sting, very similar findings with respect to histamine release were recorded. Those authors suggested that successful hyposensitization did not induce a state of immunological non-responsiveness but rather altered the magnitude and pattern of mediator release. One paradoxical finding reported by Jutel *et al.* [7] was the apparent lack of a change in dose required to evoke histamine release by allergen, anti-IgE or anti-IgE receptor, after venom immunotherapy.

Overall, these results suggest that the capacity for basophils to release preformed and/or rapidly produced mediators is only marginally moderated by specific allergen immunotherapy but that either the mediator content of the basophils present or their number (as a proportion of total granulocytes) is reduced. This leaves the question open as to how rapid desensitization of inflammatory effector cells occurs. There is the intriguing possibility that, similar to the arguments above for cytokine and immunoglobulin control, the effects of increasing concentrations of allergen interacting with IgE initially, and perhaps IgG later, serve to

directly modulate the signals transduced by FcRs. This scenario is not without precedent as signalling via FcεR1 in rat basophils has been shown to depend on receptor mobility and aggregation which in turn is determined by the concentration and valency of ligand (antigen) [29]. In this study it was shown that at maximal IgE aggregation, signalling was ineffective even when the IgE was cross linked-with monoclonal anti-IgE. Furthermore, Shalit and Levi-Schaffer [30] have demonstrated that gradually increasing exposure of rat peritoneal mast cells to antigen *in vitro* led to their refractoriness.

In conclusion, with reference to the two articles reviewed I have presented a rather unorthodox view of possible immunological mechanisms of immunotherapy. Clearly there are many omissions relating to more conventional mechanisms and to many other models of investigation, and I would refer the reader to excellent articles covering these subjects [1–5,27]. However, it is known that FcεR1 is not only expressed on mast cells and basophils but also on monocytes, eosinophils and Langerhans cells and that expression of FcRs is antagonistically regulated by Th1 and Th2 type cytokines [25,31]. The difference in speed and duration of desensitization effected at the level of IgE with basophils/mast cells or IgG with APCs and T cells may relate to the enhanced ability of IgE over IgG subclasses to 'focus' antigen and activate effector cells [25]. In the context of the direct effects of allergens on modulation of allergic inflammatory effector cells via FcRs, these observations may be of some significance; clearly more research is required in this interesting area.

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