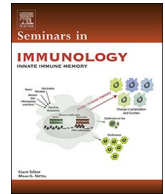




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Review

Recombinant allergen and peptide-based approaches for allergy prevention by oral tolerance

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ABSTRACT

Several studies conducted in animal models for immunologically-mediated hypersensitivity diseases have shown that oral administration of antigens early in life can prevent the development of specific humoral and cellular immune responses and thus hypersensitivity reactions to the respective antigens. Such data were also obtained in models for Immunoglobulin E (IgE)-associated allergy, the most common hypersensitivity disease affecting more than 25% of the population. Based on data obtained in animal models for allergy several clinical intervention studies have been conducted in children to study if oral administration of materials containing allergens or allergen-derived peptides early in life can prevent the subsequent development of allergy. In this article we argue that oral tolerance induction could be a potent way to prevent allergy and may be even improved regarding efficacy provided that well-defined allergen molecules and/or allergen-derivatives were used in optimized dose regimens and periods of intervention. The knowledge regarding the molecular and immunological characteristics of allergens which has been achieved in the last decades is a prerequisite for such a treatment. In fact, defined recombinant allergens/allergen derivatives and allergen-derived synthetic peptides from the most common allergen sources are now available for targeted intervention. Moreover, molecular allergy diagnosis allows deciphering the disease-causing relevant allergens for different regions in the world allowing composing cocktails of tolerogens according to the needs of populations from different parts of the world. Furthermore, it is suggested to use defined allergen molecules and epitopes in the analysis of clinical tolerance studies. This will allow understanding if clinical unresponsiveness is due to true immunological tolerance or to other mechanisms such as induction of blocking antibodies or cellular immunomodulation. Using molecularly defined tolerogens it can now be explored if oral tolerance induction is a powerful strategy to prevent IgE-associated allergy.

1. Introduction

IgE-associated allergies affect more than 25% of the population worldwide [1]. Allergic symptoms include a plethora of symptoms such as allergic rhinoconjunctivitis, bronchial asthma, skin allergy, food allergy and life-threatening anaphylaxis. The hallmark of allergy is the production of IgE antibodies against harmless antigen (i.e., allergens) (Textbox 1) which can bind to inflammatory cells such as mast cells, basophils, eosinophils and antigen presenting cells via specific Fcε receptors [2]. A subsequent contact with allergen then leads to the activation of these cells and the consecutive release of inflammatory mediators, cytokines and/or presentation of allergen to specific T cells

which causes immediate and late phase allergic inflammation. Allergen-specific IgE responses as well as T cell responses are therefore crucial for the development of allergic symptoms in sensitized patients. Soon after the discovery of IgE antibodies [3,4] serological tests, especially the radioallergosorbent test RAST, [5] were established which allowed the detection of allergen-specific IgE antibodies in allergic patients. Thus it became possible to identify allergenic molecules in the various allergen sources by their specific reactivity with IgE antibodies from patients and, after purification, to determine their allergenic activity in cellular assays and by *in vivo* provocation testing [6]. A major breakthrough regarding the characterization of the disease-eliciting allergen molecules was the introduction of molecular cloning technologies for

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Textbox 1

: Definitions

Allergen: An antigen which induces IgE-associated allergy in susceptible individuals.

Oral tolerance (OT): Inhibition of specific immune responses by oral pre-administration of an antigen. OT thus induces immunological tolerance.

Oral allergen-specific immunotherapy (OIT): Administration of allergen via the oral route to allergic patients to reduce clinical symptoms upon subsequent allergen encounter. OIT thus induces clinical tolerance.

Sublingual immunotherapy (SLIT): Administration of allergen by the sublingual route to allergic patients to reduce clinical symptoms upon subsequent allergen encounter. SLIT aims to induce clinical tolerance.

Clinical tolerance: Reduction or lack of clinical reactions towards an antigen.

Immunological tolerance: Lack of adaptive immune response towards a particular antigen used for tolerance induction while maintaining reactivity to others.

Hypoallergen: Modified allergen-derivative, often produced by recombinant expression, with reduced allergenic activity that can induce protective antibody responses in allergic patients upon immunotherapy with reduced risk of IgE-mediated side-effects.

Carrier-bound B cell epitopes: Non-allergenic peptides derived from the IgE binding sites of allergens which are linked to an unrelated carrier protein for immunotherapy of allergic patients with reduced risk of inducing IgE- and T cell-mediated side effects.

T cell epitope-containing peptides: Allergen-derived peptides, often made by chemical synthesis, that contain T cell epitopes of the allergen but have no IgE-binding capacity.

the isolation and characterization of allergen-encoding cDNAs which allowed to produce the most relevant allergen molecules as recombinant proteins. Soon after the isolation of the first allergen-encoding cDNAs, recombinant allergens became available to characterize the allergen-specific immune response in allergic patients in great detail and to develop new forms of allergen-specific diagnostics and immunotherapies based on defined allergen molecules or derivatives thereof [6]. In contrast to many other hypersensitivity diseases (e.g., autoimmune diseases, transplant rejection) where the disease-triggering antigens and the operative pathomechanisms are not fully known, IgE-associated allergy is a paradigmatic disease for exploring therapeutic and preventive antigen-specific strategies because most identified allergens are well characterized.

1.1. Why IgE-associated allergy could be prevented by oral tolerance

It has been shown in classic experiments that systemic exposure to antigens early in life when the immune system develops can induce a state of immunological tolerance which is characterized by the inability to form any adaptive effector (i.e., specific humoral or cellular) immune response [7]. Many of these classic experiments have been conducted in the field of transplantation immunology showing that transfer of cells from a foreign donor to a recipient soon after birth can induce tolerance against transplants from the donor [8,9]. In a series of elegant experiments carried out in experimental animal models it has been shown that hematopoietic stem cells can be transduced to express transplant antigens, autoantigens and allergens to induce long-lasting tolerance in the host [10–13]. Likewise it has been shown that feeding of antigens early in life can induce a similar form of immunological tolerance characterized by a loss or strong reduction of humoral and cellular immune responses against the fed antigen [14,7]. Oral tolerance induction (Textbox 1) has therefore been considered as possible intervention for immunological hypersensitivity diseases such as autoimmune disease and allergy but it turned out that the treatment cannot fully abolish an immune response which has already started. Therefore oral tolerance has limitations when it comes to its use for therapy of an already established hypersensitivity disease [15]. However, oral tolerance induction should be considered as a preventive intervention early in life before and during the development of the adaptive immune system. It requires a precise knowledge of the disease-causing antigens and the possibility to produce these antigens in large quantities and in a form which induces tolerance instead of sensitization. These prerequisites are fulfilled in the case of IgE-associated allergy because the molecular structures of most of the disease-causing allergens are known.

1.2. Animal studies to induce oral tolerance for prevention and treatment of IgE associated allergy: what can we learn from them?

Already in 1911 Wells & Osborne published a study showing that feeding of guinea pigs with cornmeal before sensitization prevented them from death upon subsequent challenge with zein protein [16]. A similar finding was reported for delayed type hypersensitivity in 1946 [17]. Guinea pigs which were fed with dinitrochlorobenzene did not develop reactions to the hapten when it was painted on their skin. Table 1 [14,18–47] and [48] provide an overview of studies in which pre-feeding of animals with antigens, allergen extracts, allergens or allergen-derived T cell epitope-containing peptides prevented or reduced subsequent production of IgE, IgG and/or T cell responses induced by sensitization. A comprehensive general overview of the history of oral tolerance induction is given by Rezende & Weiner in this issue [49]. Among the studies in Table 1, the study published by David in 1977 is quite informative because it has investigated several parameters of oral tolerance induction for prevention of allergic sensitization [19]. In this study rats were fed ragweed pollen extract or control antigens before sensitization and the effects of this treatment on subsequent allergic sensitization was measured by assessment of IgE antibody formation and the development of anaphylactic reactions. Specifically the influence of the age when the feeding was started, the duration of feeding and the effects of terminating feeding were investigated. The study demonstrated that early and frequent feeding is important to achieve immunological tolerance. Furthermore, animals could be sensitized when feeding was stopped long enough. Another important finding was that after feeding, antigen was present systemically in the animals indicating that similar mechanism of tolerance may be operative in oral tolerance induction as compared to tolerance induced by exposing antigen via stem cells or by systemic administration [50,51]. The studies summarized in Table 1 also show that oral tolerance induction for prevention of allergic sensitization is possible in different animals (e.g., rats, mice, dogs) and for different antigens (e.g., experimental antigens such as ovalbumin, albumin, food allergens, respiratory allergens). Most of the early studies were conducted with experimental antigens without clinical relevance for patients but with the characterization of allergens which are clinically relevant for allergic patients, also defined pollen allergen molecules (e.g., birch pollen derived Bet v 1, cypress pollen-derived Cry j 1, Cry j 2) [41–44] as well as house dust mite allergens [40] were studied. Another aspect to consider is how allergic sensitization is induced in experimental animal models. Since a robust induction of allergic sensitization can be best achieved by immunization with Alum-adsorbed allergens, there are

Table 1
Experimental oral immunotherapy studied in animal models for IgE-mediated allergies.

Allergen/ Allergen derivative	Animal species	Intervention	Outcome	References
Bovine serum albumin	rat	I.g. feeding of 25 mg BSA for 14 days, i.m. immunization with 1 mg BSA in CFA 1 week later	Reduction of IgE production	[18]
Ragweed pollen extract	rat	I.g. feeding of extract (0.45 mg protein) 5 days a week for 1–20 weeks, i.p. immunization with ragweed extract + <i>B. pertussis</i> 3 days later	Abolishment of IgE-antibody formation and systemic anaphylaxis; dependent on age, duration of feeding and time point of terminating feeding	[19]
Ovalbumin	mouse	I.g. feeding of 20 mg ovalbumin, immunization with 1 µg ovalbumin in alum 7 days later	No detectable IgE and IgG1 production	[14]
Ovalbumin	mouse	Ovalbumin in drinking water for two weeks, 7 days later immunization with 10 µg ovalbumin in alum	No detectable IgE and IgG1 response; tolerance transferable by Peyer's patch cells	[20]
Ovalbumin	mouse	I.g. feeding of 20 mg ovalbumin, immunization with 500 and 100 µg ovalbumin in CFA on days 7 and 21	No detectable IgG1 producing cells in spleen; no proliferation of LN cells; tolerance transferable by spleen cells; reduced response to DNP after DNP-OVA immunization, reversible after depletion of Thy 1.2+ cells; Suppression of proliferative response, transferable by LN cells;	[21]
Ovalbumin	mouse	I.g. feeding of 20 mg ovalbumin, after 7 days s.c. immunization with 100 µg ovalbumin in CFA	LN cell proliferation inhibited, no IL-2 production, reversible by previous CY application	[22]
Ovalbumin	mouse	3 mg ovalbumin on 3 consecutive days, 7 days later s.c. immunization with 100 µg ovalbumin in CFA	Abolishment of IgE and IgG production, suppression of DTH reactions	[23]
Ovalbumin/CTB conjugate	mouse	I.g. feeding of 5–150 µg ovalbumin/CTB on days –14, –11, –8, i.p. immunization with 10 µg ovalbumin in alum on days 0 and 14	Prevention of IgE and IgG production, conjunctivitis, and asthma-like responses; increased TGF-β and IL-10 secretion	[24]
Ovalbumin	dog	Daily feeding with 10 mg ovalbumin dissolved in cow's milk for 4 weeks (weeks 9–13), s.c. immunization with 10 µg ovalbumin in alum at weeks 14, 15, 17, and 19	Decrease in IgE, IgG1, IgA production, AHR, Th2 cytokine production in offspring; dependent on TGF-β	[25]
Ovalbumin	mouse	Administration of ovalbumin to lactating mothers i.n., i.g. or as aerosol, i.p. immunization of offspring (6–8 weeks) with 10 µg ovalbumin in alum, followed by aerosol challenge from day 17–21	IgE, IgG1 and IgG2a response reduced, dependent on the age at the time of intervention, reduction of BALF cells and AHR	[26]
Ovalbumin	mouse	I.g. feeding with 1 mg ovalbumin on 5 consecutive days; i.p. sensitization with 100 µg ovalbumin in alum 3 and 11 days later; i.t. challenge	Reduced IgE and IgG1 production, DTH reaction, proliferation of spleen cells, and Th2 cytokine production; increased number of CD4+ cells in LN	[27]
Ovalbumin, hydrolyzed egg protein, whole egg protein	mouse	Ovalbumin, HE, or WE in drinking water for 5 consecutive days, s.c. immunization with 100 µg ovalbumin in alum on days 10 and 23	Dose-dependent reduction of DTH and LN cell proliferation; reduced IgE and IgG response	[28]
Peanut extract, ovalbumin	mouse	I.g. application of 0.02–100 mg extract or ovalbumin, 7 days later immunization with 100 µg antigen in CFA, after 21 days with 100 µg antigen in PBS into footpad	Suppression of IgE responses dependent on the degree of hydrolyzation, suppression of mast cell degranulation and proliferation of LN cells	[29]
Milk formulas of different degree of hydrolyzation	mouse	Milk formulas in drinking water from day 1–19; s.c. immunization on day 5 with 0.1 mg βLG and ovalbumin in alum	Dose dependent reduction of IgE production, reduced DTH and proliferation of spleen cells, decrease of IL-2 and IFN-γ, increase of IL-10 and TGF-β secretion	[30]
β-lactoglobulin, whey proteins	mouse	I.g. feeding of whey proteins (3 mg/g body weight) or βLG (5 mg/g body weight), i.p. immunization with 0.08 mg βLG and ovalbumin in alum on day 5	Dose dependent reduction of IgE production, reduced DTH and proliferation of spleen cells	[31]
β-lactoglobulin-derived peptides by tryptic hydrolysis	mouse	I.g. feeding of native βLG or βLG-peptides or βLG-peptide fractions (5 mg/g body weight), i.p. immunization with 0.08 mg βLG and ovalbumin in alum on day 5	Abolishment of IgE production; decrease of DTH and proliferative response	[32]
β-lactoglobulin	mouse	I.g. feeding of βLG in biodegradable microspheres (3.8 mg βLG/100 mg polymer), i.p. immunization with 0.08 mg βLG in alum on day 5	Suppression of IgG production and proliferation of LN cells	[33]
β-lactoglobulin, raw milk	mouse	0.3 or 3% βLG solution or milk instead of drinking water for 8 weeks, i.p. immunization with 40 µg βLG in CFA/IFA on days 7, 21, 35, and 49	Decreased IgE production, increased Th1 cytokine production, dependent on βLG concentration in bacterial strains	[34]
Recombinant β-lactoglobulin or fusion proteins produced in <i>Lactococcus lactis</i>	mouse	I.g. feeding of recombinant <i>L. lactis</i> strains on 5 consecutive days, i.p. immunization with 5 µg βLG in IFA on days 26 and 47	Abolishment of IgE and IgG1 production, inhibition of cytokine secretion, reduced eosinophils and increased FcγR3+ cells in BALF	[35]
β-lactoglobulin, β-lactoglobulin-derived peptides by tryptic hydrolysis	mouse	I.g. feeding of 0.05–4 mg native βLG or βLG-peptides, i.p. immunization with 5 µg βLG in alum on day 14	Reduction of IgE and IgG1 production and acute allergic skin response in whey pretreated mice	[36]
Whey and hydrolyzed whey protein	mouse	I.g. feeding of 50 mg partial whey hydrolysate or whey on days –7 to –2, i.g. sensitization with 20 mg whey + CT on days 0, 7, 14, 21, and 28	Reduction of IgE and IgG2a production and of acute allergic skin response	[37]
β-lactoglobulin- derived synthetic peptides	mouse	I.g. feeding of peptides on days –7 to –2 to mice on prebiotic diet, i.g. sensitization with 20 mg of whey + CT on days 0, 7, 14, 21, and 28	Reduction of IgE and IgG1 production and cytokine secretion, tolerance not transferable by CD4+ CD25+ regulatory T cells	[38]
Peanut extract	mouse	I.g. feeding of 1 mg peanut extract on three consecutive days, i.p. immunization with 100 µg peanut extract in alum on days 21 and 28	Decreased proliferation of LN cells and IL-2 secretion	[39]
Der p 1, recombinant Der p 1-derived peptides	mouse	I.g. feeding of 3 mg Der p 1 or peptides on 3 consecutive days, s.c.		[40]

(continued on next page)

Table 1 (continued)

Allergen/ Allergen derivative	Animal species	Intervention	Outcome	References
fused to GST rBet v 1	mouse	immunization 7 days later with 100 µg Der p 1 in CFA I.g. feeding of rBet v 1 (100 µg) or i.n. application (10 µg), i.p. immunization with 1 µg rBet v 1 in alum and subsequent aerosol exposure to birch pollen extract for 10 days	Suppression of IgE, IgG1, and IgG2a production and cutaneous type-I skin test response, reduction of cell proliferation and cytokine secretion	[41]
rBet v 1/CTB conjugate, ovalbumin/CTB conjugate, soluble rBet v 1	mouse	Oral application of 120 µg Bet v 1/CTB or ovalbumin/CTB or rBet v 1 three times at 7-day intervals, i.p. immunization with 1 µg rBet v 1 in alum and subsequent aerosol exposure to birch pollen extract	Bet v 1/CTB increased IgE, IgG1 and IgG2a production, ovalbumin/CTB decreased IgE production, IL4 and IL-5 secretion, rBet v 1 prevented IgE, IgG1, and IgG2a production, proliferation of spleen cells and cytokine secretion	[42]
rBet v 1	mouse	I.g. feeding of 100 µg rBet v 1 on days 0, 7, and 14 to germ-free or conventional mice, s.c. immunization with 1 µg rBet v 1 in alum on days 24, 38, and 52	Reduction of IgE, IgG1 and IgA production, spleen cell proliferation and IL-5 production, independent of the microflora	[43]
Cry j 1 and Cry j 2-derived synthetic peptides	mouse	I.g. feeding of 200 µg peptide 4 times at 3–4 days intervals, i.n. immunization with 1 µg Cry j 1 and Cry j 2 + CT twice on days 0 and 14, or two i.p. immunizations of 10 µg Cry j 1 and Cry j 2 in alum on days 0 and 21	Reduction of IgE and IgG production, proliferation of LN cells, and of Th1 and Th2 cytokine secretion	[44]
Recombinant Cry j 1 and Cry j 2-derived T cell epitopes fused to glycyltin and expressed in rice seed	mouse	I.g. feeding of 200 mg fusion protein-containing rice seeds over 4 weeks, i.p. immunization at week 4 and 5 with 0.1 mg Japanese cedar pollen extract in alum	Abolishment of IgE and IgG responses, proliferation of spleen cells, Th2 cytokine secretion, histamine release and clinical symptoms	[45]
Recombinant Cry j 1 and Cry j 2-derived T cell epitopes fused to CTB and expressed in rice seed	mouse	I.g. feeding of 200 mg fusion protein-containing rice seeds for 10 days, i.p. immunization with 0.1 mg Japanese cedar pollen extract in alum on days 10 and 17	Reduction of IgE production, pollen-induced histamine release, and allergic symptoms	[46]
Cockroach extract	mouse	I.g. feeding of cockroach extract containing 16 µg Bla g 1 and Bla g 2 for 4 days, i.t. sensitization with cockroach extract containing 4 or 2 µg Bla g 1 and Bla g 2 on days 0, 14, and 21	Reduced AHR and pulmonary eosinophilia, increased IL-10 secretion in the lung	[47]

Abbreviations: I.g., intragastric; i.m., intramuscular; i.p., intraperitoneal; s.c. subcutaneous; i.t., intratracheal; LN, lymph node; DNP, 2,4-dinitrophenyl; CY, cyclophosphamide; CT, cholera toxin; CTB, cholera toxin subunit B; DTH, delayed type hypersensitivity; AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; HE, hydrolyzed egg protein; WE, whole egg protein; βLG, β-lactoglobulin; r, recombinant.

Signs of Immunological and Clinical Tolerance			
Immunological Tolerance		Clinical Tolerance	
Laboratory parameters (allergen-specific)		Clinical parameters (allergen-specific)	
Humoral	Cellular	Symptoms	Allergen Challenge
IgE	T cell activation	Rhinitis	Nasal challenge
IgG	Basophil activation	Conjunctivitis	Exposure chambers
IgA	Mast cell activation	Asthma	Ocular challenge
IgM	Cytokine production	Urticaria	Skin prick test (SPT)
		Allergic dermatitis	Atopy patch test (APT)
		Food allergy	Food challenge (DBPCFC)
		Anaphylaxis	

Fig. 1. Signs of true immunological tolerance versus clinical tolerance.

fewer studies having investigated oral tolerance in animals which have been sensitized only by respiratory or oral allergen administration. Interestingly, one study reports that the mode of secondary antigen challenge had different effects on the quality of tolerance during the neonatal period and indicates that tolerance induction may be possible even in 6–8 week old mice [52].

Of particular interest is a study published in 1994 because it showed that feeding with recombinant major house dust mite allergen Der p 1 as well as with T cell epitope-containing peptides of Der p 1 could decrease Der p 1-specific T cell responses [40]. When reviewing the studies in Table 1 it becomes apparent that oral tolerance induction has attracted the interest of the food industry because the ability of hydrolyzed food antigens, in particular of milk hydrolysates to induce tolerance to complete cows milk allergens has been studied [30,32,33,37,38]. In fact, cows milk hydrolysates with reduced IgE reactivity and allergenic activity are used in the diet of cows milk allergic children and the hope seems to be that these formulas may contain T cell-reactive milk peptides capable of inducing oral tolerance when used as an early diet [53,54]. However, a major problem in such an approach is that due to extensive hydrolyzation and use of selective formulas (e.g., whey proteins) the T cell epitope repertoire of the individual cows milk allergens varies and thus does not guarantee oral tolerance induction against all relevant cows milk allergens. Another interesting aspect of the studies displayed in Table 1 is that in certain studies the authors have developed vehicles to deliver the tolerogens. For example, *Lactococcus lactis* strains producing lactoglobulin [35] or genetically modified rice expressing cypress pollen allergens [45,46] have been produced to induce oral tolerance but the risk of such an approach is the uncontrolled release of unknown quantities of intact allergens in the gut. Another interesting finding was that protection of the cows milk allergen β -lactoglobulin in biodegradable particles allowed to reduce the antigen dose for oral tolerance induction [33] but again the release of uncontrolled amounts of intact allergen in the gut remains as a possible concern of such an approach.

The important role of T cells for preventive oral tolerance against allergens becomes apparent because several studies demonstrate that oral tolerance can be induced by feeding of T cell epitope-containing peptides [40] and that tolerance induction can be suppressed with drugs inhibiting T cell function [23]. Even in the animal models oral tolerance induction is not always complete in terms of abolishing antibody, T cell, cytokine, effector cell activation and symptoms. Furthermore, the review of the studies in Table 1 indicates that the detailed mechanisms of oral tolerance induction are not fully elucidated even in the animal models and may include central tolerance, regulatory/immune suppressive cytokines, regulatory T cells or Th3 cells.

1.3. Oral tolerance in clinical trials in humans: true immunological tolerance or “only” clinical tolerance?

Oral administration of allergens for treatment and prevention of

allergy has been and is used in several clinical studies. Three articles in this issue are focused on oral immunotherapy of allergy (OIT) and one on sublingual immunotherapy (SLIT) [55–58]. OIT and SLIT are forms of allergen-specific immunotherapy (AIT) which aim to reduce allergic symptoms in patients who are already allergic (Textbox 1). Interestingly OIT is mainly, if not exclusively conducted for food allergies and in particular for allergies to foods containing allergens that are quite resistant to digestion in the gastrointestinal tract such as milk, egg and peanut allergens. A few early studies conducted with respiratory allergens which are easily degraded in the GI tract have yielded negative or inconclusive results [59–62], presumably due to the fact that the allergens were degraded and hence did not induce systemic immune responses required to modulate the allergic immune response and thus to reduce clinical sensitivity. Thus OIT seems to be effective only for few allergen sources. Another major problem of OIT is the induction of often severe side effects due to allergic reactions to the administered allergens [63,64]. The assessment of immunological effects of OIT conducted with food allergens demonstrate that OIT is usually accompanied by an induction of allergen-specific IgG antibodies, by alterations of allergen-specific T cell and cytokine responses and by suppression of allergen-induced mast cell and basophil activation which mainly seems attributable to the induction of allergen-specific IgG antibodies which block allergen-induced cross-linking of IgE on allergic effector cells [65]. As a result of these immunological alterations a state of clinical tolerance can be observed in treated patients which is accompanied by the patients ability to tolerate higher doses of allergen. Accordingly the term “clinical tolerance induction” seems appropriate to capture the effects of OIT (Textbox 1). However, OIT does not induce true immunological tolerance which should be accompanied by a lack or reduction of allergen-specific antibody, T cell and cytokine responses as it is observed in the classical models of oral tolerance induction in the animal studies described in Table 1 (Fig. 1). By contrast, OIT leads to increases of allergen-specific IgG blocking the IgE-allergen interaction and alterations of T cells and cytokine responses. Both immunological alterations obviously lead to a reduction of clinical symptoms but the mechanisms are entirely different (Fig. 1, right part). The discrimination of true immunological tolerance from clinical tolerance becomes an even more important issue if one reviews so-called oral tolerance studies conducted in infants or children (Table 2) [66–80]. Most, if not all of these studies are conducted with the goal to prevent the development of food allergies. The major endpoints of almost all of the allergy prevention studies are clinical endpoints measuring “clinical tolerance” of the respective food allergens in terms of a reduction of clinical symptoms, the ability of consuming the food and/or a reduction of the risk of developing symptoms of food allergy (Fig. 1). Several of these intervention studies provide evidence that early introduction of food allergens may reduce the risk of developing food allergies [67–69,71,72,74,75,79,80] similar as is observed in certain populations in which certain food antigens are introduced early in the diet [81]. However, the immunological mechanisms underlying

Table 2
Oral tolerance intervention studies in humans to prevent allergy and wheat hypersensitivity

Trial name	Tolerogen	Number of subjects	Definition of subjects	Type of intervention	Time and duration of intervention	Endpoints	Results	References
Vaara, 1995	Whole cow's milk proteins, casein peptides	20	Infants from mothers with diabetes mellitus	n = 10: cow's milk, n = 10: casein until age of 9 months	Until age of 9 months	Cellular responses at 6, 9 and 12 months of age	T cell response declined	[66]
GINI (German Infant Nutritional Intervention)	Hydrolyzed whey vs. hydrolyzed casein vs cow's milk	945	Infants with at least 1 parent or sibling with a history of allergic disease	n = 256: cow's milk, n = 241: partially hydrolyzed whey, n = 238: extensively hydrolyzed whey, n = 210: hydrolyzed casein	0 until 6 months of age	Presence of allergic manifestations at the age of 1 year	Incidence of allergic manifestations was reduced by using hydrolyzed casein	[67]
GINI (German Infant Nutritional Intervention) 6 year follow-up	Hydrolyzed whey vs. hydrolyzed casein vs cow's milk	795	Infants with at least 1 parent or sibling with a history of allergic disease	n = 217: cow's milk, n = 201: partially hydrolyzed whey, n = 198: extensively hydrolyzed whey, n = 179: hydrolyzed casein	0 until 6 months of age	Presence of allergic manifestations at the age of 6 years	Long lasting preventive effect of hydrolyzed whey and casein on atopic eczema but not on asthma or allergic rhinitis	[68]
Health Nuts	egg	2589	General population	Retrospective study	Introduction of egg with 4–6 months vs. 10–12 months and > 12 months	Egg allergy defined by OFC at 11–15 months of age	Early introduction of egg into the food reduced the risk of egg allergy	[69]
Lowe, 2011	Partially hydrolyzed whey vs. cow's milk	620	Infants with first-degree relative with a history of eczema, asthma or allergy	n = 206: whey, n = 208: soy, n = 206: cow's milk until age of 12 months	0 until 12 months of age	Development of any allergies in the first 2 years of life	No difference between groups	[70]
GINI (German Infant Nutritional Intervention), 10 year follow-up	Hydrolyzed whey vs. hydrolyzed casein vs cow's milk	665	Infants with at least 1 parent or sibling with a history of allergic disease	n = 175: cow's milk, n = 168: partially hydrolyzed whey, n = 168: extensively hydrolyzed whey, n = 154: hydrolyzed casein	0 until 6 months of age	Presence of allergic manifestations at the age of 10 years	Hydrolyzed whey and casein have preventive effect on AD, but not on asthma and allergic rhinitis	[71]
STAR (Solids Timing for Allergy Research)	Egg powder vs. rice powder (placebo)	86	Infants with moderate to severe eczema (SCORAD > 15)	n = 49: egg powder, n = 37: rice powder until age of 8 months	4 until 8 months of age	IgE-mediated egg allergy at 12 months of age defined by OFC and SPT	Exposure to egg reduced the risk of egg allergy	[72]
Prevent CD (Prevent Coeliac Disease)	Gluten vs. placebo	944	Infants 0–3 months of age with first-degree family member with celiac disease and positive for HLA-DQ2 or HLA-DQ8	n = 475: gluten, n = 469: placebo until 24 weeks of age	16 until 24 weeks of age	Frequency of celiac disease at 3 years of age	No difference between groups regarding the development of celiac disease and anti-gluten antibodies	[73]
LEAP (Learning Early About Peanut allergy)	Peanut consumption vs. avoidance	542	Infants 4–11 months of age with severe eczema and/or egg allergy	272: peanut consumption, 270: peanut avoidance until 60 months of age	4–11 until 60 months of age	Proportion of participants with peanut allergy at 60 months of age confirmed by OFC	Early introduction of peanuts decreased risk of peanut allergy	[74]
LEAP-On (Persistence of Oral Tolerance to Peanut)	Peanut consumption vs. avoidance	529	Infants of the LEAP study (Infants with eczema and/or egg allergy)	N = 266: peanut consumption, n = 263: peanut avoidance until 60 months of age, followed by 12 months peanut avoidance	4–11 until 60 months of age followed by 12 months peanut avoidance	Percentage of participants with peanut allergy at age of 72 months defined by OFC	Peanut avoidance for 1 year after 5 years of consumption did not increase prevalence of peanut allergy. Fewer participants had higher specific IgE levels than in the peanut avoidance group.	[75]
EAT (Enquiring about Tolerance study)	Peanut, egg, cow's milk, sesame, whitefish, wheat consumption vs avoidance	1162	General population of 3 month old breastfed infants	n = 567: consumption of 6 allergenic foods, n = 595: exclusive breastfeeding until 6 months of age	3 until 6 months of age	Food allergy to one or more of the six foods between 1 and 3 years of age determined by OFC	Prevalence of peanut allergy and egg allergy was reduced in the consumption group. No difference was found for milk, sesame, fish and wheat.	[76]
STEP (Starting Time for Egg Protein)	Egg powder vs. rice powder (placebo)	820	Infants of 4–6 months with atopic mothers and without eczema	n = 407: egg powder, n = 413: rice powder until age of 10 months	4–6 months until 10 months of age	IgE-mediated egg allergy at 12 months defined by OFC and SPT to egg	No difference between groups	[77]
HEAP (Hen's Egg Allergy Prevention)	Egg powder vs. rice powder (placebo)	383	General population of 4–6 months old infants without sensitization to hen's egg.	n = 184: egg white powder, n = 199: rice powder until 12 months of age	4–6 until 12 months of age	Sensitization to hen's egg at age of 12 months	No protective effect of early exposure to egg for egg allergy	[78]

(continued on next page)

Table 2 (continued)

Trial name	Tolerogen	Number of subjects	Definition of subjects	Type of intervention	Time and duration of intervention	Endpoints	Results	References
BEAT (Beating Egg Allergy Trial)	Egg powder vs. rice powder (placebo)	319	Infants of 4 months with at least 1 first-degree relative with allergic disease and with SPT to egg < 2 mm	n = 165: egg powder, n = 154: rice powder until age of 8 months	4 until 8 months of age	SPT response to egg white of ≥ 3 mm at age of 12 months	Early introduction of egg reduced risk to develop egg allergy	[79]
PETT (Prevention of Egg Allergy with Tiny Amount Intake)	Egg powder vs. placebo	147	Infants of 4–5 months with eczema but without egg allergy	n = 73: egg powder (50 mg per day from 6–9 months and 250 mg per day until 12 months), n = 74: placebo	6 until 12 months of age (increasing amounts)	Proportion of participants with hen's egg allergy at 12 months defined by OFC eggs.	Reduction of prevalence of egg allergy by stepwise introduction of eggs.	[80]

this clinical tolerance remain unclear because only few studies investigated immunological endpoints and those which did, did not find signs of true immunological tolerance such as abolishment or reduction of adaptive immune response towards the administered food allergens [66,73]. It should be also mentioned that several studies report no effects of early food allergen intake on the development of food allergies [70,73,76–78] and it is difficult to say what factors were responsible for the different outcomes. In most of the oral prevention studies the intake of food allergens did not start before month 4 raising the question if this time of intervention is not too late to induce true immunological tolerance considering the results from the animal studies which indicate that early intervention is critical for a successful induction of true oral tolerance [19]. In line with this thinking are the results of the German Infant Nutritional Intervention (GINI) studies in which children were fed hydrolyzed cows milk formulas immediately after birth which led to reduced allergic manifestations even after prolonged observation of the children until 6 years of age [67,68,71] but these studies have not investigated immunological parameters and if the effect of intervention was antigen-specific. The early study by Platts-Mills regarding cat allergy actually has shown that early cat exposure in childhood does not induce immunological tolerance but rather a protective IgG responses [82].

Another early prevention study which was performed for celiac disease (CD) [73], a T cell-mediated hypersensitivity reaction to wheat, has performed a detailed analysis of immunological and clinical parameters. This study is interesting because it has investigated children who were genetically predisposed for developing CD. The major outcome of the study was that children who were fed gluten early at 4 months of age as well as children who were exposed to gluten later (i.e., after 6 months) developed at similar frequencies CD at the age of 3. The only difference between the groups was that the group of children who were fed gluten as early intervention developed gluten-specific immune responses earlier than the children without early feeding. This study thus demonstrates that feeding of intact food antigens at the age of 4 months does not induce immunological tolerance but rather an early sensitization. Whether the lack of immunological tolerance induction in this study was due to the fact that the intervention was too late, or due to the feeding of intact antigen capable of inducing an immune response remains an open question. In the case that serum samples from studies summarized in Table 2 were still available it would be interesting to subject these serum samples to a detailed analysis of antibody responses towards a comprehensive panel of defined food allergens and their epitopes as well as to other allergens to obtain more knowledge regarding the immunological effects of the interventions. Chip tests containing comprehensive panels of micro-arrayed allergens and their epitopes have been successfully used to analyze antibody responses in birth cohorts with small volumes of serum and thus would be available for further data mining in the intervention cohorts [83,84].

2. Recombinant allergens, allergen derivatives and synthetic peptides: defined tools for oral tolerance in allergy

Since the publication of the first DNA sequences of important allergens in the late 1980ties [85–87], the molecular structures of the clinically most relevant allergens have been revealed and IgE and T cell epitopes recognized by allergic patients have been mapped [6]. Using recombinant expression and synthetic peptide chemistry it has become possible to produce allergen-derivatives with defined immunological properties for targeting the immune system by specific mechanisms (Fig. 2) [88]. The term “recombinant allergen” or “recombinant wild-type allergen” defines a recombinant allergen which has been made to resemble the IgE and T cell epitopes of the corresponding natural allergen [89,90]. Such recombinant allergens are used for diagnostic purposes and replace natural allergens which often contain impurities or consist of mixtures of isoallergens with different properties. Upon administration, recombinant wildtype allergens can in principle

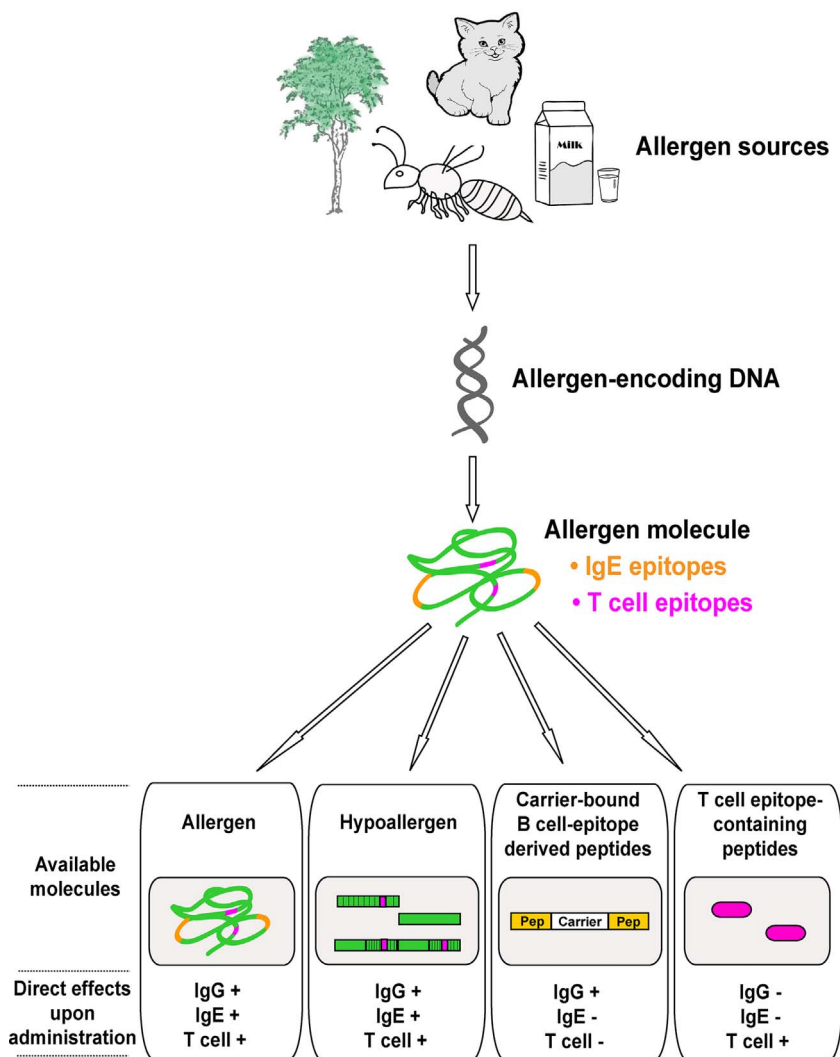


Fig. 2. From undefined allergen source-derived materials to defined tolerogens: Recombinant allergens, allergen-derivatives and allergen-derived synthetic peptides.

stimulate allergen-specific IgE, IgG and T cell responses. Wildtype allergens are extremely well-defined in terms of structure and can be administered in defined quantities. They can be used for oral tolerance induction because they resemble the epitope spectrum of the natural allergens but it is possible that they can induce also an unwanted IgE sensitization.

The term “hypoallergen” defines an allergen derivative which usually is produced by recombinant expression and has been modified from the original allergen by reducing its IgE reactivity and allergenic activity (i.e., its ability to induce IgE-mediated allergic reactions in a sensitized individual) (Textbox 1). Hypoallergens are made to preserve the T cell epitope repertoire of the corresponding natural allergen and upon administration can induce allergen-specific IgG antibodies but also to some extent IgE responses [91–94]. Recombinant hypoallergens can therefore be considered as eventually superior candidate molecules for oral tolerance induction over wildtype allergens because of their reduced IgE reactivity and reduced ability to induce IgE responses (Fig. 2).

Carrier-bound B cell-epitope-containing allergen peptides are made by grafting non-allergenic peptides from the IgE binding sites of allergens to carrier proteins which are not allergen-derived [95–99] (Textbox 1). Thus the presence of allergen-derived T cell epitopes is reduced in these derivatives (Fig. 2). They have been shown to have a profoundly reduced allergenic activity and upon administration induce allergen-specific IgG responses blocking IgE binding to the allergens without inducing IgE responses by receiving T cell help from the non-

allergenic carrier protein [97]. The carrier-bound B cell-epitope-derived peptides seem to be optimal for therapeutic allergen-specific immunotherapy because of their reduced allergenic activity and ability to induce protective allergen-specific IgG responses but the absence/reduction of allergen-specific T cell epitopes makes them less suitable for oral tolerance induction.

Synthetic peptides containing allergen-derived T cell epitopes can be produced by synthetic peptide chemistry to resemble allergen-specific T cell epitopes of the natural allergens (Fig. 2) (Textbox 1). Due to the short length these peptides usually lack secondary structure and thus show no IgE reactivity [98,100,101]. Upon administration T cell epitope-containing peptides can target allergen-specific T cells but do not induce allergen-specific IgG or IgE responses. The lack of induction of allergen-specific IgG responses may in fact have been responsible why T cell peptide-based AIT has not been clinically effective (<http://www.circassia.com/media/press-releases/circassia-announces-top-line-results-from-cat-allergy-phase-iii-study/>) but these peptides should be useful for oral tolerance induction and should bear a low risk of inducing allergic sensitization upon administration. Major advantages of the listed candidate molecules for oral tolerance induction are that they can be produced in defined quantities, amounts and with defined properties to target the immune system in a highly specific manner. These features are important for performing precise investigations in experimental animal models and for their use in clinical trials which require the use of highly defined drug products that are manufactured under GMP (Good manufacturing practice) conditions.

3. Towards a systematic and molecular approach for oral allergen-specific tolerance

3.1. Defining the optimal combination of allergens and allergen-derivatives for oral tolerance: grasping regional variations of sensitization profiles with micro-arrayed allergens

Since the first demonstration that recombinant allergens can be used for diagnosis of allergy and for identifying allergic patients molecular sensitization profiles in 1991 [102], innovative technologies for deciphering allergic patients sensitization patterns have been developed. In 2002 the first study was published demonstrating that comprehensive panels of allergen molecules can be immobilized on chips to allow simultaneous testing towards a broad variety of allergens with a few microliters of serum or plasma [103]. In the meantime the allergen micro-array technology has become not only an important tool for routine allergy diagnosis [104,105] but has also been used to investigate IgE reactivity profiles in birth cohorts and populations from different parts of the world [83,84]. Data from birth cohorts demonstrate that the IgE reactivity profiles of allergic patients are established in the first few years of life [106–112].

Once they are established they seem to remain unaltered in adult patients. In fact a study testing sera from adult allergic patients collected over a period of ten years for IgE reactivity to almost 100 allergen molecules showed that the IgE reactivity profiles did not change over the ten years observation period [113]. Depending on the local environment allergic patients acquire IgE sensitizations against certain allergen molecules which are prevalent in the region where they are born and grow up. This has been noticed already very early when the prevalence of IgE recognitions of patients from northern, middle and southern parts of Europe were tested for IgE reactivity to recombinant birch pollen allergens [114]. One recent study even demonstrated that changes in the local vegetation caused by a governmental replanting program or by natural change of flora changed the molecular sensitization profiles of the population within two generations [115]. Using the micro-array technology it is thus possible to determine the key allergens for different parts of the world (Fig. 3) and to establish a “shortlist” of the most relevant allergens. For example, it was found that sensitization to clinically relevant pollen allergens was rare in the Philippines whereas sensitization to house dust mite and pet allergens was very common [116]. Different sensitization profiles were found in Middle Europe as compared to Northern Europe regarding sensitization

to house dust mite allergens which were frequently recognized in Middle Europe and rarely in Northern Europe [117,118]. Furthermore, different sensitization profiles to various plants pollen allergens were found in a study investigating IgE reactivity profiles to micro-arrayed pollen allergens in different parts of France [119]. In the course of the European MeDALL research project [1] scientists have tested serum samples from birth cohorts from different parts of Europe using the micro-array technology and are currently working on the establishment of a molecular allergen map for Europe which will allow to define the key molecules for tolerance induction. Similar analyses are currently performed also for other regions of the world [120] so that we can expect that a quite complete world-wide map of molecular sensitization profiles will become available soon.

The detailed knowledge of the relevant allergens in different parts of the world will be very useful to design tolerogenic allergen mixtures for the different populations. Furthermore, the fact that sensitization to certain allergen molecules is highly prevalent in certain countries will be extremely helpful for the design of clinical studies investigating the effects of tolerogenic candidate molecules in humans. For example, approximately 25% of the population in Scandinavia (e.g., Sweden, Finland) and Russia are sensitized to the major allergen of birch pollen, Bet v 1 [108]. The high prevalence of Bet v 1 sensitization will thus greatly facilitate clinical trials for tolerance induction in these countries because Bet v 1 can be used as model allergen to study tolerogenic strategies aiming to suppress Bet v 1-specific sensitization and immune responses.

3.2. The possible importance of formulating the tolerogen

As summarized in Table 3, different strategies have been used in experimental animal studies to facilitate oral tolerance induction [24,25,30,32,35,41,45,46]. For example, cholera toxin has been suggested as an “adjuvant” to facilitate tolerance induction but it has also been used to induce allergic sensitization [24,41]. It is thus possible that it promotes allergic sensitization rather than tolerance induction. Another vehicle for transporting tolerogens via the oral route is to use milk or hydrolyzed milk preparations which have the potential advantage that the tolerogen can be administered in a “natural formulation” already early in life, if not immediately after birth [25,30,32]. In fact, data from experimental animal studies indicate that it is important to administer the tolerogen continuously and already early in life [19]. Other investigators have thought about engineering bacteria or

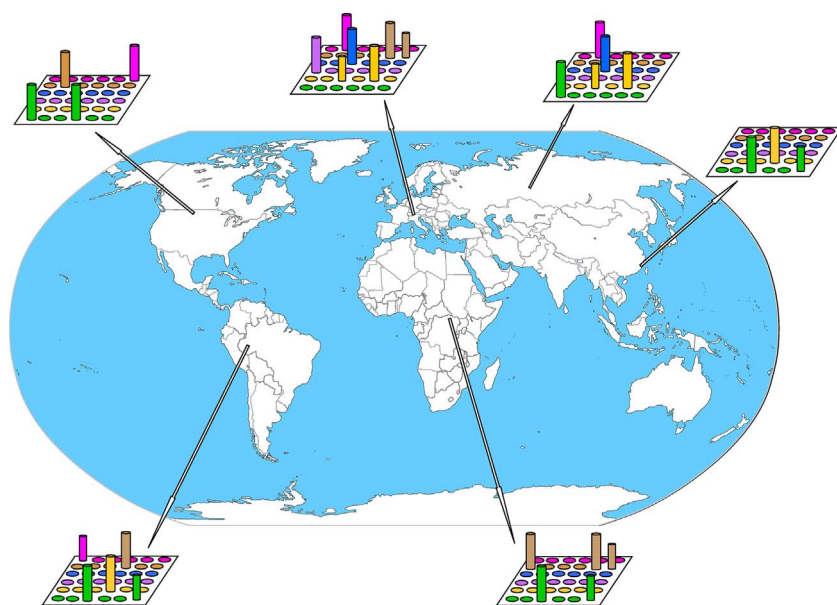


Fig. 3. Defining the relevant allergens for different regions in the world using micro-arrayed allergen molecules.

Table 3
Vehicles used for tolerogens in animal studies.

Vehicle	Tolerogen	Possible advantage	Reference
Cholera toxin subunit B	Ovalbumin rBet v 1	Mucosal adjuvant, lower amounts of allergen necessary	[24,41]
Milk	Ovalbumin Hydrolyzed milk protein	Easy manufacturing and application, less risk of sensitization by using peptides	[25,30,32]
<i>Lactococcus lactis</i>	β -lactoglobulin	Adjuvant favoring Th1 responses, Protection of antigen against intestinal proteases, no antigen purification necessary	[35]
Transgenic plant food	Cry j 1 and Cry j 2-derived peptides expressed in rice seeds	No antigen purification necessary, simple administration, less risk of sensitization by using peptides	[45,46]
D,L-PLG microspheres	β -lactoglobulin	Protection of antigen against intestinal proteases	[33]

r, recombinant; D,L-PLG, poly D,L-lactide-co-glycolide.

preparing transgenic food to express allergens for oral tolerance induction [35,45,46]. While the technology bypasses the expense of purifying recombinant allergens and could prolong the exposure to allergen it will be very difficult to control the amount of expression of the allergen in the transgenic organism. Expression of the allergen by genetic engineering in other organisms as well as encapsulation of allergens into microspheres [33] may reduce the amount of allergen which is needed for tolerance induction but on the other hand may bear the risk that large amounts of intact allergen enter the body and eventually induce allergic sensitization. It may therefore be important to combine optimal allergen derivatives and formulation strategies to obtain a safe, convenient and effective form of oral tolerance induction.

3.3. Defining the time window for oral tolerance induction

Data from birth cohorts have shown that allergic sensitization develops early soon after birth and is characterized by an initial sensitization to food allergens followed by sensitization to respiratory allergens which typically reflects the course of allergen encounter [121]. Infants are first exposed to large amount of food antigens followed by repeated exposure to respiratory allergens in much lower quantities [122]. Using micro-arrayed allergens it has become possible to analyze the evolution of allergic sensitization in birth cohorts in great detail to the level of the individual allergen molecules [1,19,84,105–112]. The birth cohort studies indicate that allergic sensitization develops in the first few years of life and then leads to an established IgE reactivity profile which does not seem to change in adults [113] (Fig. 4). In this issue Westman et al. [107] provide an overview of birth cohort studies in which the molecular sensitization profiles have been measured in children in a longitudinal manner. According to the results of these studies it appears important to make attempts for oral tolerance

induction early in life because when children are growing up they quickly establish their allergen-specific immune responses (Fig. 4). The birth cohort studies demonstrate that certain children seem to acquire “additional” IgE sensitizations up to puberty because IgE antibodies against certain allergens become detectable in follow up blood samples against which no specific IgE reactivity was detected in a blood sample taken earlier. This would indicate that there is plasticity in the development of the allergic immune response up to the period of puberty suggesting a relatively long window of opportunity for oral tolerance induction (Fig. 4). However, it is also possible that in such children sensitization has occurred already much earlier but has not yet reached the threshold for detecting specific IgE antibodies in early blood samples. In fact, allergen-specific IgG antibodies are already detectable a few months after birth when maternal IgG has disappeared from the circulation of the child (Hochwallner & Valenta, unpublished observations) which would suggest that allergen-specific immune responses develop in the first months of life. If this is correct, oral tolerance induction must be performed immediately after birth and continued during the first months of life (Fig. 4). The assumption that oral tolerance induction must take place very early to become effective and to lead to true immunological tolerance is also supported by experimental animal studies (Table 1). Data obtained in these studies indicate that oral tolerance induction is most effective if it is performed very early in life. Also data from clinical studies for inducing oral tolerance (Table 2) point in this direction. The clinical studies show that it is possible to induce to some extent “clinical tolerance” (Table 3) in children. However, a limitation of these studies is that they lack to a large extent a detailed investigation of the development of the allergen-specific immune response in the study subjects and it is therefore difficult to say if indeed immunological tolerance has been achieved. It is therefore difficult to say if the outcome of these studies could be improved by

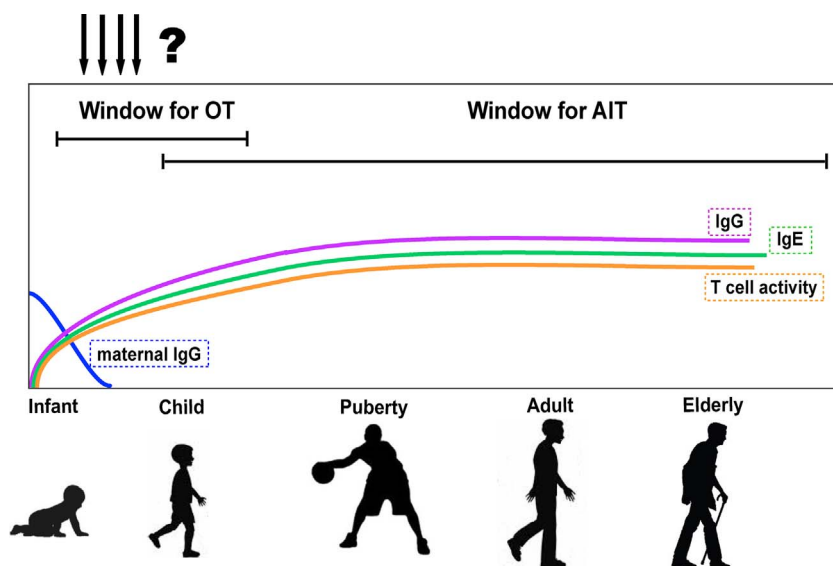


Fig. 4. Defining the time window for oral tolerance induction in allergy.

certain measures such as earlier and more frequent administration or by using defined allergen molecules. We would therefore argue that there is a need for more clinical studies investigating some very basic parameters using well-defined allergen model systems.

3.4. How to study true oral allergen-specific tolerance in clinical studies

If one follows a slightly modified definition of oral tolerance given in the article by Rezende & Weiner in this issue [49] (i.e., oral tolerance can be defined as an inhibition of specific immune responsiveness to subsequent administration of proteins to which an individual has been previously exposed via the oral route), one would suggest that an important endpoint of clinical studies investigating oral-allergen-specific tolerance is if subjects fed with allergens or allergen derivatives do not mount allergen-specific immune responses. This would include lack of production of allergen-specific IgE, T cell and cytokine responses but strictly speaking also lack of the production of allergen-specific IgG responses as shown in experimental animal studies (Table 1). This consideration is of importance when it comes to the design of clinical studies. Only a certain percentage of the general population develops allergen-specific IgE responses and of those only a limited number experiences allergic symptoms upon encounter to the corresponding allergen [108,111]. Therefore, if one chooses “absence of clinical symptoms towards a given allergen” as endpoint for a prospective clinical study one must enroll a very large number of subjects to achieve the statistical power of getting meaningful results for the comparison of the active intervention group (i.e., the subjects who receive the tolerogen) and those who receive only placebo. In this context it must be noted that many of the previously conducted studies were not conducted in a blinded manner and did not include a real placebo group for comparison. The inclusion of a placebo would require that the active tolerogen represents a well-defined substance (e.g., a purified natural or recombinant allergen molecule, allergen-derived peptides or allergen-derivatives) which cannot be discriminated from a placebo by the subjects participating in the study. Furthermore, one must consider that the development of clinical symptoms may also take considerable time in children and does not occur in all children at the same time which makes it very difficult to plan the optimal duration for the study and to obtain results in a reasonable period of time. It should therefore be considered to investigate in pilot studies immunological endpoints such as the development of allergen-specific IgE and T cell responses. Since food allergies are very rare forms of IgE-associated allergies [123,124] it is very difficult to perform adequately powered studies if one starts with a very early intervention because only few subjects will develop food allergies in a population. It will therefore be interesting to study oral tolerance induction for frequently recognized respiratory allergens. For example, more than 25% of the population in Scandinavia and Russia develop birch pollen allergy and the major allergen of birch, Bet v 1, is the culprit allergen for birch pollen allergic patients [108,125]. It should thus be relatively easy to study oral tolerance induction for the prevention of birch pollen allergy in such populations because one can focus on one major allergen molecule (i.e., major birch pollen allergen, Bet v 1) and to study Bet v 1-specific IgE and T cell responses as endpoints. Due to the high prevalence of birch pollen allergy in such populations one would require relatively small numbers of subjects in the active and in the placebo group to achieve statistical power. The number of subjects in pilot studies might even be more reduced if one considers the development of Bet v 1-specific IgG responses as a study endpoint because it has been shown in cohort studies that virtually every person, allergic or non-allergic, develops Bet v 1-specific IgG responses upon natural exposure to birch pollen [119]. If in such trials immune responses towards multiple antigens to which the individuals are exposed, for example with micro-arrayed allergens, it will be possible to study how selective tolerance induction is. The latter is interesting because it is argued that linked-immunological suppression is only possible when antigens are covalently linked [126] whereas others

consider by-stander suppression as a possible mechanism [127].

We therefore suggest to conduct clinical studies investigating the development of true immunological tolerance by OT by using defined allergen molecules to which subjects in a given population are highly exposed and to take immunological endpoints into consideration.

4. Conclusion and outlook

Oral tolerance (OT) induction has been shown to be extremely powerful for inducing antigen-specific non-responsiveness by pre-feeding of the antigen. It seems to be most effective as a very early intervention soon after birth but is less powerful in a therapeutic setting, when specific immune responses have already been established. Here we argue that OT could be ideally suited for the prevention of allergic diseases because most of the important allergen molecules have been characterized and can be produced for OT as pure molecules or in the form of defined derivatives with favorable immunological properties for OT. At the same time, new molecular tests are available which allow to measure allergen-specific immune responses such as IgE sensitization in small blood samples to study the effects of OT. Experimental animal studies are needed to investigate the parameters for optimal OT with defined allergen molecules such as the time, duration, mode, frequency of application, dose and formulation to create a solid basis for subsequent clinical studies. Clinical OT studies can be performed in populations where allergic sensitization to certain allergens is very common. The prevalence of sensitizations to defined allergen molecules in populations can be determined by multiallergen tests based on micro-arrayed allergens. The analysis of immunological endpoints in clinical studies will help to clarify if the intervention has induced true immunological tolerance or other types of immune modulation. Furthermore, assessment of immunological endpoints will be useful to conduct meaningful pilot trials in smaller numbers of subjects before large, expensive and long clinical trials are performed. Such pilot studies will show if OT can be a powerful tool for the prevention of allergy.

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References

- [1] J.M. Anto, J. Bousquet, M. Akdis, C. Auffray, T. Keil, I. Momas, et al., Mechanisms of the Development of Allergy (MeDALL): Introducing novel concepts in allergy phenotypes, *J. Allergy Clin. Immunol.* 139 (2) (2017) 388–399.
- [2] U. Blank, N. Charles, M. Benhamou, The high-affinity immunoglobulin E receptor as pharmacological target, *Eur. J. Pharmacol.* 778 (2016) 24–32.
- [3] K. Ishizaka, T. Ishizaka, M.M. Hornbrook, Physico-chemical properties of human reagenic antibody. IV. Presence of a unique immunoglobulin as a carrier of reagenic activity, *J. Immunol.* 97 (1) (1966) 75–85.
- [4] K. Ishizaka, T. Ishizaka, Identification of IgE, *J. Allergy Clin. Immunol.* 137 (6) (2016) 1646–1650.
- [5] L. Wide, H. Bennich, S.G. Johansson, Diagnosis of allergy by an in-vitro test for allergen antibodies, *Lancet* 2 (7526) (1967) 1105–1107.
- [6] R. Valenta, F. Ferreira, M. Focke-Tejkl, B. Linhart, V. Niederberger, I. Swoboda, et al., From allergen genes to allergy vaccines, *Annu. Rev. Immunol.* 28 (2010) 211–241.
- [7] R.M. Rezende, H.L. Weiner, Inducing tolerance one antigen at a time, *Nat. Biotechnol.* 34 (5) (2016) 515–517.
- [8] R.E. Billingham, L. Brent, P.B. Medawar, Actively acquired tolerance of foreign cells, *Nature* 172 (4379) (1953) 603–606.
- [9] L. Brent, Transplantation tolerance—a historical introduction, *Immunology* 147 (3) (2016) 267–268.
- [10] J.L. Bracy, D.H. Sachs, J. Iacomini, Inhibition of xenoreactive natural antibody production by retroviral gene therapy, *Science* 281 (5384) (1998) 1845–1847.
- [11] C. Tian, J. Bagley, N. Cretin, N. Seth, K.W. Wucherpfennig, J. Iacomini, Prevention of type 1 diabetes by gene therapy, *J. Clin. Invest.* 114 (7) (2004) 969–978.
- [12] U. Baranyi, B. Linhart, N. Pilat, M. Gattringer, J. Bagley, F. Muehlbacher, et al., Tolerization of a type I allergic immune response through transplantation of genetically modified hematopoietic stem cells, *J. Immunol.* 180 (12) (2008) 8168–8175.

- [13] U. Baranyi, A.M. Farkas, K. Hock, B. Mahr, B. Linhart, M. Gattringer, et al., Cell therapy for prophylactic tolerance in immunoglobulin E-mediated allergy, *EBioMedicine* 7 (2016) 230–239.
- [14] N.M. Vaz, L.C. Maia, D.G. Hanson, J.M. Lynch, Inhibition of homocytotropic antibody responses in adult inbred mice by previous feeding of the specific antigen, *J. Allergy Clin. Immunol.* 60 (2) (1977) 110–115.
- [15] T. Meyer, R. Ullrich, M. Zeitz, Oral tolerance induction in humans, *Exp. Mol. Pathol.* 93 (3) (2012) 449–454.
- [16] H.G. Wells, T.B. Osborne, The biological reactions of the vegetable proteins, *J. Infect. Dis.* 8 (77) (1911) 66–124.
- [17] M.W. Chase, Inhibition of experimental drug allergy by prior feeding of the sensitizing agent, *Proc. Soc. Exp. Biol. Med.* 61 (1946) 257–259.
- [18] H.C. Thomas, M.V. Parrott, The induction of tolerance to a soluble protein antigen by oral administration, *Immunology* 27 (4) (1974) 631–639.
- [19] M.F. David, Prevention of homocytotropic antibody formation and anaphylactic sensitization by prefeeding antigen, *J. Allergy Clin. Immunol.* 60 (3) (1977) 180–187.
- [20] J. Ngan, L.S. Kind, Suppressor T cells for IgE and IgG in Peyer's patches of mice made tolerant by the oral administration of ovalbumin, *J. Immunol.* 120 (3) (1978) 861–865.
- [21] L.K. Richman, J.M. Chiller, W.R. Brown, D.G. Hanson, N.M. Vaz, Enterically induced immunologic tolerance. I. Induction of suppressor T lymphocytes by intragastric administration of soluble proteins, *J. Immunol.* 121 (6) (1978) 2429–2434.
- [22] S.J. Challacombe, T.B. Tomasi Jr., Systemic tolerance and secretory immunity after oral immunization, *J. Exp. Med.* 152 (6) (1980) 1459–1472.
- [23] G.F. Hoynes, M.G. Callow, J. Kuhlman, W.R. Thomas, T-cell lymphokine response to orally administered proteins during priming and unresponsiveness, *Immunology* 78 (4) (1993) 534–540.
- [24] C. Rask, J. Holmgren, M. Fredriksson, M. Lindblad, I. Nordstrom, J.B. Sun, et al., Prolonged oral treatment with low doses of allergen conjugated to cholera toxin B subunit suppresses immunoglobulin E antibody responses in sensitized mice, *Clin. Exp. Allergy* 30 (7) (2000) 1024–1032.
- [25] B. Zemann, C. Schwaerzler, M. Griot-Wenk, M. Nefzger, P. Mayer, H. Schneider, et al., Oral administration of specific antigens to allergy-prone infant dogs induces IL-10 and TGF-beta expression and prevents allergy in adult life, *J. Allergy Clin. Immunol.* 111 (5) (2003) 1069–1075.
- [26] V. Verhasselt, V. Milcent, J. Cazareth, A. Kanda, S. Fleury, D. Dombrowicz, et al., Breast milk-mediated transfer of an antigen induces tolerance and protection from allergic asthma, *Nat. Med.* 14 (2) (2008) 170–175.
- [27] J.M. Birmingham, S. Patil, X.M. Li, P.J. Busse, The effect of oral tolerance on the allergic airway response in younger and aged mice, *J. Asthma* 50 (2) (2013) 122–132.
- [28] F. Hacini-Rachinel, Y.M. Vissers, R. Doucet-Ladeveze, C. Blanchard, A. Demont, M. Perrot, et al., Low-allergenic hydrolyzed egg induces oral tolerance in mice, *Int. Arch. Allergy Immunol.* 164 (1) (2014) 64–73.
- [29] J. Strid, M. Thomson, J. Hourihane, I. Kimber, S. Strobel, A novel model of sensitization and oral tolerance to peanut protein, *Immunology* 113 (3) (2004) 293–303.
- [30] R. Fritsche, J.J. Pahud, S. Pecquet, A. Pfeifer, Induction of systemic immunologic tolerance to beta-lactoglobulin by oral administration of a whey protein hydrolysate, *J. Allergy Clin. Immunol.* 100 (2) (1997) 266–273.
- [31] S. Pecquet, A. Pfeifer, S. Gaudie, R. Fritsche, Immunoglobulin E suppression and cytokine modulation in mice orally tolerized to beta-lactoglobulin, *Immunology* 96 (2) (1999) 278–285.
- [32] S. Pecquet, L. Bovetto, F. Maynard, R. Fritsche, Peptides obtained by tryptic hydrolysis of bovine beta-lactoglobulin induce specific oral tolerance in mice, *J. Allergy Clin. Immunol.* 105 (3) (2000) 514–521.
- [33] S. Pecquet, E. Leo, R. Fritsche, A. Pfeifer, P. Couvreur, E. Fattal, Oral tolerance elicited in mice by beta-lactoglobulin entrapped in biodegradable microspheres, *Vaccine* 18 (13) (2000) 1196–1202.
- [34] K. Mizumachi, J. Kurisaki, Induction of oral tolerance in mice by continuous feeding with beta-lactoglobulin and milk, *Biosci. Biotechnol. Biochem.* 66 (6) (2002) 1287–1294.
- [35] K. Adel-Patient, S. Ah-Leung, C. Creminon, S. Nouaille, J.M. Chatel, P. Langella, et al., Oral administration of recombinant *Lactococcus lactis* expressing bovine beta-lactoglobulin partially prevents mice from sensitization, *Clin. Exp. Allergy* 35 (4) (2005) 539–546.
- [36] K. Adel-Patient, S. Wavrin, H. Bernard, N. Meziti, S. Ah-Leung, J.M. Wal, Oral tolerance and Treg cells are induced in BALB/c mice after gavage with bovine beta-lactoglobulin, *Allergy* 66 (10) (2011) 1312–1321.
- [37] B.C. van Esch, B. Schouten, S. de Kivit, G.A. Hofman, L.M. Knippels, L.E. Willemsen, et al., Oral tolerance induction by partially hydrolyzed whey protein in mice is associated with enhanced numbers of Foxp3+ regulatory T-cells in the mesenteric lymph nodes, *Pediatr. Allergy Immunol.* 22 (8) (2011) 820–826.
- [38] L.A. Meulenbroek, B.C. van Esch, G.A. Hofman, C.F. den Hartog Jager, A.J., Nauta, L.E. Willemsen, et al., Oral treatment with beta-lactoglobulin peptides prevents clinical symptoms in a mouse model for cow's milk allergy, *Pediatr. Allergy Immunol.* 24 (7) (2013) 656–664.
- [39] M. Marcondes Rezende, I. Hassing, M. Bol-Schoenmakers, R. Bleumink, L. Boon, J. van Bilsen, et al., CD4(+) CD25(+) T regulatory cells do not transfer oral tolerance to peanut allergens in a mouse model of peanut allergy, *Clin. Exp. Allergy* 41 (9) (2011) 1324–1333.
- [40] G.F. Hoynes, M.G. Callow, M.C. Kuo, W.R. Thomas, Inhibition of T-cell responses by feeding peptides containing major and cryptic epitopes: studies with the Der p I allergen, *Immunology* 83 (2) (1994) 190–195.
- [41] U. Wiedermann, B. Jahn-Schmid, B. Bohle, A. Repa, H. Renz, D. Kraft, et al., Suppression of antigen-specific T- and B-cell responses by intranasal or oral administration of recombinant bet v 1, the major birch pollen allergen, in a murine model of type I allergy, *J. Allergy Clin. Immunol.* 103 (6) (1999) 1202–1210.
- [42] U. Wiedermann, B. Jahn-Schmid, M. Lindblad, C. Rask, J. Holmgren, D. Kraft, et al., Suppressive versus stimulatory effects of allergen/cholera toxoid (CTB) conjugates depending on the nature of the allergen in a murine model of type I allergy, *Int. Immunol.* 11 (10) (1999) 1717–1724.
- [43] A. Repa, H. Kozakova, T. Hudcovic, R. Stepankova, T. Hrnir, H. Tlaskalova-Hogenova, et al., Susceptibility to nasal and oral tolerance induction to the major birch pollen allergen Bet v 1 is not dependent on the presence of the microflora, *Immunol. Lett.* 117 (1) (2008) 50–56.
- [44] K. Hirahara, S. Saito, N. Serizawa, R. Sasaki, M. Sakaguchi, S. Inouye, et al., Oral administration of a dominant T-cell determinant peptide inhibits allergen-specific TH1 and TH2 cell responses in Cry j 2-primed mice, *J. Allergy Clin. Immunol.* 102 (6 Pt 1) (1998) 961–967.
- [45] H. Takagi, T. Hiroi, L. Yang, Y. Tada, Y. Yuki, K. Takamura, et al., A rice-based edible vaccine expressing multiple T cell epitopes induces oral tolerance for inhibition of Th2-mediated IgE responses, *Proc. Natl. Acad. Sci. U. S. A.* 102 (48) (2005) 17525–17530.
- [46] H. Takagi, T. Hiroi, L. Yang, K. Takamura, R. Ishimitsu, H. Kawauchi, et al., Efficient induction of oral tolerance by fusing cholera toxin B subunit with allergen-specific T-cell epitopes accumulated in rice seed, *Vaccine* 26 (48) (2008) 6027–6030.
- [47] L.J. Vaickus, J. Bouchard, J. Kim, S. Natarajan, D.G. Remick, Oral tolerance inhibits pulmonary eosinophilia in a cockroach allergen induced model of asthma: a randomized laboratory study, *Respir. Res.* 11 (2010) 160.
- [48] U. Smole, I. Schabusova, W. Pickl, U. Wiedermann, Murine Models for Oral Tolerance in Allergy, *Semin Immunol.* 2017.
- [49] R.M. Rezende, H.L. Weiner, History and mechanisms of oral tolerance, *Semin. Immunol.* (2017) (this issue).
- [50] U. Baranyi, M. Gattringer, R. Valenta, T. Wekerle, Cell-based therapy in allergy, *Curr. Top. Microbiol. Immunol.* 352 (2011) 161–179.
- [51] J.M. Crichtfield, M.K. Racke, J.C. Zúñiga-Pflücker, B. Cannella, C.S. Raine, J. Goverman, et al., T cell deletion in high antigen dose therapy of autoimmune encephalomyelitis, *Science* 263 (5150) (1994) 1139–1143.
- [52] I.T. Tobagus, W.R. Thomas, P.G. Holt, Adjuvant costimulation during secondary antigen challenge directs qualitative aspects of oral tolerance induction, particularly during the neonatal period, *J. Immunol.* 172 (2004) 2274–2285.
- [53] T. Hays, R.A. Wood, A systematic review of the role of hydrolyzed infant formulas in allergy prevention, *Arch. Pediatr. Adolesc. Med.* 159 (9) (2005) 810–816.
- [54] H. Hochwallner, U. Schulmeister, I. Swoboda, M. Focke-Tejkl, R. Reininger, V. Cijav, et al., Infant milk formulas differ regarding their allergenic activity and induction of T-cell and cytokine responses, *Allergy* 72 (3) (2017) 416–424.
- [55] A. Hamad, W. Burks, Oral tolerance and allergy, *Semin. Immunol.* (2017) (this issue).
- [56] N. Yanagida, S. Sat, M. Ebisawa, Clinical Aspects of Oral Immunotherapy for the Treatment of Allergies, *Semin Immunol.* 2017.
- [57] D.M. Hussey Freeland, M. Manohar, S. Andorf, B.D. Hobson, W. Zhang, K.C. Nadeau, Oral immunotherapy for food allergy, *Semin. Immunol.* (2017) (this issue).
- [58] P. Moingeon, L. Mascarell, Differences and similarities between sublingual immunotherapy of allergy and oral tolerance, *Semin. Immunol.* (2017) (this issue).
- [59] W. Reibien, E. Puttonen, H.J. Maasch, E. Stix, U. Wahn, Clinical and immunological response to oral and subcutaneous immunotherapy with grass pollen extracts. A prospective study, *Eur. J. Pediatr.* 138 (4) (1982) 341–344.
- [60] P.J. Cooper, J. Darbyshire, A.J. Nunn, J.O. Warner, A controlled trial of oral hyposensitization in pollen asthma and rhinitis in children, *Clin. Allergy* 14 (6) (1984) 541–550.
- [61] E. Taudorf, L.C. Laursen, R. Djurup, E. Kappelgaard, C.T. Pedersen, M. Søborg, et al., Oral administration of grass pollen to hay fever patients. An efficacy study in oral hyposensitization, *Allergy* 40 (5) (1985) 321–335.
- [62] E. Taudorf, C. Möller, M.W. Russell, Secretory IgA response in oral immunotherapy. Investigation in birch pollinosis, *Allergy* 49 (9) (1994) 760–765.
- [63] F. Mori, A. Cianferoni, A. Brambilla, S. Barni, L. Sarti, N. Pucci, et al., Side effects and their impact on the success of milk oral immunotherapy (OIT) in children, *Int. J. Immunopathol. Pharmacol.* 30 (2) (2017) 182–187.
- [64] D. Babaie, M. Mesdaghi, M. Nishino, M. Mansouri, M. Ebisawa, Oral and sublingual immunotherapy potential causes for eosinophilic gastrointestinal disorders? *Int. Arch. Allergy Immunol.* 172 (2) (2017) 89–98.
- [65] M. Ponce, S.C. Diesner, Z. Szépfalusi, T. Eiwegger, Markers of tolerance development to food allergens, *Allergy* 71 (10) (2016) 1393–1404.
- [66] O. Vaarala, T. Saukkonen, E. Savilahti, T. Klemola, H.K. Akerblom, Development of immune response to cow's milk proteins in infants receiving cow's milk or hydrolyzed formula, *J. Allergy Clin. Immunol.* 96 (6 Pt 1) (1995) 917–923.
- [67] A. von Berg, S. Koletzko, A. Grubl, B. Filipiak-Pittroff, H.E. Wichmann, C.P. Bauer, et al., The effect of hydrolyzed cow's milk formula for allergy prevention in the first year of life: the German Infant Nutritional Intervention Study, a randomized double-blind trial, *J. Allergy Clin. Immunol.* 111 (3) (2003) 533–540.
- [68] A. von Berg, B. Filipiak-Pittroff, U. Kramer, E. Link, C. Bollrath, I. Brockow, et al., Preventive effect of hydrolyzed infant formulas persists until age 6 years: long-term results from the German Infant Nutritional Intervention Study (GINI), *J. Allergy Clin. Immunol.* 121 (6) (2008) 1442–1447.
- [69] J.J. Koplin, N.J. Osborne, M. Wake, P.E. Martin, L.C. Gurrin, M.N. Robinson, et al., Can early introduction of egg prevent egg allergy in infants? A population-based study, *J. Allergy Clin. Immunol.* 126 (4) (2010) 807–813.

- [70] A.J. Lowe, C.S. Hosking, C.M. Bennett, K.J. Allen, C. Axelrad, J.B. Carlin, et al., Effect of a partially hydrolyzed whey infant formula at weaning on risk of allergic disease in high-risk children: a randomized controlled trial, *J. Allergy Clin. Immunol.* 128 (2) (2011) 360–365 (e4).
- [71] A. von Berg, B. Filipiak-Pittroff, U. Kramer, B. Hoffmann, E. Link, C. Beckmann, et al., Allergies in high-risk schoolchildren after early intervention with cow's milk protein hydrolysates: 10-year results from the German Infant Nutritional Intervention (GINI) study, *J. Allergy Clin. Immunol.* 131 (6) (2013) 1565–1573.
- [72] D.J. Palmer, J. Metcalfe, M. Makrides, M.S. Gold, P. Quinn, C.E. West, et al., Early regular egg exposure in infants with eczema: a randomized controlled trial, *J. Allergy Clin. Immunol.* 132 (2) (2013) 387–392 (e1).
- [73] S.L. Vriezinga, R. Auricchio, E. Bravi, G. Castillejo, A. Chmielewska, P. Crespo Escobar, et al., Randomized feeding intervention in infants at high risk for celiac disease, *N. Engl. J. Med.* 371 (4) (2014) 1304–1315.
- [74] G. Du Toit, G. Roberts, P.H. Sayre, H.T. Bahnson, S. Radulovic, A.F. Santos, et al., Randomized trial of peanut consumption in infants at risk for peanut allergy, *N. Engl. J. Med.* 372 (9) (2015) 803–813.
- [75] G. Du Toit, P.H. Sayre, G. Roberts, M.L. Sever, K. Lawson, H.T. Bahnson, et al., Effect of avoidance on peanut allergy after early peanut consumption, *N. Engl. J. Med.* 374 (15) (2016) 1435–1443.
- [76] M.R. Perkin, K. Logan, A. Tseng, B. Raji, S. Ayis, J. Peacock, et al., Randomized trial of introduction of allergenic foods in Breast-Fed infants, *N. Engl. J. Med.* 374 (18) (2016) 1733–1743.
- [77] D.J. Palmer, T.R. Sullivan, M.S. Gold, S.L. Prescott, M. Makrides, Randomized controlled trial of early regular egg intake to prevent egg allergy, *J. Allergy Clin. Immunol.* 139 (5) (2017) 1600–1607 (e2).
- [78] J. Bellach, V. Schwarz, B. Ahrens, V. Trendelenburg, O. Aksunger, B. Kalb, et al., Randomized placebo-controlled trial of hen's egg consumption for primary prevention in infants, *J. Allergy Clin. Immunol.* 139 (5) (2017) 1591–1599 (e2).
- [79] J. Wei-Liang Tan, C. Valerio, E.H. Barnes, P.J. Turner, P.A. Van Asperen, A.M. Kakakios, et al., A randomized trial of egg introduction from 4 months of age in infants at risk for egg allergy, *J. Allergy Clin. Immunol.* 139 (5) (2017) 1621–1628 (e8).
- [80] O. Natsume, S. Kabashima, J. Nakazato, K. Yamamoto-Hanada, M. Narita, M. Kondo, et al., Two-step egg introduction for prevention of egg allergy in high-risk infants with eczema (PETIT): a randomised, double-blind, placebo-controlled trial, *Lancet* 389 (10066) (2017) 276–286.
- [81] E. Wollmann, C. Hamsten, E. Sibanda, M. Ochome, M. Focke-Tejkl, A. Asarnoj, et al., Natural clinical tolerance to peanut in African patients is caused by poor allergenic activity of peanut IgE, *Allergy* 70 (6) (2015) 638–652.
- [82] T. Platts-Mills, J. Vaughan, S. Squillace, J. Woodfolk, R. Sporik, Sensitisation, asthma, and a modified Th2 response in children exposed to cat allergen: a population-based cross-sectional study, *Lancet* 357 (2001) 752–756.
- [83] C. Lupinek, E. Wollmann, A. Baar, S. Banerjee, H. Breiteneder, B.M. Broecker, et al., Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip, *Methods* 66 (1) (2014) 106–119.
- [84] I. Skrinko, C. Lupinek, R. Valenta, V. Hovland, S. Pahr, A. Baar, et al., The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr. Allergy Immunol.* 26 (3) (2015) 239–246.
- [85] K.Y. Chua, G.A. Stewart, W.R. Thomas, R.J. Simpson, R.J. Dilworth, T.M. Plozza, et al., Sequence analysis of cDNA coding for a major house dust mite allergen, *Der p 1*. Homology with cysteine proteases, *J. Exp. Med.* 167 (1) (1988) 175–182.
- [86] F.S. Fang, M. Vitale, P. Fehner, King, cDNA cloning and primary structure of a white-face hornet venom allergen, antigen 5, *Proc. Natl. Acad. Sci. U. S. A.* 85 (3) (1988) 895–899.
- [87] H. Breiteneder, K. Pettenburger, A. Bito, R. Valenta, D. Kraft, H. Rumpold, et al., The gene coding for the major birch pollen allergen Betv1, is highly homologous to a pea disease resistance response gene, *EMBO J.* 8 (7) (1989) 1935–1938.
- [88] R. Valenta, R. Campana, K. Marth, M. van Hage, Allergen-specific immunotherapy: from therapeutic vaccines to prophylactic approaches, *J. Intern. Med.* 272 (2) (2012) 144–157.
- [89] G. Pauli, T.H. Larsen, S. Rak, F. Horak, E. Pastorello, R. Valenta, et al., Efficacy of recombinant birch pollen vaccine for the treatment of birch-allergic rhinoconjunctivitis, *J. Allergy Clin. Immunol.* 122 (5) (2008) 951–960.
- [90] R. Valenta, B. Linhart, I. Swoboda, V. Niederberger, Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens, *Allergy* 66 (6) (2011) 775–783.
- [91] R. Valenta, The future of antigen-specific immunotherapy of allergy, *Nat. Rev. Immunol.* 2 (6) (2002) 446–453.
- [92] V. Niederberger, F. Horak, S. Vrtala, S. Spitzauer, M.T. Krauth, P. Valent, et al., Vaccination with genetically engineered allergens prevents progression of allergic disease, *Proc. Natl. Acad. Sci. U S A* 101 (2004) 14677–14682.
- [93] R. Valenta, B. Linhart, Mechanisms underlying allergy vaccination with recombinant hypoallergenic allergen derivatives, *Vaccine* 30 (29) (2012) 4328–4335.
- [94] B. Linhart, M. Focke-Tejkl, M. Weber, M. Narayanan, A. Neubauer, H. Mayrhofer, et al., Molecular evolution of hypoallergenic hybrid proteins for vaccination against grass pollen allergy, *J. Immunol.* 194 (8) (2015) 4008–4018.
- [95] R. Valenta, R. Campana, V. Niederberger, Recombinant allergy vaccines based on allergen-derived B cell epitopes, *Immunol. Lett.* (2017), <http://dx.doi.org/10.1016/j.imlet.2017.04.015> pii: S0165-2478(17)30180-3.
- [96] P. Ziegelmayer, M. Focke-Tejkl, R. Schmutz, P. Lemell, R. Ziegelmayer, M. Weber, et al., Mechanisms, safety and efficacy of a B cell epitope-based vaccine for immunotherapy of grass pollen allergy, *EBioMedicine* 11 (2016) 43–57.
- [97] C. Cornelius, K. Schöneweis, F. Georgi, M. Weber, V. Niederberger, P. Ziegelmayer, et al., Immunotherapy with the preS-based grass pollen allergy vaccine BM32 induces antibody responses protecting against hepatitis B infection, *EBioMedicine* 11 (2016) 58–67.
- [98] R. Valenta, R. Campana, M. Focke-Tejkl, V. Niederberger, Vaccine development for allergen-specific immunotherapy based on recombinant allergens and synthetic allergen peptides: lessons from the past and novel mechanisms of action for the future, *J. Allergy Clin. Immunol.* 137 (2) (2016) 351–357.
- [99] M. Focke-Tejkl, M. Weber, K. Niespodziana, A. Neubauer, H. Huber, R. Henning, Development and characterization of a recombinant, hypoallergenic, peptide-based vaccine for grass pollen allergy, *J. Allergy Clin. Immunol.* 135 (5) (2015) 1207–1217 (e11).
- [100] M. Larché, T cell epitope-based allergy vaccines, *Curr. Top. Microbiol. Immunol.* 352 (2011) 107–119.
- [101] M. Worm, H.H. Lee, J. Kleine-Tebbe, R.P. Hafner, P. Laidler, D. Healey, Development and preliminary clinical evaluation of a peptide immunotherapy vaccine for cat allergy, *J. Allergy Clin. Immunol.* 127 (1) (2011) 89–99.
- [102] R. Valenta, M. Duchene, S. Vrtala, T. Birkner, C. Ebner, R. Hirschwehr, et al., Recombinant allergens for immunoblot diagnosis of tree-pollen allergy, *J. Allergy Clin. Immunol.* 88 (6) (1991) 889–894.
- [103] R. Hiller, S. Laffer, C. Harwanegg, M. Huber, W.M. Schmidt, A. Twardosz, et al., Microarrayed allergen molecules: diagnostic gatekeepers for allergy treatment, *FASEB J.* 16 (3) (2002) 414–416.
- [104] G.W. Canonica, I.J. Ansotegui, R. Pawankar, P. Schmid-Grendelmeier, M. van Hage, C.E. Baena-Cagnani, et al., A WAO - ARIA - GA²LEN consensus document on molecular-based allergy diagnostics, *World Allergy Organ J* 6 (1) (2013) 17.
- [105] P.M. Matricardi, J. Kleine-Tebbe, H.J. Hoffmann, R. Valenta, C. Hilger, S. Hofmaier, et al., EAACI molecular allergology user's guide, *Pediatr. Allergy Immunol. Suppl.* 23 (2016) 1–250.
- [106] M. Westman, A. Asarnoj, C. Hamsten, M. Wickmann, M. van Hage, et al., Windows of opportunity for tolerance induction for allergy by studying the evolution of allergic sensitization in birth cohorts, *Sem. Immunol.* (2017).
- [107] L. Hatzler, V. Panetta, S. Lau, P. Wagner, R.L. Bergmann, S. Illi, Molecular spreading and predictive value of preclinical IgE response to *Phleum pratense* in children with hay fever, *J. Allergy Clin. Immunol.* 130 (4) (2012) 894–901.
- [108] M. Westman, C. Lupinek, J. Bousquet, N. Andersson, S. Pahr, A. Baar, et al., Early childhood IgE reactivity to pathogenesis-related class 10 proteins predicts allergic rhinitis in adolescence, *J. Allergy Clin. Immunol.* 135 (5) (2015) 1199–1206.
- [109] A. Simpson, N. Lazic, D.C. Belgrave, P. Johnson, C. Bishop, C. Mills, et al., Patterns of IgE responses to multiple allergen components and clinical symptoms at age 11 years, *J. Allergy Clin. Immunol.* 136 (5) (2015).
- [110] A. Asarnoj, C. Hamsten, K. Wadén, C. Lupinek, N. Andersson, I. Kull, et al., Sensitization to cat and dog allergen molecules in childhood and prediction of symptoms of cat and dog allergy in adolescence: a BAMSE/MeDALL study, *J. Allergy Clin. Immunol.* 137 (3) (2016) 813–821.
- [111] D. Posa, S. Perna, Y. Resch, C. Lupinek, V. Panetta, S. Hofmaier, et al., Evolution and predictive value of IgE responses toward a comprehensive panel of house dust mite allergens during the first 2 decades of life, *J. Allergy Clin. Immunol.* 139 (2) (2017).
- [112] A. Asarnoj, C. Hamsten, C. Lupinek, E. Melén, N. Andersson, J.M. Anto, et al., Prediction of peanut allergy in adolescence by early childhood storage protein-specific IgE signatures: the BAMSE population-based birth cohort, *J. Allergy Clin. Immunol.* (2017), <http://dx.doi.org/10.1016/j.jaci.2016.12.973>.
- [113] C. Lupinek, K. Marth, V. Niederberger, R. Valenta, Analysis of serum IgE reactivity profiles with microarrayed allergens indicates absence of de novo IgE sensitizations in adults, *J. Allergy Clin. Immunol.* 130 (6) (2012).
- [114] R. Moverare, K. Westritschnig, M. Svensson, B. Hayek, M. Bende, G. Pauli, et al., Different IgE reactivity profiles in birch pollen-sensitive patients from six European populations revealed by recombinant allergens: an imprint of local sensitization, *Int. Arch. Allergy Immunol.* 128 (4) (2002) 325–335.
- [115] V. Garib, E. Wollmann, G. Djambekova, P. Lemell, M. Kmenta, U. Berger, et al., Possible effect of landscape design on IgE recognition profiles of two generations revealed with micro-arrayed allergens, *Allergy* (2017), <http://dx.doi.org/10.1111/all.13169>.
- [116] C.R. Cabauatan, C. Lupinek, S. Scheibhofer, R. Weiss, M. Focke-Tejkl, P.L. Bhalla, et al., Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates, *J. Allergy Clin. Immunol.* 133 (3) (2014) 910–914.
- [117] I. Mittermann, G. Wikberg, C. Johansson, C. Lupinek, L. Lundeberg, R. Cramer, et al., IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis, *PLoS One* 11 (5) (2016).
- [118] P. Panzer, M. Vachová, P. Vítová, P. Brodská, T.A. Vlas, A comprehensive analysis of middle-European molecular sensitization profiles to pollen allergens, *Int. Arch. Allergy Immunol.* 164 (1) (2014) 74–82.
- [119] V. Siroux, C. Lupinek, Y. Resch, M. Curin, J. Just, T. Keil, et al., Specific IgE and IgG measured by the MeDALL allergen-chip depend on allergen and route of exposure: the EGEA study, *J. Allergy Clin. Immunol.* 139 (2) (2017) 643–654.
- [120] P.F. Moreira, K. Gangl, A. Vieira Fde, L.H. Ynoue, B. Linhart, S. Flicker, et al., Allergen microarray indicates pooidae sensitization in brazilian grass pollen allergic patients, *PLoS One* 10 (6) (2015), <http://dx.doi.org/10.1371/journal.pone.0128402>.
- [121] M. Kulig, R. Bergmann, U. Klettke, V. Wahn, U. Tacke, U. Wahn, Natural course of sensitization to food and inhalant allergens during the first 6 years of life, *J. Allergy Clin. Immunol.* 103 (6) (1999) 1173–1179.
- [122] M. Kulig, U. Klettke, V. Wahn, J. Forster, C.P. Bauer, U. Wahn, Development of seasonal allergic rhinitis during the first 7 years of life, *J. Allergy Clin. Immunol.* 106 (5) (2000) 832–839.
- [123] R. Valenta, H. Hochwallner, B. Linhart, S. Pahr, Food allergies: the basics,

- Gastroenterology 148 (6) (2015) 1120–1131.
- [124] A.A. Schoemaker, A.B. Sprickelman, K.E. Grimshaw, G. Roberts, L. Grabenhenrich, L. Rosenfeld, et al., Incidence and natural history of challenge-proven cow's milk allergy in European children–EuroPrevall birth cohort, *Allergy* 70 (8) (2015) 963–972.
- [125] R. Moverare, T. Petays, E. Vartianen, T. Haahtela, IgE reactivity pattern to timothy and birch pollen allergens in Finnish and Russian Karelia, *Int. Arch. Allergy Immunol.* 136 (2005) 33–38.
- [126] S. Bitoh, M. Takata, P.K. Maiti, V. Holford-Strevens, D. Kierek-Jaszczuk, A.H. Sehon, Antigen-specific suppressor factors of noncytotoxic CD8 + suppressor T cells downregulate antibody responses also to unrelated antigens when the latter are presented as covalently linked adducts with the specific antigen, *Cell. Immunol.* 150 (1993) 168–193.
- [127] C.R. Oliveira, E.A. Taniguchi, A.E. Fusaro, J.R. Victor, C.A. Brito, A.J. Duarte, et al., Bystander effect in synergy to anergy in oral tolerance of *Blomia tropicalis*/ovalbumin murine co-immunization model, *J. Clin. Immunol.* 25 (2005) 153–161.