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Modulating Th2 Cell Immunity for the Treatment of Asthma

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It is estimated that more than 339 million people worldwide suffer from asthma. The leading cause of asthma development is the breakdown of immune tolerance to inhaled allergens, prompting the immune system's aberrant activation. During the early phase, also known as the sensitization phase, allergen-specific T cells are activated and become central players in orchestrating the subsequent development of allergic asthma following secondary exposure to the same allergens. It is well-established that allergen-specific T helper 2 (Th2) cells play central roles in developing allergic asthma. As such, 80% of children and 60% of adult asthma cases are linked to an unwarranted Th2 cell response against respiratory allergens. Thus, targeting essential components of Th2-type inflammation using neutralizing antibodies against key Th2 modulators has recently become an attractive option for asthmatic patients with moderate to severe symptoms. In addition to directly targeting Th2 mediators, allergen immunotherapy, also known as desensitization, is focused on redirecting the allergen-specific T cells response from a Th2-type profile to a tolerogenic one. This review highlights the current understanding of the heterogeneity of the Th2 cell compartment, their contribution to allergen-induced airway inflammation, and the therapies targeting the Th2 cell pathway in asthma. Further, we discuss available new leads for successful targeting pulmonary Th2 cell responses for future therapeutics.

Keywords: Th2 airway inflammation, Tfh cell, asthma, cytokines, T cell

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INTRODUCTION

Asthma is a chronic lung disease characterized by breathing problems and obstructed airflow when airways swell and narrow and produce excess mucus (1). Allergic asthma is the most common form of asthma and is caused by the inhalation of allergens, which trigger the overreaction of the immune system in allergic people (1). The most common airborne allergens are pollen, fungal spores, house dust mites (HDM), and animal allergens. The characteristic pattern of inflammation in the airways of patients with allergic asthma includes the production of T helper 2 (Th2)-associated cytokines, such as interleukin- (IL-) 4, IL-13, and IL-5 by Th2 cells and type 2 innate lymphoid cells (ILC2s), the activation of mast cells, the infiltration and activation of eosinophils, and the increased production of immunoglobulin E (IgE) by B cells (2). Clinical and preclinical studies demonstrate a strong cause and effect relationship between the aberrant expansion of allergen-specific Th2 $CD4^+$ T cells and the development of asthma pathogenesis, thus leading to the idea that Th2 cells play a central role in allergic asthma (1, 2).

The development of allergic asthma occurs in two phases (1, 3). Phase one requires an initial exposure to allergen or "*sensitization*" that does not necessarily cause symptoms or pathology. Phase two is characterized by pathology development following secondary or subsequent allergen exposures or "*challenges*." Initial sensitization to airborne allergens occurs typically in early childhood, and it is characterized by the initial priming of allergen-specific $CD4^+$ T cells with a Th2-like cytokine profile. These T cells persist after the initial priming and can be subsequently reactivated upon re-exposure with the same inhaled allergen, which caused their migration to the airways, where they locally produce Th2 cytokines. The accumulation of effector Th2 cells in the lungs ultimately stimulates the hallmark features of asthma, such as mucus hypersecretion and bronchial hyperresponsiveness (1).

Most patients with asthma achieve good disease control with the principal use of inhaled corticosteroids and bronchodilators (4, 5). However, a large proportion of patients with asthma remain poorly controlled (6). The failure of conventional therapies in these corticosteroid-resistant patients justifies looking for new approaches to treat allergic asthma. In this regard, the central role of Th2 cells in regulating airway inflammation has aroused great interest in the therapeutic potential of *"anti-Th2 approaches."* As such, new biological asthma medications based on monoclonal antibodies against key Th2 mediators have been recently approved, and more are being under investigation (7). Furthermore, allergen immunotherapy, a long-term treatment that inhibits Th2-cell-mediated responses, decreases symptoms for many people with allergy disease (8), thereby evidencing the central pathogenic role of Th2 cells in the pathophysiology of allergy.

Here, we will review the available treatments for allergic asthma and discuss the potential immunological mechanisms underlying the clinical benefits of these therapies. Finally, recent studies provide evidence of a critical function of T follicular helper (Tfh) cells, a subset of $CD4^+$ T cells that help GC B cell responses, in the allergic asthma pathogenesis. Therefore, we will discuss potential therapeutic approaches to target Tfh cells and suppress IgE responses and Th2 cell-mediated allergic inflammation in asthmatic patients.

PATHOGENIC ROLES OF Th2 CYTOKINES IN ALLERGIC ASTHMA

Eighty percentage of children and 60% of adults with asthma have type 2/Th2 asthma (9), which is driven by allergen-induced production of IgE and Th2 cytokines, including IL-5, IL-13, and IL-4 (**Figure 1**). Studies in mice, initially using OVA adjuvant and adjuvant-free sensitization protocols and most recently, using natural allergens such as HDM, cockroaches, sensitizing fungi, and protease allergens, have demarcated our knowledge on Th2 cytokines in asthma. For example, IL-4 produced by T cells drives IgE class switching (10–15) and, in conjunction with IL-13, is required to produce high-affinity IgE (16). IgE mediates mast cell and basophil degranulation by FcεRI crosslinking upon allergen recognition (17–19). Activation of FcεRI results in the immediate release of preformed granular substances (e.g., histamine, heparin, and proteases) and the production of inflammatory mediators, such as cytokines and arachidonic acid metabolites. This activation drives edema, mucus hypersecretion, and bronchial hyperresponsiveness, all accompanied by a drop in airflow in the airways. In some cases, activation of FcεRI can develop into a life-threatening systemic reaction called anaphylaxis (20).

In addition to regulating IgE production, IL-13 and IL-4 are implicated in cardinal features of asthma, such as extravasation and trafficking of eosinophils into the tissue (21–27), goblet cell maturation, mucus secretion (28), bronchial hyperresponsiveness (28, 29), and tissue remodeling (30).

IL-5 is responsible for the maturation of eosinophils in the bone marrow and their release into the blood (31). As such, IL-5 production in the airways favors the production, accumulation, and activation of eosinophils in the lung (32), and ultimately, the release of a large number of mediators capable of inducing bronchial hyperresponsiveness, mucus hypersecretion via enhanced differentiation of goblet cells (33–36) and, airway remodeling (37, 38).

Although ILC2s and other cells can also contribute to Th2 cytokines production, IL-4, IL-13, and IL-5 are principally produced by Th2 cells during ongoing chronic asthmatic responses. Given the pathogenic role of Th2 cells and Th2 cytokines, treatments for patients with type 2/Th2 asthma are directed to globally suppress Th2-mediated inflammation or to specifically target the most pathogenic effector functions of the various Th2 cytokines or the IgE response.

CONVENTIONAL TREATMENTS THAT TARGET Th2-TYPE INFLAMMATION IN ASTHMA

Inhaled corticosteroids (ICS) are the most effective and commonly used long-term control drugs for asthma (4, 5). They locally suppress many aspects of Th2 cell-mediated inflammation, including Th2 cytokines (IL-4, IL-13, IL-5) epithelium-derived cytokines (TSLP, IL-33), chemotactic chemokines (IL-8, RANTES, MIP-1α, eotaxin, CCR2), and adhesion molecules (ICAM-1, VCAM-1) (4, 5, 39–41). Globally, ICS reduce the recruitment and maintenance of inflammatory cells into the airways of asthmatic patients, including dendritic cells, Th2 cells, eosinophils, and mast cells. Mechanistically, ICS suppress the production of chemotactic mediators, prevent the expression of adhesion molecules, and inhibit the survival of inflammatory cells in the airways (4, 5).

ICS mediate their effects through the glucocorticoid receptor (GR), an intracellular receptor and transcription factor belonging to the nuclear receptor family (39). In the absence of the ligands, GR is maintained in the cytoplasm by chaperone proteins. Upon ligand binding, GR becomes active and translocates into the nucleus to bind glucocorticoid response elements (GREs), thereby regulating the transcription of GR target genes. GR dimers and monomers can induce either transcriptional gene induction or gene repression (39, 42–44). Besides, GR

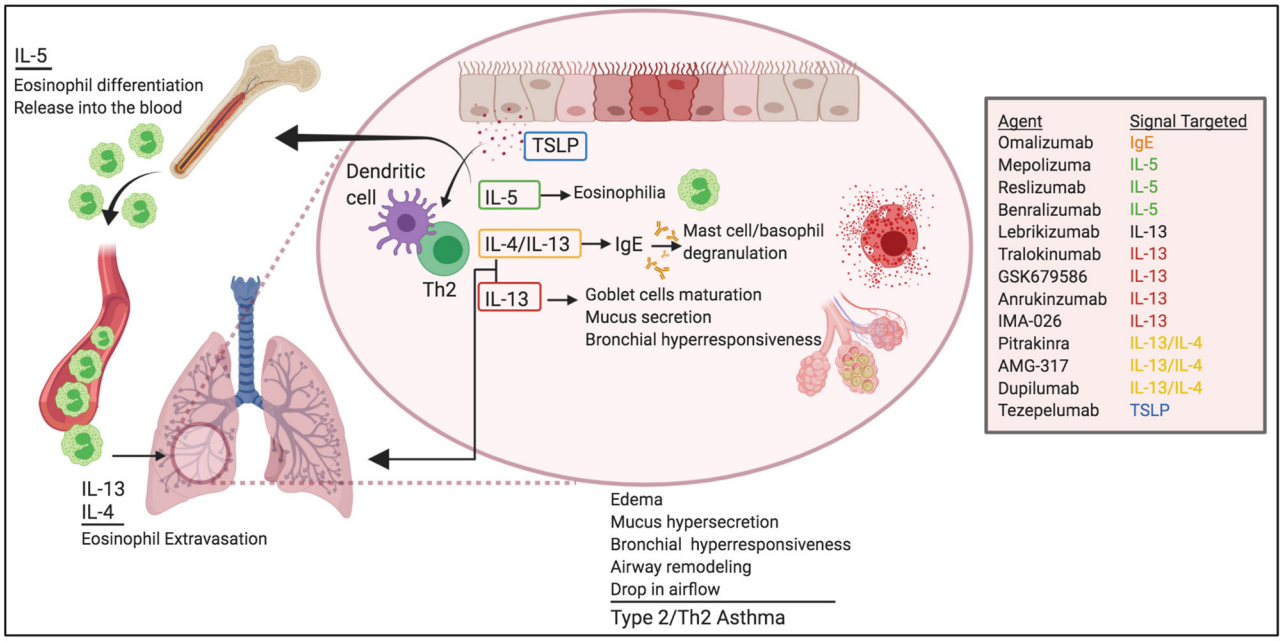

FIGURE 1 | Pathogenic roles of Th2 cytokines in allergic asthma. Th2 cytokines play critical roles in asthma pathogenesis. IL-5 promotes eosinophil egress from the BM and help survival in the lungs. IL-4 and IL-13 induce eosinophil extravasation from the blood into the tissue and promote IgE class-switching. IL-13 favors globet cell maturation, mucus secretion, and airway hyperresponsiveness. TSLP helps Th2 differentiation and DC function. Altogether, these effects lead to edema, mucus hypersecretion, bronchial hyperresponsiveness and remodeling, and ultimately drop in air-flow and asthma. Novel biological therapeutics target these pathways for the treatment of asthma.

can indirectly induce gene repression by GR interaction with DNA-bound transcription factors such as NF-κB and activator protein-1 (AP-1), resulting in the repression of the respective inflammatory signaling cascades (39, 45, 46).

The wide range of anti-inflammatory effects of ICS probably accounts for their clinical effectiveness in managing type 2/Th2-asthma. Regular treatment with ICS (alone or in combination with bronchodilators, such as long-acting β2 agonists (LABAs) or Theophylline) can effectively control chronic symptoms and prevent asthma attacks in most of the patients (4, 5). However, in patients with moderate to severe asthma, ICS are less effective. Hence, unacceptably high doses of ICS or even oral corticosteroids may be required to achieve optimal control.

Several mechanisms can contribute to the reduced responsiveness to ICS in moderate/severe asthma [for a review, see (6)]. For example, cytokines such as IL-1, TNFα, nitric oxide (NO), IL-13, and IL-4, which are overexpressed in the airways of patients with corticosteroid-resistant asthma, have been shown to reduce GR nuclear translocation and function. Ultimately, people with severe asthma are refractory to ICS treatment and experience poor symptom control. Additionally, these patients can have frequent asthma exacerbations, in which symptoms flare-up and get progressively worse, leading to respiratory failure. Therefore, new treatments have emerged for selected patients with moderate to severe type 2/Th2 asthma disease and inadequate responsiveness to ICS. These new therapeutic avenues are aimed to target cytokines and mediators that promote type 2/Th2 immunity.

Biologic Drugs That Target Th2-Type Inflammation in Asthma

The clinical characteristics of moderate/severe asthma disease are frequent asthma exacerbations (>2 episodes in 12 months period), high blood counts of eosinophils and sputum eosinophils, and poor response to high dosage ICS/ LABAs (47). These uncontrolled symptoms place patients at high risk for hospitalization and reduced health-related quality of life. Therefore, additional therapeutics are needed for those patients whose severe asthma does not respond well to conventional anti-inflammatory treatment. Several biologics designed to target specific mediators of Th2-type cell immunity have been proved to be effective as add-on treatments for severe asthma patients (**Figure 1**).

Anti-IgE Therapy in Severe Asthma: Omalizumab

Omalizumab is a humanized IgG1 monoclonal antibody that specifically binds to free IgE and prevents it from binding to the high-affinity IgE receptor (FcεRI) on basophils and mast cells. As Omalizumab depletes free IgE, it further promotes FcεRI down-regulation in basophils and mast cells, rendering those cells much less sensitive to stimulation by allergens and consequent degranulation (48–50).

Omalizumab is given by subcutaneous injection every 2–4 weeks. It is FDA-approved to treat moderate-to-severe asthma in patients over 6 years of age that have sensitivity to perennial aeroallergens (e.g., dust mites, pet dander, cockroach debris). The appropriate doses are determined on a combination of age,

IgE levels, and body weight. In clinical trials and observational studies with moderate to severe persistent asthma patients, Omalizumab has been shown to decrease the incidence of asthma exacerbations and emergency visits by 38 and 47%, respectively, compared with controls (50).

Some potential adverse reactions have been described related to long-term effects on cardiovascular and cerebrovascular events. However, the available studies limit the ability to quantify the magnitude of the risk (50, 51). Omalizumab has also been associated with life-threatening systemic allergic anaphylactic reactions; thus, anyone who gets an injection of this drug should be monitored closely by health professionals (50).

Anti-IL-5 Therapy in Severe Asthma: Mepolizumab, Reslizumab, and Benralizumab

Three different biologic drugs targeting IL-5 signaling are available, and FDA-approved. All three treatments have been consistently shown to reduce blood eosinophil counts and sputum eosinophils (47, 52, 53). ***Mepolizumab*** is a humanized IgG1 monoclonal antibody that recognizes and blocks IL-5 and prevents its binding to IL-5 receptor alpha subunit (IL-5Rα or CD125) on the surface of eosinophils. ***Reslizumab*** is a humanized IgG4 monoclonal antibody against IL-5 that likewise prevents IL-5 function in eosinophils. Finally, ***Benralizumab*** also targets IL-5-mediated effects on eosinophils, but in this case, it is via a humanized IgG1 monoclonal antibody directed against IL-5Rα/CD125. Besides, blocking IL-5/IL-5R signaling, Benralizumab induces antibody-mediated eosinophil depletion (54) and as such, very rapid eosinophil reduction in sputum, bone marrow and blood (53).

Targeting the biological activity of IL-5 with Mepolizumab, Reslizumab and Benralizumab reduces asthma exacerbations and life-threatening emergencies in corticosteroid-resistant severe eosinophilic asthma, as well as help minimize corticosteroid use (55–69). However, no consistent benefits have been shown to improve daily asthma symptoms and quality-of-life, pertaining to the use of short-acting bronchodilators, nigh awakenings, or the limitation of activities (55–57, 62, 66, 67, 70). Likewise, targeting IL-5 does not improve asthma control in patients with mild-to-moderate eosinophilia (59, 71–73). Hence, while these findings highlight the importance of eosinophils in the pathogenesis of asthma exacerbations, they also suggest that the inflammatory cues driving the day-to-day symptoms are different from the eosinophil-driven mechanisms responsible for asthma attacks. Therefore, the primary target population for these medications is limited, at best, to patients with moderate-to-severe eosinophilia and a history of frequent exacerbations.

The three current FDA-approved anti-IL-5 therapies have different administration routes and schedules. Mepolizumab is given as an at-home monthly subcutaneous injection and approved as an add-on treatment for patients 6 and older. Reslizumab is a personalized, weight-based intravenous injection given every 4 weeks and approved for use with other asthma medicines in patients aged 18 and older. Due to the risk of an anaphylactic reaction, patients should be observed after drug administration. Benralizumab is an add-on maintenance treatment for patients 12 and older and is administered once every 4–8 weeks by subcutaneous injection. A healthcare professional should oversee Benralizumab administration due to the risk of anaphylaxis.

Anti-IL-13/4 Therapy in Severe Asthma

Due to the central role of IL-13 and IL-4 in controlling critical aspects of asthma pathophysiology, several biologic drugs have been designed to block either IL-13 alone or IL-13 and IL-4 simultaneously. IL-13 signals primarily through the Type-2 IL-4 receptor, which is composed of two chains, IL-13Rα and IL-4Rα IL-4 can signal through both, the Type-2 IL-4 receptor and the Type 1 IL-4 receptor (consisting of IL-4Rα and common γ chain).

IL-13 alone blocking drugs include monoclonal antibodies against IL-13 such as ***Lebrikizumab*** (humanized IgG4), **Tralokinumab** (human IgG4), ***GSK679586*** (humanized IgG1), **Anrukinzumab** (IMA-638; humanized IgG1) and **IMA-026** (humanized IgG1). Simultaneous targeting of IL-4 and IL-13 signaling has been achieved by using a human IL-4 mutein that competes with IL-13 and IL-4 for binding to the IL-4Rα (**Pitrakinra**), and by using monoclonal antibodies against IL-4Rα (**AMG-317,** human IgG2 and **Dupilumab,** human IgG4).

IL-13 blocking agents show evidence of IL-13 pathway inhibition, such as a reduction in biomarkers of Th2/eosinophilic airway inflammation and serum IgE concentration. However, they do not consistently show clinically meaningful improvements in asthma control, pulmonary function, or exacerbations in severe asthma patients (74–83), most likely due to the inability of IL-13 blocking agents to reduce airway eosinophilia in humans significantly (79, 83). Collectively, these results do not support the use of Lebrikizumab, Tralokinumab, GSK679586, Anrukinzumab, and IMA-026 for the treatment of severe asthma.

The biologic activities of IL-14 and IL-13 significantly overlap. Thus, the relatively low efficacy of IL-13 blocking agents is likely due to the capacity of IL-4 and other inflammatory mediators to compensate for the lack of IL-13. Therefore, dual targeting of IL-13 and IL-4 has been suggested as a superior approach to reduce airway eosinophilia and other activities associated with airway inflammation, fibrosis, and mucus production (84). In agreement with this idea, local (inhaled) treatment with Pitrakinra, an IL-4 mutein that simultaneously blocks IL-13 and IL-4 signaling, has shown clinical efficacy in reducing asthma symptoms in a phase 2a study in patients with mild asthma (85). In a later larger study, inhaled Pitrakinra showed significant clinical efficacy in reducing the rate of exacerbations in patients with moderate-to-severe eosinophilic asthma (86). Despite these promising data, further development of Pitrakinra for asthma has ceased.

Additionally, two monoclonal antibodies to IL-4Rα have been developed for the dual inhibition of IL-4/13 signaling (AMG-317 and Dupilumab). AMG-317 displayed relatively poor pharmacokinetics and did not demonstrate clinical efficacy in a clinical trial with moderate-to-severe asthma patients (87). Dupilumab, however, has shown clinical improvements in reducing asthma exacerbations and asthma symptoms and control, as well as lung function in patients with persistent, moderate-to-severe asthma and elevated eosinophil levels (88–91). Besides, Dupilumab appears to have a more significant

effect in improving bronchial hyperreactivity than inhibitors of IL-5 and significantly reduce levels of Th2-associated inflammatory indicators, including markers of eosinophilic airway inflammation and IgE levels (88, 89). IL-4 and IL-13 are essential factors promoting Th2 cell differentiation and class switching into IgE in B cells (1), but at the same time, precluding the differentiation of regulatory T cells (Tregs) (92–95). Therefore, the blockade of the actions of IL-4 and IL-13 with Dupilumab could potentially alter the course of adaptive immune responses to allergens and thus cause a long-term tolerogenic effect. If this is confirmed, Dupilumab could be considered not only a Th2-targeted therapy but an immunomodulatory therapy as well.

Up until now, Dupilumab is the only FDA-approved dual inhibitor of IL-4 and IL-13. It is currently used as an add-on maintenance treatment in patients with moderate-to-severe asthma aged 12 years and older with an eosinophilic phenotype or oral corticosteroid-dependent asthma. It is also approved for inadequately controlled chronic rhinosinusitis with nasal polyposis and atopic dermatitis (96–98). The drug is administered once every 2 weeks by subcutaneous injection and is administered at home or in office.

Interestingly, though Dupilumab decreases bronchial hyperreactivity, serum IgE, and pulmonary eosinophilia, eosinophil counts in blood are elevated (88, 89). This observation is not entirely surprising since, rather than inhibiting eosinophil differentiation, the likely mechanism by which IL-4/IL-13 blockade prevents airway eosinophilia is by precluding eosinophils recruitment from the blood into the tissues (21–27). Notably, IL-5 stimulates eosinophil development, maturation, and egress from bone marrow (31). As a result, anti-IL-5-based therapies significantly reduce eosinophil numbers in both blood and sputum (47, 52, 53). Therefore, combined blockade of multiple Th2-associated cytokines (IL-13, IL-4, and IL-5) may be a better approach to overcome cytokine redundancy and gain full control of asthma symptoms, including exacerbations, lung function, and quality of life, by simultaneous optimization of airway hyper-reactivity, eosinophil, and IgE targeting (99).

Promising New Therapy in Severe Asthma Targeting the Epithelial-Cytokine TSLP: Tezepelumab

The epithelial cell-derived cytokine thymic stromal lymphopoietin (TSLP) has been described as a central regulator of Th2 cell-mediated inflammation in asthma (100–104). Several studies have shown that the airways of asthmatic patients have increased TSLP expression, which correlates with higher Th2 cell response and disease severity (100–103, 105). *In vitro* approaches and *in vivo* animal models have demonstrated that TSLP is released by the barrier epithelium in response to external insults, particularly to allergens with proteolytic activity, such as HDM, cockroaches, ragweed, *Alternaria*, *Aspergillus*, and papain (106–113). Additional preclinical studies demonstrate that the lack of TSLP signaling results in reduced Th2 cell-mediated airway inflammation (106, 114, 115). On the contrary, TSLP overexpression leads to spontaneous Th2 cell-mediated airway inflammation and an asthma phenotype (115, 116). Mechanistically, TSLP can directly stimulate naïve $CD4^+$ T cells to commit to the Th2 cell lineage (106, 114, 117) and directly stimulate dendritic cells (103, 106, 113, 115, 118, 119) and ILC2 (106, 113, 120–122) for priming Th2 cell responses.

Based on the central role of TSLP in the initiation and maintenance of Th2-cell-mediated inflammation, including not only asthma but also atopic dermatitis and food allergy (123), a human IgG2 monoclonal antibody with the ability to neutralize TSLP (***Tezepelumab***) was developed (124) and have shown promising results in severe, uncontrolled asthma (125–127). Tezepelumab was given as an add-on therapy to patients whose asthma was uncontrolled despite the use of ICS. It was found to reduce asthma exacerbations, allergen-induced bronchoconstriction, and airway inflammation indexes, including decreased levels of blood and sputum eosinophils. These findings are being further explored in an ongoing phase 2/3 trial that will produce data by early 2021. Current trials are testing *Tezepelumab* when given subcutaneously every 4 weeks. Additionally, an inhaled anti-TSLP antibody will be studied in a 652-patient Phase II study (NCT04410523) that has yet to start recruiting.

ALLERGEN IMMUNOTHERAPY OR ALLERGEN DESENSITIZATION

Allergen immunotherapy, also known as desensitization, is a long-term medical treatment that decreases symptoms and prevents the development of allergic asthma in patients with environmental allergies (128–131). Contrary to ICS, oral corticoids, LABAs, and biologic drugs, which require continuous utilization to keep asthma symptoms under control, allergen immunotherapy is a disease-modifying approach. In these therapies, patients are exposed to gradually increasing doses of environmental allergies to divert their pathogenic Th2 cell responses from pathogenic to tolerogenic. The treatment requires a significant commitment since it usually takes 3–5 years to achieve clinical benefits. However, it often leads to long-lasting relief of allergy symptoms and severity of asthma, with an observed efficacy duration of 7–12 years after treatment is stopped (129–135). Allergen Immunotherapy may also decrease the development of new sensitizations to other allergens in both pediatric and adult patients (8, 131).

Despite proven efficacy, the mechanisms of allergen immunotherapy remain not entirely understood. Multiple overlapping mechanisms, mediators, and cell types are likely responsible for re-directing the established Th2/IgE-dominant response and the restoration of the immune tolerance to the aeroallergens. Desensitization of FcεRI-bearing mast cells and basophils, accompanied by decreased activity for degranulation and anaphylactic reactions, is observed early after treatment. This effect could be mediated by the up-regulation of the histamine type 2 receptor, which has a suppressive effect on the activation of mast cells and basophils (136). As the therapy progresses, IgG-dependent inhibition of mast cell/basophil activation might contribute to sustaining inhibition of mast cell/basophil activity. In this regard, it has been shown that specific-IgE levels in blood progressively decrease during allergen immunotherapy. On the

contrary, the titters of allergen-specific IgG4 antibodies increases over time (137–142). This change in balance is thought to be the consequence of increased IL-10 production (140), which can drive allergen-specific B cells to produce IgG4 at the expense of IgE secretion (143). Although the exact clinical consequences of these changes remain unclear, it has been suggested that IgG4 can sequester antigen, thereby limiting its availability for cross-linking of receptor-bound IgE. Alternatively, IgG4 can co-stimulate the inhibitory IgG receptor FcγRIIb, which negatively regulates FcεRI signaling and cell activation (144).

Phenotypic and functional changes in the allergen-specific T cell response have been observed in the peripheral blood and nasal mucosa of treated patients. These changes included diminished production of Th2 cytokines (IL-4, IL-13, IL-5) by allergen-specific T cells (142–148) and elevated numbers of allergen-induced $Foxp3^{+}CD25^{+}$ Tregs expressing IL-10 and TGF-beta (139, 142, 146, 149–152). Whereas, the exact mechanisms through which allergen immunotherapy drives inhibition, deletion, exhaustion, replacement, or reprogramming of T cells remain elusive, changes in the cytokine milieu could partially account for these changes. For example, allergen immunotherapy triggers IL-10 induction by multiple cell types (138, 140, 153, 154). In turn, IL-10 can control Th2 cell-mediated allergic inflammation by both direct and indirect mechanisms. On the one hand, intrinsic IL-10 signaling may limit Th2 cell responses by directly inducing Th2 cell death (155). On the other hand, IL-10 might prevent Th2 cell expansion by down-regulating antigen presentation by reducing MHCII class II expression (156, 157) or via IgG4-mediated inhibition of IgE-facilitated allergen presentation (140, 158–160). The subsequent reduction in the production of Th2 cytokines, most crucially in IL-4, could favor the differentiation of allergen-specific, IL-10-producing inducible Tregs by allowing TGF-beta-dependent up-regulation of FOXP3 in responding T cells (92–95). Thus, initiating a positive feedback loop of IL-10 signaling and Treg-mediated immunosuppression that ultimately suppresses the differentiation and function of newly formed allergen-specific Th2 cells (149, 161).

In current clinical practice in the United States, immunotherapy is delivered either subcutaneously or sublingually. Additionally, other methods of allergen delivery are being tested for improving outcome.

Subcutaneous Immunotherapy (SCIT)

Subcutaneous immunotherapy (SCIT), also known as allergy shots involves receiving subcutaneous injections of a particular aeroallergen that has been identified to cause the allergic reaction. Allergen identification is based on the presence of IgE antibodies specific to that allergen (162). Injectable allergen extracts are available to treat allergies triggered by common airborne allergens such as pollen, mold, dust mites, and animal dander.

SCIT treatment consists of two phases: During the ***Build-up phase***, the antigen is given frequently (one to two times per week) in gradually increasing doses until achieving an effective targeted dose (that reduce disease severity from natural exposure). This phase usually lasts 3–6 months. During the ***maintenance phase***, the targeted dose of allergen is injected every 3–4 weeks for at least 3–5 years. Allergy shots are recommended for people with allergy symptoms who do not respond well to usual mediations, have significant side effects from their mediation, want to reduce the long-term use of allergy medication, or for whom allergies might become life-threatening (8). Although allergen immunotherapy is generally safe, it can have adverse reactions, including anaphylaxis (163, 164). For that reason, each injection is administered in a setting with trained professionals and equipment to treat anaphylaxis (8). Further, it is essential to identify any patient characteristics (such as severe uncontrolled asthma) that may increase the risk of a severe reaction (165).

Sublingual Immunotherapy (SLIT)

SLIT involves administering the allergens in a tablet form under the tongue, generally on a daily basis. Sublingual tablets are intended for the treatment of allergic rhinitis and allergic asthma. This therapeutic approach is available for different species of grass pollen and dust mites. SLIT can achieve a significant clinical improvement but shows less efficacy than SCIT, which offers earlier and robust clinical effects and induces systemic changes (166–169). SLIT only provides local changes in the oral mucosa and regional lymph nodes (170, 171). The significant advantage of SLIT over SCIT is its safety profile, which allows for administering this treatment outside of the medical setting after the first dose (131, 172). Still, as for the possibility of severe allergic reactions from SLIT, an epinephrine auto-injector is usually prescribed to treat potential severe reactions at home.

Future Approaches in Allergen Immunotherapy

Although SCIT and SLIT are efficacious in that both offer significant clinical improvements in allergic and asthma symptoms, the adherence with the current regimens is low. Most likely because of the frequency of administrations and the long duration of the therapeutic courses. Thus, there is a need for more effective allergen immunotherapy strategies, especially for patients with refractory allergic disease or those who suffer adverse drug reactions.

One of the novel approaches includes using adjuvants such as Toll-like receptor (TLR) agonists. Lipopolysaccharide (LPS), also known as endotoxin, is a major component of Gram-negative bacteria that activates the innate immune response through TLR4. Exposure to airborne allergens containing endotoxin protects against asthma by suppressing the Th2 cell differentiation program in allergen-specific T cells (173–175). In this regard, monophosphoryl Lipid A (MPL), which is a TLR4 agonist, being a derivate of Lipid A from LPS that triggers a moderate inflammatory reaction (176, 177), have been evaluated in allergen immunotherapy. Compared to conventional allergen desensitization strategies, MPL immunotherapy show lasting clinical benefits even when administered in shorter courses (178–186). These results are certainly promising and encourage further controlled studies to evaluate clinical and immunological measurements and long-term efficacy.

Outside of TLR4, other agonists targeting alternative TLRs are being investigated in the context of allergen immunotherapy, with components targeting TLR9, TLR8, and TLR7. TLR9

agonists have been shown to reduce allergic symptoms and modulate the immune response to allergens when administered as an adjuvant in allergen immunotherapy (187–189). Despite promising data, clinical trials have not yet progressed beyond initial studies. TLR7 agonists are currently being evaluated for their safety in the context of allergen immunotherapy (190–193). Future studies will determine whether those are promising adjuvants.

Finally, other routes of allergen administration have been tested. Intralymphatic immunotherapy has shown favorable results in shorten treatment duration. Hence, it might offer an alternative approach to improving allergen immunotherapy adherence and success (194). Intralymphatic immunotherapy involves the application of the allergen directly into the lymph nodes. The whole treatment consists of three ultrasound-guided injections into the inguinal lymph nodes 1 month apart. Although the clinical results are favorable, more extensive studies are needed to support long-term effectiveness.

FUTURE THERAPEUTIC TARGETS: Tfh CELLS IN ASTHMA

Experimental mouse models of allergic asthma have been instrumental in investigating the mechanisms underlying the initiation and maintenance of allergen-specific Th2 cell responses. Using these preclinical models, it has been shown that the development of allergic Th2 cell responses is more complex than initially expected. During the initial sensitization through the intranasal (i.n.) route, lung-migratory dendritic cells traffic into the lung-draining lymph nodes to prime allergen-specific $CD4^+$ T cells (3, 195). Importantly, however, this initial exposure does not typically result in the accumulation of effector allergen-specific Th2 cells in the airways (1, 3). Instead, allergen sensitization triggers a strongly biased Tfh cell response that is restricted to the lung-draining lymph nodes (1, 3, 196).

Tfh cell development depends on the expression of the transcription factor Bcl6, which functions as a transcriptional repressor that prevents the acquisition of T effector programs, thereby facilitating Tfh cell differentiation (197–199). However, the capacity of Bcl6 to repress alternative T effector fates is not absolute. As such, whereas Tfh cells were initially characterized as IL-21-producing cells (198, 199), they are more plastic than expected and can initiate secondary differentiation programs and secrete Th1 (200–202), Th2 (3, 203), and Th17 (204) effector-like cytokines when developing in high polarizing environments. Correspondingly, work by us (3, 205), and others (10–12, 16, 206–208), show that Tfh cells can produce large amounts of Th2 cytokines, including IL-4 and IL-13, in response to allergens and helminths. Notably, while early studies considered that Th2 cells

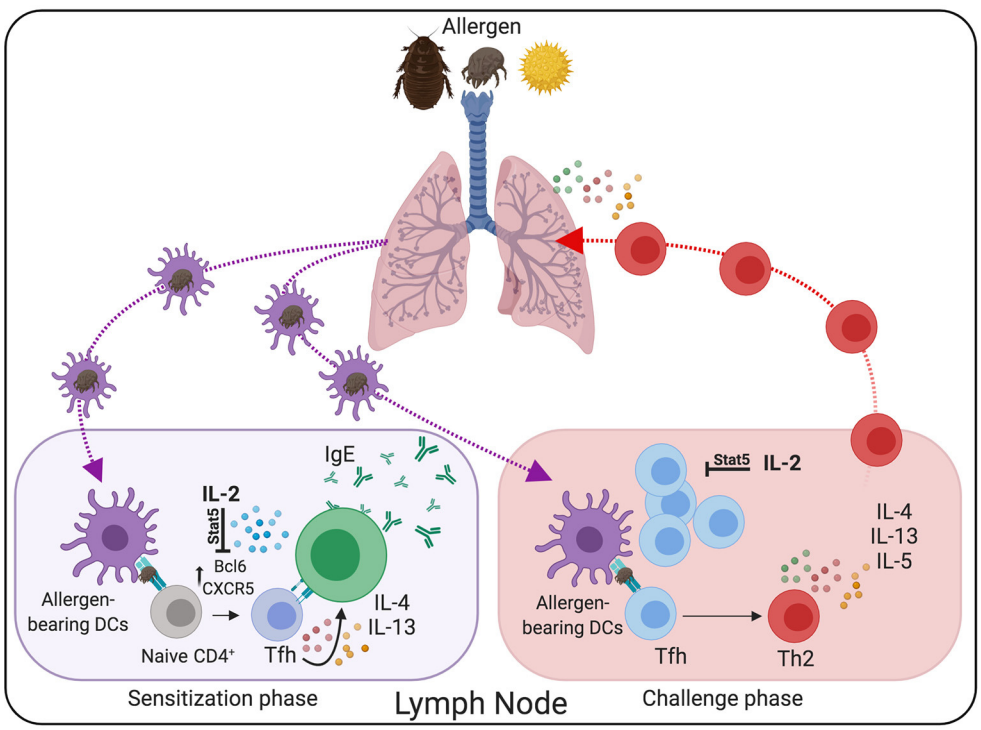

FIGURE 2 | Tfh cells are critical mediators in the pathogenesis of allergic asthma. During the sensitization phase, lung-migratory DC primed allergen-specific "Type 2" Tfh cell responses in the lung-draining lymph node. Through the interaction with B cells, Type-2 Tfh cells promote IgE secretion. Following re-challenge, Tfh cells differentiate into conventional effector Th2 cells that subsequently migrate to the lung and promote allergic airway inflammation. Treatment with rIL-2 has the potential to prevent Tfh cells differentiation and maintenance, thereby reducing asthma pathogenesis.

were the primary source of type 2 cytokines, it is increasingly accepted that Tfh cells, and not effector Th2 cells, are the main providers of IL-4 and IL-13 during the sensitization phase (3, 16). Furthermore, more recent data demonstrate that allergen-specific Tfh cells are critical mediators in the pathogenesis of allergic asthma (**Figure 2**). For example, IL-4/IL-13 producing Tfh cells are critical for the sustained production of high-affinity, allergen-specific IgE (1, 10, 16), which, as aforementioned, plays a crucial role in asthma pathogenesis. In addition, using an HDM sensitization and challenge model of asthma, we have recently found that type-2 Tfh cells survive in the lymph nodes for extended periods as memory cells and have the unique ability to give rise to effector Th2 cells upon allergen rechallenge (3). Combining fate-mapping and adoptive transfer experiments, we demonstrated that allergen-specific Tfh cells generated during the sensitization phase were the precursors of effector Th2 cells found in the lung after secondary challenge. Supporting the role of Tfh cells as progenitors of Th2 cells, depletion of Tfh cells during the sensitization phase prevented the accumulation of effector Th2 cells in the airways after challenge, thereby inhibiting asthma pathogenesis. Thus, our work establishes the lineage flexibility of Tfh cells in allergic disease and identifies these cells as a crucial long-term reservoir of Th2 cell progenitors.

All these studies collectively show a critical function of Tfh cells in allergic asthma pathogenesis, thus highlighting Tfh cells as an attractive target for the suppression of IgE responses and Th2 cell-mediated allergic inflammation. Unfortunately, there are currently no therapies to selectively target Tfh cells *in vivo*. Thus, a better understanding of the cellular and molecular mechanisms that control allergen-specific Tfh cell development and function will be critical for designing new therapeutic approaches to prevent Tfh-cell-mediated pathology in asthmatic patients. Interestingly, a large body of evidence indicates that IL-2 is a potent inhibitor of Tfh cells (3, 209–214). IL-2/STAT5 signaling prevents Tfh cell differentiation by repressing the expression of Bcl6, the master regulator of Tfh cells. As a consequence of the inhibitory effect of IL-2, Tfh cells fail to differentiate and are efficiently depleted after exogenous recombinant IL-2 treatment (3, 212, 214–217). Importantly, subcutaneous administration of low-dose recombinant human IL-2 r-IL2, (Aldesleukin/Proleukin) has potent immunosuppressive effects in patients with autoimmune disorders and can be safely administered to humans (217–220). In agreement with the role of IL-2 as an *"anti-Tfh"* agent, treatment of active Systemic Lupus Erythematosus (SLE) patients with low-dose rIL-2 resulted in reduced frequencies of Tfh cells in a recent clinical study by Jing He and colleagues (217), hence evidencing the therapeutic potential of IL-2 to prevent unwanted Tfh cell responses (**Figure 2**). Given the efficacy and safety of the low-dose IL-2- treatments and the putative role of Tfh cells in asthma pathogenesis, IL-2-based therapies, alone or in combination with other strategies, could represent a promising therapeutic approach to deplete allergen-specific Tfh cells and prevent allergic asthma pathogenesis.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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