



Review

Peptide based immunotherapy: A pivotal tool for allergy treatment

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ABSTRACT

Immunotherapies with T-cell epitope peptides have shown a promising impact over allergic diseases as a potential therapeutic tool in in vitro and in vivo conditions. It is recognized as an effective treatment with long lasting clinical effects and subsequent reduction of the allergic inflammatory reactions. In this review, we have summarized the role of peptide based immunotherapy and emphasis has been given to the recent advancement in pollen, cat, hymenoptera venom, and food allergy.

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Abbreviations: IgE, Immunoglobulin E; MHC, Major Histocompatibility Complex; ASIT, Allergen Specific Immunotherapy; APCs, Antigen Presenting Cells; DCs, Dendritic Cells.

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1. Introduction

Allergy is a serious, life threatening health concern due to the imbalances in the immune system provoked by environmental substances in the susceptible individuals. Immunoglobulin E (IgE) is the most important immunoglobulin involved in the allergic reactions. Most of the allergic reactions are IgE mediated or type I hypersensitivity reactions.

Because of its quick onset type I allergic reactions are also known as immediate type hypersensitivity. Allergens are substances responsible for induction of allergy in the susceptible individuals. These substances belong to pollens, dust mites, molds, danders, foods, medicines etc. The mechanisms of allergic reactions involve the interaction between IgE and allergen that cause release of allergic mediators like histamine, prostaglandin D₂ and leukotrienes following a cascade of molecular events [1]. The symptoms of allergy can be mild to severe including allergic rhinitis, allergic conjunctivitis, abdominal pain, vomiting, diarrhea, asthma, anaphylaxis and sometimes death. About 30–40% of populations have been found to be affected by the different allergic diseases including allergic rhinitis, asthma, allergic conjunctivitis, eczema, and food allergy [2].

The prevalence of allergy is worldwide, but still a permanent cure for allergic reactions is not available except strict avoidance of the offending allergens and symptomatic treatment of any adverse effects from accidental exposures [3]. Several therapeutic approaches are under scanner for the treatment of allergic diseases [4]. Among these, one method that's gathering popularity for allergy treatment is peptide based immunotherapy. Peptide immunotherapy is the use of peptides containing allergen-derived immunodominant T-cell epitopes that hold therapeutic promise for allergic reactions [5]. In this review, focus has been made on the mechanism of immunotherapy, overviews of immunotherapy developed for the treatment of pollen, food, insect, bee venom, and cat allergens, as well as pros and cons of T-cell epitope peptides in immunotherapy and its future perspective.

2. Different types of immunotherapy

Immunotherapy has been carried out for many years in which one of the most popular methods that are widely accepted is Allergen Specific Immunotherapy (ASIT). The ASIT includes oral, sublingual, and epicutaneous immunotherapy according to their route of administration.

2.1. Oral immunotherapy

An oral immunotherapy method induces desensitization by regular ingestion of a small amount of allergen, which increases IgG production, suppresses IgE production and ultimately suppresses allergic sensitization. The oral route of immunotherapy was reported for the first time in the 1980s [6]. The oral immunotherapy is easily administrable, carries much lower risk of anaphylaxis and requires less time to achieve therapeutic effects. Oral immunotherapy with cow's milk has been reported to modify the levels of a number of specific antibodies including IgE and IgG4 [7]. Several studies advocated administration of a single allergen at a time but a recent study by Bégin et al. (2014) reported that at preliminary level oral immunotherapy using multiple allergens simultaneously may be given under well-equipped medical supervisions [8]. But in many cases, oral immunotherapy with a relevant allergen is found to be risky and several side effects have also been reported which need to be minimised for the betterment of the therapy [9–11].

2.2. Sublingual immunotherapy

Sublingual immunotherapy is another type of immunotherapy in that allergen extracts are given as drops under the tongue of susceptible patients. It is commercialized in several parts of the world including American, European and Asian countries. Several clinical trials have shown clinical efficacy of single allergen tablets (grass and ragweed) or extract solution (ragweed) at primary level [12]. In a clinical study related to sublingual immunotherapy of house dust mite it has been reported that twelve months of treatment with 500 and 300 index of reactivity (IR) can be efficacious and well tolerated [13]. Sublingual immunotherapy is found very efficacious in children as well as in adults but, the chances of side effects cannot be ignored if not handled properly [14].

2.3. Subcutaneous immunotherapy

Subcutaneous immunotherapy is very similar to sublingual immunotherapy but in this mode of immunotherapy allergen is administered under the skin. This therapy has proven its efficacy in allergic diseases. Side effects induced by subcutaneous therapy include itchiness, swelling, and redness at the site of injection, highest systemic reaction, such as generalized itching, upper airway itchiness, coughs, or a shortness of breath [15]. Further, during the course of subcutaneous immunotherapy local reactions are very common [16].

2.4. T cell epitope peptide based immunotherapy

Considering the side effects of the above mentioned immunotherapies, there is a need of a safer and efficacious approach to combat allergic reactions. The T cell epitope peptide based immunotherapy could be one option. In this therapy, soluble peptides containing allergen-derived immunodominant T-cell epitopes are used to treat allergic patients [17]. Due to the use of T-cell epitope peptides the chance of adverse effects reduced significantly. Therefore, among various therapeutic approaches, allergen specific immunotherapy with T cell epitope peptide has been reported to be one of the most appropriate immunotherapy methods available as of now [18].

3. Mechanism of action of T-cell epitope peptide

T cell epitope is the particular region of an antigen or allergen that is immunogenic as well as antigenic in nature. T cell epitopes are mostly proteinaceous in nature. When these proteins are engulfed by antigen presenting cells (APCs), they get degraded in the proteosomal complex into small peptide fragments. These fragments bind to the Major Histocompatibility Complex (MHC) as per their binding capacity. These MHC–peptide complexes gets recognized by T cells and T-cell epitopes. Despite this, additional secondary signals are also required for activation of T cell provided by co-stimulatory molecules expressed on APCs [1]. The binding of CD28 on T cells and its two ligands B7-1 (CD80) and B7-2 (CD86) on APCs is considered as key event for T cell activation [19]. The recognition of T cell epitopes responsible for immune reactivity is necessary for the immune mechanism in response to exposure of allergens. The activated APC presents processed allergen epitopes in an inflammatory context to allergen-specific T cells which further lead to allergic reactions [1]. The Allergen Specific Immunotherapy amplifies the cross-linking of allergen specific antibodies bound to the surface of APC resulting in the activation and upregulation of pro-inflammatory genes. For cross-linking of two IgE molecules, allergens should be at least bivalent that it contained a minimum of two epitopes. Fig. 1 demonstrated an outline of the mechanism by which T-cell epitope deciphers its anti-allergic potential. It induces anergy in T-cells and IgG production. IgG further inhibits IgE-facilitated allergen uptake by Dendritic Cells (DCs) and prevents IgE-mediated allergen activation of effector cells and subsequent release of allergic mediators that finally suppress allergic manifestations [20,21].

4. T-cell epitope peptide therapy in different allergies

Immunotherapy with T-cell epitope peptide has been reported in a variety of allergies like bee and wasp venom, grass pollen, house dust mites and food allergy [22–25]. Immunotherapy has been proven as a pivotal tool in the treatment of several allergic diseases. Immunotherapy with T cell epitope peptides of cat allergens, immunotherapy with chimeric human–cat fusion proteins, immunotherapy with T cell epitope peptides of bee venom, and oral immunotherapy with T cell epitope peptide of ovalbumin (OVA) will be discussed in the next section.

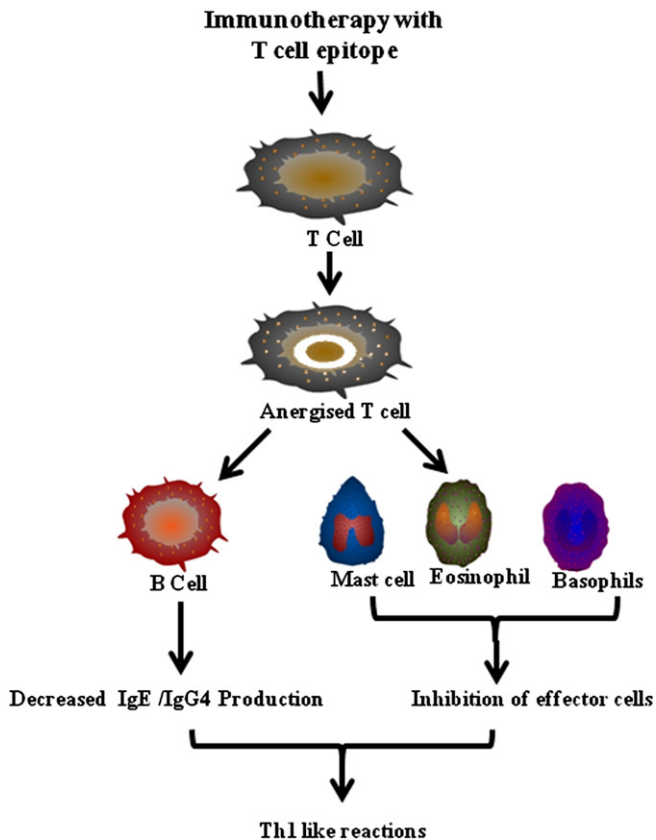


Fig. 1. A pictorial demonstration of the basic mechanism behind T-cell epitope peptide therapy in allergic reaction. Exposure of T cell epitope peptide induces anergy in T-cells, production of IgG, and inhibition of IgE-facilitated allergen uptake that ultimately suppresses allergic reactions.

4.1. Japanese cedar pollinosis

Recent years have witnessed a marked increase in Japanese cedar pollen (JCP) allergic disease or pollinosis in Japan and it was estimated that approximately 23 million patients suffer from Japanese cedar pollinosis from February to April, each year. JCP is a potent allergen, which causes seasonal allergic rhinitis, asthma, rhinoconjunctivitis, and atopic dermatitis, in spring in Japan [26–29]. JCP encoded two T cell epitope peptides; the first one is Cry j1, a basic glycoprotein of molecular mass 41 to 45 kDa, located in pollen cell walls and has pectate lyase activity [30]. The second epitope peptide of JCP is Cry j2 a 45 kDa protein, located in pollen starch granules and has polymethylgalacturonase activity [31]. The uses of Cry j1 and Cry j2 have been reported to cure JCP, frequently [32].

A new fusion protein was designed that was composed of T-cell epitopes from JCP allergens, Cry j1 and Cry j2, with soybean seed protein glycinin. Both Crp-1 and Crp-2 were introduced at 15 amino acid residues (residues 293–307 of A1aB1b) in the acidic subunit and 8 amino acid residues (residues 488–495 of A1aB1b) in the basic subunit of glycinin A1aB1b, resulting in the recombinant proteins A1aB1b-Crp-1 and A1aB1b-Crp-2 [33,34]. To control allergic diseases induced by JCP, an effective approach to prepare fusion proteins like A1aB1b-Crp-1 and A1aB1b-Crp-2 can prove to be a powerful tool to combat allergic diseases. Fig. 2A provides a pictorial summary of A1aB1b-Crp-1 and A1aB1b-Crp-2 efficacy in allergic reactions. In a preclinical model, daily oral administration of transgenic rice seeds with A1aB1b-Crp-1 and Crp-2 to mice prior to systemic challenge with JCP allergens was found to inhibit proliferative responses of allergen specific CD4⁺ T-cells, Th2 cytokines like IL-4, IL-5, and IL-13, as well as histamine release [33,34].

The CpG-DNA motifs containing Cry j2 T-cell epitope peptide p246–259 (CpG-peptide) have been prepared to treat Japanese cedar pollinosis effectively and investigated for its potential following subcutaneous, intraperitoneal or intranasal administration. Further, it has been found that the challenge of CpG-peptide with the cedar pollen allergen Cry j2 resulted in the reduced levels of specific IgE, IgG1, IL-4, IL5 and passive cutaneous anaphylaxis reactions as demonstrated in Fig. 2B [35]. In summary, it can be concluded that the use of Cry j1, Cry j2, A1aB1b-Crp-1 and A1aB1b-Crp-2 and CpG-DNA motifs containing T-cell epitope is safe and effective options to treat JCP allergy.

4.2. Cat allergy

Among all IgE mediated allergies worldwide, cat allergy is one of the most prevalent allergies. The symptoms of cat allergy comprise asthma, conjunctivitis, hay fever, sneezing, chronic sore throat, watery eyes, and nasal congestion [36]. More than five allergens from cat have been reported that are found to be responsible for inducing allergic reactions including two major allergens Fel d1 and Fel d4. The Fel d1 is a secretoglobin secreted by the cat's sebaceous glands and squamous epithelial cells [37]. The Fel d4 is an alipocalin found to be expressed in saliva, perianal, and lachrymal glands [38]. The Fel d2, an albumin, Fel d3, a cystatin, and cat IgA have been reported as other allergens involved in cat allergy [37,39,40]. One of the major allergens of cat, Fel d1, is composed of two heterodimers consisting of two chains derived from independent genes and T cell epitope peptides [41–44]. The Fel d1 peptide with altered epitopes has been found to reduce sensitivity to allergens including reduction in the Th2 cytokine responses [45]. Simons et al. showed in his experiment that the use of Fel d1 peptide can reduce skin reactivity to allergen as well as decrease Th1 and Th2 responses and increases in IL-10 levels [46]. Further, to enhance the potential of Fel d1, second generation of Fel d1 peptide vaccines has been developed to avoid the risk after the initial trials with Allervax CAT. This second generation vaccine is comprised of 12 shorter (16–17 residues) overlapping T-cell epitope-containing synthetic peptides that can bind through a number of human leukocyte antigen (HLA) molecules [47].

To combat cat allergy, human–cat fusion protein (GFD), a chimeric fusion protein containing a human IgG Fcγ1 and Fel d1, has been prepared. Mast cells and basophils possess FcεRI and FcγRIIb that are high and low affinity receptors of IgE, respectively [48]. The FcγRIIb receptor showed inhibitory effect and completely blocks the FcεRI signaling and, ultimately prevents IgE mediated allergic reactions [49]. These responses implicate that inhibitory receptors of mast cell and basophils have potential for therapeutic intervention in the treatment of cat allergen induced asthma and allergic rhinitis. Fig. 3 demonstrated the influence of human Fcγ1 chimeric gene on the levels and functionality of eosinophils, basophils and mast cells. Further, it also affects IgE mediated syk and ERK phosphorylation that impaired the mast cell degranulation. Because of these aforementioned events, the allergic reactions decrease significantly.

4.3. Bee venom allergy

The panic effect of bee venom can be easily visualized by majority of the population. ASIT with bee venom is associated with the risk of inducing systemic side-effects, redness and local swelling [50]. Moreover, administration of T cell epitope peptides of allergen of interest to ASIT reduces the risk of ASIT-related systemic reactions. The use of peptides of bee venom improves safety and efficacy and may be a useful approach to combat allergenic manifestations induced by bee venom. The bee venom phospholipase A2 or PLA (Api m 1) is one of the key allergens in bee sting allergy [51]. The PLA is characterized by three peptides (amino acid sequence 45–62, amino acid sequence 81–92, and amino acid sequence 113–124) and a glycopeptide T cell epitope (mol wt. 14–16 kDa), comprised of 134 amino acids [51,52]. Peptide immunotherapy with PLA generates IL-10 that induces specific anergy in

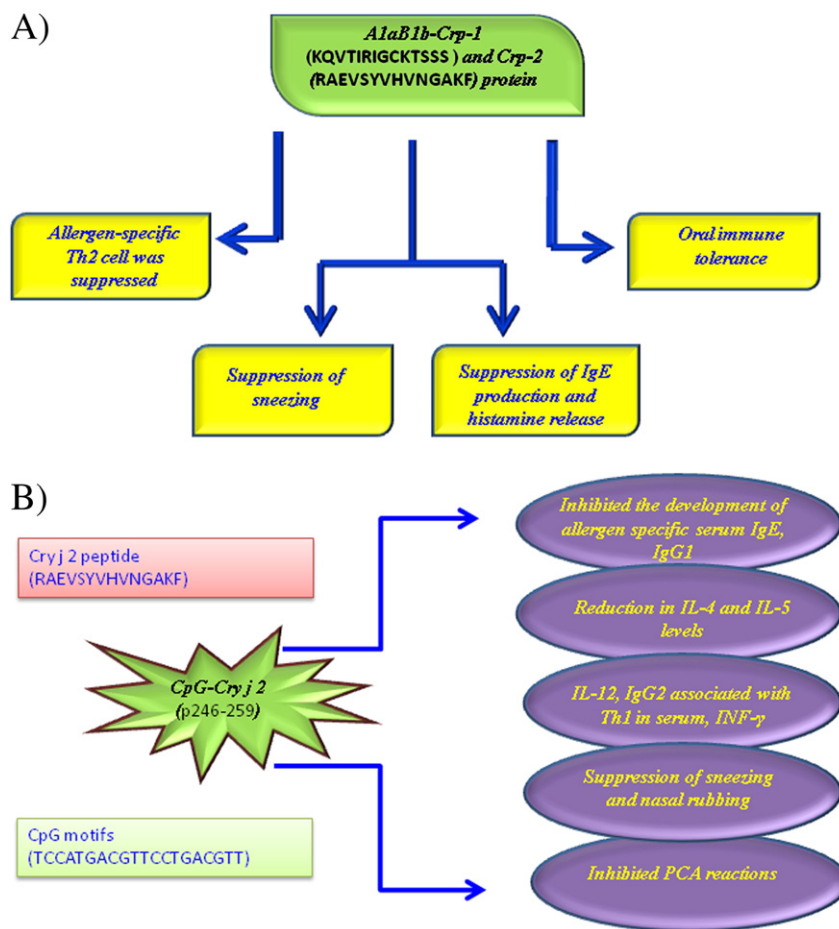


Fig. 2. (A) Effects of intradermal administration of A1aB1b-Crp-1 and -2 fusion proteins. This treatment leads to the reduced count of allergen specific T-cells and IgE and reduced release of histamine. Further, this immunotherapy reduces sneezing and insures oral tolerance in the susceptible individuals. (B) Effects of Cry j2 along with CpG-DNA adjuvant on different allergy parameters. The CpG-Cry j2 induces anti-allergic effects following its administration in the susceptible individuals. PCA = passive cutaneous anaphylaxis.

peripheral T cells and decreases IgE/IgG4 ratio and IL-4, IL-5 and IL-13 levels, a rise in the allergen-blocking IgG antibodies, especially IgG4 class after allergen challenge, decreased mast cells and eosinophil counts and release of allergic mediators [53]. The decrease in exocytosis of mediators from basophils and mast cells is found to be associated with anergy induction in T cells, since effector cells which play a

major role in allergy require priming by T cell cytokines for full activity and mediator release [54–57]. The PLA peptide did not induce any skin reaction as well as not bind to IgE antibodies and due to this property, the immunotherapy using PLA is a nice option to treat bee venom allergy [58–60]. Further, the use of PLA peptide does not cause any change in the activity of B cells that indicates the successful history of T cell

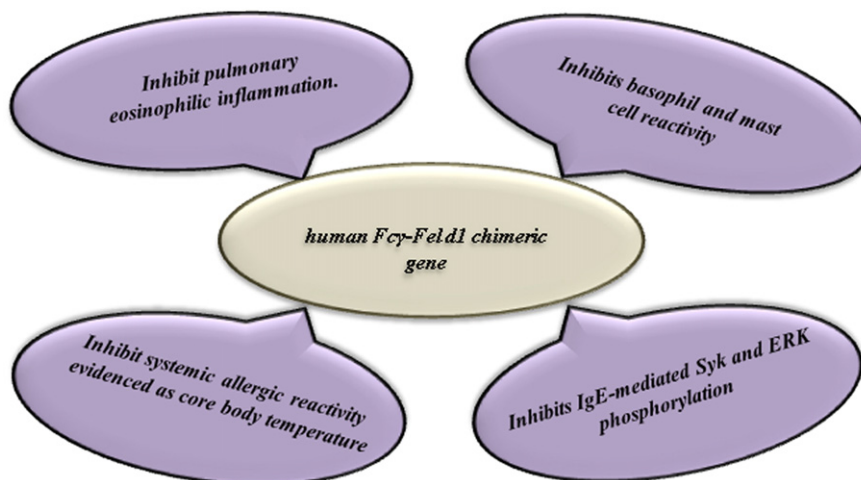


Fig. 3. Effects of intradermal administration of Fcγ1–Fcεd1 fusion protein on different allergy parameters. This chimeric gene decreases eosinophils, basophils and mast cell functionality and also impaired IgE mediated syk and ERK phosphorylation.

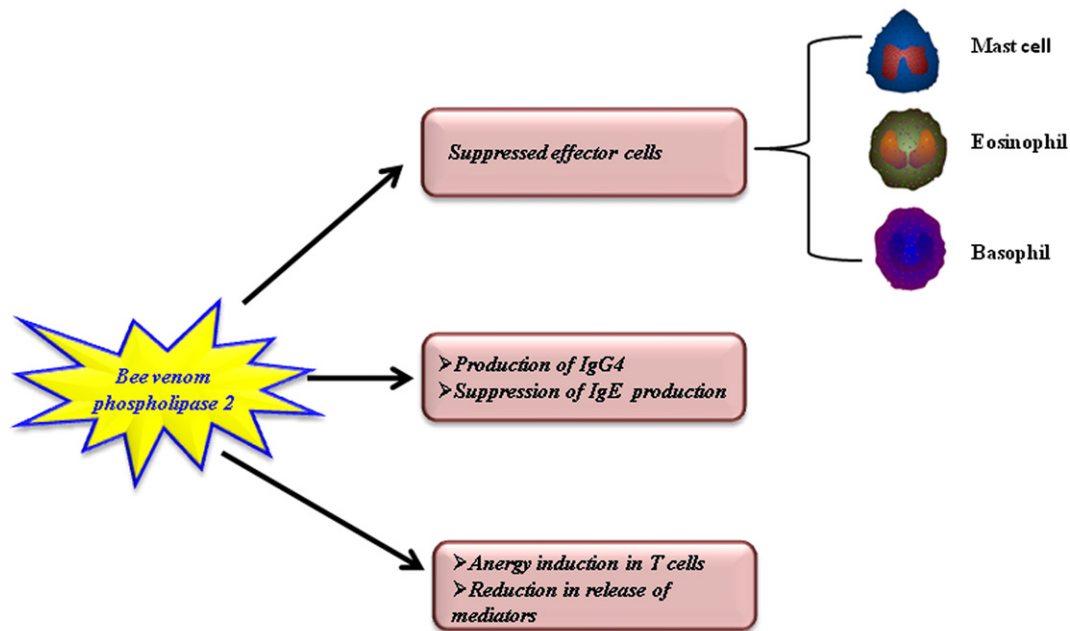


Fig. 4. Effects of subcutaneous administration of bee venom phospholipase A2 (PLA) fusion protein on different allergy parameters. Bee venom phospholipase A2 suppresses effector cells, IgE production, and release of allergic mediators.

epitope peptide of PLA without any side effects [61,62]. A schematic diagram regarding the use of T cell epitope peptide in bee venom allergy has been given in Fig. 4.

4.4. House dust mite allergy

House dust mite allergy is the most awful form of allergy causing several health complications in the susceptible individuals. These are small microscopic organisms highly abundant in all homes, especially in warm and humid environments which are the optimal conditions for their growth; Despite dry climates, house dust mites can survive and reproduce easily, especially in bedding [63]. Proteins from house dust mite are some of the most potent elicitors of hay fever and asthma along with the symptoms like itchiness, sneezing, and watering and reddening eye. About 1.2 billion people have been found to be affected by house dust mite [64]. Among 15 allergens of house dust mites, the most prominent and potent allergens are der p1 and der p2 (*Dermatophagoides pteronyssinus*). The der p1 is a cysteine protease enzyme that degrades polypeptides and affects most of the population. It has been reported that der p2 plays a vital role in immune modulation because it is recognized by the majority of house dust mite allergic patients and due to this fact, a new vaccine has been prepared that comprised of der p2 and a reduced IgE mediated side effect has also been observed during its implication [65,66]. In this regard, five der p2 peptides (P1–P5) were synthesized and lymphoproliferative, cytokine responses induced in peripheral blood mononuclear cells from mite-allergic patients with der p2 and der p2 peptides were measured. It has been found that the administration of der p2 peptides resulted in an increase in allergen specific IgG1 and IgG4 antibodies and Th1 cytokines, reduced activity of mast cells and basophils, and also reduced levels of the Th2 cytokines indicating a successful peptide immunotherapy [67–70].

4.5. Food allergy

Food allergy is an immune provocation induced by certain foods. Food allergy affects nearly 5% of adults and 8% of children, and its prevalence is increasing continuously [71]. The symptoms of food allergy include, hives itching of the mouth, lips, tongue, throat, eyes,

and skin, or other areas of swelling of the lips, tongue, eyelids, or the whole face, difficulty in swallowing, runny or congested nose, hoarse voice, wheezing, shortness of breath, nausea, vomiting, abdominal pain or stomach cramps, lightheadedness, and fainting [72]. There are several foods found to induce allergic reactions. In recent years, the oral immunotherapy with T cell epitope peptides for the treatment of peanut, milk and egg allergies have been well documented.

4.5.1. T cell epitope peptide for egg allergy

The prevalence of egg allergy has been reported from several parts of the world [73]. T cell epitope peptide immunotherapy for egg allergy is not uncommon. Ovm (mol. wt. 28 kDa) is the most potent allergen of the egg and comprises 186 amino acid residues arranged tandemly in 3 domains OMCHI1 (residues 1–68), OMCHI2 (residues 65–130), and OMCHI3 (residues 131–186) [74,75]. It has been reported that the T cell from BALB/c mice recognizes 100–110 and 157–171, in contrast to C3H/He that recognizes only 157–180 residues of Ovm. It has also been reported that the oral administration of two synthetic peptide comprising 15 mer (157–171; single peptide (SP), DNKTYGNKSNFSNAV) and 51 mer [3 repeated units of SP linked by alanine residues (157–171), 3 multiple peptide (MP)-DNKTYGNKSNFSNAVAADNKTYGNKSNFSNAVAADNKTYGNKSNFSNAV] of the Ovm induces anergy, T cell deletion or active cellular suppression mediated by regulatory T cells or Tregs as demonstrated in Fig. 5. Oral administration of these peptide vaccine induced immunological tolerance in both Th1 and Th2 cell responses against Ovm [76]. Significant reduction in histamine, IgE and IL-4 and increase in IgG2a, IgA, IL-10, and IL-12 cytokines and CD4+CD25+ and CD4+FOXP3+ regulatory T cells in SP treated group, in contrast to the MP treated group showed an alteration in specific IgE, IgA, and IL-4 production rather than the above parameters besides that the group showed suppressed clinical signs of allergy. The use of the complete T-cell epitope map of OVA has been defined in the BALB/c mice that have opened a future possibility for peptide-based immunotherapy [77].

4.5.2. T cell epitope peptide for peanut allergy

Among several legume allergies peanut allergy is the most common. Studies in the United Kingdom and Canada showed a high prevalence of peanut allergy [78]. Peanut allergy is frequently reported in several

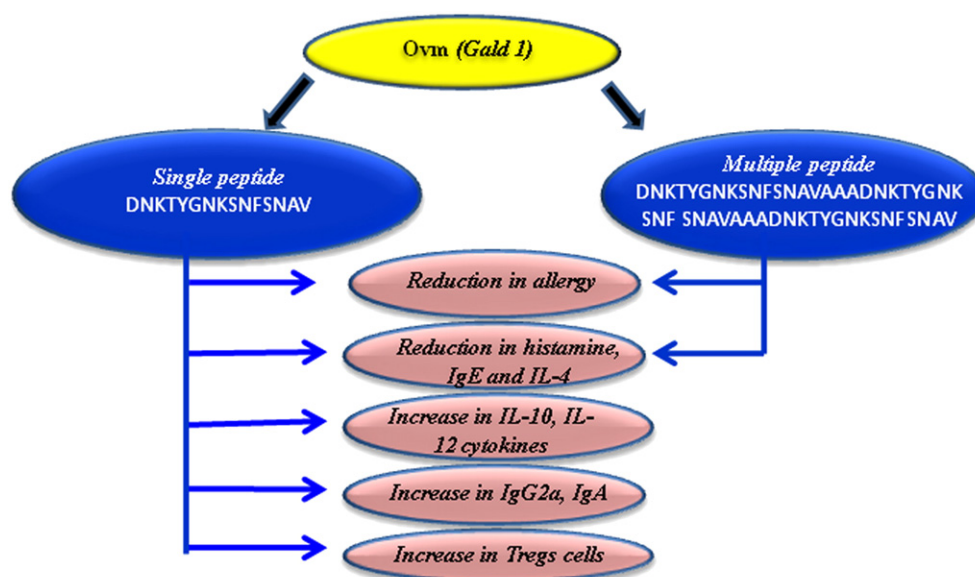


Fig. 5. Effects of oral administration of egg allergen ovomucoid (Ovm) fusion protein on different allergy parameters. The single as well as multiple peptide exposure reduce cytokine level, IgE production and allergic reactions.

parts of the worlds and therefore, effective immunotherapy for peanut allergy is highly required. Multiple Ara h 1 epitopes with defined HLA restriction and Ara h 1-reactive T cells can be effective tools for tracking peanut-reactive CD4 (+) T cells during immunotherapy [79]. In a study carried out by Prickett et al., T-cell epitope-based peptides that target allergen-specific CD4 (+) T cells but do not bind IgE have been used for peanut-specific immunotherapy [80].

4.5.3. T cell epitope peptide for cow's milk allergy

Cow's milk allergy (CMA) is a leading cause of food allergy especially in infants and children [81]. The prevalence of CMA varies between 0.25% and 4.9%, being higher in children than adults [82]. The β -lactoglobulin (BLG) is one of the major allergens responsible in CMA. Six T-cell clones (TCC) from CMA patients have been recognized in BLG and these peptides can be potential options to treat CMA [83].

5. Future perspectives of T-cell epitope peptide therapy

The treatments of immune mediated diseases with T cell epitope peptide are becoming increasingly accepted due to its powerful impact on human health. There is an increased risk of adverse side effects with various forms of immunotherapy, but at the same time the clinical trials on T cell epitope peptides have found them safe. Immunotherapy with T cell epitope peptides is associated with several features, including increases in allergen-specific serum immunoglobulin levels (particularly IgG1, IgG4 and IgA), induced T-cell anergy (functionally inactivated T cells), induced Treg cell counts, inhibited effector cells like mast cells, eosinophils and basophils, reduced infiltration of leukocytes in bronchoalveolar lavage fluid, airway hyper-reactivity, sneezing, rhinorrhea, nasal obstruction, nasal itching and anaphylactic reactions. The immunological mechanism behind these short, synthetic T-cell epitope peptides is that they have less ability to crosslink IgE bound to the surface of APC and results in APC inactivation as compared to native allergen and thus induces immune tolerance. Immunotherapy with T cell epitope peptides has emerged as an extremely attractive tool for a wide range of therapeutic applications including allergic diseases. Due to its safety and efficacy the use of T-cell epitope peptide based treatment of allergy is a favorite topic for the researchers of the allergy regimen. T-cell reactive peptides of JCP, bee venom, and Ovm are the examples of novel candidates for safe and efficacious treatment of allergy. The approach of T-cell epitope peptides may be better than the use of

fragments or folding from a variety of recombinant allergens, B-cell-epitope containing peptides, induction of blocking antibodies, and IgE-binding haptens of major allergens, etc. Treatments with T cell epitope peptide can be more effective for patients in whom avoidance of animal allergens is not fully effective. Conclusively, in the coming future the use of T-cell epitope peptides for treatment of several allergic disorders will increase in the health market.

6. Conclusion

Immunotherapy using T-cell epitope peptides has been opened to a new dimension in the field of therapeutic research for several allergic manifestations. The existing knowledge about immunotherapy needs to be elaborated up to some more extent, so that the use of this immunotherapy can be accelerated. Taken together, the use of T-cell epitope peptide based immunotherapy in allergic diseases can be a vital tool for combating unavoidable and nontreatable health concerns.

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