

Animal models of type I allergy using recombinant allergens

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Accepted 21 August 2003

Abstract

Various animal models including guinea pigs, monkeys, dogs, rats, and mice have been established in an attempt to provide insights into the complex immunological and pathophysiological mechanisms of human type I allergic diseases. The detailed knowledge of the murine genome, the various components of the murine immune system, and the generation of engineered mice has made the murine system the most attractive among all animal models. The availability of multitude technologies and reagents to characterize and manipulate immunological pathways and mediators adds to the outstanding opportunities to assess the pathology of allergic diseases and to develop novel therapeutic strategies in mice. Numerous sensitization protocols with food and aero-allergens are used to establish an allergic/asthma-like phenotype in mice. Requirements for an appropriate murine model include a close resemblance to the pathology of the disease in humans, the objective measurement of the physiologic parameters, as well as reliability and reproducibility of the experimental data. With respect to reproducible experimental conditions, it has been recognized that extract preparations from natural allergen sources can vary in their allergen-content and -composition. This might influence the degree of sensitization or the outcome of treatment strategies in dependence of the applied extract preparation. The use of recombinant allergens in experimental *in vivo* and *in vitro* systems can overcome these problems. Another aspect, that has become obvious from the experimental studies, is that allergens can differ in their immunogenicity as well as in their capacity to act as tolerogens. Therefore, it seems important that the efficacy of the different allergen-molecules to act as therapeutic agents is individually examined. In this review, examples of animal models are described, in which recombinant allergens have been used for sensitization and/or treatment of allergic responses and how they have been used to enhance our understanding of the pathology of allergic diseases.

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1. Background

1.1. Type I allergy and asthma

Allergic diseases such as bronchial asthma are involving complex interactions between exogenous factors such as allergen exposure, infections and other environmental stimuli, and genetically determined factors. Bronchial asthma is a chronic inflammatory disease defined by chronic airway inflammation, reversible broncho-obstruction, and non-specific airway hyper-responsiveness (AHR). Sensitization to normally harmless environmental antigens (pollen, house-dust mite, etc.) is thought to be the prerequisite for initiating the

inflammatory cascade in bronchial asthma. Repeated and continuous allergen-aerosol exposure causes inflammation of the airway mucosa and submucosa, which is orchestrated by Th-2 type T-cells. These cells produce Th-2 cytokines (IL-4, IL-5, IL-9, IL-13, and IL-15), which regulate both IgE production and airway inflammation. Particularly, airway inflammation is thought to be the main underlying pathological aspect of bronchial asthma, characterized by the influx of eosinophils and activated CD4+ T-cells. Tissue damage due to eosinophil degranulation products—and as a consequence airway mucosa remodelling—may contribute to severity of the disease and development of AHR. An ideal treatment of already established bronchial asthma should cure the disease. At present, as for most multifactorial and chronic conditions, this has not been achieved. Another important approach represents primary and secondary prevention. Normally, environmental allergens are recognized as

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harmless antigens. The appropriate immune response pattern can be described as development of T-cell tolerance. Inability to either develop or maintain tolerance will then result in the development of Th-2 mediated inflammation in a susceptible individual. The immune system offers a wide range of mechanisms to prevent or suppress such dysregulations including induction of counter-active Th-1 cells, CD-8 suppressors, regulatory T-cells, and production of anti-inflammatory/suppressive cytokines, including IL-10 and TGF- β .

1.2. Current treatment against type I allergy in humans

Specific immunotherapy (SIT) is performed by injection of increasing amounts of allergen extracts to induce hyporesponsiveness to the respective allergens. Even though SIT is an effective treatment in many cases of allergic rhinitis and in allergy to insect venom, certain drawbacks, such as the long duration of the treatment or the risk of anaphylactic side reactions, have to be taken into account. Moreover, the use of allergen extracts encounters the possibility of *de novo* sensitization against components present in natural allergen preparations. Therefore, the concept of a component resolved, patient-tailored therapy using the disease-eliciting allergen in recombinant form, as well as a change to less invasive routes have been suggested for enhanced treatment efficacy [1,2].

The mechanisms operating in SIT are still not completely understood. It seems that several immunological pathways are involved in the clinical improvement achieved by the common SIT schedules: (I) rise in allergen-specific IgG antibodies, in particular IgG4, which is supposed to block the allergen and IgE-facilitated allergen presentation to T-cells [3–5]; (II) generation of IgE-modulating CD8+ T-cells [6]; (III) reduction in the number of mast cells, eosinophils, and release of mediators [7,8]; and (IV) modulation of allergen-specific T cells: a shift from Th-2 to Th-1 cytokine pattern with a decrease of IL-4 and IL-5 production accompanied by an increase of IFN- γ (immune deviation) [9–11]; (V) moreover, the induction of an anergic state in peripheral T-cells (immunologic tolerance), which is mediated by IL-10 and characterized by suppressed proliferative and cytokine responses against major allergens, represents an important aspect in successful SIT [12].

1.3. Mucosal tolerance

Mucosal tolerance describes a state of an antigen-specific non-responsiveness of the immune system induced by mucosal application of soluble antigen. Based on the physiological role to inhibit hypersensitivity reactions towards harmless antigens—such as environmental inhalant allergens, food or the commensal bacterial flora—mucosal tolerance induction has been

suggested as a treatment strategy against several inflammatory diseases. In that respect experimental studies have demonstrated the efficacy of mucosal antigen administration in preventing the onset or severity of certain Th-1-based autoimmune diseases [13–15] and in suppressing Th-2 based allergic immune responses [16–18].

The mechanisms of tolerance induction seem to be very complex and depend on many factors, such as the nature of the antigen, antigen-dose, and frequency of application. High-dose antigen administration preferentially leads to T-cell anergy or deletion [19–21]. Application of low antigen-doses favours the induction of regulatory T-cells, of which several subtypes with distinct cytokine secretion pattern of TGF- β and IL-10 have been described [22–24]. Additional data suggest also a role of CD8+ Tr cells in tolerance induction [25]. Antigen-presenting cells, in particular dendritic cells (DCs) seem to play an important role in determining the outcome of an immune response: in dependence of the maturation state [26,27] and/or the phenotype of DCs [28] antigen presentation leads to suppressive immune responses or induction of inflammatory signals. Recently, distinct DC populations from different mucosal compartments were described, showing that gastrointestinal tract-DCs preferentially lead to induction of TGF- β producing Th3 cells, whereas DCs from the respiratory tract favour the activation of IL-10 producing Tr-1 cells [29,30].

2. Animal models of type I allergy and asthma

An ideal mouse model for human type I allergic diseases such as bronchial asthma should resemble the major features of the human disease including high levels of allergen-specific immunoglobulin production, immediate hypersensitivity with early and late phase responses, airway inflammation characterized by the influx of T-cells, eosinophils and some neutrophils, development of airway hyperresponsiveness, and chronic airway remodelling with deterioration in lung function. Until now no mouse model has been available, which resembles all of these features of human bronchial asthma. Especially the development of chronic airway inflammation and airway remodelling is not yet reached. In mice, exposure to allergens induces allergen-specific immunoglobulin production associated with the development of immediate cutaneous hypersensitivity reactions after allergen challenge. In addition, after repeated airway allergen challenges these mice develop acute but not persistent airway inflammation, including eosinophil accumulation and both, airway hyperresponsiveness to unspecific stimuli such as methacholine and acute broncho-obstruction during allergen-aerosol exposure [31,32]. However, the level and quality of such a Th-2

biased immune response depend largely on the genetic background of the mice, the route, dose and frequency of exposure to the allergen, and the type of adjuvant being used.

2.1. Genetic background (Table 1)

As in humans, the establishment of a full-type asthmatic syndrome in mice seems to be highly dependent on the genetic background of the used mouse strain. Based on the level of allergen-specific IgE/IgG1 production and the degree of airway inflammation following repeated airway allergen challenges, high- and low-responder mouse strains were identified. It becomes apparent that each individual mouse strain demonstrates a unique response pattern following immunization to allergens. In many studies, either BALB/c or C57BL/6 mice were used. BALB/c mice are known as IgE-high responders to many allergens (e.g., ovalbumin, OA), whereas C57BL/6 and SJL mice are characterized as intermediate and low-IgE responder animals (Table 1). All strains develop cutaneous immediate-type hypersensitivity reactions due to mast cell degranulation triggered by cross-linking of membrane-bound allergen-specific IgE and IgG1 antibodies after allergen challenge. However, the inflammatory component of the lung and the severity of altered lung function differ between the strains [33]. In OVA-sensitized BALB/c mice, a marked increase in lymphocytes, eosinophils, and neutrophils in bronchoalveolar lavage (BAL fluids) is paralleled by elevated production of IL-4, IL-5, and TNF- α in the lung. In contrast, in OVA-sensitized C57BL/6 mice, the inflammatory immune response in the lung is much weaker. Moreover, BALB/c mice demonstrated greater degree of airway hyperreactivity as C57BL/6 mice, indicating that the same allergen causes different phenotypes dependent on genetical prerequisites. Although the recruitment of eosinophils to the airways is a central feature of all mouse models of asthma, and although this cell population has been linked to the development of the asthma phenotype, the contribution of eosinophils to the development of altered airway function remains controversial. Indeed, several studies with wild-type and knock-out mice im-

plicate IL-4 as the key cytokine and suggest that increased airway resistance (AR) can occur independently of IL-5 and eosinophils [34–37], whereas other studies provide equally convincing evidence that IL-5 is the critical cytokine [38–41]. Furthermore, it has been shown that passive sensitization of BALB/c mice with allergen-specific IgE and IgG1 antibodies alone is sufficient to induce a state of increased AR and airway inflammation following airway allergen challenges [42]. Recently, it was demonstrated that early phase bronchoconstriction requires allergen-specific IgG1, but is independent of allergen-specific IgE and mast cell activity [43].

2.2. Protocols of allergic sensitization and airway inflammation

Sensitization to inhalant and/or food allergens determines the first phase in a dynamic set of events leading to allergic bronchial asthma. In mice, a wide variety of soluble proteins are used to establish an allergen-specific IgE/IgG1 immune response, which is associated with the development of cutaneous immediate-type hypersensitivity responses and airway inflammation. However, several factors directly influence the quality and quantity of the immune response to the allergen and differences in the protocols can have drastic effects on the development of the asthma-like phenotype in mice, and as a consequence appropriate interpretation of the results becomes difficult. As a model-allergen ovalbumin, a dietary allergen in humans is widely used in animal experiments, in addition to a variety of several clinically important inhalant allergens—such as those from birch and grass pollen or house-dust mite allergen, and pet allergen—used for allergic sensitization [44]. The route of allergen administration has an important impact on the quality of the immune response. The highest immune response is achieved by a combination of systemic sensitization, to induce peripheral priming of the immune response, and repeated airway allergen challenges to induce airway inflammation. This is achieved by systemic (subcutaneous, intraperitoneal) injection(s) of antigen/allergen adsorbed to the adjuvants aluminium hydroxide Al(OH)₃, long known to induce Th-2

Table 1

Development of immediate type I hypersensitivity responses to allergens, airway inflammation, and altered lung function in different mouse strains

Mouse strain	MHC class-II	OVA	Birch pollen	House-dust mite	Ragweed
BALB/c	H-2 ^d	High	High	Low	Low
C57BL/6	H-2 ^b	Intermediate	Intermediate	High	High
CBA	H-2 ^k	Intermediate	n.d.	High	High
SJL	H-2 ^s	Low	n.d.	Low	n.d.

Mice of different strains were sensitized by intraperitoneal injection of the allergen on days 1, 14, and 21. On days 26 and 27, mice received two consecutive airway allergen challenges by aerosolization of the allergen as previously described. Development of an asthma-like phenotype was assessed by measurement of allergen-specific serum IgE/IgG1 antibody titers, assessment of airway inflammation, determination of airway responsiveness to unspecific stimuli, and analysis of skin test responses. Animals were considered as “high responder” mice if all read-out systems gave positive results. Low responders were mice that did not mount a specific IgE response and had normal airway responses.

responses, followed by multiple airway allergen challenges. The allergen could be delivered either by aerosolization of the allergen or instillation by intratracheal or intranasal application. Such protocols result in a maximal and reproducible induction of an inflammatory immune response in the airways paralleled by development of airway hyperresponsiveness.

That systemic priming prior to mucosal antigen application is essential for induction of allergic immune responses has become obvious from studies by Holt et al., showing that antigen inhalation alone led to a preferential suppression of antigen-specific IgE antibodies [16,45]. The phenomenon of peripheral tolerance induction via mucosal surfaces has been recognized early in animal models after oral antigen application and ever since then it has been referred to as oral/mucosal tolerance [46].

2.3. The nature of the antigen

In the majority of animal models ovalbumin (OVA) has been used as a model antigen to study pathophysiological events and the mechanisms behind experimental and clinical immunomodulations. In particular, the availability of OVA-transgenic mice has proved to be of great advantage in studies on the inductive and effector sites of immunostimulation and suppression [47].

However, with respect to experimental models of allergic asthma, sensitization with an inhalant rather than a dietary allergen may be closer to the situation in humans. Moreover, it is well recognized that different antigens can vary in their immunogenicity as well as their capacity to act as tolerogens. As an example, we have previously noticed that OVA, applied under identical experimental conditions as the birch pollen allergen Bet v 1, was less immunogenic (in conjunction with a mucosal adjuvant) and tolerogenic than the inhalant allergen [48]. Therefore, it seems necessary that the efficacy of each allergen molecule/construct to act as potential therapeutic agent is individually examined in a suitable model of type I allergy.

2.4. Role of adjuvants

Adjuvants are frequently used in conjunction with different vaccines, including SIT. Apart from the immunoenhancing effects, certain adjuvants can also influence the quality of an immune response. Parenteral antigen administration in conjunction with Al(OH)₃ is known to promote a Th-2 response and is therefore a commonly used adjuvant for induction of Th-2 immune responses. In mice, these responses are characterized by the appearance of allergen-specific IgE and IgG1 antibodies in blood, associated with the production of Th-2 type cytokines of primed lymphocytes. Certain bacterial compounds [49,50], bacterial DNA [51,52], or CpG-ol-

igonucleotides [53,54] rather induce Th-1 responses, which belong to a new generation of immunomodulators, examined for potential use for treatment of type I allergy. Counterbalancing Th-2 inflammatory reactions can also be achieved through infection or vaccination, which stimulates the Th-1 development. Mouse experiments suggested a protective effect of *Bacillus Calmette–Guérin* (BCG) vaccination [55–57]. Although findings do not necessarily implicate a role of BCG vaccination in preventing allergy in humans, limited studies indicated that *M. vaccae* treatment in adults with asthma and rhinitis or in children with atopic dermatitis demonstrated clinical benefits as measured by reduction in use of rescue medication, severity of disease or inhibition of the allergen-induced late-phase response [58,59].

In the case of mucosally applied antigens, the use of certain mucosal adjuvants is required to induce any proper immune response. Among these, cholera toxin (CT), the enterotoxin of *Vibrio cholerae*, is one of the most potent experimentally used adjuvants [60]. We have previously shown that aerosol-treatment of allergen together with the mucosal adjuvant cholera toxin induced a Th-1 response in naïve mice and modulated an allergic immune response in sensitized mice. We proposed that also certain mucosal adjuvants could be useful tools for treatment strategies against allergies [61].

3. Animal models with recombinant allergens (Table 2)

3.1. Pollen allergy

Birch pollen allergy. We have previously established a murine model of allergic sensitization to birch pollen. BALB/c mice were identified as high IgE-responders to the major birch pollen allergen Bet v 1. The standard sensitization scheme is based on an intraperitoneal injection of recombinant (r) Bet v 1 adsorbed to Al(OH)₃, followed by an aerosol challenge with the whole birch pollen extract. This sensitization procedure leads to high allergen-specific IgE and IgG1 levels, associated with positive type I skin tests in vivo, as well as high IL-4 and IL-5 versus low IFN- γ production in vitro in response to birch pollen. Moreover, these mice develop airway inflammation, characterized by eosinophilia and IL-5 levels in lungs and bronchial alveolar lavage (BAL), as well as airway hyperresponsiveness [62,63]. Using this model immunomodulation was achieved either by injection of the immunodominant peptide [64] or by mucosal application of rBet v 1 prior to or after sensitization. Intranasal or oral application of rBet v 1 led to suppression of allergen-specific antibody levels of all isotypes, reduction of IL-4, IL-5, and IFN- γ , as well as inhibition of airway inflammation and airway hyperresponsiveness in naïve and sensitized mice [65,66]. Low dose tolerance induction prior to sensitization lasted up

to a year and at least 6 months in mice tolerized after sensitization. This indicated that Bet v 1 acts as a strong tolerogen. Further experiments were carried out to investigate the underlying mechanisms of tolerance induction. It was found that the state of tolerance was associated with increased levels of TGF- β . Furthermore, tolerance could be transferred by CD4+ T-cells, and to a lesser extent also by CD8+ T-cells, indicating active suppression as a central operating mechanism [67] (see Table 2).

Grass pollen allergy. Unlike in birch pollen allergy, several allergens have been characterized as major allergens in grass pollen allergic patients. To analyse the allergenic potency of these allergens, BALB/c mice were immunized with recombinant Phl p 1, Phl p 2, Phl p 5 or Phl p 6. It was demonstrated that only those allergens that bind high levels of IgE in humans induced high IgE and IgG1 levels in mice, indicating that the immunogenicity of the allergens is similar in humans and mice. Moreover, repeated immunizations with single recombinant allergens led to induction of protective IgG1 antibodies, since these IgG1 antibodies strongly inhibited human IgE binding to the allergen and suppressed allergen-induced histamine release from human basophils [68].

Using recombinant grass pollen allergens for intranasal tolerance induction revealed that the tolerogenic property of an allergen was not closely related to its immunogenicity: in comparison to Bet v 1, tolerance induction with Phl p 5 only led to incomplete immunosuppression, whereas sensitization with Phl p 5 led to higher humoral and cellular immune responses as well as stronger airway inflammation than in Bet v 1 sensitized mice [69].

Other models of pollen-associated allergies have been established aiming at evaluating the immune responses to recombinant and natural Ole 1 in comparison to the sensitization pattern of olive pollen allergic patients [70], or to study the efficacy of peptide-based oral immunotherapy against Japanese cedar pollinosis [71].

3.2. House-dust mite allergy

A model of allergic asthma to house dust mite was established by systemic priming and subsequent intranasal challenge with minute amounts (1 μ g) of the group 1 allergen of *Dermatophagoides pteronyssinus*, Der p 1 in C57BL/6J mice. This sensitization protocol led—in the absence of allergen-specific IgE—to extensive pulmonary eosinophilic inflammation, which shared many features of inflammation found in human asthma, such as eosinophilic influx into the large airways, mucus plugging, and formation of Charcot–Leyden crystals [72]. These results differed from our studies in the model of birch pollen allergy in BALB/c mice, where airway inflammation was only achieved by inhalation of high doses

(mg amounts) of allergen and in the presence of IgE. These differences might be related to either distinct intrinsic properties of various allergens or to genetic differences in the mouse strains used to sensitize against house-dust mite and birch pollen.

It was shown further that intranasal administration of high doses of the immunodominant T-cell epitope of Der p 1 induced long-lasting tolerance. Tolerance to the immunodominant epitope inhibited T-cell responses to all other epitopes of Der p 1 in mice challenged with the intact protein. This phenomenon was termed “linked suppression,” mediated by CD4+ cells. The mechanism of linked suppression was not associated with suppressive or anti-inflammatory cytokines, such as TGF- β or IL-10, but with the Notch signalling pathway, that generally regulates cell differentiation through cell–contact dependent mechanisms. In this case, cell–cell contact via an interaction of Delta-1 expressed on inhibitory T-cells and its ligand Notch on naïve cells may result in transmission of negative signals preventing clonal expansion [73–75].

Recently, it was demonstrated that the route/type of sensitization can influence the effects of peptide induced tolerance. Only after systemic immunization with Der p 1, but not after respiratory immunization—subsequent to intranasal peptide tolerance induction—C56BL/6J mice exhibited reduced allergen-specific IgE; immunosuppression at the cytokine level (Th-1 and Th-2 responses) was however induced by either type of sensitization [76].

A murine model of type I allergy to the group 2 allergen of *Dermatophagoides farinae*, Der f 2, was established aiming at inducing tolerance by oral application of recombinant Der f 2 [77]. Very high oral doses (20 mg) of the recombinant allergen were necessary to suppress allergen specific immune responses as well as immediate airway constriction and neutrophil influx into the airways [77]. Intranasal treatment with recombinant Der f 2 or a mutant of Der f 2 was associated with a downregulation of CD23 expression on B-cells; however, no direct changes in allergen-specific IgE/IgG1 or Th2 cytokines, such as IL-4 or IL-5, were observed [78].

3.3. Latex allergy

Mice have been shown to mount a dose and time dependent IgE response to latex proteins following topical, respiratory, and subcutaneous exposure [79]. Bronchoconstriction was only elicited after topical, intranasal or intratracheal, but not after systemic immunization with natural allergens [80]. These data indicated that different clinical manifestations of the allergic responses depend on the route of sensitization [81,82]. In another study using two major recombinant allergens of latex, rHev b 1, and rHev b 3, high levels of

allergen-specific IgE/IgG1 antibodies were induced by systemic immunization. Murine T cells recognized the same T-cell epitopes as human T-cell clones from latex allergic patients [83,84]. Intranasal tolerance induction prior to sensitization with the recombinant latex allergens led to suppression of allergen-specific IgG1/IgE levels and cytokine production (IL-4, IL-5, and IFN- γ) in vitro. In contrast to tolerance induction with the major birch pollen allergen Bet v 1, intranasal treatment with recombinant Hev b 1 or Hev b 3 was less effective in sensitized BALB/c mice, indicating that allergens have varying tolerogenic properties and require individual doses for successful treatment [84].

3.4. Cat allergy

Mice sensitized with Fel d 1, the major cat allergen, exhibited T-cell, B-cell, and mast-cell responses when challenged with the protein. Subcutaneous injections of peptides containing the immunodominant T-cell epitopes of the allergen induced T-cell tolerance in pre-sensitized mice. The fact that these tolerized mice produced a decreased amount of histamine in vivo, indicated that histamine release was not solely dependent on the reduction of allergen-specific IgE. These data showed that mast cell activity in mice with established type I allergy can be regulated through peptide-induced T-cell tolerance [85,86].

3.5. Bee venom/Wasp venom allergy

In CBA/J mice, sensitized with phospholipase A2 (PLA₂)—a major bee venom allergen—daily treatment for 6 days by i.p. injections with overlapping peptides, spanning the entire PLA₂ molecule, resulted in significant reduction of allergen-specific IgE and an increase in IgG2a antibodies. The marked T-cell unresponsiveness was characterized by a shift from a Th2 to a Th1 profile. Upon rechallenge with the native PLA₂, the treated mice were fully protected from anaphylaxis [87]. Also after intranasal application of the overlapping peptides of PLA₂ an immunological hyporesponsiveness, associated with an enhanced IgG2a response, was induced. As underlying mechanism clonal anergy was discussed, affecting more profoundly the Th-2 responses [88]. Successful immunotherapy with T-cell epitope-peptides inducing T-cell anergy was recently shown in bee venom allergic patients [89].

Concerning wasp venom allergy we have sensitized BALB/c mice with *Vespula vulgaris* venom extract without the use of adjuvant, thereby mimicking the natural route of sensitization. Mucosal pretreatment with recombinant Ves v 5, one of the major allergens of wasp venom, led to immunosuppression not only against Ves v 5 but also against the whole wasp venom extract. Unlike in the studies with bee venom PLA₂,

tolerance induction was not associated with a shift from Th-2 to Th-1 responses, but a general immunosuppression due to regulatory mechanisms [90].

4. New approaches for prophylaxis and therapy of type I allergy

4.1. Systemic immunization—induction of protective IgG

Allergen-derivatives with reduced allergenicity have been promoted to prevent anaphylactic side reactions during SIT [91]. In that respect, it was shown that immunization of mice with two recombinant fragments of Bet v 1, which failed to bind IgE due to a loss of B cell epitopes, induced the production of IgG antibodies that cross-reacted with the complete Bet v 1. The antibodies inhibited the IgE binding of allergic patients to the wild type allergen [92,93]. Similar results of induction of protective IgG antibodies were achieved after immunization of mice with peptides derived from B-cell epitopes of Phl p 1 [94], or with recombinant hybrid allergens of the major grass pollen allergens Phl p 1, Phl p 2, Phl p 5, and Phl p 6 [95].

Induction of non-anaphylactic blocking IgG antibodies has also been achieved using Bet v 1 mimotopes for immunization [96]. Conjugation of these mimotopes to monovalent fusion proteins not only generated IgG that could inhibit human IgE binding, but also presented to be safe candidates for therapy as these conjugates could not cross-link cell-bound IgE in sensitized mice [97].

4.2. Induction of mucosal tolerance

Hypoallergenic molecules have also been shown to be promising candidates for mucosal tolerance induction. Prophylactic treatment with a hypoallergenic fragment of Bet v 1 led to a comparably strong suppression of T and B cell responses and inhibition of airway inflammation as with the whole recombinant Bet v 1 molecule [67]. Recent data suggest that such fragments can also be successfully used for mucosal treatment of already sensitized mice [98]. The mechanism of tolerance induction seems to depend on the structure of the molecule, as fragment treated mice—in contrast to Bet v 1 treated mice—did not exhibit elevated TGF- β levels nor was tolerance transferable with CD4⁺ or CD8⁺ T cells [67].

Based on the fact that many allergic patients are sensitized to several unrelated allergens, tolerance induction with a panel of simultaneously applied allergens is a desirable goal. We have established a model of polysensitization with recombinant Bet v 1, Phl p 1, and Phl p 5. Intranasal tolerance with a mixture of the immunodominant peptides of the three allergens led to a marked decrease of the IgE/IgG2a ratio and a decrease in Th2 cytokines. Similarly as observed after treatment

Table 2
Examples for mouse models of type I allergy using recombinant allergens

Antigen	Mouse strain	Sensitization	Treatment	Effects		Reference
OVA	BALB/c	i.p. OVA/alum	Oral OVA prior sens.	IgE IgG1 IFN- γ	– = +	Van Halteren et al. [114]
OVA	BALB/c	i.p. OVA/Al(OH) ₃ , OVA aerosol challenge	n.d.	IgG1/IgE IL-4/IL-5 Airwayinflamm. AHR		Renz et al. [115] Herz et al. [33]
Bet v 1	BALB/c	i.p. Bet v 1/Al(OH) ₃ ; aerosol with birch pollen extract	i.n., oral rBet v 1 prior and after sens.	IgG1, IgE, IgG2a Skin tests IL-4, IL-5, IFN- γ TGF- β Airwayinflamm. AHR-	– – – + – –	Wiedermann et al. [48] Winkler et al. [66]
Bet v 1	BALB/c	i.p. Bet v 1/Al(OH) ₃ ; aerosol with birch pollen extract	i.n. Bet v 1- fragment prior sens.	IgG1, IgE, IgG2a Skin tests IL-4, IL-5, IFN- γ TGF- β Airwayinflamm. AHR	– – – – – +	Wiedermann et al. [63]
Bet v 1	BALB/c	i.p. Bet v 1/Al(OH) ₃ ; aerosol with birch pollen extract	i.n., s.c., i.p. CpG-ODN + Bet v1 prior and after sens.	IgG1, IgE IgG2a IFN- γ IL-5, eosinophils	= + + –	Jahn-Schmid et al. [104]
Bet v 1	BALB/c	i.p. Bet v 1/Al(OH) ₃	i.d. plasmid DNA (pCMV-Bet v1)+/– CpG-ODN	IgG2a/IgG1 ratio IFN- γ	+ +	Hartl et al. [101]
Bet v 1	BALB/c	i.p. Bet v 1/Al(OH) ₃	i.n. <i>L. plantarum</i> , <i>L. lactis</i> + Bet v 1 prior and after sens.	IgG2a/IgG1 ratio IFN- γ IL-5/IFN- γ ratio	+ + –	Repa et al. [112]
Ole e 1/ Olive pollen	BALB/c	i.p. Ole e 1/Al(OH) ₃	n.d.	IgG1, IgE IL-4		Batanero et al. [70]
Cry j 2/ Japanese cedar pollen	BALB/c	i.n. Cry j 2 + CT	Oral Cry j 2-peptide prior and after sens.	IgG1, IgE IL-2 IL-4 IFN- γ	– – – –	Hirahara et al. [71]
Der p 1	C57BL/6	s.c. Der p 1/Al(OH) ₃ ; i.n. Der p 1	n.d.	Eosinophils, mucus plugging, Charcot-Leyden crystals in lungs		Clark et al. [72]
Der p 1	C57BL/6	s.c. Der p 1/FCA	i.n. peptides prior and after sens.	DTH IgE IL-2, IFN- γ IL-3, IL-4	– = – –	Hoyne et al. [73–75]
Der p 1	C57BL/6J	i.p. Der p 1/Al(OH) ₃	i.n. peptide	IgE IL-4, IL-5 IFN- γ	– – –	Jarnicki et al. [76]
		i.n. Der p 1 + LTB	i.n. peptide	IgE IL-4, IL-5 IFN- γ	= – –	Jarnicki et al. [116]
Der f 2	A/J	i.p. Der f 2/FCA i.p. Der f 2/Al(OH) ₃	Oral Der f 2 after sens. i.n. peptides after sens.	IgG1, IgE Airwayinflamm. CD23	– – –	Yasue et al. [77] Yasue et al. [78]
Latex allergen	BALB/c	topical, i.n., s.c. latex allergens	n.d.	IgE Eosinophils AHR		Woolhiser et al. [81] Meade et al. [82] Thakker et al. [80]

Table 2 (continued)

Antigen	Mouse strain	Sensitization	Treatment	Effects	Reference
Hev b 1, Hev b 3	BALB/c	i.p. Hev b 1, Hev b 3 /Al(OH) ₃	i.n. Hev b1, Hev b 3	IgG1, IgE IL-4, IL-5 IFN- γ	– – – Hufnagl et al. [69]
Fel d 1	BALB/c	s.c. Fel d 1/Al(OH) ₃	s.c. Fel d 1 peptides prior and after sens.	IL-4, IFN- γ Histamine- release	– – Briner et al. [85] Treter et al. [87]
Bee venom	CBA/J	i.p. PLA ₂ /Al(OH) ₃	i.p. PLA ₂ peptides or i.n. PLA ₂ peptides prior and after sens.	IgE IgG2a IL-4 IFN- γ	– + – + Von Garnier et al. [81] Astori et al. [88]
Wasp venom	BALB/c	Wasp venom +/- Al(OH) ₃	i.n. Ves v 5 prior sens.	IgG1,IgE IgG2a IL-4, IL-5, IFN γ	– – – Winkler et al. [66]

Examples for mouse models of type I allergy using recombinant allergens. Different sensitization protocols, as well as treatment approaches are listed. Symbols (+, –, =) indicate that the immunological and inflammatory effects in response to allergic sensitization are reduced (–), enhanced (+) or remain unchanged (=) by the respective treatment intervention.

Examples for mouse models of allergic sensitization without treatment procedures (n.d., not done) are also given, for which the effects upon allergen exposure are listed without any scoring.

Abbreviations: i.n., intranasal; i.p., intraperitoneal; s.c., subcutaneous; i.d., intradermal; airwayinflamm., airway inflammation; and AHR, airway hyperresponsiveness. LTB₄, B subunit of the heat labile enterotoxin of *Escherichia coli*.

with the hypoallergenic Bet v 1 derivative, IL-10 and TGF- β levels were decreased, suggesting operative mechanisms of anergy [69]. Production of hybrid peptides or chimeric molecules, containing the immunodominant sequences of several allergens, may even enhance the efficacy of tolerance induction in polysensitized organisms and may represent a novel form of a “mucosal poly-valent allergy vaccine.”

4.3. Induction of immunomodulation—shift from Th-2 to Th-1

DNA-based vaccination represents an attractive alternative to protein-based desensitization. In that respect, it was recently shown that prophylactic vaccination with a recombinant adenovirus expressing a model allergen (β -galactosidase) led to induction of allergen-specific IgG2a antibodies and IFN- γ production in T cells. After subsequent immunization with the antigen, production of specific IgE was abolished, indicating a successful shift from Th-2 to Th1-responses. Suppression of an ongoing IgE response turned out to be the more difficult task and seemed to be strictly dependent on the immunization protocol [99]. Similar results were achieved by vaccination of plasmids encoding the latex allergen Hev b 5 [100], the birch pollen allergen Bet v 1 [101], or the Japanese cedar pollen allergen Cry 1 [102].

Along these lines, application of CpG-oligonucleotides, interacting with the Toll-like receptor 9, also results in strong Th-1 responses [103]. Such CpG motifs have been shown to prevent pulmonary inflammation and Th-2 responses in mice [54,103,104]. In vitro experiments revealed induction of Th-1 responses in

PBMCs from allergic patients [105]. Conjugation of CpG-ODNs to allergenic molecules or peptides was more effective than their co-administration [106]. Based on these promising data, the evaluation of CpG-ODNs for treatment of allergic disease in human is in progress.

On the basis of recent epidemiologic and clinical studies, a possible role of certain lactic acid bacteria (LAB) in the prevention of allergic diseases has become evident [107,108]. Recent experimental studies have shown a reduction of IgG1 or IgE when certain LAB were injected or orally applied together with the particular allergen [109–111]. In the mouse model of birch pollen allergy, we could demonstrate that intranasal co-application of certain LAB bacteria with the recombinant Bet v 1, prior and after sensitization with the allergen resulted in a shift from Th-2 to Th-1 responses, characterized by a marked reduction of the IgE/IgG2a ratio accompanied by increased IFN- γ production. We anticipated that local delivery of allergens by recombinant LAB may even enhance the protective/immunomodulatory effects against allergies [112]. Along these lines, a recent study using recombinant LAB expressing the house dust mite allergen Der p 1 could demonstrate the efficacy of such a mucosal allergen-delivery system in reducing allergic immune responses [113].

5. Concluding remarks

It is unquestionable that animal models are important tools for preclinical evaluation of new approaches for prophylaxis and therapy against different diseases including allergies. The results from the experimental studies using alternative routes of antigen administration,

application of recombinant or hypoallergenic molecules, or new antigen-carrier systems to induce counter-regulatory immune responses are generally very promising. However, it is also obvious that in dependence of the experimental model and the antigen used, the effects as well as the mechanisms of action can vary, which might indicate the complexity of predicting clinical consequences of a new therapeutic approach.

The most difficult task therefore remains the step from promising animal studies to successful use in humans, which can be bridged by characterization of immune responses towards potential therapeutic agents in healthy subjects, including studies on the mechanisms in human *in vitro* systems, prior to application in allergic patients.

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