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Review

T lymphocytes and food allergy

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Food allergy is a hypersensitivity reaction to normally harmless substances and involves humoral immune responses, mediated by immunoglobulin (IgE) synthesized by B lymphocytes, and cellular immune responses mediated by T lymphocytes. An IgE-mediated mechanism leads to clinical symptoms occurring immediately after food ingestion, *e.g.*, "the oral allergy syndrome". For delayed reactions involving the gastrointestinal tract or the skin, the underlying immune mechanisms are less clear. In order to elucidate the cellular response to food allergens, human allergen-specific T cell cultures generated *in vitro* represent helpful tools. The majority of food allergen-specific $CD4^+$ T lymphocytes isolated from food-allergic individuals was found to synthesize high levels of IL-4 and IL-13, two cytokines required for initiation of IgE synthesis. Due to selective homing profiles, food-specific T cells seem also to be involved in defining the target organ of the allergic inflammation. Recent data provide evidence that in addition to IgE-mediated inflammation, food allergen-specific T lymphocytes may also cause inflammatory responses independently of IgE-mediated mechanisms.

Keywords: Cytokines / Food allergy / T lymphocytes / Th1/Th2

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1 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, *i.e.*, an acute onset of symptoms after ingestion or inhalation of food substances. During sensitization, specific IgE antibodies directed against food allergens are produced by the immune system which bind to high affinity receptors on the surface of mast cells and basophils. Upon re-exposure to the food constitutents, cross-linking of the IgE antibodies triggers the release of preformed mediators, *e.g.*, histamine, tryptase, and tumor necrosis factor, causing the acute phase of the allergic immediate reaction. Newly synthesized mediators, such as leukotrienes, prostaglandins, and cytokines, lead to the recruitment of inflammatory cells causing IgE-mediated late-phase responses. Allergenic food proteins may also activate special subsets of T lymphocytes which cause IgE-independent inflammatory ("delayed-type-hypersensitivity") reactions like atopic eczema [2].

Clinically, IgE-mediated immediate hypersensitivity reactions occur within minutes up to 2 h after ingestion of the food. Localized clinical symptoms are confined to the site of contact with the food, *e.g.*, the "oral allergy syndrome" (OAS). This term comprises the rapid onset of pruritus, tingling, and angioedema of the lips, tongue, palate, and throat, occasionally also a sensation of tightness in the ears or in the throat, or both. Contact urticaria occurs on hands after handling food stuff and localized gastrointestinal symptoms after food ingestion are characterized by nausea, cramping, pain, vomiting, flatulence, and diarrhea. Systemic immediate symptoms develop in different organs after the consumption of food, *e.g.*, in the skin (urticaria, angio-oedema), the respiratory tract (rhinitis, asthma), or as

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Abbreviations: APC, antigen-presenting cell; **Bet v 1**, major allergen in birch pollen; **CLA**, cutaneous lymphocyte antigen; **IFN**, interferon; **IgE**, immunoglobulin E; **IL**, interleukin; **SIT**, specific immunotherapy; **TCC**, T cell clone; **TCL**, T cell line; **Th**, T helper

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anaphylaxis, the maximum variant of an immediate-type allergic reaction with cardiovascular and gastrointestinal symptoms sometimes leading to shock. Delayed symptoms can be observed in the gastrointestinal tract or in the skin, *e.g.*, a deterioration of atopic dermatitis [1, 3].

On the basis of the clinical appearance, the nature of the disease-eliciting allergens and the underlying immunological mechanisms, two forms of IgE-mediated food allergy can be distinguished. "True" food allergy mainly affects young children and can be the first manifestation of the "atopic syndrome", a term summarizing the clinical manifestations connected to the atopic constitution: atopic dermatitis, allergic rhinitis, and asthma bronchiale. The most important allergens involved in this form of food allergy are cow's milk, hen's egg, and legumes (peanuts and soybean), fish and wheat [4]. Often these allergens cause or influence atopic dermatitis, a chronically relapsing IgE-mediated skin disease. With some exceptions (peanuts, fish) this manifestation disappears later during childhood in the majority of the cases, but is often replaced by other manifestations, *e.g.*, allergic rhinitis, a clinical phenomenon known as the "atopic march" [5, 6].

Adolescent and adult individuals may develop the second form of food allergy as a consequence of a respiratory allergy to pollens of birch, mugwort or ragweed, which was termed "pollen-food allergy syndrome" [7]. Similarly, the "latex-fruit allergy syndrome" is due to the presence of an allergy to rubber latex (*Hevea brasiliensis*) [8]. Characteristically, these patients experience hypersensitivity reactions to food after having developed respiratory allergy because the respective food sources contain proteins structurally homologous to pollen allergens. Consequently, IgE antibodies specific for respiratory allergens cross-react with these food proteins.

2 T lymphocytes in Type I allergy

The development of an IgE-mediated response to a food allergen requires a series of molecular and cellular interactions involving antigen-presenting cells (APCs), T cells, and B cells. After uptake and processing of the allergen, APCs (*e.g.*, dendritic cells, monocytes) present small peptide fragments (T cell epitopes) in conjunction with major histocompatibility complex (MHC) class II molecules on their cell surface to T lymphocytes. $CD4^+$ T helper (Th) lymphocytes bearing the appropriate T cell receptor bind the peptide-MHC complex which leads to T cell proliferation and cytokine production. In turn, these T cells and their products activate B cells bearing the appropriate antigen-specific receptors and help them to generate allergen-specific antibodies. According to the synthesis of two key cytokines, interleukin (IL)-4 and interferon (IFN)-γ, two polarized forms of the heterogenous Th cell-mediated immune response were defined [9]. $CD4^+$ T cells which synthesize high amounts of IL-4, IL-13, and IL-5 but little or no IFN-γ were designated Th2. IL-4 and IL-13 are important factors that switch B lymphocytes to IgE synthesis [10, 11]. Consequently, Th2 cells play an important role for the induction and maintenance of Type I allergy. $CD4^+$ cells synthesizing high levels of IFN-γ a potent antagonist of IL-4, and tumor necrosis factor (TNF)-β which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses were designated Th1. This cell type is also involved in "delayed-type" hypersensitivity reactions. Th1 and Th2 cells can develop from the same precursor T cell (Th0) depending on the local cytokine milieu: The presence of IL-4 during T cell activation favors the differentiation of Th2 cells whereas cytokines as IL-12, IL-18, IFN-α, and IFN-γ support the differentiation of Th1 cells. Figure 1 illustrates the above-mentioned humoral and cellular responses in allergy.

However, the clear cut Th1/Th2 dichotomy as observed in mice is not unrestrictedly valid in humans and $CD4^+$ T cells producing a more heterogeneous cytokine profile were found. Nevertheless, the Th1/Th2 paradigm has provided indispensable help to describe basic immunological mechanisms underlying Type I allergy and successful allergen-specific immunotherapy [12]. Lately, a heterogenous family of $CD4^+$ regulatory T cells able to suppress effector immune responses was defined. $CD4^+$ T lymphocytes which produce transforming growth factor (TGF)-β are defined as Th3 and linked to mucosal/oral tolerance induction [13]. Type 1 regulatory T cells (so-called Tr1 cells) are defined by their ability to produce high levels of IL-10 and TGF-β, cytokines which mediate their suppressive effects [14, 15]. $CD4^+CD25^+$ regulatory T cells suppress immune responses *via* cell-cell interactions which probably involves CTLA-4 and TGF-β [16]. Based on accumulating data describing $CD4^+CD25^+$ cells, recently the determination of regulatory T cells into natural and adaptive subsets was proposed [17].

3 Characterization of allergen-specific T lymphocytes

With the increasing knowledge about the amino acid sequence of various allergens due to DNA technology the elucidation of the cellular reactivity against allergens became feasible. Because the frequency of allergen-specific T cells in the peripheral blood is estimated to be of the order of $1:10^5$, these cells need first to be enriched and expanded before proliferative and cytokine responses to specific stimulation with allergens can be determined. Figure 2 presents a schematic overview of a basic technical protocol how to establish allergen-specific oligo- and monoclonal T

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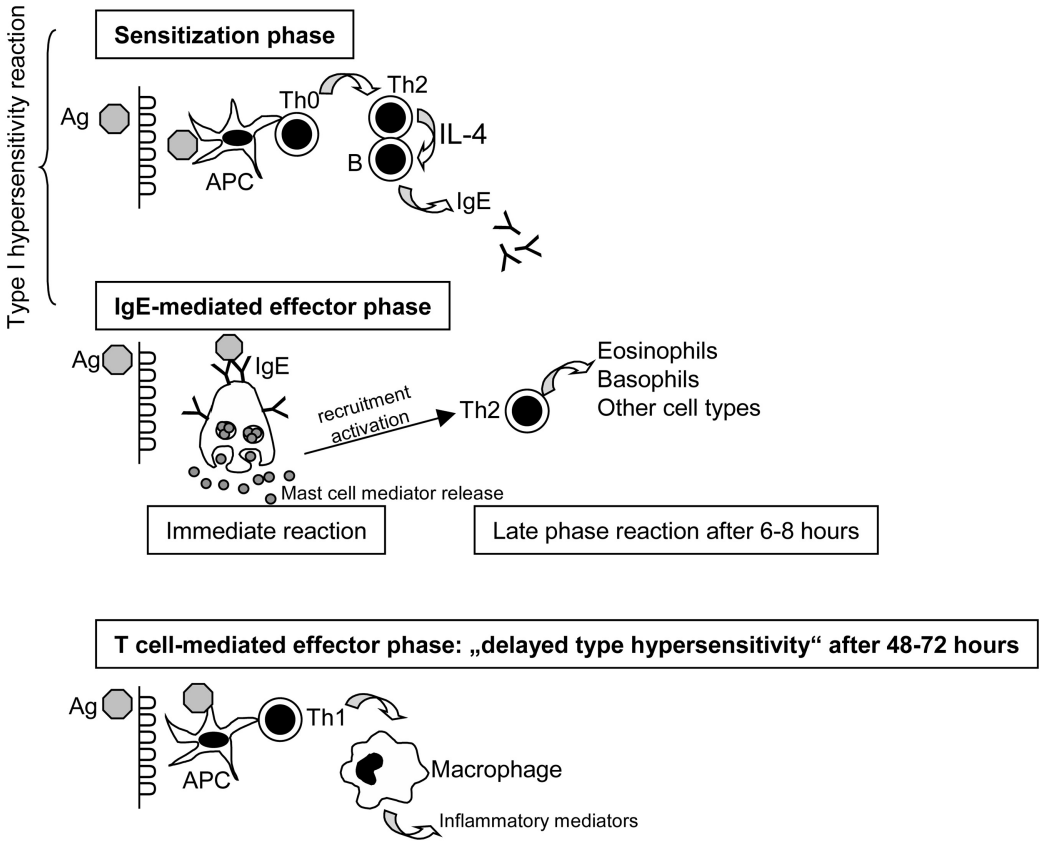

Figure 1. Humoral and cellular responses involved in allergy. In the presence of IL-4 naïve Th0 cells differentiate into Th2 cells which in turn switch B cells to the synthesis of specific IgE. IgE binds to the surface of effector cells. Upon allergen re-exposure cross-linking of the IgE antibodies triggers the immediate release of preformed mediators. Newly synthesized mediators recruite and activate inflammatory cells causing IgE-mediated late-phase reactions. The presence of IL-12, IL-18, IFN-α, and IFN-γ during priming of naïve Th0 cells supports the differentiation of Th1 cells. Upon second contact with the antigen, Th1 cells induce IgE-independent inflammatory "delayed-type-hypersensitivity" reactions. APC, antigen-presenting cell; Ag, antigen; Th, T helper lymphocyte.

cell cultures from the blood of allergic individuals. Peripheral blood mononuclear cells isolated from whole blood by density-gradient centrifugation are incubated with the respective allergen which stimulates and preferentially expands allergen-specific T cells. These activated cells can be further expanded by the addition of suboptimal amounts of growth factors, such as human IL-2. Monoclonal T cells (TCCs) are generated by limiting dilution techniques from oligoclonal T cell lines (TCLs) and after further expansion TCC can be tested for antigen specificity. Allergen-specific cultures represent useful tools to characterize their phenotype and function which comprises the analysis of the cytokine pattern produced after allergen-specific stimulation, the expression of surface markers, the T cell receptor usage and the restriction by particular MHC class II molecules. Furthermore, these cultures can be used to define T cell epitopes on allergens. For this purpose, TCLs and TCCs are stimulated with overlapping synthetic short peptides representing the complete amino acid sequence of an allergen.

This tissue culture technique has been sucessfully applied to characterize the T cell response to various respiratory allergens, *e.g.*, allergens from tree and grass pollen, house dust mites, *Hevea brasiliensis* (latex), or cat dander and allergens from insect venoms [18–26]. These studies revealed that the majority of allergen-specific TCCs were $CD4^+$ and produced Th2-like cytokines in response to specific stimulation. Most allergens characterized so far contained multiple T cell epitopes dispersed throughout the entire allergen molecule. A high intra- and interindividual diversity of recognized T cell epitopes was observed in allergic patients. Furthermore, it was shown that nonallergic and allergic individuals recognize the same repertoire of T cell epitopes but the activation of allergen-specific TCC derived from allergic individuals led to a higher ratio of IL-4 *versus* IFN-γ production [18, 27]. Studying the T cell receptor usage indicated polyclonal T cell responses in single individuals and confirmed the high diversity of the allergen-specific T cell response [28].

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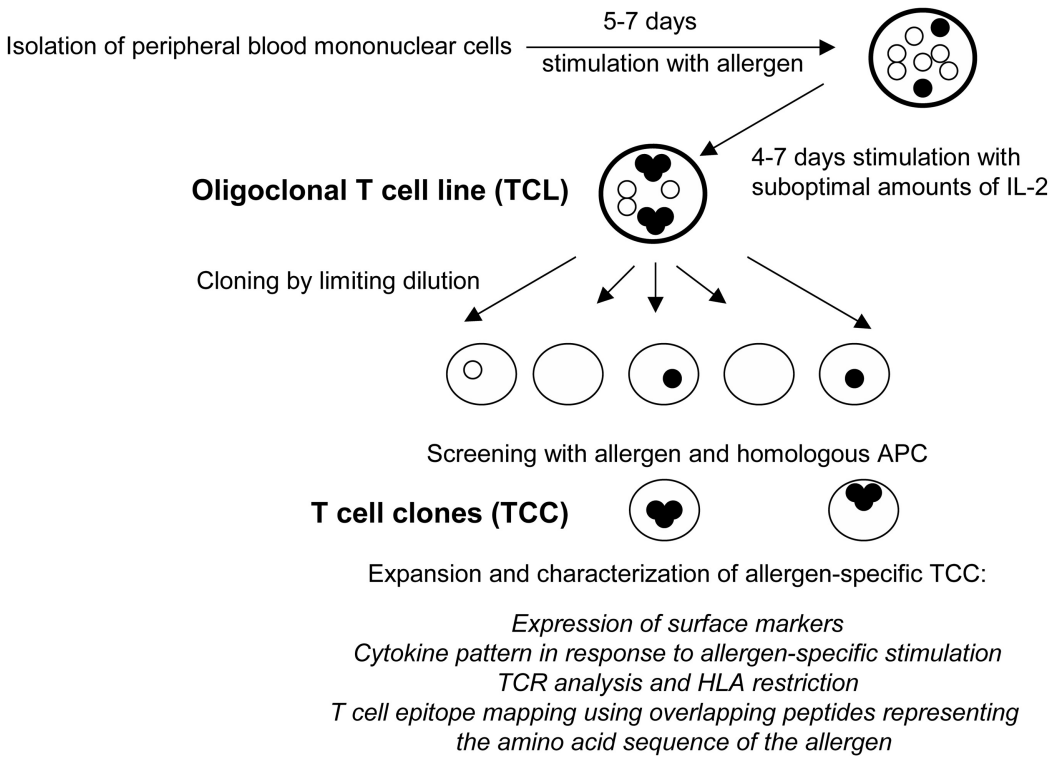

Figure 2. Protocol to generate human allergen-specific TCL and TCC. For explanation see text.

4 T cells in "true" food allergy

Foods, such as milk, eggs, peanut, soy, and wheat, account for approximately 90% of hypersensitivity reactions in children [4]. A common feature of most of these allergens is a high resistance to heat and gastric digestion [29, 30]. Therefore, primary sensitization to these food proteins seems to occur in the gut [31]. Naïve T lymphocytes encounter the antigen in Peyer's Patches where the type of APCs and the local cytokine milieu seems to determine whether T lymphocytes become tolerant or primed [32]. After priming, food-specific T lymphocytes can enter the blood stream besides resting and local trafficking in gut-associated tissues. It was demonstrated that peripheral blood lymphocytes derived from food-allergic patients displayed a higher proliferative capacity in response to food allergens as compared to nonallergic individuals [33–36]. It is assumed that these food allergen-specific T lymphocytes guided by so-called homing receptors on their cell surface can recirculate to gut-associated sites. For example, the expression of α4β7 integrin, a molecule directing T cells to intestinal tissues, was shown to be significantly increased on peripheral T lymphocytes specific for milk allergens [37]. Furthermore, food-specific T cells can migrate to the skin, which besides the gastrointestinal tract represents the most frequent target organ in food-allergic children [38]. Urticaria is one of the most common symptoms of milk allergy in young children [39]. Moreover, milk has been found to be a well-defined trigger for eczema in patients suffering from atopic dermatitis. Both, milk-induced urticaria as well as atopic eczema could be associated with circulating antigen-specific T lymphocytes which express the cutaneous lymphocyte antigen (CLA), a skin-specific homing receptor, on their surface [2, 40].

Several studies have addressed the characterization of the cellular response to allergens from hen's egg, cow's milk, and peanuts [34, 41–46]. As assumed, the majority of TCCs specific for oral food allergens isolated from the peripheral blood of allergic patients belonged to the Th2 subset. A predominance of food-antigen specific Th2 cells was also observed in the gut [47]. A recent study by Lin *et al.* [48] demonstrated that the duodenum of patients symptomatic after food challenges contained increased numbers of IL-4$^+$ T cells being associated with IgE$^+$ cells, even though these individuals had no food allergen-specific serum IgE. These findings indicate that specific Th2 cells located at intestinal sites produce IL-4 after activation by food allergens which could then induce local IgE synthesis and consequently, IgE-mediated inflammatory responses. Local IgE synthesis in response to respiratory allergens was demonstrated in the nasal and bronchial mucosa [49, 50].

It still remains a matter of discussion whether food-specific T lymphocytes could also be directly involved in local inflammatory processes. It was shown that intradermal

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injection of T cell activating peptides of the major cat allergen, Fel d 1, that did not cross-link Fel d 1-specific IgE, led to systemic late-phase reactions in cat-allergic patients [51]. This observation shows that short allergen-derived peptides lacking IgE epitopes can induce strong T cell-mediated inflammatory responses. Enzymatic digestion of food allergens in the gastrointestinal tract can create peptides which lack IgE epitopes but still contain T cell epitopes [52]. Therefore, it is possible that digested fragments of food allergens could induce systemic reactions similar to that described for the cat allergen-derived peptides. On the other hand, a role for food-specific Th1 lymphocytes in inflammatory processes was proposed. Patients suffering from atopic dermatitis reacted with a marked deterioration of their eczematous skin lesions upon food challenges [53]. The majority of food-specific TCCs isolated from the peripheral blood of these individuals belonged to the Th1 phenotype. The initial phase of this skin disease is predominated by Th2 cells which later switches into a second phase predominated by Th1 cells causing chronic eczematous skin lesions not essentially involving IgE [54]. Hence, food allergens or digestion-derived peptides of food allergens could activate specific Th1 cells which then maintain the inflammatory response in the skin independently of IgE.

5 T cells in pollen-associated food allergy

Adolescent and adult individuals often develop food allergy as a consequence of an allergic sensitization to respiratory allergens. Immunologically, this kind of food allergy is based on IgE antibodies specific for inhalant allergens which bind to homologous proteins in food sources. Therefore, it is generally assumed that primary sensitization occurs *via* respiratory sites against the inhalant allergens and immune reactivity against the respective food allergens occurs exclusively due to cross-reactivity. Nevertheless, up to now the knowledge about the T cell reactivity against these food allergens is still very limited.

The "birch-fruit-syndrome" affects up to 70% of birch pollen-allergic individuals. After the ingestion of fresh stonefruits, vegetables or hazelnuts, the patients develop oral allergy syndromes in most cases [55]. More severe reactions were described after the consumption of soybean [56]. Allergy to birch pollen is one of the main causes of allergic rhinitis from spring to early summer in central Europe and over 95% of the patients are sensitized to the major birch pollen allergen, Bet v 1 [57]. Several proteins sharing a high amino acid sequence identity with Bet v 1 were identified as major allergens in different foods, *e.g.*, Mal d 1 in apples, Pyr c 1 in pears, Pru av 1 in cherries, Cor a 1.04 in hazelnuts, Api g 1 in celeriac, Dau c 1 in carrots, and Gly m 4 in soybean [56, 58–63]. Although birch pollen contains additional allergens with homologues in food, *e.g.*, profilins or isoflavone reductase-like proteins [64, 65], cross-reactive Bet v 1-specific IgE antibodies most frequently give rise to clinical symptoms after consumption of birch pollen-related food [66].

In our studies, we focused on the characterization of the cellular cross-reactivity between Bet v 1 and its homologous proteins in food. We established TCLs and TCCs specific for the major allergens of apple and celery, Mal d 1 and Api g 1, from the peripheral blood of birch pollen-allergic individuals with concomitant food allergy [67, 68]. These T cell cultures responded by far better to stimulation with Bet v 1 than with both food allergens even though the blood samples were taken outside the tree pollen season. Thus, *in vitro* stimulation with pollen-related food allergens resulted in T cell cultures containing pollen-specific, cross-reacting T lymphocytes. This observation strongly supports the hypothesis that the corresponding inhalant allergen, Bet v 1, initializes the sensitization to the major allergens in apple and celeriac. On the other hand, both food allergens represent stimuli for Bet v 1-specific T cells. Their activating capacity included several distinct T cell epitopes of Bet v 1 even though Mal d 1 and Api g 1 show only 56% and 41% of amino acid sequence identity with the major birch pollen allergen [67, 68]. Reekers *et al.* [69] showed that the oral challenge with birch pollen-related food caused a marked deterioration of the eczema in birch pollen-allergic individuals suffering from atopic dermatitis. This clinical reaction was associated with the increased expression of the skin-homing molecule CLA on peripheral blood T lymphocytes and the presence of Bet v 1-reactive T lymphocytes in the eczematous skin. Hence, *in vivo* pollen-related food allergens are apparently capable of activating pollen-specific T cells which home to the skin and exacerbate skin lesions.

In allergic individuals, allergen-specific T cells were shown be long-lived and to exist for several years [70, 71]. In order to survive, specific memory T cells seem to require repeated contact with antigen [72, 73]. The ingestion of pollen-related food proteins capable of activating pollen-specific T lymphocytes may represent one way to stimulate these cells, in particular outside the tree pollen season. Normally, individuals who develop allergic symptoms against foods will avoid to eat these aliment. However, approximately one-third of pollen-allergic individuals does not experience a "birch-fruit-syndrome". These patients usually do not exclude birch pollen-related foods from their diet and consequently consume a repertoire of antigens able to trigger pollen-specific T cells. In our studies the majority of the food allergen-reactive Bet v 1-specific TCC were Th2-like and synthesized high levels of IL-4 in response to the food allergens [67, 68]. Therefore, the perennial uptake of pollen-related food could stimulate ongoing IL-4 synthesis and contribute to the typical maintenance of high levels of pollen-specific IgE outside the tree pollen season.

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6 T cells and specific immunotherapy

Conventional allergen-specific immunotherapy (SIT) is the only therapeutic intervention to reduce symptoms in the long-term. SIT was introduced in 1911 and consists of subcutaneous injection of incremental doses of native allergen-extract into sensitized subjects according to standardized protocols in order to achieve a state of clinical tolerance to subsequent natural allergen exposure [74]. Successful SIT could be associated with the counter-balance of the overwhelming allergic Th2 immune response due to (i) the upregulation of a Th1 immune response (immune deviation), (ii) the induction of a state of unresponsiveness (anergy) in peripheral T lymphocytes characterized by suppressed proliferative and cytokine responses against allergens, and (iii) the induction of regulatory T cells [75–78]. The modified T cell response subsequently affects the humoral allergen-specific immune response, *i.e.*, the reduction of IgE and the induction of IgG4 antibodies with the capacity to block IgE-binding to the allergen [77, 79].

Unfortunately, the administration of the allergen to the patient bears the risk to induce undesired IgE-mediated effects. This applies in particular to allergens eliciting strong IgE-mediated symptoms, *e.g.*, to allergens contained in peanuts. Indeed, the rate of systemic reactions was particularly high during SIT with peanut extract [80, 81]. Hence, an extensive search to increase the safety and efficacy of specific treatment regimens is going on [82]. One approach to reduce the risk of vaccine-induced anaphylactic adverse effects but still to achieve the desired modification of the allergic at the T cell level, *i.e.*, the downregulation of the allergic Th2-dominated immune response, is the creation of "hypoallergenic" variants of allergens by genetic engineering [83]. These recombinant proteins contain T cell epitopes and therefore a retained T cell activating capacity, but less or no IgE-binding epitopes which reduces their capacity to cross-link IgE and consequently anaphylactic symptoms. Therefore, in order to develop such hypoallergens, the identification of relevant T cell epitopes on the respective allergens is essential. This strategy was recently successfully applied to modify the major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, and the engineered hypoallergenic variants displayed a reduced binding capacity for serum IgE from peanut-hypersensitive patients but stimulated T cell proliferation and activation [84, 85].

SIT has been proven to be successful and effective for the treatment of pollen-allergy [86]. As pollen-associated food allergy is believed to be exclusively due to cross-reactivity, one would assume that successful desensitization of pollen-allergic patients could concomitantly induce tolerance against the respective foods. Indeed, a reduction of clinical symptoms to apples due to successful birch pollen-immunotherapy was reported and this clinical tolerance lasted for a longer time period [87, 88]. However, in another study some individuals developed a "birch-fruit-syndrome" after the beginning of pollen-specific immunotherapy [89].

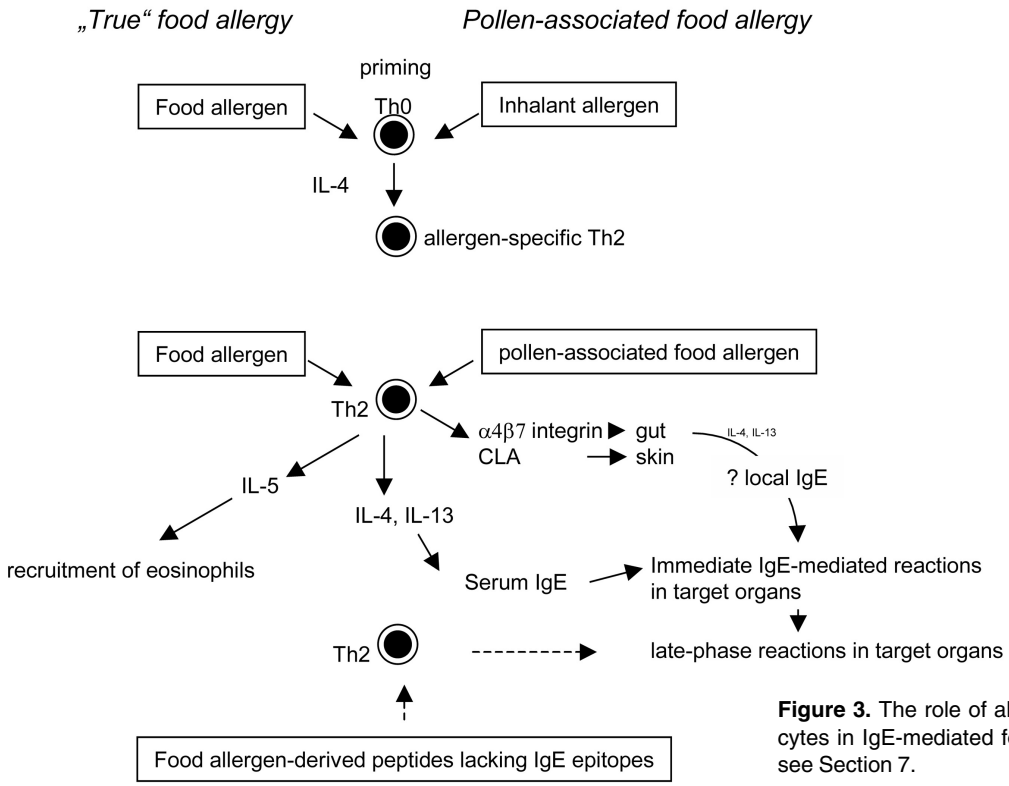

Figure 3. The role of allergen-specific Th2 lymphocytes in IgE-mediated food allergy. For explanation see Section 7.

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Thus, it remains an open question whether SIT against pollen allergy is really effective and sufficient to treat concomitant pollen-associated food allergy or whether additional and/or different treatment strategies are necessary to induce clinical tolerance to the food.

7 Conclusions

Allergen-reactive Th2 cells play a central role in the pathophysiology of food allergy (Fig. 3). In "true" food allergy which develops very early in life, naïve T lymphocytes are primed by food allergens. Repeated contact with food allergens leads to differentiation of Th2 cells into Th2 effector cells which after activation secrete cytokines and express particular homing factors. IL-4 and IL-13 induce systemic and presumably local IgE synthesis which then causes IgE-mediated inflammatory responses in target organs. In addition, IL-5 recruits eosinophils to the inflammatory site. Furthermore, food-specific T lymphocytes may also take an active part in inflammatory responses independently of IgE-mediated mechanisms. In adult patients, food allergy frequently develops as a consequence of a respiratory Type I allergy. In this case, T cells are initially primed for inhalant allergens but can be activated by proteins in food due to their structural homology to respiratory allergens. In particular, pollen-associated food allergy found special interest in the past decade probably because of the steadily increasing prevalence of respiratory allergy during this time period. It is presently not clear whether this form of food allergy represents solely an IgE-mediated symptomatic which hampers the perennial quality of life of patients with seasonal pollen allergy, or whether the uptake of pollen-associated food allergens has relevant consequences on disease-eliciting mechanisms involved in the pathophysiology of respiratory allergy.

The limited knowledge about basic cellular mechanisms underlying food allergy cannot explain the great variability in the pathophysiology of this disease. The *in vitro* characterization of human T cell cultures specific for relevant food allergens will help develop concepts for the prevention and specific treatment of food allergy and allow improvement in the clinical management of food-allergic patients.

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