

Peptide allergen immunotherapy—unraveling new pathways



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A better understanding of immunologic pathways modulated by allergen immunotherapy (AIT) is fundamental for improving its efficacy (short- and long-term), safety, and acceptability by the patient. At the same time, it will expedite the development of reliable biomarkers capable of predicting and monitoring therapeutic responses. As a consequence, AIT clinical trials should consider preplanning a thorough mechanistic analysis of the immune modulation induced by AIT in support of the clinical benefit.

Successful AIT is sustained by multiple changes on the cellular and humoral level. Early desensitization of basophils and mast cells, a shift of type 2 immune responses toward the type 1, along with an increase in regulatory T (Treg) and regulatory B (Breg) cells and IL-10, decreased activation, mediator release and tissue migration of effector cells, and lower IgE production in favor of higher levels of IgG₄ blocking antibodies are regarded as key mechanisms of AIT.¹ Recent evidence shows that AIT modulates the balance between circulating T follicular helper (Tfh) cells and T follicular regulatory (Tfr) cells, with Tfr cells as a potential biomarker for AIT efficacy.^{2,3} Upregulation of the activated allergen-specific Treg cells and downregulation of dysfunctional allergen-specific Treg-cell subset, associated with improved clinical response, were recently described.⁴ Responder status was shown to be associated with increased frequency of IgA- and IgG₄-expressing allergen-specific B cells, plasmablasts, and IL-10⁺ and/or IL-1RA⁺ Breg cells.⁵ Thus, it is becoming increasingly clear that targeting both the T and B cells, along with the follicular and peripheral effector and regulatory cells compartment, is necessary for a successful AIT intervention.

Novel AIT vaccines should respond to increasing needs for reduction in adverse effects, costs, and duration of treatment, while inducing long-term tolerance to allergens. Bypassing IgE binding, allowing the administration of higher doses of tolerogenic T- and B-cell signal with low risk of anaphylaxis, has been pursued for more than a decade, using different approaches such as fusion of recombinant and chimeric allergens, hypoallergenic hybrid proteins, non-IgE-binding fragments of major allergens, un-refolded native or recombinant allergens, polymers of major allergens, and T- and B-cell epitope peptides.¹

The first molecular approach for AIT that reached clinical application for cat, house dust mite, ragweed, and grass pollen allergens was the synthetic peptide immunoregulatory epitopes containing T-cell epitopes. Although there were some beneficial effects for cat-allergic patients in allergen exposure studies, a large phase III study failed to achieve a clinically significant benefit; thus, the T-cell peptide approach was not further pursued. The immunologic mechanism of synthetic peptide immunoregulatory epitopes is presently unclear because no induction of allergen-specific IgG blocking antibodies has been observed because the peptides are too short to induce IgG responses. No statistically significant change in the frequency of Fel d 1–specific CD4⁺ T cells, nor in their expression of chemokine receptors or memory phenotype, was observed, and the only significant reported change is a reduction in cell surface expression of CRTh2.⁶

B-cell epitope-based allergy vaccines contain intrinsically non-IgE-reactive peptides derived from the IgE-binding sites of the major allergens. These peptides are rendered immunogenic by covalently linking to a carrier protein that is not related to the allergens and provides carrier-specific T-cell help. Selective inclusion of peptides from the IgE-binding sites allows better focus on allergen-specific IgG responses toward the IgE-binding sites of allergens, which might result in better protection. Because of increased immunogenicity and low allergenicity, higher doses can be administered.

Lolium perenne pollen peptides (LPP) is an adjuvant-free mixture of peptides resulting from the enzymatic hydrolysis of *L. perenne* purified proteins. In a preliminary small study, the administration of only 5 subcutaneous injections of increasing doses of LPP was safe, well tolerated, and associated with the induction of immune-reactive and IgG-associated blocking antibodies that persisted for at least 24 weeks. In a consequent large clinical trial, a short course (8 subcutaneous injections in increasing doses in 4 visits over 3 weeks) of LPP was shown to significantly reduce the combined symptom and medication score measured over the peak pollen season and the reactivity to conjunctival provocation test.⁷ The study by Sharif et al⁸ published in this issue reports on the immunologic mechanisms of the short-course fast-acting LPP AIT evaluated in a subgroup of this trial. Besides providing evidence for fast suppression of early allergic effector cell (basophils) response, and modulation of T- and B-cell subsets with induction of peripheral FoxP3 Treg cells and IgG₄ blocking antibodies, this study reveals novel immunologic pathways modulated by the LPP immunotherapy. LPP AIT induces the release of IL-35 and inducible Treg cells secreting IL-35 cells, promoting production of IL-10 from CD19⁺ B cells, Breg-cell subsets, and Tfr cells. The induction of IL-35 by LPP endorses the increased efficacy of LPP, because classical T-cell peptide AIT does not induce IL-35. The authors show a decrease in circulating Tfr cells and an increase in Tfh cells. These cells share properties of memory cells and are distinct from their lymph node counterpart because circulating Tfr cells suppress B and Tfh cells with a much lower capacity, whereas circulating memory-like Tfh cells are more potent

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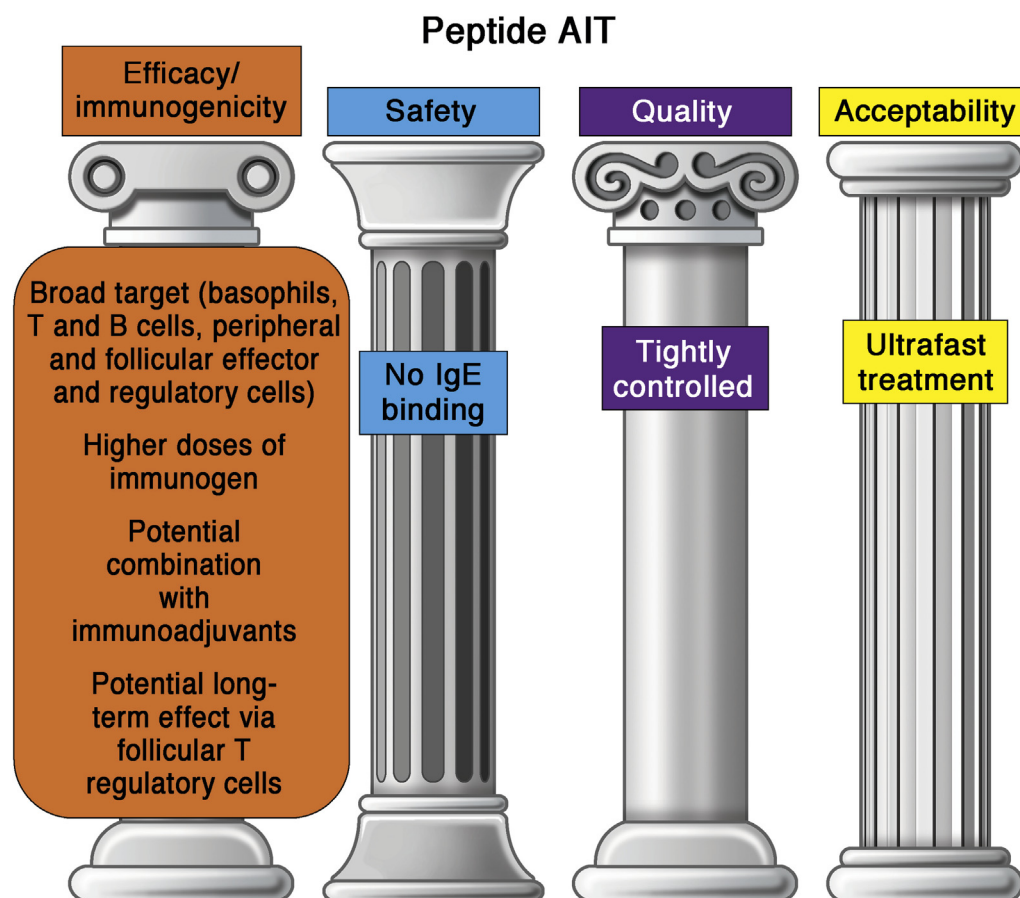


FIG 1. Peptide AIT holds significant promise as a short-course, efficacious, and safe approach to allergic diseases. Broad immunogenicity targeting both T and B cells might result in better protection as compared with regular AIT. Because of reduced allergenic activity, it is possible to administer high doses. Together with the fast-installed immunogenicity, only few doses per year will be needed for treatment and these vaccines would thus be extremely convenient for the patient.

than lymph node effector Tfh cells.⁹ In addition, circulating memory-like Tfr cells persist for long periods; thus, they could support the long-term immunomodulatory effect of peptide AIT. Functional evaluation of Treg cells via expression of glycoprotein A repetitions predominant (GARP) and special AT-rich sequence binding protein 1 (SATB1) is reported for the first time in an AIT study. Treg cells uniquely express GARP on their cell surface and GARP functions as a delivery system for latent TGF- β , which might augment the immunosuppressive role of Treg cells on effector cells.¹⁰ GARP expression was described as an activation marker of parasitic infection-induced Treg cells that strongly suppress allergic inflammation, and thus it is a novel potential mechanistic pathway for AIT. SATB1 is a genome organizer protein expressed in a lineage-specific manner in CD4⁺ T cells. During the early T_H2 cell differentiation, IL-5 expression is repressed through direct binding of SATB1 to the IL-5 promoter. Thus, SATB1 modulation might expand the impact of AIT on eosinophilic inflammation. In addition, SATB1-dependent Treg-cell-specific superenhancer activation is crucial for Treg-cell lineage specification in the thymus (for further reading, see this article's Online Repository at www.jacionline.org).

The study has a number of strengths, because the mechanistic analyses were preplanned and were included in the study protocol

with special focus on less explored immune targets such as T follicular cells and inducible Treg cells secreting IL-35 together with the thorough functional evaluation of Treg cells. Yet, the results should be interpreted with caution because of the small number of subjects enrolled from a single center.

Peptide AIT with a broad immunogenic target holds significant promise as a short-course, efficacious, and safe approach to allergic diseases (Fig 1). Nonetheless, we are left with many unanswered questions, such as selection of responders based on biomarkers, the long-term immunomodulatory changes, and the link to Treg-cell and Breg-cell responses, the functional comparison of different subsets of Treg and Breg cells generated, the impact of the innate immune response, and the specificity of the observed changes for peptide AIT. The addition of an immune-modulating adjuvant or the effect of a booster injection is also open for future research.

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