

EDITORIAL

Clinical & Experimental Allergy

Carrying peptides towards the ideal allergen-specific immunotherapy

This editorial discusses the findings of the paper in this issue by B. Linhart et al. [6] pp. 278–287.

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Allergen-specific immunotherapy was first utilized over a century ago and is still practised today with crude extracts of allergenic sources given subcutaneously or sublingually. Identification of major allergens within allergenic sources (e.g. pollens, dander, and foods) has paved the way to capitalize on molecular techniques leading to the determination of amino acid sequences and protein structures, recombinant protein production, and mutagenic strategies. With protein sequence information in hand, the peptides responsible for IgE-binding and T cell interactions have been elucidated for many allergens. These B and T cell epitopes have been strategically targeted to improve allergen-specific immunotherapy [1]. For example, engineered recombinant allergens with reduced IgE-binding capacity, but intact T cell epitopes, have shown promise as immunotherapy molecules in clinical trials [2]. Alternatively, peptides containing T cell epitopes have been used directly as immunotherapy and likely function by inducing allergen-specific regulatory T cells secreting IL-10 [3]. However, these approaches are not without hurdles, as it has been demonstrated that T cell-mediated, late-phase reactions occur in patients receiving T cell peptide-based immunotherapy [4, 5].

In this issue of the journal, Linhart et al. [6] explored an interesting approach for preventing and treating allergies in an antigen-specific fashion without using T cell epitopes from the allergen. The strategy employed was based on the work of Nobel laureate Baruj Benacerraf who conducted studies in the 1960s, leading to a detailed understanding of the hapten-carrier principle [7, 8]. Essentially, an antibody response can be

produced against a small molecule lacking T cell epitopes only when it is conjugated to a larger, carrier protein that has its own T cell epitopes. In the present study, the researchers chose Bet v 1 as their model allergen as it is well suited to their goals. Bet v 1 peptides have been previously identified that are surface-exposed, do not contain IgE-binding epitopes, and do not contain T cell epitopes [9]. Three peptides, each approximately 30 amino acids long, derived from Bet v 1 were chemically linked to keyhole limpet haemocyanin (KLH) to produce the hapten-carrier complex [6]. The authors then tested this complex in both prophylactic and therapeutic models of immunotherapy in BALB/c mice.

In a prophylactic model, three subcutaneous injections of the hapten-carrier complex adsorbed to aluminium hydroxide preceded a sensitization protocol typically used to render mice allergic to Bet v 1. The authors found that mice immunized with the peptide-KLH vaccine produced large amounts of Bet v 1-specific IgG1, comparable to levels induced by immunization with intact Bet v 1 protein. The IgG response in mice receiving the peptide-KLH vaccine was specific for the peptides conjugated to the carrier protein. In mice receiving the peptide-KLH vaccine prior to undergoing a sensitization protocol, the T cell proliferation response to Bet v 1 was significantly lower than T cells from mice receiving no vaccine prior to sensitization. These findings are exciting because the vaccine did not contain any Bet v 1 T cell epitopes, yet after the sensitization protocol, the T cells from these mice were not responsive to Bet v 1. It appears that the Bet v 1-specific IgG response, generated by T helper cells specific for KLH, was able to prevent the establishment of allergen-specific T cell responses following a sensitization protocol. These findings may lay the groundwork towards the development of a prophylactic allergen-specific immunotherapy used to prevent allergic sensitization, which impacts at least 25% of the population in industrialized countries.

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A therapeutic model was developed in which mice were sensitized to Bet v 1 and then given three subcutaneous injections of the peptide–KLH complex or a placebo. Surprisingly, the researchers found that mice given the peptide–KLH complex after sensitization to Bet v 1 had significantly reduced T cell proliferation responses to Bet v 1, despite the fact that no Bet v 1 T cell epitopes were used in the therapeutic phase. A current paradigm for the mechanism of immunotherapy leading to long-term efficacy involves changes in the allergen-specific T cell responses [10], and this novel approach appears to accomplish this goal. The authors also demonstrated that a serum factor, presumably IgG, from treated mice, but not those receiving placebo, was able to inhibit proliferation of T cells from mice sensitized to Bet v 1 when supplemented into cell culture experiments, despite not measuring statistically significant differences in Bet v 1-specific IgG following therapy. It is known that allergen-specific IgG generated during immunotherapy in humans can prevent IgE-facilitated antigen presentation via the low-affinity IgE receptor, CD23, on B cells [11] and it is probable that the same phenomenon is reported here. Interestingly, recent work has shown that although quantities of specific IgG return to near-baseline levels following discontinuation of immunotherapy in humans, there is a functional fraction of IgG from subjects developing tolerance that persists [12]. Lastly, the researchers determined that transfer of the Bet v 1 peptide-specific IgG-containing serum prevented lung inflammation in mice sensitized to Bet v 1 and challenged by intranasal administration of Bet v 1. Therefore, the IgG blocking effect was not only found effective in reducing T cell responses *in vitro*, but could also protect sensitized mice from allergen challenge.

While this study provides convincing evidence that production of allergen-specific IgG via the hapten–carrier principle can prevent and reduce established allergen-specific T cell responses, it is unclear whether this approach would be applicable to other manifestations of IgE-mediated allergy, such as food allergy. The peptides used in this study were from an IgE-binding region of Bet v 1, although the peptides themselves did

not bind IgE, as the epitope is a surface-exposed conformational epitope containing amino acids that are nonsequential in the primary protein sequence. It is usually thought that food allergens contain linear B cell epitopes so it may be difficult to identify antigenic epitopes that are incapable of binding IgE, which is problematic because IgE-binding peptides coupled to a carrier protein would cross-link IgE on mast cells and basophils leading to unwanted allergic effects. It would seem that utilizing allergens with conformational epitopes would be the best candidates for this hapten–carrier approach. Although the literature is not devoid of reports on structural epitopes being present in food allergens [13, 14], it is well established that linear peptides from food allergens bind IgE [15–17], with some allergens containing greater than 20 epitopes. Food allergens are especially difficult to render nonallergenic, as previous studies using peanut allergens that were mutated in several IgE-binding epitopes had shown promise in preclinical models of food allergy [18], yet even these molecules were capable of inducing anaphylaxis in humans [19]. From a food allergy perspective, it is fascinating to think about utilizing the authors' approach of generating an allergen-specific IgG response to prevent food allergies in children that have not been previously exposed. However, it is often reported by families that the first-known exposure to a food was symptomatic, so, again, identifying antigenic, non-IgE-binding peptides from foods for a hapten–carrier approach would first be necessary.

In summary, this paper provides a novel approach using the hapten–carrier principle to inhibit allergen-specific T cell responses and could lead to a safe and effective, 'ideal', form of immunotherapy that does not contain allergen-related T cell epitopes or IgE-binding epitopes. Time will tell whether the approach can be utilized clinically and whether it will be applicable for other forms of allergy, particularly food allergy, where the allergens themselves present distinct challenges.

Conflict of interest: The author declares no conflict of interest.

References

- Valenta R, Niespodziana K, Focke-Tejkl M *et al.* Recombinant allergens: what does the future hold? *J Allergy Clin Immunol* 2011; 127:860–4.
- Niederberger V, Horak F, Vrtala S *et al.* Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci USA* 2004; 101(Suppl 2):14677–82.
- Moldaver D, Larche M. Immunotherapy with peptides. *Allergy* 2011; 66:784–91.
- 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; 154:1623–8.
- Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reactions. *J Exp Med* 1999; 189:1885–94.
- Linhart B, Narayanan M, Focke-Tejkl M, Wrba F, Vrtala S, Valenta R. Prophylactic and therapeutic vaccination with carrier-bound Bet v 1 peptides lacking allergen-specific T cell epitopes reduces Bet v 1-specific T cell responses via blocking antibodies in a murine model for birch pollen allergy. *Clin Exp Allergy* 2014; 44:278–87.
- Paul WE, Katz DH, Goidl EA, Benacerraf B. Carrier function in anti-hapten immune responses. II. Specific proper-

- ties of carrier cells capable of enhancing anti-hapten antibody responses. *J Exp Med* 1970; 132:283–99.
- 8 Katz DH, Paul WE, Goidl EA, Benacerraf B. Carrier function in anti-hapten immune responses. I. Enhancement of primary and secondary anti-hapten antibody responses by carrier preimmunization. *J Exp Med* 1970; 132: 261–82.
 - 9 Focke M, Linhart B, Hartl A *et al.* Non-anaphylactic surface-exposed peptides of the major birch pollen allergen, Bet v 1, for preventive vaccination. *Clin Exp Allergy* 2004; 34:1525–33.
 - 10 Jutel M, Van de Veen W, Agache I, Azkur KA, Akdis M, Akdis CA. Mechanisms of Allergen-Specific Immunotherapy and Novel Ways for Vaccine Development. *Allergol Int* 2013; 62:425–33.
 - 11 van Neerven RJ, Wikborg T, Lund G *et al.* Blocking antibodies induced by specific allergy vaccination prevent the activation of CD4⁺ T cells by inhibiting serum-IgE-facilitated allergen presentation. *J Immunol* 1999; 163: 2944–52.
 - 12 James LK, Shamji MH, Walker SM *et al.* Long-term tolerance after allergen immunotherapy is accompanied by selective persistence of blocking antibodies. *J Allergy Clin Immunol* 2011; 127:509–16 e1–5.
 - 13 Albrecht M, Kuhne Y, Ballmer-Weber BK *et al.* Relevance of IgE binding to short peptides for the allergenic activity of food allergens. *J Allergy Clin Immunol* 2009; 124:328–36, 36 e1–6.
 - 14 Swoboda I, Bugajska-Schretter A, Linhart B *et al.* A recombinant hypoallergenic parvalbumin mutant for immunotherapy of IgE-mediated fish allergy. *J Immunol* 2007; 178:6290–6.
 - 15 Lin J, Bardina L, Shreffler WG *et al.* Development of a novel peptide microarray for large-scale epitope mapping of food allergens. *J Allergy Clin Immunol* 2009; 124:315–22, 322.e1–3.
 - 16 Shreffler WG, Lencer DA, Bardina L, Sampson HA. IgE and IgG4 epitope mapping by microarray immunoassay reveals the diversity of immune response to the peanut allergen, Ara h 2. *J Allergy Clin Immunol* 2005; 116:893–9.
 - 17 Jarvinen KM, Beyer K, Vila L, Bardina L, Mishoe M, Sampson HA. Specificity of IgE antibodies to sequential epitopes of hen's egg ovomucoid as a marker for persistence of egg allergy. *Allergy* 2007; 62:758–65.
 - 18 Li XM, Srivastava K, Grishin A *et al.* Persistent protective effect of heat-killed *Escherichia coli* producing “engineered”, recombinant peanut proteins in a murine model of peanut allergy. *J Allergy Clin Immunol* 2003; 112:159–67.
 - 19 Wood RA, Sicherer SH, Burks AW *et al.* A phase 1 study of heat/phenol-killed, *E. coli*-encapsulated, recombinant modified peanut proteins Ara h 1, Ara h 2, and Ara h 3 (EMP-123) for the treatment of peanut allergy. *Allergy* 2013; 68:803–8.