

T-Cell Epitopes of Food Allergens

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Abstract

During recent years, a great deal of research has focused on the identification of food allergens, their characterization at the molecular level, and the investigation of immunological mechanisms responsible for food hypersensitivity. Allergen-reactive T-helper 2 cells play a central role in the pathophysiology of allergic diseases. After activation with allergens, this subset of CD4⁺ T-lymphocytes produces a spectrum of cytokines that induce high serum levels of immunoglobulin E and eosinophilia, which, in turn, mediate the clinical symptoms. Furthermore, T-helper 2 cells are present in target organs during the late response to allergen exposure. The analysis of the allergic T-cell response, including cytokine production and the identification of T-cell epitopes, provides important information on the characteristics of food allergens. Therefore, in vitro cultures of allergen-specific T-cells derived from peripheral blood or different target organs have been of indispensable help. Results obtained from such studies may not only contribute to the understanding of cellular mechanisms underlying different types of food allergy but also to the development of novel strategies for immunotherapy of food allergy.

Index Entries

Allergic T-cell response; T-cell epitope; food allergy; human; food allergens.

Introduction

The term “food allergy” summarizes immune-mediated, nontoxic adverse reactions to foods (1). The most common form of food allergy is mediated by immunoglobulin (Ig)E antibodies and reflects an immediate-type (“type I hypersensitivity”) reaction—that is, an acute onset of symptoms after ingestion or inhalation of food substances. Within 15 to 30 min, immediate mast-cell-dependent reactions occur that resolve within 1 to 3 h. Some individuals also experience a late-phase reaction that manifests 6 to 12 h after contact with allergen and resolves within 24 h. Whereas the clinical manifestations of immediate phase are exclusively IgE-mediated, the mechanisms operative in the late-phase reaction are characterized by the recruitment, activation, and persistence of eosinophils, basophils, and activated T-cells in the respective target organs.

The development of an IgE-mediated immune response requires a series of direct and indirect cellular interactions involving antigen-presenting cells (APCs) and T- and B-lymphocytes. After uptake and processing of the allergen, APCs (e.g., dendritic cells, monocytes) present small peptide fragments containing T-cell epitopes in conjunction with major histocompatibility complex (MHC) class II molecules on the cell surface. CD4⁺ T-helper (Th) lymphocytes bearing the appropriate T-cell receptor (TCR) recognize the peptide-MHC class II complexes. TCR signaling is performed by the CD3 protein complex associated to the TCR. Additionally, co-stimulatory signals, mediated by surface antigens, such as CD2, CD4, CD45, CD40L, and CD28, are crucial for APC-mediated T-cell activation, which leads to cytokine production and proliferation. CD40, CD80, and CD86 provide these accessory signals. In turn, activated CD4⁺ cells and their products activate B-cells specific for the same antigen to generate allergen-specific antibodies (Fig. 1).

The cytokines interleukin (IL)-4 and IL-13 are important factors to switch B-lymphocytes to IgE synthesis (2). IL-5 mediates recruitment and activation of eosinophils (Fig. 1; ref. 3). Consequently, CD4⁺ cells producing these factors play an important role in the pathophysiology of atopic diseases. Initially, two subsets of Th cells were defined in mice according to the type of cytokine production: CD4⁺ T-cells synthesize high amounts of IL-4, IL-13, and IL-5, but little or no interferon (IFN)- γ are designated Th2. Conversely, Th1 cells synthesize high levels of IFN- γ , a potent antagonist of IL-4, but no Th2-like cytokines. In humans, the classification of CD4⁺ T-cells into Th1/Th2 cells describes two similarly polarized phenotypes of effector T-cells. However, CD4⁺ T-cells producing a more heterogeneous cytokine profile have also been found (e.g., Th0, which is characterized by the synthesis of high amounts of both IL-4 and IFN- γ). Nevertheless, the “Th1/Th2 paradigm” has provided indispensable help in understanding basic immunological mechanisms underlying the allergic disease (4). Allergen-specific Th2 cells have been identified in the peripheral blood and the nasal mucosa of patients with rhinitis, in skin lesions of patients with atopic dermatitis, and in the bronchial alveolar lavage fluid of patients with asthma, thus demonstrating their important role in induction and maintenance of allergy (5–7).

In nonallergic individuals, the immune response to allergens is not dominated by Th2 but is equilibrated between Th2 and Th1 lymphocytes (8,9). Recently, evidence has accumulated that allergen-specific regulatory CD4⁺ T-cells also play an important role in healthy individuals (10). Regulatory CD4⁺ T-cells suppress immune responses by the production of anti-inflammatory cytokines, such as IL-10 and transforming growth factor- β and/or via cell-cell interactions (10–12). Allergen-reactive regulatory T-cells were found to be higher in children with outgrown food allergy compared with those in children still suffering from their

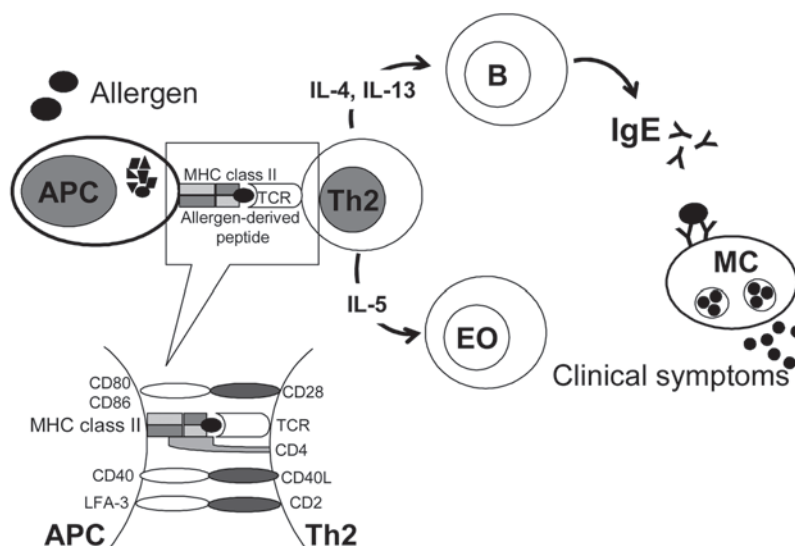


Fig. 1. Allergen-specific activation of CD4⁺ T-cells. APC, antigen-presenting cell; Th2, T-helper 2 lymphocyte; B, B-lymphocyte; EO, eosinophil granulocyte; MC, mast cell. (For explanation see Introduction.)

food allergy (13). Specific immunotherapy demonstrated the induction of similar mechanisms (as observed in healthy individuals) to counterregulate the dominating allergic Th2 response. Successful treatment could be associated with the downregulation of allergen-specific Th2 cells by regulatory T-cells and by immune deviation—that is, the modulation toward a more balanced cytokine production (14–18). Therefore, allergen-specific Th2 cells are important targets for therapeutical intervention in atopic diseases. In this context, the detailed analysis of the T-cell response against allergens, including cytokine synthesis and the identification of relevant T-cell epitopes, is an important contribution to a comprehensive characterization of allergenic proteins. This knowledge not only improves our understanding of the pathophysiology of allergic diseases, but also represents an important prerequisite to develop superior forms of immunotherapy (e.g., the use of synthetic peptides and recombinant and mutated allergens).

Identification of T-Cell Epitopes of Allergens: Experimental Approach

The introduction of recombinant DNA technology into the field of allergology has allowed the exact characterization of the immune response against allergens at the B- and T-cell levels. During the last decade, most of the common allergens have been cloned, sequenced, and produced as recombinant proteins. Instead of complete extracts from allergenic sources, which contain variable quantities of different allergenic and nonallergenic proteins, purified recombinant allergens are defined proteins of known amino acid sequence and concentration. Therefore, in addition to the characterization of antibody responses, these pure and standardizable proteins also became perfect tools for analyzing the T-cell response to allergens in allergic and nonallergic individuals.

If peripheral blood is used as a source for circulating T-lymphocytes, allergen-specific T-cells need to be enriched prior to epitope map-

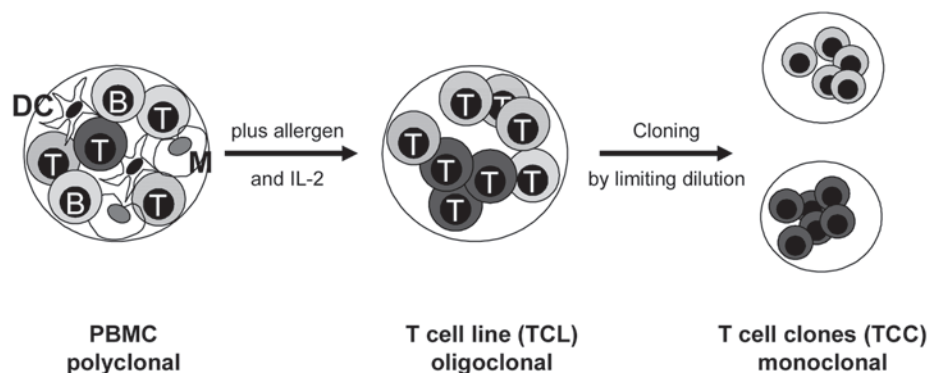


Fig. 2. Allergen-specific T-cell cultures. Peripheral blood mononuclear cells (PBMCs) contain T-cells with various specificities. Allergen stimulation induces proliferation of T-cells that are reactive with different epitopes. By limiting dilution techniques, T-cells that are specific for only one epitope are isolated. DC, dendritic cell; M, monocyte; T, T-cell; B, B-cell.

ping experiments because their frequency in the blood is rather low (estimated around $1:10 \times 5$). Therefore, peripheral blood mononuclear cells (PBMCs) isolated from whole blood by density-gradient centrifugation are incubated with an optimum concentration of the allergen under investigation. PBMCs contain dendritic cells, monocytes, and B-lymphocytes, all of which can serve as APCs and can activate allergen-specific T-cells (Fig. 2). Consequently, these T-cells express IL-2 receptors (CD25) on their surfaces. Suboptimal concentrations of IL-2 added to the cultures after 5 to 7 d support the preferential proliferation of these "privileged" allergen-specific cells. The resulting culture is called T-cell line (TCL) and contains a mixture of T-lymphocytes enriched for allergen-specific T-cells with different epitope specificities (oligoclonal). After an additional 2 to 4 d, monoclonal cultures can be generated from TCLs by limiting dilution. For this purpose, T-cell blasts are enriched by density-gradient centrifugation and are counted and seeded into 96-well plates in the presence of irradiated PBMCs and growth factors. Varying concentrations are used. Statistically, when approx 33% of the culture wells contain proliferating T-cells, a single T-cell clone (TCC) should be produced. After approx 14 d, grown

TCCs are further expanded by the addition of IL-2 and irradiated PBMCs. To test allergen specificity, TCLs and TCCs are stimulated with allergen and autologous irradiated PBMC for 24 to 48 h. The proliferative response is then determined (e.g., by measuring the uptake of [^{3} H]-thymidine and liquid scintillation counting). By stimulating TCLs and TCCs with IL-2 or allergen at intervals, the cultures can be maintained for longer time periods.

Epitope mappings of allergens can be performed using oligoclonal allergen-specific TCLs, as well as monoclonal TCCs. Because T-cell epitopes are short, linear peptides representing the primary structure of a protein, the cultures are stimulated with peptides synthesized according to the amino acid sequence of the allergen under investigation. The length of the single peptides and the number of residues shared by neighboring peptides can be individually defined. Because MHC class II molecules predominantly bind peptides of approx 12 to 15 amino acid residues, most studies have applied peptides of 10 to 20 residues. In our own studies, we have used 12-mer peptides walking along the entire amino acid sequence, with overlaps of 9 residues each (Fig. 3). Peptides that induce proliferation in T-cell cultures contain T-cell epitopes.

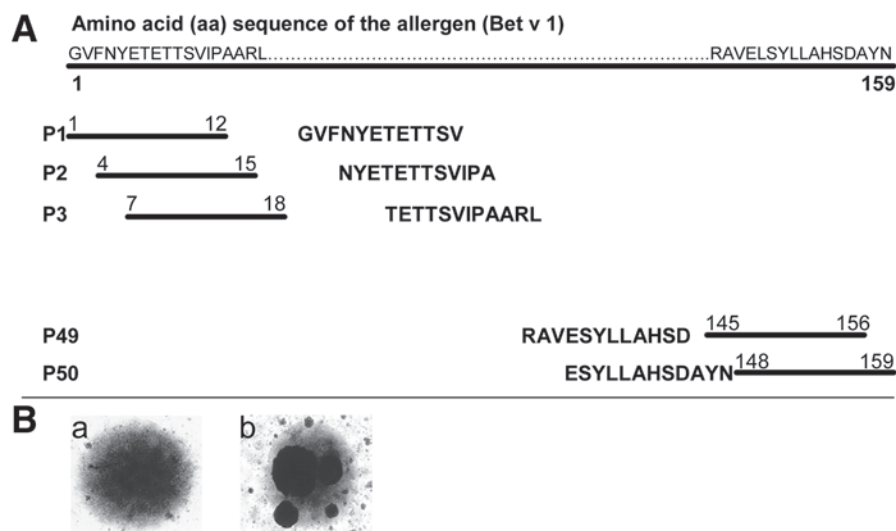


Fig. 3. T-cell epitope mapping of allergens. **(A)** According to the primary structure of an allergen (the major birch pollen allergen, Bet v 1, is shown), 12-mer peptides (P1, P2,...) overlapping for 9 amino acid residues are synthesized and used for stimulation of T-cell lines and T-cell clones. **(B)** Bet v 1-specific TCCs 24 h after stimulation with peptides. Clustering indicates that peptide (b) contains a T-cell epitope, whereas peptide (a) does not.

Allergen-specific oligoclonal TCLs are well suited to gain an overview on the different T-cell-activating regions within a particular allergen. These cultures contain T-cell subpopulations with different specificities and, therefore, reveal a broad spectrum of epitopes recognized by a single patient. By comparing the epitope recognition patterns of a large number of different individuals, immunodominant T-cell epitopes (i.e., those recognized by more than 50% of subjects) can be identified. Allergen-specific TCCs are specific for one single epitope and are well suited to characterize the expression of surface markers defining their subset (e.g., CD4, CD8, TCR- $\alpha\beta$, TCR- $\gamma\delta$) and homing markers (e.g., α/β integrins, cutaneous lymphocyte antigen). Furthermore, the expression of T-cell receptor gene families and the cytokine response to stimulation with an allergen or a peptide containing a particular epitope (e.g., IL-4 and IFN- γ) can be analyzed. The restriction of T-cells to antigen presentation by MHC class II molecules can be determined in inhi-

bition experiments using neutralizing anti-human leukocyte antigen (HLA)-DR, DQ, and DP antibodies (19,20). Finally, TCCs can be used to clarify the relevance of single amino acid residues within an antigenic site (e.g., to define the critical residues for MHC class II or TCR binding) (21,22).

The issue regarding whether TCCs obtained by in vitro propagation actually represent T-cells involved in the pathogenesis of the studied disorder has always been controversial. The methodology used in our studies is based on the selection of single clones and their expansion to adequate cell numbers, which is accomplished by the use of mitogenic stimuli and growth factors. To address this debate, we developed a special polymerase chain reaction-based T-cell tracing system, in which the hypervariable region of the TCR β -chain was used as a specific fingerprint of a single TCC. This hypervariable complementarity determining region (CDR) 3 of the TCR β -chain is encoded by the *V(D)J* junctional gene segment and is highly specific

for a single clone. Using oligonucleotides complementary to the *V(D)J* junctional gene segment, we detected the same allergen-specific Th2 clones in blood and skin of allergic individuals up to 4 yr after their first isolation (23). These findings strongly support the suggestion that in vitro propagated allergen-specific TCCs reflect clones involved in the allergic pathophysiology in vivo. However, it must be remembered that the experimental approach used in our studies is based on allergen-induced cell division of specific clones. Presumably, proliferating T-lymphocytes are not the only responding cells when PBMCs are stimulated with allergen but become the predominant population under the highly selective conditions used. In particular, regulatory and/or anergic allergen-specific T-lymphocytes, which do not proliferate in response to allergens, cannot be addressed.

T-Cell Epitopes of Food Allergens

The human diet includes a great diversity of different foods. Interestingly, only a limited number of edibles cause food allergy. According to the clinical appearance, the pattern of allergens, and the underlying immunological mechanisms, two different forms of food allergy must be distinguished. In young children, the sensitization process occurs via the gastrointestinal (GI) tract, possibly resulting from their immature GI epithelial membrane barrier (24). Food allergy can be the first manifestation of the atopic syndrome. The most important "classic" food allergen sources are cow milk, hen eggs, and legumes. These food allergens are particularly resistant to heating and enzymatic degradation (25,26). In addition to GI manifestations, classic food allergens frequently cause or influence atopic dermatitis, a chronic eczematous skin disease (27).

In the majority of cases—with some exceptions (e.g., peanuts and fish)—these manifestations disappear later in childhood but are replaced with other manifestations of the

atopic syndrome (28). The second type of food allergy, which is observed mainly in adolescents and adults, is believed to develop as a consequence of immunological crossreactivity. In all likelihood, the patients are first sensitized via respiratory sites to inhalant allergens (e.g., pollens from birch, ragweed, or mugwort; proteins from house dust mites; or rubber latex). IgE antibodies specific for these inhaled allergens bind to structurally related proteins in foods. For example, patients allergic to birch pollen frequently develop symptoms after the ingestion of fresh apples and hazelnuts. Individuals with allergy to ragweed can experience symptoms after contact with fresh melons.

Because most of these "aero-allergen-related" food allergens are easily degraded by heating and enzymes, this type of food allergy mainly manifests in immediate symptoms localized at the site of allergen contact (e.g., the oral mucosa). However, systemic (e.g., urticaria) and more severe reactions (e.g., anaphylactic shock) are occasionally observed. A potential role of aero-allergen-related food allergens for atopic dermatitis in children was recently suggested (29).

Although the IgE response to many major food allergens has been thoroughly studied, the detailed information about the T-cell response to food proteins remains scarce. Table 1 summarizes food allergens that were analyzed for their T-cell epitopes in humans. Comparable to most inhalant allergens, allergenic food proteins contained multiple T-cell-activating regions dispersed throughout the entire allergen molecule (21,30–33). The individual patients markedly differed in peptide recognition (interindividual diversity), and most individuals harbored T-cells specific for more than one epitope (intra-individual diversity). Also comparable to respiratory allergens, the same repertoire of T-cell epitopes was recognized by food-allergic and nonallergic individuals (34). Studies of the *TCR-VA* and *-VB* genes and human leukocyte antigen phenotypes indicated polyclonal T-cell responses in single

Table 1
Food Allergens Mapped for T-Cell Epitopes

Allergen	Food	Length of peptides (amino acid)	Test culture	No. of tested individuals	No. of identified T-cell epitopes	Ref.
αs1-Casein	Milk	20	TCL	2	5	78
		17–20	TCL	1	2 (7)	79
Api g 1	Celery	12	TCL, TCC	8	7	33
Ara h 2	Peanut	8	TCL	10	4	39
		20	TCL	8	8	32
Ber e 1	Brazil nut	15	CD4 ⁺ T-cells	92 ^a	4	31
β-Lactoglobulin	Milk	17–83	PBMC	13 ^a	4	34
		12–21	TCL, TCC	4	7	30
Bovine serum albumin	Beef	4–20	PBMC	4	3	38
Cor a 1.04	Hazelnut	12	TCL, TCC	17	20	77
Ovomucoid (Gal d 1)	Egg	Approx 60	PBMC	33	3	80
		55–65	TCL, TCC	4	3	81
		14–18	TCL	8	10	21
		14–22	TCL, TCC	4	6	35
Ovalbumin (Gal d 2)	Egg	10–24	TCL	1	1	82
		12–17	TCL	2	10	83

^aSum of allergic and nonallergic individuals.

TCL, T-cell line; TCC, T-cell clone; PBMC, peripheral blood mononuclear cell.

individuals and confirmed the high diversity of the allergen-specific T-cell response (30,35,36).

Interestingly, several T-cell-activating peptides of different classic food allergens also bind IgE antibodies, which is rarely true for inhalant allergens because they contain mainly three-dimensional B-cell epitopes. For example, chicken ovomucoid (Gal d 1) contains various T-cell epitopes distributed over the entire primary structure, which were shown to also represent B-cell epitopes (21). The amino acid region from 323 to 339 from ovalbumin is recognized by T- and B-cells (37). Three T-cell-activating regions of the major beef allergen bovine serum albumin were also recognized by B-cells (38). Only recently, a dominant T-cell epitope of the major peanut allergen Ara h 2 was identified at residues 19 to 38, a region which contains a dominant IgE-binding epitope at amino acids 27 to 36 (32,39).

A common feature of classic food allergens is that they still bind IgE antibodies after pepsin digestion in the stomach (25,40,41). This resistance to gastric digestion was proposed

as a marker for potential allergenicity. The fact that classical food allergens contain short linear amino acid sequences representing T- and B-cell epitopes may support the hypothesis for a sensitization via the GI tract. These short linear peptides may easily survive gastric, and even pancreatic, digestion and reach the gut-associated lymphoid tissue in a highly immunogenic form. Furthermore, these short food fragments may easily enter the circulation and be distributed throughout the body. In target organs (e.g., the skin), they can induce IgE- as well as T-cell-mediated effector functions, which may explain why classical food allergens frequently cause or influence atopic dermatitis (42).

T-Cell Epitopes of Pollen-Associated Food Allergens

A typical example for aero-allergen-associated food allergy is the “birch–fruit syndrome.” The frequency of birch pollinosis has almost tripled in Central and Northern Europe during the past 15 yr (43). Accordingly, birch-pollen-

associated food allergy has found special attention during this time period. More than 95% of individuals with allergy to birch pollen are sensitized to the major birch pollen allergen Bet v 1 (44). Bet v 1 represents one of the best-characterized aero-allergens, and its amino acid sequence, conformational structure, and B- and T-cell epitopes have been identified (9,45–48). Furthermore, it is well-documented that Bet v 1 shares IgE epitopes with proteins in fruits of the *Rosaceae* family (e.g., Mal d 1 in apples, Pru av 1 in cherries, Pyr c 1 in pears), hazelnuts (Cor a 1), vegetables of the *Apiaceae* family (e.g., Dau c 1 in carrots, Api g 1 in celery), soybeans (Gly m 4), mungbean (Vig r 1), and peanuts (Ara h 8) (49–59). For example, the tertiary structure of Pru av 1, the major cherry allergen, was found to be virtually identical to Bet v 1 (60). Consequently, up to 70% of individuals with allergy to birch pollen develop Type I hypersensitivity reactions to these foods (61–64). In most cases, the patients experience the so-called “oral allergy syndrome” (OAS) after contact with raw foods, which is characterized by swelling of the lips, itching, and irritation of the oral mucosa (65). Nevertheless, systemic and more severe reactions, such as anaphylactic shock, are not rare after the consumption of soy-containing dietary products (55).

Because the major birch pollen allergen is so well characterized, Bet v 1 and related food proteins are ideal model molecules for studying basic immunological mechanisms underlying aero-allergen-associated food allergy. Analyzing the T-cell response to the major allergens in apples and celery (Mal d 1 and Api g 1, respectively), one immunodominant T-cell epitope for each food protein was identified (33,66). Both epitopes are located in parts corresponding to relevant T-cell-activating regions of Bet v 1, and their amino acid sequence is highly identical to the respective sequences of the Bet v 1 epitopes. Accordingly, the majority of T-cell cultures initiated with Mal d 1 and Api g 1 also proliferated and secreted cytokines when stimulated with Bet v 1 (33,66). Interest-

ingly, crossreactive TCLs and TCCs responded by far more pronounced to Bet v 1 than to their original stimulus (Api g 1 and Mal d 1, respectively). Furthermore, the T-cell cultures lost their reactivity with the food allergens after a few expansion cycles in vitro but remained highly reactive to Bet v 1. Therefore, peripheral T-cells that respond to the major apple and celery allergens appear to be primarily pollen-specific, crossreacting T-lymphocytes. The fact that Bet v 1 shares important T- and B-cell epitopes with its related food allergens corroborates the concept that the major pollen allergen initializes sensitization to the food allergens.

Pollen allergens can induce flare-ups in atopic dermatitis; however, the involvement of pollen-related food allergens for such reactions is still not fully understood (67). It has been demonstrated that subgroups of birch pollen-allergic individuals with atopic dermatitis reacted with a marked deterioration of their eczema after oral challenge with birch pollen-related food (29,68). Ingestion caused an increased expression of cutaneous lymphocyte antigen on the surface of peripheral blood T-lymphocytes, and the cutaneous flare-ups contained Bet v 1-reactive T-cells (68). Hence, pollen-specific T-cells can be activated by related food allergens to migrate to the skin and exert effector functions in vivo. It is worth noting that the individuals mentioned above did not experience OAS upon oral food challenge, indicating that T-cell-mediated exacerbations of skin lesions can occur independently of IgE-mediated immediate reactions to the respective foods. In vitro, we found that pollen-related food allergens are potent stimulators of pollen-specific T-cells and TCCs specific for various different T-cell epitopes of Bet v 1 proliferated and secreted cytokines after stimulation with homologous food proteins (33,66,69). Moreover, we incubated Bet v 1-related food allergens with the enzymes pepsin and trypsin in succession in order to simulate GI digestion. Several different proteolytic fragments were isolated, which

neither bound IgE antibodies nor induced basophil mediator release, but still potentially activated Bet v 1-specific T-cells (70). Hence, although the passage through stomach and duodenum obviously destroys the ability of Bet v 1-related food allergens to induce IgE-mediated symptoms, digested allergens may be well capable to stimulate pollen-specific T-cells *in vivo*. Recent experimental data from our laboratory further corroborate that crossreactivity between Bet v 1 and related food allergens differ strikingly at the humoral and T-cell homology of each food allergen with the immunodominant T-cell epitope of Bet v 1 located at residues 142–156 in the C-terminal part of the pollen allergen (69). In contrast, IgE crossreactivity correlated with the overall aa sequence homology between each food allergen and Bet v 1 necessary for the formation of a similar tertiary structure level (69). We observed that the major allergens in celery and carrot represented very strong activators of Bet v 1-specific T-cells, although they are rare inducers of clinical symptoms after consumption. In contrast, the major apple allergen, which causes OAS in 80 to 90% of birch pollen-allergic patients, turned out to be a rather weak stimulator of Bet v 1-specific T-lymphocytes. Analyzing these differences at the molecular level, the cellular crossreactivity correlated with the aa sequence homology between each food allergen and Bet v 1 necessary for the formation of a similar tertiary structure.

Together, these findings emphasize the role of pollen-related food allergens as potent activators of pollen-specific T-cells—even in patients with no clinical history suggestive of food allergy. We speculate that consumption of pollen-related foods may have consequences for the allergic state of pollen-allergic individuals—in particular, in individuals without food allergy who do not avoid, but eat the respective foods. After consumption, crossreactive food allergens might activate pollen-specific T-cells during time periods when patients do not encounter pollen allergens, *i.e.*, outside

the pollen season. Such an intermittent perennial activation of pollen-specific T-cells might accomplish the persistence of peripheral memory Th2 cells, which has been observed in birch pollen-allergic individuals (23). Concomitantly in response to allergen-specific activation effector Th2 cells produce high levels of IL-4, which might be responsible for the typical maintenance of increased serum levels of pollen-specific IgE outside the pollen season. On the other hand, the continuous activation of pollen-specific T-cells by crossreactive allergens could also lead to the induction of peripheral tolerance. Therefore, the clinical relevance of perennial stimulation of pollen-specific T-cells by pollen-related foods still needs to be evaluated. For this purpose, and also in order to understand the immunological mechanisms underlying different manifestations of atopic diseases, the identification of T-cell epitopes in allergens is pivotal.

Conclusion

In vitro characterization of food allergens concerning allergenicity, antigenicity, epitope composition, and so forth requires the use of allergen-specific TCCs, which can be isolated from peripheral blood or from tissues infiltrated by lymphocytes during an inflammatory process (*e.g.*, the skin). The majority of these cultures are Th2s and reflect T-cells constituting a central element in the allergic disease. First, food-allergen-specific Th2 cells produce cytokines that lead to the synthesis of IgE, thus causing immediate clinical reactions, such as OAS. Second, food-allergen-specific T-lymphocytes are involved in the induction and maintenance of atopic dermatitis. Therefore, allergen-specific T-cells should be the targets for therapeutic intervention in atopic diseases, and the identification of the regions relevant for T-cell recognition within allergens is important.

Specific immunotherapy of Type I allergy is the only causative therapy that could be associated with the induction of tolerance in allergen-specific T cells (18). Conventional specific

immunotherapy consists of subcutaneous injections of increasing doses of allergen extracts. However, because of the high rate of IgE-mediated adverse reactions after injection of peanut extract, standard clinical use of this therapy for peanut allergy was not recommended (71,72). Conversely to IgE antibodies, which recognize mainly conformational epitopes dependent on the molecular tertiary structure, T-cell epitopes are short linear peptides. Therefore, different approaches based on the alteration of the allergenic tertiary structure of allergens and the preservation of T-cell-activating regions were developed to reduce IgE-mediated side effects. One approach is the use of peptides corresponding to immunodominant T-cell epitopes of the allergen (73). These peptides are too short to crosslink mast-cell-bound IgE, and, therefore, higher molar doses of T-cell-activating peptides can be delivered. Thus far, peptide immunotherapy has been successfully applied for insect venom and cat allergy. Most allergens contain several distinct relevant T-cell epitopes. A way to retain all distinct T-cell epitopes but to reduce IgE-binding is genetic engineering of "hypoallergenic variants" (74).

Selective point mutations of single amino acid residues are introduced into the complementary DNA encoding the allergen, which destroy the formation of IgE epitopes but do not alter the different T-cell-activating regions. This approach was successfully applied for the peanut allergen Ara h 3. Only four point mutations changed Ara h 3 into a variant with reduced binding capacity for IgE antibodies from patients who were hypersensitive to peanuts and retained potency to stimulate T-cell proliferation and activation (75).

Pollen-related food allergy results from crossreactivity between respiratory and food allergens at the B- and T-cell levels. Therefore, one might assume that the downregulation of specific Th2 cells during successful pollen-SIT also cures pollen-related food allergy. However, this issue remains controversial, and curative effects, as well as no impact of immu-

notherapy with birch pollen extract, were reported regarding associated food allergy (76–78). We recently investigated the T-cell response to the Bet v 1-homolog in hazelnuts, Cor a 1.04, and identified a dominant T-cell epitope that is not crossreactive with pollen allergens (79). Therefore, T-cells specific for food epitopes but not reactive to pollen allergens exist and may escape tolerance induction during pollen SIT; this could explain the ineffectiveness of this therapy on concomitant food allergy.

In summary, the knowledge regarding basic cellular mechanisms underlying food allergy remains limited and cannot explain the great variability in the pathophysiology of this disease. The characterization of the allergic T-cell response to relevant food allergens, including the identification of T-cell epitopes, represents a pivotal contribution to the understanding of this disease and is a prerequisite for the development of concepts for the prevention and specific treatment of food allergy.

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