Immunotherapy for the Treatment of Allergic Diseases

Abstract

Allergen Immunotherapy (hereafter AIT) is the only disease-modifying medication available for allergic diseases[1]. Currently, there are several AIT options available on the market, with varying routes of administration, limitations, and implications. However, possible side effects of those treatment options raise the necessity for novel options of AIT with with improved carriers, improved safety profile, or shorter treatment course duration.

This review summarizes the current types of treatments of allergic diseases available on the market, their routes of administration, and possible limitations associated with them. It also touches upon the core principles involved in allergic diseases, the main cells involved, and explains the medical need for novel AIT options. Moreover, the current AIT options are explained, and the core underlying mechanisms are described. Subsequently, the review focuses on the novel AIT options available in the market, or that are under development. Some of those novel AIT options include novel adjuvants, allergoids, or recombinant proteins.

The availability or novel carriers, such as liposomes or probiotics for AIT shows a promising outlook on the further improvement of allergic disease treatment or cure. However, most studies suggest that more research is needed for the development of a long-term safety profile, and larger scale studies are necessary, before those new active substances can enter the market.

Student: Aleksandra Behzanyan
Student Number: 5912652

Master's Program: Drug Innovation
Supervisor: Dr. Frank Redegeld
Second Examiner: Dr. L.E.M. (Linette) Willemsen
Date: 15 April 2023

Introduction

Allergies have been prevalent throughout history. One of the first mentions of intolerances or adverse effects from foods were in the Old Testament. Several foods were considered unfit for consumption, because of the possible reactions they could cause. Some of these foods included fish without scales and reptiles. Hippocrates has noticed that products, such as milk could cause a rash among some people. A distinct way of food allergy management was mentioned in the Babylonian Talmud, which described instructions for egg white preparation to prevent intestinal egg sensitivity[2,3]. Furthermore, a prime mention of Allergic Rhinitis (hereafter AR) management was found in texts from ancient Egypt and Ayurvedic traditions in India. Texts mentioned how inhalation of certain plants could be used for achieving an anticholinergic effect and treating AR-induced ailments[4,5].

It has been known that allergies have been prevalent for centuries and are still highly prevalent till this day. From 1997 to 2007, the occurrence of pediatric food allergies has increased by 18%[6]. In 2021, around 7.8% of the population has hay fever. Additionally, between 10-30% of the global population has AR. One of the causes of increased allergy occurrence is the global rapid urbanization. Urbanization induces lowered exposure to microbes, which is correlated with the increased rate of allergies[7].

In addition to high occurrence, allergic diseases cause a significant financial burden on the healthcare system. In 2005, the costs associated with allergic rhinitis in the US were estimated to be around $11,2 billion. A similar study conducted in 2007 showed that asthma associated costs to the society were $56 billion. Despite the high cost associated with allergy treatment, they only manage the symptoms associated with allergic diseases.

One of the novel treatment options that goes beyond managing allergy-related symptoms is Allergen Immunotherapy (hereafter AIT). This novel treatment option attempts to modify the disease itself and treat allergies by inducing immunity to the allergen in question.

This literature review focuses on reviewing the current state of AIT, types of AITs, its mechanism of action and challenges associated with it. Additionally, the novel versions of AIT are outlined, discussed, and evaluated. AIT was the topic of choice due to its unique nature. The well-known disease-modifying property of AIT makes it an intriguing subject of interest for the review[8]. Understanding the novel treatment options for allergic diseases might help meet the medical need for allergy treatment options.

Background Information

Allergic Diseases

Allergies are characterized by hypersensitivity of the immune system to a substance that is normally harmless. The most common allergies are hay fever, food allergies, insect stings, and venom. Main symptoms of allergies are red eyes, a rash, difficulty breathing, and swelling. Most allergic reactions of all groups are mild; however, severe cases can lead to a fatal outcome.

Allergic responses are characterized with high levels of IgE. These molecules are generated by B cells in response to the allergen entering the system. The IgE molecules circulate in blood and bind to Ig-E receptors on cells such as mast cells. Once exposed to the same allergen for the second time, an IgE receptor induced cascade of events starts; this results in an immediate Ig-E mediated immune response[9].

The core reasons why some people develop allergic diseases are not entirely clear[10]. It is, however, known, that both genetic and environmental factors can increase susceptibility, and that immune responses have a polymorphic nature[11]. Several genetic factors involved in

allergic diseases have been found during the genome-wide association studies (GWAS). An example of such factors are T-helper 2 (Th2) cytokine genes. Additional factors that were identified were genes encoding for α -chain of the high affinity receptor of IgE, RAD50, which is located next to the gene for IL-13, and signal transducer and activator of transcription 6, which is regulated by IL-4 and IL-13[12]. However, there were no overlaps in the GWAS studies. It is believed that environmental factors influence the occurrence of allergies more than genetic factors. Genetic factors are more inclined to influence the severity of allergic diseases, and how strongly they will be expressed[13,14].

Diagnosis of Allergic Diseases

Two main methods of allergy diagnosis are skin prick tests and blood tests. Both of these diagnostic techniques are cost-effective and do not cause a significant healthcare burden[15]. Skin prick testing is primarily used for diagnosing a suspected type I hypersensitivity (the immediate type). This diagnostic technique was first used by Helmtraud Ebruster in 1959; since then it was used as a standard for allergic diagnosis[16]. In contrast with skin prick testing, first patch tests were performed in 1895; since then, scholars worked on standardizing this method of testing.

Skin prick testing shows immediate results, that are reproducible if the test is performed correctly. It can take about half an hour before results are shown[17]. As mentioned previously, skin prick testing is used for IgE mediated responses. On the contrary, patch testing is used for diagnosing allergies that cause non-IgE mediated responses. It tests delayed reactions, that may take hours or days after allergen exposure. The recommended site for patch test is the upper back. Patches are applied to the tested area and checked after approximately 48 hours. Circular or square-shaped patches with diameters between 8-10 mm are used for this diagnostic technique. Larger patch units are used for detecting weak sensitization to some allergens[18,19]. Other methods, such as blood testing are available for patients that cannot undergo the abovementioned tests.

Blood tests for allergies focus on measuring IgE levels in blood against allergens.[20]. Blood tests are sometimes the preferred diagnostic option because other techniques come with possible inaccuracies. Previous studies show that combination of medical history evaluation in combination with physical examination do not have an accuracy level of above 50%[21]. During blood tests, IgE levels are measured via several main types of immunoassays. Some of these assays include enzyme-linked immunosorbent assays (ELISAs), fluorescent enzyme immunoassays (FEIAs), and radioallergosorbent assays (RASTs)[20]. These assays use IgE binding labelled with radioactive isotopes for quantifying the IgE levels in the blood. The final IgE levels are calculated from the amount of fluorescent antibodies present in blood[22,23].

It is worth mentioning that while diagnosing food allergies it is crucial to determine whether the allergic reaction is IgE mediated or non-IgE mediated. Medical history of the patient is used to establish the type of allergic reactions. If the symptoms are evident after a few minutes of exposure, it is a sign of an IgE mediated food allergy. Some of these symptoms could be erythema, vomiting, anaphylaxis, or diarrhea. Non-IgE mediated responses involve symptoms that my take hours to show, such as eczema or frequent loose stools[24].

Once the nature of the allergic reaction is established, medical practitioners can proceed with the diagnosis. If the patients' medical history implies it was a non-IgE mediated allergic reaction, the allergen is eliminated from the diet and reintroduced weeks later[25]. The diagnosis of FAs may be challenging, since food allergies are at times confused with FAs[26].

Similarly, diagnosis of AR may be challenging among children in the first few years of their lives. In the first 2-3 years of life, children frequently get viral respiratory infections, which have similar symptoms to AR. This high occurrence of viral respiratory infections makes it challenging for practitioners to diagnose toddlers with AR[27].
Currently, there are several main ways of managing allergies. More novel allergy management options are allergen-specific immunotherapy and peptide-based immunotherapy.

Pathophysiology of Allergic Diseases

There are several main types of allergies; they have a lot in common when it comes to their mechanisms. This section describes general mechanisms and cells involved in allergic reactions and allergic diseases.
Allergic diseases, such as allergic rhinitis or food allergies, are a combination of symptoms induced by inflammatory reactions. These inflammatory reactions can be categorized into IgE-mediated, non-IgE mediated, and mixed reactions. The reactions may vary on the region, for instance the nasal mucosal layer during allergic rhinitis, or the lips during food allergies. The first step of allergies is the exposure to the allergen. Some common allergens for AR are seasonal pollens, molds, pets, dust mites, and pests[28]. For food allergies, some common allergens are cow's milk, chicken eggs, and shellfish[29].
During Ig-E mediated responses, allergic reactions happen within minutes after exposure. They are induced by Th2 cells, that play an irreplaceable role in allergic inflammation; they release cytokines such as IL-4, IL-5, IL-9, and IL-13 and initiate allergic inflammation[27,30,31]. Th2 cells have an irreplaceable role in allergic inflammation; they release cytokines such as IL-4, IL-5, IL-9, and IL-13 and initiate allergic inflammation[30]. Once the exogenous allergen, e.g., pollen is inhaled, they are broken down by DCs and present T-cell epitopes to naïve T cells. Simultaneous activation of epithelial cells by nonantigenic pathways release epithelial cytokines, which transition the sensitization process into a Th2 response. During this transition, type2 innate lymphoid cells (ILC2) and basophils release Th2-producing cytokines, i.e., IL-13 and IL-4. Th2 cells differentiate after IL-4 is released. Once DCs present the allergen, Th2 activate B cells, which respond and produce IgE, which later sensitizes mast cells and basophils by binding to the high-affinity IgE (FCER1) receptor.
Mast cells and basophils release mediators, such as histamine or cysteinyl leukotrienes that cause symptoms of AR, such as mucous production. During AR, IL-9, and stem-cell factor (SCF) aid mucosal mast cells' recruitment to the mucosal layer. SCF cells are released from epithelial cells. SCF and mast cells contact via SCF's tyrosine kinase receptor (c-Kit)[32,33]. Once Th2 sensitivity is developed, the subsequent exposure to the same allergen result in Ig-E induced hypersensitivity reactions, and AR symptoms. These symptoms are caused by the activation of sensory nerves and vasodilation, which cause obstruction of the nasal passages.[34–37].
In contrast with Ig-E mediated responses, non-IgE mediated responses take longer to occur and have a chronic nature[38]. In essence, non-IgE mediated responses precipitate allergic reactions involving other parts of the immune system other than IgE antibodies. Ig-E mediated responses cause an immediate reaction, which is often noticeable minutes after exposure, whereas non-IgE mediated responses cause delayed reactions, that show symptoms days or weeks after exposure. It is crucial to establish the nature of the allergic reaction to accurately and optimally treat its symptoms[39]. Examples of non-IgE mediated FAs are food protein-induced enterocolitis syndrome, food-protein enteropathy, and food

protein0induced enterocolitis syndrome. Most of these disorders have gastrointestinal symptoms that also extend to respiratory symptoms[40]
Evidence from clinical and pre-clinical trials shows that basophils and mast cells play a significant role in food allergic reactions. Examples of mixed IgE and non-IgE reactions are eosinophilic esophagitis, or atopic dermatitis.

Current Treatment Options

Currently, allergies do not have a cure. Conventional treatment options for allergies include antihistamines, glucocorticoids, adrenaline, or mast cell stabilizers. In this section, some of the common treatment options are described, and some side effects are mentioned.

Antihistamines

Antihistamines are mostly prescribed for AR. An example of antihistamines are intranasal antihistamines, that are prescribed as a first-line treatment for AR. Intranasal antihistamines have been shown to suppress numerous immune response mediators, such as histamines, leukotrienes, cytokines, chemokines, mast cells, eosinophils, and neutrophils. Higher concentrations of antihistamines show anti-inflammatory effects[41]. Because of its local delivery, antihistamines are shown to have high effectivity treating the symptoms of AR[42].
There are several types of antihistamines, such as intranasal, first generation and second-generation antihistamines.
First generation antihistamines lack compound specificity. They lack recognition by the P-glycoprotein efflux pump, which grants them permeability. They tend to cross the blood-brain barrier and cause sedation due to their lipophilic nature[43]. Some examples of first-generation antihistamines include alimemazine, chlorphenamine, or clemastine.
In contrast, second generation antihistamines have lower affinity for muscarinic receptors, resulting in poor penetration into the CNS. They also have a higher molecular weight and affinity for the P-glycoprotein efflux pump[43].

Decongestants

Decongestants are mostly prescribed to patients with AR that also actively experience congestion. This type of medication is more relevant for patients with seasonal AR. In contrast with other treatment options, decongestants can be obtained without a prescription, which makes it a more accessible option for some patients. The main therapeutic agents in this group are phenylephrine and pseudoephedrine, that are received orally. There are also the options of topical sprays and an inhaler, that has to be used on a regular basis[44]. These agents mostly work by stimulating α-adrenergic receptors, reducing edematous mucosal tissue volume and reduce mucus production, that result in the alleviation in AR-induced symptoms. Even though decongestants are safe for most patients, they include side effects, such as stimulation of the CNS[45].

Antileukotriene agents

Leukotrienes are molecules released by mast cells during an asthma attacks and AR, that induce bronchoconstriction[46]. They are released after triggering events, such as antigen-antibody reactions, cold, or similar events that increase intercellular calcium[47,48].
These agents were first approved for treatment of asthma, however, later they were also approved for the treatment of AR. The main agents in the US are montelukast and zafirulast,

which inhibit leukotriene receptors. Another agent is zileuton, which inhibits leukotriene synthesis[49].
Antileukotriene causes very few side effects, however, reports of agitation, aggression, suicidal thoughts and depression have been made by Merck and Co-the manufacturer of Montelukast[44]. This combination of side effects makes it an unavailable option for patients with mental health problems.

Intranasal Steroids
This method of treatment is first-line therapy that reduces AR symptoms, such as inflammation and nasal congestion. Like decongestants, most intranasal steroids require prescription in the Netherlands.
The most common side effects of intranasal steroids are headache and pharyngitis[50,51]. Those side effects can be minimized by proper application technique. However, regular use of intranasal steroids among children is associated with greater is of cataracts and ocular hypertension[44].

Topical Steroids
Topical steroids are commonly used for managing skin-related symptoms induced by allergic reactions; some of these symptoms are eczema and dermatitis. There are multiple side effects associated with topical steroid application. Locally, it can cause acne and rosacea[52]. Chronic use of highly concentrated topical steroids can induce steroid rosacea, or steroid withdrawal. These side effects can significantly affect one's quality of life[53,54].

Medical Need for Treatment Options

The evolutionary high occurrence of allergic diseases does not only induce a significant burden on the healthcare system, but also results in a lower standard of life among a significant fraction of the population. Allergic diseases, such as AR, also affect the corporate environment, by greatly contributing to health-related absenteeism and lowering productivity levels among workers. Research by Lamb et al. concluded that AR-induced productivity and opportunity cost loss is the highest among employees in the United States[55].
High frequency of allergic disorders in combination with the lethal outcome of severe allergic reactions raise the medical need for novel treatment options for allergic disorders. When it comes to food allergies, the most common allergies among children are milk allergies and egg protein allergies. The standard of care for children and adolescents with allergies can be overwhelming for parents. It is crucial to manage potential nutritional deficits and growth impairment among children[56], which can be challenging depending on accessibility to healthcare. Like AR, FAs can be a burden for the guardians of children with allergies, since they are encouraged to read labels and regularly consult nutritionists. Moreover, they should always carry epinephrine, and watch out for severe allergic reactions, which causes stress and anxiety. It is also worth mentioning that currently available pharmaceutical agents only manage the symptoms, meaning the symptoms will persist after the administration of medication is discontinued[10].
A potentially disease-modifying therapeutic agent could reduce the patient burden, and potentially cure allergic disorders. Even though AIT was shown to be effective in clinical studies for both AR and FAs, it comes with challenges, and requires novelties, which will cause less side effects.

In contrast to the current treatment options described previously, AIT is the only potentially disease-modifying treatment option, which can possibly cure allergic disorders, which makes it a relevant subject for research.

Search Strategy

Major search engines, such as PubMed were used. Keywords Allergen-specific immunotherapy, allergies, allergic rhinitis, food allergies were used. For more thorough research, keywords, such as peptide-specific immunotherapy, novel immunotherapy options were used. For more detailed explanation of immunotherapy treatments, keywords such as intranasal steroids, antihistamines, and corticosteroids were used.

Immunotherapy for Allergies

Immunotherapy for allergic disorders is not historically new. There are records of food desensitization attempts from the early 20th century. Some of these attempts include Finkelstein conceptualizing oral immunotherapy (OIT), by desensitizing infants by giving drops of milk and gradually increasing the dose[57]. Another well-known example of AIT involved the treatment of grass pollen allergy during seasonal hay fever. Leonard Noon treated seasonal hay fever with grass pollen extract. He injected increasing doses of the allergen for several years once the maintenance dose was established. Evidence suggested that this method was effective for treating seasonal hay fever, cat allergies and insect venom allergies[58–60].

Allergen-specific immunotherapy is a novel treatment option for several allergic diseases, such as allergic rhinitis, asthma, and sting insect hypersensitivity[61]. AIT has also been used for the treatment of FAs, e.g. peanut allergy among children and adolescents[62].

It generally works by gradually increasing the dose of the allergen and administering maintenance doses for the next three years. AR patients that still experience symptoms after pharmacotherapy and avoidance are good candidates for the AIT treatment. Patients with uncontrolled or severe asthma, however, are not eligible for the treatment[63]. Comorbidities should be taken into consideration before prescribing AIT, which is why diagnosis is done on individual basis.

It is a type of therapy that can be used on a specific group of patients. It is used for patients that have specific IgE antibodies, that are specific to the allergens in question.

The most widely used AIT types are subcutaneous (SCIT), sublingual (SLIT) with unmodified allergen extracts. Among the two most popular types of AITs, SLIT was shown to be the more patient-friendly route of administration, which also helped saving patient time in the hospitals. Since these are new emerging treatment options, both of those methods require supervision in the beggining[64]. SLIT can also be administered by daily drops or tablets of the allergen extract, which can be later self-administered by the patient at home, which reduces hospitalization burden. Both of these routes of administration have been shown to be effective for AR[65].

For FA, the main routes of AIT administration are oral, epicutaneous, sublingual, and subcutaneous. The most researched route of administration was OIT. Most OIT trials focus on milk, egg, and peanut allergies. On the contrary, the SCIT method using unmodified food extracts was not further researched because of the severe allergic reactions it showed after administration[66,67]. Like AR, the patients are given the increased dose of the allergen throughout several years to achieve constant unresponsiveness to the allergen after remission. OIT for FA usually has a higher starting dose compared to SLIT and EPIT, which

are administered as drops under the tongue. The low doses make SLIT and EPIT safer for patients, however, they are also less efficacious methods[68]. Table 1 summarizes the main routes of AIT administration.

A study conducted by Durham et al. in 2016 compared the efficacy of SLIT to that of montelukast (a leukotriene receptor antagonist), desloratadine (an H_1 antagonist), and mometasone (an intranasal steroid) for management of seasonal and perennial AR. Overall, grass and ragweed-based SLITs were shown to be 16.3% and 17.1% more effective than placebo respectfully. On the other hand, montelukast, desloratadine, and mometasone were shown to be 5.4, 8.5, and 22.2% respectively. The main conclusion of the analysis was that SLIT options were nearly as effective as conventional therapies for treating seasonal and perennial AR. SLIT was shown to be more effective than montelukast and desloratadine, and nearly as great as mometasone. It was concluded that SLIT offered an additional long-term efficacy to the treatment, and makes it a favorable option for the treatment of AR[69].

Table 1: *Main routes of AIT administration.*

Route of Administration	Advantages	Disadvantages
Epicutaneous (EPIT)	• Administration does not require needles, making it more suitable for children[70]. • Overall well established safety profile[71].	• Larger antigen doses can induce mild drug-related adverse effects, e.g. eczema[72–74].
Intralymphatic (ILIT)	• Reduced number of sessions, and treatment duration[75]. • Is shown to be a safe treatment option[76].	• Requires physicians for administration, and is ultrasound guided[39].
Oral (OIT)	• Effective treatment option for FAs[77,78].	• Can cause adverse reactions to the treatment[79]. • Efficacy is not well established[80].
Subcutaneous (SCIT)	• Established safety and efficacy profiles. • Can be used for venom immunotherapy[81].	• Must be administered repeatedly. • Relatively long course of treatment[82]. • May result in hypersensitivity reactions, such as redness or pruritus[83].

		• Requires accessibility to hospitals[71].
Sublingual (SLIT)	• More economically favorable than SCIT[84].	• Relatively low efficacy compared to SCIT[85].

Mechanism of Action

Even though the mechanism of action of AIT is not fully known, the core principle of AIT is the gradual exposure to the antigen to gradually induce desensitization to the antigen[86]. AIT It is the only clinically effective disease-modifying treatment for AR[87,88]. The mechanism of action it may vary depending on the route of administration.

In essence, the mechanism of AIT encapsulates early desensitization effects, in combination with T- and B-cell response modulation and inhibition of eosinophil, basophil and mast cell migration to tissues[89].

The underlying mechanism and effects of AIT can be categorized in three stages: the initial reaction to AIT, intermediate effects, and long-term effects. The summary of AIT effects is shown in Figure 1.

Initially, administration of the AIT dose hinders basophil and mast cell reactivity, which results in lower reactivity from the patient. The mechanisms of the induction of this low reactivity are not fully known, however, mast cell desensitization has been shown in multiple studies. In a study conducted by Woo et al., mice were desensitized to penicillin V via oral AIT. In their results, oral desensitization to penicillin V was achieved, and antigen-specific mast-cell desensitization was associated with it[90].

Once AIT is injected, a rapid increase in IgE is noticed, which decreases later throughout the duration of the treatment.

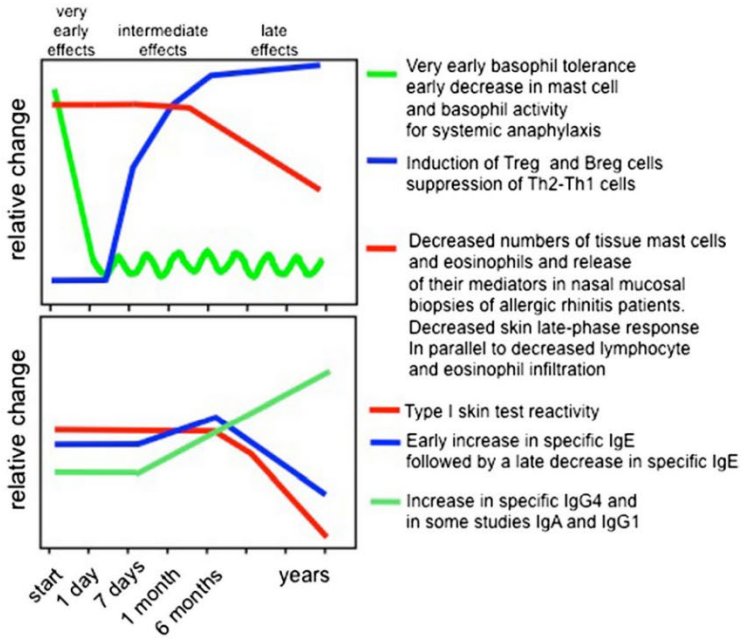

Figure 1: *Events taking place during different stages of AIT*[89].

During AIT, increased levels of anti-inflammatory cytokines IL-10 and TGF-β are produced by Treg cells, which launch peripheral T-cell tolerance[91]. The increased levels of IL-10 possibly

suppress stronger allergic responses and cause IgG4 production. In addition to increased IgG4 production, there is an increase in antigen-specific IgA, IgG1, and IgE production. However, in later stages of AIT, IgE production is decreased in the long-term, which is effective especially for seasonal AR. These changes in IgE production are usually noticeable after 12-18 months of AIT. AIT induces lowered mononuclear cell production of histamine-releasing factors, mast cells, and eosinophils[92]. As a result, patients show lowered skin prick test reactivity[93].

During subcutaneous immunotherapy for different allergies, elevations of IgG4 were detectable in the serum. These changes could possibly be caused by the allergen-immune system interactions taking place in the oral mucosa in the regional lymph nodes. A study by Scadding and Durham describes AIT mechanism of action for patients with AR. The main focus of the therapy is to reduce the seasonal increase of pollen allergen specific IgE concentrations and reduce the increase of allergen-specific IgG4 in serum[94,95].

Challenges and Limitations Associated with AIT

AIT is a unique disease-modifying treatment option for patients with IgE-induced allergic diseases, however, it comes with challenges. Despite its high efficacy, some patients do not respond well to the treatment, and experience relatively severe allergic reactions[96].

Evaluation of AIT effectiveness is challenging at times. Various clinical trials define successful outcome of therapy differently. For instance, when it comes to OIT in FAs, the perfect outcome is the complete incorporation of allergen-containing foods in the diets of participants. However, assuming how much the product will be incorporated in one's diet it relatively challenging and is not always incorporated in studies[93].

Additionally, more research is required for identifying better biomarkers for confirming clinical responsiveness of AIT. Currently, there are a few indicators, such as basophil responsiveness, amount of IL-10 producing regulatory T and B cells, and IgE/IgG4 ratio. However, more research is required to establish causation, and to show the relationship between these markers and AIT effectiveness[7].

Other limitations of AIT include the heterogenous nature of patients with perennial AR, and possible severe allergic reactions to the allergen extract. A study by Biregani et al points out that patients may be sensitive to the components in the allergen extract, which might precipitate an IgE response, resulting in more inflammation and allergic reactions[96].

Various studies have evaluated the disadvantages associated with AIT. A study by Linda Cox outlined the practical considerations when it comes to AIT. For example, SCIT can easily cause adverse side effects due to its narrow therapeutic window. Therefore, it requires medical supervision and a longer wait period[97].

Even though AIT is the only disease-modifying treatment option for IgE-mediated allergies, the side effects can be of severe nature. Some of the severe side effects include anaphylaxis resulting in lethal outcomes. The number of AIT associated anaphylactic reactions has increased after the introduction of standardized and more potent extracts. Some of the causes of the sensitivity reactions are errors in allergen dosage. The potential risk factors and the long commitment duration result in patients discontinuing the therapy.

A limitation worth mentioning is the possible enzymatic degradation associated with OIT. Once the medication surpasses through the stomach, it might be dissolved, resulting in decreased absorption, and lower efficacy. To overcome this limitation, novel strategies of AIT delivery are currently being developed, e.g., nanoparticles, that can protect the encapsulated antigen. Once the OIT vaccine is protected by a nanoparticle capsule, it can

survive the harsh conditions of the gastrointestinal tract, such as high acidity of the stomach[98].

Novel AIT Options

Even though AIT comes with challenges, there have been developments to make advancements to it. The increasing understanding of T-cells' role in allergy caused an increased interest in the use of short T-cell peptides as an alternative to AIT. Evidence suggests that IgE-binding epitopes were redundant in immunotherapy, and removing them could increase safety and efficacy[10]. A review by Rajakulendran et al. outlines nine novel strategies in immunotherapy[8]. This section discusses some of the relatively novel approaches to AIT.

Peptide-based Immunotherapy (PIT)

PIT is a novel method for treating allergic diseases. In contrast to conventional AIT, T-cell epitopes are used instead of B-cell epitopes. Conventional AIT uses B cell epitopes, which results in adverse reactions. In PIT, the lack of B-cell epitopes is believed to result in less adverse reactions to the allergen[96]. The effectiveness of PIT varies through clinical trials. PIT is further researched and developed for allergic and autoimmune diseases[10].

One of the main distinctions between PIT and AIT is the use of T cell epitopes in PIT. To understand mechanisms involved in PIT, it is important to understand the role of molecules involved in a T-cell mediated immune response. Generally, T cells recognize the epitopes presented by major histocompatibility complex (hereafter MHC) on the surface of cells. They recognize the harmless "self" peptides and peptides that come from pathogens, or allergens. After recognition of harmful epitopes, T cells undergo activation to initiate an immune response[99]. In contrast to B-cell induced immune responses, T-cells antigen receptors can only recognize the antigen when it is bound to cell surface molecules that were encoded by the MHC. This differentiation between B- and T-cells was first observed from studies conducted by Gell and Benacerraf in 1950s, when lymphocytes were not divided into varying classes. They hypothesized that the recognition of antigens relied on the tertiary configuration of the immunizing protein, the cells effecting the delayed type hypersensitivity response had the ability to recognize peptides defined by the primary amino acid sequence[100]. In short, T-cell activation starts after a ternary complex consisting of the T-cell receptor, nominal antigen, and class-I or -II MHC molecules is formed[101,102]. Additional molecules necessary for T-cell activation are CD4 and CD8 co-receptors, that are present on $CD4^+$ and $CD8^+$ receptors respectfully[103]. Moreover, the CD3 complex is necessary to aid the transduction of signals in T-cell activation[104]. Lastly, CD28 co-receptor that binds to B7, which is a molecule present in nucleated antigen presenting cells[105,106].

A study by Tarzi et al. evaluates the effectiveness of HLA-DR based phospholipase2 (hereafter PLA_2) vaccine in individuals with a mild honeybee allergy in an open, controlled study. HLA-DR is one of three variants of class 2 MHC (hereafter MHC) molecules that are expressed by human antigen presenting cells. It is a type 1 membrane glycoprotein that presents peptides; these peptides are later recognized by T-cell receptors and helper T cells[107]. PLA_2 is a glycoprotein, that is the main allergen found in bee venom[108].

12 participants with sensitivity to bee venom were selected; these volunteers received injections containing PLA_2 peptides.

The effectiveness of the vaccine was evaluated by the size of the late-phase reaction to the allergen, peripheral blood mononuclear cell (hereafter PBMC) proliferation, cytokine release, and gene expression[109].

Allergoids

Traditional allergen extracts are made from natural allergen sources, and come with disadvantages, such as an undefined amount of nonallergenic materials, that can cause Th2 responses. Allergoids are allergen extracts that have been chemically modified by glutaraldehyde or formaldehyde. First, allergens are depigmented with acid, then they are polymerized with glutaraldehyde[110]. The chemical modifications result in a higher molecular weight and cause less sensitivity. Modified allergens were developed because they are less likely to cause severe allergic reactions, yet could be used for retaining immunogenicity[111]. They are modified to be used in desensitization procedures and inducing tolerance. A significant advantage of allergoids is the opportunity of faster increase of the dose during allergen therapy[112]. The latter results in a shorter duration of treatment.

A study by Henmar et al. compared allergenicity of four grass pollen allergoid with three grass pollen allergen vaccines used during SCIT *in vitr*o. Allergenicity was measured via IgE inhibition and basophil activation assays. The allergoids were found to cause lower allergenicity as well as lower T-cell activation rates. It was concluded that the commercially available allergoids did not contain high doses of immunogenic ingredients[113].

A study by Gallego et al studied the effects of a mixture of two types of dust mite (*Dermatophagoides pteronyssinus* and *Dermatophagoides farina*) allergoids for the treatment of asthma patients. The allergoid had the commercial name Depigoid. After receiving the treatment for approximately a year (54 weeks), reduced use of emergency medication was reported. Additionally, less respiratory and ocular symptoms were present among participants compared to the placebo group[114].

Currently, grass pollen allergoid vaccines are commercially available; several studies are evaluating this methods cost-effectiveness compared to conventional AIT, which is believed to be more immunogenic[115].

Another example of allergoids is Pollinex Quattro- a four-injection allergoid therapy used for the treatment of AR and allergic asthma. A study by Rosewich et al. describes the steps of allergoid preparation and the immunological changes after the treatment. Allergoids are prepared based on patient needs and the types of pollen they are sensitive to. Natural allergens are extracted and diafilterd via nominal molecular weight and treated with glutaldehyde to form allergens Once the allergoids are synthesized based on patients' needs and sensitivities, they receive gradually increasing doses of the allergoid throughout a four-week treatment. Once the treatment is finished, it suppresses the allergy-induced IgE production, and therefore decreases patient sensitivity. [116]. Results from 12 patients were analyzed after they received Pollinex Quatro. Patients that took the allergoid therapy showed decreased expression of CD34, DC54 and HLA-DR- II on B cells compared to the subjects from the placebo group; the decreased expression is an indication of reduced B-cell activation, and therefore decreased immunogenicity. The clinical efficacy of this therapeutic agent was also clinically shown[117]. After the Pollinex Quattro treatment, patients show less sensitive skin-prick sensitivity reactions[118–120].

Immunostimulatory Sequences (ISS)

Immunostimulatory sequences (ISS) work by inducing a strong Th1 response. It also acts as an immunotherapy adjuvant. Immunostimulatory DNA that contains unmethylated CpG motifs is recognized by toll-like receptor9 (TLR9), which result in the activation of innate

immune responses. The activation of innate immune responses results in the subsequent activation of adaptive immune responses[121].
Once pathogen exposure takes place, pattern recognition receptors (PRRs), such as TLRs, recognize the pathogen. The most studied TLR is TLR9, which is expressed on APCs, such as DCs, B cells, or macrophages[122]. Once TLR9 encounters a pathogen-associated molecular pattern (PAMP), such as a pathogen lipid or a peptide, it moves to endosomal compartments. PAMPs are by nature unmethylated CpG-containing pathogenic DNA. CpG is a dinucleotide that is necessary for TLR9 activation[123]. TLR9 ligands can include DNA from viruses, bacteria, plasmids, or short, synthetic oligodeoxynucleotide (ODN) sequences, that are modified with one or more CpG units. The ODN sequences engineered with CpG units are the ones that are referred to as ISSs. Those ISS ODN units are used as a core constituent of vaccinations[124–126].
Small-scale clinical studies for ISS show improvements in symptoms among subjects with AR[127]. Pre-clinical *in vitro* studies using food allergen sequences resulted in lower suppressed IgE levels[128,129].

Monoclonal Antibodies
Monoclonal anti-IgE and anti-IL-4 antibodies (hereafter mAb) are generally used for treating allergy-related asthma[8]. In recent studies, mAbs such as Omalizumab have been studied as potential treatment options for FA in children. In FA, Omalizumab is conventionally used for managing reactions, such as anaphylactic shock[130]. A study by Guilleminault et al. has combined conventional OIT with Omalizumab to explore its effects on the quality of life and safety of OIT administration. The choice of Omalizumab in this study could be explained by two major reasons. First, Omalizumab is one of the more extensively studied mAbs; there is high abundance of literature explaining its mechanism of action. Second, Omalizumab's effect on IgE has been studied in detail[131,132]. The effects of Omalizumab in combination with conventional OIT have been summarized in Figure 2. The main results of the study were that OIT in combination with Omalizumab could decrease the risk of post-administrative severe adverse reactions, by therefore increasing the day-to-day quality of life. In essence, the combination of mAbs with conventional OIT can increase the patient pool eligible for the treatment. One of the downsides of conventional OIT is the cause of severe hypersensitivity reactions after exposure. Administering Omalizumab pre-OIT can also alter the threshold of reactivity and potentially prevent severe reactions.

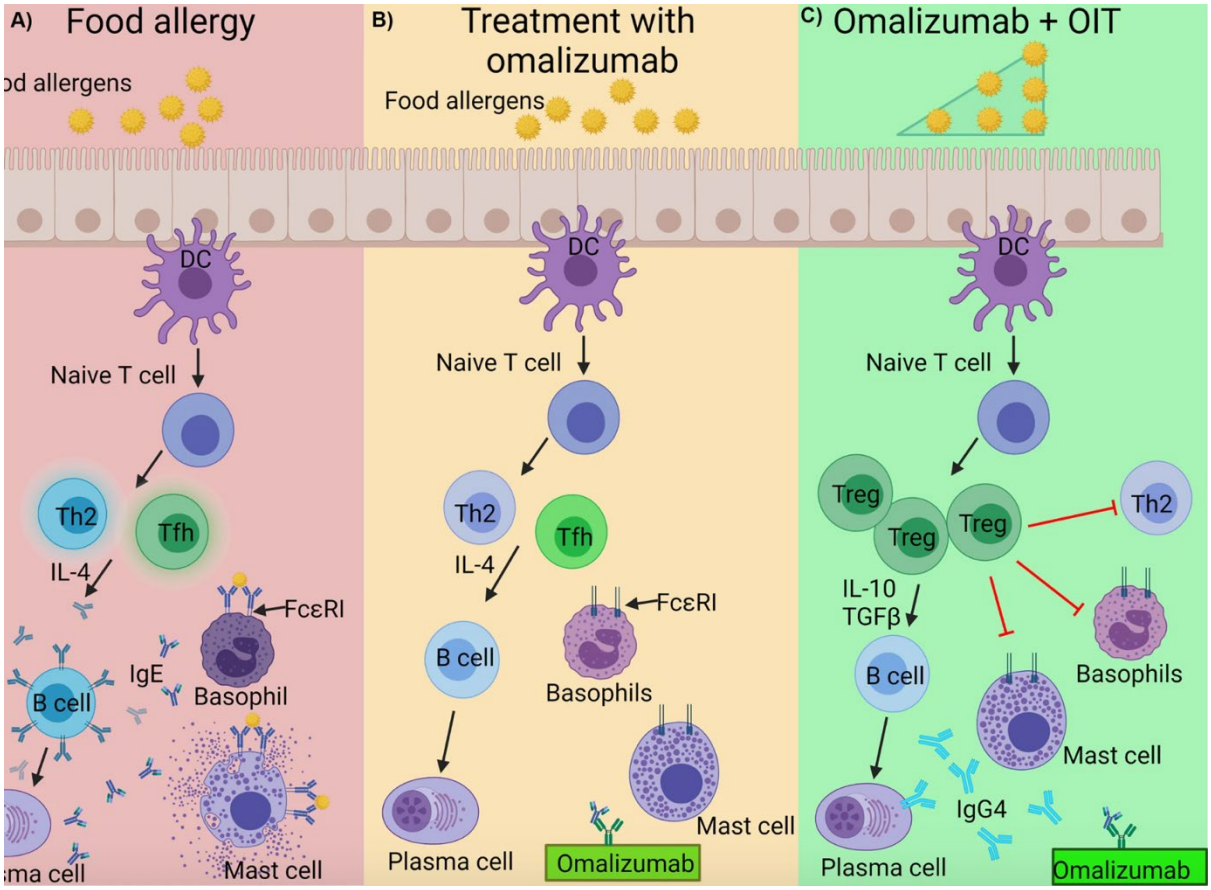

Figure 2: *Changes in the immune system during FA (A), treatment with Omalizumab (B), and Omalizumab in combination with OIT (C). During FA (A), the allergen is engulfed by DCs, and allergen-derived peptides are presented, resulting in T cell differentiation into CD4+ Th2 cells. This leads to recombination of B cells towards Ig-E producing plasma cells. Once there is re-exposure to the same antigen, histamine and other inflammatory mediators are released. When treating FAs with only Omalizumab (B), the anti-IgE mAB, the binding of IgE to FcεRI is inhibited, resulting in mast cell and basophil not showing sensitivity to the allergen. Lastly, when combining OIT with Omalizumab, subsequent decrease of IgE production, and inhibition of mast cell and basophil activation is shown. The risk of adverse reactions shown during OIT is reduced while Omalizumab is used as a pre-treatment for OIT.*[133].

Because of the high cost of mAbs, this option is not a convenient modality of first-line treatment for most patients. However, it is a more suitable option for patients that have shown hypersensitivity to conventional OIT and are not eligible for first line OIT. To reduce the financial burden of this novel treatment option, the patient pool can be selected based on biomarkers and previous history of severe reactions associated with FAs. As mentioned in studies, the combination of mAbs with OIT seems promising particularly for the treatment of FAs. Lastly, more research is required for the approval of those new active substances for the purpose of alleviating FA-associated hypersensitivity reactions.

Carriers

Currently, AIT is administered using various carriers or adjuvants to ensure highest efficacy and best possible delivery route. Like adjuvants in vaccines, adjuvants in AIT aim to establish a healthy immune response in the long run and ameliorate the state of the pathology. Some of the available adjuvants in the market are alum, calcium phosphate, microcrystalline

tyrosine, and monophosphoryl lipid A [134,135]. Most of those adjuvants have been used for decades in AIT, with alum being the most prevalent adjuvants due to its convenience, easy preparation, and high stability[136]. Even though the alum adjuvant has been in use for decades, it does cause concern when it comes to long-term application. If the alum adjuvant is ingested during a full OIT course, it exceeds the recommended oral intake levels by World Health Organization. A full OIT course takes between three and four years. Ingestion of alum during an AIT course, such as OIT or SCIT for a substantial period may result in toxicity. The long-term excision of alum should be avoided if there are advanced alternatives of AIT[137]. Incorporating novel carriers into AIT can potentially improve drug delivery and therefore improve treatment efficacy. Some of the novel carriers for AIT include nanoparticles, liposomes, virus-like particles (hereafter VLPs), and chitosan.

Nanoparticles have been utilized previously in fields such as oncology for improving the safety and the efficacy of the treatment[138]. Recently, nanoparticles have been proposed for the treatment of allergic diseases, due to their properties, such as easy tissue permeation, defined size, or relatively easy production[134]. Studies have been conducted for comparing the efficacy of nanoparticles to that of microparticles. For instance, a study by Palmer et al. investigated the effect of transdermal delivery of amorphous silicon dioxide nanoparticles (hereafter SiNPs) during contact dermatitis. During the study, the effect of SiNPs was reported on healthy mouse skin and mouse skin with contact dermatitis. The results showed that SiNPs showed almost no effect on healthy mouse skin, whereas there was an immunomodulatory effect on the skin with contact dermatitis. The main conclusion of the study was the confirmation of SiNPs to treat allergic diseases, such as contact dermatitis, yet more research is required[139]. Another study by Hirai et al. compared the skin permeation and localization of monodisperse amorphous silica nanoparticles in mice. The study showed that nanoparticles result in improved localization of AIT. The studies show AIT's ability to permeate the skin barrier[140]. Upon improved and more detailed research is conducted regarding nanoparticles and AIT, it can be used for relatively novel AIT administration routes, e.g., the intranasal route[141].

Like nanoparticles, liposomes are a novel carrier option for allergens in AIT. Studies have been conducted for studying liposomal ability to alleviate allergy symptoms. Due to liposomal nature, they can encapsulate both hydrophobic and hydrophilic antigens, prevent them from degranulation, and release the antigen at a the designed rate[142]. A study by Kawakita et al. used oligomannose-coated liposomes (hereafter OMLs) in allergen-sensitized mice to prevent allergic diarrhea. The study was based on the previously established connection between CD8+ regulatory T cells and the prevention of allergic diarrhea. CD8+ regulatory T cells have the established ability to prevent allergic diarrhea. Therefore, the ability of OMLs to induce CD8+ regulatory T cells for the prevention of those allergic symptoms was studied. The results of the study suggested that intranasal immunization of mice can be effective against FAs, since it prevented allergic diarrhea symptoms[143].

Efficacy of liposomes was also shown in a study conducted by Arora and Gangal, where they studied the efficacy of pollen allergen combination with liposomes for downregulation of sensitivity reactions in mice. The study resulted in mice treated with liposomal antigens showing higher immunogenicity and higher IgG levels. This novel approach works by preventing IgE synthesis and downregulate the immune response among infected mice. It was concluded that liposomal ability of entrapping antigens and delivering them could play an important role in managing type1 hypersensitivity reactions[144]. In essence, liposomes are a promising approach to drug delivery during AIT.

Moreover, virus-like particles (hereafter VLPs) have also been studied as a novel carrier for allergens during AIT. Previously, VLPs have shown high tolerance and effectiveness in vaccines, such as hepatitis B, or human papillomavirus (HPV); they are still being applied in clinical research[145]. Due to their convenience, they have also been used for development of unconventional vaccines, such as a vaccine against nicotine addiction[146]. VLPs are a suitable target for allergen delivery due to their low allergenicity, high immunogenicity, and relative ease at preparation. An example of such VLPs is the bacteriophage Qbeta, which is conventionally used for increasing antibody count in cancer immunotherapy. Research suggests it can also be used during allergy immunotherapy. For instance, a study by Beeh et al. investigated the clinical efficacy of bacteriophage Qbeta-derived VLP containing CpG-motif G10 (hereafter QbG10) in patients with persistent allergic asthma. The results showed improvement in all the parameters they used for measurement patient wellbeing. The placebo group of the study showed deterioration in their condition. After a two-week period, two-thirds of patients in the experimental group had their asthma under control and managed the symptoms relatively well. The main conclusion of the study was that QbG10 had potential to aid patients in asthma control[147]. Overall, studies demonstrate that further long-term research is required for establishing the safety profile and the long-term effects[148].

Lastly, chitosan was proposed as a carrier for AIT for alleviating side effects and local irritation induced by non-invasive inhaled antihistamines. Even though AR is well managed by antihistamines, such as ketotifen (hereafter KT) or cetirizine (hereafter CTZ), it results in local irritation and raising the medical need for improved less allergenic treatment options. A study by Sun et al. incorporated KT and CTZ decorated hydroxybutyl chitosan nanoparticles (hereafter CNPs) for long term AR treatment and management. The results of the study demonstrated that CNP use resulted in longer inhibition of histamine release compared to that of free KT and CTZ molecules without chitosan nanoparticles. CNP and free KT and CTZ administration resulted in 24- and 12-hour inhibition of histamine release respectfully. In short, CNPs showed comparative efficacy to that of free form antihistamines, i.e., KT and CTZ. However, they showed those results after a reduced number of administrative treatments, and with a lower dose. The study came to the conclusion that CNPs application is effective in low doses for alleviating the symptoms associated with AR therapy, and could possibly be a more advanced alternative to already available free antihistamines[149].

Recombinant Proteins

The core principle behind recombinant proteins application lies in cloning allergens and using them during AIT. Even though AIT has been applied for about a century, AIT with recombinant allergens has been used in clinical practice for about a few decades[150]. In the last decade, the genetic code of hundreds of allergens has been deciphered. The replicated allergen is intended to make AIT safer due to their lower allergenic nature[151]. In contrast to conventional preparation of allergens, recombinant proteins have a more precise molecular composition, resulting in more optimal treatment. For instance, a study by Focke et al. has used commercially available timothy pollen allergen extracts from eight different sources to compare their molecular composition. The results showed composition differences, and some allergens were either degraded or almost undetectable. The study suggested that using recombinant grass pollen allergen can reduce those differences in allergen amount and composition. It was stated in the discussion that this difference in compositions was

overcome in another study by using a synthesized recombinant allergen instead of previously approved free allergens. In a study by Lundberg et al., recombinant allergen was used for treating medical latex allergy. Recombinant allergens showed to be an effective and a more advanced way of AIT preparation. The conclusion of the study mentioned that sometimes it can be challenging to reproduce some allergens, yet the available antigens are potentially solving the problem of heterogenicity of allergens in AIT[152,153].
Multiple studies have shown the effectiveness of recombinant allergens for AIT. For instance, a study by Niederberger et al. used a recombinant-protein based allergy vaccine for the treatment of major birch pollen allergy. The results of the double-blind placebo-controlled study showed that the use of genetically engineered allergens induced the production of protective IgG antibodies that later inhibited inflammatory reactions. Patients showed less pollen-induced IgE production[150]. The basic principle of recombinant protein application in AIT is depicted in Figure 3.

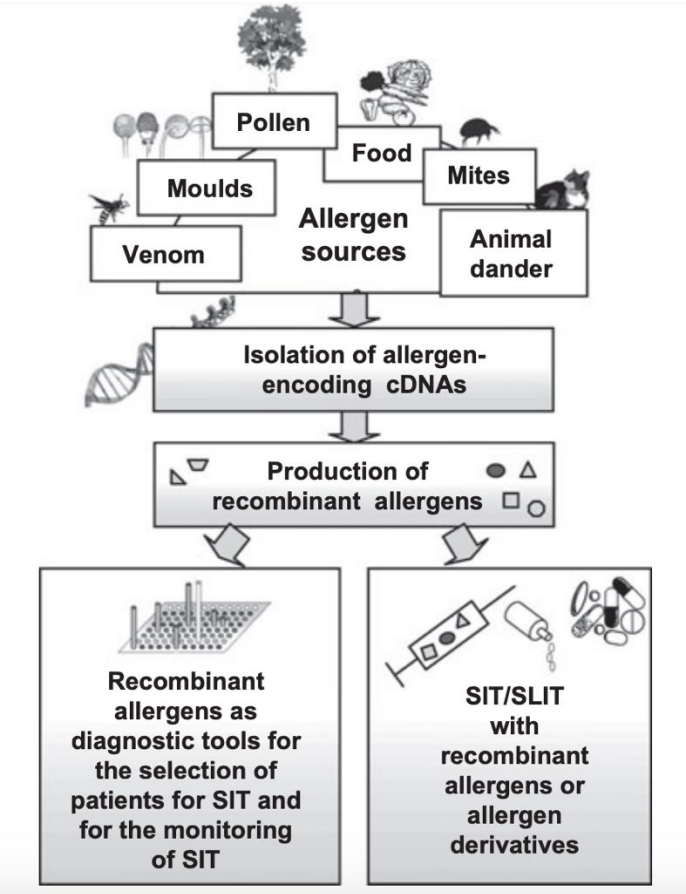

Figure 3: *Encapsulation of cDNA production and its application in AIT diagnosis and treatment. cDNA is produced based on the allergy in question. First, the allergen is selected, and the allergen encoding cDNA is isolated. Subsequently, the recombinant allergens are synthesized and used for diagnosis and treatment of Specific Allergen Immunotherapy (hereafter SIT) and AIT*[151].

Probiotics

Lastly, probiotics have been used as adjuvants for AIT. They have been also prominent for their ability to boost the immune system of patients[154]. Their potential as AIT adjuvants has been demonstrated in multiple recent studies. A study by Liu et al. studied the efficacy of AIT in combination with probiotics to free AIT. They have suggested that using probiotics,

such as *Clostridium butyricum* (hereafter CB) increases the efficacy of AIT for asthma patients. The results of the study showed that AIT alone helped asthma patients, yet other biomarkers did not change their value. On the contrary, AIT in combination with CB, serum levels of those markers, such as (IL)-4, IL-5, IL-13 were improved. The duration of the therapeutic effect was also significantly different between the control and experimental groups. In the control group, the patient's therapeutic effect lasted only for two months. However, patients that received both AIT and CB experienced the therapeutic effect for about 12 months, which is a significant improvement. The main difference between these two treatments was that CB was capable to induce IL-10+ B cells (hereafter B10), that resulted in the conversion of antigen specific B cells to antigen specific regulatory B cells. The production of B10 cells is crucial in the process of AIT since they can produce mast-cell blocking IgG4 cells[155]. The study showed that using a combination of AIT with probiotics such as CB is beneficial for patients with asthma due to events, such as B10 production, and inhibition of inflammatory effects for a longer period of time[156].

Conclusion and Future Insights

AIT is the only disease-modifying treatment option for allergic diseases, such as AR or FA. Despite the possible limitations and downsides among various types of IT, novel options are in development and in use to overcome those limitations. This review outlined the current AIT options and focused on describing the novel options that are in clinical development. More research is required for investigating the side effects of novel AIT options. Currently, there are many AIT options in development; most studies show that there are better AIT options for patients with allergic diseases. However, all studies conclude that more research is required before we can apply those AIT options.

For instance, more studies could be conducted for establishing factors that can reduce immunogenicity of AIT, or reduce the severity of side effects. Additionally, more allergens could be isolated for the production of recombinant proteins for AIT and for allergy diagnosis. As mentioned above, about 200 of the main allergens were isolated for this novel AIT option. The production of more variants of recombinant proteins could increase the patient pool eligible for recombinant AIT.

Studies also mention novel carrier options, such as parasites, herbal medicine or vitamin D for AIT. However, currently there is not enough research to suggest those carriers' viability and safety. More research into the mechanisms behind those carriers associated with AIT could open up new possibilities for the patients who could not use conventional AIT. In conclusion, multiple AIT variations are being developed to make allergy treatment accessible for all patients with allergic diseases due to their high prevalence and the need of management.

References

1. Pavón-Romero GF, Parra-Vargas MI, Ramírez-Jiménez F, Melgoza-Ruiz E, Serrano-Pérez NH, Teran LM. Allergen Immunotherapy: Current and Future Trends. *Cells*. 2022;11(2). doi:10.3390/cells11020212
2. Goldstein GB, Heiner DC. Clinical and immunological perspectives in food sensitivity. A review. *J Allergy*. 1970;46(5):270-291. doi:10.1016/0021-8707(70)90068-7
3. Zukiewicz-Sobczak WA, Wróblewska P, Adamczuk P, Kopczynski P. Causes, symptoms and prevention of food allergy. *Postep Dermatologii i Alergol*. 2013;30(2):113-116. doi:10.5114/pdia.2013.34162
4. Cohen SG. Landmark Commentary Asthma in Antiquity: The Ebers Papyrus. Published online 1992:147-154.
5. Anderson PJ. History of Aerosol Therapy : Liquid Nebulization to MDIs to DPIs Ceramic Inhalers (19th Century). *Respir Care*. 2005;50(9):1139-1149.
6. Branum AM, Lukacs SL. Food allergy among children in the United States. *Pediatrics*. 2009;124(6):1549-1555. doi:10.1542/peds.2009-1210
7. Głobińska A, Boonpiyathad T, Satitsuksanoa P, et al. Mechanisms of allergen-specific immunotherapy: Diverse mechanisms of immune tolerance to allergens. *Ann Allergy, Asthma Immunol*. 2018;121(3):306-312. doi:10.1016/j.anai.2018.06.026
8. Rajakulendran M, Tham EH, Soh JY, Van Bever H. Novel strategies in immunotherapy for allergic diseases. *Asia Pac Allergy*. 2018;8(2):1-18. doi:10.5415/apallergy.2018.8.e14
9. Pichler WJ. Immune mechanism of drug hypersensitivity. *Immunol Allergy Clin North Am*. 2004;24(3):373-397. doi:10.1016/j.iac.2004.03.012
10. Larché M. Peptide immunotherapy for allergic diseases. *Allergy Eur J Allergy Clin Immunol*. 2007;62(3):325-331. doi:10.1111/j.1398-9995.2006.01309.x
11. Haider S, Simpson A, Custovic A. Genetics of Asthma and Allergic Diseases. *Handb Exp Pharmacol*. 2022;268(1):313-329. doi:10.1007/164_2021_484
12. Weidinger S, Gieger C, Rodriguez E, et al. Genome-wide scan on total serum IgE levels identifies FCER1A as novel susceptibility locus. *PLoS Genet*. 2008;4(8). doi:10.1371/journal.pgen.1000166
13. Akhabir L, Sandford AJ. Genome-wide association studies for discovery of genes involved in asthma. *Respirology*. 2011;16(3):396-406. doi:10.1111/j.1440-1843.2011.01939.x
14. Barnes PJ. Pathophysiology of allergic inflammation. *Immunol Rev*. 2011;242(1):31-50. doi:10.1111/j.1600-065X.2011.01020.x
15. Cox L. Overview of serological-specific IgE antibody testing in children. *Curr Allergy Asthma Rep*. 2011;11(6):447-453. doi:10.1007/s11882-011-0226-3
16. Untersuchun- U, Globuline S-. The prick test, a recent cutaneous test for the diagnosis of allergic disorders. 1960;38:22-24.
17. Heinzerling L, Mari A, Bergmann KC, et al. The skin prick test - European standards. *Clin Transl Allergy*. 2013;3(1):1-10. doi:10.1186/2045-7022-3-3
18. Kostant GH. Patch testing. In: *Journal of Occupational Medicine*. Vol 6. ; 1964:381-382. doi:10.5005/jp/books/12813_4
19. Foussereau J. History of epicutaneous testing: the blotting-paper and other methods. *Contact Dermatitis*. 1984;11(4):219-223. doi:10.1111/j.1600-0536.1984.tb00987.x
20. Siles RI, Hsieh FH. Allergy blood testing: A practical guide for clinicians. *Cleve Clin J Med*. 2011;78(9):585-592. doi:10.3949/ccjm.78a.11023

21. Williams PB, Ahlstedt S, Barnes JH, Söderström L, Portnoy J. Are our impressions of allergy test performances correct? *Ann Allergy, Asthma Immunol*. 2003;91(1):26-33. doi:10.1016/s1081-1206(10)62054-6
22. Hamilton RG. Clinical laboratory assessment of immediate-type hypersensitivity. *J Allergy Clin Immunol*. 2010;125(2 SUPPL. 2):S284-S296. doi:10.1016/j.jaci.2009.09.055
23. Hamilton RG, Franklin Adkinson N. In vitro assays for the diagnosis of IgE-mediated disorders. *J Allergy Clin Immunol*. 2004;114(2):213-225. doi:10.1016/j.jaci.2004.06.046
24. Sherwood E, Boyd A. Food Allergy in Children and Young People. *InnovAiT Educ Inspir Gen Pract*. 2012;5(2):76-82. doi:10.1093/innovait/inr221
25. Walsh J, O'Flynn N. Diagnosis and assessment of food allergy in children and young people in primary care and community settings: NICE clinical guideline. *Br J Gen Pract*. 2011;61(588):473-475. doi:10.3399/bjgp11X583498
26. Boyce JA, Jones SM, Rock L, et al. *Guidelines for the Diagnosis and Management of Food Allergy in the United States: Report of the NIAID-Sponsored Expert Panel*. Vol 126. Elsevier Ltd; 2010. doi:10.1016/j.jaci.2010.10.007
27. Zhang L, Han DM. [An introduction of allergic rhinitis and its impact on asthma (ARIA) 2008 update]. *Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi*. 2008;43(7):552-557.
28. Wheatley LM, Togias A. Clinical practice. Allergic rhinitis. *N Engl J Med*. 2015;372(5):456-463. doi:10.1056/NEJMcp1412282
29. Matsuo H, Yokooji T, Taogoshi T. Common food allergens and their IgE-binding epitopes. *Allergol Int*. 2015;64(4):332-343. doi:10.1016/j.alit.2015.06.009
30. Barnes PJ. Immunology of asthma and chronic obstructive pulmonary disease. *Nat Rev Immunol*. 2008;8(3):183-192. doi:10.1038/nri2254
31. Rapiejko P, Jurkiewicz D, Pietruszewska W, Zielnik-jurkiewicz B, Woroń J. Treatment strategy of allergic rhinitis in the face of modern world threats. Published online 2018:1-12. doi:10.5604/01.3001.0011.8057
32. Jensen BM, Akin C, Gilfillan AM. Pharmacological targeting of the KIT growth factor receptor: A therapeutic consideration for mast cell disorders. *Br J Pharmacol*. 2008;154(8):1572-1582. doi:10.1038/bjp.2008.204
33. Reber L, Da Silva CA, Frossard N. Stem cell factor and its receptor c-Kit as targets for inflammatory diseases. *Eur J Pharmacol*. 2006;533(1-3):327-340. doi:10.1016/j.ejphar.2005.12.067
34. Sokol CL, Chu NQ, Yu S, Nish SA, Laufer TM, Medzhitov R. Basophils function as antigen-presenting cells for an allergen-induced T helper type 2 response. *Nat Immunol*. 2009;10(7):713-720. doi:10.1038/ni.1738
35. Yoshimoto T, Yasuda K, Tanaka H, et al. Basophils contribute to TH2-IgE responses in vivo via IL-4 production and presentation of peptide-MHC class II complexes to CD4+ T cells. *Nat Immunol*. 2009;10(7):706-712. doi:10.1038/ni.1737
36. Galli SJ. Mast cells and basophils. *Curr Opin Hematol*. 2000;7(1):32-39. doi:10.1097/00062752-200001000-00007
37. Galli SJ, Kalesnikoff J, Grimbaldeston MA, Piliponsky AM, Williams CMM, Tsai M. Mast cells as "tunable" effector and immunoregulatory cells: Recent advances. *Annu Rev Immunol*. 2005;23:749-786. doi:10.1146/annurev.immunol.21.120601.141025
38. Pelz BJ, Bryce PJ. Pathophysiology of Food Allergy. *Pediatr Clin North Am*. 2015;62(6):1363-1375. doi:10.1016/j.pcl.2015.07.004

39. Assa'Ad A. Novel Administration routes for AIT. *Pediatr Ann*. 2014;43(8):312. doi:10.3928/00904481-20140723-06
40. Nowak-Węgrzyn A, Katz Y, Mehr SS, Koletzko S. Non-IgE-mediated gastrointestinal food allergy. *J Allergy Clin Immunol*. 2015;135(5):1114-1124. doi:10.1016/j.jaci.2015.03.025
41. Kaliner MA, Berger WE, Ratner PH, Siegel CJ. The efficacy of intranasal antihistamines in the treatment of allergic rhinitis. *Ann Allergy, Asthma Immunol*. 2011;106(2 SUPPL.):S6-S11. doi:10.1016/j.anai.2010.08.010
42. Lieberman P. Intranasal antihistamines for allergic rhinitis: mechanism of action. *Allergy Asthma Proc*. 2009;30(4):345-348. doi:10.2500/aap.2009.30.3263
43. Mahdy AM, Webster NR. Histamine and antihistamines. *Anaesth Intensive Care Med*. 2011;12(7):324-329. doi:10.1016/j.mpaic.2011.04.012
44. Malone M. Review: Side Effects of Some Commonly Used Allergy Medications (Decongestants, Anti-Leukotriene Agents, Antihistamines, Steroids, and Zinc) and Their Safety in Pregnancy. *Int J Allergy Medicat*. 2017;3(1):1-6. doi:10.23937/2572-3308.1510024
45. Kaya Z, Tuncez A. Adverse cardiac effects of decongestant agents. *Eur J Gen Med*. 2013;10(SUPPL.1):32-35. doi:10.29333/ejgm/82301
46. Löfdahl CG, Reiss TF, Leff JA, et al. Randomised, placebo controlled trial of effect of a leukotriene receptor antagonist, montelukast, on tapering inhaled corticosteroids in asthmatic patients. *Br Med J*. 1999;318(7202):87-90. doi:10.1136/bmj.319.7202.87
47. Scow DT, Luttermoser GK, Dickerson KS. Leukotriene inhibitors in the treatment of allergy and asthma. *Am Fam Physician*. 2007;75(1):65-70.
48. Salvi SS, Krishna MT, Sampson AP, Holgate ST. The anti-inflammatory effects of leukotriene-modifying drugs and their use in asthma. *Chest*. 2001;119(5):1533-1546. doi:10.1378/chest.119.5.1533
49. Wardle EN. Leukotriene antagonists and synthesis inhibitors: New directions in asthma therapy. *Lancet*. 1976;308(7986):628. doi:10.1016/S0140-6736(76)90692-9
50. Sastre J, Mosges R. Local and systemic safety of intranasal corticosteroids. *J Investig Allergol Clin Immunol*. 2012;22(1):1-12.
51. Zitt M, Kosoglou T, Hubbell J. A Review of Safety and Systemic Effects. 2007;30(4):317-326.
52. Mooney E, Rademaker M, Dailey R, et al. Adverse effects of topical corticosteroids in paediatric eczema: Australasian consensus statement. *Australas J Dermatol*. 2015;56(4):241-251. doi:10.1111/ajd.12313
53. Hengge UR, Ruzicka T, Schwartz RA, Cork MJ. Adverse effects of topical glucocorticosteroids. *J Am Acad Dermatol*. 2006;54(1):1-15. doi:10.1016/j.jaad.2005.01.010
54. Coondoo A, Phiske M, Verma S, Lahiri K. Side-effects of topical steroids: A long overdue revisit. *Indian Dermatol Online J*. 2014;5(4):416. doi:10.4103/2229-5178.142483
55. Lamb CE, Ratner PH, Johnson CE, et al. Economic impact of workplace productivity losses due to allergic rhinitis compared with select medical conditions in the United States from an employer perspective. *Curr Med Res Opin*. 2006;22(6):1203-1210. doi:10.1185/030079906X112552
56. Christie L, Hine RJ, Parker JG, Burks W. Food allergies in children affect nutrient intake and growth. *J Am Diet Assoc*. 2002;102(11):1648-1651. doi:10.1016/S0002-

8223(02)90351-2
57. Freier S. KB. Allergy in infants and children. *Nebr State Med J*. 1954;39(5):199-202. doi:10.1001/archpedi.1920.01910240021003
58. Bousquet J, Lockey R, Mailing HJ, et al. Allergen immunotherapy: Therapeutic vaccines for allergic diseases. *Ann Allergy, Asthma Immunol*. 1998;81(5 I):401-405. doi:10.1016/S1081-1206(10)63136-5
59. Varney VA, Edwards J, Tabbah K. Clinical efficacy of specific immunotherapy to cat dander: A double-blind placebo-controlled trial. *Am J Rhinol*. 1997;11(6):493. doi:10.1046/j.1365-2222.1997.1220903.x
60. Kevin H. A controlled trial of mmunotherapy in insect hypersensitivity. Published online 2010:2015.
61. Mueller RS. Allergen-Specific Immunotherapy. *Vet Allergy*. 2013;7(Suppl 1):85-89. doi:10.1002/9781118738818.ch12
62. Vereda A, Casale TB, Beyer K, et al. AR101 Oral Immunotherapy for Peanut Allergy. *N Engl J Med*. 2018;379(21):1991-2001. doi:10.1056/nejmoa1812856
63. Moote W, Kim H, Ellis AK. Allergen-specific immunotherapy. *Allergy, Asthma Clin Immunol*. 2018;14(s2):1-10. doi:10.1186/s13223-018-0282-5
64. Cox L, Compalati E, Kundig T, Larche M. New directions in immunotherapy. *Curr Allergy Asthma Rep*. 2013;13(2):178-195. doi:10.1007/s11882-012-0335-7
65. Scadding GW, Calderon MA, Shamji MH, et al. Effect of 2 years of treatment with sublingual grass pollen immunotherapy on nasal response to allergen challenge at 3 years among patients with moderate to severe seasonal allergic rhinitis: The GRASS randomized clinical trial. *JAMA - J Am Med Assoc*. 2017;317(6):615-625. doi:10.1001/jama.2016.21040
66. Nelson HS, Lahr J, Rule R, Bock A, Leung D. Treatment of anaphylactic sensitivity to peanuts by immunotherapy with injections of aqueous peanut extract. *J Allergy Clin Immunol*. 1997;99(6 I SUPPL.):744-751. doi:10.1016/S0091-6749(97)80006-1
67. Oppenheimer JJ, Nelson HS, Bock SA, Christensen F, Leung DYM. Treatment of peanut allergy with rush immunotherapy. *J Allergy Clin Immunol*. 1992;90(2):256-262. doi:10.1016/0091-6749(92)90080-L
68. Tordesillas L, Berin MC, Sampson HA. Immunology of Food Allergy. *Immunity*. 2017;47(1):32-50. doi:10.1016/j.immuni.2017.07.004
69. Durham SR, Creticos PS, Nelson HS, et al. Treatment effect of sublingual immunotherapy tablets and pharmacotherapies for seasonal and perennial allergic rhinitis: Pooled analyses. *J Allergy Clin Immunol*. 2016;138(4):1081-1088.e4. doi:10.1016/j.jaci.2016.04.061
70. Dorofeeva Y, Shilovskiy I, Tulaeva I, et al. Past, present, and future of allergen immunotherapy vaccines. *Allergy Eur J Allergy Clin Immunol*. 2021;76(1):131-149. doi:10.1111/all.14300
71. Kucuksezer UC, Ozdemir C, Cevhertas L, Ogulur I, Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy and allergen tolerance. *Allergol Int*. 2020;69(4):549-560. doi:10.1016/j.alit.2020.08.002
72. Espa S, Scheurer S, Toda M. Epicutaneous immunotherapy. 2017;45(3).
73. Senti G, Graf N, Haug S, et al. Epicutaneous allergen administration as a novel method of allergen-specific immunotherapy. *J Allergy Clin Immunol*. 2009;124(5):997-1002. doi:10.1016/j.jaci.2009.07.019
74. Senti G, Von Moos S, Tay F, et al. Epicutaneous allergen-specific immunotherapy

ameliorates grass pollen-induced rhinoconjunctivitis: A double-blind, placebo-controlled dose escalation study. *J Allergy Clin Immunol*. 2012;129(1):128-135. doi:10.1016/j.jaci.2011.08.036
75. Senti G, Freiburghaus AU, Larenas-Linnemann D, et al. Intralymphatic Immunotherapy: Update and Unmet Needs. *Int Arch Allergy Immunol*. 2019;178(2):141-149. doi:10.1159/000493647
76. Senti G, Crameri R, Kuster D, et al. Intralymphatic immunotherapy for cat allergy induces tolerance after only 3 injections. *J Allergy Clin Immunol*. 2012;129(5):1290-1296. doi:10.1016/j.jaci.2012.02.026
77. Manabe T, Sato S, Yanagida N, et al. Long-term outcomes after sustained unresponsiveness in patients who underwent oral immunotherapy for egg, cow's milk, or wheat allergy. *Allergol Int*. 2019;68(4):527-528. doi:10.1016/j.alit.2019.02.012
78. Itoh-Nagato N, Inoue Y, Nagao M, et al. Desensitization to a whole egg by rush oral immunotherapy improves the quality of life of guardians: A multicenter, randomized, parallel-group, delayed-start design study. *Allergol Int*. 2018;67(2):209-216. doi:10.1016/j.alit.2017.07.007
79. Anagnostou A. Weighing the benefits and risks of oral immunotherapy in clinical practice. *Allergy Asthma Proc*. 2021;42(2):118-123. doi:10.2500/AAP.2021.42.200107
80. Reier-Nilsen T, Michelsen MM, Lødrup Carlsen KC, et al. Feasibility of desensitizing children highly allergic to peanut by high-dose oral immunotherapy. *Allergy Eur J Allergy Clin Immunol*. 2019;74(2):337-348. doi:10.1111/all.13604
81. Jarkvist J, Salehi C, Akin C, Gülen T. Venom immunotherapy in patients with clonal mast cell disorders: IgG4 correlates with protection. *Allergy Eur J Allergy Clin Immunol*. 2020;75(1):169-177. doi:10.1111/all.13980
82. Shakya AK, Lee CH, Gill HS. Microneedle-Mediated Allergen-Specific Immunotherapy for the Treatment of Airway Allergy in Mice. *Mol Pharm*. 2020;17(8):3033-3042. doi:10.1021/acs.molpharmaceut.0c00447
83. Zhang W, Deng Y, Tong H, et al. Adverse reactions to subcutaneous immunotherapy in patients with allergic rhinitis, a real-world study. *Eur Arch Oto-Rhino-Laryngology*. 2021;278(11):4353-4360. doi:10.1007/s00405-021-06736-2
84. Hardin FM, Eskander PN, Franzese C. Cost-effective Analysis of Subcutaneous vs Sublingual Immunotherapy From the Payor's Perspective. *OTO Open*. 2021;5(4). doi:10.1177/2473974X211052955
85. Kim JY, Jang M jin, Kim DY, Park SW, Han DH. Efficacy of Subcutaneous and Sublingual Immunotherapy for House Dust Mite Allergy: A Network Meta-Analysis–Based Comparison. *J Allergy Clin Immunol Pract*. 2021;9(12):4450-4458.e6. doi:10.1016/j.jaip.2021.08.018
86. Rachid R, Umetsu DT. Immunological mechanisms for desensitization and tolerance in food allergy. *Semin Immunopathol*. 2012;34(5):689-702. doi:10.1007/s00281-012-0333-9
87. Arasi S, Corsello G, Villani A, Pajno GB. The future outlook on allergen immunotherapy in children : 2018 and beyond. Published online 2018:1-9.
88. Walker SM, Varney VA, Gaga M, Jacobson MR. Grass pollen immunotherapy: efficacy and safety during a 4-year follow-up study. *Allergy*. 1995;50(5):405-413. doi:10.1111/j.1398-9995.1995.tb01170.x
89. Akdis CA, Akdis M. Mechanisms of allergen-specific immunotherapy and immune

tolerance to allergens. *World Allergy Organ J*. 2015;8(1):1-12. doi:10.1186/s40413-015-0063-2
90. Woo HY, Kim YS, Kang NI, et al. Mechanism for acute oral desensitization to antibiotics. *Allergy Eur J Allergy Clin Immunol*. 2006;61(8):954-958. doi:10.1111/j.1398-9995.2006.01147.x
91. Akdis CA, Akdis M, Blesken T, et al. Epitope-specific T cell tolerance to phospholipase A2 in bee venom immunotherapy and recovery by IL-2 and IL-15 in vitro. *J Clin Invest*. 1996;98(7):1676-1683. doi:10.1172/JCI118963
92. Şahin E, Bafaqeeh SA, Güven SG, et al. Mechanism of action of allergen immunotherapy. *Am J Rhinol Allergy*. 2016;30(5):S1-S3. doi:10.2500/ajra.2016.30.4367
93. Lanser BJ, Wright BL, Orgel KA, Vickery BP, Fleischer DM. Current Options for the Treatment of Food Allergy. *Pediatr Clin North Am*. 2015;62(6):1531-1549. doi:10.1016/j.pcl.2015.07.015
94. Scadding G, Durham S. Mechanisms of sublingual immunotherapy. *J Asthma*. 2009;46(4):322-334. doi:10.1080/02770900902785729
95. Nouri-Aria KT, Wachholz PA, Francis JN, et al. Grass Pollen Immunotherapy Induces Mucosal and Peripheral IL-10 Responses and Blocking IgG Activity. *J Immunol*. 2004;172(5):3252-3259. doi:10.4049/jimmunol.172.5.3252
96. Farhadi Biregani A, Khodadadi A, Doosti A, Asadirad A, Ghasemi Dehcheshmeh M, Ghadiri AA. Allergen specific immunotherapy with plasmid DNA encoding OVA-immunodominant T cell epitope fused to Tregitope in a murine model of allergy. *Cell Immunol*. 2022;376(April):104534. doi:10.1016/j.cellimm.2022.104534
97. Cox L, Nelson H, Lockey R, et al. Allergen immunotherapy: A practice parameter third update. *J Allergy Clin Immunol*. 2011;127(1 SUPPL.):S1-S55. doi:10.1016/j.jaci.2010.09.034
98. Virkud Y V., Wang J, Shreffler WG. Enhancing the Safety and Efficacy of Food Allergy Immunotherapy: a Review of Adjunctive Therapies. *Clin Rev Allergy Immunol*. 2018;55(2):172-189. doi:10.1007/s12016-018-8694-z
99. Schaap-Johansen AL, Vujović M, Borch A, Hadrup SR, Marcatili P. T Cell Epitope Prediction and Its Application to Immunotherapy. *Front Immunol*. 2021;12(September):1-11. doi:10.3389/fimmu.2021.712488
100. Gell PG, Benacerraf B. Studies on hypersensitivity. II. Delayed hypersensitivity to denatured proteins in guinea pigs. *Immunology*. 1959;2(1):64-70.
101. Livingstone AM, Fathman CG. The structure of T-cell epitopes. *Annu Rev Immunol*. 1987;5:477-501. doi:10.1146/annurev.iy.05.040187.002401
102. Schwartz RH. T-lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. *Annu Rev Immunol*. 1985;3:237-261. doi:10.1146/annurev.iy.03.040185.001321
103. Wiesel M, Oxenius A. From crucial to negligible: Functional CD8 + T-cell responses and their dependence on CD4 + T-cell help. *Eur J Immunol*. 2012;42(5):1080-1088. doi:10.1002/eji.201142205
104. Otsuji M, Kimura Y, Aoe T, Okamoto Y, Saito T. Oxidative stress by tumor-derived macrophages suppresses the expression of CD3 ζ chain of T-cell receptor complex and antigen-specific T-cell responses. *Proc Natl Acad Sci U S A*. 1996;93(23):13119-13124. doi:10.1073/pnas.93.23.13119
105. Prezzemolo T, Guggino G, La Manna MP, Di Liberto D Di, Dieli F, Caccamo N.

Functional signatures of human CD4 and CD8 T cell responses to Mycobacterium tuberculosis. *Front Immunol*. 2014;5(APR):1-13. doi:10.3389/fimmu.2014.00180
106. Linsley PS, Ledbetter JA. The role of the CD28 receptor during T cell responses to antigen. *Annu Rev Immunol*. 1993;11:191-212. doi:10.1146/annurev.iy.11.040193.001203
107. Andersson G. Evolution of the human HLA-DR region. *Front Biosci*. 1998;3(August 1998). doi:10.2741/A317
108. Jilek S, Barbey C, Spertini F, Corthésy B. Antigen-Independent Suppression of the Allergic Immune Response to Bee Venom Phospholipase A 2 by DNA Vaccination in CBA/J Mice. *J Immunol*. 2001;166(5):3612-3621. doi:10.4049/jimmunol.166.5.3612
109. Tarzi M, Klunker S, Texier C, et al. Induction of interleukin-10 and suppressor of cytokine signalling-3 gene expression following peptide immunotherapy. *Clin Exp Allergy*. 2006;36(4):465-474. doi:10.1111/j.1365-2222.2006.02469.x
110. Casanovas M, Gómez MJ, Carnés J, Fernández-Caldas E. Skin test with native, depiqmented and glutaraldehyde polymerized allergen extracts. *J Investig Allergol Clin Immunol*. 2005;15(1):30-36.
111. Valenta R, Niederberger V. Recombinant allergens for immunotherapy. *J Allergy Clin Immunol*. 2007;119(4):826-830. doi:10.1016/j.jaci.2007.01.025
112. Marsh DG, Lichtenstein LM, Campbell DH. Studies on “allergoids” prepared from naturally occurring allergens. I. Assay of allergenicity and antigenicity of formalinized rye group I component. *Immunology*. 1970;18(5):705-722. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1455593&tool=pmcentrez&rendertype=abstract
113. Henmar H, Lund G, Lund L, Petersen A, Würtzen PA. Allergenicity, immunogenicity and dose-relationship of three intact allergen vaccines and four allergoid vaccines for subcutaneous grass pollen immunotherapy. *Clin Exp Immunol*. 2008;153(3):316-323. doi:10.1111/j.1365-2249.2008.03710.x
114. Gallego MT, Iraola V, Himly M, et al. Depigmented and polymerised house dust mite allergoid: Allergen content, induction of IgG4 and clinical response. *Int Arch Allergy Immunol*. 2010;153(1):61-69. doi:10.1159/000301580
115. Reinhold T, Brüggenjürgen B. Cost-effectiveness of grass pollen SCIT compared with SLIT and symptomatic treatment. *Allergo J*. 2017;26(1):26-35. doi:10.1007/s40629-016-0002-y
116. Rosewich M, Lee D, Zielen S. Pollinex Quattro: An innovative four injections immunotherapy in allergic rhinitis. *Hum Vaccines Immunother*. 2013;9(7):1523-1531. doi:10.4161/hv.24631
117. DuBuske LH, Frew AJ, Horak F, et al. Ultrashort-specific immunotherapy successfully treats seasonal allergic rhinoconjunctivitis to grass pollen. *Allergy Asthma Proc*. 2011;32(3):239-247. doi:10.2500/aap.2011.32.3453
118. Drachenberg KJ, Wheeler AW, Stuebner P, Horak F. A well-tolerated grass pollen-specific allergy vaccine containing a novel adjuvant, monophosphoryl lipid A, reduces allergic symptoms after only four preseasonal injections. *Allergy Eur J Allergy Clin Immunol*. 2001;56(6):498-505. doi:10.1034/j.1398-9995.2001.056006498.x
119. Mothes N, Heinzkill M, Drachenberg KJ, et al. Allergen-specific immunotherapy with a monophosphoryl lipid A-adjuvanted vaccine: Reduced seasonally boosted immunoglobulin E production and inhibition of basophil histamine release by therapy-induced blocking antibodies. *Clin Exp Allergy*. 2003;33(9):1198-1208.

doi:10.1046/j.1365-2222.2003.01699.x
120. Rosewich M, Schulze J, Eickmeier O, et al. Tolerance induction after specific immunotherapy with pollen allergoids adjuvanted by monophosphoryl lipid A in children. *Clin Exp Immunol*. 2010;160(3):403-410. doi:10.1111/j.1365-2249.2010.04106.x
121. Bode C, Zhao G, Steinhagen F, Kinjo T, Klinman DM. CpG DNA as a vaccine adjuvant. *Expert Rev Vaccines*. 2011;10(4):499-511. doi:10.1586/erv.10.174
122. Boonstra A, Rajsbaum R, Holman M, et al. Macrophages and Myeloid Dendritic Cells, but Not Plasmacytoid Dendritic Cells, Produce IL-10 in Response to MyD88- and TRIF-Dependent TLR Signals, and TLR-Independent Signals. *J Immunol*. 2006;177(11):7551-7558. doi:10.4049/jimmunol.177.11.7551
123. Latz E, Schoenemeyer A, Visintin A, et al. TLR9 signals after translocating from the ER to CpG DNA in the lysosome. *Nat Immunol*. 2004;5(2):190-198. doi:10.1038/ni1028
124. Lund J, Sato A, Akira S, Medzhitov R, Iwasaki A. Toll-like receptor 9-mediated recognition of Herpes simplex virus-2 by plasmacytoid dendritic cells. *J Exp Med*. 2003;198(3):513-520. doi:10.1084/jem.20030162
125. Krug A, French AR, Barchet W, et al. TLR9-dependent recognition of MCMV by IPC and DC generates coordinated cytokine responses that activate antiviral NK cell function. *Immunity*. 2004;21(1):107-119. doi:10.1016/j.immuni.2004.06.007
126. Mogensen TH. Live Streptococcus pneumoniae, Haemophilus influenzae, and Neisseria meningitidis activate the inflammatory response through Toll-like receptors 2, 4, and 9 in species-specific patterns. *J Leukoc Biol*. 2006;80(2):267-277. doi:10.1189/jlb.1105626
127. Tulic MK, Fiset PO, Christodoulopoulos P, et al. Amb a 1-immunostimulatory oligodeoxynucleotide conjugate immunotherapy decreases the nasal inflammatory response. *J Allergy Clin Immunol*. 2004;113(2):235-241. doi:10.1016/j.jaci.2003.11.001
128. Yu JE, Sicherer SH. Immunotherapy With a Ragweed-Toll-Like Receptor 9 Agonist Vaccine for Allergic Rhinitis. *Pediatrics*. 2007;120(Supplement_3):S152-S152. doi:10.1542/peds.2007-0846gggg
129. Zhu FG, Kandimalla ER, Yu D, Agrawal S. Oral administration of a synthetic agonist of Toll-like receptor 9 potently modulates peanut-induced allergy in mice. *J Allergy Clin Immunol*. 2007;120(3):631-637. doi:10.1016/j.jaci.2007.05.015
130. Mumm J, Mahr TA. Randomized trial of omalizumab (anti-IgE) for asthma in inner-city children. *Pediatrics*. 2011;128(SUPPL. 3):1005-1015. doi:10.1542/peds.2011-2107PPP
131. Wright JD, Chu HM, Huang CH, Ma C, Wen Chang T, Lim C. Structural and Physical Basis for Anti-IgE Therapy. *Sci Rep*. 2015;5:1-14. doi:10.1038/srep11581
132. Davies AM, Allan EG, Keeble AH, et al. Allosteric mechanism of action of the therapeutic anti-IgE antibody omalizumab. *J Biol Chem*. 2017;292(24):9975-9987. doi:10.1074/jbc.M117.776476
133. Guilleminault L, Michelet M, Reber LL. Combining Anti-IgE Monoclonal Antibodies and Oral Immunotherapy for the Treatment of Food Allergy. *Clin Rev Allergy Immunol*. 2022;62(1):216-231. doi:10.1007/s12016-021-08902-0
134. Johnson L, Duschl A, Himly M. Nanotechnology-based vaccines for allergen-specific immunotherapy: Potentials and challenges of conventional and novel adjuvants under research. *Vaccines*. 2020;8(2). doi:10.3390/vaccines8020237
135. Apostólico JDS, Alves V, Lunardelli S, Coirada FC, Boscardin SB, Rosa DS. Adjuvants:

Classification, Modus Operandi, and Licensing. 2016;2016.
136. Gamazo C, D'Amelio C, Gastaminza G, Ferrer M, Irache JM. Adjuvants for allergy immunotherapeutics. *Hum Vaccines Immunother*. 2017;13(10):2416-2427. doi:10.1080/21645515.2017.1348447
137. Kramer MF, Heath MD. Aluminium in allergen-specific subcutaneous immunotherapy - A German perspective. *Vaccine*. 2014;32(33):4140-4148. doi:10.1016/j.vaccine.2014.05.063
138. Yan S, Zhao P, Yu T, Gu N. Current applications and future prospects of nanotechnology in cancer immunotherapy. *Cancer Biol Med*. 2019;16(3):486-497. doi:10.20892/j.issn.2095-3941.2018.0493
139. Palmer BC, Jatana S, Phelan-Dickinson SJ, DeLouise LA. Amorphous silicon dioxide nanoparticles modulate immune responses in a model of allergic contact dermatitis. *Sci Rep*. 2019;9(1):1-11. doi:10.1038/s41598-019-41493-7
140. Palmer BC, DeLouise LA. Nanoparticle-enabled transdermal drug delivery systems for enhanced dose control and tissue targeting. *Molecules*. 2016;21(12):7-9. doi:10.3390/molecules21121719
141. Hirai T, Yoshikawa T, Nabeshi H, et al. Dermal absorption of amorphous nanosilica particles after topical exposure for three days. *Pharmazie*. 2012;67(8):742-743. doi:10.1691/ph.2012.1853
142. Jongejan L, van Ree R. Modified Allergens and their Potential to Treat Allergic Disease. *Curr Allergy Asthma Rep*. 2014;14(12):1-10. doi:10.1007/s11882-014-0478-9
143. Kawakita A, Shirasaki H, Yasutomi M, et al. Immunotherapy with oligomannose-coated liposomes ameliorates allergic symptoms in a murine food allergy model. *Allergy Eur J Allergy Clin Immunol*. 2012;67(3):371-379. doi:10.1111/j.1398-9995.2011.02777.x
144. Arora N, Gangal S V. Efficacy of liposome entrapped allergen in down regulation of IgE response in mice. *Clin Exp Allergy*. 1992;22(1):35-42. doi:10.1111/j.1365-2222.1992.tb00112.x
145. Senéjoux A. Quadrivalent vaccine against human papillomavirus to prevent anogenital diseases: Commentary. *Colon and Rectum*. 2007;1(3):220-221. doi:10.1007/s11725-007-0044-x
146. Maurer P, Jennings GT, Willers J, et al. A therapeutic vaccine for nicotine dependence: Preclinical efficacy, and phase I safety and immunogenicity. *Eur J Immunol*. 2005;35(7):2031-2040. doi:10.1002/eji.200526285
147. Beeh KM, Kanniess F, Wagner F, et al. The novel TLR-9 agonist QbG10 shows clinical efficacy in persistent allergic asthma. *J Allergy Clin Immunol*. 2013;131(3):866-874. doi:10.1016/j.jaci.2012.12.1561
148. Klimek L, Kündig T, Kramer MF, et al. Virus-like particles (VLP) in prophylaxis and immunotherapy of allergic diseases. *Allergo J Int*. 2018;27(8):245-255. doi:10.1007/s40629-018-0074-y
149. Sun M, Qin D, Fan P, Chen X, Liu Y. Chitosan-centered nanosystems as sustained therapeutics for allergic rhinitis intervention: Inhibition of histamine-induced cascades. *J Control Release*. 2021;335(May):422-436. doi:10.1016/j.jconrel.2021.05.048
150. Niederberger V, Horak F, Vrtala S, et al. Vaccination with genetically engineered allergens prevents progression of allergic disease. *Proc Natl Acad Sci U S A*. 2004;101(SUPPL. 2):14677-14682. doi:10.1073/pnas.0404735101

151. Valenta R, Linhart B, Swoboda I, Niederberger V. Recombinant allergens for allergen-specific immunotherapy: 10 years anniversary of immunotherapy with recombinant allergens. *Allergy Eur J Allergy Clin Immunol*. 2011;66(6):775-783. doi:10.1111/j.1398-9995.2011.02565.x
152. Lundberg M, Chen Z, Rihs HP, Wrangsjö K. Recombinant spiked allergen extract. *Allergy Eur J Allergy Clin Immunol*. 2001;56(8):794-795. doi:10.1034/j.1398-9995.2001.056008794.x
153. Focke M, Marth K, Flicker S, Valenta R. Heterogeneity of commercial timothy grass pollen extracts. *Clin Exp Allergy*. 2008;38(8):1400-1408. doi:10.1111/j.1365-2222.2008.03031.x
154. Bubnov R V., Spivak MY, Lazarenko LM, Bomba A, Boyko N V. Probiotics and immunity: Provisional role for personalized diets and disease prevention. *EPMA J*. 2015;6(1):1-11. doi:10.1186/s13167-015-0036-0
155. Akdis M, Akdis CA. Mechanisms of allergen-specific immunotherapy: Multiple suppressor factors at work in immune tolerance to allergens. *J Allergy Clin Immunol*. 2014;133(3):621-631. doi:10.1016/j.jaci.2013.12.1088
156. Liu J, Chen F, Qiu S, Yang L, Zhang H, Liu J. Probiotics enhance the effect of allergy immunotherapy on regulating antigen specific B cell activity in asthma patients. 2016;8(12):5256-5270.