

Anti-T-cell strategies in the treatment of allergic disease

Specific allergen immunotherapy (SIT) has been shown to be effective in modulating allergic responses in diseases such as rhinitis and asthma. However, the ability of whole allergen to cross link mast cell bound IgE, resulting in release of mediators such as histamine, has limited the application of this therapy to carefully selected patients who have failed conventional pharmacotherapy. The use of peptide sequences corresponding to T cell epitopes of the allergen has been postulated as an alternative to SIT in which high molar doses of T cell epitope can be delivered over a shorter time period and with improved safety. Using peptides from the sequence of the major cat allergen, Fel d 1, we have demonstrated the ability to induce transient T cell activation, resulting in isolated late asthmatic reactions, which are followed by prolonged periods of allergen-specific hyporesponsiveness, both to peptide re-challenge and to cutaneous challenge with whole allergen. Thus, peptide therapy may prove safe and efficacious in the treatment of allergic diseases.

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Introduction

Challenge of allergic individuals with allergen results in a bimodal response consisting of a classical Type I hypersensitivity reaction (Gell & Coombs classification) followed by a Type IV reaction with Th2 cytokine characteristics (1). In the asthmatic individual, inhaled bronchial challenge with allergen extract results in an early asthmatic reaction (EAR) which peaks at 10–15 min and resolves after approximately 1 h. The EAR is followed (in some but not all individuals) by a late asthmatic reaction (LAR) beginning at about 3 h, peaking at 6–9 h and resolving by 24 h. The EAR is mediated by cross linking of allergen specific IgE on the surface of the mast cell. It is likely that the LAR results from a combination of a number of mechanisms occurring at the same time including cross linking of allergen-specific IgE, release of neurokinins, release of leukotrienes and T lymphocyte activation. Cross linking of specific IgE on the mast cell surface relies upon a degree of conformational integrity within the allergen molecule (or fragment) and the presence of at least two contiguous epitopes in order to bridge adjacent IgE molecules.

In contrast, T cell receptors do not require tertiary structure but recognize short linear peptides presented in the context of MHC molecules. In order to determine whether peptides, without IgE cross linking capacity, could induce LAR in the absence of the EAR, we challenged cat-allergic asthmatic subjects with short peptides derived from the sequence of the major cat allergen Fel d 1. Following previous reports from murine models in which T cell hyporesponsiveness

had been demonstrated following a transient T cell activation event (2–4), we hypothesized that the peptides would induce MHC class II restricted T cell-mediated reactions in allergic subjects, which would be followed by allergen-specific T cell hyporesponsiveness.

Materials and methods

Peptides

Peptides were synthesized by 9-fluorenylmethoxy carbonyl (Fmoc) chemistry at The Advanced Biotechnology Centre, Imperial College School of Medicine, London, UK. Following purification by high performance liquid chromatography (HPLC), peptides were solubilized, mixed and diluted into vials for single patient administration (Nova Laboratories, Leicester, UK). Studies were approved by the Ethics Committee of the Royal Brompton & Harefield Hospitals Trust and performed under a Doctors and Dentists Exemption Certificate from the Medicines Control Agency, UK.

Patient selection

Subjects were recruited through the Allergy Clinic, Royal Brompton Hospital or by advertisement. Subjects were aged 18–55 years, of either gender or any ethnic group with a clear history, during the previous 12 months or more, of sneezing, rhinorrhoea or nasal blockage on exposure to cats, in addition to a clear history of asthma, e.g. cough, wheeze, dyspnoea on exposure to cats. Subjects also gave a positive skin prick test to whole cat dander allergen extract (ALK, Horsholm, Denmark; sum of the longest and orthogonal diameters of the wheal produced was at least 6 mm greater than that of the diluent control). All subjects were able to provide written informed consent.

Intradermal allergen challenge

Whole cat dander allergen extract (30BU ALK) was injected intradermally into the volar aspect of the forearm at the same

time as a diluent injection on other arm. The injection site was inspected at 15 min and 6 h. Any observed reaction was traced, and the outline recorded, using transparent adhesive tape. The mean between the longest and the orthogonal diameters of the wheal or induration was calculated.

Intradermal peptide challenge

Peptides or diluent were injected intradermally into the volar aspect of the forearm. Skin reactions were recorded at 15 min and 6 h as for allergen challenge. Spirometry was recorded at 5, 10, 15, 30, and 60 min and hourly thereafter for 6 h.

Results

Following intradermal injection of 80 µg of a mixture of three peptides from the major cat allergen Fel d 1, nine out of 40 cat-allergic asthmatics had a greater than 20% fall in FEV1 which started at 3–4 h and reached a plateau by 6 h (5). No immediate skin or lung reactions were observed. Subjects were HLA typed and the individuals who developed isolated late asthmatic reactions were found to express at least one DR1, DR4 or DR13 allele. For example, four out of nine responders (as opposed to one of 31 nonresponders) expressed a DR13 allele. Murine L-cells transfected with either DRA, DRB1*1301 or DRA, DRB1*1302 were used to assess whether either of these two molecules could bind and present any of the three FCIP peptides to allergen-specific T cell lines derived from the peripheral blood of reactors prior to peptide administration. One of the FCIP peptides (FCIP3) was capable of being presented by both DR13 molecules. Using fibroblasts expressing DR1, the same peptide was shown to be capable of stimulating DR1-restricted T cell proliferation and production of IL-5. Furthermore, FCIP2 was capable of being presented by DR4. Since induction of LAR appeared to be MHC-restricted, it was likely that the bronchoconstriction characterizing these reactions was mediated by direct T cell activation through an, as yet, unidentified mechanism.

In agreement with murine studies, transient peptide-dependent activation of T cells was followed by marked hyporesponsiveness both to further peptide challenge and also challenge in the skin with whole allergen. Individuals who experienced a LAR were challenged with a second and in some cases a third injection of the same dose. We demonstrated that rechallenge between 2 and 8 weeks of the initial challenge was accompanied by a state of hyporesponsiveness to peptides (as measured by a fall in FEV1 after intradermal challenge), which lasted for a period of months. However, rechallenge after a period of 12 months or more was characterized by the induction of LAR of similar magnitude to the initial challenge.

In order to study the reactions further it was necessary to address the issue of MHC restriction of

T cell responses. Since the original preparation of three peptides represented approximately 25% of the overall length of the Fel d 1 molecule, a larger number of peptides was required to account for other MHC-binding peptides in other parts of the sequence. A total of 16 overlapping peptides were generated which spanned the entire molecule, both chain 1 and chain 2. Due to poor solubility, four of the 16 were excluded. Thus a mixture of 12 peptides was generated for *in vivo* use. A dose ranging study was performed to determine whether the induction of LAR was dose dependent and to establish the lowest dose of peptide that was capable of inducing bronchoconstriction.

Three groups of eight cat-allergic asthmatics were challenged with three different doses of peptide preparation (MOP; multiple overlapping peptides). In the group challenged with 1 µg, one out of eight individuals experienced a LAR with a reduction in FEV1 of 20%. A dose of 2.5 µg resulted in two LAR of greater magnitude and the highest dose of 5 µg produced four LAR which were greater still in magnitude. On the basis of these findings, together with data relating to the induction of hyporesponsiveness to re-challenge, we have hypothesized that for therapeutic purposes an escalating dose regimen, starting at a very low dose of peptide (similar to conventional immunotherapy), may be used to induce antigen-specific T cell hyporesponsiveness without the induction of LAR (6).

The magnitude of the cutaneous early and late phase responses to whole allergen was evaluated before and after peptide administration. Statistically significant reduction in the magnitude of the cutaneous late phase response to whole allergen has been shown to be associated with successful SIT (7, 8). Several months of SIT for grass pollen sensitivity was accompanied by a reduction in the cutaneous late phase reaction of approximately 60% (7, 8). A comparable effect was achieved following administration of 5 µg of MOP. A statistically significant reduction of approximately 50%, in the cross-sectional area of the cutaneous late phase response was observed. No change in the early cutaneous response at 15 min was observed. Furthermore, in related studies, reduced responses to cutaneous whole allergen challenge in individuals who experienced no isolated LAR after peptide administration suggesting that significant down-regulation of the response to whole allergen can occur in the absence of isolated LAR.

In addition to measurement of clinical parameters, *in vitro* analysis of T cell responses demonstrated that following a single injection of 5 µg of MOP, T cell proliferative responses were reduced both to the peptides and to whole allergen extract. In contrast, no change was seen in response to culture with the recall antigen PPD (purified protein derivative of *Mycobacterium tuberculosis*). Similarly, cytokine

responses were generally suppressed following peptide injection. Levels of IL-4, IL-13 and IFN γ production were all reduced following peptide challenge (unpublished data) in both the peptide and whole allergen challenged cultures but not in the PPD challenged cultures. The fact that reductions in the Th1 cytokine IFN γ were observed suggests that the mechanism underlying reduced skin reactivity to whole allergen challenge is not a shift from Th2 to Th1 but rather the induction of a regulatory response, clonal anergy or cell death.

Discussion

Peptides have been shown to prevent the induction of disease and to modulate ongoing disease in murine models following subcutaneous, intranasal and intravenous administration. Peptide-induced tolerance has been demonstrated in models of experimental autoimmune diseases (9–13) and more recently, in models of allergic disease (14, 15).

The polymorphism displayed by both the human major histocompatibility complex (MHC) in comparison to inbred strains of mice has given rise to concerns that peptide immunotherapy may be impractical in humans since it will not be possible to accommodate the large number of potential epitope–MHC combinations involved in disease pathogenesis. The issue of MHC polymorphism is particularly relevant to allergic diseases since, unlike many autoimmune diseases, there are few strong human leukocyte antigen (HLA) disease associations. The same is not true of autoimmune where clear associations between HLA haplotype and disease are seen.

Peptide-based immunotherapy has recently been evaluated in subjects with allergic disease induced by cats (16). Norman and colleagues administered two 27 amino acid peptides from the cat allergen Fel d 1 to cat-allergic subjects. Clinical efficacy was observed only at the highest dose of peptide ($4 \times 750 \mu\text{g}$). Both nasal and lung symptom scores improved in response to peptide therapy. Treatment was associated with a significant incidence of adverse events which occurred a few minutes to several hours after peptide injection. Further studies (17) demonstrated reduced IL-4 production in peptide-specific T cell lines following therapy. A similar reduction in IL-4 production was reported by Pène and colleagues (18). A reduction in allergen PD₂₀ was also observed in this study in response to both high and medium (150–450 μg) dose regimens when compared to baseline, but not to placebo. In another placebo-

controlled study of 42 individuals, Simons and colleagues found no change in cytokine secretion patterns of PBMC before and after therapy (19). No change in early and late-phase skin responses to whole allergen were observed at several time points after treatment. Maguire and colleagues reported an improvement in pulmonary function in subjects who had diminished baseline FEV1 but only at a single time point (3 weeks) post therapy (20). A large number of adverse events were reported in this study including some that required the use of epinephrine.

Müller and colleagues identified three T cell peptide epitopes in bee venom phospholipase A₂ (PLA₂) molecule and administered these peptides in an up-dosing protocol to five allergic subjects (21). Peptides were well tolerated and despite the differing MHC backgrounds of the subjects, T cell responses to all three peptides were observed suggesting that the peptides may have promiscuous MHC binding characteristics.

Our own studies suggest that the adverse events experienced in the studies described by Norman, Pène, Simons and Maguire, employing two 27 amino acid peptides derived from Fel d 1, occurred as a result of cross linking of allergen-specific IgE (in the case of early reactions requiring epinephrine) and induction of T cell-dependent LAR (in the case of late onset symptoms). We have demonstrated that peptide-induced LAR following the administration of short peptides (16/17 amino acids which do not cross link IgE) are dose related both in frequency and magnitude and also that reductions in skin responses to whole allergen challenge following peptide administration are not dependent upon the subject experiencing a LAR. Taken together, these observations suggest that it will be possible to design up-dosing peptide immunotherapy protocols, such as that employed by Muller and colleagues, which will result in the induction of allergen-specific T cell hyporesponsiveness which will not be accompanied by early or late onset adverse events.

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References

1. KAY AB. Concepts of allergy and hypersensitivity. In: KAY AB, editor. *Allergy and Allergic Diseases*. Oxford: Blackwell, 1997;23–35.
2. WEBB S, MORRIS C, SPRENT J. Extrathymic tolerance of mature T cells: clonal elimination as a consequence of immunity. *Cell* 1990;**63**:1249–1256.
3. VIDARD L, COLARUSSO LJ, BENACERRAF B. Specific T-cell tolerance may be preceded by a primary response. *Proc Natl Acad Sci USA* 1994;**91**:5627–5631.
4. HOYNE GF, ASKONAS BA, HETZEL C, THOMAS WR, LAMB JR. Regulation of house dust mite responses by intranasally administered peptide. transient activation of CD4+ T cells precedes the development of tolerance in vivo. *Int Immunol* 1996;**8**:335–342.
5. HASELDEN BM, KAY AB, LARCHÉ M. IgE-independent MHC-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999;**189**:1885–1894.
6. SHIRLEY KE, OLDFIELD WLG, KAY AB, LARCHÉ M. Attenuation of cutaneous and bronchial late allergic reactions by short allergen-derived peptides is associated with a reduction in peptide and whole allergen-induced T cell effector function. *J Allergy Clin Immunol* 2001;**107**:225 (Abstract).
7. VARNEY VA, GAGA M, FREW AJ, ABER VR, KAY AB, DURHAM SR. Usefulness of immunotherapy in patients with severe summer hay fever uncontrolled by antiallergic drugs. *BMJ* 1991;**302**:265–269.
8. VARNEY VA, HAMID QA, GAGA M, et al. Influence of grass pollen immunotherapy on cellular infiltration and cytokine mRNA expression during allergen-induced late-phase cutaneous responses. *J Clin Invest* 1993;**92**:644–651.
9. CLAYTON JP, GAMMON GM, ANDO DG, KONO DH, HOOD L, SERCARZ EE. Peptide-specific prevention of experimental allergic encephalomyelitis. *J Exp Med* 1989;**169**:1681–1691.
10. GAUR A, WIERS B, LIU A, ROTHBARD J, FATHMAN GC. Amelioration of experimental autoimmune encephalomyelitis by myelin basic protein synthetic peptide-induced anergy. *Science* 1992;**258**:1491–1494.
11. KU G, KRONENBERG M, PEACOCK DJ, et al. Prevention of experimental autoimmune arthritis with a peptide fragment of type II collagen. *Eur J Immunol* 1993;**23**:591–599.
12. STAINES NA, HARPER N, WARD FJ, MALMSTROM V, HOLMDAHL R, BANSAL S. Mucosal tolerance and suppression of collagen-induced arthritis (CIA) induced by nasal inhalation of synthetic peptide 184–198 of bovine type II collagen (CII) expressing a dominant T cell epitope. *Clin Exp Immunol* 1996;**103**:368–375.
13. VAYSBURD M, LOCK C, McDEVITT H. Prevention of insulin-dependent diabetes mellitus in non-obese diabetic mice by immunogenic but not tolerated peptides. *J Exp Med* 1995;**182**:897–902.
14. BRINER TJ, KUO MC, KEATING KM, ROGERS BL, GREENSTEIN JL. Peripheral T-cell tolerance induced in naive and primed mice by subcutaneous injection of peptides from the major cat allergen Fel d I. *Proc Natl Acad Sci U S A* 1993;**90**:7608–7612.
15. HOYNE GF, O'HEHIR RE, WRAITH DC, THOMAS WR, LAMB JR. Inhibition of T cell and antibody responses to house dust mite allergen by inhalation of the dominant T cell epitope in naive and sensitized mice. *J Exp Med* 1993;**178**:1783–1788.
16. NORMAN PS, OHMAN JL Jr, LONG AA, et al. Treatment of cat allergy with T-cell reactive peptides. *Am J Respir Crit Care Med* 1996;**154**:1623–1628.
17. MARCOTTE GV, BRAUN CM, NORMAN PS, et al. Effects of peptide therapy on ex vivo T-cell responses. *J Allergy Clin Immunol* 1998;**101**:506–513.
18. PÈNE J, DESROCHES A, PARADIS L, et al. Immunotherapy with Fel d 1 peptides decreases IL-4 release by peripheral blood T cells of patients allergic to cats. *J Allergy Clin Immunol* 1998;**102**:571–578.
19. SIMONS FE, IMADA M, LI Y, WATSON WT, HAYGLASS KT. Fel d 1 peptides: effect on skin tests and cytokine synthesis in cat-allergic human subjects. *Int Immunol* 1996;**8**:1937–1945.
20. MAGUIRE P, NICODEMUS C, ROBINSON D, AARONSON D, UMETSU DT. The safety and efficacy of ALLERVAX CAT in cat allergic patients. *Clin Immunol* 1999;**93**:222–231.
21. MULLER U, AKDIS CA, FRICKER M, et al. Successful immunotherapy with T-cell epitope peptides of bee venom phospholipase A2 induces specific T-cell anergy in patients allergic to bee venom. *J Allergy Clin Immunol* 1998;**101**:747–754.