



Associate editor: P.S. Foster

Peptide therapy for allergic diseases: Basic mechanisms and new clinical approaches

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Abstract

Desensitising allergen immunotherapy has been practised for many decades. Although time consuming, this form of therapy is antigen-specific and disease-modifying, in contrast to palliative pharmacotherapy. However, the use of allergen extracts containing native allergen molecules frequently results in allergic adverse reactions to treatment. Several strategies to reduce the allergenicity of therapeutic preparations, while maintaining their therapeutic benefit, are being developed. Peptide immunotherapy is one such approach. Short synthetic peptides, comprising T cell epitopes of major allergens, were unable to crosslink allergen-specific IgE molecules on basophils in vitro. Treatment of allergic volunteers with allergen peptides resulted in reduced skin, lung and nasal sensitivity to allergen challenge and improved their subjective ability to tolerate allergen exposure. Peptides reduced pro-inflammatory cytokine secretion from peripheral blood cells, whilst increasing the immunosuppressive cytokine IL-10. Furthermore, peptide therapy was associated with the induction of a population of CD4⁺ T cells with a suppressive functional phenotype. Thus, peptide therapy may be suitable for the antigen-specific treatment of allergic diseases.

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Keywords: Asthma; Epitope; Immunological tolerance; Immunology; Major histocompatibility complex

Abbreviations: EPR, early-phase reaction; FEV₁, forced expiratory volume in 1 sec; IL, interleukin; LAR, late asthmatic reaction; LPR, late-phase reaction; MOP, multiple overlapping peptides; PBMC, peripheral blood mononuclear cells; PC₂₀, provocative concentration inducing a 20% reduction in FEV₁; PD₂₀, provocative dose inducing a 20% reduction in FEV₁; SOCS, suppressor of cytokine signalling.

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1. Introduction

The ability of the immune system to provide protective immunity to the host relies on the ability to distinguish self (host-derived) from non-self antigens. The regulation of self-reactive T-cell specificities is achieved at 2 levels: central tolerance, occurring in the thymus during T-cell development, and peripheral tolerance. Extensive discussion of the mechanisms of central and peripheral tolerance is beyond the scope of this review. However, it is clear that both mechanisms are prone to failure under certain circumstances, and that this failure leads to immunity, which is destructive rather than protective.

Autoimmune and allergic diseases are examples of a failure of immune regulation. In autoimmunity, autoaggressive T-cell specificities appear to escape central tolerance in the thymus and, for reasons that are incompletely understood, are inadequately regulated in the periphery. The result is a proinflammatory response to host-derived tissue components. The immunopathology of allergic diseases displays certain similarities. In particular, recent studies have demonstrated inadequate peripheral regulation of allergen-specific T-cells in individuals with allergic diseases (Akdis et al., 2004; Ling et al., 2004). A notable difference between autoimmune and allergic disease processes is the lack of central tolerance to allergens since these are exogenous, non-host-derived factors that are not expressed in the thymus. Spontaneous remission of both autoimmune and allergic diseases has been well documented and provides evidence that adequate regulation can be reinstated. However, the mechanisms underlying such events are poorly understood. An important goal in ameliorating these diseases is the understanding of the processes that govern peripheral tolerance induction. With this insight, it may be possible to provide curative, rather than merely palliative, intervention for a number of major chronic inflammatory diseases. Moreover, the ability to manipulate peripheral tolerance mechanisms holds the promise of resolving issues of graft intolerance in transplantation.

The field of allergic diseases has historically provided a setting in which therapeutic approaches may be validated. This review will focus on data from recent studies of peptide immunotherapy that may hold promise as a universal approach to immune modulation.

2. Whole allergen immunotherapy

Whole allergen, or “specific” immunotherapy (SIT), has been practised, in a form largely unchanged since its first description, for almost a century (Noon, 1911). At the time of its development, the causal agents in summer hay fever were believed to be “toxins” to which it was necessary to induce immunity to achieve a cure. Thus, small quantities of allergen extract were administered parenterally with a view to inducing protective immunity. While allergens are now known not to be “toxins” in the standard sense of the word, the notion that protective immunity was induced during SIT was entirely correct.

SIT is a highly effective treatment when administered appropriately to carefully selected patient populations. Its efficacy has been shown to persist for many years following the discontinuation of treatment, and in this sense, SIT can be seen to be an effective cure for certain allergic diseases such as “hay fever” (Varney et al., 1991; Durham et al., 1999). Early investigations of the mechanisms underlying SIT described the induction of Th1 immunity to the allergen at the relative expense of Th2 (Secrist et al., 1993; Varney et al., 1993). Thus, following SIT, fewer Th2 cells and eosinophils were observed at sites of allergen challenge (Durham et al., 1996). Additionally, numbers of T-cells producing Th1 cytokines were elevated after therapy (Varney et al., 1993; Hamid et al., 1997). These observations gave rise to the concept of “immune deviation” and led to a decade of research into the development of therapeutic interventions designed to redirect a Th2-dominated response towards a Th1 response. In the field of autoimmunity, where many diseases are characterised by a pathogenic Th1 response, a parallel quest for deviation towards Th2 was seen.

More recently, the significance of immune regulation in the suppression of pathogenic responses has been increasingly recognised. Rekindling of interest in suppressor or “regulatory” T-cell function has led to a reexamination of the mechanisms of immunotherapy. Treatment of bee venom allergic individuals with allergen extract was associated with the up-regulation of interleukin (IL)-10 expression (Akdis & Blaser, 2001). Grass pollen immunotherapy for summer hay fever was associated with the elevation of IL-10 mRNA and protein in the nasal mucosa during (but interestingly not outside) the

pollen season (Nouri-Aria et al., 2004). These data imply the induction of regulatory mechanisms during periods of allergen challenge postimmunotherapy. The induction of IL-10 responses was also seen following immunotherapy for house dust mite sensitivity (Jutel et al., 2003). IL-10 is a pleiotropic cytokine with a number of immunosuppressive activities. Of particular relevance to allergic disease are the observations that IL-10 down-regulates mast cell function (probably through the up-regulation of suppressor of cytokine signalling [SOCS]-1 and -3; Royer et al., 2001), regulates T-cell responses (Groux et al., 1996), and serves as an isotype switch factor for IgG4, a prominent antibody isotype following immunotherapy (Jeannin et al., 1998). Allergen-specific IgG4 has been regarded by many as a blocking antibody that prevents allergen reaching and cross-linking the relatively less abundant allergen-specific IgE.

3. Specific immunotherapy: novel approaches

Despite the efficacy of SIT, concerns persist with respect to the safety of this form of therapy and with the difficulty of standardisation of natural allergen extracts. SIT is associated with a relatively high incidence of adverse events, the majority of which are the result of allergic reactions following mast cell and basophil activation by allergen cross-linking IgE on their surface. Numerous strategies have been developed to reduce the allergenicity of immunotherapy preparations while maintaining their ability to induce beneficial immune modulation. Such approaches include alteration of the tertiary structure of the allergen molecules through chemical modification (such as the generation of “allergoids” with glutaraldehyde; Marsh et al., 1970; Marsh, 1971), or gene/protein engineering (Ferreira et al., 1996; Okada et al., 1998; Hage-Hamsten et al., 1999; Vrtala et al., 1999; Pauli et al., 2000), conjugation of bacterial DNA oligomers to purified allergen molecules (Creticos et al., 2000), and generation of allergen multimers (Pauli et al., 2000).

Synthetic peptides corresponding to T-cell epitopes of the allergen have also been evaluated in a variety of studies. In comparison to many other approaches, peptides offer the advantages of inexpensive production, ease of purification/standardisation, and storage. The application of peptide immunotherapy to allergic diseases fulfils the 2 basic requirements for an improved therapeutic in that peptides lack the tertiary structure of the parent protein, yet retain the ability to stimulate T-cells. Thus, peptides of the appropriate size should display reduced allergenicity while still capable of interacting with MHC and T-cell receptors. The actual size of peptides employed in immunotherapy may be critical. In order to reduce interaction with IgE as far as possible, peptide length must be minimal, without compromising minimal T-cell epitopes.

4. Peptide immunotherapy

4.1. *In vitro* and animal models

Early in the 1980s, cloned CD4⁺ helper T-cells were rendered refractory to antigen challenge by pretreatment with supraoptimal concentrations of cognate ligand (peptide). Lamb et al. (1983) described this phenomenon as “antigen-specific non-responsiveness”. The absence of antigen presenting cells from the experimental system implied that the presentation of peptide to T-cell receptors was being achieved through binding to MHC class II molecules expressed by the T-cells themselves; T–T antigen presentation. These observations were among the first to suggest that T-cell nonresponsiveness (also referred to as T-cell anergy or T-cell tolerance), could be achieved through the inappropriate presentation of antigenic peptide.

Peptide-induced, antigen-specific nonresponsiveness, or hyporesponsiveness, was subsequently demonstrated in several murine models of both autoimmunity and allergy. These included models of experimental autoimmune encephalomyelitis (Clayton et al., 1989; Gaur et al., 1992; Metzler & Wraith, 1994; Anderton et al., 1998; Burkhart et al., 1999), experimental autoimmune neuritis (Stienekemeier et al., 2001), systemic lupus erythematosus (Monneaux et al., 2003; Monneaux & Muller, 2004), arthritis (Ku et al., 1993; Anderton et al., 1995; Staines et al., 1996; Prakken et al., 1997; Prakken et al., 2002; van Bilsen et al., 2002), and allergic disease. Briner and colleagues sensitised mice to the cat allergen Fel d 1 and subsequently demonstrated the ability of allergen-derived peptides to inhibit T-cell cytokine and antibody production (Briner et al., 1993). Hoyne et al. (1993) administered Der p 2 peptides intranasally (i.n.) to mice, resulting in the down-regulation of T-cell and antibody responses to intact protein. Bauer administered a dominant T-cell epitope of Bet v 1 to CBA/J mice both prophylactically and therapeutically. T-cell proliferative responses were inhibited by both treatments, but no change in antibody isotype was observed (Bauer et al., 1997). In another model, mice were sensitised to bee venom allergen phospholipase A₂ (PLA₂; Api m 1) and treated by intraperitoneal (i.p.) or i.n. administration of a mixture of 3 polypeptides spanning the whole molecule. Following i.p. administration, mice were protected from anaphylaxis. A significant reduction in specific IgE was observed together with a reduced Th2/Th1 ratio. The administration of peptide prior to priming resulted in a failure to develop IgE sensitisation to subsequent immunization with the whole molecule (Astori et al., 2000).

Together, the studies described above supported the use of peptides to modulate T-cell responses. This has provided a logical basis for the translation of peptide-based therapy into human subjects.

4.2. Clinical studies

Peptide immunotherapy has been evaluated in a number of small clinical studies. The majority of studies have focussed on individuals with allergic disease induced by cats (see below). Limited studies have been undertaken in autoimmune disease, including multiple sclerosis (Warren et al., 1997; Bielekova et al., 2000; Kappos et al., 2000) and rheumatoid arthritis (Prakken et al., 2004).

The safety and efficacy of 2 polypeptides from the cat allergen Fel d 1 were evaluated for the treatment of cat-induced allergic disease by Norman and colleagues. An equimolar mixture of 2 peptides (27 amino acids each in length) was administered in 4 injections (over a 2-week period) to 95 cat-sensitive patients in 3 dose groups (7.5, 75, and 750 µg per injection; Norman et al., 1996). Statistically significant, albeit modest, changes in symptom scores were observed in the high dose group. Both nasal and lung symptom scores improved although a prominent placebo effect was observed. Treatment was associated with a significant incidence of adverse events, which occurred a few minutes to several hours after peptide injection. Further studies demonstrated reduced IL-4 production in peptide-specific T-cell lines following therapy (Marcotte et al., 1998).

A reduction in IL-4 production was also reported by Pene et al. (1998) together with a reduction in allergen provocative dose, inducing a 20% reduction in FEV₁ (PD₂₀) in subjects treated with higher dose regimens when compared with baseline (within group), but not when compared with placebo (between group).

Evaluating the same 2 peptides but with a different dosing regimen, Simons et al. (1996) performed a double-blind, parallel group study administering 4 weekly injections of 250 µg of the peptide preparation to 42 (21 active and 21 placebo) individuals with cat-induced rhinitis and/or asthma. Late onset symptoms of rhinitis, asthma, and pruritis were observed following treatment. Peripheral blood mononuclear cell (PBMC) cytokine secretion profiles remained unaltered after therapy. No change in early- and late-phase skin responses to whole allergen was recorded at several time points (2, 6, and 24 weeks) after treatment.

In a multicentre study of 133 subjects receiving 8 injections of 750 µg of the same peptides, Maguire et al. (1999) reported an improvement in pulmonary function but only in those who had diminished baseline forced expiratory volume in 1 sec (FEV₁). Moreover, improvements were evident at only a single time point (3 weeks) posttherapy. A large number of adverse events were reported, including the occasional requirement for the use of epinephrine. As in other studies with the same peptides, late onset adverse reactions diminished with successive doses of peptide, suggesting the induction of hyporesponsiveness.

In summary, treatment of cat-allergic subjects with two 27-amino-acid peptides (from the amino terminal half of chain 1 of Fel d 1; IPC-1/IPC-2) resulted in modest improvement in clinical and surrogate outcome measures.

Treatment was associated with a high frequency of adverse events. Early adverse events may have been due to the cross-linking of IgE. The length of the peptides and their capacity to form homo- and heterodimers through oxidation of cysteine residues may have resulted in cross-linking of adjacent IgE molecules on the surface of mast cells and/or basophils. Other, more common adverse events were characterised by symptoms of chest tightness and wheeze, with an onset several hours after peptide administration. Based on the descriptions and kinetics of such reactions, it is likely that these events were T-cell-dependent, IgE-independent, isolated late asthmatic reactions (LARs; a delayed and prolonged reduction in forced expiratory flow rate, in the absence of acute bronchoconstriction), the latter being defined by our own studies at the time (Haselden et al., 1999).

4.3. IgE-independent, MHC-restricted isolated late asthmatic reactions

Cat-allergic, asthmatic individuals were challenged, by intradermal (i.d.) injection, with peptides representing T-cell epitopes of Fel d 1, defined in earlier studies (Haselden et al., 2001). It was hypothesised that direct activation of allergen-specific T-cells would lead to bronchoconstriction through mechanisms that did not involve IgE. Forty subjects, with mild to moderate disease, were challenged by i.d. injection of 80 µg of a mixture of 3 peptides. Nine of the 40 subjects developed delayed, prolonged reduction in FEV₁ representing isolated LAR (Haselden et al., 2001). The response to the peptide mixture was believed to be mediated by CD4⁺ T-cells that have been shown to be present in increased numbers in bronchial biopsies from asthmatic individuals.

It was hypothesised that similar reactions would occur if peptides were delivered by the inhaled route. Peptides were nebulised in a small volume of saline for inhaled delivery. Inhaled challenge induced isolated LAR in cat-allergic asthmatics with identical kinetics to the intradermal route. A proportion of individuals developed isolated LAR at doses very similar to those given intradermally (Ali et al., 2004).

4.4. Induction of T-cell hyporesponsiveness following peptide challenge

4.4.1. Open study: Fel d 1 peptides

Interestingly, when individuals experiencing LAR following peptide challenge received a second dose via the same route, a reduced or absent response was observed. Thus, a second peptide challenge within a few weeks was associated with a marked attenuation of LAR, implying the induction of peptide-specific hyporesponsiveness or “T-cell tolerance”. In contrast, rechallenge after ~1 year or more was associated with the induction of LAR similar to the initial challenge, implying that hyporesponsiveness was long-lived but transient (Haselden et al., 1999). The results lead to the conclusion that peptide administration induced a

transient T-cell activation leading to an isolated LAR. After a period of a few days, responsiveness to a second challenge with peptide was markedly reduced or absent. Precedents for the induction of immunological tolerance in the T-cell compartment (in mice) following transient T-cell activation have been reported previously in animal models (Webb et al., 1990; Vidard et al., 1994; Hoyne et al., 1996).

Late asthmatic reactions were followed by several months of relative hyporesponsiveness to rechallenge. Reactivity (bronchoconstriction) gradually returned between 2 and 6 months after tolerance induction (Oldfield et al., 2001). Additional surrogate markers of systemic reactivity to allergen, such as the cutaneous late-phase reaction (LPR) to intradermal challenge with cat dander allergen extract, were evaluated. Cat-allergic asthmatics were challenged intradermally with whole cat dander allergen extract, before and after a single injection of 5 µg of each of 12 peptides in a mixture. The 12 peptides (termed MOP; multiple overlapping peptides) spanned ~80% of the Fel d 1 molecule and encompassed most of the major T-cell epitopes. The results demonstrated that the magnitude of the cutaneous late phase response to allergen could be markedly reduced even after a single administration of peptide. Furthermore, several subjects who had received peptide but had not experienced an LAR also displayed equivalent reductions in the size of the cutaneous late response to whole allergen, further emphasising the fact that LAR were not required to achieve T-cell hyporesponsiveness to whole allergen.

4.4.2. Double-blind,

placebo-controlled study: Fel d 1 peptides

Fel d 1 peptide immunotherapy with MOP was evaluated in a small double-blind, placebo-controlled clinical trial (Oldfield et al., 2002). The primary outcome measure of the study was the magnitude of the late-phase cutaneous reaction to intradermal challenge with purified Fel d 1 and also with whole cat dander allergen extract, as described earlier. Secondary outcomes were the allergen PD₂₀ and the histamine provocative concentration inducing a 20% reduction in FEV₁ (PC₂₀). Twenty-four cat-allergic asthmatic subjects with moderate to severe asthma were subjected to cutaneous allergen challenge, inhaled methacholine PC₂₀, and inhaled allergen PD₂₀ together with quality of life evaluated by questionnaire (Global evaluation to cat exposure). Two outcome assessments were performed after treatment, at which baseline parameters were repeated. The first follow-up was 4–8 weeks after therapy, and the second, 3–9 months after treatment. Subjects were treated with a total of 90 µg of each of 12 peptides (MOP; 16–17 amino acids in length mixed together in saline). Doses were administered at 3- to 4-day intervals. As the subjects were asthmatic, it was expected that ~50% of those on active treatment would develop peptide-induced, isolated LAR during peptide up-dosing (based on previous observations, described above). Twice as many subjects were recruited into the treatment arm of the study in order to test the hypothesis

that the induction of isolated LAR was not a prerequisite for the induction of hyporesponsiveness. When LAR was induced, repeat injections of the same dose were administered until changes in FEV₁ during the 6-hr period after injection were less than 20% from baseline. Up-dosing was then reinstated with adjustments made in order to deliver the same cumulative dose to all subjects. A total of 8 subjects on active treatment experienced LAR following the first or subsequent peptide injections. In each case, the magnitude of the reaction diminished on successive injections. Two of the placebo group also experienced a reduction in FEV₁ of greater than 20%, indicating the relatively severe nature of the disease in the group as a whole.

Reduction in early-phase reaction (EPR) size was not evident at the first follow-up, but was observed at the second follow-up. Peptide treatment resulted in statistically significant reductions in the magnitude of both early- (3- to 9-month follow-up only) and late-phase cutaneous reactions (both follow-up points) to intradermal challenge with purified Fel d 1 when compared with placebo. Reactions to whole cat dander allergen extract were also significantly reduced from baseline in treated patients. The results implied a dissociation between the modulation of the IgE-mediated EPR and the LPR, which may be largely T-cell-driven. Thus, it may be that T-cell responses are modified rapidly through, for example, reduction in IL-4 and IL-13 secretion or the induction of regulatory T-cells.

4.4.3. Controlled open study: Api m 1 peptides

Müller and colleagues evaluated 3 phospholipase A₂ (Api m 1) peptides in a small open study in which 5 bee venom allergic subjects received divided incremental doses of peptides at weekly intervals, to a total dose of nearly 400 µg. Following therapy, subjects were able to tolerate challenge with 10 µg of whole Api m 1, and 3 out of 5 tolerated a live bee sting challenge without reaction (Muller et al., 1998).

Following a similar protocol, but using peptides from Api m 1 that were selected on the basis of direct MHC binding characteristics (Texier et al., 2000), Tarzi and colleagues (unpublished data) performed a controlled, open, single-blind study of peptide therapy in subjects with mild bee venom allergy. Peptide therapy was well tolerated and resulted in a significant reduction in the magnitude of the late-phase cutaneous reaction to both whole bee venom and Api m 1 following challenge. In vitro findings were similar to those following Fel d 1 peptide therapy (see Section 4.3).

5. In vitro evaluation of immune modulation

The effects of peptide administration on peripheral blood responses to allergen were evaluated in vitro. Paired blood samples (before and after peptide treatment) were collected from individuals receiving either single or multiple administrations of peptides. PBMC were cultured with whole

allergen extract, with individual peptides from the challenge mixture, and in certain studies, with a small number of peptides that were not included in the challenge material. Consistent results were obtained in several studies (Oldfield et al., 2001, 2002, and unpublished observations). Peptide treatment resulted in reduced allergen-specific proliferation of PBMC together with reductions in both Th2 (IL-4 and IL-13) and Th1 (IFN- γ) cytokine production. Interestingly, levels of IL-10 in culture supernatants were found to be significantly elevated after therapy with peptides from both Fel d 1 and Api m 1. Similar patterns of cytokine expression have been observed in earlier Api m 1 peptide studies (Akdis et al., 1996).

6. Hyporesponsiveness in the absence of late asthmatic reaction

The induction of LAR following peptide administration was shown to be related to dose (Oldfield et al., 2001) and appeared to be attenuated during dose escalation (Oldfield et al., 2002). LARs were likely the result of transient T-cell activation by peptide presented under certain conditions (e.g., presentation of antigen by nonprofessional APC). LARs were followed (temporally) by a reduced or absent reaction following readministration of the same dose indicating the induction of anergy/hyporesponsiveness, or perhaps, removal of the cells by clonal deletion. The apparent temporal linkage of the 2 observations, induction of LAR and of hyporesponsiveness, may imply a relationship between the 2 events: transient activation of a population of allergen-specific effector T-cells, followed by their removal or paralysis. Alternatively, the 2 events may be separate, with transient activation occurring in the memory effector T-cell compartment in the target organ (lung), and the “hyporesponsiveness” occurring systemically in the lymphatic system and targeting central memory and naive T-cells.

Indeed, in certain subjects, it was possible to induce hyporesponsiveness in the absence of LAR, suggesting that it is possible to dissociate these 2 events. In an open study of Fel d 1 peptide therapy, 8 cat-allergic asthmatic individuals received a mixture of overlapping peptides by intradermal injection. The dosing regimen was incremental (0.1, 1.0, 5, 10, and 25 μ g), with a 2-week dose interval. None of the 8 subjects experienced an LAR (reduction in FEV₁ of 20% or greater). The magnitude of the cutaneous LPR was markedly reduced, and nonspecific bronchial hyperactivity (measured by histamine challenge) was also significantly improved (Alexander et al., 2001, 2005). Thus, it is possible to induce tolerance in the absence of transient T-cell activation. Tolerance induction appears to require lower doses of peptide than transient T-cell activation and each of the 2 events occurs with distinct kinetics: activation taking 2–3 hr and tolerance taking a number of days to be achieved.

7. Differential induction of hyporesponsiveness through the intradermal and inhaled routes

Fel d 1-derived T-cell peptides induced LAR following both intradermal (into a distal site such as the forearm) and inhaled administration. However, only intradermal challenge was followed by bronchial hyporesponsiveness to peptide rechallenge and other manifestations of tolerance described previously. Repeated inhalation of peptides was not associated with attenuation of the LAR, even with inhalation of multiple doses on consecutive days (Ali et al., 2004). Additionally, inhaled peptides did not inhibit the cutaneous late-phase reaction (LPR) to whole cat dander. Thus, unlike the intradermal route, LARs provoked by inhalation did not induce whole allergen or peptide-specific hyporesponsiveness. This observation provides compelling evidence that the route of administration of peptides may be critical in “tolerance” induction.

Inhaled (intranasal) delivery of peptides in several murine models has been shown to be associated with the induction of tolerance. The administration of peptides via the nose may result in a qualitatively different immune response. Alternatively, the success of the intranasal route in animals may, in reality, be associated primarily with the relatively high doses of peptide employed. Thus, a critical factor in the induction of immunological tolerance may be achieving a sufficiently high plasma concentration of peptides. This may be achieved through the inhaled route, but much larger doses of peptide may be required. A recent study of peptide immunotherapy for rheumatoid arthritis employed high doses of peptides delivered orally (25 mg/dose/day for 6 months; Prakken et al., 2004). Although clinical data have yet to be reported from this study, surrogate markers of tolerance induction support an immunomodulatory effect of the peptide intervention. Increased production of IL-4 and IL-10 was observed after treatment, while T-cell proliferation was reduced together with IL-2, IFN γ and TNF α production.

8. Mechanisms of peptide-induced tolerance

Based on the ability of peptides to induce peptide- and allergen-specific hyporesponsiveness in the absence of the induction of LAR in allergic asthmatics, it appears that the 2 events are not causally linked. Thus, the activation of memory T-cells in the airways may result in bronchoconstriction, while other mechanisms may be involved in induction of hyporesponsiveness. Additionally, while activation of T-cells manifest as a LAR can be observed within a few hours, hyporesponsiveness appears to take several days to become established.

Following intradermal administration, some peptide may enter the peripheral circulation via the cutaneous capillary bed, traffic to the lung, and activate effector T-

cells. When administered directly to the lung, sufficient peptide may be present to activate local effector T-cell populations. Additionally, peptides may be taken up by dendritic cells in the peripheral lymphatics as they migrate to draining lymphoid tissue. The low-dose, high-solubility, and monomeric interaction with allergen-specific antibodies on the surface of antigen presenting cells may render the peptides “tolerogenic”. Recent studies have suggested that the cross-linking of IgE on the surface of antigen presenting cells (e.g., by allergen) results in the activation of these cells and the release of cytokines (Novak et al., 2001). Furthermore, peptides may bind to MHC class II peptide binding grooves on the cell surface without providing an activation/maturation signal. Previous studies have demonstrated the presence of a significant percentage of MHC class II molecules, without bound peptides, on the surface of immature “steady-state” dendritic cells (Santambrogio et al., 1999b). Furthermore, this is associated with active HLA-DM, a chaperone and peptide editing protein capable of loading exogenous peptides into empty MHC class II binding grooves (Santambrogio et al., 1999a). As a result, when the immature antigen presenting cell recycles through lymphoid tissue, it presents allergen peptides to T-cells while in a state of incomplete maturation and activation. In contrast, peptides delivered in small amounts by inhalation or by ingestion may be degraded rapidly and may not reach the lymphatics or peripheral circulation in sufficiently high concentrations to induce hyporesponsiveness.

Recently, we have demonstrated the existence of a CD4⁺ population of T-cells that are induced during peptide immunotherapy. These cells are able to suppress proliferative responses of CD4^{neg} target cells obtained before therapy (baseline). When combined with data from our earlier studies and those of others, these results strongly support the notion that adaptive regulatory T-cells can be induced by therapeutic administration of T-cell peptide epitopes (Verhoef et al., 2005).

9. Summary and conclusions

Synthetic peptides representing T-cell epitopes of auto-antigens and allergens have been demonstrated to induce antigen-specific hyporesponsiveness or “tolerance” in both in vitro and in vivo experimental models. Administration of peptides to human subjects either by intradermal injection or inhalation resulted in transient T-cell activation and subsequent bronchoconstriction. The latter may be due to direct interaction between T-cells and smooth muscle cells or may be effected through an intermediate. The delivery of peptides by injection (but not inhalation at comparable doses) leads to reduced reactivity to allergen that was accompanied by a modulation of T-cell function in favour of a regulatory phenotype. The production of IL-10 was increased while the PBMC secretion of IFN γ , IL-4, and

IL-13 was reduced. Hyporesponsiveness was transient but required several months to wane.

Thus, peptide therapy was well tolerated and allowed delivery of molar-equivalent allergen doses that far exceed those in conventional allergen immunotherapy. Favourable responses to therapy have been documented for 2 major allergens, and reports of the application of peptide therapy to autoimmune diseases are beginning to appear. Peptide immunotherapy appears to reduce both Th1 and Th2 responses (probably through IL-10-related mechanisms) and may therefore be clinically efficacious in autoimmune and allergic diseases (Larche & Wraith, 2005).

Acknowledgments

Some of the studies described in this review were funded by the Medical Research Council UK, Asthma UK, and the British Medical Association (BMA TV James Fellowship). The author is an Asthma UK Senior Research Fellow. The following individuals are gratefully acknowledged for their substantial contributions to the studies presented in this review: Prof. A. B. Kay, Drs. B. M. Haselden, W. L. G. Oldfield, C. Alexander, F. R. Ali, M. Tarzi, T. Smith, A. Verhoef, D. S. Robinson, and E. Syrigou, Ms. K. Shirley, and Ms. J. Arbery.

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