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Allergen Specific Immunotherapy (AIT)

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Abstract

Allergic/atopic patients do not develop immune tolerance to certain substances in the environment which are harmless to non-allergic individuals. These patients are hypersensitive to these substances-allergens. Specific Allergen Immunotherapy (AIT), also known as Allergy Shots or Desensitization, is a therapeutic approach used to treat allergic conditions, such as allergic rhinitis, allergic asthma, and insect sting allergies. The primary goal of AIT is to reduce the allergic response to specific allergens by gradually exposing the patient to increasing doses of the allergen over time. This exposure helps the immune system build tolerance to the allergen, leading to a decrease in allergic symptoms and a long-term improvement in clinical outcomes. Research has confirmed the long-term clinical efficacy of AIT, and its ability to induce antigen-specific tolerance. In this review, we aim to discuss the immunological mechanisms involved in different types of AITs, their efficacy and safety.

Keywords: Allergen Immunotherapy; Allergy; Immune tolerance; Immune system; Allergic rhinitis

1. Introduction

1.1. Allergen-specific Immunotherapy (AIT)- The Centenary

In 2011, the world celebrated one hundred years of immunotherapy for allergic-atopic diseases. In 1911, Leonard Noon conducted the first study showing that repeated injections of crude grass pollen extract into individuals with hay fever reduced immediate sensitivity to grass pollen and improved symptoms [1]. In 1954, Frankland [2] confirmed the efficacy of subcutaneous grass pollen injection therapy for seasonal asthma and identified the high molecular weight protein-containing component of the allergen extract as responsible for the therapeutic effect and in 1978, Norman and Lichtenstein [3] demonstrated that AIT was allergen-specific, showing that a ragweed pollen extract was effective during the ragweed season but not during the grass pollen season.

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Over the past 100 years, subcutaneous immunotherapy (SCIT) has remained the primary method of AIT, involving weekly injections followed by monthly maintenance injections over several years. However, it requires specialist supervision and presents a high-riskallergic adverse effects including anaphylaxis [4]. During this period several alternative routes have been proposed, tested, evaluated in clinical trials and developed to improve efficacy and safety. In recent years, there has been increasing interest in sublingual immunotherapy (SLIT) as a safer alternative to SCIT and today, the development of Local Nasal Immunotherapy (LNIT) has also gained acceptance and it has been proposed as a safe and effective alternative for SCIT although other methods have been emerging such as Intralymphatic immunotherapy (ILIT) with good results [5, 6].

Today, AIT still represents the only currently available treatment with sustained efficacy for allergic rhinitis, and allergic asthma [7-9] that targets the underlying pathophysiology of allergic rhinoconjunctivitis (AR) and may have a disease-modifying effect [4]. The advancements in knowledge in Immunology and Immunopathology have substantially enabled us to understand the mechanisms underlying factors involved in Immunotherapy. This understanding has facilitated the widespread practice of this therapy with allergens in clinical immunology. However, issues related to the safety and efficacy of the treatment [10, 11], as well as its duration, standardization in the production of allergens [12], and routes of application [13, 14], continue to be subjects of study and are topic of discussion in the annual congress of European Academy of Allergy and Clinical Immunology (EAACI) [15].

Allergen-specific immunotherapy is used for immunomodulation and desensitization in allergic diseases, mainly prescribed in cases of allergic rhinitis, allergic conjunctivitis, allergic asthma, atopic dermatitis, and food allergy, considering that individuals with allergies exhibit an abnormal hypersensitive response, leading to the production of immunoglobulin E (IgE) inappropriately [16]. Initially, this production occurs in a nonspecific manner until it becomes specific when the allergen selects the lymphocyte clone responsible for producing this immunoglobulin, which supports the theory of clonal selection. Thus, Allergen immunotherapy (AIT) plays a fundamental role in the treatment of allergic patients by modulating the underlying immune mechanisms. While this treatment is integrated into practice patterns globally, significant variations exist in the implementation of AIT at the national and international levels due to diverse methods. Clinical recommendations also differ across various regions of the world.

The two global regions, Europe and the United States, serve as significant examples of both differences and similarities in crucial aspects of AIT application. Firstly, there are several regulatory variations. Secondly, discrepancies can be observed in manufacturing practices and formulations of AIT products. Thirdly, while clinical administration patterns within current guidelines exhibit similarities in indications and contraindications of AIT, they also diverge in certain practical aspects [17].

In relation to polysensitization for example, the focus in the US is often on mixtures of extracts immunotherapy.This approach is based on identifying and targeting individual allergens through allergy testing, and subsequent immunotherapy treatment [17-18]. On the contrary, in Europe, there is more emphasis on using one or a few allergens deemed to be most clinically relevant [10-12, 18].

There is an ongoing debate as to whether single- or multiple-allergen formulations of AIT should be prescribed. There is more emphasis on using mixtures of allergens in immunotherapy, especially for patients with polysensitization. This approach, known as polyvalent or mixed-allergen immunotherapy, involves combining multiple allergens into a single treatment to address multiple sensitivities simultaneously and provide a comprehensive therapeutic effect by addressing multiple sensitivities simultaneously.

The prevalence of polysensitization varies between populations and regions, with estimates suggesting that 50% to 80% of patients consulting allergists are polysensitize. It is important to note that the current evidence suggests that single-allergen AIT is effective in polysensitized patients, but not necessarily in polyallergic patients. A detailed molecular diagnosis can add value when determining whether AIT is appropriate for a given patient and, if so, which allergen(s) should be administered. However, there is a need for well-powered, specifically designed clinical studies comparing the efficacy and safety of single-allergen AIT, AIT with two or three allergen extracts (preferably delivered separately and at high dosage levels), and AIT with a high number of allergen extracts [19].

In conclusion, the prevalence of monosensitization and polysensitization varies across populations and regions, with different treatment approaches being utilized in the USA and Europe. Further research is needed to determine the most effective treatment strategies for polysensitized patients, particularly in terms of the number of allergens included in AIT formulations.

1.2. Standardization of allergens used in Allergen Immunotherapy (AIT).

Standardization of allergens used in Allergen Immunotherapy (AIT) is an essential aspect of ensuring the safety and efficacy of this treatment approach. It is a well-established practice in the field, involving the process of preparing allergen extracts for AIT to ensure consistent potency, purity, and quality. This process aims to minimize variations in allergen extracts and guarantees that they contain the appropriate amount of allergenic components necessary to achieve a therapeutic effect. During this process, the major allergenic components in the extracts are characterized and quantified using techniques such as immunoassays and protein analysis. National and International entities provide recommendations for determining allergenic activity and standardization [20 – 22].

The Standardized Quality-Units (SQ-U) of allergens used in Allergen Immunotherapy (AIT) serve as a measure of the potency and standardization of allergen extracts utilized in AIT. However, the SQ-U value or dosage for AIT is not determined by the administration route, but rather by the specific allergens to be used in the patient's treatment, the patient's sensitivity to those allergens, and the treatment protocol prescribed by the allergist or immunologist. Standardization ensures consistency in the composition and potency of allergen extracts, enabling accurate dosing and reliable therapeutic outcomes. The entire process, therefore, ensures the safety and efficacy of various AIT products in clinical trials and facilitates regulatory approval and marketing authorization.

1.3. Efficacy and safety and cost-effectiveness of AIT

The efficacy and safety of allergen-specific immunotherapy (AIT) has been extensively studied and demonstrated in several clinical studies and through laboratory techniques. However, it is important to highlight that they may vary depending on the specific allergen, the treatment protocol and the individual characteristics of each patient. Thus, randomized controlled clinical trials designed to answer specific questions about the efficacy and safety of a therapeutic intervention has been conducted in different allergic conditions such as:

- Studies in patients with allergic rhinitis: evaluating the effectiveness of AIT in reducing nasal symptoms, improving quality of life and decreasing medication use [20-21].
- Studies in patients with allergic asthma: investigating whether AIT can reduce the frequency and severity of asthma symptoms, decrease the need for rescue medications, and improve lung function [23, 24].
- Studies in patients with food allergies: exploring whether AIT can induce tolerance to specific food allergens and reduce the severity of allergic reactions [25 – 27].

Another approach explored to assess the efficacy and safety of AIT involves trials based on the route of administration, but several trials have been published by various authors using different designs, allergens, patients with different types of allergies, and diverse patient populations.

Alongside the clinical benefits, economic evaluations of immunotherapy in comparison with pharmacotherapy for assessing the cost-effectiveness of AIT have been explored and demonstrated in recent literature [28, 29]. Interestingly, Richter A.K. et al. (2018) compared the cost-effectiveness of SCIT in addition to standard therapy versus the use of SCIT alone, demonstrating that increasing treatment rates of SCIT is a cost-effective strategy with an incremental cost-effectiveness ratio. [30].

1.4. Immunology – the basis of AIT

Immunology plays a fundamental role as the basis of AIT that aims to modulate the immune response to allergens, shifting it from an allergic or hypersensitive state to a more balanced and tolerant state. Immunology is indispensable for Allergen Immunotherapy (AIT), also known as allergy shots [31]. The immune system, particularly adaptive immunity, plays a central role in AIT through the recognition of specific non-self antigens, the generation of pathogen-specific immunologic effector pathways, and the development of immunologic memory [32].

Immunopathology, for example, which refers to disorders caused by defects or malfunctions in the immune response, is a key area of study in AIT. Hypersensitivity reactions, autoimmunity, and immunodeficiency can all impact the efficacy and safety of AIT. Understanding the immunopathological mechanisms underlying these conditions helps in designing targeted interventions and optimizing treatment outcomes. Among the mechanisms of action of allergens used is the modulation of T and B lymphocytes, which is desired for effective immunomodulation. These cells have a genetically encoded defect, leading to dual activities: hyperactivity at one moment and a decrease in their intercellular controls at another. In this case, B cells exhibit a stimulatory response to IgE antibody isotype production.

In allergic-atopic mechanisms, the pathophysiology of the immune system's response is subject to a complex mechanism, influenced by genetic factors. This genetic state, known as atopy, results in an individual having a profile of specific responses that are unusual when exposed to innocuous proteins. The atopic complexity of the atopic individual encodes immune responses that are influenced by factors such as the route of exposure to the allergen, the dose of the allergen, and its three-dimensional molecular structure [33].

The three-dimensionality of molecules is what confers specificity to the allergen and, consequently, to the IgE antibodies. Allergic-atopic patients exhibit behavioral changes in inflammatory cells, including basophils, mast cells, and eosinophils [34]. The latter cells are resistant to apoptosis, leading to their effective multiplication (eosinophilia). They are considered responsible for the disordered multiplication in characteristic allergic-atopic processes, resulting in undesirable inflammatory effects.

T lymphocytes, with their markers and receptors, including CD4 surface markers (CD4+ lymphocytes or TH2 subpopulation), are responsible for producing lymphokines characteristic of allergic processes, such as IL-4, IL-5, IL-13, and IL-10. These lymphokines induce a shift from the primary production of IgM by B-lymphocytes/plasmocytes to IgE. At the same time, these lymphocytes produce IL-5, a potent chemotactic factor for eosinophils [35]. Mast cells, which are sensitized with at least two molecules of IgE through their high-affinity receptors (FcεRI), can release substances like histamine, the prototypical substance of anaphylactic processes, immediately upon activation. Additionally, substances with late and chronic effects, such as prostaglandins and leukotrienes, are produced through the action of phospholipases on cell membrane phospholipids [12].

In the thymus, subpopulations of T lymphocytes receive surface markers that represent their phenotypes and, consequently, have characteristic genotypes. Among them are TH2 CD4+ and CD25+ cells, which are phenotypically Foxp3 producers. Foxp3 is a transcription factor capable of inducing DNA to produce IL-10, IL-35 and transforming growth factor-β, (TGF-β), which are responsible for negative antiproliferative control of the cells that make up the immune system. These T cells, also known as regulatory T cells (Treg), represent the dominant subpopulation that reacts to foreign substances in non-allergic individuals, leading to the production of IgM and IgG [36]. Additionally, among the mechanisms involved in immunosuppression are the interruption of the metabolic pathways involving the histamine receptor 2 (HR2), CD39, and CD73 [37], suppression of dendritic cells by programmed death 1 (PD1), cytotoxic T lymphocyte antigen 4 (CTLA-4) and cytolysis by granzymes A, B, and K [38, 39].

The goal of specific immunotherapy (SIT) is to achieve immunomodulation and control of Th2CD4+ lymphocytes, which are the inducers of IgE production and inflammatory substances in allergic-atopic individuals. The primary objective is to induce a state of Th2CD4+ cell tolerance to the specific allergen. This tolerance is mainly achieved through the specific induction of Treg cells, which in turn inhibits the proliferation of Th2 and Th1 cells responsible for producing specific IgE to the allergen in question. Additionally, Treg cells inhibit the production of pro-inflammatory allergy substances [35]. As Th2 is inhibited, there is stimulation of the production of IgG4, IgG1, and allergen-specific IgA, leading to a decrease in IgE levels. Consequently, there is a concomitant decrease in the tissue infiltration of mast cells, eosinophils, basophils, and lymphocytes. This phenomenon occurs because basophils, mast cells, and eosinophils have surface receptors, with high and low affinity, respectively, for IgE [33].

AIT works by reducing the immediate hypersensitivity response triggered by the binding of allergen-specific IgE to mast cells. When an allergen encounters specific IgE antibodies on sensitized mast cells, it leads to the cross-linking of IgE receptors, and the subsequent release of inflammatory mediators stored in the granules of mast cells. These mediators include histamine, which is a potent mediator of immediate allergic responses [40]. By modulating the immune response through AIT, the release of histamine and other pro-inflammatory substances stored in mast cell granules can be inhibited. This helps to reduce the intensity of allergic symptoms associated with immediate hypersensitivity reactions. AIT also acts by inhibiting the formation of newly synthesized cysteine, leukotrienes (such as LTC, D, and E4) and prostaglandins [41]. These lipid mediators play significant roles in inflammation and immune responses associated with allergic diseases.

Finally, advancements in immunology have led to a better understanding of the immune system's complexity, including its cross talk with other systems like the endocrine and nervous systems. These developments have fueled interest in manipulating the immune response for therapeutic purposes. Immunotherapy, including AIT, has expanded rapidly in recent years, utilizing monoclonal antibodies, cytokines, immunomodulatory nano particles, and cellular immunotherapy. Ongoing research in immunology aims to address challenges and develop safer, more effective and specific immunotherapeutic approaches [42].

1.5. The Signals and Symptoms

Individuals with atopic allergies have a genetic predisposition that allows them to become sensitive to environmental allergens, a phase known as sensitization [43]. Th2CD4+ cells constitute the largest population of the inflammatory cellular infiltrate in target organs, such as nasal mucosa, sinus, conjunctival, bronchial, dermo-epidermal, gastroenteric, and neurological. It is important to clarify that allergen proteins are harmless to the non-atopic population. After the specific antigen sensitization phase that triggers the production of inflammatory cytokines and the activation and recruitment of pro-inflammatory cells in mucosal target organs, signs and symptoms of allergic inflammatory processes, including edema, cutaneous reactions, hypotension, bronchoconstriction, and anaphylaxis can be observed in various target organs, such as the nose, skin, lungs, eyes, gastrointestinal tract, or even systemic reactions with varying degrees of anaphylaxis, highlighting the different organ systems that can be affected by allergens and the various symptoms they can cause [44].

The CD4+ T cells are activated by food proteins, aeroallergens, and bacterial superantigens. Examples of this mechanism include atopic dermatitis, asthma, rhinitis, and autoantigens. Sensitization can be classified as activation, homing to specific organs, or cellular reactivation with inflammatory action. The CD4+ T cells are influenced by a network of cytokines in the skin, lungs, and nose, gastrointestinal tract with leukocytes being selectively recruited with specific receptors for each organ. Effector cells subsequently induce hyperproduction of IgE, prolonged lifespan of eosinophils, increased mucous production, and interaction of organ epithelial cells with smooth muscles, leading to activation and even programmed cell death (apoptosis). Prolonged survival of allergic inflammatory cells in tissues is observed, leading to reactivation in subepithelial tissues.

The tolerance of peripheral CD4+ T cells to specific allergens can overcome all these pathological events of allergic inflammation, which is the primary goal of specific immunotherapy (AIT). Understanding of AIT mechanisms has been deepened by the role of CD4+/CD25+ Treg cells, stimulating and inducing specific tolerance to the allergen used in specific immunotherapy (AIT). The balance between Treg and Th2 cells is crucial in the development of allergies, just as the ideal response to allergens is essential for overall health [45].

1.6. Sequence of Events During Allergen-Specific Immunotherapy (AIT)

1.6.1. Mechanisms of Action

The immediate effects of AIT are related to the desensitization of mast cells and the intermediate effects are observed with changes in allergen-specific T cells, while the late effects involve B cells and IgE, as well as mast cells, basophils, and eosinophils [46]. This statement highlights the multi-faceted nature of AIT, targeting various components of the immune system at different stages to achieve desensitization. It suggests that AIT aims to provide long-term relief from allergies by reprogramming the immune system's response to allergens. While a definitive decrease in levels of IgE antibodies and IgE-mediated skin sensitivity usually requires years of treatment (AIT), most patients experience protection from the first AIT injection against anti-pitoxin (hymenoptera), resulting in decreased mast cell degranulation and basophil activity, reducing the risk of systemic anaphylaxis.

Studies have shown that the mediators of anaphylaxis (histamine and leukotrienes) are released during AIT without inducing systemic anaphylactic responses, systemic reactions occur in approximately 1-4% of patients on subcutaneous allergen immunotherapy, and fatal anaphylactic reactions are rare, occurring in an estimated 1 in every 8 million doses of immunotherapy administered [47]. This gradual release remains below the levels necessary to induce systemic anaphylaxis, but it can reduce the content of granules and mediators and affect the degree of degranulation of mast cells and basophils when they are activated by allergens. This characteristic has been demonstrated by *in vitro* analysis shortly after the onset of AIT.

Though there are fluctuations and risks associated with triggering anaphylaxis during AIT, the suppression of mast cells and basophils continues to be affected by other immune mechanisms, such as the generation of allergen-specific T-regulatory (Treg) cells and decreased production of specific IgE [48].

1.6.2. Generation of Regulatory T Cells (Treg) and Peripheral T Cell Tolerance

The induction of a state of tolerance of peripheral T cells is an essential step in allergen-specific immunotherapy (AIT). Peripheral T cells are characterized by the generation of allergen-specific Treg cells). Tregs are a subset of T cells that play a crucial role in maintaining immune tolerance and preventing excessive immune responses. They suppress cellular proliferation and cytokine response against major allergens that trigger allergic reactions, contributing to the

overall reduction of allergic inflammation and symptoms [49]. During AIT, allergen-specific immunotherapy, the immune system is exposed to gradually increasing doses of the allergen [50].

By inducing a state of tolerance in peripheral T cells, AIT aims to reprogram the immune system's response to allergens. This process helps to reduce the severity of allergic reactions and improve the individual's tolerance to allergens over time [51]. The groups of Treg with distinct phenotypes and mechanisms of action include those naturally selected by the thymus, CD4+CD25+ Treg cells, and the inducible type 1 regulatory T cells (Tr1). In Allergy, peripheral Treg cells are blocked by the autocrine action of IL-10 and TGF-ß, which are produced in increased amounts by allergen-specific T cells [52]. The Helper T cells 2 (Th2), which are negatively regulated by Treg cells, do not produce interleukins (IL)-4 and IL-13, thus, they do not stimulate IgE production and cannot produce the cytokines IL-3, IL-4, IL-5, and IL-9, which are necessary for the differentiation, survival, and activity of mast cells, basophils, eosinophils, and mucus-producing cells [49].

In AIT, Th0/Th1 cells, which are involved in promoting inflammation, are unable to mediate tissue damage mechanisms through the production of interferon gamma (IFN-γ), tumor necrosis factor (TNF), and Fas ligand (FasL) that induce apoptosis of keratinocytes and bronchial epithelial cells. This is because these Th0/Th1 cells are suppressed by regulatory T cells (Tregs) to prevent excessive inflammation and tissue damage. By suppressing Th0/Th1 cells, Tregs help to maintain immune homeostasis and prevent excessive tissue damage in allergic inflammation. This suppression prevents the release of inflammatory mediators that can cause damage to essential tissues, such as keratinocytes in the skin and bronchial epithelial cells in the airways.

Studies on the mechanisms by which immune responses to non-pathogenic environmental antigens lead to allergies or non-harmful immune responses demonstrate that Treg cells are dominant in healthy individuals. "If a response occurs, specific Tr1 cells (regulatory) for common environmental allergens represent the dominant subpopulation in healthy individuals." Both healthy individuals and allergic individuals exhibit all three types of allergen-specific lymphocyte subpopulations, TH1, TH2, and Tr1, but in different proportions. A change in the dominant subpopulation and the balance between TH2 cells and Treg cells can lead to the development of allergies or a healthy immune response [53]. Another study on the healthy immune response to allergens demonstrated the involvement of CD4+CD25+ Treg cells in spontaneous remission of allergy. Children with cow's milk allergy who outgrew their allergy (tolerant children) had higher frequencies of circulating CD4+CD25+ T cells and decreased in vitro proliferative response to bovine β2-lactoglobulin protein compared to children who continued to have clinically active allergy. Similar findings have been reported in other diseases. The in vitro proliferative response to nickel of CD4+ human T cells from healthy, non-allergic individuals was significantly increased when CD4+CD25+ Treg cells were depleted [54].

1.6.3. Immunological Modifications During Specific Immunotherapy (AIT)

Although there is significant variation among protocols, from the first day of Immunotherapy, there is an early and immediate reduction in the activity of mast cells and basophils, as well as their degranulation. There is a decrease in the tendency for systemic anaphylaxis. The immediate phase is followed by an intermediate phase with the generation of allergen-specific Treg cells and the suppression of allergen-specific Th1 and Th2 cells. There is an early increase in the production of specific IgE, but its reduction occurs in the late phase of Immunotherapy. Additionally, an increase in IgG4 is observed during the intermediate phase, and in some studies, an increase in IgG1 and IgA is also observed. A significant decrease in the IgE/IgG4 ratio is seen after several months of Immunotherapy, as well as a delayed reduction in skin prick test responses (type I). The decrease in tissue mast cells and eosinophils, as well as the release of their mediators, is detected after a few months [55].

The most probable reason for the decrease in the IgE/IgG4 ratio during AIT would be related to the shift from specific TH2 cells towards the predominance of Treg cells. However, even though the generation of Treg cells occurs within days, a significant reduction in the IgE/IgG4 ratio takes years to occur. The reason for the long period between the change in T cell subpopulations, but not the levels of IgE/IgG4, is not easily explained by the half-life of antibodies [56].There might be several factors contributing to this delay, including the complexity of immune regulatory mechanisms and the time required for the immune system to establish a new balance between TH2 and Treg responses. Additionally, AIT is a gradual and long-term process, typically lasting several years, and changes in the IgE/IgG4 ratio may take time to become noticeable. The role of Treg cells is not limited to the suppression of Th2 cells; they employ various mechanisms to induce peripheral tolerance in normal individuals, preventing allergies and controlling allergen-specific immune responses in five main ways: a) suppression of antigen-presenting cells that induce the generation of effectors Th1 and Th2 cells; b) suppression of both Th2 and Th1 cells; c) suppression of allergen-specific IgE and induction of IgG4/IgA antibodies; d) suppression of mast cells, basophils, and eosinophils; and e) interaction with resident cells and remodeling.

In patients with allergies, the allergen-specific IgE, as well as specific IgE in the serum and on the surface of mast cells and basophils bound to FcεRI, it's a characteristic of atopic disease. Although peripheral T cell tolerance is rapidly induced during Allergen-Specific Immunotherapy (AIT), there is no evidence for B cell tolerance at the beginning of AIT. The natural exposure of allergic patients to a relevant allergen is often associated with an increase in IgE synthesis. Similarly, Specific Immunotherapy (SIT) induces a transient increase in serum IgE, followed by a gradual decrease over a period of months or years of treatment.

In pollen-sensitive patients, desensitization prevents an elevation in serum IgE during the pollen season. However, changes in IgE levels cannot explain the decreased specific allergen response because of SIT (Specific Immunotherapy) because the decrease in serum IgE is relatively late and does not correlate with clinical improvement after SIT. The antibodies induced during SIT are functionally heterogeneous, which may explain the conflicting data regarding the effects of IgG4 subclass protectors. IgG4 is produced after induction by AIT (Allergen Immunotherapy) and binds to the allergen in question before it reaches the IgE bound to the effector cell, preventing the activation of mast cells and basophils. However, the relationship between the effectiveness of AIT and the induction of allergen-specific IgG subtypes remains a controversial issue; an increase in serum concentrations of allergen-specific IgG4 is correlated with clinical improvement.

When allergen-specific IgG is directed against the same epitopes that induce IgE, it results in direct competition for allergen binding, leading to the desired blocking effect. However, if IgG is induced against other epitopes that do not cause allergy in the patient, it may fail to effectively compete with IgE, even when present in excess. The concept of blocking antibodies has recently been reevaluated. Analysis of induced IgG subtypes by AIT showed specific increases in IgG1 and IgG4, particularly with levels increasing from 10 to 100 times. Additionally, IgA induction and suppression of mast cells, basophils, and eosinophils also occurred. The results suggest that successful AIT is associated with an increase in blocking IgG activity, which does not solely depend on the quantity of IgG antibodies. It appears to be more relevant to assess allergen-specific IgG blocking activity or subtypes of IgG, particularly IgG4 and IgG1, rather than just their levels in the serum [35, 48].

Pre-treatment with anti-IgE monoclonal antibodies (mAb) can enhance the safety of AIT for allergic rhinitis and serve as an effective strategy to allow more rapid and concentrated doses of allergens in Immunotherapy. The non-inflammatory role of IgG4 can be explained by unique structural features in the hinge region of IgG4, resulting in lower affinity for Fcγ receptors. Additionally, IgG4 does not activate complement, does not cause tissue damage, and can inhibit the formation of immune complexes by other immunoglobulin isotypes, demonstrating anti-inflammatory characteristics [57, 58]. Studies on AIT mechanisms should be conducted with individual and specific allergens. IgG4 antibodies can be considered markers of the ideal allergen dose used in AIT and have the capacity to modulate the immune response, influencing the clinical response to the allergen.

1.6.4. The Role of Interleukin 10 (IL-10) in ATI

In the healthy immune response of a non-atopic individual to the main allergen, Der p1, from the Dermatophagoides mite, there is an increase in specific IgA and IgG4, small amounts of IgG1, and nearly undetectable levels of IgE antibodies in the serum. The increase in serum-specific IgA and IgG4 coincides with the increase in serum levels of TGF-β and IL-10, respectively, which could explain the role of IgA and TGF-β, as well as IgG4 and IL-10, in peripheral mucosal immune responses to allergens in healthy individuals. In AIT for mites, there is no decrease in IgE levels even after seventy days of treatment; however, there is a significant and specific increase in IgA, IgG1, and IgG4. Apparently, specific immunotherapy for mites or other allergens induces an increase in allergen-specific IgG antibodies, which can block IgE recognition of allergens.

IL-10 reduces pro-inflammatory cytokines released by mast cells, negatively regulates the functional activity of eosinophils, and suppresses the production of IL-5 by resting human TH0 cells and TH2 cells. Additionally, IL-10 reduces mast cell density and degranulation, thereby limiting their growth and resulting in a decrease in the release of pro-inflammatory substances from their granules. It also decreases the concentration of local histamine, suppresses the growth of mast cells, and prevents their degranulation [59, 60].

Allergen Specific immunotherapy (AIT), when used for a long time, is associated with a reduction in both the immediate response and the late-phase reaction (LPR) to the allergen. The immediate response is primarily mediated by mast cells, while the LPR involves the recruitment, activation, and increased half-life of eosinophils and activated T cells at the sites of allergen exposure. Successful AIT increases the concentration of allergen required to induce both the immediate and LPR responses in the target tissues. Additionally, AIT decreases the responses to nonspecific stimuli [61]. Bronchial,

nasal, and conjunctival hyperreactivity to non-specific stimuli reflects inflammation in the underlying mucosa, which decreases after AIT and correlates with clinical improvement.

1.7. Effects of Specific Allergen Immunotherapy (AIT) on Clinical and Immunological Parameters

Some key effects of AIT on clinical and immunological parameters can be resumed as: a) Clinical Improvement: AIT has been shown to significantly reduce the symptoms associated with allergic conditions. Patients often experience decreased nasal congestion, sneezing, itching, and improved lung function in the case of allergic asthma. The clinical improvement is usually evident after a few months of AIT, and the benefits can persist even after the treatment is completed [62] b) Decrease in Medication Use: As AIT helps control allergic symptoms, patients may need fewer medications to manage their allergies. This reduction in medication use can lead to cost savings and a better quality of life. c) Long-term Efficacy: AIT can provide long-lasting effects even after the treatment is completed. Some studies have shown that the benefits of AIT can persist for several years, providing sustained relief from allergy symptoms; d) Modulation of the Immune Response: AIT induces a shift in the immune response from an allergic Th2-type response to a more tolerogenic Th1-type response. This shift is associated with a decrease in the production of allergy-promoting cytokines (such as IL-4, IL-5, and IL-13) and an increase in regulatory cytokines (such as IL-10 and TGF-β), promoting immune tolerance to the allergen, e) Changes in Allergen-specific Antibodies: AIT can lead to changes in the production of allergen-specific antibodies. The levels of allergen-specificIgE, which are responsible for allergic reactions, may decrease over time. In contrast, there is often an increase in allergen-specific IgG4, which is associated with a protective effect against allergic reactions [52].

AIT can have various effects on different immune cells involved in the allergic response. However, although these effects can vary depending on several factors, in general, they can be summarized as: a) Basophils and Mast Cells: AIT can lead to a decrease in the activation and degranulation of basophils and mast cells. As a result, there is a reduced release of pro-inflammatory mediators, such as histamine and leukotrienes, which are responsible for causing allergy symptoms like itching, sneezing, and swelling; b) Eosinophils: AIT has been shown to reduce the recruitment and activation of eosinophils, which are key cells involved in allergic inflammation. This leads to a decrease in eosinophilic inflammation and tissue damage, particularly in conditions like allergic asthma and eosinophilic esophagitis; c) T Cells: AIT can promote the development of regulatory T cells (Tregs), which play a crucial role in immune tolerance. Tregs help control and dampen the allergic response by suppressing the activation of other immune cells, such as Th2 cells, which are responsible for promoting allergy. By increasing Treg populations, AIT helps maintain immune balance and prevent excessive allergic reactions; d) B Cells: AIT can result in changes in B cell responses to allergens. Specifically, there may be a decrease in the production of allergen-specific IgE antibodies, which are central to allergic reactions. At the same time, AIT may promote an increase in allergen-specific IgG4 antibodies, which are thought to have a protective effect against allergies; e) Dendritic Cells: AIT can influence dendritic cell function, leading to a shift in their ability to present allergens to T cells. This altered dendritic cell presentation may contribute to the promotion of tolerance and reduced allergic responses; f) Monocytes: AIT can impact the function of monocytes, which are precursors to macrophages. Monocytes play a role in inflammation, and AIT may help reduce their pro-inflammatory properties, contributing to decreased allergic inflammation. It's important to note that the effects of AIT on these immune cells may vary depending on the individual's specific allergic condition, the allergen being targeted, and the duration of treatment. AIT is a personalized therapy, and its mechanisms of action can be complex and multifaceted. These immune cell changes collectively contribute to the overall clinical improvement and tolerance development observed in patients undergoing AIT for allergic conditions [63].

In this context, the assessment of the efficacy and safety of allergen immunotherapy (AIT) is typically monitored through clinical evaluations of patients in vivo at various time points. This monitoring involves the use of instruments to measure comorbidity symptom scores (CMSS), administer the rhinosinusitis quality of life questionnaire (RQLQ), and employ visual analog scale (VAS) questionnaires to assess potential symptom modifications. Additionally, the investigation of immunological changes induced by AIT is carried out by monitoring biomarkers of response to ongoing AIT [64]. This in vitro assessment involves the identification and measurement of various biomarkers, such as basophil activation (BAT) for the identification of basophil activation markers, enzyme-linked immunosorbent assay (ELISA) for serum IL-10 levels, and allergen-specific IgE. Notably, a new method called the Allergy Explorer (ALEX) [65], which comprises a microarray containing both extracted 'whole' allergens and molecular components, is now available for the identification of allergen-specific IgE. In certain circumstances, nasal cytology is also performed at different stages of AIT to identify inflammatory nasal cells using a semi quantitative grading system [66].

1.8. Non - Injectable Immunotherapy

The AIT can be administered in various ways through several routes, injectable, including intravenous (IV) and Subcutaneous Immunotherapy (SCIT) or non-injectable, oral, pills, capsules or drops (OIT) [67, 68], topical (cream),

intravesical (directly into the bladder), sublingual (SLIT) [69, 70] bronchial (LBIT): aerosolized material is vacuumed and nasal (LNIT) [14, 71 -73].

Until the 1970s, non-injectable immunotherapy was rarely used, but systematic studies of the oral and nasal routes were conducted during that period. In 1986, the American Academy of Allergy Asthma & Immunology and the British Committee for Safety of Medicines reported deaths caused by subcutaneous immunotherapy (SCIT), which raised concerns about the safety of this form of immunotherapy and sparked increased interest in non-injectable routes [13]**.** Sublingual immunotherapy (SLIT) was initially introduced in 1986 and later reintroduced in 1993. The European Academy of Allergy Asthma & Immunology (EAACI) called for more research into non-injectable routes, and in 1998, the World Health Organization (WHO) declared the Sublingual immunotherapy (SLIT) and local nasal immunotherapy LNIT (nasal) as viable alternatives to subcutaneous immunotherapy (SCIT) [74].

1.8.1. The Sublingual Immunotherapy

Indeed, the original concept behind sublingual immunotherapy (SLIT) was to achieve rapid absorption of the allergen vaccine through the sublingual mucosa (under the tongue) to bypass possible degradation in the gastrointestinal tract. However, research has shown that there is limited direct absorption of the allergen extracts through the sublingual mucosa. [75] Contrary to the initial hypothesis, studies have indicated that the sublingual route does not provide significant systemic absorption of the allergens. Instead, the immune response is primarily localized in the mucosa of the mouth and throat, where the allergens meet the immune cells. Even though SLIT does not lead to significant systemic absorption, it still induces changes in the immune system that result in immune tolerance to the allergen. The allergen exposure through the sublingual route interacts with dendritic cells and other immune cells in the oral mucosa, leading to the activation of regulatory T cells (Tregs) and a shift in the immune response from a Th2 pro-allergic response to a more tolerogenic Th1/Treg response. This shift ultimately results in a reduction of allergy symptoms and an improvement in clinical outcomes. So, while the direct systemic absorption of allergens through SLIT is limited, the localized immunomodulatory effects in the oral mucosa play a crucial role in achieving the therapeutic benefits of immunotherapy for allergies.

Sublingual Immunotherapy (SLIT) is generally considered a safe and well-tolerated treatment for allergic conditions, particularly allergic rhinitis and allergic asthma particularly in reducing symptoms and medication usage in allergic individuals [76].Most adverse effects reported in the literature are mild and localized to the site of administration (sublingual/oral mucosa). The most frequent adverse effect is post-dose sublingual or oral pruritus, which means itching in the mouth or under the tongue. This effect is generally mild and transient, resolving on its own without the need for intervention. The incidence and severity of adverse effects with SLIT are typically lower compared to subcutaneous immunotherapy (SCIT), which involves injections. High doses of antigens used in SLIT could increase the risk of gastrointestinal side effects. Studies have shown that when very high doses of allergens are administered in SLIT, there is a higher likelihood of experiencing gastrointestinal symptoms such as abdominal pain, nausea, and gastrointestinal discomfort. It's essential for healthcare providers to carefully select the appropriate dose and titration regimen to minimize the risk of adverse effects while maximizing the treatment's efficacy. SLIT is favored by many patients due to its non-invasive nature, ease of administration, and good safety profile. Today, SLIT is available in the form of tablets (SLIT tablets) and liquid formulations (SLIT drops). In general, SLIT tablets are currently available as freeze-dried or compressed formulations of allergen extracts [7, 77] and SLIT drops are available as aqueous solutions of allergen extract, typically formulated with glycerin [78] Both SLIT tablets and drops are administered under the tongue and held there until swallowed or spit out.

1.8.2. Immunological Aspects of Local Immunotherapy

The immunological effects of local immunotherapy (IT), which includes sublingual immunotherapy (SLIT) and intranasal immunotherapy (LNIT), can differ from those of subcutaneous immunotherapy (SCIT) in several aspects such as: a) the Target immune response; b) site of administration; c) immunomodulatory effects; d) absorption and systemic effects and e) safety profile [23, 79].

Local immunotherapy (SLIT and LNIT) primarily targets the mucosal immune system in the oral cavity (for SLIT) or the nasal passages (for **L**NIT). On the other hand, subcutaneous immunotherapy (SCIT) targets the systemic immune response. Because of local administration, directly to the mucosal surfaces, the allergens meet the immune cells of the oral or nasal mucosa inducing a more localized immune response in the oral or nasal mucosa. This leads to the activation of regulatory T cells (Tregs) and a shift in the immune response from a pro-allergic Th2 response to a more tolerogenic Th1/Treg response.

In terms of absorption and systemic effects, in local immunotherapy, there is limited direct systemic absorption of the allergens. The immune response is primarily localized to the mucosal tissues of the mouth or nose, resulting in a reduction of allergic symptoms in those areas. SCIT, however, involves systemic absorption of the allergens, leading to a more widespread immune response and potential effects beyond the targeted site. Finally, comparing the safety profile of Local immunotherapy (SLIT and LNIT) versus SCIT, local immunotherapy is considered safer since it reduces the risk of severe systemic reactions, making it a preferred option for patients who may be at higher risk for anaphylactic reactions. According to Bandeira LW, there is absorption in the nasal mucosa, and this pathway could even be considered for asthma [14].

1.8.3. Intralymphatic immunotherapy (ILIT)

Intralymphatic allergen administration is a relatively new approach in the field of immunotherapy and is still considered experimental. It involves the direct injection of allergen extracts into the lymph nodes, aiming to induce a more targeted and potent immune response. [80 - 84].

The concept behind Intralymphatic administration is to deliver the allergen directly to the lymph nodes, where immune cells are activated, and the immune response is initiated. By targeting the lymph nodes, the hope is to achieve a more robust and specific immune tolerance to the allergen, potentially leading to faster and longer-lasting results compared to traditional routes like subcutaneous or sublingual immunotherapy [85]. However, Intralymph node injection is still in the early stages of research and clinical evaluation.

There are several ongoing and already finished clinical trials and studies investigating its safety and efficacy compared to conventional AIT methods. Some authors describe ILIT as a potential alternative to overcome the challenges of long-term subcutaneous immunotherapy (SCIT) and sublingual immunotherapy (SLIT). ILIT requires only three ultrasound-guided injections into inguinal lymph nodes over a two-month period, which is significantly fewer injections compared to SCIT. Thus, ILIT would reduce the resource overhead for patients, physicians, and reimbursement parties, and may improve treatment adherence [5, 86]

A recent systematic review and meta-analysis carried out by Wang W et al, [87] concluded that ILIT treatment for Allergic Rhinoconjunctivitis (AR) is safe and effective with high compliance to SCIT. On the contrary, Aini NR et al, 2021, also in a systematic review and meta-analysis had already shown that although ILIT could be safe, it was not effective for AR treatment. Further studies and trials with a larger number of patients are necessary to finally confirm finds to date [88].

1.9. Epicutaneous Immunotherapy

Epicutaneous Immunotherapy (EPIT) is an emerging approach in the field of allergen immunotherapy that involves the administration of allergens through the skin using a specialized patch or patch-based delivery system. The goal of EPIT is to induce immune tolerance to specific allergens and reduce allergic symptoms in individuals with allergic conditions such as allergic rhinitis, allergic asthma, and food allergies [89 - 92].

The EPIT works by gradually exposing the immune system to small amounts of allergens through the skin. The specialized patch contains allergen extracts that come into contact with immune cells in the skin's outermost layer. This exposure aims to modulate the immune response, shifting it from a pro-allergic Th2 response to a more balanced and tolerant response. The mechanisms of action underlying EPIT are not fully understood, and additional research is needed to fully elucidate the intricate processes underlying EPIT's effects on the immune system.

The effectiveness of EPIT can vary based on factors such as the specific allergen, the dosage used, and individual patient responses. However, the literature data suggests several keyways in which it influences the immune system and induces tolerance to allergens. Here we summarize these possible mechanisms as: a) Local Immune Modulation:The patch delivers allergen extracts to the outermost layer of the skin, where it interacts with immune cells known as Langerhans cells and dendritic cells, epithelial cells, Keratinocytes and Type 2 Innate Lymphoid Cells (ILC2s). These cells play a role in presenting antigens to the immune system and determining the type of immune response. The local exposure to allergens is thought to trigger a shift in the immune response from a pro-allergic Th2 response to a more balanced Th1/Treg response. This shift promotes immune tolerance and reduces the allergic response to the allergen [93, 94]; b) Induction of Regulatory T Cells (Tregs): EPIT is believed to induce the development of regulatory T cells (Tregs), which are immune cells responsible for suppressing excessiveimmune responses. Tregs play a crucial role in maintaining immune balance and preventing overreactions to harmless substances. The presence of Tregs is associated with a reduced Th2-driven allergic response [95 – 97]; c) Blocking Antibodies (IgG4): EPIT may lead to an increase in allergen-specific IgG4 antibodies. The IgG4 antibodies are thought to compete with allergen-specific IgE antibodies, which are

responsible for initiating allergic reactions. IgG4 antibodies can neutralize the allergen and prevent it from triggering an allergic cascade [98]; d) Local Cytokine Production: The skin's immune response involves the production of various cytokines. EPIT may lead to the secretion of anti-inflammatory cytokines such as IL-10 and TGF-β, which further contribute to the development of immune tolerance and suppression of allergic responses [99] and ILC2s produce cytokines, including IL-33, IL-22, and IL-31. e) Mast Cell Modulation: Mast cells are immune cells involved in allergic reactions. EPIT might help modulate mast cell behavior, reducing their tendency to release pro-inflammatory mediators like histamine upon allergen exposure [100]; f) Changes in Dendritic Cell Activity: Dendritic cells are key players in shaping the immune response. EPIT might influence dendritic cells' ability to present allergens to immune cells, steering the response toward tolerance [101]; EPIT offers potential benefits, including being a non-invasive approach compared to other immunotherapy methods like injections. This can make it more appealing, especially to pediatric patients. EPIT also provides a reduced risk of systemic reactions. Because it focuses on the skin, the likelihood of systemic allergic reactions is generally lower compared to methods that involve the direct injection of allergens. Additionally, due to its patch-based delivery system, EPIT allows for convenient at-home administration, reducing the necessity for frequent clinic visits.

1.9.1. Clinical Trials and Research:

Several clinical trials and research studies have been conducted to evaluate the safety and efficacy of EPIT for various allergens, including pollen, dust mites, and food allergens. These studies have shown promising results in terms of reducing allergic symptoms and improving patients' quality of life [102, 103].As a result, regarding the regulatory status, EPIT has been approved for certain allergens by regulatory authorities in some regions but, the availability of EPIT may vary depending on the specific allergen and the region. EPIT is not suitable for all allergens and conditions.

1.10. Local Nasal Immunotherapy – (LNIT)

Local Nasal Immunotherapy (LNIT) is an alternative Non injection immunotherapy method, that entails directly administering allergen extracts into the nasal passages. Like Sublingual Immunotherapy (SLIT) and Subcutaneous Immunotherapy (SCIT), LNIT aims to induce immune tolerance to allergens and alleviate allergic symptoms in individuals with allergic rhinitis (nasal allergies) and related conditions. The concept of intranasal immunotherapy was first proposed by Dunbar, WP in 1913 [104]. However, it wasn't until the 1970s that the use of the intranasal route for immunotherapy began to be more extensively investigated [105, 106].

The suppression of IgE responses following antigen inhalation was published by Patrick G. Holt et al. in 1987 [107]. The authors suggested the existence of a back-up immune mechanism which prevents sensitization to antigens which cross epithelial barriers.

The first indication that passive antigenic stimulation of the respiratory mucosa may serve a protective function came from the induction of DTH following intratracheal administration of metal salts to guinea pigs. This procedure induced a state of specific immunological unresponsiveness. In subsequent studies with mice and rats showed that repeated inhalation of aerosols containing ovalbumin or ragweed antigen 6 induced transient IgE responses which spontaneously switched off despite continuing aerosol exposure. Studies from this laboratory, employing the ELISA-plaque assay for the enumeration of IgE-secreting cells have also established that the same respiratory tract regional lymph nodes contain many IgE-secreting cells and that these lymph nodes are also the site in which IgE production is initiated in response to antigen inhalation [108]. Suggesting that the downregulation of potentially pathogenic responses to inhalant allergens occur not within central lymphoid organs such as the spleen, but rather within the lymph nodes which drain the respiratory mucosa.

In LNIT, allergen extracts are administered directly into the nasal passages using various approaches, such as drops, sprays, gels, or tablets. As the nasal mucosa serves as the gateway for pathogens and contains various lymphoid organs, LNIT has emerged as a favorable alternative for inducing immune tolerance. It has shown promising results in reducing symptoms and the need for medication in patients with allergic rhinitis.

The allergen extracts delivered through LNIT meet immune cells in the nasal mucosa, including dendritic cells and T cells. This exposure triggers a controlled immune response, leading to the development of regulatory T cells (Tregs) and a shift from a pro-allergic Th2 response to a more balanced and tolerant immune response.

Despite challenges such as difficulties in adjusting antigen dosage, local reactions, and mucosal distribution, which have made this approach less popular, LNIT has continued to be a subject of study. In fact, in 1998, LNIT was considered by the World Health Organization (WHO), along with SLIT, as one of the only viable alternatives to therapy via injection [109]. In the early 2000s, a new approach has been attempted with local nasal immunotherapy using allergen-coated

strips of mite placed in the nose cavity [110]. The authors speculated that this would be a better alternative in reducing nasal reactions and avoiding difficulties in application for dry powder or solution. Patients with dust mite-induced allergic rhinitis were recruited to receive four months of local nasal immunotherapy and were instructed to place the mite-coated strips over the septum nose for 10 minutes each week for four months. The treatment resulted in a decrease in sneezing, runny nose, nasal congestion when compared with the placebo reinforcing the use of LNIT.

Recent advances in intranasal drug delivery systems warrant re-examination of LNIT as a viable option for the treatment of the allergic airway disease [111]. In a recent review and meta-analysis by Kasemsuk N et al. (2022), after analyzing 20 studies involving 698 participants, the authors concluded that the LNIT group exhibited greater post-treatment improvements in the total nasal symptom score (TNSS), symptom-medication score (SMS), and medication score compared to the control (placebo) group [112], important parameters to be evaluated for, in general, determining efficacy of AIT.

1.11. Biodistribution of Radiolabeled Allergen in LNIT (Nasal)

Several studies have focused on understanding the distribution of allergen in LNIT. After nasal application, dynamic scintigraphy showed that a considerable fraction of the radioactivity in the nasal cavities shifted towards the upper pharynx, likely due to mucociliary clearance, and then into the esophagus, stomach, and intestines. Plasma radioactivity began to increase in the initial minutes. No radioactive accumulation was observed in the bronchial tree, minimizing the risk of asthma attacks. Persistent presence of the radioactive allergen in the nasal mucosa was noted for up to 40 hours like SLIT (sublingual immunotherapy), with relatively high radioactivity (approximately 10% of the administered dose). The locally retained fraction is higher than that absorbed in the intestinal tract, providing an optimal efficacy/safety ratio and a more convenient allergen introduction route.

Clinical trials and research conducted over the past 15 years have demonstrated the clinical efficacy and safety of SLIT (sublingual) and LNIT (nasal). The safety and clinical efficacy of LNIT suggest that this therapy could be beneficial in treating allergic rhinitis in adults, and particularly in children. Several research on LNIT are still ongoing, with efforts to optimize dosing regimens, improve allergen extracts, and better understand its mechanisms of action. Although the optimal allergen dosage has not yet been determined. Studies indicate that it varies by up to 375 times that of SCIT (subcutaneous immunotherapy), and there is no significant data suggesting a specific dose as the best option. Likewise, there is no evidence that efficacy is dependent on the dose (28, 31-34).

Recently, Bandeira LW et al. [14], have published the results of a retrospective five-years use LNIT in patients with perennial rhinitis in Rio de Janeiro, Brazil. According to the authors the LNIT performed with full concentrations of the allergen, did not show secondary reactions with risks to patients and the effect of inducing tolerance to the antigens of *Dermatophagoides sp*. was achieved, based on the observation of the decrease in the use of control medications for signs and symptoms and mainly by the improvement in the patients' quality of life, without adverse effects. In another study, Sacarpa A et al., 2023, used LNIT for patients with allergic rhinitis (allergy to mites, to Graminaceae or Parietaria pollen) from Salerno, Italy [113]. It was shown that LNIT is a valid alternative to the most used local routes of AIT (subcutaneous and sublingual administration). Additionally, administration is easy, and both, systemic and local reactions, were few and mild. These results show the safety and efficacy of LNIT in different patient populations across the globe and for different allergens.

2. Conclusion

Immunology provides the scientific framework for Allergen Immunotherapy by elucidating the mechanisms underlying allergic responses, immune tolerance, and immunomodulation. This understanding allows clinicians and researchers to design effective AIT strategies, monitor treatment outcomes, and ultimately improve the quality of life for individuals suffering from allergic diseases.

Compliance with ethical standards

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