

Design and Modifications of Allergens for Improving Specific Immunotherapy

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Abstract: Specific immunotherapy is a clinically effective causative treatment for allergic conditions. However, the reagents used for immunotherapy are crude extracts, prepared from natural sources, and as such may cause potential life-threatening anaphylactic side effects. Our increasing knowledge of mechanisms that lead to allergy and of the mechanisms of successful immunotherapy have introduced new ideas for the treatment of allergic diseases.

Techniques of biochemistry and molecular biology have made it feasible to design novel therapeutic approaches for improved and safer forms of allergen-specific immunotherapy. The purpose of this review is to examine different approaches in designing novel allergen formulations for specific immunotherapy. These include chemically modified forms of allergens, genetically modified allergens (hypoallergens), hybrid allergens and allergens bearing immunostimulatory molecules, or adjuvants, which foster Th 1 immune responses.

There is great interest in newly designed allergens and their derivatives to improve the efficacy and safety of allergen immunotherapy. A better understanding of immunological mechanisms and further clinical trials utilizing new allergen formulations are needed.

Keywords: Immunotherapy, allergen, modification, allergoid, hypoallergen.

1. ALLERGEN-SPECIFIC IMMUNOTHERAPY

The most common treatments of allergy include pharmacotherapy (mainly antihistamines and corticosteroids) and specific immunotherapy. Although pharmacotherapy is very efficient in relieving symptoms of allergic inflammation in patients, it does not treat the pathogenesis of allergy. So far, the only causative treatment of allergic diseases, able to influence the natural progression of allergy, has been allergen-specific immunotherapy. The treatment was introduced almost 100 years ago by Noon, who was the first to try desensitizing an allergic patient by repeated subcutaneous injections of the causative allergen (grass pollen extract).

2. MECHANISM OF ALLERGEN-SPECIFIC IMMUNOTHERAPY

Although allergen specific immunotherapy is currently the only curative approach for treating allergic diseases and has been in use for almost a century, its mode of action still remains not completely understood.

It is believed that the mode of action of a successful immunotherapy involves modulation of antigen presenting cells (APCs), T cell and B cell responses to the allergen accompanied by an increase in allergen-specific serum antibodies of the IgG class (so called blocking antibodies) [1]. Its effectiveness, especially in the treatment of allergic rhinitis, is well documented. [2] The so called blocking antibodies, mainly of IgG4 class, are considered to act in competition with IgE for the binding sites on an allergen. The hindering of IgE-binding epitopes by blocking IgG antibodies could hamper cross-linking of bound IgE by allergen molecules at the surface of effector cells, thus preventing its degranulation

and subsequent histamine-release. The specific IgG antibodies also prevent the IgE facilitated uptake and presentation of allergen by antigen-presenting cells to T-cells [3]. The mode of action includes immunomodulation, i.e. skewing of the Th2 polarized immune response towards Th1, with an upregulation of IL-10 secretion, probably by APCs and T regulatory cells. The role of regulation in immune response and immunotherapy is the focus of the most recent research in this field [4-6]. It has been shown that allergen-specific immunotherapy increases the proportion of CD25 expressing regulatory T-cells and IL-10-secreting T-cells [7-9].

Thus, new strategies in the design of allergens, with improved properties for better and safer immunotherapy, are focusing on B cells, T cells and antigen-presenting cells as targets for modified allergens or derivatized allergens.

3. NEW STRATEGIES

Even though it has been used for decades, allergen vaccination can be accompanied by severe side-effects and can sometimes be followed by anaphylactic reactions in a patient receiving treatment. In addition, a recent study has shown that severity of adverse reactions depends on the type of allergen used, with grass pollen or cat dander being associated with a higher frequency of side-effects during immunotherapy than wasp venom or birch pollen extract [10]. For these reasons, preparations of allergens that are effective in treating the disease but exhibit reduced side effects have been introduced into clinical practice and new formulations are still the subject of research.

The aim is to modify allergens in order to reduce IgE binding, while preserving structural features necessary for T-cell recognition and induction of allergen-specific IgG antibodies. Various forms of allergoids (derivatives obtained by chemical modification) [11-13] or adjuvanted, conjugated, changed by chemical means [14-16] and hypoallergenic preparations (derivatives of allergens obtained by recombi-

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nant technology) have been described as having those features and have proved promising in treating allergic diseases [17].

Simple, straight-forward methods leading to desirable properties of an allergen, e.g. reduction of allergenicity but with the potential to stimulate the immune system, are of great interest as means of providing new tools to improve allergy vaccination. Such formulations have been made by recombinant technology or by coupling different immunomodulatory compounds to the allergen itself. There are hybrid vaccines for grass pollen and venom major allergens [18-21] that are able to induce higher titers of IgG antibodies during immunization than mixtures of individual allergens. CpG motifs, immunomodulatory agents acting on Toll-like receptors (TLR) 9, that drive the immune response towards the Th1 type, have recently been launched into clinical trials and represent a promising new approach to treating allergies [22-26].

An additional approach includes hybridization of an allergen to an inhibitory molecule by recombinant technology [27].

4. MODIFICATIONS OF ALLERGENS BY CHEMICAL MEANS

The incidence of severe side-effects during the conventional specific immunotherapy intensified the need for exploring new and safer ways for administering allergenic extracts.

4.1. Polymerized Allergoids

The first forms of modified allergens were of higher molecular weights, designed for subcutaneous immunotherapy [12, 13, 28, 29]. The mode of action of chemical agents, used for preparing chemically modified polymerized allergoids, involves a cross-linking reaction with formaldehyde or glutaraldehyde which mainly act on amine groups of proteins. The results are highly polymerized and modified proteins designated as allergoids.

The T-cell reactivity of allergoids depends on the type of antigen-presenting cell (APC). With dendritic cells and macrophages as APCs, allergoids exhibit a pronounced and reproducible T cell-stimulating capacity. Responses were superior to those with PBMC, and isolated B cells failed to present allergoids [30].

With growing tendencies towards using local forms of immunotherapy, some low-molecular weight allergoids that would be able to pass mucosal barriers and reach the patients immune system *via* mucosal tissue were considered of interest.

4.2. Monomeric Allergoids

The low-molecular weight (LMW) derivatives of allergens are modified allergens of a same (native size) as unmodified ones. Two principal approaches were taken into account: carbamylation and acid anhydrides treatment. The carbamylation of allergens leads to ureido group formation, but it does not lead to cross-linking of proteins [31-33]. The results are derivatized allergens with a slightly reduced net charge. The acid anhydrides treatment introduces a negative charge (acyl group) at the place of a positive charge in a pro-

tein (amine groups of lysine), thus generating highly acidic proteins. All three types of allergoids exhibited low allergenicity in the experiment of SPT on allergic patients [34, 35]. They were not able to induce skin reactions of such magnitude as the native extract while retaining their immunogenicity in animals.

Additionally, the experiments of pharmacokinetics of these derivatives suggested that carbamylated allergoids might be protected against proteolytic cleavage and that they reached even higher levels in the blood samples after buccal administration than the unmodified proteins [31]. All acyl derivatives were efficiently cleaved by proteolytic enzymes, with modified residues remaining stable in simulated fluids of gastric and intestinal digestion [36, 37].

Chemical modification of allergens may provide a mean to reduce allergenicity and introduce immunomodulatory properties into a protein by a single process. Maleylation and succinylation, by blocking positively charged amino groups, renders treated proteins negatively charged. The molecular basis of recognition by a family of pattern-recognition receptors (PRRs), called scavenger receptors (SRs) expressed mainly on cells involved in early stages of the immune response to foreign antigens, has not been characterized in detail, but it is known that recognition requires negatively charged moieties on a ligand [38, 39]. Similarly to TLRs, these receptors can bridge the innate and adaptive immunity and can induce adaptive immune responses to their ligands [40]. Many proteins are known to become SR ligands when chemically modified to enhance their negative charges with acetic or maleic anhydride, or physiologically with malondialdehyde [41]. As a result of enhanced uptake by APCs *via* SRs (and perhaps more efficient antigen processing), their immunogenicity is increased together with a dominantly Th1 type of immune response [42-44]. From the aspect of immunomodulation of an already established Th2 immune response, targeting scavenger receptors has potential in immunotherapy of allergies. So far, no data exist on clinical efficacy of these derivatives.

4.3. Immunotherapy with Modified Allergens

Allergoids have been used in immunotherapy for decades. Their application in subcutaneous immunotherapy was proved to be safe and efficient both in adults and children [14, 45-47].

The physicochemical modifications of allergens leading to allergoids provide a mean to administer higher allergen doses in accelerated schedules. Clinical studies conducted with chemically modified allergen extract in an accelerated schedule proved its safety along with dramatical immunological changes during the course of immunotherapy [48].

The first clinical trials of monomeric allergoids in local immunotherapy, conducted with carbamylated derivatives of *Dermatophagoides pteronissinus* [49] allergens, showed promising features. The mode of action involved down regulation of allergen-specific IgE and increased both INF-gamma and IL-10 production [50]. The newest clinical data consistently prove the effectiveness of allergoid immunotherapy in adults and children [51-56].

The obstacle for using allergoids may be extensive modification of the primary structure of the protein, that might

disrupt T-cell epitopes, thus leading to reduced immunogenicity of the allergoids, when compared to the unmodified extracts, as well as lower cross-reactivity with the unmodified proteins. Some recent data showed reduced immunogenicity in some of the commercially available allergoid preparations [57, 58].

5. GENETICALLY MODIFIED ALLERGENS - HYPOALLERGENS

Hypoallergenic forms of natural allergens may exist in nature. If an allergen from some source is present in several isoforms, some of them may be less prone to binding IgE from allergic patients. Those molecules are known as natural hypoallergens and for Bet v 1, the major birch allergen, they are characterized in details [59-62]. Knowledge gained from studying natural hypoallergenic molecules was of great help in designing engineered recombinant hypoallergens.

Otherwise, hypoallergenic molecules are produced by genetic engineering. The rationale behind producing hypoallergens is basically the same as for allergoids. The aim is to reduce IgE binding, while preserving immunological properties of the molecule, especially its immunogenicity and ability to provoke a protective IgG antibody response. The technology behind the production of hypoallergens is sophisticated and allows us to fine tune protein structure, as well as its IgE binding properties. The main difference between a robust chemical approach and a fine tuned protein engineering is that the former may be applied on crude extracts, while the later requires a target protein, or its gene, cloned and expressed in a heterologous system. The knowledge about the structure, IgE binding epitopes and allergenicity of the target allergen should preferably be tremendous, which is not an obstacle, considering that the majority of important allergens are defined at molecular level in detail.

The starting point is cloning the gene that codes the allergen. The gene is isolated from the allergenic source and cloned into a suitable vector that will allow heterologous expression, preferably in *E. coli*, due to its simplicity and large number of vectors, cloning strategies and systems developed and commercially available for bacterial expression. Other expression systems, like yeast, insect cells and plants, may also be used [63].

At the gene level, the genetic modification is introduced by various molecular biology approaches, such as error-prone PCR, site-directed mutagenesis, gene deletion or insertion, or gene-shuffling technology, depending on the strategy that is to be applied and the amount of information available from structure analysis by bioinformatics tools. Introducing mutations into a molecule may also be used for mapping of the IgE epitopes [64].

Three different approaches exist for modifying target genes: random, semi-rational and rational design of hypoallergenic molecules.

5.1. Gene-Shuffling Strategy

A random approach includes introduction of mutations by PCR, or by gene-shuffling. Directed molecular evolution by DNA shuffling and screening provides a means by which to evolve proteins having novel or improved functional properties without knowledge of structure-function relation-

ships of the target molecules. The next step is screening and characterization of the best potential candidates for immunotherapy, on the basis of several criteria, such as the reduction in allergenicity, ability to evoke immune response or immunogenicity even stronger than the original protein. Following that is a detailed *in vitro* characterization of the obtained hypoallergenic derivatives, before being introduced in an immunotherapy trial on allergic patients.

The gene shuffling strategy has been applied to mite group 2 allergens [65] and tree pollen allergens [66].

5.2. Re-Design of Allergens

A re-design of an allergen, in a way as it was recently applied to Phl p 12, an allergen of timothy pollen, resulted in reduction of allergenicity. The approach preserved the complete structure of the protein, while shuffling N-terminus onto C-terminus (tail-to-head redesign). The resulted loss in IgE binding was a consequence of a changed folding pattern and disruption of conformational epitopes. The T-cell stimulating capacity, i.e. immunogenicity, was preserved [67]. A similar approach was applied to Der p 2 [68], whose structure was fragmented and redesigned. The obtained derivative had all of the hypoallergenic features and may be a promising candidate for the immunotherapy of mite allergies.

5.3. Folding Variants of Allergens

Quite interestingly, the so-called folding variants of recombinant allergens also have promising features as potential candidates for immunotherapy. These molecules have completely preserved primary structure, as their natural counterparts, while the folding pattern was impaired, resulting in a miss-folded recombinant protein. The folding variant of Bet v 1 that was investigated for IgE-binding and histamine release potential, showed significant reduction in *in vitro* allergenicity, also as a consequence of disruption of conformational epitopes on the molecule [69]. The T-cell stimulating capacity and ability to induce immune response in animals was comparable to a wild type protein. It was shown recently that vaccination with folded and unfolded recombinant allergen derivatives of Bet v 1 induced IgG antibodies against new epitopes [17].

5.4. Site-Directed Mutagenesis and Rational Design

The rational-design approach requires a detailed knowledge of the IgE binding epitopes and three-dimensional structure of an allergen. The mutations are introduced by site-directed mutagenesis and the resulting protein, expressed and obtained in a protein form that folds properly, binds significantly less IgE than the natural counterpart. The approach may also include a rational-design for disrupting disulfide bridges, mainly responsible for correct folding of a protein, as it has been applied for Fel d 1 [70] and Hev b 6.01 allergens [71]. The approach includes replacement of cysteine (Cys) residues, which are able to form covalent linkages, by structurally similar, but unable to link into a bridge, serine (Ser) or alanine (Ala) residues. The resulting mutants may have an impaired folding property which consequently disrupts the majority of conformational epitopes on a target allergen [67, 72-74]. As a consequence of changed conformation, the disruption of IgE-binding epitopes results in reduction of allergenicity, which was remarkable in the case of triple Fel d 1 mutant.

Deletion mutants of Phl p 5, another important allergen of *Phleum pratense* pollen [75] and Ole e 1, a major allergen of *Olea europea* pollen [76] have recently been produced in *E. coli* and *Pisichia pastoris*, resp. The deletion of IgE bearing epitopes generated molecules of reduced allergenicity, that were still able to induce proliferation of T-cells in PBMCs established from allergic donors.

IgE epitope mapping data of allergenic fragments applied on Phl p 6, and selection of best IgG boosting candidate, gave a mutated protein with hypoallergenic features [77]. The lack of structural fold was proved by CD spectroscopy.

Ovomucoid third domain mutation generated a hypoallergen, able to ablate an allergic immune response and desensitize in a murine model [78, 79].

Point mutations introduced in Mal d 1, a major apple allergen, resulted in a hypoallergen, that induced lower skin reactivity in apple allergic patients, as demonstrated by double-blind placebo-controlled food challenge [80]. Deletion of B-cell epitopes and hybridization of allergenic fragments of molecules was applied on Par j 1 and Par j 2, major allergens of *Parietaria judaica* pollen [81].

Site-directed mutations in a functionally important domain of a protein, was applied on calcium-binding domains of fish parvalbumine, a major fish allergen [72] and a potential ligand-binding domain of Der f 2 [82]. The applied approach resulted in a mutated protein that was able to bind less IgE, but had a preserved globular fold, i.e. three-dimensional structure.

5.5. Hybridization of Allergens

By applying molecular biology techniques, the genes coding for different allergens may be linked in a PCR reaction, giving as a result a single protein comprised of two or more different allergens. Such a chimeric protein is known as a hybrid molecule. As a result of bridging more allergens into a single protein that folds in a manner which is not always possible to predict the IgE binding may be reduced, by many of the conformational epitopes being destroyed or hidden in a hybrid molecule, but quite interestingly, the immunogenicity of the hybrid molecule is usually raised compared to the individual allergens. Moreover, the IgE binding of such molecules may resemble the full spectrum of IgE epitopes of constituent molecules and boost its application both in diagnosis [83] and perhaps immunotherapy of allergic disorders [20].

Hybrid vaccines are constructed for timothy pollen and bee venom major allergens [21, 84] and were able to induce higher titers of IgG antibodies during immunization than mixtures of individual allergens. The recombinant hybrid molecule consisting of grass pollen allergens permitted accurate and specific *in vivo* diagnosis of grass pollen allergy in grass pollen allergic patients [83]. Hybrid molecules were synthesized of genes coding for Par j allergens [81, 85].

Hybrid allergens may also consist of dominant T-cell peptides, derived from non-cross-reactive allergens. This has been applied for the design of a hybrid molecule composed of dominant peptides derived from Bet v 1, Phl p 5 and Phl p 1 [19].

5.6. Synthesis of T-Cell Peptides

Allergen-specific T cells play a pivotal role in initiating and regulating the immune response to allergens, and T cell targeted strategies show promise for improved specific immunomodulation of the adverse immune response in allergic diseases. Atopic allergic individuals respond to allergen stimulation by dominant secretion of IL-4 and IL-5 (Th2-type cytokines) in contrast to non-atopic individuals where there is predominant IFN-gamma secretion (Th1-type). The epitopes of allergens recognized by T cells and antibodies are not identical. To identify T cell epitopes, antigen-specific T cell lines and T cell clones are generated. Overlapping synthetic peptides spanning the known sequence of the allergen molecule are screened for T cell reactivity.

By synthetic peptide chemistry, a non-anaphylactic, T-cell epitope bearing peptides may easily be obtained. Several recent studies have described immunodominant epitopes from major allergens that may form candidate peptides for use in peptide vaccination. The peptides have been characterized and produced for many important allergens, such as cat allergy [86], dog allergy [87], milk allergy [88], japanese cedar pollinosis [89]. The pre-clinical evaluations of these allergen derivatives were promising and they have been included in several clinical trials so far [90]. In mice, the administration of peptides based on dominant T cell epitopes induced specific T cell tolerance.

5.7. Immunotherapy with Hypoallergenic Derivatives of Allergens

First immunotherapy trials were conducted with hypoallergenic derivatives of allergens. Peptides comprising T-cell epitopes were administered to induce T-cell tolerance in patients, but though the peptides had very reduced IgE-binding capacity, they induced late phase systemic side-effects [90, 91].

Data from early clinical trials of peptide vaccination revealed that therapy was associated with a modest improvement in allergic disease, and was accompanied by a high frequency of adverse reactions [91]. More recent studies have demonstrated improved safety, and have defined the mechanisms of adverse events observed in earlier studies [92, 93].

Mechanisms of peptide vaccination include the hyporesponsiveness of allergen-specific responses and the induction of regulatory T cells and cytokines [90]. Novel peptide design has allowed the generation of fragments that contain T-cell stimulatory epitopes, lack B cell epitopes, and can induce protective IgG responses in both mice and humans. Peptides that specifically induce regulatory cytokine production would also enhance peptide vaccines [94].

The manipulation of peptide epitopes may provide a strategy for the rational design of peptide allergy vaccines, further improving safety and efficacy.

The first immunotherapy trials with recombinant hypoallergenic derivatives were performed with the rBet v 1 fragments and rBet v 1 trimer adsorbed to aluminium hydroxide [95]. The treatment showed that beneficial immunomodulatory effects, reduction of clinical symptoms and

inhibition of IgE memory responses, were associated with the induction of IgG antibodies which inhibited IgE binding to the allergens and allergen-induced basophile degranulation [96, 97].

6. IMMUNOSTIMULATORY DNA SEQUENCES (ISS) IN IMMUNE MODULATION OF ALLERGIC DISEASES

Bacterial and synthetic DNAs containing CpG dinucleotides in specific sequence contexts activate the vertebrate immune system through Toll-like receptor 9 (TLR9). The presence of CpG motifs of unmethylated DNA are potent stimulators of the innate immune system, modulating the immune response towards Th1 type. The Th1 and Th2 responses are counter-regulated and immune deviation of the allergenic Th2 immune response towards Th1 may reestablish a balance [22, 98].

In a murine model of asthma, coadministration of CpG ISS with the antigen, prevented airway eosinophilia, Th2 cytokine induction, IgE production, and bronchial hyperreactivity. Significantly, in a previously sensitized mouse, CpG dinucleotides rich sequences can prevent allergen-induced airway inflammation [99].

CpG motifs may be added as adjuvants during immunization, as it was shown to be very effective for Bet v 1 and mite allergy [100, 101], or chemically conjugated to the allergen itself, as investigated in ragweed pollen allergy [24]. The later proved to express even more profound effects on the immunogenicity and allergenic potential of the treated allergen. The effect of CpG motifs *in vivo* depends on a variety of parameters, like the nature of the antigen and the immunization modality. A combination of CpG plus DNA immunization may also be effective in antagonizing Th2 responses as the combination of CpGs plus protein immunization [102].

In mice, the loss of APC function after ISS treatment correlates with a reduced expression of costimulatory molecules, including programmed cell death ligand (PD-L)1, PD-L2, CD40, CD80, CD86, and inducible T cell costimulator, and of major histocompatibility complex class II on CD11c(+) APCs from the airways of ISS-treated mice. The second important action of ISS is inhibition of immunoglobulin E-dependent release of Th2 cytokines, especially interleukin 4, from basophiles and/or mast cells in the airways of Th2-primed mice. Thus, inhibition by ISS of allergic responses can be explained by two mechanisms that culminate in the inhibition of the principal sources of type 2 cytokines in the airways [103].

The conjugation of the CpG motifs to an allergen may, as a result of the coupling reaction, give a derivatized allergen that also exhibits hypoallergenic features. The conjugated molecule, like Amb a 1, a major allergen of short ragweed pollen, when conjugated to the ISS DNA sequence, besides being able to induce higher titers of specific IgG antibodies, may reverse an established airway hypersensitivity [104] and is also less allergenic than the unconjugated Amb a 1 [22, 24].

Hypoallergenic fragments that are able to stimulate innate immunity by up-regulating TLR 2 and TLR 4 receptors, may also have potential in immunotherapy. Such a hy-

poallergenic fragment, that was able to interfere with innate immunity cells, was shown for *Parieatria judaica* allergen, Par j 2, fragment [105].

Immune system stimulation may also be achieved with an allergen conjugated or adjuvanted to monophosphoryl lipid (MPL) A [106, 107] as it was shown for pollen allergoids adjuvanted with a MPL A to be very efficient in short-term specific immunotherapy of children and adolescents.

6.1. Immunotherapy with Immunostimulatory DNA Sequences

CpG motifs, immunomodulatory agents acting on Toll-like receptors (TLR) 9, that drive the immune response towards the Th1 type, have recently been launched into clinical trials and represent a promising new approach to treating allergies [22, 23].

One approach is based on vaccinations using live or killed bacteria or their components, CpG oligodeoxynucleotides or DNA vaccination, which all induce allergen-specific or unspecific Th1 responses. Th1 responses lead to the production of IFN-gamma, IL-12, IL-18 and IL-23, thereby inhibiting Th2 responses and thus the development of asthma. A further strategy, both for the prevention and therapy of asthma, is the induction of T regulatory (Tr) cells. Tr cells have also been shown to suppress allergic Th2 responses, however, in contrast to Th1 cells through a cell/cell contact mediated mechanism or by the secretion of the anti-inflammatory cytokines IL-10 and/or TGF-beta. Furthermore, there is growing information on how to induce Tr cells both in animals and humans. The data show that animals can be protected from developing asthma by immune stimulation leading to Th1 or Tr responses [98].

A recent study showed that the ISS is safe and pharmacologically active in the respiratory tract of asthmatics but, at the selected dose regimen, did not inhibit a fall in FEV(1) or other key features of the response to inhaled allergen challenge. This suggests that induction of IFN and IFN-inducible genes alone is not sufficient to inhibit allergen-induced responses in asthmatic subjects [108].

Clinical trials in ragweed allergic patients showed that allergen-immunostimulatory oligodeoxynucleotide conjugates are well tolerated, less allergenic and induce allergen specific IgG more rapidly than allergen extracts, without significantly increasing the immunoglobulin E titer [22]. Allergen-DNA conjugates are a novel type of allergoid that have great potential for a safe and potent form of allergen specific immunotherapy.

7. Hybrid Molecules Comprising an Inhibitory Molecule

By targeting the dominant-negative signaling receptor FcgammaRIIb expressed on pro-allergic cells, two novel forms for the treatment of IgE-mediated allergic disease have been developed [27, 109].

First is a genetically engineered bifunctional human fusion protein GE2, which is comprised of the Fc portions of human IgE and IgG1 with a flexible linker, designed as an allergen-nonspecific therapy [110]. GE2 blocks the effector phase of the IgE response *in vitro* in mice and human subjects [111] and *in vivo* in the skin and airway in mice and monkeys.

The second form is designed to provide a safer form of allergen-specific immunotherapy and consists of genetically engineered chimeric human Fcγ-allergen proteins, with Fcγ₁-Fel d 1 being the first such molecule constructed [112]. The allergen portion binds to specific IgE on FcεR1s, whereas the Fcγ₁ portion coaggregates inhibitory FcγRIIb and drives inhibition of allergic reactivity. Fcγ₁-Fel d 1 blocked human mast cell FcεR1-induced allergic reactivity *in vitro* and *in vivo* in murine models while functioning as an immunogen but not as an allergen [113].

8. CONCLUDING REMARKS AND FUTURE DIRECTIONS

Mainly because of a large availability of cloned allergen sequences, deduced allergen structures and growing knowledge on mechanisms of specific immunotherapy, it is possible to design novel therapeutic means for improved and safer forms of allergen-specific immunotherapy on a basis of allergen structure manipulation. Nowadays, different strategies exist in designing novel allergen formulations for specific immunotherapy. These include chemically modified forms of allergens, genetically modified allergens (hypoallergens), hybrid allergens and allergens bearing immunostimulatory molecules, or adjuvants, which foster Th 1 immune responses. Many of them have been proven as safe and reliable in clinical trials. The manipulation of B cell or T cell epitopes may provide a strategy for the rational design of new allergy vaccines further improving safety and efficacy. Growing knowledge on regulation of allergic diseases with a pivotal role of regulatory T cells may be a new preferential target in further improvements of allergen specific immunotherapy.

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