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REVIEW



Biomarkers of immunotherapy response in patients with allergic rhinitis

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ABSTRACT

Introduction: Allergen immunotherapy represents the only disease-modifying therapy available for immunoglobulin E-mediated diseases such as allergic rhinitis and asthma. Allergen immunotherapy induces allergen tolerance by interfering with the immune-pathogenic mechanisms of the allergic response and is potentially able to provide long-term relief of symptoms of allergic rhinitis and asthma and alter the natural course of allergic diseases.

Areas covered: Since allergen immunotherapy (AIT) is actually considered an individualized treatment on patient's clinical and immunological profile, the identification of specific biomarkers, which may guide diagnosis, management, and predict response to AIT treatment in allergic rhinitis (AR) patients, is essential and is currently an active field of research.

Expert commentary: The identification and validation of biomarkers of successful AIT for AR is an urgent need to definitively establish the role of AIT as a therapeutic tool of personalized medicine.

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Allergic rhinitis; allergen immunotherapy; biomarkers; IgE; allergy; allergen tolerance

1. Introduction

Allergic rhinitis (AR) is an inflammatory disorder of the nose and eyes initiated by an immunoglobulin E (IgE)-driven response to inhaled allergens in sensitized individuals [1]. AR, with or without conjunctivitis, represents a global health problem, affecting around a fifth of the general population and occurring in both developed and developing countries and across all ethnic groups and ages [2–4]. Increasing trends in prevalence and incidence of AR has been recorded in the last few decades [5]. AR symptoms include itching, sneezing, watery nasal discharge, and nasal congestion; commonly, there are associated ocular symptoms such as watery, red, and/or itchy eyes [1]. If left untreated, AR may significantly affect quality of life and impair productivity and school performance [6,7]. In addition, AR may be associated with various comorbidities, including asthma; in particular, AR is an independent risk factor for the development of asthma and uncontrolled AR affects asthma control [8]. It has been demonstrated that childhood AR increases the likelihood of new-onset asthma after childhood and the likelihood of having persisting asthma from childhood into middle age [9]. Thus, an appropriate and early treatment of AR may reduce asthma burden in later life.

The management strategy of AR includes allergen avoidance, pharmacotherapy to control symptoms, and allergen immunotherapy (AIT) [8]. Allergen avoidance and environmental control, when possible, may lead to a reduction in allergen levels, but with no significant effect on symptoms [10]. The main pharmacological agents for AR include oral or topical antihistamines and intranasal corticosteroids; however, these therapies have several limitations, including side effects and/or incomplete therapeutic

response [11]. Differing from symptomatic pharmacotherapy, AIT aims to induce allergen tolerance by interfering with the immune-pathogenic mechanisms of the allergic response and is potentially able to provide long-term relief of symptoms of AR and asthma and alter the natural course of allergic diseases [12–15], in particular preventing the progression from AR to asthma in children [16]. Considering its unique immunological rationale, AIT is today considered a therapeutic model for precision medicine, aiming to individualize treatment on patient's clinical and immunological profile [17,18]. Thus, the identification of specific biomarkers, which may guide diagnosis, management, and predict response to AIT treatment in AR patients, is currently an active field of research.

2. Overview of the mechanisms of AIT

AIT represents the only disease-modifying therapy available for IgE-mediated diseases such as AR and asthma. Through the action on both innate and adaptive immune responses, AIT modulates cellular and humoral effectors of the allergic molecular pathway [19–21]. In this context, the upregulation of allergen-specific T-regulatory (Treg) cells and B-regulatory (Breg) cells along with the consequent down-regulation of the Th2 response represents a pivotal immune-modulating mechanism of AIT [19]. AIT-induced Treg and Breg cells produce regulatory cytokines, such as interleukin (IL)-10 and transforming growth factor- β (TGF- β), which suppress the allergic inflammation, predominantly by inhibiting the activation of allergen-specific Th2 lymphocytes and inducing a shift toward a Th1-mediated immune response [22]. Moreover, AIT induces the polarization of dendritic cells (DCs) to a specific regulatory phenotype (DCregs) producing IL-12, IL-27, and IL-10,

which favor preferential Treg cell development [19,21]. Both the two distinct phenotypes of Treg cells, forkhead box P3 (Foxp3)⁺ Treg and inducible Treg (iTreg) cells, are able to suppress allergic response through direct and indirect mechanisms. Tregs release regulatory cytokines as IL-10, TGF- β , and IL-35 that suppress both activity and proliferation of Th2 cells and innate lymphoid cells type 2 (ILC2), thus reducing the production of pro-inflammatory cytokine release from Th2 cells (including IL-4, IL-5, IL-9, and IL-13) consequently decreasing the activation of eosinophils, basophils, and mast cells. Moreover, regulatory cytokines suppress activation of allergen-specific inflammatory response downregulating the expression of FC ϵ RI receptors on mast cells, decreasing allergen-specific IgE synthesis, and promoting B-cell production of IgA and IgG₄ antibody [19,21,23].

IgG₄ antibodies competitively bind to the same site epitopes recognized by IgE, resulting in the inhibition of mast cell and basophil degranulation, and inhibit IgE-mediated facilitation of allergen presentation to T lymphocytes [22]. Breg cells can directly induce the synthesis of IgG₄ blocking antibodies, and, through the production of IL-10, TGF- β , and other factors, including membrane-associated proteins and intracellular signaling molecules, suppress Th2-dependent inflammation, as well as promote T-cell expression of Foxp3 and generation of functional Treg cells [24]. Through the mechanisms described, AIT modulates the allergic inflammatory response inducing tolerogenic effects on cellular and molecular pathways underlying the IgE-mediated immune cascade. The improvements in the understanding of immunological effects of AIT have paved the way for the identification of possible AIT biomarkers.

3. Candidate biomarkers for AIT

The identification and validation of predictive biomarkers of AIT response is a topic of major interest in this field of research and could improve the selection and the clinical management of patients receiving AIT. Biomarkers are defined as quantitative measurements that allow clinicians to diagnose, assess the disease stage, predict clinical outcomes, and monitor the treatment effects [25]. In particular, ideal biomarkers for AIT should favor patient selection and identification of responders and predict clinical and immunological response during treatment and after discontinuation of treatment [21,26].

The recently published European Academy of Allergy and Clinical Immunology (EAACI) Position Paper reviewed all candidate biomarkers used in clinical trials of AR patients with or without asthma. The biomarkers have been classified into seven domains including IgE (total IgE, specific IgE, and slgE/total IgE ratio), IgG subtypes (slgG1, slgG4 including SlgE/IgG4 ratio), serum inhibitory activity for IgE (IgE-FAB and IgE-BF), basophil activation, cytokines and chemokines, cellular markers (Treg cells, Breg cells, and DCs), and *in vivo* biomarkers (including nasal and chamber provocation tests) (Table 1) [21,26].

3.1. IgE (total IgE, specific IgE, and specific IgE/total IgE ratio) and IgG subclasses (slgG1, slgG4, and slgE/IgG4 ratio)

Increased serum allergen-specific IgE levels (slgE), along with a history of symptoms on exposure to allergen, represent the sole

Table 1. Overview on the pragmatical biomarkers for allergen immunotherapy.

Domain	Candidate biomarkers	Categories	Possible applications
Antibodies	IgE (slgE, tlgE, slgE/tlgE)	Biomarkers for	diagnosis
slgE/total IgE	Prediction of disease severity and/or progression	Biomarkers of AIT efficacy	Prediction of patients' compliance
	IgG subclasses (slgG ₁ , slgG ₄ , slgE/IgG ₄)		
	Prediction of clinical response		
Basophil Biomarkers for diagnosis	Prediction of disease severity and/or progression	CD63, CD203c, DAO, basophil	histamine release
Serum	Biomarkers predictive of AIT safety	Reduced risk of side effects and	improvement of patients' compliance to treatment
	inhibitory activity for IgE	IgE FAB, IgE-BF	Biomarkers of AIT efficacy
Prediction of clinical response	Chemokines	CCR3, ECP, eotaxin, IFN- γ , IL-2, IL-2R, IL-4/5/6, IL-8/9/10, IL-13/18, MCP-1, TARC,	transthyretin
Cytokines and		Not known	
	determined		
Not yet	Cellular biomarkers	DCs, Breg, Treg	Prediction of immunological response
	<i>In vivo</i> biomarkers	SPT, Id, In, chamber studies	Prediction of clinical response

BF, binding factor; Breg, B regulatory cell; CCR, chemokine receptor; CD, cluster of differentiation; DAO, diamine oxidase; DC, dendritic cell; ECP, eosinophil cationic protein; FAB, facilitated antigen binding; Id, intradermal test; IFN, interferon; Ig, immunoglobulin; IL, interleukin; In, intranasal test; MCP, monocyte chemoattractant protein; slg, allergen-specific Ig; SPT, skin prick test; TARC, thymus and activation-regulated chemokine; tlg, total Ig; Treg, T regulatory cell.

major inclusion criteria for initiation of immunotherapy [27]. Moreover, the application of recombinant allergen technology through the identification of specific IgE to the major allergen determinants improved patient selection, allowing to recognize irrelevant cross-reacting allergens. Considering their utility in follow-up of AIT-treated patient, long-term studies of subcutaneous immunotherapy showed that levels of slgE are transiently increased during treatment and followed by blunting of the seasonal increases; moreover, a gradual decrease in slgE levels over several years has been observed [28]. However, no evident association between changes in slgE levels and the grade of the clinical response has been identified [29]. In addition, the correlation between elevated ratio of slgE/total IgE (tlgE) in serum at baseline and positive clinical outcome of immunotherapy is a potential predictive marker for AIT but more data are needed for confirmation of its utility and to validate cutoff values for sensitivity and specificity [30,31].

During immunotherapy allergen-specific IgG subclasses, in particular IgG1 and more consistently IgG4, have been

reported to be increased compared with baseline values. The increase in serum allergen-specific IgG1 or IgG4 levels is likely due to high allergen exposure, indicating an immunological response during AIT treatment [32]. However, a correlation with clinical response to treatment has been described in some but not all studies [33]. Furthermore, the need to perform the assays with titrations of sufficiently representative allergen components, as well as the nature of IgE response, should be taken into account when specifically evaluating clinical outcome of AIT (e.g. subjects with a dominant response to profilin would need to show an anti-profilin responses). Finally, the sIgE/IgG4 ratio has been reported to decrease in some but not all AIT studies [34,35].

3.2. Serum IgE inhibitory activity (IgE-FAB and enzyme-linked immunosorbent-facilitated antigen-binding assay)

Previous studies demonstrated the induction of IgG 'blocking' antibodies inhibiting IgE-facilitated allergen presentation by B cells and regulating T cell responses, overall resulting in a reduction in allergic responses *in vivo* in subjects on AIT [36]. In order to evaluate IgG-associated IgE-inhibitory activity, a flow cytometry-based assay (IgE-FAB) has been validated [36]. This assay quantifies the capacity of IgG-containing serum obtained after AIT to inhibit the FcεRII-dependent binding of allergen-IgE complexes to B lymphocytes. Although reproducible, the complexity of this assay limits its use to specialized centers or laboratories. Recently, an alternative solid-phase assay has been developed (ELIFAB) [37]. Promising data show that IgE-FAB and ELIFAB results correlate with clinical response to immunotherapy [38–40]. However, further studies are needed to evaluate the role of IgE-FAB as a predictive biomarker for AIT; in particular no data are available on the relationship between serum inhibitory activity for IgE-FAB in responders and nonresponders to AIT.

3.3. Basophil activation

Basophil activation can be studied through flow cytometry-based assays, evaluating the expression of surface markers, such as CD63 and CD203c [41]. Moreover, a new functional assay based on intracellular staining of phycoerythrin-conjugated diamine oxidase (DAO) is available [42]. The data from the application of basophil activation during AIT in placebo-controlled trials are still conflicting. Some studies report a reduction in basophil activation following AIT that is possibly due to serological factors [43,44], while others failed to demonstrate suppression of basophil activation in successful trials of AIT [31]. A hypothesis for these contrasting findings is the different route of immunotherapy, with SLIT being possibly less effective in inhibiting basophils than SCIT. Considering the possible role of basophil activation in patients' follow-up after AIT, several studies show that basophil activation (including DAO) decreases after AIT, with one study demonstrating persistent basophil suppression after AIT discontinuation [42,45].

3.4. Cytokines and chemokines

Despite their speculated role in demonstrating the shift from a dominant Th2 and inflammatory response (IL-4, IL-13, IL-9, IL-17, eotaxin, or tumor necrosis factor-α) toward a Th1 and regulatory response (interferon (IFN)-γ, IL-12, IL-10, TGF-β) during and after AIT, no serum cytokines and chemokines showed correlation with the clinical response [26]. However, promising results have been reported dosing local nasal cytokines during AIT. The measurement of cytokines and chemokines in nasal fluid through miniaturized multiplex cytokine analysis with improved technology showed an increase in response to allergen provocation and are modified by immunotherapy [46,47]. However, considering the available data so far, cytokines and chemokines are not applicable as a biomarker; in particular, further studies of local nasal cytokines during AIT are required.

3.5. Cellular markers

AIT has been associated with the induction of cellular responses within regulatory T cells (Tregs), regulatory B cells (Bregs), and DCs [48].

Tregs appear to be fundamental in the immunological processes of AIT, mainly shifting the Th2 to Th1 immune response [49]. Two specific subsets of Tregs have been studied: Foxp3+ regulatory T cells (nTregs) and inducible regulatory T cells (iTregs) that produce regulatory cytokines such as IL-10, TGF-β, and IL-35 [50]. During AIT, the expansion of Tregs has been associated with delayed immune deviation from a Th2-pattern response to a Th1-type response. In the nasal mucosa, the increased numbers of Tregs after AIT is associated with clinical efficacy; the reduction of local allergic inflammation suggests a role for Tregs in the induction of allergen-specific tolerance [51,52].

Similar to T cells, B cells can be classified into functional subsets according to the cytokines and some of the surface markers that they express. One of the major alterations in the course of AIT is the modulation of allergen-specific B cells in the direction of Breg cells [53,54]. Bregs are a subset of CD19+ B cells that promote immune tolerance state through production of IL-10, IL-35, and TGF-β. IL-10 producing CD25⁺CD71⁺CD73[−] Breg subset has been shown to be induced following AIT. They regulate pro-allergic immune responses by inhibiting antigen-specific CD4⁺ T cell proliferation and produce anti-inflammatory IgG4 antibodies [55].

Tregs and Bregs have been shown to be modified during immunotherapy, principally by using flow cytometry. In clinical trials, these markers have shown to correlate with clinical response [56,57]. However, the complex flow cytometry for multiple T and B cell-associated markers is a limit for their application in clinical practice.

Changes in markers associated with DCs polarization, supporting the differentiation of either effector Th1, Th2, Th17, or Treg lymphocytes, could represent an early and more persistent signature for the shift from a Th2 to a regulatory immune response during AIT [26]. When triggered by an allergen, immature DCs polarize into DC1s, DC2s, DC17s, or DCregs, which in turn can differentiate T cells into Th1 cells, Th2 cells, Th17 cells, or regulatory T cells.

DCregs preferentially express C1QA, FcεRIIIA, FTL, and SLCO2B1, which favor preferential Treg cell development, whereas DC2s express CD141, GATA-3, OX40 ligand, and RIPK4, polarizing naive T cells into TH2 cells [58,59]. Gueguen et al. demonstrated that, after 4 months of AIT in patients with AR, the four markers of DC2 cells were decreased, whereas the expression of the four DCreg cell markers was increased in the peripheral blood; interestingly, these changes were associated with clinical efficacy [58]. Moreover, it has been demonstrated that, after 2 and 4 months of AIT, the optimal combination of five molecular markers of which three DC2 (CD141, GATA3, and RIPK4) and two DCreg (C1QA and FcγRIIIA) allowed to efficiently distinguish clinical responders from nonresponders with a sensitivity of 90.48% and a specificity of 61.9% [58]. However, these promising findings need to be confirmed in large clinical studies and then in clinical practice.

3.6. *In vivo* biomarkers

In vivo biomarkers derive from the use of allergen provocation tests to evaluate patients' allergen-specific response before and after treatment. Allergen provocation tests include skin prick tests, intradermal tests, and direct evaluation of target organ responses with conjunctival provocation tests, nasal provocation tests (NPTs), and environmental exposure chambers (EEC). NPT have been more extensively studied. During NPT, subjective nasal symptom scores, measurements of peak nasal inspiratory flow and evaluation of cells and mediators in nasal fluid can be performed. With NPT, several data demonstrated that AIT successfully reduced early and late nasal responses [60,61]. In particular, grass pollen AIT was associated with reduced increases in tryptase, eotaxin, and Th2 cytokines (IL-4, -5, -9, and -13) in nasal fluid in comparison to allergic controls not receiving AIT [46]. EEC is a possible alternative to NPT that more closely simulates real exposure. Jacobs et al. demonstrated a correlation between symptoms induced in the EEC compared to natural seasonal exposure [62]. The European Medicinal Agency guideline recommends provocation tests for proof of concept or phase II dose-finding trials in AIT [63,64]. However, validation of the different provocation models is required to standardize the results.

4. Conclusions

The improvements in the understanding of immunological effects of AIT have paved the way for the identification of possible related biomarkers. Biomarkers could assist in patient selection, identification of responders, and prediction of clinical and immunological response during treatment and after its discontinuation. To date, no validated biomarkers are available. However, there is a constantly increasing body of literature evaluating potential candidate biomarkers. Further studies are needed to raise the evidence level for the described biomarkers and to evaluate and interpret their association with the clinical and immunological response during AIT and after its discontinuation.

5. Expert commentary

Currently, in case of a clinical history suggestive of AR, serum sIgE level is the single most relevant biomarker for selection of patients for AIT; moreover, the application of recombinant allergen technology improved this selection, allowing to recognize irrelevant cross-reacting allergens. However, at present, sIgE resulted useless to predict or monitor the clinical response to AIT. In order to monitor patients' adherence to AIT, serum allergen-specific IgG4 levels could be used as a biomarker for compliance that would be particularly useful in patients receiving sublingual immunotherapy. Candidate biomarkers for clinical outcome are sIgE/tIgE ratio and IgE-FAB. The correlation between elevated ratio of sIgE/tIgE in serum at baseline and positive clinical outcome of immunotherapy is a potential predictive marker for AIT but more data are needed for confirmation of its utility and to validate cutoff values for sensitivity and specificity. Moreover, promising data show that IgE-FAB and ELIFAB results correlate with clinical response to immunotherapy, although no data are available on the relationship between serum inhibitory activity for IgE-FAB in responders and nonresponders to AIT. The diffusion of cellular assays is limited by the complexity of their procedure. Although several studies have shown that basophil activation (including DAO) decreases after AIT, data from the application of basophil activation during AIT in placebo-controlled trials are still conflicting. Tregs and Bregs have been shown to be modified during AIT and, in clinical trials, these markers correlate with clinical response. However, they have been unable to distinguish responders from nonresponders or predict response in individual subjects. Emerging biomarkers for clinical response are the molecular markers identified in DC2 (CD141, GATA3, and RIPK4) and DCreg (C1QA and FcγRIIIA) induced by AIT; they show the promising characteristics of separating clinical responders and nonresponders to grass pollen sublingual immunotherapy.

6. Five-year view

The identification and validation of biomarkers of successful AIT for AR is an urgent need to definitely establish the role of AIT as a therapeutic tool of personalized medicine. Careful patient selection and early identification of responders are essential to target intervention at those who will benefit and to exclude those who are less likely to respond to AIT, with additional advantages over health cost savings. Furthermore, the identification of timing of suspension of AIT and the prediction of an eventual 'allergic' relapse could both enhance the clinical management of AR patients.

The EAACI Position Paper on biomarkers for AIT recommended further exploration of the use of allergen-specific sIgG4 as a biomarker for compliance and proposed sIgE/tIgE ratio and IgE-FAB as candidate biomarkers for clinical outcomes. As for the standardization of clinical outcomes for AR in clinical trials that has been recently implemented, we expect guidelines to conduct biomarker studies with a novel approach in design (i.e. responders vs. nonresponders), both for the registration of novel products of AIT and the optimization of existing treatment regimens. Furthermore, a comparison of the candidate

biomarkers in children and adult population would be useful to possibly differentiate AIT treatment through age groups.

Key issues

- Allergen immunotherapy (AIT) represents the only disease-modifying therapy available for immunoglobulin E (IgE)-mediated diseases such as allergic rhinitis (AR) and asthma.
- Interfering with the immunopathological mechanisms of the allergic response, AIT provides long-term relief of symptoms of AR and asthma and may alter the natural course of allergic diseases, in particular the progression from AR to asthma in children.
- The identification and validation of biomarkers for AIT could enhance the clinical management of AR patients. Biomarkers could assist in patient selection, identification of responders, and prediction of clinical and immunological response during AIT treatment and after its discontinuation.
- To date, no validated biomarkers for AIT are still available in clinical practice.
- sIgE/tIgE ratio and IgE-FAB have been proposed as candidate AIT biomarkers for clinical outcomes.
- Allergen-specific sIgG4 has been proposed as a candidate biomarker for AIT patients' compliance.
- There is an urgent need to implement guidelines to conduct biomarker studies with a novel approach in design, both for the registration of novel products of AIT and the optimization of existing treatment regimens.

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Declaration of interest

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