

An Update of Immunotherapy for Specific Allergies

Susan L. Prescott* and Catherine A. Jones

Department of Paediatrics, University of Western Australia, Princess Margaret Hospital, PO Box D184, Perth, Western Australia 6001



Abstract: Allergic diseases are common, disabling and potentially life threatening. The processes that lead to production of excessive allergen-specific IgE production are highly complex and heterogeneous. While current treatment strategies are limited, recent technological advances have provided a better understanding of underlying disease processes and offered new potential therapeutic targets. Optimal treatment strategies permanently modify underlying inflammatory allergic immune responses (immunotherapy) with long term alleviation of symptoms and minimal side-effects.

Although these processes are still not completely understood, methods of modifying allergen recognition by the immune system have already been successful. Here, we review recent developments and future directions in allergen immunotherapy and adjunctive therapies. Specifically, we address the molecular mechanisms of allergen immunotherapy and new techniques including allergen modification, allergen gene vaccination, CpG immunostimulation, and peptide immunotherapy. Other non-allergen specific molecular targets (including receptor, cytokine and IgE targets) which may complement specific immunotherapy are also discussed. Ideally these methods will eventually be replaced by strategies targeting the prevention of allergic responses (immunoprophylaxis).

1. OVERVIEW OF UNDERLYING IMMUNOLOGICAL PROCESSES AND POTENTIAL THERAPEUTIC TARGETS FOR IMMUNOTHERAPY

Many allergens have been found to have enzymatic and other biological activity [1] yet these antigens elicit a tolerance response in the majority of people. Unfortunately, for an increasing number of individuals allergens trigger a type I hypersensitivity characterised by a strong humoral response dominated by IgE. Irrespective of the atopic status of the individual, antigen is taken up by antigen presenting cells (APCs; namely dendritic cells, monocytes/macrophages and B cells) and processed for presentation by MHC Class II molecules to CD4+ T cells. In an atopic individual, the resulting allergen-specific T cells have a Th2 skewed phenotype with the cytokine profile dominated by IL4, IL-5 and IL-13. In contrast, the response from the non-atopic person is characteristically Th1 skewed, being dominated by the prototypic Th1 cytokine IFN γ . Numerous factors favour the development of Th2 type antigen-specific responses including the amount and affinity of antigen and the HLA genotype of the responder, the type of APC and the prevailing cytokine milieu.

Th2 type cytokines support the allergic response in many ways. IL-4 and IL-13 (in conjunction with other cognate interactions between T and B lymphocytes) induce class switching and production of IgE by B cells. Allergen can then cross link specific IgE bound to high affinity IgE

receptors (Fc RI) on mast cells and basophils inducing the release of vasoactive and inflammatory mediators, including histamine and cytokines. It is these molecules that mediate the early reaction characterised by vascular leakage and inflammation. IgE can also facilitate the uptake of small quantities of allergen by B cells via IgE-mediated antigen focusing utilising the low affinity IgE receptor (CD23) [2]. More recently, dendritic cell populations, such as Langerhans cells have been described to use the high affinity receptor for this process [3]. CD23-mediated IgE-facilitated antigen focusing by B cell is postulated to activate T cells and induce to release of proinflammatory cytokines that mediate the late reaction. The other classical Th2 cytokine, IL-5, supports that maturation and survival of eosinophils that contribute to the inflammatory environment within the tissue.

Thus there are numerous targets for alleviating or abrogating allergic inflammatory responses. Widely used pharmacological interventions generally target the biological mediators released by basophils, mast cells and eosinophils, hence the use of anti-histamine therapy and, more recently, of anti-leukotriene therapy. These treatments are not curative whereas immunotherapy directed to any of the numerous targets identified in the brief description above (and reviewed by [4]) offer the best hope of achieving cure, or at least long term alleviation of clinical symptoms.

Study of the immunological response to immunotherapy has provided some information about the immunological mechanisms underlying successful immunotherapy. It is only as the use of immunotherapy becomes more widespread (more allergens targeted/different routes of administration) that we will be able to fully understand the pivotal

*Address correspondence to this author at the Department of Paediatrics, University of Western Australia, Princess Margaret Hospital, PO Box D184, Perth, Western Australia 6001; Ph: (618) 9340 8171; Fax: (618) 9340 8121; e-mail: susanp@ichr.uwa.edu.au

mechanisms and how regulation of these may be critical for successful treatment of different allergies.

2. CURRENT USE OF ALLERGEN VACCINATION IN ESTABLISHED ALLERGIC DISEASE

The therapeutic benefits of administering increasing quantities of allergen in sensitised patients was established early last century [5]. Using many varied adaptations of this technique, "vaccination" with allergen [6] has been subsequently used to desensitise patients with allergic rhinitis / conjunctivitis, asthma or insect venom allergy. Until very recently there was no clear understanding of the underlying mechanisms of action, or use of standardised procedures and extracts [6]. Vaccination strategies remain the only established forms of therapy which modify disease progress by altering underlying immune responses.

2.1 Mechanisms of Action (of Subcutaneous Immunotherapy)

There are considerable variations in protocols for allergen vaccination, and the mechanisms of action are likely to vary with differences in allergens, adjuvants, doses, duration and routes of administration. Additional host factors, including the pattern of disease expression are also likely to affect the clinical and immunological response. Allergens have been variously administered through oral, sublingual, or subcutaneous routes. There is very limited information about the comparative efficacy of these different routes. Most of the studies evaluating mechanistic pathways in humans relate to subcutaneous allergen vaccination.

Local tissue effects are also seen early in immunotherapy, and are likely to be the result of mechanisms different from those responsible for the long lived changes in cellular responses. There is early reduction in the numbers and activity of local inflammatory effector cells, including mast cells [7, 8] eosinophils [9, 10] and their inflammatory mediators [11] following nasal allergen challenge. Similar findings are documented in the lower respiratory tract [12].

It is now widely held that the immunological changes which lead to sustained clinical improvement are mediated by changes in T cell function. With the recognition of functional dichotomy in T lymphocyte subsets, it was hypothesised [13] that immunotherapy resulted in relative shift towards Th1 responses (with reciprocal inhibition of allergic Th2 responses). There is now evidence to support this. A number of studies have now shown a decrease in the production of Th2 cytokines (IL-4 and IL-5) and an increase in Th1 (IFN γ) responses with immunotherapy [14-16].

In the initial phases of immunotherapy high dose allergen leads to allergen-specific T cell anergy. This transient anergic state has been seen following allergen vaccination with bee venom, wasp, rag weed, cat, grass pollens (reviewed in [17]). Inhibition of lymphoproliferation and both Th1 and Th2 cytokine responses is apparent within 60 days of commencing treatment [17, 18]. This is an active process, associated with increased CD25 expression and

increased IL-10 and IL-12 signalling from tissue macrophages and other antigen presenting cells [19]. IL-10 is believed to play an important role in inducing and maintaining T cell anergy (reviewed in [17]). IL-12 [19] has further inhibitory effects on Th2 cytokine production. Although vaccine induced T cell anergy is transient, when allergen responsive T cell are reactivated their subsequent pattern of cytokine response is crucial and determines if allergen vaccination is successful (Th1) or not (Th2).

Inhibition of T cell function does not immediately prevent the production of IgE which may increase transiently. However, within weeks total and specific IgE levels fall in favour of IgG4 production. Reported alterations in allergen-specific IgE with allergen vaccination are varied and do not correlate well with the therapeutic effects [20-22].

Increased levels of allergen-specific IgG1 and IgG4 subclasses [23] were initially proposed to mediate the effects of immunotherapy by "blocking" IgE [24-26]. There is more recent evidence that IgG antibodies may actually inhibit IgE mediated T cell activation [18]. In untreated allergic individuals allergen-specific IgE facilitates CD23 mediated antigen presentation to CD4+ T cells, promoting activation at extremely low allergen concentrations. Following allergen vaccination, allergen-specific IgG inhibits this process so that a significantly higher concentration of allergen is required for T cell stimulation.

Physicochemical properties of immune interactions are a fundamental determinant of T cell activation. It appears that the strength of the association between the MHC class II, allergen peptide and the T cell receptor dictates the pattern of resulting cytokine responses. Factors which favour strong signalling (high allergen dose / high affinity / high density of MHC) promote Th1 responses (reviewed in [17]). This may in part explain the induction of Th1 responses by high allergen doses as well as the HLA associations, and observed heredity of bee venom allergy.

2.2 Sublingual Immunotherapy

There are ongoing efforts to determine the efficacy of other less invasive delivery routes for vaccination, including oral, intranasal and sublingual vaccination. This is probably of greatest relevance to the growing paediatric population with allergic diseases. While a number of double-blind randomised control trials have demonstrated the safety and clinical benefits of sublingual immunotherapy [27, 28, 29, 30, 31], other studies have shown no clinical improvement [31] or effects that were only seen in a subgroups of patients [32]. Relatively few of these studies have involved children [28, 33, 34]. The immunological effects of sublingual allergen vaccination are still not well documented, even though this treatment is in therapeutic use in many centres. Even studies demonstrating clinical improvement have not shown any associated changes in IgE or IgG levels [34]. Although these therapies are attractive because they are less invasive and have a lower risk of systemic reactions, further research is required to assess the mechanisms and to optimize treatment regimes. Still less is known about the underlying mechanisms of intranasal and oral immunotherapy in humans.

2.3 Recent Developments in Existing Techniques for Allergen Vaccination

Recombinant DNA technology has provided the basis for large scale production of well defined purified allergens. This has also enabled identification of proteins and epitopes with highest allergenicity, and facilitated the development of peptide vaccines (below). Standardisation of existing extracts and administration protocols has also lead to improved safety.

3. NEW STRATEGIES FOR IMMUNOTHERAPY

3.1 Allergen Gene Vaccination

The administration of plasmid DNA (pDNA) encoding allergic epitopes has produced encouraging results in animals [35-37]. Because of fewer side effects these strategies are attractive for human application. In mice, immunisation with allergen-*gene* vaccines produces Th1 responses, whereas allergen-*protein* vaccines favour Th2 responses [38]. The main reason for the strong "Th1 biasing effect" of gene vaccination is the presence of highly Th-1 immunostimulatory bacterial CpG motifs in the plasmid vectors (reviewed by Spielberg [38]). This is discussed further below. Parenteral gene vaccinations have been administered both subcutaneously and intramuscularly, but the subcutaneous route appears more efficient in evoking a Th1 response [38] because of the greater number of antigen presenting cells in cutaneous tissues. Allergen gene-vaccination via the oral route also appears effective and has obvious advantages, particularly in paediatric populations. In animals, the oral administration of peanut allergen-genes has already been shown to modify immune responses to this allergen [39]. Although these strategies may be very attractive, the regulation of allergen gene transcription remains a concern. In one recent study (in mice), latex gene vaccination (Hev b1) resulted in widespread expression of transcripts throughout many lymphoid and non-lymphoid tissues [40]. As many allergens have potentially toxic characteristics, levels of expression need to be regulated. This is likely to delay human trials.

3.2 CpG Motifs as Immunostimulants in Allergen Vaccination

Conjugation of allergens to immunostimulatory bacterial DNA is a new promising development in allergen-specific vaccination. A number of strategies have been employed including conjugation of the allergenic proteins to adjuvant bacterial oligonucleotides, or incorporation of allergen encoding cDNA which will be transcribed by the host (reviewed by [41, 42]). These techniques are more effective and better tolerated than conventional allergen-vaccination. Conjugation renders allergen proteins less anaphylactogenic. Alternatively, allergen transcribed from cDNA is processed by intracellular pathways of CD8+ T cells, and is also unlikely to induce IgE mediated reactions. Preliminary studies indicate that a combination of CpG + DNA vaccination may be more effective in antagonising Th2 responses than the combination of CpG + protein immunisation [43].

The use of bacteria as adjuvants was reported in the 1960's, and proved to be effective although the reasons were then uncertain [44]. It is now evident that this was probably due to bacterial DNA which contains repeated immunostimulatory sequences (ISS) of unmethylated cytosine and guanosine (CpG motifs). These motifs (which are conserved among bacteria) evoke an efficient innate immune response as part of an important evolutionary defence mechanism. CpG motifs activate antigen presenting cells (especially dendritic cells) and natural killer cells, and promote the production of pro-Th1 signaling in the form of IFN γ , IFN α , TNF α , IL-12 and IL-18. This has been shown to enhance Th1 maturation and inhibit IgE production, although other mechanisms including IL-10 and CD8+ T cells may be involved. It was proposed that these adjuvants could suppress Th2 responses to specific allergens. This has been demonstrated in animal models [45], and does appear to be sustained. In mice, the administration of ISS oligodeoxynucleotides (ISS-ODN) coupled to rag weed allergen (Amb a 1) induced a Th1 biased (IFN γ) response to Amb a 1, with concurrent IgE suppression [45]. Although, it appears that this strategy will be more effective in down-regulating developing rather than established immune responses [45], these techniques are likely to proceed to human trials. Again, the possible long term effects are not known. As with other forms of Th1 simulation, there have been concerns about Th1 mediated complications including the production of endogenous complement fixing IgG, and associated disease.

3.3 Modified Allergens

Molecular technology has also allowed the generation of modified allergens (without disulfide bonds) that do not have tertiary structure. These have maintained immunogenicity (T cell stimulation), but significantly reduced allergenicity (IgE binding) with fewer side effects. A number of these allergens are under investigation in humans.

3.4 Allergen-Peptide Immunotherapy

One main problem of immunotherapy is adverse side effects especially anaphylaxis to whole native allergen, and for this reason it has not been advocated for treatment of food allergies, especially peanuts. Further, the lack of standardised reagents has also limited the application of this approach. Modern DNA technology and protein chemistry can overcome many of these problems. Many allergens, including those of peanut, bee venom, pollen, mites and animal dander have been cloned, sequenced, expressed and epitope mapped. Recombinant forms of allergens are becoming more widely used for skin prick testing and intradermal injection and are increasingly considered the best source for allergen immunotherapy [46,47]. Furthermore, the native sequence and structure can be manipulated to generate hypoallergenic mutants that no longer bind IgE but retain T cell epitopes, ie loss of allergenicity with maintenance of immunogenicity. A number of methodologies are being considered to generate hypoallergenic forms of allergens and these are discussed below.

Site-Directed Mutagenesis

Modern DNA technology allows the manipulation of the nucleotide coding sequence and therefore the resulting protein. Typically, substitutions and deletions of nucleotide bases are used to change the cDNA encoding an allergen leading to an altered amino acid sequence upon production of the recombinant protein. This modifies the tertiary structure and thereby the functional properties of the protein. Loss of native tertiary structure has a central role in loss of allergenicity as IgE epitopes are often discontinuous, being dependent on the juxtaposition in space of different parts of the molecule. In contrast, T cell epitopes being composed of a discrete string of amino acids, are generally not affected by changes in structure. There are a number of examples of the use of this technology. A Der f2 mutant (C8/119S) that has lost an intramolecular disulphide bond leading to structural changes no longer binds IgE from patients serum and instead induces a strong Th1 (IFN γ) response from the blood of atopic donors [48]. More recently three major peanut allergens (Ara h1, h2 and h3) were cloned, characterised and subjected to site-directed mutagenesis [49]. Amino acids critical to the IgE binding epitope of these allergens were identified and targeted for site-directed mutagenesis. The mutants were generally poor competitors for binding of peanut-specific IgE compared to the wild type and binding by IgE from patient serum was reduced. The mutant allergens also retained the ability to stimulate proliferation by most patients and they, or their derivatives, may be suitable candidates for use in immunotherapy.

Short Peptide Therapy

Hypoallergenic peptide fragments (approximately 20 amino acids) that retain the ability to stimulate a T cell response are also being investigated for usefulness in immunotherapy. These fragments are generally produced using commercially available peptide synthesisers using now standard techniques. Preliminary analysis of peptides confirmed that priming with an appropriate peptide or peptide pool could tolerate for subsequent challenge with the whole antigen. This was demonstrated using the immunodominant T cell epitope of Der p1 [50] which also limited production of all T cell cytokines but had no effect on IgE production [51]. Two peptides (27 amino acids in length) derived from chain 1 of the major cat allergen Fel d1 (IPC-1 and IPC-2) corresponding to amino acids 7 - 33 and 29 - 55, respectively were as effective as entire chain 1 in limiting (prophylactically and therapeutically) the response by mice to Fel d1 [52]. These peptides were administered intranasally or sub-cutaneously, respectively, making them candidates for immunotherapy in humans. As yet there have been only limited human studies of their efficacy and safety. Although Der p1 peptides have not been trialed in humans, Fel d1 peptide therapy has proved of limited success. Reduced allergy scores (nasal and lungs) were recorded 6 weeks after the completion of treatment (s.c. injection of IPC-1 and 2 each week for four weeks) [53] and IL-4 production was significantly lower in the treatment compared to placebo group but there was no change in IE, IgG or IFN γ [54]. These effects were not seen with treatment lower than 750 g/dose [53-55]. Strikingly, nearly 70% of

subjects in the treatment groups had adverse reactions, predominantly a late asthmatic reaction characterised by allergic rhinitis and asthma symptoms. There was no immediate or late phase skin reactivity. In fact the response to shorter peptide fragments of Fel d1 (16/17 amino acids) has been used to characterise this late asthmatic reaction [56]. Intradermal injection of pooled Fel d1 fragments did not induce an immediate reaction but in a subset of donors there was delayed reaction characterised by reduced FEV₁. This late asthmatic reaction had some association with the HLA genotype of the subjects and supports a central role for T cells in the late asthmatic response.

Long Peptide Therapy

In addition to the reported adverse reactions to short peptides, one of the major shortcomings of short peptide immunotherapy is the requirement for information about the subject's T cell epitope usage. These will vary with the individual's tissue type (HLA genotype) and the short peptide pool may need to be tailor made to suit the donor.

Long peptide technology, as the name suggests, utilises long peptides that span the entire sequence of the protein thereby ensuring all T cell epitopes are available in the absence of IgE binding. Knowledge of all the T cell epitopes within an allergen aids in the design of the fragments but details of the subject's HLA genotype are not required because all epitopes are present. These fragments can be generated as described for short fragments or by PCR using the wild type cDNA as the template then cloning into a plasmid for expression by *E. coli* and purification of the protein. The two best examples being considered for use in immunotherapy are PLA₂ and Bet v1.

PLA₂

Three overlapping fragments of PLA₂ have been generated corresponding to amino acids 1 - 60, 47 - 99 and 90 - 134. These fragments have been used successfully (intranasal and intra-peritoneal administration) in mice sensitised to PLA₂ in alum leading to reduced specific IgE and IgG1, increased IgG2a (Th1 response in mice) and decreased IL-4:IFN γ [57,58] Thus a shift from Th2 to Th1 responsiveness was induced. Intra-nasal or intra-peritoneal administration of the fragments also prevented anaphylaxis. Further elaboration of the immunological effect of these fragments was provided by the ability to transfer specific T cell tolerance to naïve mice using CD4⁺ T cells [59]. Although further phenotyping was not conducted this observation suggests that an anergic or regulatory population was produced during successful immunotherapy with long peptide fragments paralleling the observations made using current therapy modalities in humans.

Preliminary data is available on the potential usefulness of these molecules for treating humans. Long PLA₂ fragments, unlike shorter overlapping peptides, induced vigorous T cell proliferative responses from all PLA₂ sensitised allergic patients undergoing conventional venom immunotherapy, and did not bind IgE or trigger skin reactions on intradermal injection [60, 61].

Bet v1

In contrast to PLA₂ two non-overlapping long fragments of Bet v1 have been engineered; an N-terminal fragment corresponding to amino acids 1 – 74 and a C-terminal fragment that corresponds to amino acids 75 – 160. The breakpoint has been designed to correspond to a point outside all known epitopes. Neither fragment could bind IgE but the C-terminal fragment induced greater proliferation and favoured a potent IFN response whereas the N-terminal favoured IL-4, IL-5 and IL-13 production. Administration of the fragments to animals led to the production of IgG that inhibited IgE binding by serum from allergic patients [62]. Thus these Bet v1 fragments, especially fragment C, have the properties desired in a candidate for successful immunotherapy. These fragments have also been tested by skin prick test and intradermal injection. Lower reactivity than wild type Bet v1 was observed with greater than 100 fold more fragment needed to induce immediate type skin reactions [63]. A comparison of wild type Bet v1 and peptide derivatives has been undertaken in a human skin chamber model using birch pollen sensitised subjects. Chamber fluid was collected 2 and 8 hours after administering the peptides or wild type Bet v1 and examined for various mediators. Although the levels of eotaxin and the number of eosinophils recruited to the fluid were similar for the two groups, cellular activation was reduced in the peptide treated group; lower levels of histamine, granulocyte/macrophage-colony stimulating factor (GM-CSF) and eosinophil cationic protein (ECP) were recorded and eosinophil expression of CD69 (a common activation marker) was diminished [64]. Animal models indicate that peptide pools can be used, prophylactically and therapeutically, for alleviating the allergic response to Bet v1. Mucosal administration (intranasal) of Bet v1 peptides reduced specific responses by B and T lymphocytes both *in vivo* and *in vitro*, limited eosinophil infiltration of the airways and decreased airways hyperresponsiveness in subsequently sensitised animals. This response was comparable to the prophylactic effect of the administration of intact wild type Bet v1 prior to sensitisation of the animals [65].

Thus, long peptide fragments have a promising future in immunotherapy although assessment of the long-term benefits is essential especially as PLA₂-specific IgE gradually increased in the long peptide treated mice in the studies described above. However, reversibility provides further support that T cell anergy rather than deletion is central to immunotherapy success.

Other Protein Manipulations

Structural mimotopes are discovered using random phage display peptide libraries. This technology has been used to yield IgG that blocks rather than enhances IgE binding/cross-linking which otherwise is not predictable. By panning with a murine antibody that enhances IgE binding to Bet v1 and using the mimotope to immunise mice, the investigators generated IgG that also enhanced this reaction. Conversely, by panning for mimotopes recognised by Bet v1-specific IgE from a birch pollen allergic patient and immunising mice

with the identified mimotope, resulted in IgG that blocked IgE binding *in vitro* [66].

Other modifications of protein allergens have been considered. Addition of a maleyl group to a protein targets the scavenger receptors on macrophages which is postulated to bias towards Th1 responses. This has been attempted with tropomyosin, the major shrimp allergen. In a mouse model administration of maleylated tropomyosin led to decreased IL-4 and increased IFN production compared to the native antigen [67]. Prophylactic and therapeutic oral administration of cholera toxin B (CTB) coupled with ovalbumin reduced specific IgE levels in plasma from appropriately sensitised mice despite CTB being considered to down-regulate Th1 rather than Th2 responses [68].

3.5 Anti-IgE Antibodies

As a central mediator in allergic disease, IgE is a logical therapeutic target. There has been intense interest in the development of therapeutic antibodies that can bind IgE without inducing histamine release. There are a number of strategies which have been investigated including:

Passive:

- i) Allergen-specific passive immunisation with (anti-idiotype) IgG antibodies to variable regions of IgE,
- ii) Nonspecific passive immunisation with antibodies to constant (Fc regions of IgE (targeting all IgE),

Active:

- iii) Vaccination to induce active production of anti-idiotype antibodies to target allergen-specific IgE,
- iv) Vaccination to induce active antibodies to all IgE (Fc),
- v) The use of allergen-antibody immune complexes to down regulate production of specific IgE.

The principal challenge for all these techniques has been to bind and inhibit IgE activity without inducing IgE cross linking and mast cell degranulation. The use of antibodies to Fc for passive immunisation is the most developed technique (already used in human clinical trials) but does have some limitations. While this therapy achieves dramatic (non-specific) reductions in total serum IgE levels and clinical improvement (summarised briefly below) it requires repeated administration of high doses of monoclonal anti-Fc antibodies, with obvious cost. More recently, techniques for safely inducing sustained endogenous production of anti-Fc antibodies have been explored. [69], although these studies are still limited to animal models. Strategies to selectively inhibit the activity of allergen-specific IgE are also not yet well developed in humans. Because these techniques rely on binding at the variable allergen recognition regions of IgE, these anti-idiotype antibodies cannot prevent binding of IgE to mast cells. The risks of inducing IgE crosslinking and mast cell degranulation [70] are a major limitation. The development of humanised mouse IgG4 antibodies (which are less likely to crosslink

IgE) may overcome this, and vaccine strategies to induce naturally occurring anti-idiotypic IgG4 responses may have more sustained benefits. However, because of the demonstrated greater safety of anti-Fc antibodies in passive vaccination, and remaining concerns that antibodies to other portions of IgE may be anaphylactogenic, most techniques for active vaccination are focused on inducing antibodies to Fc domains [69]. In animals there have been successful attempts to neutralise the effects of IgE by vaccination with C 3 and C 4 epitopes.

Antibodies to IgE Fc

The identification of monoclonal antibodies (mAb) to human IgE lead to the development of humanised non-anaphylactogenic antibodies to IgE. These have shown beneficial effects in human trials (reviewed in [71-74]). Although the effects of these antibodies are not allergen-specific, they are relevant to this discussion because of the potential benefits and complimentary effects in the treatment of many specific allergies.

These antibodies (CGP 51909 and rhu mAb E25) bind to the Fc (C 3) domain of IgE that normally binds to mast cells via the high affinity IgE receptor Fc R1. This achieves a very efficient reduction in the free serum IgE (by more than 90% in some studies [75], and prevents binding to mast cells (and basophils) and the downstream inflammatory effects. The resulting immune complexes (with E25) are very small [76] and do not activate complement or accumulate in any body organs [77]. As the B cell binding domain on IgE is distinct from the C 3 mast cell binding region, these mAb can still recognise IgE on B cells and suppress IgE production. The immunological benefits of anti-C 3 mAb do not appear to be limited to reducing the acute phase response [78]. Anti-IgE mAb also prevents IgE binding to Fc RII (CD23) receptors on monocytes, dendritic cells, epithelial cells and possibly eosinophils (reviewed by [74]). These changes have been associated with clinical improvement and a significant reduction in medication requirements for both asthma [75, 79-81] and allergic rhinitis [82, 83]. It is worth noting that immunological parameters improved more consistently than clinical symptoms, and few patients show complete resolution of their symptoms. The reason for heterogeneity in the clinical response despite immunological improvement is unknown. It is likely that other IgE independent pathways are involved in disease expression, as noted in IgE/B cell deficient mice [84, 85]. While systemic administration of anti-C 3 mAb reduces circulating levels of IgE, there may be little effect in the end-organ tissues where disease is expressed. However, efforts to deliver anti-C 3 mAb topically to mucosal sites of atopic inflammation have been ineffective so far [86].

As administration of anti-C 3 mAb is a form of "passive" immunisation, the clinical and immunological effects of anti-IgE (rhu mAb E25) are also not sustained after therapy is discontinued. The development of newer strategies to promote endogenous production of anti-(C 3) IgE mAb by "active immunisation" may be a way of achieving better end-organ effects and a more sustained effects. This has been recently achieved in animals [69]. Oral administration of bacteriophage expressing homologous IgE structures (C 3

and C 4) can induce systemic anti-IgE responses to. The potential of this strategy in humans is uncertain.

Although anti-C 3 mAb have been given safely and effectively in humans, the long term consequences are unknown. Potential antigenicity of murine derived antibodies has been minimised by removing nonessential murine residues [87], with fewer than 5% mouse sequences in the remaining products (CGP51901 and E25). To date there are no reported cases of sensitisation to the remaining murine residues. Initial concerns that inhibiting IgE may compromise the host response to parasites also appear unfounded. In fact the efficient clearance of parasites by infected animals treated with anti-IgE mAb [88, 89] has challenged the previously held assumptions about the role of IgE in parasitic disease.

These antibodies will be soon available for clinical use. It is important that there are clear guidelines and indications for use. At this stage anti-(C 3) IgE mAb appear inappropriate as monotherapy for asthma. As demonstrated in clinical studies [90] these antibodies have a steroid sparing effect in patients with moderate to severe asthma and may be useful in this context. This non-allergen specific therapy may have a role in patients with multiple sensitivities for whom vaccination for all specific allergens is not practical. IgE monoclonal antibodies may also enable a wider application of allergen-specific immunotherapy. Used in conjunction with allergen vaccination, anti-(C 3) IgE mAb may reduce the risk of systemic reactions and possibly enhance clinical responses. This requires further investigation.

Anti-Idiotypic Antibodies

Antibodies to the variable antigen-recognition region of IgE have been largely discussed above. At present these strategies have limited application in humans.

Antibody-Allergen Immune Complexes

The administration of autologous antibody-allergen complexes was used as a treatment strategy in the early 1990's and has shown clinical benefits in allergic patients [91-95]. This is highly allergen specific, and appears to mediate a reduction in the level of circulating antibodies with specificity for the administered allergen [96, 97], with reduction in both IgG and IgE antibodies. Furthermore, these antibody-allergen complexes appear to boost the production of anti-idiotypic antibodies, which may be one mechanism of their action. Research in this area has been overshadowed by the implementation of human trials with anti-(C 3) IgE mAb.

3.6 Molecular Strategies (Cytokines) for Immune Modulation

The following methods of immunotherapy are not antigen-specific but offer alternative targets for immunotherapy, especially for the multi-sensitised patient. Subcutaneous administration of recombinant native human IL-12 has been trialed clinically with mild allergic asthmatic patients [98]. Eosinophil numbers in blood and sputum were significantly reduced compared to placebo but differences in

histamine-induced airways-hyper-responsiveness and the late asthmatic reaction to inhaled allergen were not significant. Similarly a single intravenous infusion of monoclonal anti-IL-5 prevented allergen-induced eosinophilia (sputum and blood) but did not affect airways hyper-responsiveness or the late asthmatic reaction [99]. These studies provide a cautionary tale: effectively targeting one component of the allergic response may not alleviate clinical symptoms. Obviously we have a long way to go before we fully understand the role of individual components, especially eosinophils, in the disease process. This is amplified because of the complexity, interplay and apparent redundancy within the immune system.

The development of cytokine variants that have improved or altered functional properties is another area of investigation. This requires an intimate and detailed knowledge of the structure of ligand and receptor. Functional variants can be generated in two ways. Site-directed mutagenesis, as discussed above, can be used to modify the cytokine's amino acid sequence so that interaction with the receptor is altered. For example, antagonistic IL-4 (hIL-4Y124D) has been designed that inhibits IL-4 and IL-13 induced IgE synthesis [100]. An alternative approach is molecular breeding/DNA shuffling which can yield novel variants of cytokines especially as it bypasses the need for assumptions at the design stage [101]. This technology is, however, in its infancy.

Another target for novel strategies is Fc RI expressed on mast cells and basophils. The cross-linking of high affinity IgE receptors by IgE binding of multi-valent allergen triggers the release of biological mediators. Preventing degranulation will limit the early response. The intracellular component of Fc RI contains an immunoreceptor tyrosine based activation motif (ITAM). This introduces the possibility that activity of this receptor can be negatively regulated by co-aggregation of Fc RI with an ITIM (I= inhibitory) containing receptor. An example of an ITIM containing receptor expressed by mast cells that co-aggregates with and negatively regulates Fc RI signal transduction under physiological conditions is Fc RIIB (CD32b), the low affinity IgG receptor (reviewed by [102]). Importantly, IgE induced release of biological mediators by mast cells and basophils can be inhibited by cross-linking Fc RI and Fc RIIB under physiological conditions. Co-aggregation of these two receptors in mouse mast cells reduces the secretion of serotonin and TNF [103]. Moreover, mice deficient in Fc RIIB have enhanced IgE-mediated anaphylactic responses indicating that Fc RIIB has a physiological role in regulating the response triggered via the high affinity IgE receptor [104]. Theoretically, specific IgG or allergen complexes can be engineered to enhance the inhibitory interaction of Fc RI and Fc RIIB and limit degranulation of mast cells and basophils.

4. PROGRESS IN IMMUNOTHERAPY FOR TREATMENT OF SPECIFIC ALLERGIES

4.1 Inhalant Allergies

While inhalant immunotherapy is well established a number of new strategies may improve available therapy.

Peptide immunotherapy and DNA vaccines may offer safer alternatives to protein extracts. Extracts more relevant to different geographical locations also need to be developed. Better mucosal vaccines may also become more important, particularly for primary prevention (see below).

4.2 Insect Venom Allergies

Whereas traditional immunotherapy for bee venom uses whole venom, the identification of short peptides of the immunodominant allergen PLA2 (phospholipase A2) holds future promise as an effective but safer alternative (see peptide vaccination). In sensitised mice the administration of long overlapping peptides spanning the whole PLA2 molecule has resulted in successful modulation of cellular responses and also fully protected from anaphylaxis [58]. Similar strategies are being developed for humans.

4.3 Food Allergy

Although many food allergies are transient, there is still an urgent need for new therapies for persistent life threatening allergies to foods such as peanuts. Immunotherapy for food allergy is not generally accepted because of the high risk of serious, potentially life threatening IgE mediated systemic reactions. Although there are isolated reports of desensitisation for peanut allergies [105], serious side effects are common, there are no standardised protocols and this is considered too dangerous by most.

More recently, site-directed mutagenesis of major peanut allergens has rendered peanut allergens more "hypoallergenic" [49]. These modified allergens are less likely to produce systemic reactions but remain immunogenic, and may lead to safer immunotherapeutic options in the future. Anti-IgE mAb may be also be of theoretical benefit (in conjunction with allergen-specific vaccines) by reducing serious adverse reactions to food allergens. The anticipated development of allergen peptide immunotherapy, and allergen-gene vaccination may offer future hope. Small peptides or transcribed allergen genes will theoretically induce tolerance at a cellular level without evoking IgE reactions. In animals it has been possible to induce systemic tolerance to a protein using a single epitope [50]. There has unfortunately been slow progress for food allergy in humans.

4.4 Drug and Latex Allergy

Rush desensitisation protocols are used widely, particularly for antibiotics when the drug is highly indicated for a hypersensitive patients. This remains a dangerous procedure and unfortunately there are few more definitive approaches on the horizon. There may be a role for non-specific IgE inhibition anti-(C 3) IgE mAb prior to rush protocols. To our knowledge, this has not been examined.

Extracts for latex desensitisation are now available, and can induce tolerance in sensitised individuals [106, 107]. However, systemic side-effects remain a serious concern. In 1997, Slater *et al.* [35, 108] reported favourable response in latex sensitised mice which were vaccinated with plasmid

DNA encoding latex allergen Hev b 5, with a 23% drop in the levels of allergen-specific IgE after 10 days. This form of allergen-gene vaccination offers new hope for may allergies with no conventional immunomodulatory therapy.

5. ALLERGEN VACCINATION FOR PRIMARY PREVENTION

With the escalating incidence of allergic disease there is a growing need to develop strategies to prevent the development of Th2 responses. The use of allergen vaccines has been proposed as one method of primary prevention [109]. While the therapeutic benefits of "allergen vaccines" in existing disease are well established, the role in disease prevention is still largely theoretical in humans. Potential strategies involve utilising and enhancing the natural processes which in most cases efficiently terminate IgE responses to allergens in infancy. Accordingly, vaccines for primary prevention would need to be administered in early infancy, when immune responses are still "plastic" and not "committed". This is not without concerns (discussed further below). In murine systems neonatal administration of allergen can inhibit the development of Th2 type airways disease, but the dose and delivery method appear crucial [110].

The enteric mucosal immune system plays an extremely efficient and pivotal role in the development of tolerance. Repeated exposure to allergen through the gastrointestinal tract during this period of life leads to the development of tolerance, even in highly atopic individuals (reviewed in [109]). It is proposed that exposure to aeroallergens through this route may promote the local (IgA) immune responses which promote persistent systemic tolerance, preventing the emergence of pathogenic Th2 responsive memory T cells. In animal models, early antigen feeding induces tolerance possibly mediated by allergen-specific suppressive + CD8+ T cells [111] as well as the non-specific effects of TGF . It now also appears that a population of CD25+ CD4+ T regulatory cells also have an important role in regulating systemic tolerance [112, 113]. A number of studies are currently addressing the effects of these strategies in humans, including the effects of intranasal administration of allergen, which may theoretically have similar benefits. Parenteral administration of allergen with an appropriate Th1 inducing adjuvant is also being considered for promoting selective Th1 responses during immune development. It is possible that the effects described in adults with established disease may be more effective in infants without established patterns of T cell response.

The main concerns about allergen vaccination in infants, particularly while the precise mechanism of action are uncertain, are potential unforeseen consequences on other aspects of natural immune development. It is possible that bystander effects could mediate altered patterns to other antigens. The parenteral administration of Th1-enhancing adjuvants raises theoretical concerns about Th1 mediated autoimmune reactivity. While there is a very high risk of atopic sensitisation (almost 40% in industrialised countries) [114] many will only develop mild disease, and it may be more desirable to target invasive prevention strategies to

infants who are most likely to develop severe disease. It is currently not possible to accurately predict clinical outcomes. Although allergen vaccination may become a useful strategy, at this stage it is more critical to determine the underlying reasons for the dramatic increase in allergic disease, and the mechanisms of immune dysregulation in this group.

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