



Mucosal IgE immune responses in respiratory diseases

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IgE is the less abundant immunoglobulin isotype in serum and displays higher affinity for its cognate Fc receptor (FcεRI) than the rest of antibody isotypes. Moreover, the class switch recombination and the generation of memory responses remarkably differ between IgE and other isotypes. Importantly, class switch recombination to IgE can occur in the mucosae, preferentially through the sequential switching from IgG. Therefore, resident effector cells get rapidly sensitized, and free IgE can be found in mucosal secretions. All these aspects explain the involvement of IgE in respiratory diseases. In allergic rhinitis and allergic asthma, the IgE-sensitization to environmental allergens triggers an eosinophilic inflammation of the airway mucosa of atopic patients. In recent years, growing evidence indicates that some non-atopic patients with nasal reactivity to allergens display nasal eosinophilic inflammation, which could be triggered by the local production of allergen-specific IgE. This phenotype has been termed local allergic rhinitis. Mucosal IgE is also implicated in the pathophysiology of chronic rhinosinusitis with nasal polyps, even though the mechanisms for IgE synthesis might differ in this case. The role of IgE as mediator of airway diseases identify this marker as a therapeutic target. Some biologicals antagonize IgE-mediated inflammation of the airway mucosa, but they have not shown a beneficial long-term effect after discontinuation. In contrast, allergen immunotherapy does not only control the symptoms of airway allergy, but it also induces a long-lasting effect after discontinuation, thus modifying the natural course of the disease.

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Introduction

The production of high-affinity IgE is tightly regulated at the molecular and cellular levels, resulting in serum concentrations, which are several orders of magnitude lower than those of IgG or IgA [1,2]. In contrast, the proportion of IgE in the respiratory secretions is much higher than in the bloodstream, and this relative abundance is especially significant in the case of IgE directed against aeroallergens [3]. Of note, the respiratory and intestinal mucosae harbour the largest populations of IgE-producing plasma cells (PC) in the human body [3], and a significant proportion of circulating IgE is believed to originate in the mucosae with subsequent spill over through the lymphatic system [4]. Unlike other immunoglobulin isotypes, IgE exerts its main biological functions after binding to its specific receptors [2], which display a very high affinity/avidity for IgE [5]. This fact explains why in physiological conditions most IgE molecules in the human body are found attached to its receptors in mucosal effector cells, rather than free in serum [3]. In this review, we aim to analyse the novel discoveries about IgE biology and regulation, with a strong focus on the roles of mucosal IgE in several respiratory conditions. Finally, the different approaches to modulate IgE biology in human disease will be discussed.

Regulation of mucosal IgE production and activity

Generation of IgE responses

Class switch recombination to IgE (εCSR) in germinal centre (GC) B cells has a stronger dependence on T follicular helper (Tfh) cell-collaboration than other immunoglobulin isotypes [6]. Activated Tfh cells stimulate IgM+ naïve B cells via CD28/CD86, CD40 *ligand* (L)/CD40, *inducible co-stimulator* (ICOS)/ICOSL, and *programmed death* (PD)1/PD1L interactions [7,8]. Tfh cells also provide IL-4, the key inducer of εCSR, and IL-21, which potently boosts antibody synthesis in activated B cells. In the absence of functional Tfh cells, the production of IgE in response to mucosal antigens is greatly impaired [9]. Moreover, several subsets of T regulatory cells, such as IL-10+ or TGFβ+ inducible Treg (iTreg) cells have the ability to antagonize εCSR in activated B cells [10]. Recently, the capacity of IL-35+ iTreg cells to inhibit IgE responses has been demonstrated, as they favour the generation of IgA+ and IgG4+ PCs and of IL-35 + B regulatory cells [11••].

Switched IgE+ B cells in the dark zone of GCs cannot efficiently traffic to the light zone in order to undergo

their positive selection after somatic hypermutation [12]. This fact determines an increased apoptosis of IgE⁺ B cells in the dark zone of GCs and an impaired formation of IgE⁺ memory B cells (Figure 1). These factors result in a low frequency and an insufficient affinity maturation of GC-derived IgE antibodies [13^{*}]. In contrast, high-affinity IgE can be produced by IgG⁺ memory B cells in the mucosae following ϵ CSR [14]. This sequential ϵ CSR (from IgM to IgG followed by from IgG to IgE) probably represents an additional checkpoint for IgE production reflecting that strong evolutionary pressure operates to limit, but not eliminate, the production of high-affinity IgE [6]. Although it is not clear whether mucosal ϵ CSR is necessarily associated to GC-like structures, mucosal lymphoid aggregates were detected in patients with type 2-dominated nasal inflammation [15^{*}].

IgE exerts its biological functions by binding to its high-affinity and low-affinity receptors (Fc ϵ RI and CD23, respectively) [14]. The allergen-induced cross-linking of two IgE/Fc ϵ RI complexes on the cell surface, triggers a potent activation of mast cells and basophils [14]. Monomeric IgE and IL-3 favour the expression/stabilization of Fc ϵ RI on the cell surface [16,17], whereas Fc ϵ RI activity is regulated by other surface receptors like Fc γ RIIB [18]. There is a positive correlation between the density of CD23 molecules on B cells, the serum IgE level, and the IgE-facilitated presentation of allergens to T cells [19]. Several mediators compete with IgE for the binding to allergens, thus preventing the formation of allergen/IgE/receptor complexes. The most potent competitors are antibodies with the same specificity but different class (IgG₄ or IgA) [20,21,22^{**}], but also

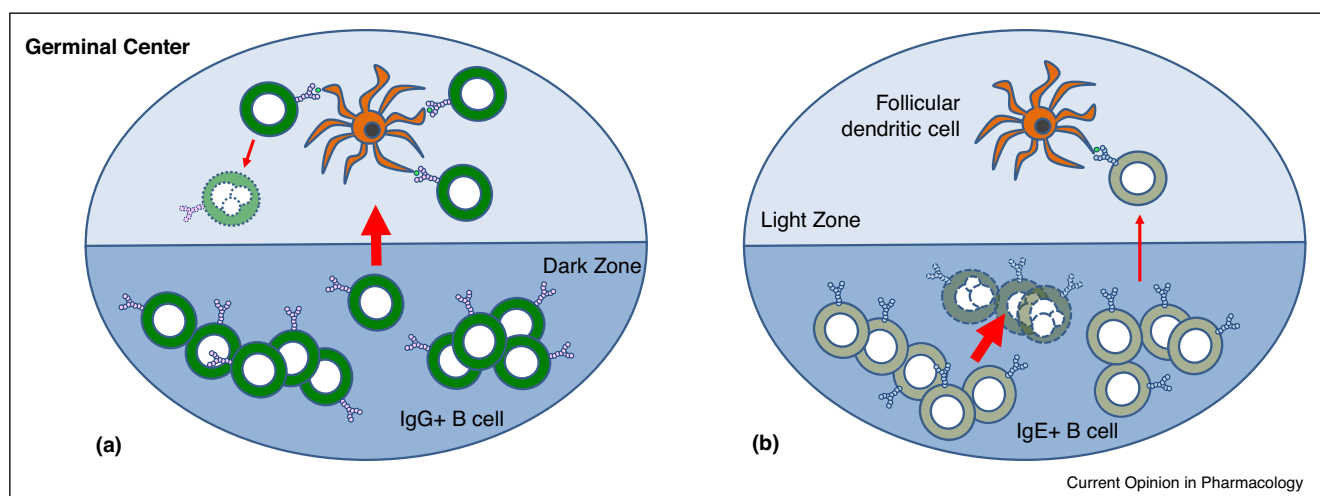
unspecific compounds, like surfactant protein D [23], can exert a similar blocking activity. These mediators are able to inhibit the IgE-dependent activation of basophils and other effector cells, and to reduce IgE synthesis by B cells and Th2 cell proliferation [20,21,22^{**},23].

Memory IgE responses

Serum IgE in mice is mostly synthesized by short-lived PCs, with a minor contribution of long-lived IgE-producing PC/memory B cells [24–26]. Initial studies suggested that IgE⁺ GC cells could account for the majority of IgE-producing PCs in mice [27]. However, later evidence demonstrated that memory IgE responses were maintained by IgG₁⁺ GC or memory B cells [28,29^{**}], which undergo sequential CSR upon interaction with IL-4-producing T cells. Importantly, a study analyzing mice with impaired CSR to IgG₁ showed a deficient affinity maturation process for IgE antibodies that was partially restored after adoptive transfer of IgG₁⁺ B cells in the presence of T cell-derived IL-4 and IL-13 [30,31].

The studies about human IgE immune responses are largely consistent with those performed in mice, yet a greater contribution of long-lived PCs to the pool of IgE antibodies might exist [32]. Of note, IgE-producing memory B cells are present in the blood of allergic patients [33]. Nevertheless, human studies with varied experimental designs have demonstrated the role of IgG⁺ cells as source of IgE-producing cells: investigation of the specificities of IgG₁, IgG₄ and IgE antibodies in allergic individuals [34], sequencing of the S μ –S ϵ switch regions of human IgE genes [35,36], or repertoire analysis of Ig genes in peripheral blood B cells [37]. Moreover, nasal

Figure 1



Differential germinal centre reaction in IgE immune responses: (a) after somatic hypermutation, IgG⁺ or IgA⁺ B cells migrate at high rates from the dark zone to the light zone of the germinal centres. In the light zone, follicular dendritic cells re-expose B cells to their cognate antigens. Those cells not having increased their affinity die by apoptosis in the light zone, whereas the rest of the B cells continue their maturation process (positive selection); (b) mutated IgE⁺ B cells do not efficiently traffic from the dark zone to the light zone. Therefore, most of them die by apoptosis in the dark zone of the germinal centers without being able to carry out the positive selection process.

allergen exposure increases circulating IgE in allergic patients, indicating that the allergen is the major trigger of memory IgE responses [38].

Mucosal IgE in airway diseases

Allergic rhinitis (AR)

AR patients are defined by chronic rhinitis symptoms, clinical reactivity to allergens (positive nasal allergen challenge (NAC)), and the presence of atopy (positive skin prick test (SPT) and/or serum allergen-specific (s) IgE) [39]. The nasal exposure to the allergen in AR individuals induces an early activation of resident mast cells, followed by a later increase of eotaxin and type 2 cytokines in mucosal secretions [40]. This local effect is paralleled by a peripheral response characterized by an early activation of basophils, and a later increase of circulating T effector cells, innate lymphoid cells 2 (ILC2), and dendritic cells [41,42]. The allergen exposure also triggers *activation-induced cytidine deaminase* (AID) expression and sequential ϵ CSR in the nasal mucosa of AR patients [43,44,45]. This phenomenon results from the allergen-induced release of IL-4 and IL-13 by mucosal resident cells [45]. Moreover, in the nasal mucosa of AR patients there are significantly more B cells and PC expressing sIgE than in healthy non-atopic controls [46]. The natural exposure to the allergen is associated in AR patients with an expansion of mucosal IgE repertoires, yet all of them remain closely related (oligoclonality) [47,48]. Moreover, the allergen-specific clones had higher likelihood of having undergone sequential ϵ CSR than the rest of the IgE repertoire [49]. The allergen-driven expansion of mucosal IgE repertoire could reflect either ongoing mucosal somatic hypermutation, or that different cells derived from a single B cell progenitor undergo independent rounds of somatic hypermutation in the GC, and subsequently and independently, experience ϵ CSR in the nasal mucosa [47,49]. Furthermore, mucosal IgE changes are paralleled by a less prominent repertoire expansion in peripheral IgE antibodies [47]. Because AR patients display a clonal overlap between IgE antibodies in blood and in nasal mucosa, this observation suggests a mucosal origin of circulating sIgE in these individuals (Figure 2).

Local allergic rhinitis (LAR)

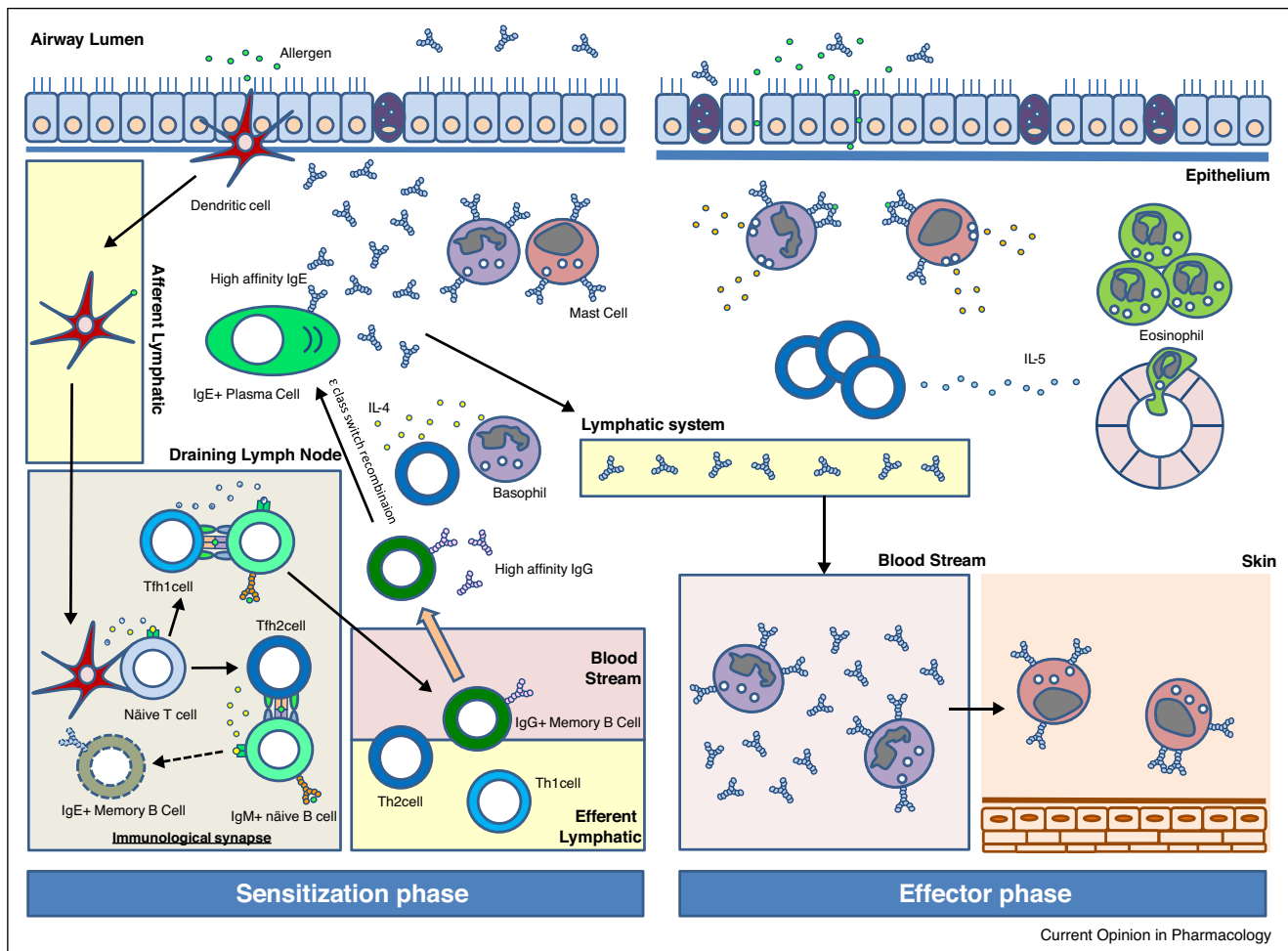
LAR is a new rhinitis phenotype characterized by the positivity of NAC and the negativity of SPT and serum sIgE [50]. The nasal secretions of LAR patients display signs of type 2 inflammation including increased eosinophils and mast cells, together with elevated activation markers (eosinophilic cationic protein and tryptase, respectively) [51,52,53]. Nevertheless, the involvement of sIgE in LAR pathophysiology is still controversial. The presence of house dust mite (HDM)-sIgE in nasal secretions of non-atopic patients with chronic rhinitis and positive NAC was reported as early as 1975 [54]. A later study demonstrated the presence of grass-sIgE in the nasal mucosal of 3 of 10 non-atopic rhinitis patients, 7 of

11 AR individuals, and in none of the 12 non-atopic healthy controls [55]. In contrast, nasal HDM-sIgE was detectable in six AR subjects, but in none of the non-atopic patients or controls [55]. Nevertheless, the study protocol did not include a NAC, and thus the clinical relevance of the allergens in those patients could not be established. More recently, several authors reported the presence of sIgE (against pollens, HDM and *Alternaria*) in the nasal secretions of highly variable proportions (9–100%) of LAR patients [51,52,56–59]. Yet the presence of nasal sIgE has been demonstrated out of the pollen season [51,56], kinetic studies in LAR individuals indicate a fast and progressive increase of sIgE in the nasal secretions during the first hours after a NAC [52,58]. Furthermore, though the differences in the proportion of patients with detectable nasal sIgE may be partially explained by dilutional effects or methodological aspects, a heterogeneity of mechanisms driving nasal allergen reactivity in LAR individuals cannot be excluded. Moreover, the source of the nasal sIgE in LAR patients remains undefined, as the local synthesis of IgE has not been demonstrated.

Chronic rhinosinusitis with nasal polyps (CRSwNP)

Most non-Asian subjects with CRSwNP display eosinophilic nasal polyps (NP), together with a marked inflammation of the mucosa lining the nostrils and the paranasal sinuses. The eosinophilic inflammation in CRSwNP is orchestrated by type 2 cytokines, especially IL-5 [60]. In the NPs, increased numbers of B cells and PCs are present, together with vast amounts of IgE [61]. The nasal cavity of these individuals is often colonized by *Staphylococcus aureus* (SA), whose enterotoxins (SAE) behave as superantigens and drive the polyclonal activation of T and B cells resulting in a preferential, yet not exclusive, synthesis of type 2 cytokines and IgE [61]. Moreover, these patients often display detectable systemic and local sIgE directed against aeroallergens and SAE [62]. Though it is unclear whether this sIgE arises from a superantigen-induced polyclonal activation or from a GC-reaction, it has been demonstrated that local grass pollen-sIgE and SAE-sIgE can efficiently mediate the activation of effector cells [63]. This observation suggests that the affinity of local sIgE in CRSwNP patients is high enough to bind allergens in a functional manner. In this regard, markers of ongoing affinity maturation of IgE antibodies have been demonstrated in eosinophilic NPs [15,64]. Recently, it was suggested that the allergen-triggered IgD-mediated activation of mast cells might favour IgE synthesis in ϵ -switched B cells from eosinophilic NPs, through the release of IL-4, IL-13, *B-cell activating factor* (BAFF) and IL-21 [65]. Yet several aspects remain unclear (is local affinity maturation restricted to GC reaction-activated B cells, or is it also occurring in superantigen-activated B cells?), sIgE (and maybe sIgD) seems to function as an amplifier

Figure 2



Mucosal synthesis of IgE in patients with respiratory allergy: During the sensitization phase of airway allergy, allergen-activated dendritic cells traffic through the lymphatic vessels to the germinal centre of the draining lymph nodes. Within this location, dendritic cells activate allergen-specific naïve T cells to generate T helper (Th) and T follicular helper (Tfh) 1 and 2 cells. Tfh1 cells subsequently stimulate allergen-specific IgM+ naïve B cells which undergo class switch recombination (CSR) to IgG and somatic hypermutation to give rise to IgG+ plasma cells (not shown) and IgG+ memory B cells. Allergen-specific Th1 and IgG+ B cells exit the lymphoid system through the efferent vessels and reach the blood stream. Activated Tfh2 cells in the germinal centres also stimulate IgM+ naïve B cells which undergo CSR to IgE. Nevertheless, IgE+ B cells cannot efficiently carry out their somatic hypermutation, and die by apoptosis before exiting the germinal centres. Conversely, Th2 cells reach the blood stream and extravasate at the lamina propria of the airway mucosa, together with Th1 cells (not shown) and IgG+ memory B cells. In the lamina propria IgG+ memory B cells can undergo sequential CSR to IgE upon allergen re-encounter (not shown) and stimulation with IL-4 provided by Th2 cells and basophils among other cells. This process results in the generation of IgE+ plasma cells releasing vast amounts of high-affinity IgE to the lamina propria. Mucosal IgE can bind FcεRI on resident basophils and mast cells and sensitize them for activation upon allergen re-encounter. After saturating the receptor system of the mucosa, free IgE can reach the blood stream through the lymphatic vessels. In the blood stream, IgE binds first to FcεRI on circulating basophils. In patients with airway allergy, IgE saturates the receptor system of blood basophils and subsequently binds FcεRI on mast cells residing at the different peripheral tissues including the skin. Moreover, after saturating the receptor system on mast cells, IgE can be found at a free state in serum or in the respiratory secretions. During the effector phase of airway allergy, the re-exposure to the allergen triggers the IgE-mediated and FcεRI-mediated activation of resident mast cells and basophils. Moreover, memory Th2 cells are locally reactivated to release IL-5 among other cytokines. IL-5 mediates the recruitment of circulating eosinophils to accumulate in the respiratory mucosa, to activate, and to contribute to tissue remodeling.

mechanism of local inflammation in patients with eosinophilic CRSwNP.

Asthma

Asthma is an inflammatory disease of the bronchi defined by chronic or chronically relapsing respiratory symptoms,

together with airway hyperreactivity [66]. The condition comprise different phenotypes defined by the corresponding inflammatory endotypes, of which the allergic/atopic type is the commonest [66]. Patients with eosinophilic asthma display markers of εCSR [67] and high levels of IgE+ [68] and FcεRI+ [69] cells in the

bronchial mucosa regardless of their atopic status. Nevertheless, there is conflicting data about the specificity and functionality of bronchial IgE in non-atopic asthma patients. HDM-sIgE was found in the sputum of 26 of 27 non-atopic asthmatics, yet the patients failed to develop a positive bronchial allergen challenge (BAC) [70[•]]. In contrast, sputum HDM-sIgE from three non-atopic asthmatics was able to activate peripheral basophils *in vitro* [70[•]]. Moreover, sputum HDM-sIgE was also detectable in 39 of 39 non-atopic asthmatics in a different work from the same group [71]. Conversely, a study analyzing bronchial mucosa homogenates, demonstrated HDM-sIgE in 10 of 10 atopic patients, but in none of the 10 non-atopic asthmatics included, yet the later individuals were on doses of inhaled corticosteroids >10 times higher than the atopic subjects [72]. Because the non-atopic asthma phenotype includes individuals with diverse inflammatory endotypes [66], it seems logical to focus the efforts for the characterization of bronchial IgE on those individuals with positive BAC responses.

Interventions to modulate IgE immune responses

Allergen immunotherapy

Allergen immunotherapy (AIT), both through subcutaneous (SCIT) and sublingual (SLIT) routes, offers long-term clinical benefit for individuals with airway allergy, and is able to prevent asthma onset in patients with AR [73,74[•],75,76]. A prolonged treatment period of SCIT or SLIT has been associated with the modulation of IgE responses, including an early and transient rise in serum sIgE and sIgG, followed by a sustained and progressive decrease of circulating sIgE and a blunting of its seasonal increase in the case of pollen allergens [77,78^{••},79]. In pollen-AR patients, the initial rise in serum sIgE is the mechanism driving the blunting of the seasonal increases during the natural exposure to the allergen, and mediates the early priming of Th2 cells, a process speculated to be crucial for the success of AIT [80,81]. In contrast, the AIT-induced sustained decrease in serum sIgE has no clear association with either the response to the treatment or its magnitude [82,83]. However, a minimum treatment period of three years is needed for sustained IgE regulation, as only AR patients who received a four-year course of SCIT, but not those who received a two-year cycle, displayed reduced levels of serum sIgE at the end of the treatment period [84]. In a double-blind placebo-controlled study involving three-years of daily treatment with grass-SLIT, the rise in serum sIgE was apparent from 2 months of AIT, with a decrease to baseline after 22 months [79]. Moreover, the capacity to induce a sustained reduction of serum sIgE might be restricted to the subcutaneous route, as only SCIT-treated AR individuals, but not patients receiving SLIT, had significantly less serum sIgE than the placebo group after a three-year period of AIT (GRASS Trial) [85]. Analyzing the groups who received a two-year course of AIT in the

same study, it was observed that the IgE/IgG₄ ratio was significantly higher in the SCIT-treated compared to the SLIT-treated patients, whereas one year after therapy discontinuation, the AIT-induced clinical improvement was equally lost in both SCIT and SLIT groups [86]. Moreover, the IgE-facilitated allergen binding to B cells returned to baseline one year after AIT discontinuation in both treated groups, yet it was still significantly lower than that of the placebo group [86].

In addition to conventional SCIT and SLIT with whole allergen proteins, other AIT approaches have been investigated. Short-course subcutaneous administration of linear *Lolium perenne* peptides (LPP) increased serum grass-sIgE during and after the treatment period, compared to both baseline and placebo [87]. Moreover, pre-seasonal intradermal immunotherapy with grass pollen reduced skin late-phase reaction and increased serum sIgE, but induced no clinical benefit in AR patients as compared to placebo [88]. Despite the numerous and consistent evidences on the AIT effect over IgE immune responses, the specific mechanisms involved in this modulation remain to be fully identified.

Biological drugs

Omalizumab is a humanized anti-IgE Cε3 domain monoclonal antibody (mAb) approved for severe allergic asthma and chronic urticaria. The drug primarily acts by preventing the binding of free IgE to its receptors, but it is not able to recognize IgE molecules attached to FcεRI and CD23 on the cell surface. Additional mAbs have been developed to inhibit IgE-mediated inflammation. The Fab fragment of the rat anti-IgE Cε2 domain mAb (Fab-6HD5) was shown to antagonize IgE binding to FcεRI in a mouse study [89]. The murine mAb 8D6 binds to human IgE Cε3 domain, but unlike omalizumab, it can also recognize IgE molecules bound to CD23 on the surface of B cells [90]. *Designed ankyrin proteins* (DARPin) are products of protein engineering, which have been used to antagonize IgE-mediated activation of effector cells [91[•]]. Most DARPins function as disruptive inhibitors with the ability to both prevent the formation of IgE/receptor complexes and dissociate pre-formed IgE/FcεRI complexes [91[•]]. Interestingly, omalizumab can also detach IgE from FcεRI expressed on effector cells *in vitro* [92], but at much higher concentrations than those achieved by therapeutic doses [93]. Recently, a bispecific DARPin (D11) targeting FcγRIIB and FcεRI-bound IgE was shown to inhibit basophil activation *in vitro* [94]. The inhibitory potential of D11 does not rely on the disruption of IgE/FcεRI complexes, but on the simultaneous activation of FcγRIIB and FcεRI [94]. Yet the *in vitro* studies with DARPins are promising [95], their therapeutic potential still needs to be established.

Given the prominent role of IL-4 in IgE synthesis, several mAbs have been developed to block the effects of this

cytokine. Dupilumab is a fully human anti-IL4R α mAb, approved for recalcitrant atopic dermatitis [96] whose effects in asthma seem promising [97]. In contrast, a humanized anti-IL-4 mAb (VAK694) in combination with AIT provided no additional benefit over AIT alone in the reduction of the late-phase reaction upon intradermal allergen injection [98]. In the same study, total and sIgE in serum experienced an early increase in the AIT+IL-4-treated group, followed by a progressive decrease until the end of the study [98].

Conclusion

The mucosal synthesis of allergen-sIgE with the subsequent sensitization of resident effector cells is one of the main disease mechanisms of airway allergy. The SAE-driven polyclonal synthesis of IgE is a prominent phenomenon amplifying nasal inflammation in patients with CRSwNP. Moreover, mucosal IgE might be involved in the pathophysiology of LAR and of some forms of non-atopic asthma. All these evidences identify IgE as a relevant therapeutic target for several human respiratory diseases. Novel biological drugs such as anti-IgE or anti-IL-4 mAbs or DARPins might antagonize several IgE-mediated disease mechanisms, yet they lack a long-term effect after discontinuation and thus raise cost-efficiency concerns. In contrast, AIT is able to modulate the allergen-specific immune response and to antagonize IgE-mediated mucosal inflammation in a sustained manner, thus providing long-term benefits to patients with allergic respiratory disease.

Conflict of interest statement

Nothing declared.

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