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Review

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Review

Clinical and experimental treatment of allergic asthma with an emphasis on allergen immunotherapy and its mechanisms

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Summary

Allergen immunotherapy (AIT) is currently the only form of treatment that modifies allergic asthma. Pharmacotherapy alone seeks to control the symptoms of allergic asthma, allergic rhinitis, and other atopic conditions. In contrast, AIT can induce long-term physiological modifications through the immune system. AIT enables individuals to live improved lives many years after treatment ends, where they are desensitized to the allergen(s) used or no longer have significant allergic reactions upon allergen provocation. The leading forms of treatment with AIT involve injections of allergen extracts with increasing doses via the subcutaneous route or drops/tablets via the sublingual route for several years. Since the initial attempts at this treatment as early as 1911 by Leonard Noon, the mechanisms by which AIT operates remain unclear. This literature-based review provides the primary care practitioner with a current understanding of the mechanisms of AIT, including its treatment safety, protocols, and long-term efficacy. The primary mechanisms underlying AIT include changes in immunoglobulin classes (IgA, IgE, and IgG), immunosuppressive regulatory T-cell induction, helper T cell type 2 to helper T cell type 1 cell/cytokine profile shifts, decreased early-phase reaction activity and mediators, and increased production of IL-10, IL-35, TGF-β, and IFN-γ. Using the databases PubMed and Embase, a selective literature search was conducted searching for English, full-text, reviews published between 2015 and 2022 using the keywords (with wildcards) "allerg*," "immunotherap*," "mechanis*," and "asthma." Among the cited references, additional references were identified using a manual search.

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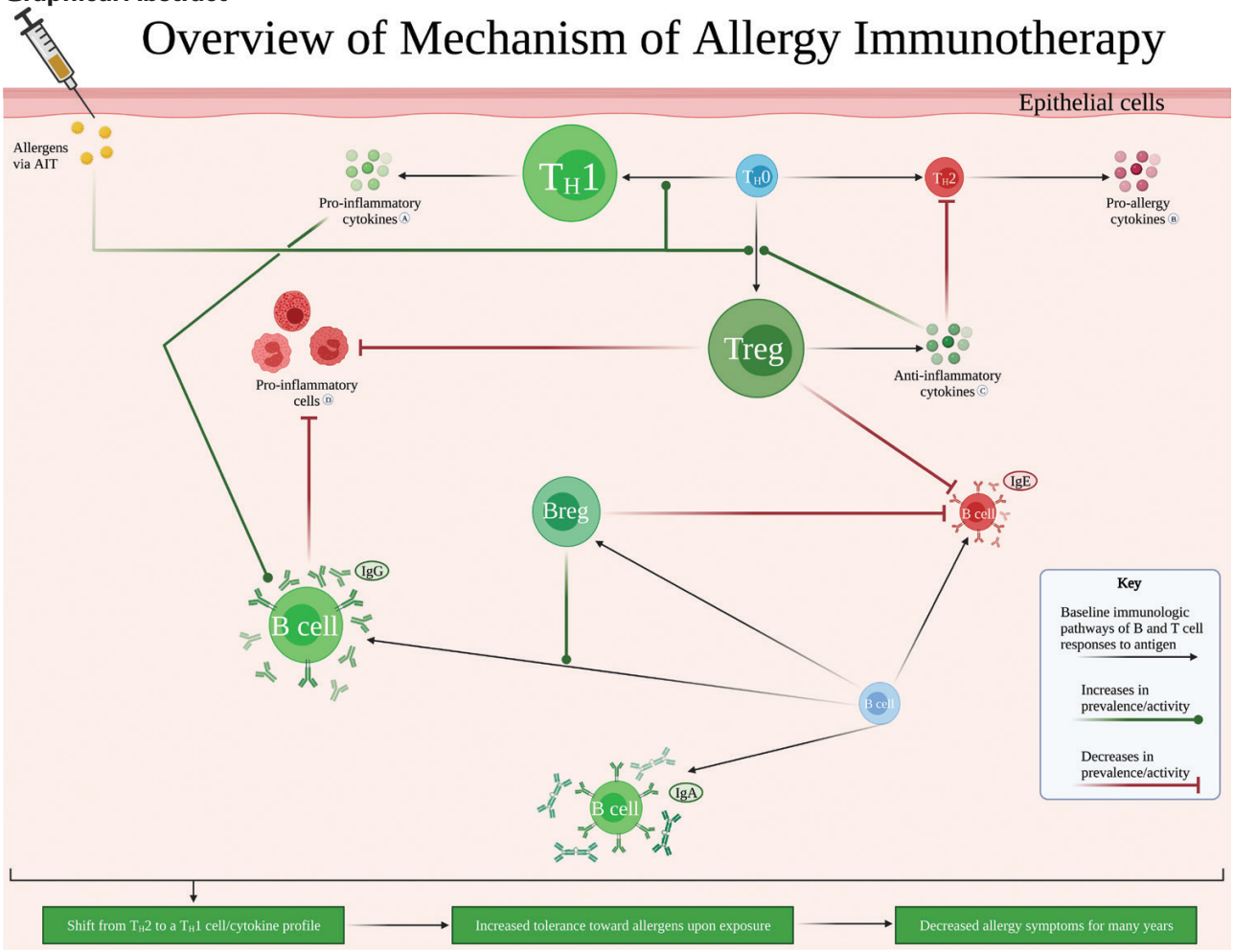
Graphical Abstract



Keywords: allergic asthma, allergen immunotherapy (AIT), regulatory T (Treg) cell, subcutaneous allergen immunotherapy (SCIT), sublingual allergen immunotherapy (SLIT)

Abbreviations: AA: allergic asthma; AD: atopic dermatitis; AIDS: acquired immunodeficiency syndrome; AIT: allergen immunotherapy; APC: antigen presenting cell; AR: allergic rhinitis; Breg: regulatory B cell; DC: dendritic cell; DCreg: regulatory dendritic cell; EPIT: epicutaneous allergen immunotherapy; Fab: fragment antigen-binding; Fc: fragment crystallizable; FcγRII: fragment-crystallizable-gamma-receptor-2; FcεRI: fragment-crystallizable-epsilon-receptor-1; FEV_1: forced expiratory volume in one second; GI: gastrointestinal; H2R: histamine type 2 receptor; HDM: house dust mite; HIV: human immunodeficiency virus; IFN: interferon; IgE: immunoglobulin E; IL: interleukin; IL-4R: interlukin-4 receptor; IL-5Rα: interleukin-5 receptor alpha; ILIT: intralymphatic immunotherapy; iTreg: induced regulatory T cell; LABA: long-acting beta-adrenergic agonist; LRA: leukotriene receptor antagonist; mAb: monoclonal antibody; MCT: microcrystalline tyrosine; MHC: major histocompatibility complex; MPL: monophosphoryl lipid A; NKC: natural killer cell; PLGA: poly lactic-co-glycolic acid; SABA: short acting beta-2-adrenoceptor agonist; SCIT: subcutaneous allergen immunotherapy; sIgE: allergen-specific immunoglobulin E; SIT: specific allergen immunotherapy; SLIT: sublingual allergen immunotherapy; TGF-β: transforming growth factor-β; T_H: helper T cell; T_H0: naïve T cell; T_H1: helper T cell type 1; T_H17: helper T cell 17; T_H2: helper T cell type 2; TLR4: toll-like receptor 4; Treg: regulatory T cell; VLP: virus-like-particle.

Introduction

Asthma is a chronic inflammatory disorder characterized by the constriction of the bronchioles, mucus hypersecretion, and an oversensitive cough [1, 2]. It is estimated that more than 330 million people are affected worldwide with some form of asthma [3–6]. Asthma is a public health problem that affects people of all ages, socioeconomic statuses, and countries. The burden of the disease and its impact on the quality of life is enormous [5, 7]. Various medications are available to treat asthma, yet only temporarily reduce allergy symptoms for as long as a patient is willing to take the medication. Currently, the only known form of disease-modifying treatment to provide long-term physiological changes to combat the effects of allergic asthma (AA) is allergen immunotherapy (AIT). However, this treatment only works for allergy/atopy-based [asthmatic] patients. AIT is a multi-year treatment consisting of increasing doses of an allergen given to a patient weekly, followed by a maintenance dosage monthly for several years, with the goal of "reprogramming" the immune system.

Immunopathology and mechanisms of allergic asthma

Allergic asthma is characterized by an inappropriate immune response upon exposure to an allergen, or an otherwise harmless substance (e.g. pollen, animal dander, foods, etc.). The immune system recognizes the allergen—an antigen—and reacts to it as if it were a pathogen. When the allergens breach the tight junctions of the epithelial cells lining the respiratory tract, those allergens are taken up by antigen-presenting cells (APC). The APCs (e.g. dendritic cells [DC], macrophages, and

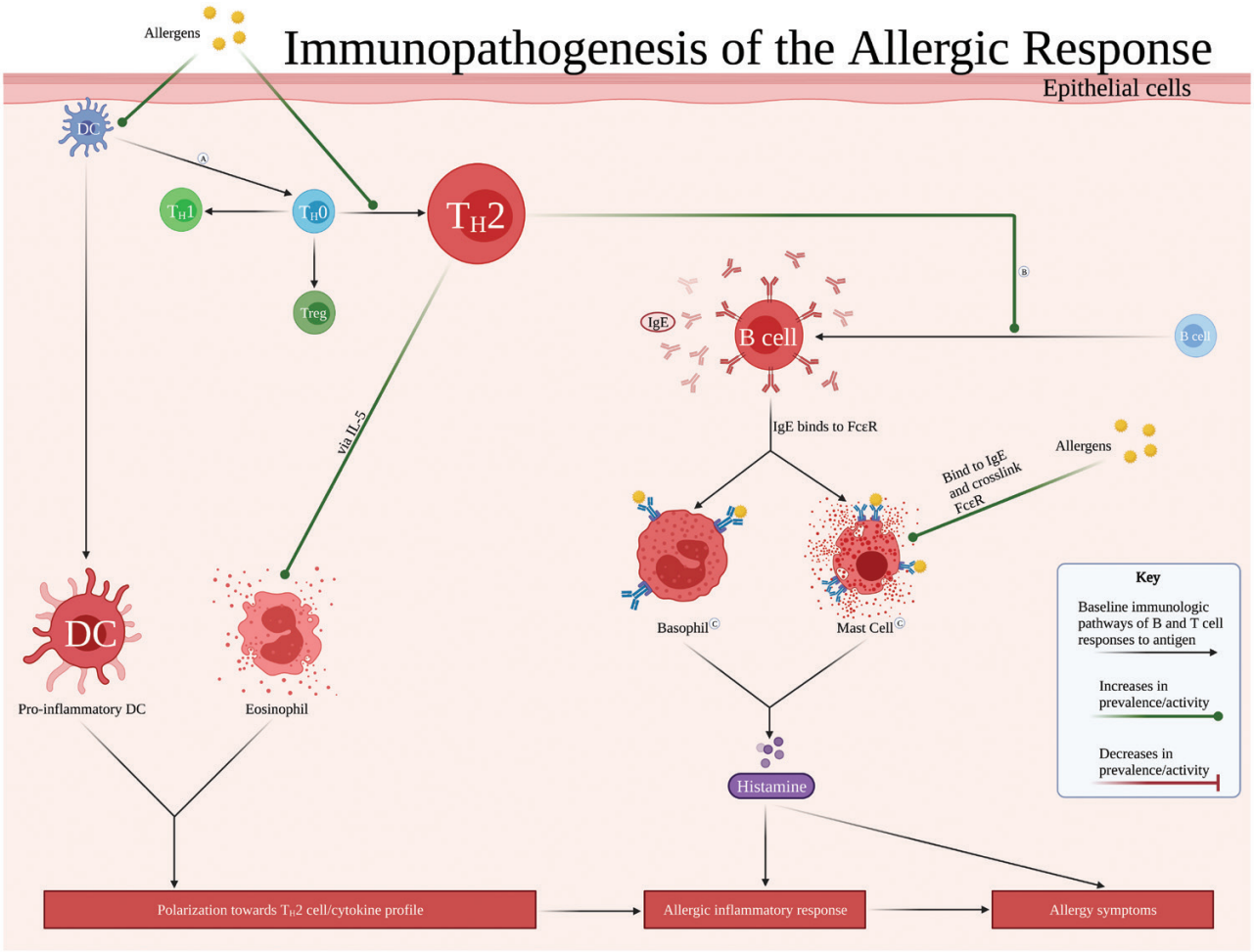


Figure 1: Overview of the allergic response immunopathogenesis. (A) DCs capture allergen and induce T_H2 proliferation from T_H0 cells; (B) IL-4, IL-5, IL-9, IL-13; (C) IgE crosslink via their receptor "FcεR." T_H0, naïve helper T cell. Created with BioRender.com

B cells) beneath the epithelial cells enzymatically process the allergens that cross the barrier and display peptide fragments via the Major Histocompatibility Complex (MHC) molecules. The presentation of these antigens via the MHC induces helper T (T_H) cells to differentiate into helper T cell type 2 (T_H2). T_H2 cells produce various cytokines (e.g. interleukin-4 [IL-4], IL-5, IL-9, IL-13) that have a cascade of events culminating in the production of molecules that result in bronchoconstriction, inflammation, edema, and epithelial desquamation [8, 9]. The T_H2 subset predominates with the augmented immunoglobulin E (IgE) and eosinophilic responses in atopic conditions, along with the defense against helminths [10]. IL-4, IL-5, IL-9, IL-10, and IL-13 tend to characterize the type 2 or T_H2 mediated response [11]. Furthermore, IL-5 produced by the T_H2 cells results in the proliferation and activation of eosinophils. Upon being triggered by an allergen, DCs can polarize into several subsets: DC1, DC2, DC17, and regulatory dendritic (DCreg) cells; these differentiated DCs can polarize naïve T cells to differentiate into T_H1, T_H2, T_H17, and regulatory T (Treg) cells, respectively [12].

Cytokines released from the T_H2 cells promote the differentiation of naïve B cells into IgE-producing plasma B cells. The IgE produced from these B cells binds to the high-affinity IgE receptor, fragment-crystallizable-epsilon-receptor 1 (FcεRI), on basophils and mast cells and is available to bind allergen epitopes and subsequently available to crosslink FcεR. The crosslinking of IgE/FcεRI complexes via allergen binding results in a rapid cascade of events denoted by the extensive extracellular release of chemical mediators; the most notable and immediate is histamine release. Fig. 1 depicts an overview of the immunopathogenesis of the allergic response.

Individuals with allergic asthma have difficulty breathing due to hyperreactivity to allergens, which leads to bronchoconstriction. They exhibit symptoms of chest tightness, shortness of breath, wheezing, and coughing, which may occur multiple times a day or only a few times a week [13]. Physical exercise, extreme emotions, strong odors, and smoke (among others) can trigger an asthma attack or exacerbate the asthmatic airways [14]. Physiologically, asthmatic airways are generally defined by bronchospasms, inflammation, mucus hypersecretion, and a hypersensitivity to triggers. The respiratory tissue is characterized by a disruption of the tight junctions between epithelial cells lining the respiratory tract, smooth muscle hyperplasia, and airway remodeling [9]. In response to chemical mediators released following allergen exposure, the smooth muscle surrounding the bronchi contracts and decreases the airway diameter. Swollen and irritated airways may lead to airway occlusion. Inflamed airways produce an excess of mucus that further clogs the already narrowed airways [14].

Table 1: Common medications used to treat allergic asthma

Long-term medications	
Medication	Mechanism of action
Corticosteroids	• Block late-phase allergic reaction • Inhibit inflammatory cell activation and migration • Reduce airway hyperresponsiveness • Examples include fluticasone and budesonide
LABAs	• Generally used with inhaled corticosteroids; they are bronchodilators with durations typically lasting at least 12 hours after a single dose • Examples include formoterol and salmeterol
LRAs	• Act as bronchodilators with some anti-inflammatory properties, such as reducing inflammatory mediator release upon allergen provocation • Examples include zafirlukast and montelukast [19]
Short-term medications	
Medication	Mechanism of action
SABAs	• Used as rescue medication during an asthma attack to quickly dilate the bronchioles • Bronchodilators, such as albuterol, like LABAs but with shorter-lasting effects
Anticholinergics	• Fast-acting bronchodilators such as ipratropium bromide

Long-term medications are used to prevent further exacerbations [14]. Short-term medications are used to treat acute symptoms.
LABA: long-acting beta-adrenergic agonist; LRA: leukotriene receptor antagonist; SABA: short-acting beta-2-adrenoceptor agonist.

The clinical classification of the severity of asthma varies depending on the symptom frequency, forced expiratory volume in one second (FEV_1), and peak expiratory flow rate [15]. Based on symptoms, asthma may be defined as poorly controlled, well-controlled, or somewhere in between; this can impact the best form of treatment for a patient. Allergic, or atopic, asthma is the specific form of asthma that AIT seeks to treat. Notably, allergic reactions may develop upon exposure to a single allergen (a monosensitivity) or to multiple allergens (a polysensitization); roughly 50–80% of patients with allergies are polysensitized [3, 16].

Following allergen exposure, there is an early phase for an allergic reaction and usually a late phase response. The exact time frame for both phases may vary depending on the source and the location of the allergic response, whether it is local (occurring in/on a specific region of the body—nose, lungs, skin, etc.) or systemic (occurring throughout the entire body). Nevertheless, the early phase response can be characterized as developing within minutes upon exposure to an allergen reaching a maximum by 30 min, followed by a resolution within 1–2 h. The early phase response involves the degranulation of histamine along with cytokines and other proinflammatory molecules by mast cells [17]. In contrast, the late phase response, if any, may reach a maximum within roughly 6–12 h and resolve by 24 h post-initial exposure. This phase is suggested to arise from the recruitment of specific cells from the circulation, including eosinophils, basophils, and T cells [17].

Current treatment methods

Symptomatic relief

Currently, there is no cure for AA. Some of the most common ways to manage asthma include lifestyle modifications after identifying the trigger(s) along with various medications. If one is going to make a lifestyle change, it is essential to identify the allergen(s). This enables the proper steps to be taken so excessive lifestyle changes are not undertaken and unnecessary resources are not wasted. The medications listed in Table 1 currently provide temporary symptomatic relief, but do not provide a cure for the patient; one study states that these treatments fail to attenuate symptoms in 30–60% of patients [18].

Overview of AIT treatment

AIT, or specific [allergen] immunotherapy (SIT), is, to date, the only disease-modifying treatment for AA (and other atopic conditions, such as allergic rhinitis [AR]). Reasons for undergoing AIT treatment include not responding well to usual allergy medications, a desire to reduce allergy medication use, having life-threatening allergies, or having significant side effects from one's current medications [4]. A helper T cell type 1 (T_H1) or T_H2 response refers to conditions that polarize the immune system into producing a specific set of T_H cell cytokines with the respective effector functions—with the imbalance between them thought to contribute toward the abnormal conditions seen with atopic conditions. AIT strives to reprogram the immune system from a T_H2 to a T_H1-mediated immune response over the course of months to years of allergen deposits into the body: from a state of allergen-specific sensitization to a more tolerogenic state. After 3–5 years of continuous treatment, a patient should be "protected" from an adverse immune response upon an allergen encounter between 3 and 12 years [20, 21]. The most common ways to deposit the allergen in a patient are via the subcutaneous or sublingual route. Subcutaneous allergen immunotherapy (SCIT) involves injecting allergen extracts (colloquially referred to as "allergy shots"), or a soluble solution of allergen(s), into the arms. On the other hand, sublingual allergen immunotherapy (SLIT) involves using a drop/tablet of allergen extract(s) that sit under the tongue and dissolve for a few minutes before swallowing the drop/tablet.

AIT is grouped into two phases. The build-up, up-dosing, or induction phase (i.e. phase one) consists of 1–2 (with some studies indicating up to three) weekly injections of an allergen mixture over 3–6 months for SCIT [22]. Allergens for SCIT

are used as physically adsorbed (depot) or aqueous extracts [23]. The first dose is the lowest concentration; the dose increases over several months until an effective targeted dose is achieved. Compared to natural exposure to the allergen, or baseline, the targeted dose is determined empirically to identify a concentration that reduces the disease severity [4]. In the case of SLIT, there is no build-up phase. Instead, the patient self-administers a drop or tablet to dissolve under the tongue for 2–3 minutes every day over 3–5 years [24, 25]. Generally, compressed or freeze-dried formulations of allergen extracts comprise the tabular form, whereas the drops are available as aqueous solutions of allergen extract (traditionally formulated with glycerin) [26]. Most SLIT regimens suggest swallowing the drop or tablet after 2–3 min if it has not dissolved already [27, 28]. Patients should be instructed not to ingest a beverage or food within 5 min of complete dissolution or swallowing of the tablet with SLIT [29]. The second phase of SCIT (i.e. the maintenance phase) consists of one injection every 4 weeks (with some studies suggesting an interval as long as every 8 weeks) for inhalant allergens and 4–6 weeks for venom over 3–5 years; this 4-week interval between doses appears to be a universal consensus [22, 30, 31]. However, the best regimen has not been defined yet. During the maintenance phase, higher, acceptable doses are ideal for maintaining a tolerogenic state during the post-treatment period [32].

A significant issue with AIT is the adherence to the schedule, with one study stating SCIT has an adherence ranging from 13% to 89%, with SLIT being 30% to 85% [33, 34]. Other than the high [initial] cost and duration, factors that may account for this discontinuation include recurrent medical visits and the poor perception of quick symptoms. Another study suggests that for patients who received AIT treatment for 3 years, cost savings were found to be as high as 80% compared to pharmacotherapy alone upon a follow-up 3 years after the treatment ended [3, 24, 35].

Other than the standard AIT procedures, cluster or rush protocols can be used during the build-up phase to reach the maintenance phase as quickly as possible [32]. On non-consecutive days, 2–3 injections with increasing doses are given successively on one treatment day for cluster immunotherapy until the maintenance dose is achieved [22]. The administration of incremental allergen doses at intervals between 15 and 60 min within 1–3 days until the maintenance dose is achieved is used for rush immunotherapy [22]. Interestingly, some reviews discussed the combination of SCIT and SLIT to yield the most effective and patient-friendly AIT treatments [3]. SCIT would be used for the build-up phase and SLIT for the maintenance phase. One small study involving 51 house dust mite (HDM)-sensitive asthmatic children over the course of 18 months noted that the only group that demonstrated significant improvement in visual analog scores for rhinitis was the group that received combination SCIT/SLIT therapy (compared to the individual SCIT, SLIT, and pharmacotherapy groups) [36]. Ultimately, it was found that this combination of SCIT/SLIT was as effective as SCIT alone for AA and AR in children (5–12 years old) and avoided related side effects and injections during the maintenance phase; the combination of SCIT/SLIT demonstrated the advantages of SCIT such as rapid onset and potency paired with the safety and avoidance of injections that accompanies a SLIT regimen. Another study focusing on 120 individuals with seasonal allergic rhinitis suggests a synergistic aspect of combination SCIT/SLIT, as the combination group saw a 70% decrease in rhinoconjunctivitis and food allergy symptoms compared to the SCIT and SLIT groups which saw decreases of 40% and 25%, respectively [37]. Nonetheless, abundant studies focusing on a combination AIT treatment using multiple allergens appear to be lacking [38]. Regardless of the variation of AIT, one study claims that when multiple allergens are used in the AIT regimen, the efficacy of each allergen is reduced; although there is evidence supporting administering mixtures of unrelated allergens in SCIT that demonstrates clinical efficacy with AA and AR [39, 40].

The future directions of treatment

Though not as common as SCIT and SLIT, intralymphatic immunotherapy (ILIT) is a novel method for the direct administration of allergens into the lymph nodes requiring three intralymphatic (e.g. inguinal lymph node) injections that are recommended one month apart throughout the entire treatment [4, 41]. This technique requires experienced personnel to administer the allergens under ultrasonic guidance, which currently appears to be a significant disadvantage. ILIT is characterized by a good safety profile and the fast elimination of symptoms, yet with hypersensitized patients, there is a possibility that ILIT may provoke severe systemic or local reactions [42]. Additionally, compared to SCIT, ILIT enhances the secretion of IL-1, IL-4, IL-10, and interferon-γ (IFN-γ).

Epicutaneous allergen immunotherapy (EPIT) has roots dating back to 1921 but has recently garnered increased attention for use with food allergies and aeroallergens. EPIT generally involves the use of an adhesive dermal patch (left on for 24–48 h and replaced daily) containing allergens that allow APCs (e.g. epidermal Langerhans cells) in the epidermis to internalize these allergens; some studies may utilize a skin pre-treatment consisting of an adhesive tape-stripping or abrasion of the skin [43–45]. As this form of AIT is not as widely used as other forms of AIT, the exact duration the patch stays on the skin and the dose of allergen used have not been agreed upon. However, several clinical studies have demonstrated the safety profile (especially due to the delivery of allergens to non-vascularized tissue and thus reducing the likelihood of systemic adverse reactions) and efficacy of EPIT (though the allergen dosage is claimed to have a significant effect on overall efficacy) [44, 45].

Recently, it has been suggested to use nanoparticles, with a diameter between 1 and 100 nm, in AIT as adjuvants/delivery systems. Nanoparticles have a low allergenic potency with a strong immunogenic effect; they merge the potential of optimal allergen presentation with inherent adjuvant properties [46]. Nanoparticles are easy to reproduce with defined sizes, can be functionalized, and are capable of being tailored (and thus be potentially more efficacious for novel AIT aqueous solutions) [47]. They also can be made [non]biodegradable, possess dimensions that are a fraction of a cell diameter, have patterned surfaces effectively giving them the capacity to provoke strong immune responses such as the targeting of APCs and other coordinated signals, and can protect an encapsulated antigen from degradation (especially from the oral mucosa in SLIT). One example of a currently FDA-approved nanoparticle with extensive research behind it for use in vaccines, tissue engineering, and drug delivery is Poly lactic-co-glycolic acid (PLGA) [47]. PLGA has been synthesized to load purified allergenic molecules (e.g. the major birch allergen—Bet v 1, amongst others) and has successfully been shown to modify the T_H2 response.

Engineered cellular therapies are a different approach to improving AIT. While still in preclinical development, they may include but are not limited to, liposomes and virus-like-particles (VLPs). Liposomes are synthetic spheres composed of lipid bilayers and have a dual purpose when encapsulating allergens, enabling them to act as delivery systems and adjuvants [46]. One randomized, double-blind, placebo-controlled study with 55 patients with AA demonstrated increased IgG populations, lower sputum eosinophils, and lower clinical scores that were treated with a liposome-encapsulated extract of HDM [48]. A high number of viral capsid protein copies constitute VLPs; allergens can be conjugated onto these VLPs, eventually being recognized as pathogen-associated molecular patterns [49]. In studies where HDM was combined with VLPs, increases in IgG populations and decreases in medication use were observed, and AA and AR symptoms were improved; overall, clinical studies have shown VLP with AIT to be safe and well-tolerated in clinical trials [46].

Mechanisms of AIT in sustaining long-term tolerance

Whereas the treatment protocols can be divided into two phases, the underlying mechanisms of AIT display three phases: rapid desensitization, early tolerance, and sustained tolerance. The first phase includes a decrease in the degranulation of mast cells and basophils, possibly resulting from the rapid upregulation of histamine type 2 receptors (H2R) [46, 50]. The second phase is characterized by a reduced number of IL-4-secreting T_H2 cells and an amplified production of IL-10-secreting induced regulatory T (iTreg) cells and regulatory B (Breg) cells [50]. This is where the transition from a T_H2 response to a T_H1 response, along with increased IL-10 and transforming growth factor β (TGF-β) production, is likely to occur [46]. The third phase corresponds to changes in memory B cells and T cells. Treg cells stimulate the production of new IgG-producing B cell populations instead of IgE-producing B cells. The current understanding is that these AIT-modified B cells now produce allergen-specific IgG4 tolerogenic high-affinity "blocking antibodies" that compete with the already-existing allergen-specific IgE antibodies for binding to allergens, thus reducing the possibility of IgE crosslinking leading to allergic immune responses [46, 50]. Fig. 2 depicts the mechanisms of AIT.

As AIT progresses, a significant factor in its ability to confer a tolerogenic state in a patient revolves around the decreased ratio of IgE to IgG4 antibodies [42]. The elevated post-AIT IgG4 levels serve several purposes when competing with IgE for allergens: (1) IgG4 contributes toward keeping basophil activation low as the IgG4 prevents allergen sequestration by IgE antibodies; (2) IgG4 prevents crosslinking of FcεRI on basophils and mast cells which ultimately lead to the inhibition of histamine release; (3) the allergen-IgG4 complexes bind simultaneously to the inhibitory FcγRIIB (via the fragment crystallizable [Fc] domain of the IgG4 molecules) and through the allergen binding to the B cell receptor—this leads to the downregulation of IgE antibody production by that cell [4, 7, 20]. Furthermore, AIT does not aim to reduce IgE levels per se but instead induces a protective immune state—a tolerogenic state. From the second year of AIT onward, the sustained allergen-specific tolerance, or unresponsiveness, arises from the induction of modifications in specific T and B cells mediating the predominance of Treg cells and IgG4-secreting B cells over T_H2 cells and IgE-secreting B cells [41]. A minimum of 3 years of treatment enables these changes to be epigenetically imprinted, thus prevailing as the mechanism that drives the continued tolerance after treatment cessation [60]. Additionally, successful long-term tolerance includes T_H2 cell suppression, decreased IgE production, increased IgG1, IgG4, and IgA production, decreased T cell migration into tissues, induction of IL-10-producing DCs, and raised thresholds for mast cell, eosinophil, and basophil activation. Furthermore, the long-term efficacy of AIT correlates with decreased nasal, bronchial, and conjunctival hyperreactivity, accompanying a decrease in underlying mucosal inflammation [61]. An overview of the changes observed from successful AIT is shown in Table 2, whereas Fig. 3 depicts the time course of AIT.

Differences between SCIT and SLIT

SCIT and SLIT differ in the mechanisms by which the allergen first encounters the immune system, yet the sequence of immunologic responses that follows the initial exposure appears to be similar [38, 39, 65]. Fig. 4 describes how the allergen first encounters the immune system in SLIT. Comparisons between studies of SCIT and SLIT are not always straightforward due to the various studies, methods, and forms of data acquisition used in each experiment. However, several studies have claimed that the efficacy of SCIT may be greater than SLIT; for example, a meta-analysis showed that SLIT was beneficial compared to a placebo, but not to the extent as SCIT was efficacious [66]. Nevertheless, compared to SCIT, SLIT enables accessibility to a broader patient population—including children—due to its convenience of at-home administration and improved safety profile, which is why SLIT is still very common [65]. In general, SLIT is considered safer than SCIT; there are no reported fatalities with SLIT and fewer reported cases of anaphylaxis [65].

Presently, the optimal dosage and desired schedule for many allergens have not yet been established [21, 68]. However, according to various estimates, the cumulative dose of allergen given via SLIT ranges from 2.4 to 100-fold higher than SCIT [28, 30]. There are currently four SLIT formulations approved by the FDA; they are for the 5-grass mix, timothy-grass, HDM, and ragweed [65].

Adjuvants

Multiple studies have used adjuvants to help increase the efficacy or efficiency of AIT and decrease the possibility of an adverse reaction to the treatment. Currently, the only four compounds marketed for use in AIT are shown in Table 3 [46]. Although the last three adjuvants listed are considered first-generation adjuvant delivery systems with a depot effect, monophosphoryl lipid A (MPL) is not considered a delivery system yet is the only second-generation adjuvant still in use [46]. The depot effect centers around a slow release of allergen that increases the time the allergen is exposed to the immune system, with higher exposure times resulting in the stimulation of high and sustained antibody titer production [46].

MPL, the TLR4 agonist, is commonly used in the Pollinex Quattro allergy vaccine on individuals over the age of six [69]. It can be delivered orally at doses 15 times higher than that given in SCIT and is observed to be a potent inducer of the T_H1 response. MPL induces the T_H1 response via an IL-12 and monocyte-dependent fashion *in vitro* [70]. Whereas basophil activation and seasonally boosted IgE production decreased

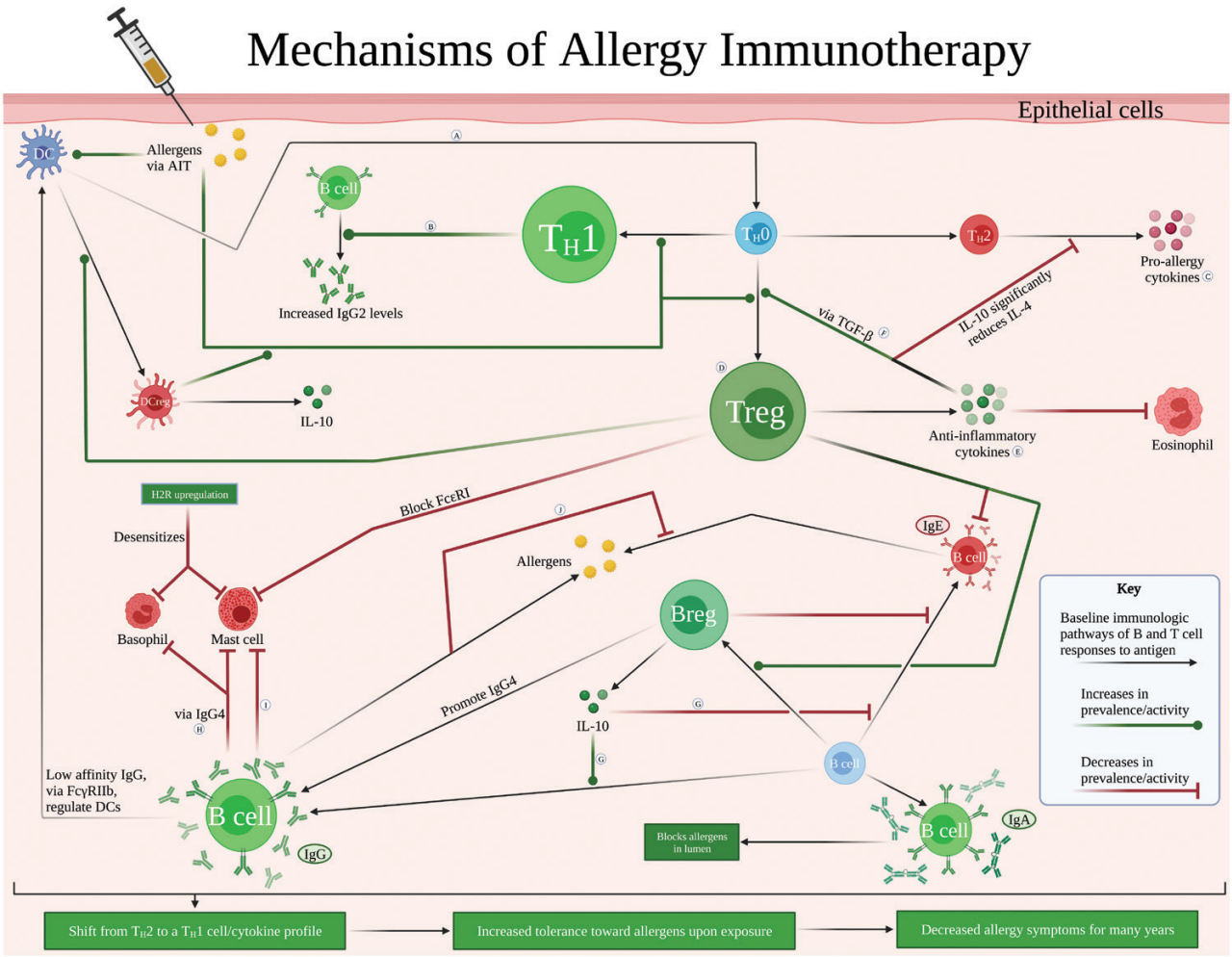


Figure 2: Mechanisms of successful AIT treatment as they are currently understood. Breg cells promote allergen-specific IgG4 antibodies and Treg cells in addition to producing IL-10, IL-35, and TGF-β [20, 51, 52]. IL-10 has several critical roles, including inhibiting IgE-dependent mast cell activation, inhibiting eosinophil production of specific proinflammatory molecules, suppressing IL-4/IL-5 on T_H2 cells (and so indirectly suppresses IgE), and inducing naïve B cell differentiation into IgG-producing B cells [21, 53]. IL-35 may have an autocrine role that can enhance FoxP3+ Treg cells [51]. (A) DCs capture allergen and induce T_H1 proliferation from T_H0 cells; (B) via proinflammatory cytokines IL-2, TNF, IFN-γ; (C) IL-4, IL-5, IL-9, IL-13; (D) includes iTregs and nTregs; (E) IL-10, IL-35, TGF-β; (F) decreased IL-4 creates a favorable microenvironment for TGF-β to upregulate FoxP3 in T_H0 cells to form Tregs; (G) promotes isotype switching from IgE-producing to IgG-producing plasma cell populations; (H) IgG4 prevents crosslinking of FcεRI; (I) low-affinity IgG inhibition via FcγRIIB of mast cells; (J) high-affinity IgG compete with IgE for allergen binding (they act as "blocking antibodies") [3, 4, 7, 10, 11, 20, 21, 23, 51–59]. *FcγRIIb*, fragment-crystallizable-gamma-receptor-2b. Created with BioRender.com

in vivo, IgG4 and IgG2 antibody populations increased. Of note, the Pollinex Quattro allergy vaccine is an ultra-short course version of SCIT consisting of a four-injection regimen that should be administered yearly for at least 3 years [71]. MCT can also be given in combination with MPL to allow for synergistic effects. Particularly, MPL has an established safety profile, is completely metabolized by the body as it is biodegradable, and generates sustained and robust IgG populations with no enhancement of IgE populations, which have made it an excellent adjuvant for use in AIT [72, 73].

Aluminum hydroxide and calcium phosphate are generally seen with subcutaneous versions of AIT. One study states that aluminum salts are the adjuvant of choice in 75% of AIT treatments that utilize an adjuvant, primarily because of its depot effect and long-cited potency [74]. Aluminum salts are also more efficacious than other adjuvants, such as calcium phosphate and tyrosine, but their long-standing use is questionable due to their nonbiodegradability and neurotoxic effects [75]. One murine study compared aluminum salts to MCT as an adjuvant for AIT and found that both MCT and aluminum salts induced high and sustained IgG titers, yet MCT stimulated less IL-4 and IgE and allergic reactions than aluminum salts [76]. Another paper states that aluminum salt adjuvants garner reduced symptom and drug requirements for individuals allergic to grasses [77]. However, it was also mentioned that some reports include granulomas forming in the skin and severe reactions when aluminum salt adjuvants were used. Evidence of calcium phosphate as an adjuvant is even more limited, though it is said to lead to improved nasal symptoms and IgG4 levels, with some individuals developing double subcutaneous local reactions [77].

Monoclonal antibodies (mAbs) can also be used as an adjuvant with AIT. However, the enormous cost of mAbs, the requirement for parenteral administration, and the potential side effects have restricted their use [78]. Omalizumab is the first anti-IgE mAb used to treat patients with severe asthma

Table 2. Observable changes seen in patients during and after successful AIT treatment compared to treatment onset

Overall changes seen during AIT	
Before AIT (baseline levels)	After [successful] AIT
• Immune system skewed toward T_H2 cytokine profile • Excessive IgE crosslinking upon allergen binding	• Immune system skewed toward T_H1 cytokine profile • Upregulation of IL-10 (by monocytes, macrophages, B cells, and T cells) [53] • Increased expression of TGF-β [53] • Changes in populations of B cells from IgE-producing to IgA- and IgG-producing B cell populations [53] • Increased Treg cell activity and abundance [53] • Decreased mast cell activity (e.g. the degranulation of proinflammatory mediators) [53] • Marked reduction in number of eosinophils and neutrophils that are recruited to the site(s) of allergen exposure [53] • Allergen-specific IgA levels in the circulation were found to increase during some forms of AIT (e.g. food-allergy SLIT) [59] • Correlation between increasing intestinal IgA and a diminished risk of IgE-associated allergic diseases [59] • Improved response correlated with increased IL-10-secreting Treg cells [58] • Decreased mast cell and basophil tissue infiltration and mediator release correlate with the late effects of AIT [42] • Induction of IL-10-producing Breg cells • A reduction is seen for the following: the eosinophil and T_H2 cell count at sites of allergen challenge, the basophil/eosinophil seasonal increase in the mucosae, and the number of mast cells in the skin [53]

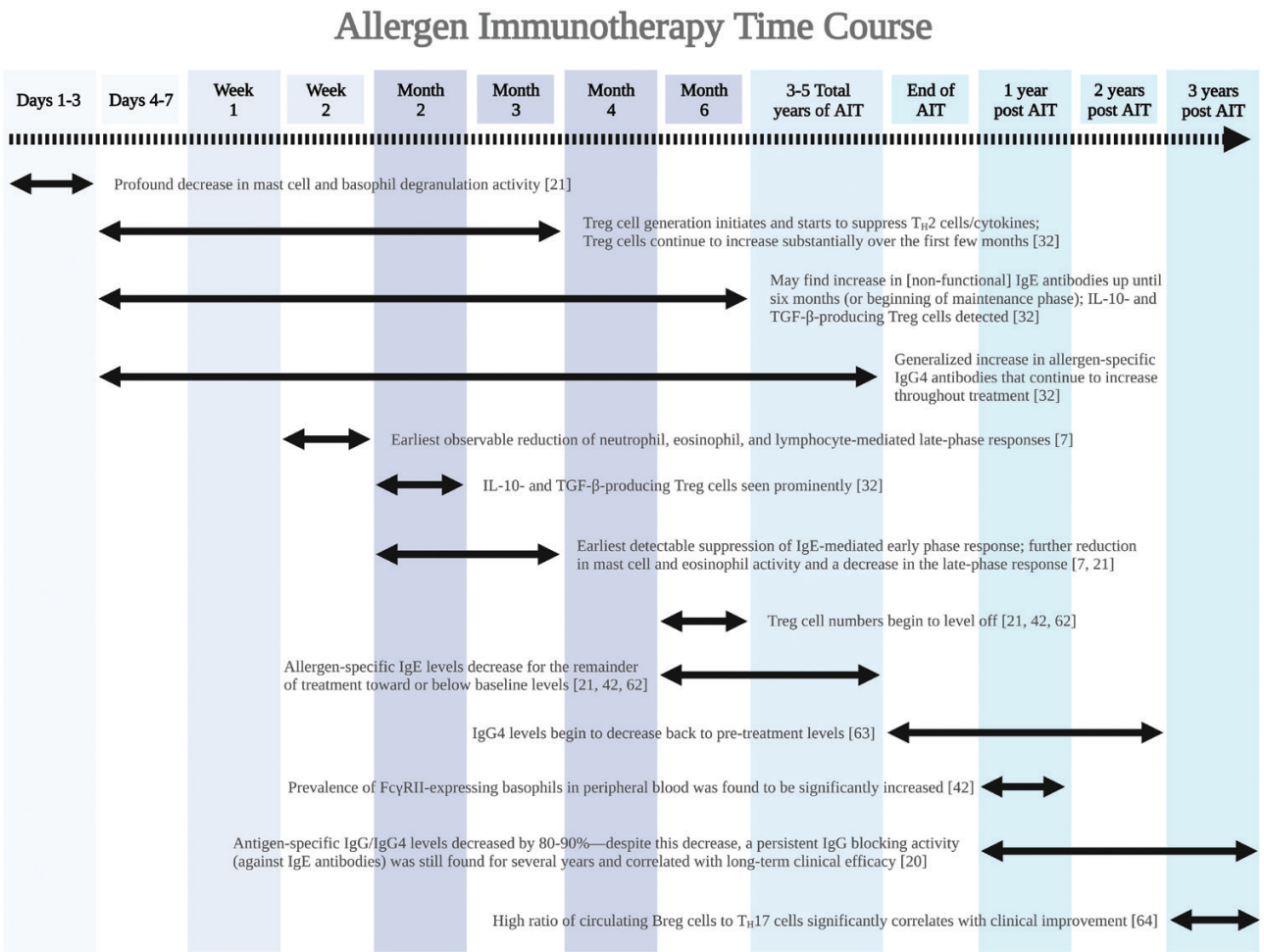


Figure 3: Time course of AIT depicting the prominent cell, cytokine, and other changes observed during AIT treatment and after cessation [62–64]. The double arrows indicate the window of time where the activity of the observed characteristics is noted. T_H17, helper T cell 17. Created with BioRender.com

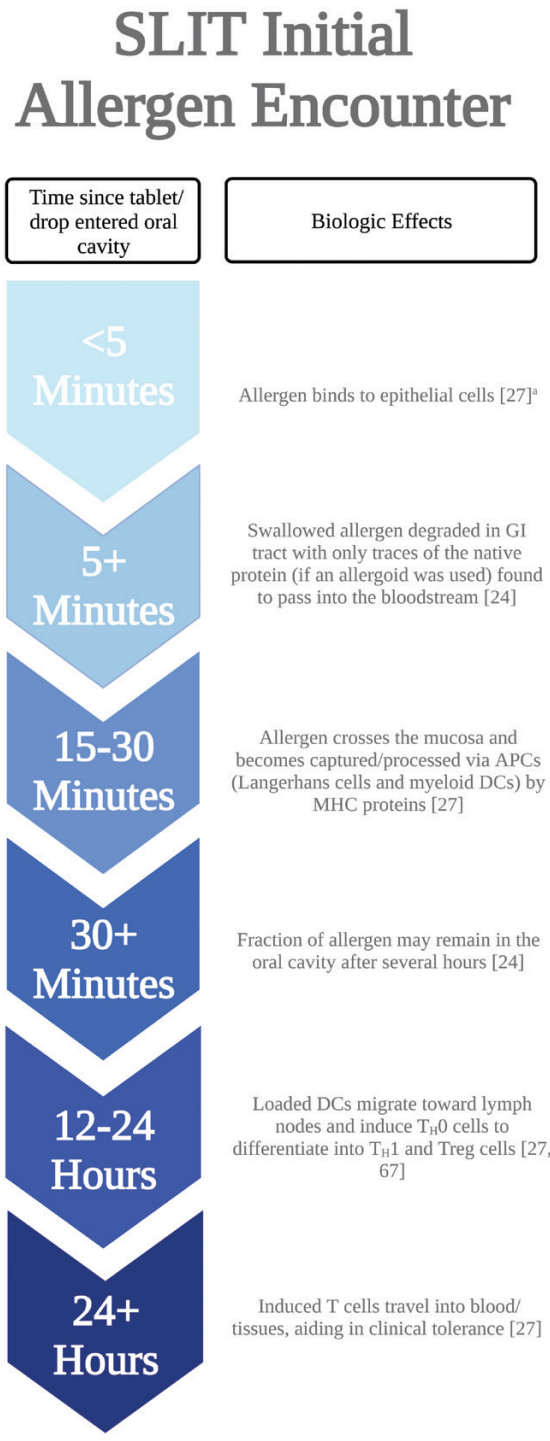


Figure 4: Initial allergen encounter with the immune system during SLIT [67]. GI: gastrointestinal. Created with BioRender.com[a] Some DCs were reported to capture allergens on the buccal mucous membrane at this point.

and is licensed for moderate to severe AA in people 6 years and older with IgE levels greater than 30 IU/L [1]. In addition to omalizumab, Table 4 lists the other four approved mAbs.

SCIT efficacy increased substantially when combined with omalizumab compared to SCIT alone. For the combination therapy for children with allergic rhinoconjunctivitis, one study observed the reduction in days with nasal and ocular symptoms to be 76% and 38%, respectively, followed by a 28% reduction in rescue medication use post-treatment [80]. It was also found that individuals who developed anaphylactic reactions to Hymenoptera AIT could undergo successful treatment with concomitant administration of omalizumab [81–83]. One paper states that omalizumab lessens systemic side effects—decreasing symptom scores up to 48%, yet does not improve the efficacy of AIT [84]. In addition, omalizumab has been shown to induce Treg cells via the restoration of plasmacytoid DCs *in vitro* [85]. It is suggested that the chances of anaphylaxis would be lesser in an individual undergoing AIT combined with dupilumab rather than omalizumab, as omalizumab was reported to have a larger number of anaphylactic events [86]. It should be noted that these anaphylactic events were not combination therapies of a mAb and AIT; instead they were independent events pertaining to either AIT, the use of omalizumab, or dupilumab. Lastly, most available data, if any, pertains to omalizumab, with clinical efficacy comparing the other mAbs to AIT appearing to be lacking.

The critical observation surrounding the use of AIT with concurrent omalizumab administration is that omalizumab lessens allergic/immunotherapy-associated side effects, which increases tolerability but does not appear to improve clinical efficacy [78]. Specifically, when omalizumab is given in combination with AIT, it allows patients to receive higher doses of allergen earlier and allows AIT to be given to higher-risk patients with AA [3]. In addition, allergoids—modified molecules or allergen multimers—can be used for AIT. By modifying the allergen(s)/molecule(s), IgE-binding epitopes are reduced while preserving T-cell epitopes [32].

Safety and contraindications for AIT

AIT is not for everyone. The treatment may prove ineffective for some individuals or too dangerous for others. Allergic reactions resulting from AIT tend to encompass two types: local (occurring at or near the injection/deposition site) and systemic (such as anaphylaxis, which in rare cases could result in death) [53]. Moreover, various AIT contraindications fall under the category of absolute (i.e. absolutely inadvisable) or relative (i.e. potentially inadvisable), as seen in Table 5 [87].

Importantly, studies have stated that SLIT can result in adverse local oral reactions, usually within the first 2 weeks of treatment, that appear to last for 30–60 min upon allergen exposure [28, 89]. Local adverse reactions such as nausea, diarrhea, heartburn, uvular edema, abdominal pain, pruritis/swelling of the mouth, tongue, or lips, and throat irritation seem to develop frequently in roughly 70–80% of patients: the most frequent reactions consist of oral pruritis (17%), throat irritation (14%), and ear pruritis (10%) [28]. Another study reported that 26–86% of patients that receive SLIT experience some form of a local reaction, especially within the first 2 weeks of starting AIT [90]. Individual large local reactions in patients do not predict a systemic reaction and should not be considered a basis for adjusting the dose. However, patients with frequent large reactions have an increased risk for systemic reactions [39]. The local adverse reactions from AIT tend to occur in the initial treatment period when the allergen doses increase; they predominantly persist for a short time, resolve spontaneously, and only in irregular cases require treatment [28].

AIT is primarily limited to individuals aged 5 years and older (with no upper limit), which seems to be based on more

Table 3: Four currently approved adjuvants for use with AIT

Adjuvant	Pros	Cons
Aluminum hydroxide	• Enhances allergen immunogenicity and tolerability [46]	• Can raise IgE and IgG titers • Induces T_H2 responses (which would not be optimal for AIT) • Leads to aluminum toxicity if used chronically [46]
Calcium phosphate	• Biodegradable • Potential to absorb antigen • Does not induce IgE production • Raises IgG levels [46]	• Induces local adverse reactions in animal models [46]
MCT	• Can enhance IgG production [46]	• Has a half-life of two days • Contraindicated in tyrosine metabolism disorders • Yields limited IgE level increases [46]
MPL[a]	• Interacts with TLR4 on immune cells [46] • No toxicity [46] • Once MPL interacts with TLR4, it stimulates DCs to mature and produce cytokines (IL-12), which stimulates naïve T cells to mature into T_H1 cells	• Not reported

MCT, microcrystalline tyrosine; TLR4, toll-like receptor 4.
[a]Detoxified derivative of a lipopolysaccharide from Salmonella minnesota R595.

practical than evidence-based reasons; repeated injections of SCIT may be seen as traumatic in small children [25, 87]. Moreover, children younger than 6 years old may have difficulty cooperating with the strict regimen and/or injection schedule, with early signs of a possible anaphylactic reaction challenging to discern earlier [22, 40].

Due to the possible adverse effects (e.g. anaphylaxis) when starting AIT, especially with SCIT, the patient should be under the care of an expert clinician in a facility that is prepared to properly deal with an anaphylactic reaction or any other related emergency procedures that may be needed [91]. Patient observation for AIT administration, especially at the start of treatment, should last longer than the 30 min that current recommendations state, as delayed anaphylaxis may occur (greater than 2 h after administration) [87]. Importantly, biphasic anaphylaxis, or recurrence of symptoms hours after the initial event, usually within 8 h (but can still vary), may be a possibility for up to one-fifth of anaphylactic reactions; thus, continued monitoring of the patient is crucial [92]. The FDA mandates that any patient undergoing SLIT be prescribed and trained on auto-injectable epinephrine use [39]. Systemic reactions are also higher in individuals with uncontrolled AA and with rush protocols [33]. Since SLIT is less likely to cause severe systemic reactions, it would be more reliable to use SLIT than SCIT on children. Moreover, the fact that SLIT can be done via self-administration at home makes it more attractive for children (and adults) who have limited time due to other obligatory places (e.g. school or work) [33].

To be considered a suitable candidate for AA AIT, asthma symptoms must be controlled with an FEV_1 being 70% or greater than predicted at baseline [22]. Patients with severe or uncontrolled asthma by pharmacotherapy have an increased risk of severe systemic reactions to aeroallergens, hence the reason for poorly controlled or uncontrolled asthma being cited as a contraindication [87].

Interestingly, one study noted that the summer period (May–September) in North America is considered to be the right time to start AIT since this period correlates with most patients having their lowest level of symptoms and can also be attributed as a "wash-out" period for the steroid effect; though, this does not apply to all allergens [93]. Some reviews note that the maintenance dose during seasons with high allergen concentrations can be reduced to lessen the chances of a systemic reaction (only if it is based on a seasonal allergen) [40]. Thus, if a patient has seasonal allergies, it might be best to start a "pre-seasonal" treatment (e.g. 3–4 months before seasonal allergies start) [29].

Long-term efficacy of AIT

Due to heterogeneous studies, forms of AIT, allergens, and parameters used to define the "endpoint" at which AIT long-term efficacy no longer appears to exist, AIT typically lasts a minimum of 3 years and a maximum of 12 years after treatment ends [20, 21, 40, 94]. A more generalized [estimated] treatment duration with a minimum of 3 years of continuous treatment appears to be between 5 and 7 years once AIT ends for patients [25, 28, 94]. It should be noted that for some studies that demonstrated a "carry-over" effect upon AIT discontinuation (which enabled the positive effects of AIT to be cited to last up to 12 years), some of the studies used for this observation had high rates of dropouts, and so the observations surrounding these long-lasting effects need to be scrutinized in future studies [4, 35].

Other than helping to combat AA, some AIT treatments seek to reduce the progress of the allergic march. The "atopic march" (or allergic march) defines the "natural history" or prototypical sequential progression of atopic manifestations that usually begin early in life. These manifestations include allergic dermatitis (AD), AR, and AA as a general order of events [54, 95]. One study stated that 3 years of SCIT had

Table 4: Monoclonal antibodies (mAbs) used for alternative biologic treatment of severe AA

mAb	Mechanism of action
Omalizumab	• Binds to the Fc region of IgE antibodies • Prevents free IgE binding to FcεRI and FcεRII • Interrupts extensive amounts of IgE-dependent cellular and molecular events [1, 79] • Decreases cellular infiltration of T cells, B cells, and eosinophils • Downregulates IgE receptors on mast cells, DCs, basophils, and monocytes [78][a]
Mepolizumab	• A humanized IgG1 mAb that targets IL-5 with high affinity [1, 79]
Reslizumab	• Similar to mepolizumab, except that it is a humanized IgG4 mAb [1, 79]
Benralizumab	• A humanized IgG1 mAb that binds, via its Fab fragments, to IL-5Rα • Through its constant Fc region, it interacts on NKCs via FcγRIIIA, resulting in the triggering of eosinophil apoptosis via antibody-dependent cell-mediated cytotoxicity, which can be quite rapid and so eosinophil reduction in blood, bone marrow, and sputum may be observed [4, 79]
Dupilumab	• A fully human IgG4 mAb that acts as a dual receptor antagonist of IL-4 and IL-13 via inhibition of the biological effects of IL-4 (and IL-13) by recognizing and binding to the alpha subunit of the IL-4R [79]

Fab: fragment antigen-binding; IL-4R: interleukin-4 receptor; IL-5Rα: interleukin-5 receptor alpha; NKC: natural killer cell.
[a]Basophils and mast cells become less sensitive to stimulation by allergens and degranulation due to omalizumab promoting the downregulation of FcεRI as it continues to deplete free IgE levels further [4].

Table 5: Contraindications for AIT [3, 31, 87]

Absolute contraindications	Relative contraindications
Uncontrolled asthma	Partially controlled asthma
AIDS	HIV infection
Children <2 years old	Cardiovascular diseases
Eosinophilic esophagitis[a]	Use of immunosuppressive drugs
Cancer	Immunodeficiencies[b]
Serious immunologic diseases	Pregnancy[c]
Chronic infections	Acute gastroenteritis[d]
Lack of compliance	Acute oral inflammation, injury, or surgical intervention
Severe psychological disorders	Patient in remission with an autoimmune disorder[e]
Patient with an active autoimmune disorder[b]	

AIDS: acquired immunodeficiency syndrome; HIV: human immunodeficiency virus.
[a]Primarily for SLIT.
[b]Some physicians feel uncomfortable administering AIT to patients with autoimmune disorders, immunodeficiency syndromes, or malignant diseases; yet, there was no convincing evidence that AIT would harm those patients [22].
[c]It is currently understood that if a patient becomes pregnant while undergoing AIT treatment, it is considered safe to continue with the maintenance dose [with caution] throughout pregnancy as long as the dose is well tolerated [87]. However, starting AIT or increasing the dose during pregnancy is not recommended due to the risk to the fetus of anaphylaxis [88].
[d]Temporary contraindication for SLIT.
[e]One study described limited data on AIT with autoimmune disorders; yet, it explained that guidelines agree that in the case of the development of an autoimmune disease, AIT should be terminated [87].

been observed to prevent asthma development in children for up to 7 years post-treatment [96]. After 5 years since AIT discontinuation with children, improved lung function, decreased use of relievers, and reduced asthma episodes were observed in one study [33]. Furthermore, children re-evaluated 9 years after discontinuing AIT showed a lower risk of frequent asthma symptoms than controls by three-fold.

The recommended duration of AIT is at least 3 years [25]. Numerous studies state that 2 years of SCIT or SLIT is insufficient to provide long-term benefits [22, 78, 97, 98]. After 2–3 years of AIT, it was observed that non-specific airway hyperresponsiveness appeared to gradually decrease and, for some patients, even returned to normal in one study [91]. They suggested that this observation indicated the long-term necessity for AIT, as symptom improvements might occur earlier than airway inflammation amelioration. Another study with different patient groups receiving SLIT for 3, 4, and 5 years noted that clinical benefits persisted for 7 years for the 3-year SLIT group and 8 years for the 4 and 5-year SLIT groups [99]. Thus, they suggested that a 4-year treatment duration is optimal as a 5-year regimen added only marginal benefit.

Notably, there is currently no universally accepted biomarker to indicate AIT efficacy or patient eligibility. However, elevated allergen-specific IgE (sIgE) levels are considered to be the gold standard for both the diagnosis of an allergy and AIT recruitment; it should be noted that this biomarker does not reliably predict or monitor a patient's clinical response to AIT [7, 18, 20].

Conclusion

Since AIT is the only currently available disease-modifying treatment for AA (and can be used for AR/other atopic conditions), it is more necessary than ever to understand the mechanisms of this treatment to ensure the best long-term clinical outcome for individuals seeking AIT. Treatment should be personalized as many factors can affect the patient's safety and [long-term] clinical efficacy. The decision between SCIT and SLIT depends on many aspects that are not limited to the following: cost, physician/patient preference, characteristics of the patient, product availability, relative safety profile (e.g. relative and absolute [contra]indications), and the ability of the patient to consistently return to the facility where treatment is being given [3]. The exact mechanisms by which AIT mediates its anti-inflammatory effects are not entirely defined due to heterogeneous treatment protocols, administration routes, and allergen preparations in different studies [53]. Though, as the mechanisms of AIT are better understood, more efficacious treatment plans may be available for the 330 million people worldwide that this inflammatory disease plagues with its never-ending financial and social burdens on life [4].

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Author Contributions

S.F. collected all of the relevant literature to prepare the draft of the manuscript and prepared all figures and tables. H.B.F. provided guidance and direction throughout the preparation of the manuscript and reviewed and revised the manuscript. Both authors have reviewed and approved the final version of the manuscript.

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Not applicable

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