

Peptide-mediated regulation of the allergic immune response

GERARD F HOYNE and JONATHAN R LAMB

Infection and Immunity Section, Department of Biology, Imperial College of Science, Technology and Medicine, London, United Kingdom

Summary A major key to successful immunotherapy may depend on altering the qualitative nature of the immune response in allergic patients. In this review we examine how immune responses to environmental allergens are regulated, and the mechanisms used by the immune system to prevent allergic sensitization. We also discuss future prospects of using allergen-derived peptides in immunotherapy and the possibility of 'reprogramming' the immune responses by immunizing under conditions that promote Th1 responses instead of Th2 responses.

Key words: allergy, cytokines, desensitization, immunotherapy, peptides, T cells, tolerance.

Introduction

Cytokines secreted by activated CD4⁺ T cells have an important influence on the qualitative and quantitative nature of immune responses made to foreign antigens.¹ Functional subsets of murine CD4⁺ T cells can be distinguished on the basis of the pattern of cytokines they secrete when activated.² Th1 cells preferentially secrete IL-2, IFN- γ , TNF- β and participate in delayed-type hypersensitivity responses, whereas Th2 cells are more efficient in promoting humoral immune responses by secreting IL-4, IL-5 and IL-6 which promote the growth and differentiation of B cells and induce isotype class switching towards IgG1 and IgE. It is also established that the cytokines secreted by each subset are mutually antagonistic. IFN- γ secreted by Th1 cells can inhibit the growth and differentiation of Th2 cells.³ Alternatively, IL-4 and IL-10 secreted by Th2 cells can prevent the activation and growth of Th1 cells.^{4,5}

There is increasing evidence that supports a role for Th2-type cells in allergic sensitization in humans.^{6–11} Unlike their murine counterparts, human CD4⁺ T cells in general do not display such polarized patterns of cytokine secretion, but Th1(IFN- γ ⁺)-dominant or Th2(IL-4 γ ⁺)-dominant phenotypes have frequently been observed in certain disease states. Allergen-reactive T cells can be isolated from both allergic and non-allergic individuals but there are qualitative differences in the types of cytokines produced by T cells of the two populations and this seems to be a decisive factor in determining whether patients display clinical symptoms.^{8,11} Allergen-specific T cells isolated from atopic patients show an IL-4^{hi}/IFN- γ ^{lo} (Th2) phenotype and these cells can support the production of antigen-specific IgE *in vitro*. T cells from non-atopic patients cannot induce IgE synthesis and display an

IFN- γ ^{hi}/IL-4^{lo} phenotype. Thus a predominance of Th2-like cells during an allergic response could provide the stimulus to drive and maintain a persistent allergen-specific IgE antibody response *in vivo*.¹² In order to further support a role for Th2-like cells *in vivo* during an allergic response recent studies have reported a high frequency of lymphocytes which stain positive for IL-2, IL-3, IL-4, IL-5 and GM-CSF mRNA in clinical biopsy samples obtained from allergic individuals.^{13–15} The allergic response is accompanied by the infiltration of inflammatory cells such as mast cells, basophils and eosinophils, into the airways which release histamine, leukotrienes and other inflammatory mediators.¹⁶ The influx of eosinophils is dependent on the release of IL-5 from activated Th cells,¹⁷ further supporting the role of Th2 cells in the allergic immune response. Although there is considerable heterogeneity in the cell types and mediators that contribute to allergic inflammation it does however provide several potential targets in immunotherapy. A major goal of specific immunotherapy is to reduce the severity of clinical disease in patients, by immunization with allergens or their derivatives, without compromising their ability to develop immunity to other pathogens. Although allergen desensitization has been practised for decades, information on the immunological mechanisms that are required to give rise to clinical improvement is limited. Recent studies have indicated that successful desensitization is accompanied by a decrease in Th2-type cytokine production by antigen-specific Th cells present in the peripheral blood,¹⁸ while others have reported a corresponding rise in IFN- γ secretion.^{19,20} Even though the levels of antigen-specific IgE do not fall dramatically in the short term,¹⁸ patients who become desensitized show improvement in clinical symptoms. Therefore, reducing or altering the pattern of cytokine secretion by allergen-reactive CD4⁺ T cells appears to be a primary event in clinical desensitization. In this review we shall examine some of the recent advances in immunotherapy especially those based on the use of immunogenic peptides. We shall also consider future directions for immunotherapy based

Correspondence: Dr Gerard F Hoyne, Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB, United Kingdom.

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on 'reprogramming' the immune response by altering cytokine responses *in vivo*.

In vivo models of allergic immune responses

Animal models have been useful for the understanding of the nature of cellular immune responses to inhaled antigens. Experimental animals exposed to a nebulized protein antigen, ovalbumin, generate a transient IgE response that is down-regulated with time and, once modulated, animals fail to produce IgE on subsequent exposure to antigen.²¹⁻²⁵ The transient IgE response was subsequently shown to coincide with activation of CD4⁺ T cells that secrete Th2-type cytokines but these are replaced by CD4⁺ T cells and CD8⁺ T cells that secrete high levels of IFN- γ and thus inhibit the growth and differentiation of Th2 cells.²⁶ The CD8⁺ T cells induced in this model system express the $\gamma\delta$ ⁺ TCR and can suppress IgE synthesis when adoptively transferred to a naive host.^{27,28} Thus, under normal circumstances, allergic sensitization to aeroallergens may be avoided by a process of immune deviation of T cell effector function.²⁶ It has been proposed that a similar set of mechanisms may operate during infancy or in early childhood to prevent allergic sensitization in humans.²⁹ The lungs are in constant contact with environmental antigens and therefore immune responses at this site need to be strictly controlled. It has been known for many years that resident pulmonary alveolar macrophages (PAM) exert lymphostatic properties that maintain an immunosuppressive environment within the lung.^{30,31} In the presence of IFN- γ , PAM can secrete nitric oxide which can effectively abrogate T cell proliferative responses *in vitro*.³² In addition, PAM can prevent the maturation of dendritic cells,³³ which are the most potent APC of the immune system, and this would have the effect of reducing T cell priming to inhaled antigens.

In another animal model, mice receiving daily exposure to nebulized ovalbumin produce specific IgE antibodies and develop an airways hyper-responsiveness that is similar to that induced during allergic responses in humans. Two populations of peripheral CD4⁺ T cells were identified that displayed distinct effector functions. The predominant population of T cells elicited following ovalbumin exposure were V β 8⁺ T cells that could support IgE synthesis, while V β 2⁺ cells were also activated that suppressed IgE synthesis when adoptively transferred to naive recipients.³⁴ In addition, exposure to ovalbumin led to activation of CD8⁺ T cells that could suppress IgE synthesis *in vivo*.³⁵

Modulating the allergic immune response

Mucosal tolerance

There is approximately 400 m² of surface area of mucosal surfaces within the body and thus is a major site of antigenic challenge. The mucosal immune system has the extraordinary potential to discriminate between antigens that are harmless (e.g. allergens and dietary antigens) and

those that are associated with microbial pathogens.³⁶ Normally soluble proteins delivered through mucosal surfaces do not elicit a strong systemic immune responses but instead induce a transient local immune response that is replaced by long-term peripheral unresponsiveness or tolerance.^{36,37} This, therefore, poses significant problems for the generation of mucosal vaccines, but the natural tolerogenic properties of the mucosal immune system can be exploited in immunotherapy as a means of controlling immune based diseases.^{38,39} There are various mechanisms used by the immune system to maintain peripheral tolerance to self antigens (Table 1). Clonal deletion and clonal anergy are frequently used to maintain tolerance of self-reactive T cells in the periphery.⁴⁰⁻⁴⁵ Alternatively, self-reactive T cells may be unable to enter tissues which express the target antigen expression, or the self antigen may be expressed in such low levels that it does not induce T cell responses. Therefore, peripheral tolerance is established through clonal ignorance.^{46,47} Experimental studies have shown that autoreactive T cells may exist in the peripheral circulation and maintain antigen reactivity for the life of the animal.⁴⁸ The development of tolerance in this latter situation may depend in part on the pattern of cytokine secretion of self-reactive T cells. Those cells that secrete inflammatory Th1 type cytokines (e.g. IFN- γ) can promote disease while T cells secreting Th2-type cytokines can protect from autoimmune destruction of tissues.⁴⁹ It has been reported that tolerance to extrathymic antigens may in some cases result in the down-regulation of the TCR,^{50,51} and continual exposure to self antigen may drive T cells further into tolerance so that eventually the self-reactive T cells will be deleted.⁵² It is possible that similar mechanisms that are used to control responses to self antigens will also be employed to regulate peripheral immune responses to foreign antigens. Feeding antigens to experimental animals can lead to the development of oral tolerance, which is characterized by suppression of both cellular and humoral immune responses to the fed antigen.^{37,39} Recent studies suggest that oral tolerance may be generated by distinct mechanisms depending on the level of antigen administered.³⁹ Oral administration of high doses of protein can lead to clonal deletion in the short term⁵³ and those T cells which remain become anergic.^{8,19,54,55} Alternatively, oral tolerance to low doses

Table 1 Mechanisms of peripheral T cell tolerance

Mechanism	References
Clonal anergy	44 43 48
Clonal exhaustion/deletion	40 42 60
Clonal ignorance	46 47
TCR down-regulation	50 51
Immune deviation	49 70

of antigen is thought to be mediated by the activation of suppressor CD4⁺ or CD8⁺ T cells that secrete inhibitory cytokines such as IL-4 and TGF- β 1.^{56,57} Immunogenic peptides derived from self antigens under some conditions have been shown to induce oral tolerance,⁵⁸ but this may not be a general rule for all self peptides.⁵⁹ It appears that oral tolerance to self proteins, which are major targets for autoimmune disease, can protect animals from disease or reduce the severity and/or delay the onset of clinical symptoms. Similarly, peptides derived from autoantigens when administered parenterally in tolerogenic form can induce unresponsiveness through the deletion of specific T cells and/or clonal anergy.^{60,61} In addition, animals can also be protected from autoimmune disease by the delivery of self proteins or peptides through the intranasal route.^{59,62,63} Therefore, mucosal delivery of target antigens appear to be useful in modulating the effector function of self-reactive T cells *in vivo*.

Peptide based immunotherapy

Using a murine model of house dust mite (HDM) reactivity we have shown that CD4⁺ T cell responses to the HDM allergen Der p 1 may be modulated by pretreating mice with a high dose of an immunodominant peptide given either intranasally or orally.^{64,65} Peripheral tolerance can be achieved with microgram quantities of peptide antigen when given intranasally,⁶⁴ whereas the development of oral tolerance requires the administration of large quantities of antigen (i.e. >1 mg of peptide).⁶⁵ Moreover, intranasal tolerance can be induced in both naive and sensitized mice.⁶⁴ It was also observed that treatment with a single immunogenic peptide, either orally or intranasally, had the potential to abrogate responses to the whole allergen, such that T cell responses to all epitopes were down-regulated.^{64,65} The phenomenon of intramolecular (or cross) epitope suppression is only induced when the whole allergen is used for immunizing, and T cell responses to minor epitopes are unaffected if the relevant peptides are used for priming (G.F. Hoyne, unpubl. data, 1996). Tolerance to the inhaled peptide can last beyond 6 months and does not require the presence of CD8⁺ T cells.⁶⁶ Recently, Briner *et al.* demonstrated that it was possible to down-regulate a Th2 response in a murine model of cat allergy by the subcutaneous administration of peptides derived from the Fel d 1 antigen.⁶⁷ Peptide treatment down-regulated both Th1- and Th2-type cytokines and treatment with one peptide appeared to modulate responses to the whole allergen.⁶⁷ It was suggested that peptide treatment resulted in the development of T cell tolerance; however, the immunological mechanisms by which subcutaneous peptide induces tolerance remains to be defined. We have studied in detail the immunological consequences of treating mice with high doses of inhaled peptide. These studies revealed that the immunodominant peptide induces a transient CD4⁺ T cell response that leads eventually to the down-regulation of both Th1- and Th2-type cytokines.⁶⁶ During the early response to the inhaled peptide, antigen-specific T cells were capable of secreting IL-2 suggesting that they

receive costimulatory signals from APC during priming.⁶⁶ However, these T cells must receive an additional negative signal from APC that eventually overrides the positive signals for IL-2 production. The nature of these inhibitory signal is not known. Thus it would appear that peripheral tolerance to the inhaled peptide does not arise as a consequence of immune deviation, which has been previously observed with tolerance to nebulized antigens.²⁶ In addition, unresponsiveness does not seem to be due to a lack of costimulation. The concept that transient T cell responsiveness can precede the development of peripheral T cell tolerance has been observed with tolerance to other foreign and self antigens and appears to affect both CD4⁺ and CD8⁺ T cells (Table 2).^{40,42,68-70} However, it is not clear if the initial burst of T cell proliferation is essential for tolerance induction, rather it may be important for inducing apoptosis.⁷¹ Since antigen-specific T cells lose the capacity to secrete IL-2 following intranasal peptide treatment it appears that cells may become anergic.⁶⁶ This raises an interesting paradox; if tolerized T cells cannot secrete IL-2 then how are they maintained for long periods of time *in vivo*? Future experiments with TCR transgenic mice may allow the fate of antigen-specific T cells to be studied in more detail during the induction of tolerance to mucosally delivered antigens.

The induction of peripheral tolerance to the inhaled peptide develops within 2 weeks of antigen delivery as assessed by *in vitro* responses to the peptide antigen.⁶⁶ However, animals remain responsive to a further challenges to the same peptide when given intranasally,⁶⁶ namely, the peptide elicits a further transient CD4⁺ T cell response of similar magnitude and kinetics to the primary response. Antigen-specific T cells that are activated on rechallenge are able to secrete IL-2 when challenged *in vitro*.⁶⁶ Nevertheless despite this transient response, animals remain refractory to an antigenic challenge with the whole antigen. Similar results have been obtained following periodic feeding with soluble protein antigens that normally leads to the development of oral tolerance.⁷² Thus although animals may appear to be phenotypically tolerant after a single treatment with either protein or peptide, they remain fully responsive to these antigens, merely the effector response of the tolerized T cells have changed. The major role of these tolerized cells is now diverted away from acting as classical 'helper' T cells and

Table 2 Transient activation precedes T cell tolerance

Nature of antigen (references)	Antigen	T cell population
Self/super antigens	MIs 1a	CD4 ⁺
40	H-Y	CD8 ⁺
42		
Protein/peptides	LCMV peptide	CD8 ⁺
60	OVA peptide	CD4 ⁺
69	OVA	CD4 ⁺
70	HDM peptide	CD4 ⁺
66		

they assume a role in which they can suppress the growth and activation of other antigen-specific T cells presumably through the secretion of inhibitory cytokines.

Peptides in human clinical trials for allergen immunotherapy

Clinical desensitization with allergen extracts can have variable success and one of the problems is the potential variation in the level of different antigenic components in the extracts, and the presence of variant isoforms of allergens. Thus for multideterminant allergens like house dust mite allergy, some antigenic components may not be in significant levels in order to achieve clinical desensitization. Another problem posed by the use of native allergen extracts is the risk of anaphylaxis, and thus allergen-derived peptides which are recognized by specific CD4⁺ T cells may be of potential benefit in the long term. Based on successful preliminary studies in mice, a multicentre trial has been established to evaluate the use of peptide therapy in cat allergic patients.⁷³ Using a panel of overlapping synthetic peptides specific for the major cat allergen Fel d 1, putative CD4⁺ T cell epitopes were identified by screening peripheral blood lymphocytes from cat allergic patients. Early results indicate that patients tolerated the subcutaneous peptide treatment well with minimal side effects. It was recently shown that the allergen-derived peptides do not possess IgE binding activity *in vitro*.⁷⁴ Unfortunately, no immunological data is available from these patients to indicate whether peptide treatment induced tolerance *in vivo* or whether clinical improvement resulted from immune deviation by shifting the ratio of IL-4/IFN- γ secretion by CD4⁺ T cells *in vivo*. In another pilot study, patients sensitized to bee venom received a pool of three immunogenic peptides derived from the allergen phospholipase A2.⁷⁵ These peptides have been shown to stimulate PLA-2-specific CD4⁺ T cell clones *in vitro*.⁷⁶ The peptides were delivered subcutaneously and patients showed minimal side effects. Patients receiving the PLA-2-specific peptides demonstrated a decrease in allergen-specific IgE and a corresponding rise in IgG levels, and most patients reported a significant improvement in clinical symptoms.⁷⁵ Those individuals who received control peptides maintained high levels of IgE and had no diminution of clinical symptoms.⁷⁵

Patients who have been desensitized normally display a decrease in Th2 cytokine production, and clinical improvement usually correlates with a decrease in immediate and late phase skin reactivity with a long-term rise in IgG levels, particularly IgG₄, and a decrease in specific IgE.^{77,78} Yet in the short term (e.g. the first 3 months), the levels of specific IgE and IgG may actually rise.^{78,79} The effects that we have observed with peptides in murine studies may help explain the clinical findings.⁶⁶ Each time a patient receives an antigen challenge during the course of immunotherapy this may cause a transient production of cytokines by allergen-reactive T cells. Activation of memory Th cells is likely to induce IL-4 secretion and this may help propagate IgE synthesis in the short term. However, as treatment progresses IL-4 secretion by specific T

cells may be modulated and thus fall below a critical threshold that is essential for the maintenance of IgE synthesis *in vivo*. In addition, clinical desensitization may involve the replacement of IL-4-secreting cells with specific CD4⁺ T cells that secrete IFN- γ which would help to down-regulate ongoing Th2 responses *in vivo*.

Future directions

Reprogramming the immune response

A major key to successful immunotherapy may depend on altering the qualitative nature of the Th cell response in allergic patients. Although allergen fragments may be used therapeutically to induce systemic tolerance,^{64,65,67} it may not be a straightforward task to modulate ongoing Th2 responses in human patients with chronic allergic disease. Because decreasing the functional response of Th2/Th0 cells can lead to improved clinical symptoms,^{18,19} provides hope that strategies aimed at targeting specific CD4⁺ T cells *in vivo* may be beneficial. It may also be possible to combine different approaches to further increase the efficacy of immunotherapy. For example, mucosally delivered antigens could be used to functionally silence existing memory Th2/Th0 cells, and then it may be possible to reprogramme the immune response towards a Th1 phenotype. Reprogramming the immune system could be achieved by co-administering allergen in the presence of IL-12 or IFN- γ , or by immunizing with recombinant live vaccine vectors such as mycobacteria expressing defined allergens or fragments.⁸⁰ Issues concerning the longevity of the tolerant state and potential 'leakiness' of the reprogrammed immune response, would need to be addressed to determine the viability of such approaches to immunotherapy.

The observation that analogues of immunogenic peptides, termed altered peptide ligands (APL) can dissociate particular effector functions of T cells have generated interest in the potential application of APL in immunotherapy of immune-based diseases. Evavold and Allen originally showed that TCR recognition of a variant peptide by cloned Th2 cells could uncouple IL-4 secretion from T cell proliferation.⁸¹ Moreover, the same group reported that it was possible to induce clonal anergy in a Th2 clone in the presence of live APC.⁸² At present, the use of APL in animal models has been restricted to the study of autoimmune diseases.^{83,84} Recently, it was shown that an APL of proteolipid protein could alter the cytokine profile of autoreactive T cell clones *in vitro* and when adoptively transferred to naive mice they failed to cause disease. Therefore it was proposed that the APL was able to prevent disease in mice by inducing immune deviation of cytokine secretion by self-reactive T cells.⁸³ It may be possible to identify APL of specific allergens that when recognized by allergen specific CD4⁺ T cells alters the ratio of IL-4/IFN- γ secretion by the cell. Such analogues may prove to be useful in immunotherapy of allergic disease. Recent studies in our laboratory have revealed that APL can reduce IL-4 but maintain IFN- γ secretion in human Th0 clones *in vitro*.

Experimental studies have shown that immunoregulatory CD8⁺ T cells are activated as part of the normal T cell repertoire during immune responses to aeroallergens.^{26,28,35} These CD8⁺ T cells have the capacity to down-regulate IgE synthesis and secrete Th1-type cytokines. Therefore if it were possible to prime specifically for antigen-specific CD8⁺ T cells this may provide a novel way of modulating the allergic immune response. Alternatively, there is evidence from experimental models that some CD4⁺ T cell populations can specifically down-regulate IgE synthesis, presumably by secreting cytokines that abrogate production of antibodies.³⁴ Whether this will be true for all antigens remains to be determined.

Conclusions

Although preliminary results from clinical trials using allergen-derived peptides in immunotherapy are encouraging, there are a number of problems that need to be addressed before peptides can make the transition from experimental systems to clinical application. Results from experimental systems indicate that peptides containing immunodominant epitopes are more potent tolerogens than those containing minor epitopes.⁸⁵ Therefore, if peptides are to be effective in immunotherapy it will be important to incorporate immunodominant peptides into a therapeutic protocol. In order to obtain information on the distribution of immunodominant T cell epitopes for a particular allergen it will be necessary to perform detailed epitope analyses on the peripheral repertoire of a large panel of allergic patients of known HLA haplotypes. It will also be important to evaluate the stability and longevity of peripheral tolerance induced by specific peptides. The development of peripheral tolerance in memory Th2 cells *in vivo* has been controversial,^{86–88} and little is known about the plasticity of the human peripheral repertoire following desensitization. Experimental studies have indicated that it is much easier to manipulate the effector function of naive cells rather than memory T cells³⁷ and that allergic sensitization frequently occurs during infancy or early childhood.²⁹ Therefore it may be beneficial to target naive T cells by vaccinating children who are at high risk of developing an allergic disease due to a family history of allergy.²⁹ Vaccination could either induce peripheral tolerance or induce allergen-specific Th1-type responses.

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