Allergen immunotherapy for allergic airway diseases

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Allergen immunotherapy for allergic airway diseases: Use lessons from the past to design a brighter future

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ABSTRACT

Allergic respiratory diseases, such as allergic dermatitis, food allergy, allergic rhino conjunctivitis and allergic asthma, are chronic inflammatory diseases with increasing prevalence. Symptoms include such as watery or itchy itching of the mouth, skin, or the eyes, swelling of the face or throat, sneezing, congestion or vomiting, wheezing, shortness of breath and coughing. For allergic asthma, additional symptoms include tightness of chest, cough, wheezing, and reversible airflow limitation. These symptoms can be triggered by inhalation of allergens such as food allergens or airborne allergens such as those from tree- or grass pollen and house dust mites. Pharmacological intervention in allergic disease includes the use of antihistamines, immune suppressive drugs and in case of asthma, the use of (long acting) beta-agonists for relaxation of the constricted airways. These treatment options merely suppress symptoms and do not cure the disease. Allergen immunotherapy (AIT), in contrast, has the capacity of inducing long-term tolerance, with symptom relief persisting decennia after discontinuation of treatment, despite recurrent re-exposure to the allergen. However, AIT is not effective for all allergic disorders, and treatment for several years is required to obtain long-term protection. Moreover, some forms of AIT have safety concerns, with risk of mild to severe allergic reactions. To improve safety and efficacy of AIT, the underlying mechanisms have been studied extensively in the clinic as well as in experimental models of allergic airway inflammation.

Despite more than a century of clinical experience and a vast body of experimental and translational studies into the immunological and cellular mechanisms underpinning its therapeutic potential, AIT is still not implemented in routine clinical care for allergic asthma. This review provides an overview of the substantial developments that contribute to our knowledge of the pathogenesis of allergic airway diseases, the mechanism of action of AIT, its treatment routes and schedules, the standardization of extracts and use of adjuvantia. Moreover, the main conclusions from experimental models of AIT with regard to the safety and effectiveness of the treatment are

Abbreviations: AHR, Airway hyperresponsiveness; AIT, Allergen immunotherapy; Alum, mixture of aluminium hydroxide and magnesium hydroxide; APCs, Antigen-presenting cells; BALF, bronchoalveolar lavage fluid; Bet v1, major allergen in white birch pollen *Betula pendula*; BP, Basophils; Bregs, Regulatory B-cells; CaP, calcium phosphate; CCL, C-C Motif Chemokine Ligand; CCR4, CCR7, and CCR8, C-C chemokine receptors; CD4, CD25, CD40, CD80/86, CD127, Cluster of differentiation; CRTH2, Chemoattractant receptor-homologous molecule on Th2 cells; CTLA-4, Cytotoxic T lymphocyte-associated protein 4; CXCLs, chemokine (C-X-C motif) ligands; DCs, Dendritic cells; Der P, *Dermatophagoides pteronyssinus* (extract); Der p1/ p2, major allergens in *Dermatophagoides pteronyssinus* extract; Der f, Dermatophagoides farina; EAACI, European Academy of Allergy and Clinical Immunology; EO, Eosinophils; EMA, European Medicines Agency; EPIT, Epicutaneous immunotherapy; FcεRI, High-affinity receptor for IgE on mast cells and basophils; FcεRII, CD23, Low-affinity receptor for IgE on amongst others activated B cells; FDA, Food and Drug Administration; FEV1, forced expiratory volume in one second; FOXP3, Transcription factor forkhead box P3; Gal10, Galactin10; GINA, Global initiative for Asthma; GITR, Glucocorticoid-induced TNFR-related proteins; GM-CSF, Granulocyte-macrophage colony-stimulating factor; GP, Grass pollen; H2R, Histamine receptor-2; HDM, House dust mites; HRQL, Self-rated health-related quality of life; ICS, inhaled corticosteroid; IDO, indoleamine 2,3 dioxygenase; IFN-y, Interferon- y; (sp)IgA, IgE, IgG1, IgG2a, IgM, (specific) Immunoglobulin A, E, G1, G4, G2a, M;; IL-4, IL-5, IL-10, IL-13 etc., Interleukins; ILC2s, Innate lymphoid cells type 2; ILIT, Intralymphatic immunotherapy; i.n., Intranasal; i.p., Intraperitoneal; iTregs, Inducible Tregs, like IL-10$^+$ Tr1 cells; i.v., Intravenous; LABA, long-acting beta2-agonist; LBIT, Local bronchial immunotherapy; LDLNs, Lung draining-lymph nodes; LN, Lymph node; LNIT, Local nasal immunotherapy; LTs, Leukotrienes; LTD4, Leukotriene D4; MC, Mast cells; MCT, microcrystalline tyrosine; MHC-II, Major histocompatibility complex class II; MPLA, monophosphoryl lipid A; nTregs, Thymic or natural CD4$^+$CD25$^+$FoxP3$^+$ Tregs for tolerance to self-antigens; OIT, Oral immunotherapy; OVA, Ovalbumin; PC, IgE producing plasma cells; PLGA, polylactideco-glycolic acid; Phl p, *Phleum pratense*; PRRs, Pattern recognition receptors; RDBPC, Randomized double-blinded placebo-controlled trial; s.c., Subcutaneous; SCIT, Subcutaneous immunotherapy; s.l., Sublingual; SPT, Skin prick tests; TCR, T-cell receptor; TCS, Total combined score; Tfh, Follicular T helper cells; Tfr, Follicular regulatory T cells; TGF-β, Transforming growth factor-β; Th0, Naïve CD4$^+$ T cells; Th1, Th17 or other T helper 1 or 17 cells; Th2, CD4$^+$ T helper 2 cells; TLRs, Toll-like receptors; TMS, Total score for medication use; TNF, Tumor necrosis factor; Tr1, Anti-inflammatory IL-10-producing Tregs; Tregs, Regulatory T cells; TSLP, Thymic stromal lymphopoietin; TSS, Total symptom score; VIT, Venom immunotherapy; VitD3, 1,25$(OH)_2D_3$, 1,25-dihydroxy Vitamin D3..

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summarized, and future directions for further improvements are outlined. AIT urgently requires further improvements in order to increase its efficiency and shorten the treatment duration while remaining safe and cost-effective.

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1. Allergic asthma

Allergic respiratory diseases, such as allergic rhino conjunctivitis (hay fever) and allergic asthma, are chronic inflammatory diseases with symptoms such as watery or itchy eyes, sneezing, congestion, and coughing. For allergic asthma, additional symptoms include tightness of chest, cough, and reversible airflow limitation. Allergic asthma is characterized by eosinophilic airway inflammation, elevated levels of Th2 cytokines, presence of allergen-specific IgE, airway hyperresponsiveness (AHR), and airway remodeling including subepithelial matrix deposition, airway smooth muscle hyperplasia and hypertrophy and goblet cell metaplasia. These symptoms can be triggered by inhalation of aerosolized allergens such as those from tree- or grass pollen (GP) and house dust mite (HDM) (Bousquet et al., 2019). These environmental factors not only trigger the symptoms of disease, but in concert with genetic susceptibility also contribute to inception of the disease. It is estimated that the heritability of asthma is around 30–50% and epidemiological studies identified atopy is the highest risk factor for asthma inception, particularly the expression of IgE to indoor allergens (Backman et al., 2017; Del Giacco et al., 2017; Ober & Yao, 2011). Studies using genome-wide associations identified multiple genes associated with an increased risk of disease (Bønnelykke, Sparks, Waage, & Milner, 2016; Demenais et al., 2018). For example, numerous genes located in the 5q region have been associated with asthma (Holloway et al., 2001). These genetic approaches identify the genes that contribute to the mechanisms of allergic asthma inception and can indicate how the response to the disease-triggering environmental factors differs between someone who will go on to develop allergic rhinoconjunctivitis and/or asthma and someone who remains healthy. Recent genetic insights indicate that a reduced barrier function of the airway epithelium and the immunological activity of this epithelium in inducing a type-2 immune response are important determinants of the susceptibility to develop allergic rhinoconjunctivitis or allergic asthma (Bønnelykke et al., 2016).

The global prevalence of allergic airway diseases has increased tremendously throughout the last decennia (ranging up to 18% of the population in several countries (Bateman et al., 2008) and is significantly affecting the quality of life for a large proportion of the population (To et al., 2012). Around 10 to 20% of the population suffers from allergic rhinitis, which equals to over 500 million patients worldwide (Broek et al., 2010). Asthma affects a minimum of 300 million people around the world, which is thought to increase to 400 million by 2025, with an estimated worldwide mortality rate of 250,000 deaths every year (Dharmage, Perret, & Custovic, 2019; Varghese & WHO., 2018). Several studies indicate an association between allergic rhinitis and allergic asthma, since the incidence of asthma in people with allergic rhinitis varies from 10 to 40% (Bergeron & Hamid, 2005). In addition, allergic rhinitis is a risk factor for asthma.

Allergic disorders are often referred to as atopic diseases to stress the shared susceptibility and pathogenesis of these conditions. Atopy is defined as 'a personal or familial propensity to produce IgE antibodies and sensitization in response to environmental triggers' (Johansson et al., 2004). The etiology of atopic disorders is complex, and not yet fully elucidated. Hereditary causes in interaction with environmental factors play a crucial role in pathophysiology of allergic airway diseases. Allergic disease often starts in early childhood, and often shows a specific sequence of atopic disorders during the first few years of life referred to as the '*atopic march*' (Spergel & Paller, 2003). Children with a susceptibility for allergic disorders are often diagnosed with atopic dermatitis (AD), as young as 1-year-old, often in combination with allergy to cow milk. These children often progress to develop allergic rhinitis and eventually asthma, which can be diagnosed from around 6–7 years of age (Čelakovská et al., 2020). Some children will retain episodes with asthma symptoms for decades to come, while others may see a gradual decline in symptoms or even complete resolution of disease, referred to as asthma remission, with increasing age. Moreover, it has been reported that children that suffered from early-life virally (respiratory syncytial virus; RSV) induced bronchiolitis, have higher rates of asthma, indicating that while genetic factors determine susceptibility, environmental triggers can induce inception of the disease (Mackay, 1985). The concept of the atopic march has been endorsed by longitudinal and cross-sectional studies (Johansson et al., 2004; Ricci et al., 2006; van der Hulst, Klip, & Brand, 2007) and verified by experimental evidence from animal models (He et al., 2009). However, more recent evidence from longitudinal studies suggests that the atopic march merely reflects one of the possible patterns of allergic comorbidity that can occur in the first years of life, and other combinations of allergic disorders are equally likely to occur (Belgrave et al., 2014).

The global increased prevalence of allergic airway diseases is, at least in part, explained by changes in our environment and lifestyle. Environmental changes that contribute to the increasing prevalence of allergic airway diseases may include greater international travel and climate change (D'Amato et al., 2017), although other factors are also thought to contribute. In 1989, Strachan suggested that allergic diseases might be prevented by early childhood viral infections, particularly in the airways, transmitted through close sibling contact in large families, and

later reinfections might confer additional protection against hay fever (Strachan, 1989). Declining family size, improved amenities in households and personal hygiene may therefore contribute to the observed increased incidence of atopic disease, such as allergic asthma. This line of thought culminated in the '*hygiene hypothesis*', which states that there is a direct correlation between the absence of infections during childhood and an increased prevalence of allergies (Lambrecht & Hammad, 2017; von Mutius, 2007). Allergic inflammation is mediated by that part of the immune system that contributes to the immune response to infection with enteric helminths, or cutaneous responses to bites from ticks or venomous snakes, for example (Fallon & Mangan, 2007). In the pathophysiology of allergic disease, this part of the immune system responds to otherwise irrelevant allergens very much in the same way as it does to the tissue damage induced by snake venom, ectoparasites or certain viruses (Yazdanbakhsh, Kremsner, & Van Ree, 2002). Several mechanisms have evolved to suppress the type-2 immune response after resolution of the infection or in the absence of tissue damage, including the induction of immunosuppressive IL-10 producing regulatory T cells (Tregs) (van der Hulst et al., 2007). For allergic conditions, these regulatory processes are assumed not to evolve entirely, to be damaged or to be overcome by inflammatory mediators (Galli, Tsai, & Piliponsky, 2008). In the last years, new adaptations to the hygiene hypothesis have placed emphasis on the potential depletion or reduction of our microbiome diversity (lung, gut and skin), which causes susceptibility to chronic inflammatory disease (Lambrecht & Hammad, 2017). Such effects are thought to be also enhanced by our 'Western' way of living, characterized by a decline in physical exercise and structural changes in diet.

2. Pathogenesis of allergic airway disease

The lung is in constant contact with the external environment, resulting in a continuous exposure to a vast array of allergens, bacteria, viruses, and noxious particulate matter. The airway epithelium acts as a chemical, physical, and immunological barrier to inhaled particles and infectious micro-organisms (Hallstrand et al., 2014) (Fig. 1). Tight junctions between epithelial cells provide the structural adhesive forces which maintain the integrity of the airway epithelial barrier. The airway epithelium also has a role in immune surveillance, expressing a broad range of sensory receptors, including domain-like receptors and Toll-like receptors which can detect pathogen-associated molecular patterns (Lambrecht & Hammad, 2012), and connect innate and adaptive immune responses by releasing cytokines and chemokines (Castillo, Zheng, & Yang, 2018; Georas & Rezaee, 2014). Inhaled allergens, like the HDM *Dermatophagoides pteronyssinus* (Der p) allergen, disrupt epithelial integrity due to their proteolytic properties (Post et al., 2012). The airway epithelium responds to loss of cell-cell contacts by releasing a range of cytokines and chemokines that stimulate innate and adaptive immune cells (Holt, Strickland, Wikström, & Jahnsen, 2008), including the alarmins IL-25 (IL-17E), IL-33, and thymic stromal lymphopoietin (TSLP) (Lambrecht & Hammad, 2012) (Fig. 1). A major feature of epithelial-derived alarmins is their ability to activate both innate and adaptive immune responses (Georas & Rezaee, 2014). For instance, TSLP is considered an inflammatory biomarker and associated with chronic eosinophilic inflammation and increased levels of exhaled nitric oxide in allergic asthma patients (Hallstrand et al., 2014). IL-33 is well known as a pro-allergic alarmin that plays a central role in asthma inception and exacerbations and found to be highly expressed in the asthmatic airways (Grotenboer, Ketelaar, Koppelman, & Nawijn, 2013). All three epithelial alarmins can drive the activation of innate lymphoid cells type 2 (ILC2s). (See Table 1.)

ILC2s were originally found in the gut-associated mucosal tissues, producing high levels of IL-5, IL-6, and IL-13 in response to IL-25, IL-33, and TSLP derived from the epithelium (Klose & Artis, 2016). IL-5 and IL-13-producing ILCs have been suggested to play a role in eosinophilic inflammation of the asthmatic lungs (Bartemes et al., 2012; Wolterink et al., 2012). In asthma patients as well as in murine models of allergic asthma, a significant expansion of ILC2s has been shown, when compared to healthy controls (Fallon et al., 2006). Studies performed in mouse models have shown that ILC2 cells can contribute to airway eosinophilia and inflammation and epithelial repair in response to viral infections (Fallon et al., 2006). Monticelli *et al.* demonstrated that accumulation of lung ILCs after infection with influenza virus in mice and administration of lung ILC-derived amphiregulin restored airway epithelial integrity and tissue homeostasis (Monticelli et al., 2012). By producing IL-5 and IL-13 in response to alarmins released upon epithelial damage, ILC2s contribute to eosinophil recruitment and airway wall remodeling and mucus production, respectively.

One of the hallmarks of allergic asthma is the presence of increased numbers of eosinophils in blood and lung tissue. Eosinophil numbers correlate with disease severity, implicating the eosinophil as one of the main effector cells in the persistent inflammation in the airways (Fulkerson & Rothenberg, 2013). Eosinophils are innate immune cells present in most mucosal barrier tissues and can be identified by their granule structure and contents. Upon degranulation, eosinophils can cause harm to the airway mucosa through the release of granule-associated basic proteins, lipid mediators and reactive oxygen species (McBrien & Menzies-Gow, 2017). In addition, eosinophils release multiple fibrogenic mediators and growth factors that stimulate remodeling of the airways, such as transforming growth factor-β (TGF-β) (Fulkerson & Rothenberg, 2013; McBrien & Menzies-Gow, 2017). Their maturation, differentiation, translocation, and survival depend entirely on chemokines (like eotaxin), and cytokines (such as IL-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), and IL-5) (Acharya & Ackerman, 2014).

In addition to the first line of defense provided by the airway epithelium and the effector cells of the innate immune system, allergic asthma is characterized by an adaptive immune response to inhaled allergens (Larché, Akdis, & Valenta, 2006). The adaptive immune response in allergic asthma is driven primarily by $CD4^+$ T helper type 2 (Th2) lymphocytes, that contribute to allergic sensitization, late allergic responses, and chronic airway inflammation.

Allergic sensitization is the humoral immune response leading to production of allergen-specific IgE that sets the stage for an allergic response upon subsequent allergen re-exposure. Allergic sensitization requires activation of a specific class of antigen-presenting cells (APCs), the dendritic cells (DCs) (Deckers, De Bosscher, Lambrecht, & Hammad, 2017; Schuijs, Hammad, & Lambrecht, 2019) (Fig. 1). DCs regulate immune responses to a wide range of inhaled antigens and are uniquely equipped to induce adaptive immune responses. In the respiratory mucosa, DCs capture and process inhaled antigens, including allergens, mainly through phagocytosis, receptor-mediated endocytosis and/or micropinocytosis (Deckers et al., 2017; Lambrecht et al., 2000). Upon activation by pattern recognition receptor (PRR) signaling, DCs upregulate CCR7 which allows them to migrate through the afferent lymphatics to T cell-enriched regions of the mediastinal- or lung draining-lymph nodes (LDLNs). In the lymph nodes, the now mature DCs present processed antigen-derived peptide fragments in the context of major histocompatibility complex-II (MHC-II) molecules and co-stimulation (CD80/86-CD28) to naïve $CD4^+$ T cells (Larché et al., 2006) (Fig. 1). During naïve T cell activation in the draining lymph nodes, DCs also induce T-helper cell polarization into a Th1, Th2, or Th17 phenotype to establish immunity, or induce differentiation of naïve Th cells into adaptive or induced regulatory T cells (Tregs) to establish tolerance. $CD4^+$ T helper 1 cells (Th1) are characterized by the production of IL-2 and interferon- y (IFN-y), whereas Th2 cells produce IL-4, IL-5, and IL-13 (Larché et al., 2006), while Tregs predominantly produce high levels of Il-10, IL-35 and TGF-β. Importantly, the airway epithelium can control the activation and phenotype of the DC prior to the migration towards the draining lymph node, and thereby indirectly influence the phenotype of the $CD4^+$ T cell response. For instance, airway epithelial cells can promote the induction and Th2-cell polarizing

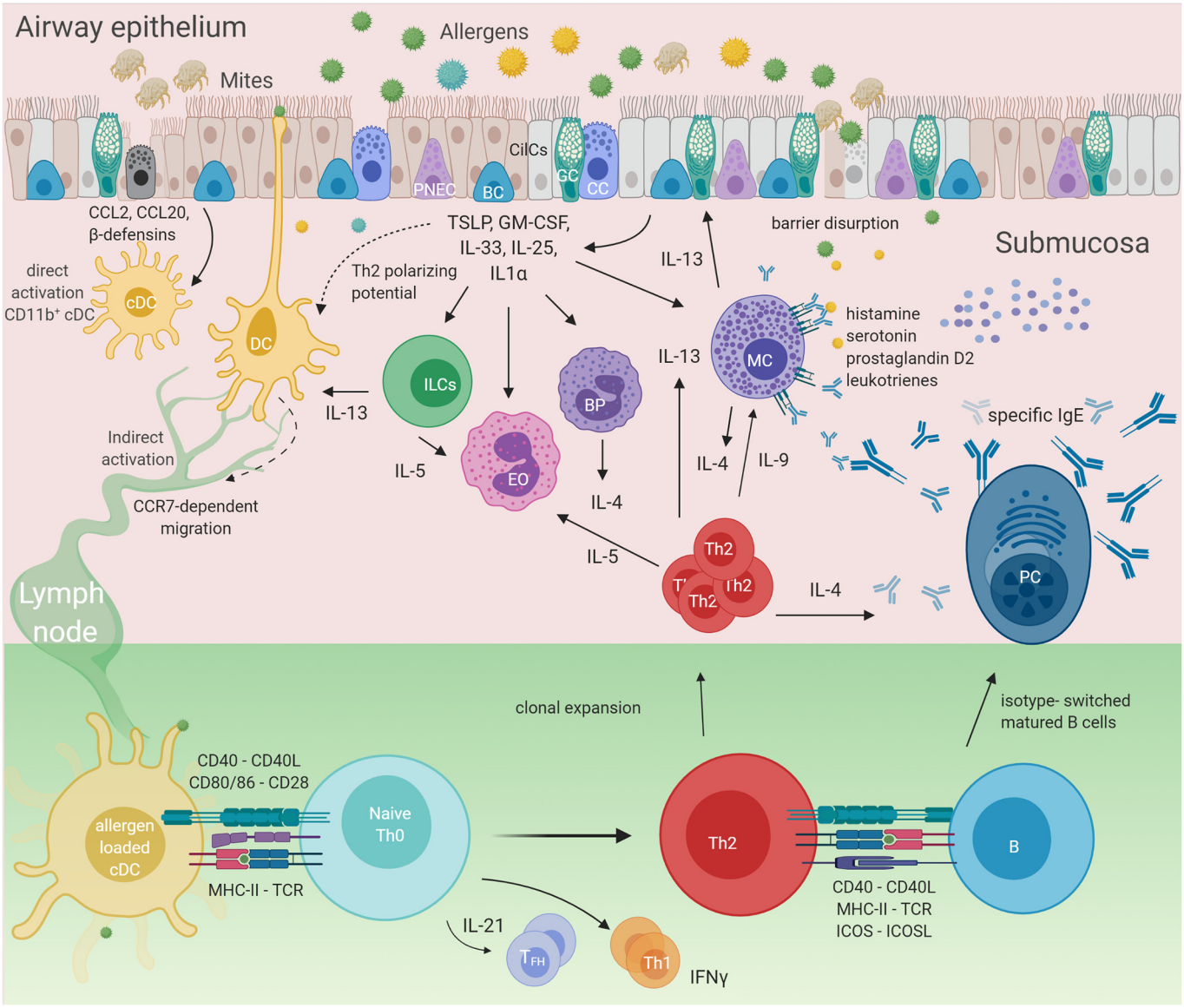

Fig. 1. Allergic sensitization in the airways. Allergens initial contact with the epithelial barrier trigger an innate immune response, releasing CCL2, CCL20, β-defensins, attracting and directly activating immature dendritic cells (DCs). Moreover, release of chemokines and cytokines such as TSLP, GM-CSF, IL-25, IL-33, and IL1α provide an additional danger signal and influence DC activation and Th2 maturation and migration. Allergens are taken up by antigen-presenting cells (APCs, dendritic cells, DCs) processed and presented to naïve T cells in the draining lymph nodes. Activation of naïve Th cells in the presence of IL-4 induced differentiation and clonal expansion of allergen-specific T helper 2 (Th2) cells. Moreover, IL-4 induces immunoglobulin class switching from IgM to IgE and clonal expansion of naive and IgE^{+} memory B-cell populations. The epithelial alarmins and cytokines also activate other innate immune cells such as innate lymphoid cells, basophils, mast cells and eosinophils. IgE binds to innate effector cells such as mast cells and basophilic granulocytes through the high-affinity IgE receptor. Cell-types: cDC, conventional dendritic cells; Th0, naïve T helper zero cells; Th1, T helper 1 cells; Th2, T helper 2 cells; Tfh, Follicular helper T cells; B, B lymphocytes; PC, IgE producing Plasma cells; MC, Mast cells; BP, Basophils; EO, Eosinophils; ILCs, Innate lymphoid cells; CC, Club Cell; GC, Goblet cell; BC, Basal cell; CilC, Ciliated cells; PNEC, Pulmonary neuroendocrine cells. Figure was created using BioRender.com.

potential of DCs by generating cytokines such as TSLP and GM-CSF (Hammad & Lambrecht, 2008).

Finally, the differentiated effector Th cells migrate via the efferent lymph and the thoracic duct into the circulation. Based on their tissue-homing receptor signature, effector T cells can migrate into inflamed tissues to exert their effector functions, including the bronchial mucosa, by exiting the bloodstream in the postcapillary venules. Th2 cells are the predominant effector Th cell type in patients with allergic asthma and have recently been formally shown to be present in airway wall of asthma patients (Vieira Braga et al., 2019). Numerous studies in humans and mice demonstrated the central role of allergen-specific Th2 cells in the pathogenesis of allergic asthma (Fig. 2).

Activation of the adaptive immune system during allergic sensitization will yield a humoral immune response characterized by production of IgG4 and IgE. Serum levels of these antibodies are controlled by memory B cells that promptly respond to antigen re-exposure by generating new plasma cells and memory B cells, as well as long-lived plasma cells that secrete antigen-specific antibodies (Larché et al., 2006). The IgE response induced by type-2 immunity protects from toxins and enhances immunity to helminthic parasites (Larché et al., 2006), but also plays a key role in allergies (Gould & Sutton, 2008; Mukai, Tsai, Starkl, Marichal, & Galli, 2016). In secondary lymphoid tissues, co-stimulation of follicular B cells by activated follicular Th cells results in the differentiation of isotype-switched, affinity matured B cells (Larché et al., 2006). The isotype conversion of B cells to IgE production is the basis for allergic responses. In the presence of IL-4, immunoglobulin class switching from IgM to IgE occurs in the germinal center B cell activated by CD40/CD40L interactions (Holgate, 2012) (Fig. 1). The germinal center

Table 1
Effects of AIT on immune and inflammatory cells. HR2: Histamine receptor 2; nTreg: natural T regulatory cells; iTregs: inducible Tregs; Tfr: Follicular regulatory T cells; IgE, IgG: immunoglobulins; IL-10, IL-35: Interleukins; TGF-β: Transforming growth factor β; CRTH2: Chemoattractant receptor-homologous molecule on Th2 cells; CD127: IL-7 receptor; ILC2s: Innate Lymphoid cells type 2; C1Q: Complement component 1; STAB1: Stabilin-1.

Immunomodulatory effects of AIT		
Cell type	Effects	Reference
Mast cells and Basophils	Early desensitization demonstrated by weaker responses to allergens	Novak et al., 2012
	Decreased basophil numbers	Larche et al., 2006
	Upregulation of histamine receptor 2 (HR2) on basophils	
T cells	Peripheral tolerance induction in allergen-specific Th cells	Sakaguchi et al., 1995
	Deletion of allergen-specific (pathogenic) Th2 clones from the T cell repertoire	Wambre et al., 2012 Wambre et al., 2014
	Switch from Th2 phenotype to Th1 phenotype	Ebner et al., 1997
	Induction and activation of allergen specific Tregs (nTreg, iTreg, Tfr)	Schulten et al., 2018 Weiner, 2001
B cells and plasma cells	Decline in specific IgE production	Scadding, Calderon, et al., 2017, Scadding, Kariyawasam, et al., 2017
	Increase in specific IgG4, neutralizing/blocking antibodies	Wachholz et al., 2003 Jutel et al., 2005
	Induction of B regulatory cells producing IL-10, IL-35 & TGF-β	van de Veen et al., 2013
Innate Lymphoid cells	Reduced numbers and activity of IL-5 producing CRTH2$^+$CD127$^+$ILC2s	Kubo, 2017 Lao-Araya, Steveling, Scadding, Durham, & Shamji, 2014 Fan et al., 2016
Dendritic cells	Induction of a tolerogenic phenotype	Funes et al., 2019
	Biomarkers such as C1Q and STAB1 indicate early tolerance induction	Zimmer et al., 2012
Eosinophils	Reduction in numbers and activity of eosinophils	Hogan et al., 2003 Rak et al., 1988

reaction will induce both memory B cells and antibody-secreting short-lived plasma cells.

Memory B cells are long-lived cells that do not secrete antibody and highly express the B cell receptor on their surface (Larché et al., 2006). Memory B cells are capable of responding to a next allergen challenge by quickly giving rise to antigen-specific antibody-secreting plasma cells. Plasmablasts may remain in the mucosal barrier tissues or migrate to the bone marrow, where they differentiate into plasma cells. Plasma cells (PCs) are incapable of self-renewal and bone-marrow resident long-lived plasma cells provide long term allergen-specific IgE production (Fig. 1 and 2).

IgE antibodies mediate allergic responses through their capacity to bind to high-affinity receptors on mast cells and basophilic granulocytes, and cause degranulation of these innate effector cells after cross-linking of the FcεRI/IgE complexes by allergens. Two receptors for IgE can be distinguished: the high-affinity IgE receptor FcεRI, and FcεRII (CD23), the low-affinity receptor for IgE that is expressed on the surface of, amongst others, activated B cells (Palomares, Akdis, Martín-Fontecha, & Akdis, 2017). Allergen-binding leads to cross-linking of the cell surface-bound IgE (IgE-FcεRI) and degranulation of basophils and mast cells. Release of mediators contained in the granules, such as histamine, serotonin, prostaglandin D2, and leukotrienes, induce the acute allergic reaction and cause vasodilatation, increased vascular permeability, bronchoconstriction, and mucus production in the airways (Lambrecht & Hammad, 2015) (Fig. 1 and 2).

This early or immediate allergic response is fully dependent on IgE, starts within seconds after allergen exposure and resolves usually within 1–2 h. This early response is often followed by a late-phase reaction, which is dependent on Th2 cells that have infiltrated the (now inflamed) tissue and activate other infiltrated and tissue resident immune cells, leading to a second wave of release of lipid mediators as well as cytokines and chemokines, leading to bronchoconstriction and tissue inflammation. The cytokines produced by the activated effector Th2 cells will further enhance local IgE production by B cells, eosinophilic inflammation, increased mucus production and airway hyper reactivity. IL-4 contributes to allergic inflammation through the differentiation of Th2 cells, the expansion of mast cells and basophils, and B cell activation. IL-5 contributes to allergic inflammation through the production, maturation, activation, and survival of eosinophils. These eosinophilic infiltrates can (in)directly cause bronchial hyperresponsiveness via abundant proteins in their granules (like eosinophil cationic protein and peroxidases), and upon activation secrete high concentrations of Galactin10 (Gal10) (Persson et al., 2019). At high concentrations, the secreted Gal10 undergoes a transition to form crystalline structures, called Charcot-Leyden crystals, which have been shown to directly actively promote key features of asthma, including airway obstruction (Persson et al., 2019). Basophils and mast cells degranulate upon a high-affinity FcεRI-dependent activation and release their mediators (like histamine and tryptase) and type 2 cytokines from intracytoplasmic granules, that enhance allergic inflammation. Also, arachidonic acid-derived mediators within the lipid membrane include sulphidopeptide leukotrienes (LTs), prostaglandin D2, and platelet-activating factor, all contributing to the vasodilatation, mucus secretion, edema, and neurogenic activation as part of an immediate type I hypersensitivity response. Tissue-resident mast cells are critical in the immediate allergic response after allergen crosslinking of the IgE on their cell surface, followed by degranulation and mediator release, which upon intracutaneous injection of the allergen results in a wheel and flare in the dermis from 10 to 30 min after injection of the allergen (Borriello, Iannone, & Marone, 2017). Moreover, mast cell-derived cytokines contribute to allergic inflammation in the late response after allergen challenge.

Prolonged or repeated allergen exposure (e.g. an entire pollen season) causes chronic inflammation of the respiratory tract, characterized by epithelial shedding, airway remodeling (airway smooth muscle hyperplasia and hypertrophy, goblet cell hyperplasia and mucus hypersecretion, increases in fibroblast and myofibroblast numbers and subepithelial matrix deposition), airway hyperreactivity, edema, skin erythema, and infiltration of inflammatory cells in bronchial tissue. Chronic inflammation and repeated early and late phase allergic responses may result in irreversible damage to the airways and increased airway sensitivity for various non-allergenic stimuli (Fig. 2) (Larché et al., 2006).

3. Treating allergic asthma

Asthma cannot be cured. Treatment is focused on reducing symptoms and has roughly two main aspects: suppression of inflammation and inducing or maintaining bronchodilation. The Global initiative for Asthma (GINA) provides guidelines for the diagnosis, prevention, and treatment management for asthma (Global Initiative for Asthma, 2020). Asthma management aims to achieve symptom control, minimize future risk of asthma-related mortality, persistent airflow limitation, exacerbations, and side-effects of treatment (Global Initiative for Asthma, 2020). Asthma medication falls into three main categories: Controller medication, such as low-dose ICS-formoterol for mild asthma; Reliever (rescue) medication; and Add-on therapies for severe asthma patients with persistent symptoms. Preventive long-term asthma control medication, taken daily, reduces airway inflammation and is considered to be the cornerstone in asthma treatment (Tamesis & Covar, 2008). However, some patients with persistent asthma continue to have exacerbations despite maximal treatment doses. In clinical practice to reduce the risk of exacerbations, asthma medications are

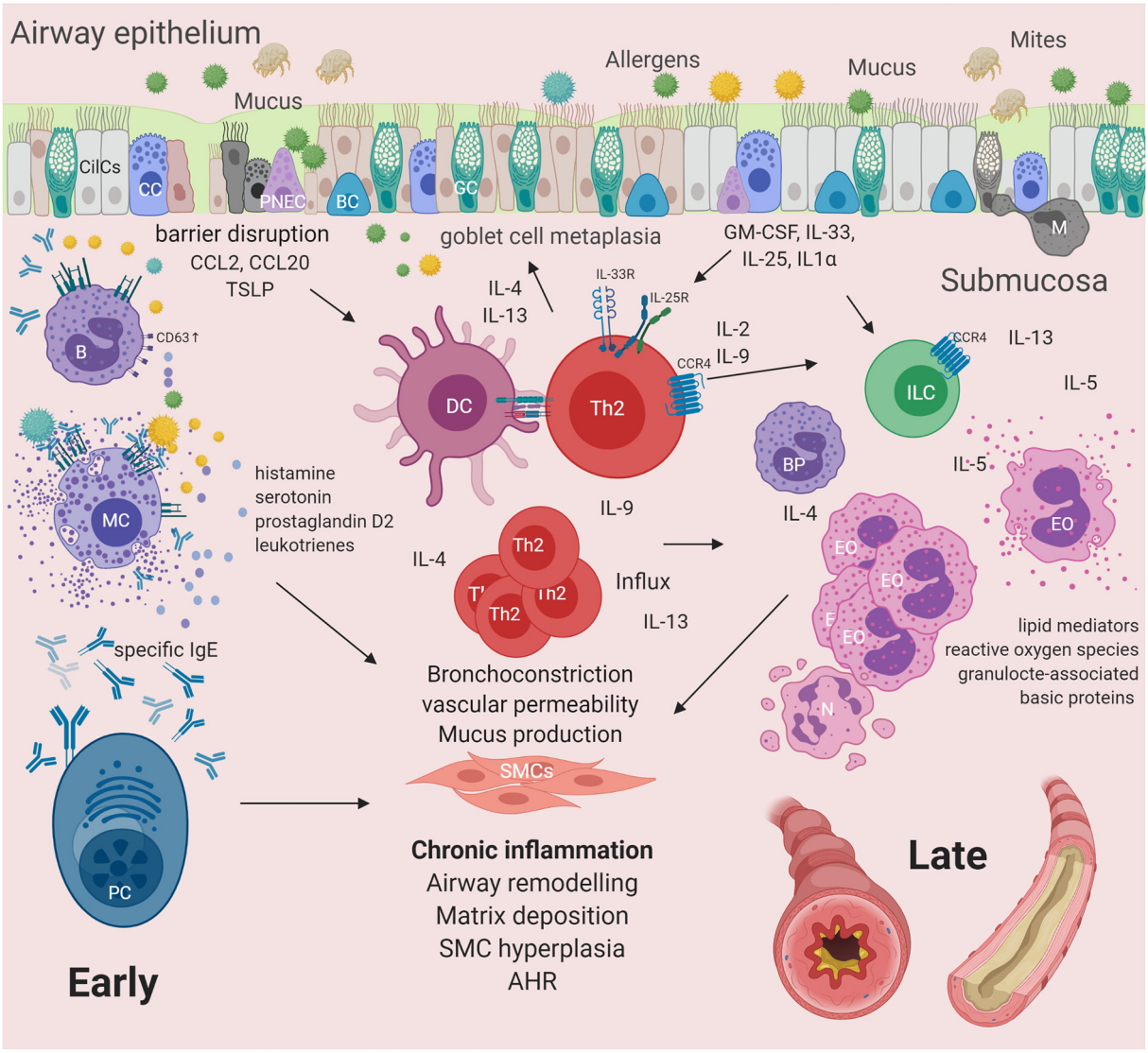

Fig. 2. Allergic inflammation in the airways after allergen exposure. Crosslinking of mast-cell and basophil cell-surface FcεRI-bound IgE by allergens leads to early-phase allergic response through release of vasoactive amines (such as histamine), lipid mediators (such as prostaglandin D2, LTC4, LTD4 and LTE4), chemokines (CXCL8, CXCL10, CCL2, CCL4 and CCL5) and other cytokines (IL-4, IL-5, and IL-13). The early phase response to influx of Th2 effector/ memory cells, as well as innate effector cells such as neutrophilic and eosinophilic granulocytes. In the inflamed airway wall, the Th2 effector cells are activated by the tissue resident or inflammatory DCs presenting the allergen, leading to cytokine release, activating the structural cells of the airway wall, the local B cells, recruiting eosinophils and activating innate effector cells. Local IgE production is seen in allergic rhinitis and asthma. Eosinophils are one of the main inflammatory cells (constituting up to 50% of the cellular infiltrate) in the lungs of asthmatic individuals. Activation of mast cells and basophils, which release histamine, chemokines, and other cytokines, also contributes to the late-phase allergic reaction. Chronic repeated exposures to allergens leads to among others: chronic inflammatory responses, airway remodeling, matrix deposition. Cell-types: DC, dendritic cells; Th2, T helper 2 cells; PC, IgE producing plasma cells; MC, Mast cells; B, Basophils; EO, Eosinophils; ILCs, Innate lymphoid cells; CC, Club Cell; GC, Goblet cell; BC, Basal cell; CilC, Ciliated cells; PNEC, Pulmonary neuroendocrine cells; M, Macrophage; N, neutrophil. Figure was created using BioRender.com.

optimized by identifying and treating modifiable risk factors for these exacerbations of disease (Global Initiative for Asthma, 2020).

In addition to these pharmacological therapies focused on immune suppression and bronchodilation, specific treatments to combat the allergic component in asthma have also been developed. Allergen avoidance is the first preventive technique that may help and improve asthma control in patients with allergic asthma and includes sending children to holiday homes and admission to a hospital. Although this strategy will allow relief of symptoms temporarily, studies showed that complete avoidance of allergenic triggers was impractical and unsuccessful in maintaining asthma control (De Blay, Barnig, & Ott, 2009). Besides, the main pollination period for the different tree and grass species spans over 6 months, from spring to autumn in Europe. As allergen avoidance strategies are not always possible, pharmacotherapy specific for the allergic component of asthma such as antihistamines, intranasal corticosteroids and nasal decongestants are routinely used in allergic rhinitis (Melvin & Patel, 2011). These treatments, however, are not effective for allergic asthma and more than 40% of patients claim to have poorly controlled symptom management after medium/high-dosage inhaled corticosteroid/long-acting beta2-agonist (ICS/LABA) treatment (Davis, Trudo, Siddall, & Small, 2019). GINA guidelines indicate that allergen exposure in sensitized patients can be one of the modifiable risk factors and sublingual immunotherapy (SLIT) can be considered as add-on treatment in HDM-sensitive asthma patients with allergic rhinitis despite ICS, provided FEV1 is >70% predicted (Global Initiative for Asthma, 2020). Moreover, in patients with allergic asthma, subcutaneous immunotherapy (SCIT) can achieve a reduction in medication use and symptom scores (Klimek, Fox, & Thum-Oltmer, 2018). Analysis of safety data for HDM allergoid-SCIT in 6 randomized controlled trials (more than 500 patients), showed no increased risk of adverse events and tolerability was comparable to tablets for SLIT (Klimek et al., 2018). The (European Academy of Allergy and

Clinical Immunology) EAACI's clinical practice guidelines recommend using add-on HDM SCIT for both adults and children and SLIT drops for children with partially controlled allergic asthma (Agache et al., 2019; Pfaar et al., 2019).

In addition to allergen-specific immunotherapy, biologicals such as omalizumab (anti-IgE, Xolair) have also been applied to suppress allergen-induced symptoms and exacerbations of disease (Tortajada-Girbés et al., 2018). Omalizumab is a monoclonal anti-IgE antibody, preventing allergen induced IgE crosslinking and subsequent activation of effector cells. According to the GINA guidelines, Omalizumab can be prescribed as an add-on treatment for patients with severe allergic asthma for more than 6 years (Global Initiative for Asthma, 2020). However, this treatment has the disadvantage of intervening at the stage of the early allergic response, and therefore achieves suppression of the allergic symptoms, but does not address the root cause of disease, and consequently does not cure the allergy. Given the great inconvenience for patients, the rising global prevalence, and the lack of success in allergy prevention, effective therapy that permanently alters the unwanted immune response in allergy remains an important unmet medical need (Zainab, Akram, Daniyal, & Riaz, 2019).

4. Allergen-specific immunotherapy

In contrast to other medication for allergic disorders, allergen specific immunotherapy (AIT) has the unique capacity to modify the natural course of disease, and to induce a long-term or even permanent state of tolerance to the causative allergen, resulting in disease remission (Jacobsen, Wahn, & Bilo, 2012). AIT is '*the repeated administration of specific allergens to patients with IgE-mediated conditions to protect against the allergic symptoms and inflammatory reactions associated with natural exposure to these allergens*' (Cox et al., 2011). AIT can modify the allergen-specific Th2 immune responses and aims to achieve tolerance by inducing Treg activity and secretion of immunosuppressive cytokines. Successful AIT is characterized by a lack of symptoms upon subsequent re-exposure to the allergen (Durham et al., 1999). Moreover, this treatment can prevent new sensitizations to unrelated allergens (Des Roches et al., 1997), and prevent progression to asthma in patients with allergic rhinitis (Jacobsen et al., 2007; Jacobsen et al., 2012). AIT treatment involves repetitive administrations of increasing dosages of allergens, for up to three to five years for successful therapy in patients with allergic rhinitis, asthma, (bee and wasp-) venom and drug allergy and, more recently, food allergies and atopic dermatitis (Čelakovská, Ettlerová, Ettler, & Bukač, 2015; Passalacqua & Canonica, 2016).

4.1. The early history of AIT

Although the seasonal recurrence of hay fever was already recognized and described in 1819 by Bostock (Bostock, 1960), AIT was introduced into clinical practice almost a century later, in June 1911, by Leonard Noon in St. Mary's hospital London, with the aim of '*vaccinating*' against hypothetical '*aerogenic toxins*' from the flowering timothy grass (Freeman, 1911; Noon, 1911) (Fig. 3). In doing so, he carried out the first study of active immunization in the form of subcutaneous immunotherapy (SCIT) with timothy grass pollen extracts (*Phleum pratense, Phl p*), a treatment that was effective in reducing hay fever symptoms. Noon was the first to describe the induction of active immune modulation towards allergens by injecting a small dose of causative allergen, resulting in a protection from symptoms of hay fever during seasonal exposure to the timothy grass pollen (Passalacqua & Canonica, 2016). In September 1911, J. Freeman continued the work of Noon and published the first results of their clinical experiments, wherein he concluded that there had been a distinct amelioration of symptoms in several forms; the attack was not so bad as in former years, and the attack subdued sooner (Freeman, 1911). Robert Cooke introduced this therapy in his report published in 'Laryngology and Rhinology' of the New York Academy of Medicine, calling it '*active immunization*' in 1914, as he injected hay fever allergic patients with allergen preparations based on rough extracts of dried pollen either prophylactic (before attack) or phylactic (during attack) (Cooke, 1915). Later, he suggested the term '*hyposensitization*' as a better name for this treatment strategy. In 1918, it was accepted that exposure to allergens resulted in the production of antibodies that in turn induced hay fever, asthma, and anaphylaxis (Cooke, 1918). In the decennia thereafter, the use of SCIT steadily increased and was more and more extended to other allergens (Ring & Gutermuth, 2011). Treatments mainly consisted of weekly injections containing extracts of pollen and animal dander.

SCIT became internationally more recognized in the 1950s as a result of the close international collaboration in allergy that was inspired by the 1st European Congress on Allergy, Paris (Muraro, Papadopoulos, & Akdis, 2014). The first clinical SCIT studies were published in 1954 by Frankland and Augustin, who showed that hyposensitization was effective for treating hay fever using a whole GP extract as well as purified pollen proteins (after ultrafiltration) (Frankland & Augustin, 1954). Both the extract and the proteins were effective in reducing symptoms compared to treatment with matching diluent controls alone (Durham & Nelson, 2011). In 1961, a new *in vitro* technique was developed to study histamine release after whole blood cell stimulations with allergens before and after AIT, introducing a new quantitative parameter of allergy diagnosis as well as the first biomarker for immune suppression upon successful AIT (Van Arsdel Jr. & Middleton, 1961). Moreover, and of great importance in the 1960s, new sensitive techniques for the identification and separation of proteins allowed for new approaches to serology and finally made the discovery of IgE possible (Ishizaka, Ishizaka, & Hathorn, 1964; Rowe & Fahey, 1965). Traditional IgE antibody tests like the *in vitro* specific IgE (spIgE) tests, or the skin prick tests (SPT), were routinely used in the 1970s (How & Cambridge, 1971), based on the results from responses of basophils and mast cells.

Although the first observations on positive (blocking) serological responses upon intradermal allergen injections were already made in 1937 (Cooke, Loveless, & Stull, 1937), Lichtenstein and Osler in 1966 developed an assay for the antigen-neutralizing capacity of serum from allergic patients and reported on the relationship between the antigen-neutralizing activity and the presence of 'blocking' antibodies after successful AIT treatment (Levy & Osler, 1966). In 1968, a few years later, Johnstone and Dutton proposed that AIT treatment in children with asthma could alter the natural history of respiratory allergy and might offer protection against the disease (Johnstone & Dutton, 1968), but these studies were not followed up on (Jacobsen et al., 2007). In parallel, in 1970s, Levine and Vaz published results on experimental immunization in mice and other animals, highlighting the first experimental models on allergen immunotherapy (Ishizaka & Ishizaka, 1978; Levine & Vaz, 1970; Neta & Salvin, 1974a; Whicker, Neel III, Kern, & Reynolds, 1973). The value of these models was further supported by the rapidly evolving field of immunology and allergology. Rocklin was the first to demonstrate a link between altered T-cell responses and allergen immunotherapy, in 1973 (Rocklin, Pence, Kaplan, & Evans, 1974), whereas identification of the relevant T cell subsets (Mosmann & Sad, 1996; Romagnani, 1991) and the crucial role of Treg cells and IL-10 was proposed by Akdis and colleagues many years later (C. A. Akdis, Blesken, Akdis, Wuthrich, & Blaser, 1998; Jutel et al., 2003).

In the last three decennia, many advances in AIT formulations, administration routes, and improved knowledge of the mechanisms of action allowed for a broader acceptance and applicability of AIT. In 1986, Mosmann and Coffman described the existence of polarized Th1 and Th2 cell subsets, which differed from each other in their pattern of cytokine production and their functions (Mosmann & Coffman, 1989). The phenomenon of AIT-induced immune deviation of allergen-specific Th2 responses in favor of Th1 responses was described by Jutel *et al.*, who showed IFN-γ responses after restimulation of allergen-specific T cells *in vitro* and suggested a clear shift towards a more Th1-like response (Jutel et al., 1995a). Next, besides using pollen, in 1978, the efficacy of purified venoms in SCIT for *Hymenoptera* venom allergy

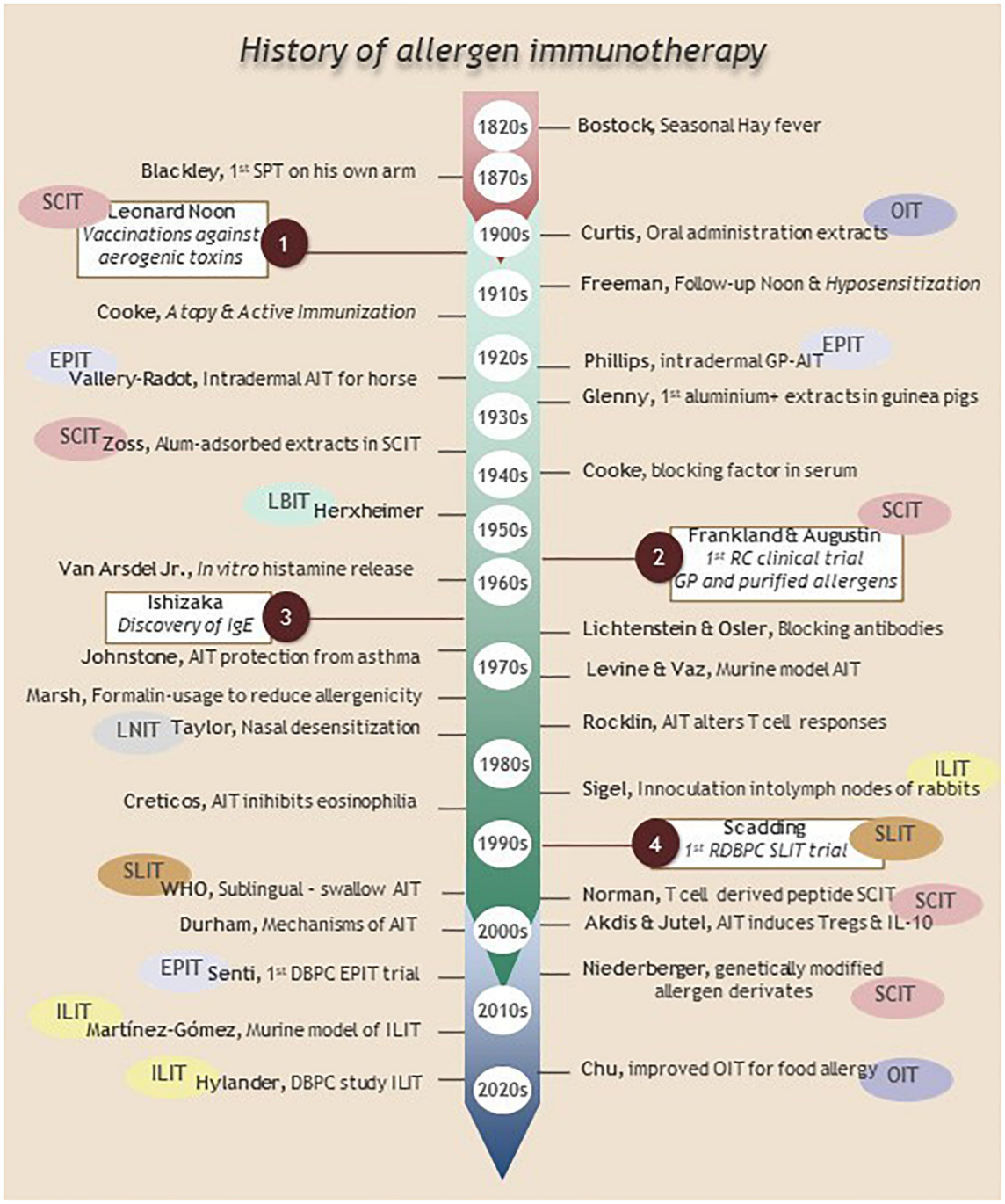

Fig. 3. Overview of important steps in the development of AIT in a timeline. SCIT: subcutaneous immunotherapy, SLIT: sublingual immunotherapy, EPIT: epicutaneous immunotherapy, OIT: oral immunotherapy, ILIT: intralymphatic immunotherapy, LNIT: local nasal immunotherapy.

was shown for the first time in a randomized, double-blinded, placebo-controlled (DBPC) trial (Hunt et al., 1978). This study was later followed by many different trials, thoroughly demonstrating both efficacy and safety of venom immunotherapy (VIT), which is now commonly used according to well standardized procedures (Schiener, Graessel, Ollert, Schmidt-Weber, & Blank, 2017).

4.2. Administration routes

4.2.1. Subcutaneous immunotherapy: SCIT

Throughout the century, a wide variety of allergen extracts (including pollen from grasses and trees, house dust mites) have become available for SCIT treatment, using non-standardized units to quantify the allergen content (Toogood, 1987). Traditionally, SCIT involves the administration of extracts obtained from the allergens of a single species (for instance, grass pollen from *P. pratense*). These allergen extracts are applied at increasing dosages, starting at low dosages, and steadily building up the dosage during weekly applications, up to a high standard dose which is reached after several weeks of treatment, and then maintained at plateau level (Cox, 2006). This dose is injected monthly for 3–5 years to induce long-term remission of allergic symptoms. Currently, SCIT is successfully used for a large number of allergens including wasp and bee venom, pollen and HDM, and its efficacy is considered to be clinically important when allergic symptoms are reduced by 30% or

more compared to placebo treatment (Canonica et al., 2007). During treatment, up to 22% of patients develop a low to moderate systemic reaction and very rarely anaphylaxis. However, SCIT is considered completely safe when patients are selected properly, outpatient clinic facilities are suitable for AIT, well-trained staff members perform the injections, and emergency treatment is available (Scadding et al., 2017).

In the 1960s, the absence of a standard quality unit for allergen extracts used in clinical practice made treatment regimens difficult to interchange or even compare (Aaronson & Gandhi, 2004). All desensitizing allergen extracts can induce allergic reactions, the most severe of which are bronchospasms and anaphylaxis, and some patients died from anaphylactic responses induced by treatment protocols that were not sufficiently standardized (Bernstein, Wanner, Borish, & Liss, 2004; C. James & Bernstein, 2017). Moreover, a number of these cases were due to human errors that could have been avoided (erroneous prescription, inaccurate administration, wrong dosage applied). In the early 1970s, attempts were made to reduce the allergenicity of the extracts that was causing these adverse reactions, by modifying purified extract using formalin resulting in a 90% reduction in allergenicity (Marsh, Lichtenstein, & Campbell, 1970). However, the reduced allergenicity was only shown *in vitro* and in guinea pigs and not in human clinical trials. In addition, short allergen peptides lacking conformational structure were developed for peptide-AIT, aimed at targeting the allergen-specific T cells without running the risk of IgE crosslinking. In 1996 Norman *et al.* published the first treatment of cat allergy using T-cell reactive peptides (Norman et al., 1996). Next, in 2004, Niederberger *et al.* were the first to clearly show the beneficial effects of using genetically engineered derivates of Bet v1 (recombinant fragments) in a multi-center DBPC clinical study in Birch-pollen allergic patients (Niederberger et al., 2004). The risk of severe side effects of the subcutaneous route also prompted the search for alternative administration routes for allergen immunotherapy, including mucosal application routes.

4.2.2. Sublingual immunotherapy: SLIT

The first randomized DBPC trial with sublingual allergen immunotherapy (SLIT) appeared in 1986 by Scadding *et al.* using HDM preparations in a small group of patients (G. K. Scadding & Brostoff, 1986). In 1998, the world health organization acknowledged that SLIT ('sublingual-swallow' AIT) was a successful alternative to SCIT (Bousquet et al., 1998). SLIT or oral allergy drops involves administering allergen formulations under the tongue daily, and is considered safe, effective, and does not require a build-up phase. SLIT is indicated in ragweed, GP, and HDM allergic rhinitis patients and the sublingual route is considered to also have a preventive effect on new sensitizations as well as the progression of rhinitis to allergic asthma (Okamoto et al., 2017). The successful implementation of SLIT in the clinic was made possible by a number of double-blind clinical studies, including hundreds (~250 to >800) of patients, wherein the optimal maintenance dose for each of the tested formulations was evaluated, with favorable results and good tolerance of a large number of allergen extracts (Hoffmann et al., 2017; Nolte et al., 2016; Passalacqua, Bagnasco, & Canonica, 2019). Compared to SCIT with its safety concerns, SLIT can be used by a larger group of allergic patients (Frati et al., 2009). The application on the sublingual surface has been shown to be convenient for efficient allergen uptake by the dendritic cells and induction of tolerance. Apart from some reported stomach aches, SLIT is considered a safe treatment for allergic rhinitis that reduces symptoms and medication use in allergic rhinitis (Passalacqua et al., 2019). In 1998, the first SLIT clinical trial (20 patients) with HDM tablets confirmed clinical efficacy and a decrease of allergic inflammation (Passalacqua et al., 1998). Besides, the introduction of fast-dissolving tablets for SLIT, rather than using droplets containing allergen extracts, further improved efficacy and convenience. The formal acceptance of SLIT ultimately resulted in 2009 in a first position paper prepared by the World Allergy Organization (Ingram & Kraft, 2012) reviewing 60 RDBPC trials, followed by an upgraded version in 2013 including an additional 77 trials (Canonica et al., 2014). From 2000 onwards multiple milestones for SLIT were published, including SLIT treatment combining multiple allergens in a single droplet application, proof for a long-lasting effect after a three-year treatment duration, and clinical effect in several allergic diseases, including asthma (Passalacqua et al., 2019). Although widely marketed as a viable treatment for allergic asthma, significant variation is still seen in treatment schedules, allergen dosages, and cumulative duration of SLIT. The 2020 GINA guidelines state that SLIT can be considered as add-on therapy in HDM-sensitive asthma patients with allergic rhinitis despite ICS, provided FEV1 is >70% predicted (GINA 2020, 2020).

The only standardized SLIT regimens currently approved by the Food and Drug Administration (FDA), European Medical Agency and Paul Ehrlich Institute include: Oralair™ (Stallergenes), Grazax-Grastek™ (Alk-Abellò), Acarizax (Alk-Abellò) and Ragwitek™. Because SLIT is self-administered and self-managed, the clinical outcome of these treatments depends mainly on patient adherence or compliance throughout the long treatment duration (Incorvaia, Mauro, Leo, & Ridolo, 2016). Adherence in clinical trials is high, but real-life adherence is very variable, from <50% to up to 90% (Senna et al., 2009). In the 3rd year of treatment, according to the sales data from the manufacturer, only around 15% of patients persevere in their daily intake of tablets or droplets (Savi, Peveri, Senna, & Passalacqua, 2013).

4.2.3. Oral immunotherapy: OIT

Based on published observations from Charles Harrison Blackley in 1873, who performed the first skin prick test on his arm (Blackely, 1873), the idea of taking allergenic extracts orally was suggested in 1900 by Curtis (Curtis, 1900), and the first clinical pilots started a few years thereafter. Since then, oral immunotherapy (OIT) was intended to generate a systemic effect, but how to calculate an effective dose for OIT is a major difficulty, since allergens are digested in the gastrointestinal tract. Several OIT trials in the 1980s failed (Bjorksten & Dewdney, 1987), whereas others have demonstrated some efficacy (Bjorksten & Dewdney, 1987; Croner, Gustafsson, Kjellman, & Säwedal, 1986). Positive-outcome studies typically used very high allergen doses and enteric-coated (polymer barrier) tablets, allowing safe passage of the allergens through the stomach. One such example is a study by Taudorf *et al.* in which adults with birch pollenosis were treated daily for 18 months with a birch pollen extract in enteric-coated capsules, using a cumulative dose ~200 times higher than that used in SCIT (Taudorf, 1992). The outcome of this treatment was a significant improvement in different parameters of conjunctivitis, although no improvement was achieved in the parameters of rhinitis. Next, in an OIT study using a mix of crude extracts of four different grass pollen in enteric-coated tablets for daily administration in adults with hay fever, treatment efficacy could not be demonstrated (Mosbech et al., 1987; Taudorf & Weeke, 1983). Here, the cumulative dose was more than 4000 times that of conventional SCIT. Studies on the influence of duodenal juice on birch and timothy GP allergens *in vitro* showed that both are hydrolyzed quickly, with around 10% of allergenic activity left after 30 min (Einarsson, Renck, & Taudorf, 1988). Although in ensuing years patient improvement was reported in a few OIT trials, the drawbacks including high dosages, variable absorption, gastrointestinal symptoms, increased respiratory symptoms, and urticaria remained (Oppenheimer, Areson, & Nelson, 1994). For such reasons, OIT is not considered to be a cost-effective alternative to SCIT or SLIT, and its clinical use was practically discontinued in the early 1990s, although there is currently a renewed interest for desensitization through the oral route specifically for food allergy (Chu et al., 2019).

4.2.4. Local bronchial immunotherapy & local nasal immunotherapy: LBIT & LNIT

In 1951, local bronchial immunotherapy (LBIT) was proposed by Herxheimer (Herxheimer, 1951, 1952) and involves inhaling aerosolized allergens from a closed spirometer. Two DBPC LBIT trials in adults

were performed using HDM extracts, the first showing no significant clinical improvement (Crimi et al., 1991) and the second showing some improvement in lowered symptoms scores and improved bronchial tolerance. However, the latter study also indicated safety concerns for LBIT, as some patients required bronchodilator therapy due to experiencing bronchospasms (Tari, Mancino, & Monti, 1992). These findings show that the clinical effectiveness of LBIT is not proven, and it has a poor safety profile. Although LBIT has been abandoned altogether, it is in use as an interesting experimental model for more fundamental research on mucosal immunity and serological responses to allergens (Holt, Reid, Britten, Sedgwick, & Bazin, 1987).

The initial reports on local nasal immunotherapy (LNIT) were published in the 1970s and this administration route of AIT involves the spraying of allergens directly into the nasal cavity, avoiding the lower airways (Mehta & Smith, 1975; Taylor & Shivalkar, 1971, 1972). LNIT was used for the delivery of pollen allergens as well as HDM. Several formulations were tested: natural extracts, allergoids, micronized powders, and extract-coated strips (Andri et al., 1993; Bertoni, Cosmi, Bianchi, & Di Berardino, 1999; Georgitis et al., 1983; Johansson, Deuschl, & Zetterström, 1979; Passalacqua, Albano, Riccio, Scordamaglia, & Canonica, 1997). Allergic rhinitis patients treated with LNIT showed long-lasting protection from symptoms upon allergen re-exposure. In 1993, Andri *et al.* evaluated the efficacy of LNIT with *Dermatophagoides farinae* (Der f) and *D. pteronyssinus* (Der p) extracts applied as a powder in adult patients with perennial rhinitis for a treatment duration of one year. The use of powders was thought to overcome the disadvantages of aqueous preparations (e.g., poor stability due to container absorption and self-digestion) (Andri et al., 1993). These studies showed that patients treated with LNIT had significantly lower total symptoms scores, medication use, and a decrease in nasal reactivity to the allergen. LNIT generally consists of a build-up phase followed by a maintenance phase, but a simpler treatment regime with a single and constant dose was shown equally efficacious (Bertoni et al., 1999). Although there have been some successful controlled LNIT trials, this treatment has never really become part of standard care, as this local therapy has other drawbacks: a therapeutic response is probably restricted to the upper airways, and repeated topical allergen application increases the likelihood of symptom exacerbation (increased non-specific bronchial reactivity or 'nasal priming'). Moreover, LNIT's particular administration technique requires frequent nasal sprays, high patient compliance, and a very precise control mechanism for the exact dose of allergens administered.

4.2.5. Epicutaneous immunotherapy & intralymphatic immunotherapy: EPIT & ILIT

In 1921, Vallery-Radot published the first case study on epicutaneous allergen immunotherapy (EPIT), which showed that allergen administration on scratched skin decreased allergic symptoms in horse-allergic patients (Vallery-Radot, 1921). Thereafter EPIT, also called intradermal AIT was applied for the treatment of pollen allergy (Scheurer & Toda, 2017). Phillips *et al.* began intradermal injections using pollen in 1923, and demonstrated symptom relief after three injections, and reported that intradermal AIT is both safe and efficacious (Phillips, 1926). During 1950 and 1960 French allergologists explored the use of EPIT, and although not entirely efficient at the time, the concept of epicutaneous administration of allergens to desensitize patients with allergic asthma was proposed (Pautrizel, Cabanieu, Bricaud, & Broustet, 1957). EPIT may mitigate adverse side effects observed in SCIT by minimizing the penetration of allergens into the vasculature, as well as shorten the duration of therapy by increasing immunogenicity of the allergen. Many years later, in 2009, Senti *et al.* performed the first DBPC EPIT study with the aim of (i) improving EPIT towards a safer administration route than that of SCIT and (ii) achieving more adherence than SLIT (Senti et al., 2009). This study reported significantly lowered symptom scores in nasal provocation tests in EPIT treated patients, whereas eczema was observed on the location of the patch as an adverse treatment effect. Currently, EPIT can provide a higher safety and adherence profile, and remains an interesting alternative to SCIT and SLIT for aerosol- as well as food allergies (Esposito et al., 2018).

In the 2005, Johansen *et al.* looked for the most effective immunization route to achieve neutralizing antibody production against purified proteins (Johansen et al., 2005). To avoid severe reactions, nanogram quantities of peptides, were applied by direct injection into lymph nodes under ultrasound guidance. Next to using only very low amounts of allergens, intralymphatic immunotherapy (ILIT) was efficient in mice after only a small number of injections and induced an increased release of IL-2, IL-4, IL-10, and IFN-γ compared to subcutaneous administration, suggesting that ILIT may not polarize the allergen specific response, but induce an improved Th1, Th2, and T-regulatory response overall (Martínez-Gómez et al., 2009). Next to animal models, clinical studies imply that ILIT is not only efficient and safe for the patients but also correlated with a lower risk of systemic adverse effects (Senti et al., 2008). Hylander *et al.* reported that with as little as 3 ILIT injections during the pollen season, symptom relief can be obtained which is equivalent to that obtained after 3 year-SCIT treatment (Hylander, Latif, Petersson-Westin, & Cardell, 2013). However, despite the need for fewer doctor visits, fewer injections, and significantly lower doses of allergens reported in ILIT clinical trials and the reported immunological changes which can be interpreted as *proof of concept*, there is so far no routine use of ILIT in the treatment of allergies, nor is there an authorized ILIT treatment available commercially in any country (Senti et al., 2019).

4.3. Mechanisms of AIT

The essential goal of AIT is to restore immunological tolerance to allergens, by inducing several immunological mechanisms that inhibit both early- and late-phase allergic responses. AIT therefore aims to achieve a decrease in eosinophil, basophil and mast cell numbers and activity, a neutralizing antibody response and induction of peripheral and local allergen-specific regulatory T-cells (Tregs) and regulatory B-cells (Bregs). As such, successful AIT is associated with a change from allergic inflammation driven by Th2 cells to an allergen-tolerant state that is dominated by Tregs, and to lesser extend Th1 cells, and is characterized by reductions in levels of cytokines and chemokines such as IL-4, IL-5, IL-9, and IL-13, and eosinophil chemotactic protein, eotaxin, while levels of IL-10 are increased. Next to the T cell responses, the antibody production from B cells is altered by successful AIT, with a long-term decrease in allergen-specific IgE (after an initial increase upon the start of AIT), and increased levels of neutralizing antibodies such as IgG1, IgG4, and IgA. Such blocking antibodies have inhibitory effects on allergen-binding and complex formation by IgE bound to the cell surface of effector cells. Upon starting AIT, early effects are usually detectable in a decrease in basophil and mast cell activity upon allergen administration. Then, suppression of Th2 occurs with induction of Tregs and Bregs along with an initial decrease in allergen-specific lymphocyte proliferation. In the later phases of AIT, increased blocking antibodies and decreased inflammation in and around the airways is shown. Long term clinical effects of AIT include improvement in quality of life, decrease in medication use, reductions in nasal symptoms (also during natural exposure), accompanied by persistent immunological changes as described above.

4.4. Basophils and mast cells: early responders

Detailed knowledge of the mechanisms involved in the allergen desensitization achieved shortly after starting AIT is of great interest in improving therapeutic approaches in AIT and to overcome unwanted side effects, that are mainly mediated by the allergen-induced IgE-dependent activation of effector cells such as mast cells and basophils (Mukai et al., 2016; Schmetzer, Valentin, Church, Maurer, & Siebenhaar, 2016). In spite of the clinical relevance, limited information is available on the mechanisms by which low-dose allergen extracts can

change the immune responses of basophils and mast cells during the build-up phase of AIT, during the first few hours after the start of treatment by allergen administration. Basophils and mast cells degranulate upon FcεRI-dependent activation and release their mediators (like histamine and tryptase) from intracytoplasmic granules and secrete type 2 cytokines that promote allergic inflammation and IgE production. Mast cell degranulation has been described to occur in either an anaphylactic (full-blown, acute) mode or in a so-called piecemeal-degranulation fashion (Bradding, Walls, & Holgate, 2006). The latter is a form of chronic, low-level degranulation and involves release through intermediate vesicles without the need of secretory granule-transport to the cell cortex and granule-fusion with the plasma membrane (Gangwar, Landolina, Arpinati, & Levi-Schaffer, 2017).

Successful AIT accomplishes early desensitization of both mast cells and basophils, as demonstrated by weaker responses to allergen challenge despite the high allergen-specific IgE levels that are still present during the early phases of the therapy (Novak et al., 2012). The mechanism of this early desensitization of mast cells and basophils might depend on co-aggregation of FcεRI with low affinity IgG receptors (FcgRIIa and FcgRIIb), alerting the activation potential of allergen-mediated IgE crosslinking (MacGlashan Jr. & Hamilton, 2016). Moreover, Novak *et al.* proposed two pathways that would be responsible for early suppression of basophil activation in patients receiving venom AIT (Novak et al., 2012). First, a decrease in overall basophil counts due to increased cell-deletion, and second upregulation of the histamine receptor-2 (H2R) on basophils, mediated by allergens. This study provided comprehensive data for attenuated activation and mediator release of basophils after selective H2R stimulation, which suggests that H2R exerts immunosuppressive effects early during AIT. Overall, it is the general perception that successful AIT, administered either subcutaneous and or sublingual, is directly correlated to a decrease in mast cell and basophil numbers and activity (Larché et al., 2006), whereas the underlying immunological mechanism needs to be further elucidated (Fig. 2).

4.4.1. Responses of the T cell family: from Th2 to Th1, while Tregs dominate

Although Rocklin *et al.* described lymphocyte responses upon AIT as early as in 1973 (Rocklin et al., 1974), an effect of AIT on T cells has long been considered highly speculative (M. Akdis et al., 2004). Nevertheless, peripheral T cell tolerance induction is currently considered to be essential for successful AIT (M. Akdis & Akdis, 2014). The generation of adaptive immunity comprises antigen-specific activation, clonal expansion, differentiation and acquisition of effector functions of Th cells. Naïve Th cells can only by activated by dendritic cells delivering 2 discrete signals through cognate receptor interactions: the MHCII-TCR interaction (signal 1) and the CD80/CD86-CD28 interaction (signal 2) (Jonuleit, Schmitt, Schuler, Knop, & Enk, 2000; Valenta et al., 2010). Without the costimulatory signal delivered through CD28, a state of long-lasting unresponsiveness can be induced in the Th clone, limiting both cellular and humoral immune responses (Wells, Walsh, Bluestone, & Turka, 2001). This cell state of specific unresponsiveness or anergy in peripheral T cells might also be induced by AIT as a marked decrease in allergen-induced T cell proliferation and cytokine production after AIT treatment has been observed (Ebner et al., 1997). Moreover, AIT might result in loss of pathogenic Th2 cell clones (Wambre et al., 2012) (Fig. 4). AIT resulted in deletion of a specific subset of the allergen-specific clones from the T cell repertoire after AIT (Wambre et al., 2014). Interestingly, the clones that were found to be deleted after AIT were the same clones as those present only in atopic, but not in non-atopic, individuals, supporting their contribution to the allergic response to the allergen. The mechanism of selective depletion of individual allergen-specific clones from the T cell repertoire is of great interest but has so far not been elucidated.

Numerous mechanistic studies in humans, as well as in animal models, provide convincing evidence that allergen-specific Th2 cells play an essential role in allergic inflammation (Głobińska et al., 2018). Immune deviation and active immune suppression of these Th2 cells have also been postulated to contribute to AIT. Early studies on AIT using allergen-specific T cell clones show that the Th2 phenotype of the allergen-specific Th response switches towards a Th1 phenotype (O'Brien, Byron, Varigos, & Thomas, 1997). This immune deviation through induction of Th1-type responses was proposed as a mechanism of AIT in several studies, with the Th1 cells counterbalancing or suppressing the Th2 cell activity underlying the IgE-mediated allergy (Durham et al., 1996; Jutel et al., 1995b; Maggi, 1998; O'Brien et al., 1997; Varney et al., 1993). These early studies showed that AIT can result in control of the Th2 immune response, as measured by lowered numbers and activity of Th2 cells and their associated cytokines (IL-4, IL-5, and IL-13, Fig. 2 and 3).

After the discovery of $CD4^+CD25^+$ T cells that contribute to sustaining self-tolerance in 1995 (Sakaguchi, Sakaguchi, Asano, Itoh, & Toda, 1995), a role for regulatory T cells (Tregs) in AIT became a focus in the field. Allergen-specific Tregs can suppress Th2-driven immune responses and the balance between Th2 and Treg cells is essential for the induction or suppression of allergic inflammation. Several distinct subpopulations of Tregs exist, including thymic or natural Tregs (nTregs) which originate from thymic T cell development and recognize self-antigens, and inducible Tregs (iTregs, such as $IL\text{-}10^+$ Tr1 cells), which are induced in the periphery and are generally specific for non-self-antigens (Palomares et al., 2017; Weiner, 2001). Natural Tregs demonstrate their suppressive ability through direct intercellular contact, express FOXP3, CD25 (IL-2 receptor) and have low expression of CD127 (IL-7 receptor), and these cells function via surface glucocorticoid-induced TNFR-related proteins (GITR), cytotoxic T lymphocyte-associated protein 4 (CTLA4), and surface bound TGF-β (Nakamura, Kitani, & Strober, 2001; Sakaguchi, Vignali, Rudensky, Niec, & Waldmann, 2013). In healthy non-allergic people, these nTregs may regulate allergen specific T cell responses as well (M. Akdis et al., 2004; Ling et al., 2004). After successful immunotherapy, both through sublingual and subcutaneous routes, nTregs were found to be increased in numbers and activity (G. W. Scadding et al., 2010; Radulovic, Jacobson, Durham, & Nouri-Aria, 2008). Inducible Tregs exert their regulatory properties via production of IL-10 (Tr1 cells) and TGF-β. Tr1 cells were shown to suppress proliferative Th cell responses and Th2 type cytokine release induced by allergens, whereas TGF-β production limits Th2 cell differentiation via downregulation of the transcription factor GATA-binding protein 3 (GATA3) and induces FOXP3 and CTLA-4 expression on nTregs (Gorelik, Fields, & Flavell, 2000). Moreover, increased levels of TGF-β after AIT can stimulate B cells to produce IgA (Pilette et al., 2007). Finally, in 2011 a specific subset of follicular regulatory T cells (Tfr cells) was identified and reported to have a significant capacity to produce IL-10 after AIT (Schulten et al., 2018). These Tfr cells would thereby be able to directly regulate the AIT-induced antibody responses.

4.4.2. B regulatory cells

Regulatory B cells (Bregs) are immunosuppressive IL-10 secreting B cells and are capable of inhibiting inflammation. Besides production of IL-10, they are capable of secreting high concentrations of IL-35 and TGF-β, contributing to their suppressive capacity (Rosser & Mauri, 2015). The first findings identifying the immunosuppressive capacity of B cells date back to the 1970s and were largely the result of experiments on the sensitization of the immune system using OVA in guinea pigs (Neta & Salvin, 1974b). In 2013, Van de Veen *et al.* demonstrated the existence of Bregs that produce IL-10 (B_R1) in bee venom allergic patients and showed two important contributions for allergen tolerance *in vivo*: B_R1 cells produce specific IgG4 and production of IL-10 contributes to T cell suppression (Schulten et al., 2018; van de Veen et al., 2013).

4.4.3. Responses of the humoral immunity

During successful AIT, the humoral immune response significantly contributes to reducing allergic reactions by the induction of

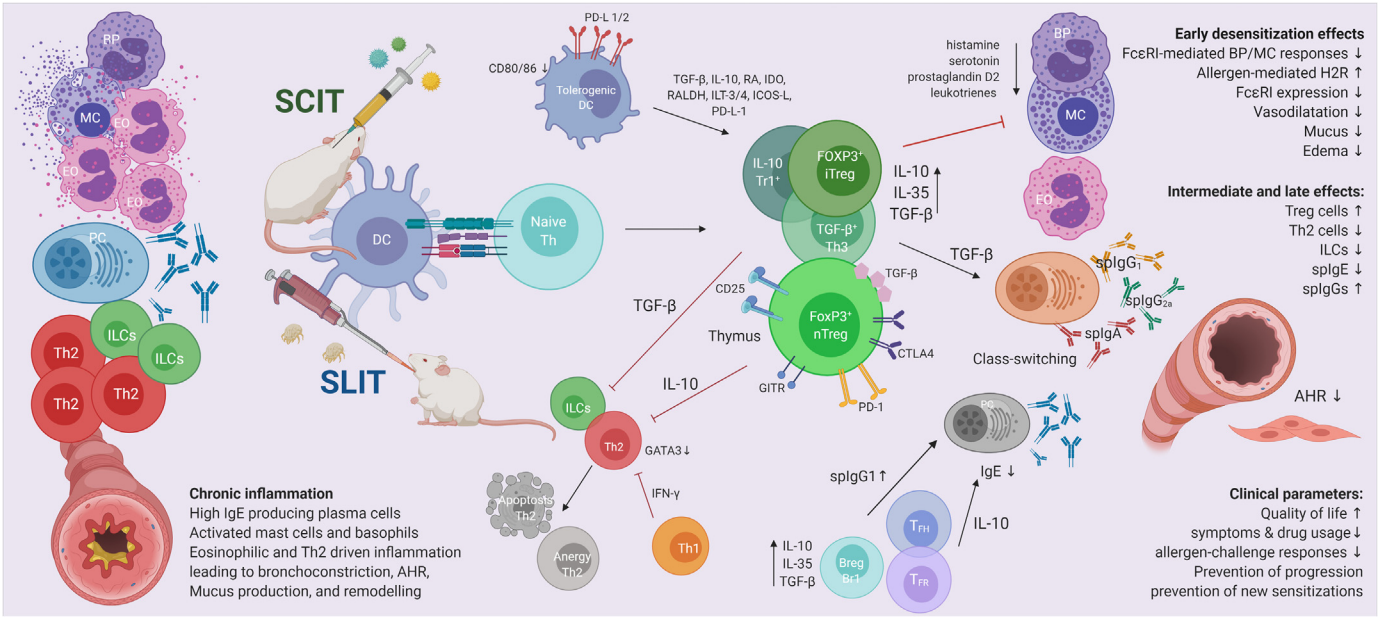

Fig. 4. The effects of Allergen-specific immunotherapy (AIT) in chronic inflammation. Subcutaneous AIT (SCIT) and sublingual AIT (SLIT) is associated with improved tolerance to allergen challenge, with a decrease in immediate- and late-phase allergic responses. AIT is disease-modifying and modifies cellular and humoral responses to allergen. The ratio of T helper 1 (Th1) cytokines to Th2 cytokines is increased following AIT, and functional Treg cells are induced. The production of IL-10 by monocytes, macrophages, B cells, and T cells is increased. The expression of TGF-β is increased and, together with IL-10, TGFβ contributes to Treg function and immunoglobulin class switching to IgA, IgG1, and IgG4. These immunoglobulins compete with IgE for allergen binding, decreasing the allergen capture and presentation that is facilitated by IgE in complex with the high-affinity receptor for IgE (FcεRI) or the low-affinity receptor for IgE (FcεRII). AIT reduces the numbers and the ability of mast cells to release mediators, and the recruitment of eosinophils and neutrophils to sites of allergen exposure. Successful AIT results in improved quality of life, reduced symptoms and drug usage, and prevents progression and new sensitizations. Cell-types: BP, Basophil; MC, Mast cell; EO, Eosinophil; PC, Plasma cell; ILCs, Innate Lymphoid cells; Th2, T helper 2 cells; Th1, T helper 1 cells; Th0, T helper 0 cells; Treg, T regulatory cells; Tfh, T follicular helper cell, Tfr, follicular regulatory cell; Breg, Br1, B regulatory cells. Figure was created using BioRender.com.

neutralizing antibodies and the suppression of specific IgE. In order to achieve desensitization to the allergen, adequate control of the B cell response during AIT is crucial (Larché et al., 2006). Independent of the administrative route, AIT usually results in a rapid but transient initial rise of specific IgE levels in serum. Long-term AIT, however, will result in a decline in serum allergen-specific IgE levels, which promotes long-term clinical tolerance (Scadding et al., 2017). In addition, overwhelming evidence from numerous studies show increases of 10- to 100-fold in serum IgG (total) and allergen specific IgG4 (spIgG4) upon successful AIT, and these are often referred to as the neutralizing or blocking antibodies capable of dampening IgE mediated responses (Jutel et al., 2005; Wachholz, Soni, Till, & Durham, 2003). It was shown that in nasal fluids, spIgG4 is capable of trapping allergens before they can crosslink surface bound IgE on mast cells and basophils, thereby inhibiting release of inflammatory mediators (Shamji et al., 2019). Moreover, spIgG4 may interact with the inhibitory FcγRIIb IgG receptor on the cell surface and suppress IgE-mediated signaling (L. K. James & Till, 2016). SpIgG4 can also repolarize macrophages of the allergic M2a phenotype to a tolerogenic M2b phenotype, characterized by secretion of IL-10 and CCL1 (Bianchini et al., 2019). Although levels of spIgG produced by AIT do not have a strong correlation with reduced symptom scores, a strong association has been reported when comparing symptom improvement with the ability of blocking IgG to compete with IgE for allergen binding and prevent effector cell activation (Shamji et al., 2012).

Similarly, allergen spIgA has important functions to capture antigen at the respiratory mucosa, as well as to control antigen collection by APCs within the oral mucosa (such as Langerhans cells, myeloid and plasmacytoid DCs) and modulate certain immune cell subsets (MacPherson, McCoy, Johansen, & Brandtzaeg, 2008). Serum IgA is present in its monomeric form and originates from long-lived plasma cells in the bone marrow, whereas secretory (or salivary) IgA (S-IgA) is present in a dimer form, containing a joining (J) chain and produced by activated B cells locally at the mucosal tissues. In 2002, Böttcher *et al.* reported that higher levels of S-IgA in Fel d1 sensitized infants have a protective effect on allergic symptoms (Böttcher, Häggström, Björkstén, & Jenmalm, 2002). TGF-β is considered the main cytokine to induce B cell class switching in favor of IgA, representing a key factor in regulatory tolerogenic responses. Pilette *et al.* evaluated serum levels of Phl p5-spIgA1, Phl p5-spIgA2, and polymeric (J-chain positive) IgA antibodies before and after GP-SCIT in allergic patients and found that AIT-induced IgA responses are selective to the IgA2 subclass and associated with levels of IL-10 and increased local expression of TGF-β (Pilette et al., 2007). These findings suggest a significant contribution for spIgA2 antibodies in the tolerance induction upon AIT.

4.4.4. Innate lymphoid cells' response to AIT

Although type-2 innate lymphoid cells make an important contribution to allergic inflammation in barrier tissues (Kubo, 2017), limited data are available on the effects of AIT on ILC2s. Lao-Araya *et al.* report a reduction of lineage negative $CRTH2^{+}CD127^{+}$ILC2s in blood from patients with allergic rhinitis undergoing SCIT during the pollen season, and concluded that it was plausible that ILC2 suppression contributes to tolerance observed upon pollen exposure after SCIT (Lao-Araya, Steveling, Scadding, Durham, & Shamji, 2014). Moreover, HDM allergic patients treated with HDM-SCIT were found to have reduced numbers of ILC2s in peripheral blood after a minimum of one-year of treatment (Fan et al., 2016). In a mouse model of SCIT using birch pollen, numbers of IL-5^{+} ILC2s were significantly reduced in broncho-alveolar lavage fluid and lung tissue after SCIT, when compared to control treated mice (van Rijt, Logiantara, Canbaz, & van Ree, 2018). All in all, these findings suggest that ILC2s might also be suppressed after successful AIT, although this needs to be confirmed in the upper or lower airway mucosa rather than peripheral blood in patients with rhinitis and allergic asthma, respectively.

4.4.5. Dendritic cells: underestimated as regulators of tolerance

Dendritic cells (DCs) play an essential role in determining the outcome of any immune response: they govern the decision of induction of immunity versus tolerance (Funes et al., 2019). Whereas their role in the immune system is well-defined, their tolerogenic role in the response to inhaled allergens was first highlighted in 2001, in a report describing the capacity of pulmonary DCs to produce IL-10 (Akbari, DeKruyff, & Umetsu, 2001). This capacity is essential for tolerance induction and pulmonary DCs can induce tolerance to aerosolized antigen exposures (Akbari et al., 2001). DCs use cues from both the innate and adaptive immune system to induce tolerance or immunity. Lack of damage or pathogen associated molecular patterns, the presence of anti-inflammatory cytokines or cognate interactions with Tregs will all contribute to tolerance induction by DCs. Tolerogenic DCs interact with $CD4^+$ naïve T cells to induce anergy or adaptive Tregs differentiation. Although the capacity of DCs to induce tolerance to inhaled antigens in naïve T cells is of great interest, the situation in AIT is quite different, given the presence of Th2 cell memory for the allergens. Hence, for tolerance induction during AIT treatment, it is in fact necessary to suppress the previously polarized and activated memory Th2 cells. This likely involves active suppression by an IL-10 producing $CD4^+$T cell population (Kunz et al., 2016) in addition to the aforementioned depletion of allergen-specific T cell clones (Wambre et al., 2014), the cellular mechanism of which is yet to be elucidated.

4.4.6. Absence of Eosinophils decreases inflammation in the late phase reaction

In successful AIT a significant reduction of the skin's immediate response to allergen injection as well as signs of the reduction in the late phase reaction in the mucosal layers of the upper airways become apparent after 6–8 months (C. A. Akdis & Akdis, 2011). The late-phase-response is dependent on influx and activation of Th2 effector/memory cells followed by activation and maintenance of eosinophilic inflammation in allergen-exposed tissues (Hogan et al., 2003). Effects of AIT on the late phase responses are important to establish (long term) clinical efficacy, and in case of allergic asthma have been shown correlate to decreases in airway hyper responsiveness, as well as eosinophil numbers and activity (Rak, Löwhagen, & Venge, 1988). GP-AIT in rhinitis patients was shown to decrease numbers of eosinophils and basophils in the nasal epithelium during the pollen season (Wilson et al., 2001).

4.4.7. Clinical outcome parameters of successful AIT

To determine clinical efficacy of AIT, an objective assessment of overall clinical outcomes is important for both clinical trials as well as routine clinical practice. Certain clinical outcome parameters are widely used to evaluate the therapeutic success of AIT treatment, including medication use and symptom scores, as well as quality of life (Fig. 2). Since a patients' personal disease burden and quality of life cannot be completely assessed by classic measurements, a self-rated health-related quality of life (HRQL) is very often used to evaluate efficacy of AIT. The recommended primary outcome indicator in clinical trials is total combined score (TCS): the sum of the total symptom score (TSS) and total score for medication use (TMS), according to the guidelines issues by the European Medicines Agency (EMA) in 2008 (European Medicines Agency, 2009). Although comparison of score-outcomes is widely used in well-powered clinical trials for AIT to describe treatment efficacy, a recent review highlighted great variability between the implementation of different scoring systems, and more importantly differences in interpretation of the results and difficulty to assess standard treatment regimens in daily practice (Makatsori, Pfaar, & Calderon, 2014).

5. Unmet needs in optimizing AIT formulations and adjuvant use

Throughout the past decade of AIT implementation and research, several important limitations to this treatment strategy have become apparent that need to be addressed. Besides improvements in treatment efficacy (early onset of tolerance as well as sustained effects), and product safety (no risk of anaphylaxis or late phase side effects), there is a high need for improved patient adherence (few doses, preferably no injections). Moreover, AIT should have no sensitizing potential (hence be suitable as prophylaxis). Next, from a pharmaceutical perspective, AIT should be applicable for all allergen sources (preferably combined allergen mixes) and easy to produce (Valenta, Campana, Focke-Tejkl, & Niederberger, 2016). Approaches that aim to overcome many of these limitations have resulted in the evaluation of new administration routes, improved standardization of allergen extracts, use of purified major allergens, recombinant allergens or even allergen-derived peptides. Moreover, regardless of the administration route, AIT can be supplemented with an adjuvant to improve its treatment efficacy and thereby shorten treatment duration.

5.1. Adjuvants

With regard to improvements in safety and efficacy of AIT, many research efforts have been focused on developing novel adjuvants (Latin, '*Adjuvare*' = to help) for the treatment. The use of adjuvants is a promising approach that could well help boost efficacy, safety, and shorten the duration of treatment. In general, adjuvants can be characterized as substances with the potential to stimulate or potentiate the therapeutic effect of allergen immunotherapy through various mechanisms without being an immunogenic substance in itself.

Currently four approved, safe and marketed adjuvants are available that are used in AIT treatments (Zubeldia, Ferrer, Dávila, & Justicia, 2019). These are the most commonly used aluminium hydroxide ($Al(OH)_3$, or Alum), as well as microcrystalline tyrosine (MCT), calcium phosphate (CaP) and monophosphoryl lipid A (MPLA). Results from experimental murine models as well as patient studies have provided insight into the mechanism of action of adjuvant supplementation in AIT treatment. The general mechanism of action of adjuvants in AIT is to concurrently improve the immunogenicity of the allergen, whilst precipitating and retaining the allergen at the site of injection to minimize the risk of systemic allergen release leading to anaphylaxis. In the case of Alum, the allergens can be absorbed to the surface of the Alum which functions as a depot, inducing a slow-release system into the body from near the injection site (Jensen-Jarolim, 2015).

Alum as an adjuvant in AIT improves immunogenicity and tolerability of allergens but also increases titers of IgG and IgE and was shown to have the capacity to induce a Th2 cell response en experimental models. In mouse models, the mechanism of Alum has been elucidated in detail (Kool et al., 2008; Kool et al., 2008). All-in-all, three possible mechanisms can explain the enhanced humoral responses induced by Alum supplemented AIT: (i) alum serves as a slow-release depot, (ii) alum alters soluble allergens into particulate form for phagocytosis by APCs, (iii) alum induced local inflammation leads to recruitment, activation, and enhanced antigen capture by APCs (Lambrecht, Kool, Willart, & Hammad, 2009). Indeed, upon injection of an alum-absorbed allergen preparation, an immediate inflammation at the injection site is caused by release of chemokines and cytokines that recruit eosinophils, neutrophils, and monocytes (Jensen-Jarolim, 2015; Kool, Pétrilli, et al., 2008). Irrespective of its widespread use in the clinic, alum does possess several adverse effects, including a strong immunostimulatory capacity - including its potential to induce Th2 cells - which might in theory counteract clinical efficacy in AIT. Moreover, results from several studies show that alum use can result in the formation of itching nodules and can lead to hypersensitivity reactions to alum itself (Kramer & Heath, 2014). Moreover, an individual's predisposition to an exceptionally high aluminium concentration, such as achieved in long-term SCIT, has been postulated to have the ability to induce toxicity (Kramer & Heath, 2014). In a recent study by Benito-Villalvilla *et al.*, use of alum in AIT treatment using allergoids was shown to decrease PD-L1 expression and IL-10 production by human DCs and increase the levels of pro-

inflammatory cytokines (Benito-Villalvilla et al., 2020). Moreover, alum use resulted in reduced numbers of functional FOXP3$^+$ Tregs and promoted Th1/Th2/Th17 responses (Benito-Villalvilla et al., 2020). Taken together, the use of alum in the clinic for long-term AIT injection is not optimal, and safer alternatives are needed.

Several adjuvants are currently under development for use in AIT. These experimental adjuvants mainly target innate immune receptors, such as Toll-like receptors (TLRs) on macrophages and DCs. Different TLRs can direct DCs to induce different Th cell populations: Th1-type responses are enhanced by TLR-4 and TLR-9, and Th2 responses by TLR-2 (Netea, Van Der Meer, Sutmuller, Adema, & Kullberg, 2005). One example of an experimental adjuvans is the use of synthetic immunostimulatory oligodeoxynucleotides that stimulate TLR9 (Wang, Rayburn, & Zhang, 2005). Another example is the use of Virus-like particles (VLPs), nanoparticles of about 20–200 nm in size that are biodegradable and can be engineered or produced to carry allergens on their surface. The use of VLPs to improve treatment efficacy has been demonstrated in many studies, as reviewed by Anzaghe *et al.* (Anzaghe, Schülke, & Scheurer, 2018). In addition, liposomes composed of lipid bilayers can be used to encapsulate allergens and function as adjuvants as well as carrier. Although one RDBPC trial in patients with allergic asthma treated with liposome encapsulated HDM extract showed promising results in inducing blocking IgG responses and lowered eosinophil numbers, no safety data were reported in this study (Zubeldia et al., 2019). Finally, biodegradable polymeric nanoparticles, like chitosan and polylactideco-glycolic acid (PLGA), are excellent delivery vehicles and considered nontoxic, completely biocompatible, and improve macromolecule penetration across the mucosal layer. Liu *et al.* illustrated beneficial effects of using chitosan as a chitosan-*Der f* Nanovaccine in a mouse model of intranasal AIT (Liu et al., 2009). Also in a murine model, Saint-Lu *et al.* showed chitosan formulations to have mucoadhesive properties, induced enhanced uptake of OVA when applied sublingually and enhanced tolerance induction via lowered AHR, eosinophils, as well as specific Th2 responses (Tourdot et al., 2011). More recently, PLGA-Nano particles were used to enhance efficacy of SLIT in a murine model of allergic rhinitis (Hajavi, Hashemi, & Sankian, 2019). Herein, PLGA-formulated rChe a3 (recombinant *chenopodium album* Protein/ polcalcin) SLIT reduced Th2 inflammation, eosinophilia, and increased numbers of Tregs when compared to control treated mice (Hajavi et al., 2019).

5.2. Other adjuvants as modulators of adaptive immune responses

Natural Tregs have the capacity to suppress allergen-specific T cell responses in part via cell-surface proteins such as CTLA4 (Rivas & Chatila, 2016). After successful AIT, nTregs were found to be increased in number and activity (G. W. Scadding et al., 2010; Radulovic et al., 2008). Inducible Tregs on the other hand, exert their regulatory properties via production of IL-10 and TGF-β. TGF-β production then limits Th2 cell differentiation via downregulation of GATA3 and by enhancing FOXP3 and CTLA-4 expression on nTregs (Gorelik et al., 2000) (Fig. 4). CTLA-4 is a CD28 homolog constitutively expressed on nTregs, while only transiently expressed on effector CD4 and CD8 T cells after activation, and acts as a co-inhibitory signal to restrain T cell immune responses (Maazi et al., 2013). Fusion proteins between extracellular domain of CTLA-4 and the Fc portion of the immunoglobulin molecule, or CTLA-4Ig, have potent immunosuppressive properties in animal studies of transplantation and autoimmunity, and have therapeutic potential in chronic inflammatory conditions. For instance, Abatacept® is an FDA-approved drug (based on the CTLA-4Ig fusion protein) for the treatment of rheumatoid arthritis (Picchianti Diamanti et al., 2014). CTLA-4Ig is thought to interact with CD80/CD86 on antigen-presenting cells to avoid CD28 dependent co-stimulation of naïve and memory Th cells (Anzaghe et al., 2018). In addition, CTLA-4Ig may partly exert direct effects on DCs through CD80/CD86 signaling leading to activation of the alternative NF-κB pathway and increased expression of indoleamine 2,3 dioxygenase (IDO), a tryptophan catabolizing immunoregulatory enzyme (Maazi et al., 2013). IDO activity in turn has been shown to enhance nTreg cell number and activity, leading to suppression of Th cell effector functions (Yan et al., 2010). Previously, in an OVA- SCIT mouse model of allergic asthma, it was shown that IDO plays a role in the induction of tolerance after successful SCIT (Taher et al., 2008). Moreover, the same mouse model of allergic asthma was used to show that co-administration of CTLA-4Ig in OVA SCIT suppressed AHR, airway eosinophilia, and spIgE levels (Maazi et al., 2013). Since this effect in the mouse model of allergic asthma was independent of IDO activity, it was concluded that CTLA-4Ig mainly acts via blocking of the CD28 costimulatory signal on T cells.

5.2.1. 1,25-dihydroxyvitamin D3

Modulation of the adaptive immune response is an interesting approach to enhance the induction of Treg cells and achieve effective immune suppression in AIT. 1,25-dihydroxyvitamin D3 (VitD3) has been shown to have the capacity for immune modulation. VitD3 in its physiologically active form binds to the vitamin D receptor (VDR), a nuclear hormone receptor, to exert its biological effects. VitD3 enhances the processing of calcium in the intestines and helps control calcium and phosphate levels in serum contributing to bone metabolism. In addition, VitD3 has immunoregulatory properties that converge on the induction of tolerogenic DCs. VitD3 can be acquired via the diet, through specific food components such as fatty fish. In addition, large amounts of active VitD3 are synthesized in the skin from 7-dehydrocholesterol after UVB exposure. After hydroxylation in either the skin or the liver, 25-hydroxy-vitamin D3 is further hydroxylated in the kidneys by the enzyme 1-α-hydroxylase resulting in the physiologically active metabolite VitD3. VitD3 binds to the nuclear receptor VDR, which hetero dimerizes with nuclear receptors of the retinoic X receptor family and binds to VitD3 response elements in the promoters of VitD3-responsive genes (Colotta, Jansson, & Bonelli, 2017). The skin, lung, colon, lymph nodes and the main cells of the immune system express both the rate-limiting enzymes 1-α-hydroxylase needed for the final hydroxylation to render biologically active VitD3 and the vitamin D receptor. Therefore, DCs, macrophages, and B and T cells are capable of producing VitD3 locally, which can act on immune cells in an autocrine or paracrine manner by VDR binding.

VDR expressing APCs are considered to be the primary target cells for the immunomodulatory effects of VitD3. In these cells, VitD3 has been shown to inhibit expression of NF-kB, preventing the maturation of DCs resulting in relatively low expression of MHC-II and costimulatory molecules (CD40, CD80, CD86) and to enhance IL-10 production (Adorini, Giarratana, & Penna, 2004). This DC phenotype is regarded to be tolerogenic, facilitating the generation of adaptive Treg cells (Table 2). Interestingly, although plasmacytoid DCs have an intrinsic capacity to induce Treg cells upon cognate interaction (De Heer et al., 2004), VitD3 is able to selectively induce tolerogenic capacities also in conventional myeloid DCs (Penna et al., 2007). The physiological serum levels of VitD3 (40-130pM), however, are unlikely to be sufficient activate VDR signaling in DCs, indicating that local VitD3 production is key in inducing tolerogenic DCs through this pathway.

In addition, VitD3 has also been shown to directly affect T-cells *in vitro* and *in vivo*. Boonstra *et al.* demonstrated that VitD3 can enhance Th2 differentiation and cytokine production when present during T-cell activation *in vitro* (Boonstra et al., 2001) (Table 2). A similar effect on Th2 cells *in vivo* may be unwanted, as this could reduce the potential adjuvant effect of VitD3 during AIT by enhancing Th2 cell differentiation (Cantorna, Snyder, Lin, & Yang, 2015). However, it has been shown that VitD3 in combination with Dexamethasone (a synthetic anti-inflammatory glucocorticosteroid) induces IL-10 producing Treg cells *in vitro* (Barrat et al., 2002). In human volunteers, oral VitD3 administration has been shown to selectively increase IL-10 expression by CD4$^+$ T-cells (Urry et al., 2009). Furthermore, Jeffrey *et al.* demonstrated that stimulation of human CD4$^+$CD25$^-$ T-cells in the presence of VitD3

Table 2
Effects of VitD3 on immune and inflammatory cells. CD40, CD80, CD86: Cluster of Differentiation membrane proteins; MHC-II: Major Histocompatibility complex II; IL-10, IL-12: Interleukins; TGF-β: Transforming growth factor β; IFN-γ: Interferon gamma; Treg: Regulatory T lymphocytes; CCR5, CCR10: C-C chemokine receptors; IgA, IgM: Immunoglobulins; VDR: Vitamin D receptor.

Immunomodulatory effects of VitD3		
Cell type	Effects	reference
Monocytes	CD40, CD80, CD86 ↓ Proliferation ↑ Differentiation ↑	Di Rosa et al., 2011
Dendritic Cells	MHC-II ↓ CD40, CD80, CD86 ↓ IL-10, IL-12, TGF-β ↑ Tolerance ↑ Maturation ↓	Adorini et al., 2004 De Heer et al., 2004
$CD4^+$ T cells	Th1, Th9, Th17, Th22 ↓ IL-4, IL-10 ↑ IL-2, IFN-γ, IL-17 ↓ Proliferation ↓ Cytotoxicity ↓ Treg ↑ CCR10, CCR5 ↑	Barrat et al., 2002 Urry et al., 2009 Jeffery et al., 2009 Gorman et al., 2010
B cells	IgA, IL-10 ↑ IgM, IgE ↓ Proliferation ↓ Differentiation ↓	Colotta et al., 2017 Chen et al., 2007
Macrophages	VDR ↑ Mediators ↓	Di Rosa et al., 2011 de Groot et al., 2019

in vitro induces CTLA4 and Foxp3 expression and inhibits cytokine production (Jeffery et al., 2009). These T-cells were able to suppress proliferation of activated effector T-cells, demonstrating that the VitD3-induced Treg cells has suppressive capacity. Interestingly, topical application (dorsal skin surface) of VitD3 in OVA-induced asthmatic BALBc mice enhanced the suppressive effect of $CD4^+CD25^+$ T-cells in draining LNs towards Th2 cells (Gorman, Judge, Burchell, Turner, & Hart, 2010). Moreover, passive transfer of $CD4^+CD25^+$ T-cells from VitD3-treated mice into OVA-sensitized mice suppressed antigen-induced airway inflammation and mildly decreased AHR. Therefore, VitD3 seems to have context-dependent effects on Th cells, and might be used to suppress memory Th2 cell responses *in vivo*.

Macrophages are most-well known for their capacity to release cytokines such as IL-1 and TNF-α to induce local inflammation and recruit effector cells for the elimination of pathogens to the site inflammation. Classically divided into M1 and M2 phenotypes, M1 macrophages release pro-inflammatory mediators (NO, TNF-α, IL-23, IL-12, and IL-1b), eliminate pathogens and promote Th1 and Th17 effector functions. Alternatively activated macrophages, or M2 macrophages are associated with Th2 responses, wound healing and produce IL-10 which has anti-inflammatory properties (de Groot et al., 2019). VitD3 is able to stimulate the differentiation of monocytes into macrophages with an anti-inflammatory M2 phenotype (Table 2) (Di Rosa, Malaguarnera, Nicoletti, & Malaguarnera, 2011).

In B cells, which upon activation express 1-α-hydroxylase and VDR, VitD3 has anti-proliferative effects on multiple stages of B cell differentiation. In addition, VitD3 stimulates IL-10 production by mature B cells and downregulates CD86 expression, suggesting a reduced stimulation capacity for Th cells (Colotta et al., 2017). Moreover, recent studies have shown that VitD3 directly inhibits IgG secretion and the generation of memory and plasma cells and promotes the apoptosis of B cells (S. Chen et al., 2007).

In general, Vitamin D insufficiency is widespread, and has been postulated to contribute to allergy and asthma (Pfeffer & Hawrylowicz, 2018). In some cases, supplementation of VitD3 in clinical studies has had positive effects on certain outcome parameters. For instance, VitD3 supplementation during pregnancy reduced the risk of recurrent wheeze and acute respiratory tract infections in early life (Pfeffer & Hawrylowicz, 2018; Wolsk et al., 2017). Moreover, VitD3 supplementation in asthma patients has been shown to reduce the rate of asthma exacerbations requiring treatment with systemic corticosteroids (Jolliffe, Greenberg, & Hooper, 2018). A systematic review by Martineau *et al.* clearly described that the average number of asthma attacks per patient per year went down from 0.44 to 0.28 with VitD and VitD supplementation reduced the hospitalization risk with 50% (from 6 per 100 to 3 per 100) during an attack (both high quality evidence) (Martineau et al., 2016). The mechanism of action is thought to include both immune modulation towards a more tolerogenic response, as well as reinforcing the barrier and antiviral properties of the bronchial epithelium (Pfeffer & Hawrylowicz, 2018). In recent clinical studies using allergen based SCIT and SLIT treatment protocols, conflicting data were reported with regard to effectiveness of VitD supplementation during AIT (Baris et al., 2014; Jerzynska et al., 2016; Joudi et al., 2019). Baris *et al.* showed only minor effects of VitD3 supplementation on HDM-SCIT in 50 children with allergic asthma, reporting an increase in $FoxP3^+$ T cells and a reduced asthma symptom score as the only improvements compared to control HDM-SCIT treatment (Baris et al., 2014). In contrast, modulation of serum VitD levels in adult patients with allergic rhinitis resulted in favorable outcomes of SCIT, as measured by the quality-of-life questionnaires and Sino-nasal outcome tests, only when VitD status was sufficient (Joudi et al., 2019). Moreover, VitD3 supplementation of GP-SLIT was reported to suppress nasal and asthmatic symptoms in comparison to the control GP-SLIT treated group (Jerzynska et al., 2016). The discrepancy between these studies might be due to differences in allergen used (HDM vs. GP), duration of treatment (12 vs. 5 months) or the route of application of the allergen vaccine.

In addition to the clinical studies, experimental mouse models of allergic airway disease have been used to study the effect of VitD3 levels on parameters of the disease (Hufnagl & Jensen-Jarolim, 2018). In these models, perinatal VitD deficiency in mice has immunomodulatory effects such as Th2 skewing and reduced numbers of $IL\text{-}10^+$ Tregs, which were exaggerated upon allergen challenges (Vasiliou et al., 2014). Murine SCIT models exploring efficacy of VitD supplementation are rather scarce. In one study, Heine *et al.* showed that VitD deficiency promoted allergic sensitization, and co-administration of 25(OH)D in OVA-SCIT reduced airway inflammation, Th2 cytokines, and AHR after allergen challenges (Heine et al., 2014). Moreover, VitD supplemented Der p2-allergoid SCIT in BALB/c mice reduced Th2 cytokines and airway eosinophilia and increased numbers of lung residing Tregs (Petrarca et al., 2016). Next, in two similar studies, increasing the standard VitD3 supplementation in chow from 2000 IU/kg to 10,000 IU/kg resulted in decreased AHR and airway inflammation, indicating that systemic levels of VitD3 do affect airway inflammation and AHR (Agrawal, Gupta, & Agrawal, 2013; Fischer, Hall, & Agrawal, 2016). Finally, VitD3 was proven effective as an adjuvant in OVA-SCIT mouse model of allergic inflammation on airway eosinophilia, Th2 related cytokines, and lowered specific IgE levels (Taher, van Esch, Hofman, Henricks, & van Oosterhout, 2008).

6. Formulations: crude extract allergens, recombinants, and peptides for use in AIT

For optimal treatment response in AIT, it is of critical importance to select the most appropriate form for administration of the allergen, or a derivative thereof. Traditionally, allergologists around the world are familiar with treatment regimens based on application of crude allergen extracts, which are easy to produce, prepare and market against low prices. Crude extracts contain multiple major allergens from a specific source and are easy to combine when patients are poly-sensitized. Despite the clinical success of crude extracts in SCIT and SLIT, the safety of this treatment has always been a concern and standardizing natural allergen extracts remains a major challenge. Use of crude allergen extracts for AIT and for diagnostic purposes has several disadvantages: production of the crude extracts has high batch-to-batch variation,

including that of the percentage of major allergen; extracts may contain contaminations from other sources; and crude extracts always contain a mixture of allergenic and non-allergenic substances. Timothy GP extract, for instance, consists of a variety of allergenic and non-allergenic proteins and glycoproteins along with other components like nucleic acids, carbohydrates, and numerous small metabolites. The majority of the adverse events in SCIT are the result of IgE-dependent basophil and mast cell activation upon injection or administration of crude allergen extracts (Valenta et al., 2016), that retain their allergenicity.

Numerous attempts have been made to reduce the allergenicity of the immunotherapy treatment while retaining the potential to suppress Th2 activity and restore tolerance to the allergen (Fig. 5). Examples include modulation of the tertiary allergen structure through chemical modifications, recombinant DNA engineering strategies for proteins, bacterial DNA oligomer-conjugation to purified allergens, or allergen multimer-production (Larché, 2005). Already in the 1970s, Marsh *et al.* investigated the use of purified allergenic component of group I rye GP in guinea pigs for the possibility of destroying selectively the allergenic properties, while retaining its original immunizing capacities (Marsh et al., 1970). Results, however, showed that allergoids had no residual hapten-like activity (i.e. did not bind to cell-bound IgE) and were unable to inhibit histamine release after allergen-exposure (Pickl, 2012).

In 1999 Valenta *et al.* reviewed a new concept of using recombinant allergens for diagnostic and treatment in AIT (Vriala et al., 1999). This concept builds on the shortcoming of the use of crude extracts for diagnostic purposes in determining the sensitization pattern against the individual components of that allergen of an allergic subject. The specific IgE reactivity profile of a patient can be quantitatively defined by the use of recombinant allergens in an *in vitro* diagnostic tool, such as an recombinant allergen array (Lupinek et al., 2014). Cross-reactive IgE can also be quantified in this approach, when a large array of individual components is used on the diagnostic array, which can help to detect clinically important sensitization patterns to a large variety of allergens for each individual patient. In addition, such component-resolved diagnosis would also allow for the selection of the major allergens to be used for specific immunotherapy in a highly personalized fashion, paving the way for precision AIT. In 2005, Jutel *et al.* reported results of a RDBPC SCIT study using 5 recombinant GP allergens in allergic patients with or without asthma. Herein, symptom medication scores and specific immunoglobulin responses (lowered IgE and increased IgG4) showed treatment effectiveness and a good safety profile after (Jutel et al., 2005). Thereafter, numerous experimental as well as clinical studies were performed using purified and or recombinant allergens for AIT most of which with success, as outlined by Zhernov *et al.* in 2019 (Zhernov, Curin, Khaitov, Karaulov, & Valenta, 2019).

In 2000, results obtained from allergic patients from Swedish and French populations with genetically engineered hypoallergenic birch pollen allergens used in skin prick tests showed lower reactivity with the modified allergens (Pauli et al., 2000; van Hage-Hamsten et al., 1999). Genetically modified derivates with hypoallergenic features also had reduced risk of anaphylactic side-effects and therefore represent new candidates for AIT with reduced allergenicity (Fig. 5). However, although allergenicity was shown to be reduced, some studies still reported side effects due to late-phase allergic responses, and not all DBPC clinical AIT studies using genetically modified allergens showed effectiveness (van Hage-Hamsten et al., 1999). Moreover, Puhorit *et al.* reported some late phase side effects and weak trend towards improvement of symptom scores and increased blocking antibody responses, after genetically modified-Bet v1 derivate SCIT in birch pollen allergic patients (Purohit et al., 2008).

In general, an allergen particle holds several distinct epitopes, as defined in 1960 by Jerne (Fig. 5) (Jerne, 1960). A sensitized immune system has specific IgE antibodies produced against certain epitopes that are part of the allergen (Prickett, Rolland, & O'Hehir, 2015). These antibodies recognize part of the protein in its native, 3D folded structure, a so-called conformational epitope. However, the same antigen is also recognized by $CD4^+$ T cells. The $CD4^+$ T cells recognize a short, linear peptide presented in the context of MCH-II on antigen-presenting cells. Hence, the allergen has both B cell and T cell epitopes, which are distinct, and in case of B cell epitopes non-linear parts of the allergen. T cell epitopes are generally short (~15–30 amino acids), lack conformational structure, and are unable to link cell-bound IgE or activate mast cells/ basophils, whereas B cell epitopes do cross-link spIgE and cause degranulation and mediator release (Pomés, 2010). Both linear (for food AIT) and conformational (for inhaled allergens) B cell epitopes were classified and studied to understand the immune regulation of B cell allergenic determinants and the nature of AIT using recombinants (Fig. 5). In a sensitized patient, use of the T cell epitopes in AIT might have the capacity to safely modulate the T cell responses. This has encouraged the production of advanced therapies to guide the cellular immune response to peripheral tolerance. Currently, short peptides are developed to include immune dominant T cell epitopes with minimal IgE-binding and insufficient stimulating capacity for inflammatory

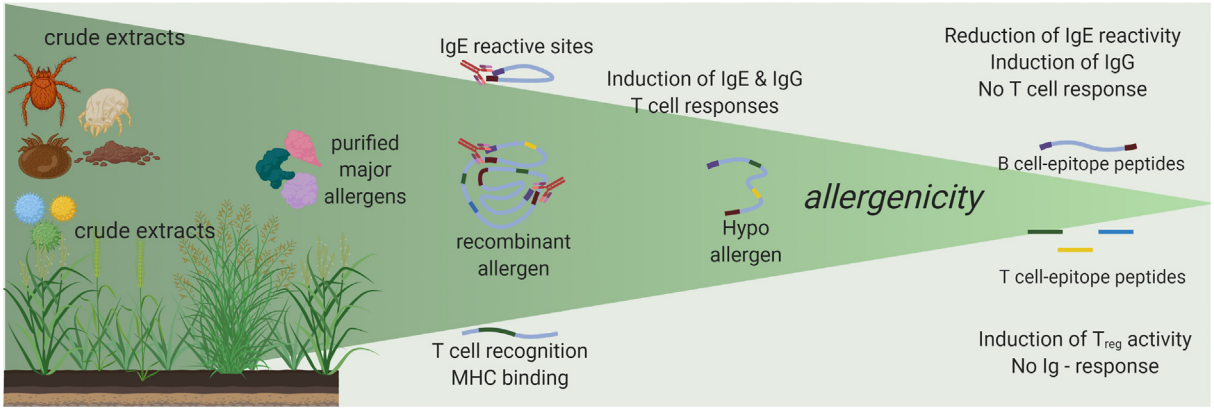

Fig. 5. Overview of the reduction in allergenicity. From Left to right: Crude allergen extracts, like pollens, house dust, animal dander, spores of mold, cosmetics, feathers etc. can be identified as highly capable of inducing an allergic reaction upon sensitization. Crude extracts contain major allergens that can be naturally purified, or its isolated mRNA can be used to produce recombinant allergens with reduced allergenicity. Usually, both purified and recombinant allergens maintain T cell recognition sites as well as IgE binding sites, whereas an hypoallergen is characterized by a reduction of IgE reactivity and maintenance of T cell epitopes. Shorter peptide sequences containing T cell recognition can be identified and isolated using T cell epitope mapping from allergen specific T cell clones. To obtain B-cell epitope peptides, peptide-sequences are selected without IgE binding capacity nor T cell stimulation capacity, however, possess strong IgG inducing responses. These B-cell epitope peptides promise to be very safe vaccines and hold great potential in allergy prevention. Figure was created using BioRender.com.

cells. After only a short course of treatment, their presentation in a non-immunogenic form induces long-lasting, specific T cell unresponsiveness (Sandrini, Rolland, & O'Hehir, 2015). In 2018, Niederberger *et al.* reported positive results obtained in a multicenter RDBPC SCIT study, wherein BM32 was used. BM32 is a vaccine containing fused non-allergic B cell epitope containing peptides, derived from GP allergen-IgE binding sites, using the hepatitis B PreS protein (Niederberger et al., 2018). SCIT using BM32 was well tolerated, induced specific IgG levels, and improved clinical symptoms after two years, when compared with placebo treated patients. Next, a RDBPC Phase II-trial using Fel d1-derived peptides (Cat-PAD) in patients with rhinoconjunctivitis showed improved treatment efficacy and safety up to 1 year after start of the treatment (Patel et al., 2013). In a follow-up of the same study, subjects exposed to cat allergens in an environmental exposure chamber two years after start of the treatment and still showed reduction in symptoms of rhinoconjunctivitis (Couroux, Patel, Armstrong, Larché, & Hafner, 2015). However, adequate phase III trials (more than 300 patients) using peptides in either SCIT or SLIT regimens are currently lacking.

When compared to treatment efficacy of AIT using crude extracts as routinely in the clinic, the use of purified or recombinant allergens or allergen-derived peptides in AIT has not yet proven to be more effective. Hence, development of a superior allergen immunotherapy is currently topic of ongoing research (Gunawardana & Durham, 2018).

7. Future perspectives

Although AIT has been used successfully for more than a century, patients with allergic airway diseases still have a lot to gain from improvements in AIT treatment, not only through a better quality of life and

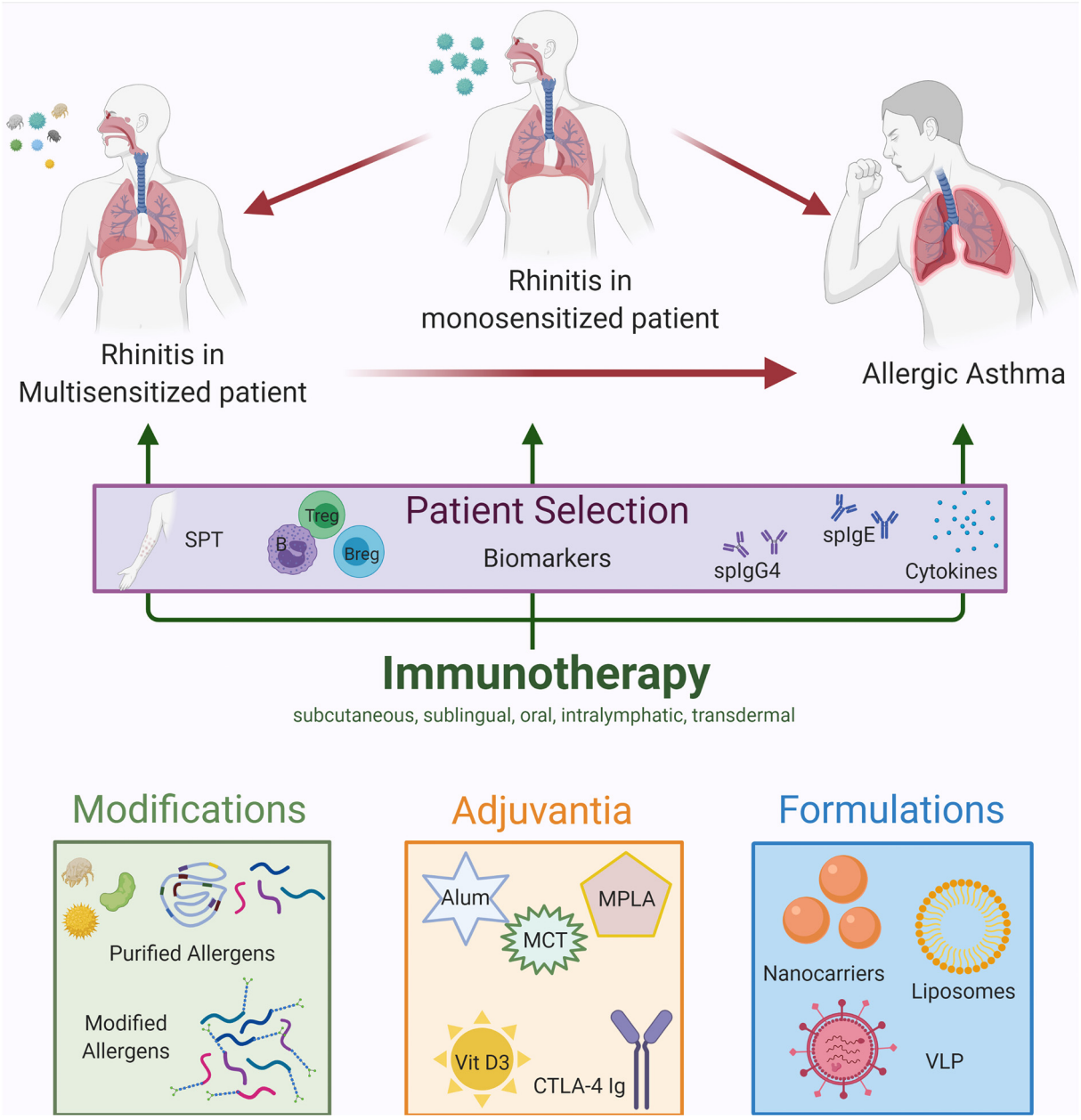

Fig. 6. Perspectives on precise diagnostics and inclusion, possibilities, and strategies in the broad field of allergen immunotherapy. Abbreviations: SPT: Skin prick test; B: Basophil, Treg: T regulatory cell; Breg: B regulatory cell; sIgE: Specific Immunoglobulin E; sIgG4: specific Immunoglobulin 4; Alum: Aluminium hydroxide; MCT: microcrystalline tyrosine; MPLA: monophosphoryl lipid A; Vit D3: Vitamin D3; CTLA-4 Ig: Cytotoxic T lymphocyte-associated antigen-4-Ig; VLP: Virus like particles. Figure was created using BioRender.com.

cheaper treatments with increased efficacy or safety and shorter treatment duration, but also from an earlier diagnosis and start of treatment. Treatment of allergic diseases with AIT from their inception in early childhood, including treatment of children with atopic dermatitis, might be tested for its capacity to prevent the atopic march and other patterns of allergic comorbidities (Čelakovská et al., 2020). Current strategies to improve the treatment of allergic disorders using AIT are focused on several aspects including: (i) prevention strategies: allergen vaccination strategies to prevent progression from rhinitis to asthma or from mono-sensitization to multi-sensitization; (ii) strategies for selection of patients that will benefit most from AIT through the (identification of the) right biomarkers; (iii) strategies to increase the safety profile and thereby the tolerability of the treatment regimens, that can be increased via purification, use of recombinant allergens and or allergen-derived peptides; (iv) to improve treatment efficacy that can be enhanced via the use of adjuvantia, like CTLA-4 and VitD3; or via antigen-targeting, like the use of glycated products; or via improved treatment formulation by using carriers, like liposomal formulated depots, that encapsulate allergens, whether or not supplemented with an adjuvants (Fig. 6).

An improved AIT would be safe and efficacious. While technological advances such as use of adjuvantia, formulations and targeted delivery can improve the efficacy of AIT, another approach to optimization of AIT treatment is patient selection and development of a precision AIT, with multiple possible treatment regimens that depend on the individual patient profile. Treatment of mono-sensitized patients suffering from upper airway symptoms versus multi-sensitized patients and/or those that are involved of the lower airways likely requires different AIT treatment protocols. One approach would be to combine component-resolved diagnostics with a 'toolbox' of AIT peptides with decreased allergenicity and improved immunogenicity. In addition, other biomarkers than allergen specific IgE are urgently needed that predict treatment response to AIT, further allowing an improved patient selection for AIT treatment.

Other approaches to develop novel, safe therapeutic allergy vaccines for use in AIT for HDM, GP, or any other allergen include the generation of recombinant hypoallergenic combination vaccines, which were shown to have limited IgE reactivity, whilst retaining its T cell epitopes and the ability to induce neutralizing antibody response in experimental models that could block IgE (Asturias et al., 2009; K. W. Chen et al., 2012). The use of these hypoallergenic recombinant vaccines holds the promise of inducing fewer side effects during therapy. For example, Der p1 peptides have also been delivered on virus-like particles, inducing IgG responses within 4 weeks after a single injection in healthy subjects (Kundig et al., 2006). These approaches involve the use of recombinant hypoallergenic proteins or peptides, while the use of purified natural proteins, with high purity and pharmacologically well defined, retains the capacity to crosslink IgE. Although hypoallergenic proteins are considered to have a better safety profile during treatment, is currently unknown whether hypoallergenic vaccines have a comparable therapeutic efficacy compared to IgE-activating allergens. Initial studies show that hypoallergenic proteins can induce neutralizing antibodies that inhibit allergen-mediated crosslinking of IgE (C. A. Akdis & Akdis, 2011; K. W. Chen et al., 2012). IgE crosslinking vaccines might have some additional therapeutic efficacy due to so-called piecemeal degranulation of mast cells and basophils, which is thought to contribute to the immediate desensitization and protection against allergic responses especially during the early phase of treatment due to inactivation or exhaustion of these effector cells (C. A. Akdis & Akdis, 2011). Further research will need to establish whether purified natural allergens, that can be produced in relatively high quantities under strictly controlled conditions at relatively low costs and address both effector cell responses, B and T cell activity, or recombinant hypoallergenic or peptide vaccines that require far higher productions costs and mainly address the T cell response, will be the most optimal treatment for AIT.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

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