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Epitope-specific T-cell responses and allergic phenotypes: implications for T-cell peptide therapy

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In the 1990s, elucidation of the primary amino acid sequence of several major allergens using molecular cloning techniques opened the door to T-cell epitope mapping studies. Such analyses underscored the complexity of the allergen-specific T-cell repertoire and the challenges to using allergen-derived peptides to identify epitope-specific differences associated with allergic and nonallergic responses. This review highlights important factors that may influence the nature of epitope-specific T-cell responses observed *in vitro*. These include the properties of the allergen, genetics of the host and selection of patients with defined allergic phenotypes based on serum antibody profiles and skin test reactivity. By taking these factors into account, T-cell epitope-specific differences associated with distinct allergic phenotypes can be identified. Observations at the T-cell epitope level undermine the Th1/Th2 paradigm as a model for the development of allergic versus nonallergic responses. Instead, they support the mounting data that point to a network of interactions between T helper cells and regulatory T cells, which controls the allergic response. The ability of peptides that localize to polypeptide chain 2 of the major cat allergen, Fel d 1, to preferentially induce interleukin-10 and interferon- γ is discussed. Mechanisms whereby specific allergen-derived peptides may modify the T-cell repertoire and influence the immune outcome are also outlined. Further investigation of allergen-derived T-cell epitopes is warranted in order to optimize the design of peptide vaccines for the treatment of allergic disease.

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A discrete subset of T helper (Th) lymphocytes that secrete a characteristic profile of cytokines (interleukin [IL]-4, -5 and -13), have long been recognized as pivotal to the initiation and persistence of allergic responses. Such Th2 cells have pleiotropic effects, which include IL-4-mediated antibody isotype switching to immunoglobulin (Ig)E antibody (Ab) production, which is a major risk factor for the development of asthma [1–3]. Additional effects of Th2 cytokines include the recruitment of eosinophils to sites of allergic inflammation through IL-5 and induction of airway hyper-responsiveness (AHR); however, the key cytokine(s) responsible for AHR remains an area of hot debate [4–7]. The Th1/Th2

paradigm dictates that such Th2-driven allergic processes are inhibited by the induction of interferon (IFN)- γ -secreting Th1 cells, based on the mutually inhibitory properties of Th1- and Th2-derived cytokines [8,9]. The implication is that preferential induction of Th1 responses protects against the development of allergic disease. However, an increasing volume of evidence supports the view that Th cell involvement in the allergic response embodies a variety of effector cell types.

In the 1990s, molecular cloning techniques led to rapid elucidation of the primary amino acid sequence of numerous allergens, and this work has proved invaluable in advancing our knowledge of allergens from diverse sources [10].

This work facilitated T-cell epitope mapping studies, that were designed to identify defined regions of allergen molecules targeted by CD4⁺ T lymphocytes. Such studies held much promise for the design of peptide vaccines for the treatment of allergic disease. Initial studies identified immunodominant T-cell epitopes of several major allergens and the development of peptide vaccines quickly ensued. However, administration of allergen-derived peptides resulted in systemic side effects in some individuals [11]. Adding to this, failure of a majority of studies to identify marked differences in T-cell epitope recognition for a variety of common allergens among allergic and nonallergic individuals may have limited further investigation of allergen-derived T-cell epitopes. In the last few years, analyses of allergen-derived peptides have yielded important observations, using both *in vitro* and *in vivo* human-based systems. These findings have underscored the complexity of T-cell responses to allergens and the promise of peptide vaccines as a strategy for the treatment of allergic disease.

This review provides an overview of T-cell epitope mapping studies of allergens and details recent studies that have revealed differences at the T-cell epitope level among subjects with distinct allergic phenotypes. Identification of allergen-derived peptides that preferentially induce Th1 or regulatory cytokines provides an avenue for exploring the interplay between allergen-specific T-cell subsets and their relevance to the development of distinct allergic phenotypes. Furthermore, the author suggests that such peptides are strong candidates for new peptide vaccines.

T-cell responses to allergens in humans: thinking beyond the Th1/Th2 paradigm

Numerous studies have evaluated *in vitro* proliferation and cytokine responses of human T cells to allergens with a view to correlating findings with atopic status or allergic disease. The majority of these studies used primary peripheral blood mononuclear cell (PBMC) cultures. In general, allergens induce T-cell proliferation in the majority of cultures established from individuals with allergen-specific IgE Ab (i.e. sensitized subjects); however, the magnitude of the response may vary considerably among different individuals [12–17]. Furthermore, although T-cell proliferative responses have been shown to be higher in sensitized compared with nonsensitized individuals [18], this is not always the case [19,20]. Indeed, some have argued that T-cell responses to ubiquitous allergens, such as those derived from house dust mites, are universal [20].

In the early 1990s, the isolation of allergen-specific T-cell clones from atopic donors that secreted Th2 cytokines was reported by several groups [21,22]. These clones exhibited distinct cytokine profiles compared with T-cell clones specific for bacterial antigens or allergen-specific clones derived from nonatopic donors. More recently, clones specific for a variety of allergens have been described, often with heterogeneous cytokine profiles (Th1, Th2 or Th0) [23–25]. Indeed, induction of the Th1 cytokine, IFN- γ , is common for a variety of allergens or allergen-derived peptides [21,23,25–28]. Such observations have challenged

the view that the dichotomy between Th1 and Th2 responses is clear cut. Consistent with this, there is mounting evidence that Th1 responses may contribute to the initiation phase of the allergic response by enhancing the priming of Th2 cells, and also to the effector phase, by exacerbating allergic inflammatory responses in the lung [29,30]. This concept of a mixed T-cell landscape in allergic disease is further complicated by emerging evidence in human-based systems of a role for novel effector and regulatory T-cell (Tregs) subsets in the generation of Th2 responses [31]. Such cells exhibit atypical cytokine profiles, that deviate from the classical Th1/Th2 or regulatory T-cell profiles. These observations highlight the necessity to delineate different types of allergen-specific T cells and their relevance to allergic disease.

Several studies have examined the patterns of T-cell proliferation and cytokine induction to allergen-derived peptides using PBMC cultures, T-cell lines or clones from allergic and nonallergic individuals. Where differences were observed for individual peptides, increased proliferation and secretion of Th2 cytokines were evident among atopic subjects [12,32,33]. In other studies, differences in cytokine secretion were only identified when data were analyzed collectively for a panel of peptides derived from the same allergen [34]. One interpretation of this latter finding is that T-cell responses to allergens generated *in vivo* are skewed by the cytokine microenvironment present during T-cell priming and that this is largely independent of the intrinsic properties of T-cell peptides. Thus, the prediction would be that selective induction of specific cytokines does not localize to a defined region of the allergen molecule, but is instead distributed uniformly among all T-cell epitopes derived from that molecule. As an aside, it is interesting to note that T-cell proliferation and cytokine responses to allergen-derived peptides can be dissociated; thus cytokine secretion can occur in the absence of proliferation [34,35]. This may arise from suboptimal signaling to T cells as a result of low affinity/avidity interactions of specific peptide–major histocompatibility complex (MHC) complexes with the T-cell receptor (TCR), with subsequent uncoupling of distinct signaling pathways that mediate cytokine gene expression and proliferation [36].

In some studies, the inability to identify marked differences in T cell-derived cytokine patterns in immune cells derived from subjects with distinct allergic phenotypes may be attributable, at least in part, to a lack of understanding of the nature of nonallergic responses. It is noteworthy that common inhalant allergens do not induce delayed-type hypersensitivity (DTH) skin tests, the hallmark of a classical Th1 response, in nonallergic individuals. Thus, rather than being the antithesis of the allergic response, the nonallergic response could reflect tolerance, immune ignorance or an immune response involving undefined effector T-cell populations distinct from the Th1 subset. In order to understand how distinct allergic phenotypes develop and persist, it is necessary to think beyond the constraints of the Th1/Th2 paradigm. In the last few years, much attention has focused on the role of Tregs in allergic and nonallergic responses. These cells, which constitute a highly heterogeneous population, have been proposed to control the balance between Th1 and

Th2 subsets, and thus, influence development of the allergic response. An overview of Tregs and their potential role in allergic disease has been described elsewhere [37,38]. The prevailing view is that increased suppression of Th2 responses mediated by Tregs is protective. Reports of increased IL-10 production coupled with diminished secretion of Th2 cytokines or enhanced production of IFN- γ in patients receiving conventional or rush immunotherapy, support this theory [39–42]. Nevertheless, there are still many questions that deserve further study in relation to the nature of Tregs involved in allergic disease. Specifically:

- How do these cells relate to Tregs described previously, including naturally occurring and adaptive CD25⁺CD4⁺ T cells and type 1 regulatory (Tr1) cells?
- Do these cells exert their suppressive effects through secreted mediators (e.g., IL-10 or transforming growth factor [TGF]- β), through cell contact via surface molecules (e.g., cytotoxic T-lymphocyte antigen [CTLA]-4 or by a combination of both mechanisms)?
- What is the principal effector cell population targeted by Tregs?
- Are these Tregs allergen-specific and, if so, can they exert an effect on T cells with different antigen specificities (i.e., bystander suppression)?

This final point is pertinent to whether an immune response to one allergen can influence the immune response to an unrelated allergen. Although these questions have been addressed in several studies, some of the systems described have used Tregs that may be contaminated with effector T cells. In other studies, the effects of purified Tregs were tested on impure responder T-cell populations. Such study designs make the data obtained open to interpretation.

The idea that Tregs can influence the balance between Th1 and Th2 cells, and thus allergic phenotype, is likely to be overly simplistic. As new effector T-cell subsets are defined, our view of the Th1/Th2 paradigm is expected to further evolve. It is also important to consider alternatives to the branching model for Th-cell differentiation. This model dictates that a common precursor naive CD4⁺ T cell has the potential to develop, under different conditions, into either a Th1 or a Th2 cell. A contrasting linear model of T-cell maturation has been proposed recently by Loza and colleagues, whereby Th1 cells arise from a proliferating pool of Th2 cells [43]. In that model, factors influencing the proliferative capacity of Th2 cells may alter the frequencies of Th1 and Th2 cells within the allergen-specific T-cell repertoire. A central role for Tregs, acting to influence the proportion of Th1 and Th2 cells, fits with both the branching model and the linear maturation model for Th cell differentiation. Nevertheless, since the Th1/Th2 paradigm has been central to the interpretation of many experimental findings in the field of allergy, the plasticity of this paradigm should not be underestimated.

Trichophyton as a model for evaluating the Th1/Th2 paradigm

Many *in vitro* studies aimed at defining the nature of allergen-specific T-cell responses in humans have utilized purified natural and recombinant allergens. These antigen preparations may contain

bacterial products such as endotoxin, which can influence the generation and activation of effector T cells. In a murine model of allergic sensitization, the dose of lipopolysaccharide (LPS) coadministered with ovalbumin was shown to influence the nature of the Th response generated *in vivo* [44]. While high-dose LPS favored induction of a Th1 response, low-dose LPS resulted in an enhanced Th2 response to inhaled antigen. It is difficult to evaluate the relative contribution of allergen and bacterial products to the quality of T-cell responses generated *in vitro*. By contrast, allergen-derived peptides, which are endotoxin-free, provide an alternative approach for analyzing allergen-specific T-cell responses in the absence of adjuvant effects. Nevertheless, a direct effect of peptides on the antigen-presenting cell (APC) cannot be excluded and this may also modulate the properties of T cells expanded *in vitro*.

Given the low precursor frequency of circulating allergen-specific T cells, which may be in the order of approximately one in 10⁵ cells, analyses using allergen-derived peptides can pose a challenge. The author's group designed a system for analyzing patterns of T-cell epitope recognition, which was optimized for detecting differences among allergic and nonallergic subjects. Features of this system included the selection of subjects with distinct allergic phenotypes based on the presence of immediate hypersensitivity (IH) and DTH to the same antigen, the use of allergen-derived peptides with significant amino acid overlap for stringent mapping of T-cell epitopes, and the incorporation of 12 replicate assay wells for each peptide in order to increase statistical power. These studies were performed on the *Trichophyton* allergen, Tri r 2 [14]. Antigens derived from the dermatophyte fungus *Trichophyton* exhibit unusual immunological properties based on their ability to elicit both IH and DTH skin tests in different individuals [14,45,46]. Consistent with this, the *Trichophyton rubrum* allergen, Tri r 2, was shown to elicit positive skin prick tests in individuals who exhibited IH responses to a commercial *Trichophyton* extract. These subjects also had measurable serum IgE Ab and high titer IgG Ab to Tri r 2. By contrast, DTH reactions to Tri r 2 were associated with DTH to *Trichophyton* extract and no specific IgE Ab to Tri r 2. Thus, in contrast to inhalant allergens, analysis of T-cell responses to Tri r 2 provided an opportunity for defining differences in the allergen-specific T-cell repertoire associated with dichotomous immune responses.

T-cell epitope mapping studies using 28 overlapping peptides of Tri r 2 identified distinct differences in the pattern of T-cell epitope recognition between IH and DTH responders. Specifically, an immunodominant epitope of Tri r 2, designated peptide (P)5, which mapped on to the amino-terminal region of the molecule, stimulated strong T-cell proliferative responses *in vitro* in peripheral blood monocytes (PBMC) cultures established from DTH but not IH responders. Use of a statistical method termed cross-validated linear discriminant analysis allowed comparison of the pattern of T-cell epitope recognition between IH and DTH responders. By this method, two regions of the Tri r 2 molecule (aa 51–90 and 231–270) were shown to contain peptides that induced different T-cell proliferative

responses between IH and DTH groups. Indeed, this method provided a 95% predictive accuracy for the classification of subjects into IH or DTH groups [14]. These observations indicated distinct differences in the TCR specificity of T cells within the allergen-specific T-cell repertoire associated with IH and DTH responses. The authors theorized that this reflected differences in the production of cytokines associated with distinct Th subsets. However, the analysis of cytokine responses to Tri r 2 peptides revealed a paradoxical finding: peptide 5 induced IL-5 and IL-10 production in PBMC from DTH but not IH responders. Thus, the DTH-associated major epitope, P5, preferentially induced the type 2 cytokine, IL-5, in DTH but not in IH responders. Moreover, levels of peptide-stimulated IFN- γ secretion by PBMCs from IH and DTH subjects were not significantly different [35]. Such complex patterns of cytokine responses may reflect the genetic heterogeneity of humans compared with other animal systems that have been used to establish the Th1/Th2 paradigm. In support of this, the regulation of IL-5 production by MHC has been demonstrated in murine models of contact sensitivity [47,48]. In those studies, congenic mouse strains producing high or low levels of IL-5 exhibited contact sensitivity. Similarly, in the author's studies, levels of IL-5 production were highly variable among DTH responders. These studies provided strong evidence that DTH and IH responses in humans that were directed against a single allergen did not adhere to classical Th1 or Th2 profiles. Consistent with this, a role for both Th1 and Th2 cytokines has been demonstrated for delayed skin reactions, including those associated with Th1-mediated (nickel sensitivity) and Th2-mediated (atopic dermatitis) allergic conditions [49–55].

Studies of T-cell responses to cat allergen: insights to protective epitopes

Although major T-cell epitopes have been identified for common inhalant allergens from diverse sources, such observations have provided little insight into differences in the allergen-specific T-cell repertoire associated with distinct allergic phenotypes. One possible explanation for this is the inappropriate design of *in vitro* systems for analyzing epitope-specific responses. In addition, many studies have failed to consider the complex role that genetics plays in T-cell recognition of allergens. This latter point is illustrated by the localization of immunodominant T-cell epitopes to different regions of the same allergen molecule in murine- and human-based systems [56]. Another important consideration is that the nonallergic phenotype has been difficult to define. Typically, this is characterized as the absence of specific IgE Ab to a given allergen. However, the recent description of the modified Th2 response to the major cat allergen, Fel d 1, has highlighted how the nonallergic phenotype encompasses at least two distinct immunological states; one associated with the presence of Fel d 1-specific IgG Ab (modified Th2), and one without [57]. Since the modified Th2 response is present almost exclusively in individuals who currently live with a cat who do not experience allergic symptoms, this immune response may represent a form of high-dose immunological

tolerance. A key question that remains is whether the modified Th2 response represents a variant of the allergic phenotype that develops with high-dose exposure to cat allergen. Numerous anecdotal reports of the development of allergic symptoms after decreased allergen exposure (e.g., removal of cats from the home) lend credence to this theory.

The Fel d 1 molecule is a heterodimeric molecule consisting of two polypeptide chains. In the mid-1990s, T-cell epitope mapping studies identified an immunodominant region within Fel d 1 chain 1, while peptides within chain 2 were reported to elicit minimal T-cell reactivity *in vitro* [58]. These experiments, which used polyclonal T-cell lines from cat-allergic subjects, formed the basis for the development of a peptide vaccine [59]. However, injection of two peptides (27 amino acids each), spanning the immunodominant region of chain 1, was associated with adverse reactions [59,60]. Most adverse reactions were classified as late events occurring approximately 6 h after administration of the vaccine. Subsequently, injection of a mixture of shorter chain 1 peptides (~14–17 amino acids) was shown to provoke late asthmatic reactions in a subset of cat-allergic individuals and these reactions appeared to be T cell-dependent and IgE-independent [61]. At the T-cell level, decreased production of IL-4 or IL-5 and increased IL-10 secretion, along with decreased proliferation in allergen- and peptide-stimulated PBMC cultures, have been reported after peptide therapy [62–66]. The mode of action of peptide vaccines is complex and may involve the induction of tolerogenic responses through a variety of mechanisms, including T-cell anergy, clonal deletion, or suppression. Moreover, the dosage of peptides administered coupled with the route of administration are likely to be important factors in the nature of the immune response generated. Identification of CD4⁺ cells with suppressive capacity after Fel d 1 peptide immunotherapy supports a role for Tregs in the immune response generated after injection of allergen-derived peptides [65]. However, at this point, alternate mechanisms cannot be excluded. Regardless of the mechanism, recent data indicate a clinical benefit of immunotherapy with peptides derived from Fel d 1 [67].

If, indeed, the goal of immunotherapy is to induce or enhance Tregs, several lines of evidence suggest that Fel d 1-derived peptides may be ideally suited to this approach. Induction of IL-10 is a feature of CD4⁺ T cells in PBMC cultures established from both allergic and cat-tolerant (modified Th2) individuals [17]. This may indicate the presence of Tregs within the Fel d 1-specific T-cell repertoire, irrespective of the allergic status of the host. Thus, the degree of control exerted by Tregs may be a key determinant in the development of allergy. Consistent with this view, Akdis and colleagues have proposed that allergic status is dictated by a fine balance between allergen-specific Th2 cells and Tregs [68]. In a different study, Ling and colleagues reported diminished suppressive capacity of CD25⁺CD4⁺ putative Tregs in atopic versus nonatopic donors with the least suppression in atopic patients who were currently experiencing symptoms [69]. An implication of these findings is that T-cell regulation is graded depending on atopic status and disease activity.

Using a rigorous T-cell epitope mapping approach, the author's group identified two immunodominant regions of the Fel d 1 molecule: one mapping to the aminoterminal of polypeptide chain 1 and the other mapping to the aminoterminal of chain 2 [17]. Peptides used in these studies were designed as 17-mers with a ten amino acid overlap for stringent mapping. Peptides spanning each immunodominant region of Fel d 1 elicited strong T-cell proliferation in PBMC cultures established from both allergic and modified Th2 responders. Interestingly, induction of the Th2 cytokine, IL-5, was a feature of chain 1 major T-cell epitopes, while overlapping major epitopes of chain 2 preferentially induced IL-10 (P2:1) and IFN- γ (P2:2). Thus, discrete patterns of cytokine induction localized to distinct regions of the Fel d 1 molecule (FIGURE 1). Initial analyses showed increased induction of IL-5 by chain 1 peptides in PBMC cultures from allergic subjects. Thus, expansion of chain 1 epitope-specific Th2 cells may be a feature of the allergic response. Flow cytometry studies confirmed that CD4⁺ T lymphocytes stimulated with chain 2 epitopes secreted IL-10, indicating that these epitopes may be targeted by Tregs. The localization of IFN- γ - and IL-10-inducing epitopes to the same region of Fel d 1 may facilitate interactions between Th1 cells and Tregs at the surface of the APC. In support of this, blocking IL-10 using an anti-IL-10 monoclonal Ab (mAb) or an anti-IL-10R mAb resulted in enhanced T cell proliferation and production of IFN- γ in PBMC cultures stimulated with pooled chain 2 peptides. In fact, chain 2 peptides stimulated unusually high levels of IFN- γ secretion (up to 10 ng/ml) when IL-10 was blocked, and these levels were up to eight-fold more than those induced by whole allergen [17]. By contrast, there was little or no effect on Th2 cytokines. Similar observations were made irrespective of the allergic status of the donor.

Analysis of epitope-specific T-cell responses in cat-allergic patients who received conventional immunotherapy (IT) revealed marked increases in IL-10 and IFN- γ secretion within the first 4 months of treatment, which localized to the amino-terminus of chain 2. By contrast, levels of peptide-stimulated IL-5 and IL-13 generally remained unchanged [17]. These findings are consistent with alterations in the distribution of effector and regulatory T-cell subsets with defined TCR specificities within the Fel d 1-specific T-cell repertoire during immunotherapy.

Activation of T cells that target chain 2 epitopes during IT in conjunction with modulation of the Fel d 1-specific serum antibody profile towards a modified Th2 response (IgE^{high}IgG^{med} to IgE^{low}IgG^{high}) supports a pivotal role for chain 2 epitopes in tolerance induction. However, analysis of T-cell responses to chain 2 epitopes did not reveal any enhanced recognition in

cat-tolerant subjects with diverse human leukocyte antigen (HLA) types. By contrast, analysis of T-cell responses in the context of the major HLA-DR allele, *0701, identified enhanced IL-10 production to P2:1 in PBMC cultures from DR7-positive cat-tolerant subjects. Interestingly, the *0701 allele was expressed at increased frequency in cat-tolerant (50%) compared with allergic (7%) subjects. Subsequent studies showed that, in atopic dermatitis (AD) patients with very high IgE Ab levels to cat allergen, T-cell reactivity to chain 2 epitopes was altered. In those individuals, chain 2 epitopes failed to induce IFN- γ and the pattern of IL-10 induction differed [70]. Thus, altered T-cell reactivity to a putative regulatory region of Fel d 1 is a feature of aberrant IgE responses. It is notable that IL-10 blockade in AD patients did not restore IFN- γ production to chain 2 epitopes, indicating a failure of T-cell control mechanisms in the immune response to cats in these individuals.

The view that allergen-derived peptides have intrinsic properties that favor selective induction of specific cytokines is controversial. Studies performed by the author's group showed consistent segregation of cytokine responses to distinct regions of the Fel d 1 molecule, irrespective of allergic status. The ability of chain 2 epitopes to favor induction of IL-10 and IFN- γ could be explained by co-exposure to Th1/IL-10-skewing adjuvants during T-cell priming. However, if this were the case, chain 2 epitopes would be expected to induce only weak IL-10/IFN- γ responses in T cells derived from allergic subjects, and this is not the case. Studies that have examined the effects of altered peptide ligands on the T-cell response have demonstrated the ability of peptides carrying single or multiple residue substitutions to alter triggering through the TCR, resulting in antagonistic or partial agonistic effects. Such effects

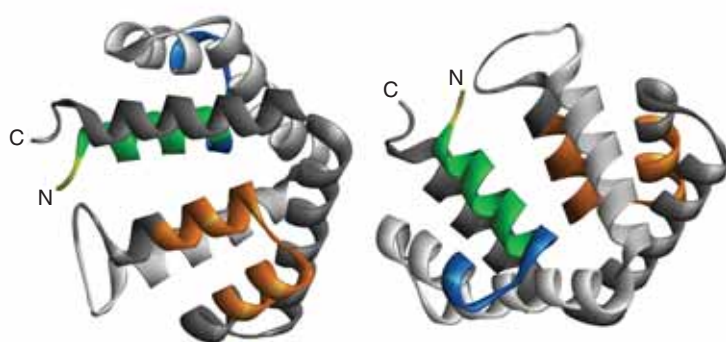


Figure 1. Molecular model of Fel d 1 showing localisation of major T-cell epitopes. Polypeptide chains 1 and 2 are shown in dark and light gray, respectively. Model shows localization of the chain 1 epitope, P1:2 (orange), and chain 2 epitopes, P2:1 (yellow), and P2:2 (blue). Overlapping residues of P2:1 and P2:2 are shown in green.

Structure obtained from Protein Data Bank file 1puo.pdb. Figure provided courtesy of A Pomes and copyright of INDOOR Biotechnologies, Inc.

may alter intracellular signaling events within the T cell with subsequent alterations in cytokine induction [71]. For altered peptide ligands derived from allergens, it has been proposed that the mode of interaction between the TCR/peptide–MHC complex may control cytokine secretion by the APC, which in turn influences cytokine induction by the T cell. Regardless of the mechanism, the implication is that a given T-cell epitope may favor preferential induction of specific cytokines. In addition to intrinsic properties of the peptide, T-cell responses generated to a specific epitope may be influenced by epitope copy number on the surface of the APC through altering the avidity of the interaction between the T cell and APC. It is conceivable that the dose of allergen encountered in the environment is relevant in this regard. Indeed, antigen dose has been shown to influence the T-cell avidity of *in vitro*- and *in vivo*-expanded T-cell populations [72]. This may affect the antigen-specific T-cell repertoire by favoring selection of T cells with defined

TCR specificities based on avidity. Thus, it could be theorized that high-dose exposure to Fel d 1 narrows the TCR repertoire diversity by focusing on the expansion of chain 2 epitopes.

Model for T-cell epitope-specific regulation of distinct allergic phenotypes

It is well established in murine asthma models that high-dose allergen exposure can induce IL-10-producing Tregs and respiratory tolerance [73,74]. The author proposes that high-dose exposure to cat allergen in the context of DR7 favors enhanced recognition of chain 2 epitopes and development of the modified Th2 response. In the cat system, IL-10 appears to act preferentially on IFN- γ by suppressing Th1 cells that target the T-cell epitope P2:2. The interplay between chain 2 epitope-specific Th1 cells and Tregs may be critical to determining the balance of effector T cells within the Fel d 1-specific T-cell repertoire.

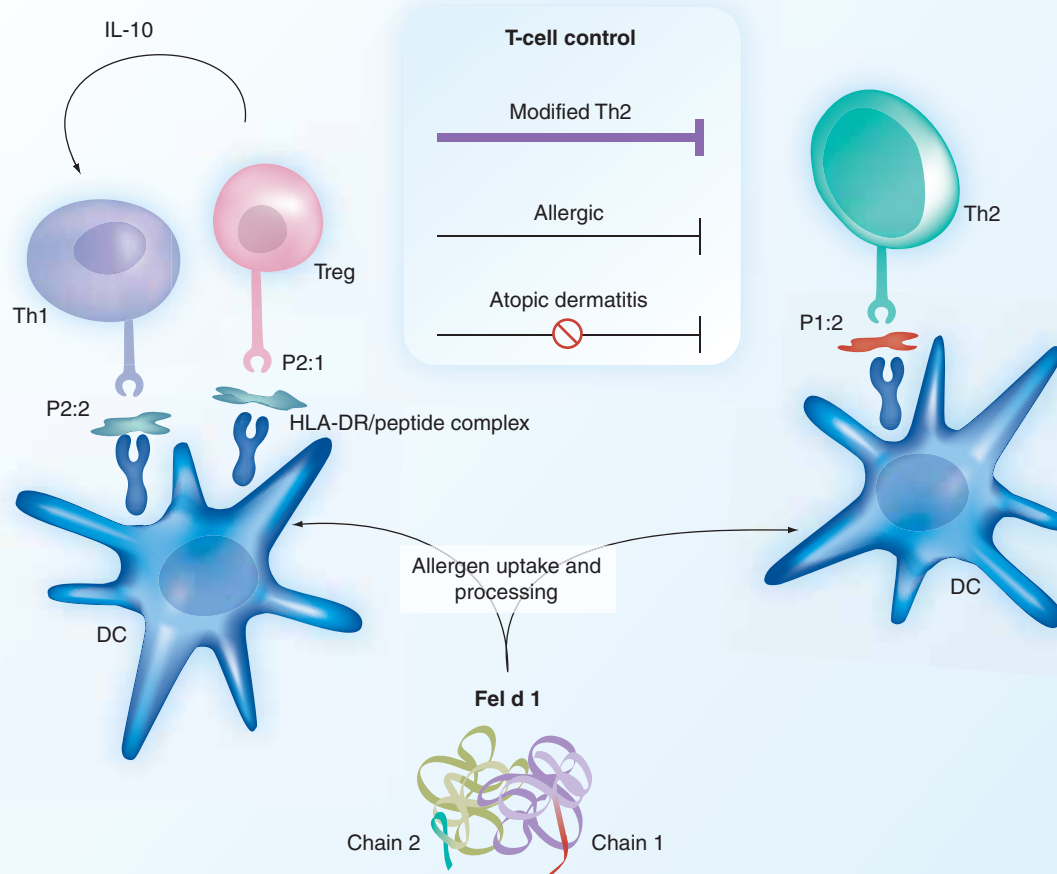


Figure 2. Model of T-cell epitope-specific regulation of Th2-driven allergic responses to Fel d 1. The author proposes a model whereby co-ordinated regulation mediated by IL-10-producing Tregs and Th1 cells, which recognize chain 2 epitopes, controls both allergic and nonallergic responses. The chain 1 peptide P1:2 preferentially induces the Th2 cytokine, interleukin (IL)-5, while chain 2 peptides preferentially induce IL-10 (P2:1) and IFN- γ (P2:2). Interaction between Th1 and regulatory T cells specific for chain 2 epitopes may influence the Th2 compartment by regulating the balance of different T cell subsets within the Fel d 1-specific T-cell repertoire. Distinct allergic phenotypes (i.e. allergic or modified Th2) represent different 'degrees' of T-cell control. The fact that epitopes that induce IL-10 and IFN- γ map to the same region of the molecule may be significant in facilitating an interaction between Treg and Th1 subsets at the surface of the antigen presenting cell (APC). The effects of IL-10 on Th1 cells may be direct or indirect, acting through the APC. Given that Fel d 1 peptides are presented to CD4⁺ T cells in the context of HLA-DR molecules, expression of a given HLA type (e.g. DR7 in subjects with a modified Th2 response) could enhance this mechanism. DC: Dendritic cell; IFN: Interferon; HLA: Human leukocyte antigen; P: Peptide; Th: T helper; Treg: Regulatory T cell.

Although IL-10 does not appear to act directly on Th2 cytokines, chain 2 epitope-specific Tregs may influence the Th2 compartment indirectly through effects on Th1 cells and, possibly, through effects on novel effector T-cell populations, which remain to be defined. This regulatory mechanism operates in both allergic and cat-tolerant individuals to control the magnitude and quality of the T-cell response (FIGURE 2). Such a mechanism would be necessary for a ubiquitous antigen that has the capacity to prime T cells in all individuals, irrespective of allergic status, thereby limiting deleterious allergic inflammatory responses. The author speculates that binding of chain 2 peptides to DR7 results in optimal activation of Th1 cells and Tregs directed against these epitopes, and increased control in DR7⁺ modified Th2 subjects compared with allergic subjects. By contrast, markedly elevated IgE Ab to Fel d 1 in patients with atopic dermatitis reflects failure of this control mechanism (FIGURE 2). Based on this model, treatment strategies that enhance the interactions between Tregs and Th1 cells may be efficacious. Given the ability of chain 2 epitopes to induce T-cell reactivity in individuals with diverse HLA types, administration of high-dose chain 2 peptides in DR7-negative allergic subjects could preferentially target Fel d 1-specific Tregs and Th1 cells, thereby downregulating a Th2 response.

Summary & conclusions

T-cell responses directed against allergens are complex. Although there is evidence of Th2 skewing in allergic individuals, adherence of distinct allergic phenotypes to the Th1/Th2 paradigm is not apparent at the T-cell epitope level. In rigorous assay systems, differences in T-cell epitope recognition can be identified; however, careful consideration of the clinical status of the patient, as well as the nature of the allergen, is imperative. Systems designed to examine allergen-specific T-cell responses in humans generally focus on established T-cell responses in adults, which represent the culmination of years of dynamic interactions between distinct Th subsets. Such immune events will be influenced by the genetics of the host and environmental exposure to allergen. Thus, a limitation of human-based systems is that they offer a snapshot view of events at the T-cell level. This may explain, at least in part, the heterogeneity of allergen-specific T-cell responses observed among subjects with the same allergic phenotype. The value of T-cell epitope-based studies performed on patients receiving IT

cannot be overstated. Such studies offer much promise for defining the mechanisms of T-cell control of allergen-specific responses. Advancement of our knowledge of the role that Tregs play in this process may open the door to the development of new treatments.

Expert commentary

Immunotherapy with allergen-derived peptides is an appropriate strategy for treating allergic disease since it avoids the IgE-mediated side effects associated with conventional IT. However, few peptide vaccines have been studied to date and studies designed to evaluate the clinical benefits are sorely needed. Detailed analyses of epitope-specific T-cell responses to allergens are clearly warranted in order to gain insight into the nature of effector T-cell and Treg populations involved in both allergic and nonallergic responses. The application of evolving experimental techniques that utilize flow cytometry and multiplex assay systems may prove invaluable in this pursuit. Such methodologies allow the direct identification and phenotyping of allergen-specific T cells and the examination of a broad panel of T cell-derived cytokines. Fine mapping of immunodominant epitopes is needed in order to identify core epitopes and their MHC-binding properties and to evaluate the influence of flanking amino acid residues on the cytokine-inducing properties of these epitopes. It will be necessary to think beyond the constraints of the Th1/Th2 paradigm if we are to identify T-cell populations that are suitable targets for therapy. Identification of Th-IL-17 cells, a new lineage of CD4⁺ Th cells involved in Th1-mediated inflammatory diseases, underscores the importance of broadening the scope of T-cell subsets studied in allergic inflammatory processes [75].

Five-year view

Increased understanding of the nature of Tregs in humans, including their mode of induction and function, as well as allergen specificity is anticipated within the next 5 years. These factors, coupled with further characterization of newly identified Th subsets and their associated cytokines, should facilitate a better understanding of the interactions between defined T-cell subsets and their relevance to distinct allergic phenotypes. The identification of allergen-derived peptides that are targeted by specific T-cell subsets is relevant to the design of peptide vaccines with minimal side effects and maximal protective effects.

Key issues

- T cells from subjects with distinct allergic phenotypes exhibit differences in allergen recognition in *in vitro* culture and these differences are epitope-specific.
- T-cell epitope-induced cytokine profiles observed in peripheral blood mononuclear cell (PBMC) cultures from allergic and non-allergic individuals do not fit with dichotomous T helper (Th)2 and Th1 responses, respectively.
- The nonallergic phenotype may constitute different types of immune response (delayed-type hypersensitivity or tolerance) depending on the nature of the allergen.
- Defining regions of allergen molecules that are targeted by anti-allergic cell types will be pivotal to the design of new or improved peptide vaccines for treatment of allergic disease

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