A review of the mechanisms and biomarkers of allergen immunotherapy

Przegląd mechanizmów działania i biomarkerów immunoterapii alergenowej

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Abstract

Allergen immunotherapy has been shown to be successful in numerous clinical trials, even leading to complete relief of allergic symptoms. However, the molecular mechanisms behind these outcomes remain unclear, substantially hindering introduction of more efficient and safer protocols. The current gold standard of assessing the effects of allergen immunotherapy is a challenge test, which might be both unpleasant and dangerous for patients. Better understanding of the molecular background of allergen immunotherapy would allow us to substitute challenge tests with biomarkers. These might also be used to predict a patient's response to the treatment. Here, we review current knowledge regarding immune responses to allergen immunotherapy and its potential biomarkers.

Streszczenie

Immunoterapia alergenowa wykazała skuteczność w licznych badaniach klinicznych, prowadząc nawet do całkowitego ustąpienia objawów alergii. Mimo to, precyzyjne mechanizmy jej działania są niewyjaśnione, co wyraźnie utrudnia wdrożenie nowych, skuteczniejszych i bezpieczniejszych protokołów terapeutycznych. Obecnie złotym standardem w ocenie skuteczności immunoterapii jest przeprowadzenie próby prowokacyjnej z danym alergenem, co może być zarówno niekomfortowe, jak i niebezpieczne dla pacjenta. Lepsze zrozumienie komórkowych podstaw immunoterapii umożliwiłoby zastąpienie prób prowokacyjnych zastosowaniem biomarkerów, które mogłoby również posłużyć do przewidywania odpowiedzi danego pacjenta na leczenie. W niniejszej pracy autorzy dokonują przeglądu obecnej wiedzy na temat mechanizmów działania, przyczyn niepowodzenia, a także potencjalnych biomarkerów immunoterapii alergenowej.

Introduction

Allergen-specific immunotherapy (AIT) is the only effective method of causal treatment in pollen, insect, and venom allergy, but it is still a non-established method in food allergy. The oral (OIT), subcutaneous (SCIT), or sublingual (SLIT) routes may be used. AIT involves the repeated use of gradually increasing doses of allergen at regular intervals, which leads to desensitization, which is a decreased immune response to the allergen. After the therapy, a challenge test is conducted to assess the level of the patient's response to the allergen.

AIT can have 3 main outcomes: desensitization, sustained unresponsiveness (SU), and failure. Desensitization is a temporary suppression of the immune response to an antigen and persists only during constant exposure to the allergen. Sustained unresponsiveness, on the other hand, is a persistent state of clinical non-reactivity achieved after successful immunotherapy, which is independent from constant dosing of allergen.

Goal

Precise molecular mechanisms of AIT remain unelucidated, hampering the development of new, safer, and more efficient protocols, as well as reliable biomarkers of immunotherapy efficacy. The aim of this review is to summarise current knowledge regarding the mechanisms behind success and failure of desensitization and SU, and to identify potential biomarkers of immunotherapy. The role of the major cells of the immune system and antibodies was investigated along with epigenetic changes induced by immunotherapy, to explore the contribution of each factor to the final AIT outcome. At the same time, the use of each factor as a potential biomarker was inspected.

Objectives

The aim of the study was: to investigate the effect of AIT on basophils and mast cells in creating desensitization, to determine the role of T and B lymphocytes in successful desensitization, along with immunoglobulins G and A, to identify possible mechanisms of creation of sustained unresponsiveness instead of simple desensitization, to discuss potential factors that contribute to the failure of AIT, to describe the use of basophils, mast cells, IgA, IgG, IgE, innate lymphoid cells, dendritic cells, and methylation of T cells' DNA as potential biomarkers of AIT efficacy.

Material and methods

A search using electronic databases of PubMed and Google Scholar search engine up to 14.02.2021 was conducted in order to identify articles discussing molecular mechanisms of immunotherapy, its biomarkers, and the role of the main immune cells and their products in the immunotherapy. Search queries were "allergen immunotherapy", "mechanisms of immunotherapy", "desensitization", "sustained unresponsiveness", "biomarkers of immunotherapy", and "food allergy". No search filters were used except for publication date: 10 years, although after the first screening we decided to add several studies from beyond that filter. Screening of titles and abstracts of received results was performed to discern relevant publications. At the beginning we found 81 articles from which we chose 36 of the latest and best meeting the following criteria: a) research exploring molecular changes occurring in the immunotherapy; and b) research regarding biomarkers of the immunotherapy. Full texts of the articles were then carefully read and evaluated. During the assessment of the publications new relevant articles were identified, screened by title and abstract, and included if they met the aforementioned criteria.

Molecular mechanism of desensitization

Basophils and mast cells

Several studies suggest that repeated anaphylaxis-sub-threshold dosing of the allergen leads to intrinsic changes in MCs and basophils, and therefore their reduced activity, contributing to desensitization seen in the first phase of AIT [1]. The possible mechanism behind these alterations of basophil activation patterns are changes in antibody levels, such as an increase in sIgG4 and a decrease in sIgE, which in turn inhibit elements of basophil intracellular transduction pathways, possibly FcεR and spleen tyrosine kinase (SYK). SYK is a crucial component of the FcεR pathway that leads to degranulation of basophils along with MCs, and sub-threshold allergen-stimulated cross-linking of FcεRs on the surface of cells was shown to induce downregulation of SYK [2].

Internalization of MCs' surface-bound allergen-specific IgE was proposed as responsible for the desensitization [3]. On the other hand, a different study by Ang *et al.* showed that mast cells from desensitized mice express sufficient amounts of surface-bound IgE to trigger degranulation. The same study indicated that it is the alteration of the actin cytoskeleton, resulting in impaired Ca^{2+} mobilization, that inhibits degranulation of MCs [1]. It is consistent with the observation that the sIgE level may temporarily increase during immunotherapy and that desensitization can be achieved with sIgE levels close to baseline [4]. A different novel research also showed that the mast cells' phenotype can change toward a regulatory one during AIT. These desensitized, regulatory MCs were proven to produce immunosuppressive IL-10 and IL-2, which resulted in the inhibition of allergic reactions and in expansion of regulatory T cells (Tregs) [5].

T cells

T cells seem to be essential in both the pathophysiology of allergies and in creating desensitization and sustained unresponsiveness. AIT affects allergen-specific T cells by altering their phenotype toward an anergic one and by inducing both proliferation and specific changes of induced Tregs (iTregs) [6–8].

Tregs form a population of suppressive cells that seem to be essential in maintaining natural tolerance and in creating desensitization. They can be divided into natural Tregs (nTregs) and iTregs, which express FOXP3 transcription factor and expand in response to specific antigens [6].

iTregs possibly influence allergic responses through production of immunosuppressive IL-10, IL-35, and TGF-β and through direct suppression, inhibition of proliferation, deletion, or promoting anergy of T effector (Teff) cells. They were also demonstrated to suppress the proliferation and activation of B cells [9, 10] and dampen local inflammation in the gut [8]. Importantly, hypomethylation of CpG regions in *FOXP3* of iTregs and the subsequent increase in FOXP3 expression were clearly associated with better outcomes of OIT [6]. It is consistent with the fact that immunosuppressive IL-35 is secreted specifically by FOXP3+ Tregs.

Moreover, pretreatment Tregs of allergic patients are skewed toward defect Th2 phenotype with detectable IL-4 secretion. OIT is capable of restoring their correct suppressor function and of shifting allergen-specific CD4+ cells toward an anergic phenotype. It is noteworthy that these changes were observed within the 3 first months of AIT, indicating that T cells might also have an important influence on desensitization achieved early in immunotherapy [7].

B cells

Although there is little research regarding mechanistic changes in B cells induced by OIT, it was shown

multiple times that immunotherapy creates specific changes in patients' antibody repertoire. Peanut OIT was shown to cause expansion in the memory allergen-specific IgE-secreting B cells, which might explain the transient increase in sIgE levels at the beginning of OIT. On the other hand, allergen-specific class-switched B cells that produce IgG and IgA arise as soon as in the 7th week of peanut OIT. Van de Veen *et al.* revealed an OIT-induced regulatory clone of B cells that secrete IL-10 and is associated with the production of IgG4 [11]. Within a couple of months of the therapy IgG4 levels actually increase significantly and may remain above baseline level even after the end of OIT [12].

IgG and IgA

Allergen-specific IgG (sIgG) probably works in multiple ways in alleviating reactivity toward allergens. One is by binding to allergen molecules and creating steric blockade, which limits IgE-allergen interactions (IgE-facilitated allergen binding – IgE-FAB) and subsequent degranulation of basophils and MCs [13]. IgG also binds to inhibitory FcγRIIb receptors on mast cell membrane, which interferes with the allergen-IgE-FcεR activation pathway, which in turn leads to reduced calcium influx and stops degranulation [13]. Moreover, IgG-FcγRIIb ligation was shown to inhibit basophils' and MCs' IL-4 secretion [13]. It has also been established that administration of sIgG to sensitized mice induces T-cell phenotype shift toward FOXP3+ with concomitant decrease in Th2 cell frequency [13], which is consistent with the aforementioned research regarding phenotype-skewing of these cells. Regarding IgA, it is possible that an increase in mucosal antigen-specific secretory IgA production dampens allergic responses through binding to allergens, therefore preventing their absorption to GALT cells, which would be followed by local inflammation.

Molecular mechanism of sustained unresponsiveness

Specific mechanisms explaining the phenomenon of SU have not been elucidated yet. It is clear that basophil activation and SPT are decreased long term in patients who have achieved SU [14]. It is possible that this is the result of a culmination of the described multiple synergic changes in many types of cells. However, bearing in mind the essential role of T cells in allergic reactions and the multitude of ways AIT affects them, it is likely that SU is heavily dependent on changes regarding these lymphocytes. Anergy, deletion, and phenotype-skewing of Th2 cells, accompanied by induction of Tregs, might result in the creation of a suppressive milieu that prevents allergic reactions in the long term. On the molecular level, such long-lasting tolerance would be underlaid with both a wide spectrum of immunosuppressive interleukins and increased IgG-dependent serum inhibitory activity for IgE-FAB generated thanks to T cells. Such speculations are supported by the fact that frequency of iTregs and increased expression of FOXP3 were very good markers differentiating between patients who achieved SU during OIT and those who did not [6]. From this point of view, SU would depend mostly on the longevity of AIT-induced iTregs and memory B cells that produce IgG and IgA instead of IgE. Nonetheless, more research investigating the mechanisms of AIT is necessary to explain this phenomenon and improve both the efficacy and safety of oral immunotherapy.

Possible mechanism of failure of OIT

Maintained responsiveness of both MCs and basophils, despite OIT, is the essence of failure to desensitize. We do not know what exactly is responsible for this phenomenon, but, based on existing OIT research, it is possible to make some assumptions.

It was shown that IgG4 levels are on average lower in patients who do not pass an oral food challenge after OIT [15]. It suggests that non-responding patients fail to produce specific T and B cells responsible for the synthesis of IgG4. It is also possible that patients who become "re-sensitized" produce iTregs, which are short-lived, as Chinthrajah *et al.* proposed [16], or lose their immunoregulatory function soon after cessation of allergen dosing. Syed *et al.* revealed that increased methylation of *FOXP3* was associated with lost tolerance after previously achieved desensitization [6]. A possible cause of these unfavourable events is inflammation occurring throughout OIT or perhaps shortly after the end of the immunotherapy.

Inflammation was shown to be able to impede the function of immunoregulatory FOXP3+ cells and convert them toward a proinflammatory phenotype, resembling Th2 and Th17 cells [17]. Although these investigations concerned arthritis and immunity to helminths, it can be assumed that similar molecular mechanisms are activated in allergic response as well. We also know that IL-4 secretion under inflammatory conditions can override IgG-generated reduction of mast cell growth and restore their pre-OIT frequency in the intestine. The same effect was observed for the intestinal Th2 cells, with a concomitant decrease of Treg frequency, suggesting that IL-4 promotes conversion of Tregs to Th2 cells [13].

On the mast cells level alterations of the actin cytoskeleton might account for failure of immunotherapy. Ang *et al.* revealed that remodelling of previously desensitized MCs' actin can restore their responsiveness to allergic stimulus [1]. However, the reasons for such phenomena, possibly occurring *in vivo*, remain undefined.

Potential markers of immunotherapy efficacy

Mast cells

An easy way to check the activity of MCs is a skin prick test (SPT). In comparison to double-blind, placebo-controlled, food challenge (DBPCFC), which represents the current standard for the diagnosis and evaluation of FA, SPT is much safer, quicker, and simpler. Mean wheal diameter above 3 mm is regarded as a positive reaction [18]. However, invariable prediction was reached when wheal diameter cut-offs were ≥ 8 mm, ≥ 7 mm, and ≥ 8 mm to cow's milk and peanut, respectively [19–22].

Due to SPT outcome variation across the extracts and allergens used, the methods and techniques by which the tests are performed and measured, and each person's intrinsic skin reactivity, several studies have evaluated the use of skin indexes, which are supposed to be immune to these variables. Some non-negligible differences were identified in the mean wheal diameter and skin index ($p = 0.03$ and $p < 0.001$, respectively) of patients with positive and negative food challenge outcome [23]. Conversely, an additional study in 172 children with cashew nut sensitization found no significant difference in the ability of mean wheal diameter or skin index to predict the outcome of food challenge to cashew [24].

Basophils

Two main markers of basophil activation are CD63 and CD203c. Recently, the expression of fluorochrome-labelled diamine oxidase (DAO) in basophils has been identified as a potential biomarker to assess AIT efficacy [25]. While numerous studies suggest associations between reduction in basophil activation, especially in the reduced expression of CD63, CD203c, and DAO [9, 12], others do not report a change of basophil activation in successful trials of AIT [6]. In the study of peanut OIT, early decreases in allergen-induced basophil activation were found in subjects who went on to develop SU, suggesting the potential use of these markers to determine AIT prognosis [26].

Innate lymphoid cells

Among several groups of ILCs, ILC2s are most closely tied to type 2 allergic reactions. Grass pollen SCIT was demonstrated to weaken seasonal increases of CD117+ILC2s and IL-13+ILC2s [27]. In contrast, studies utilizing SLIT have failed to show a reduction in ILC2s [20].

Dendritic cells

In a study of peripheral blood monocytes (PBMCs) from patients undergoing SLIT for grass pollen allergy, the expression of DCreg-associated complement component 1 (C1Q) and stabilin-1 (STAB1) were found to be significantly greater in clinical responders than in non-responders or in the placebo group [21]. A follow-up study [28] of PBMC-derived DC2 and DCreg-associated markers indicated that 4 months of SLIT promoted down-regulation of the DC2-associated markers CD141, GATA2, OX40 ligand, and receptor-interacting serine/threonine-protein kinase 4 (RIPK4) in the PBMCs of responders. Apart from this down-regulation, a significant increase in the expression of the DCreg-associated complement C1Q subcomponent subunit A (C1QA), FcgRIIIA, ferritin light chain (FTL), and solute carrier organic anion transporter family member 2B1 (SLCO2B1) in responders was observed. Changes in these DC2 and DCreg-associated markers were associated with clinical efficacy after 4 months of AIT; meanwhile, changes in FcgRIIIA were also found to correlate with the onset of clinical efficacy in 2 months of AIT. A combination of 5 of these markers, CD141, GATA3, RIPK4, C1QA, and FcgIIIA, was able to identify responders from non-responders with a sensitivity and specificity of 90.48% and 61.9%, respectively. While these markers of efficacy still need to be validated in larger cohorts of patients, they show promise in the early prediction of adaptive immune responses and immunotherapy efficacy.

IgE

A number of studies have claimed that during the AIT there is an initial, transient growth in allergen-specific IgE serum levels without significant clinical change. However, progressive decline of sIgEs after 6–12 months of therapy has been well documented [4, 19]. In a recent milk OIT study, high-throughput microfluidic assays were used to quantitate milk protein binding epitopes by IgE and IgG4 antibodies before and after therapy. Although this study reports a small sample size, epitope repertoire mapping allowed prediction of therapy outcome, suggesting its use as a potential biomarker [29]. Furthermore, the ratio of sIgE to total IgE has been shown to be more predictive of food challenge outcomes than specific IgE alone [30].

IgG4, IgA, and IgE blocking activity

The serum inhibitory activity for IgE-FAB was found to be well correlated with clinical outcomes, with increases in activity corresponding with positive clinical outcomes. The activity remained elevated even post-treatment [31]. However, a study on AIT in the therapy of allergic asthma and rhinoconjunctivitis found no correlation between the serum inhibitor activity for IgE-FAB of responders and non-responders [32].

sIgG4 was shown to remain increased in active treatment groups, but its levels decrease after treatment cessation [32]. While sIgG4 responses seem to be

significant at the cohort level, studies demonstrated that elevated production of sIgG4 during the course of AIT does not correlate with clinical outcomes, suggesting it should not be used alone as a biomarker [11].

A study of IgA during egg OIT found that all 3 IgA isotypes were increased within a 2-year period. Egg white-, ovalbumin-, and ovomucoid-specific IgA, IgA1, and IgA2 were all elevated during OIT [33]. Interestingly, subjects who developed SU had higher levels of sIgA than those who were transiently desensitized.

DNA methylation

Berni Canani *et al.* demonstrated that CpG methylation patterns in the promoting regions of specific genes associated with Th2 and Th1 cytokines of PBMCs were able to clearly distinguish those with active cow's milk allergy from those who outgrew the allergy. The greatest predictor of active allergy was the methylation rates of the IL-5 associated region. Similar findings have been demonstrated in the assessment of SU following successful desensitization of peanut allergy after 24 months of peanut OIT. sIgE or basophil activation markers were unable to discriminate tolerant and responsive patients; however, those who were immune tolerant had significantly higher numbers iTregs and expression of FoxP3. Additionally, the loss of tolerance after therapy was associated with an increased methylation at the same sites [34]. It should be noted that these associations between immunotherapy and changes in DNA methylation do not seem to be limited to OIT. In a study by Swamy *et al.*, subjects successfully treated with dual SLIT to timothy grass and dust mite produced higher levels of allergen-specific suppressive memory Tregs characterized by reduced CpG methylation within their *FOXP3* promoter regions [35].

In a recent study, Martino *et al.* determined a DNA methylation signature of 96 CpG sites able to predict clinical outcomes of AIT with significantly greater accuracy than sIgE and SPT. These 96 allergy-associated sites had significantly higher levels of methylation in subjects with positive SPT and OFC (classified as food allergic) compared to those with positive SPT but no reaction at food challenge (classified as food sensitized). The established methylation cut-off value was able to predict OFC outcomes with 96.55% and 89.66% specificity and sensitivity, respectively [36]. Regardless of the cell type and cut-off values used, the use of methylation data appears to be the most useful potential biomarker.

A summary of the discussed potential biomarkers of immunotherapy can be found in Table 1.

Table 1. Potential biomarkers of immunotherapy

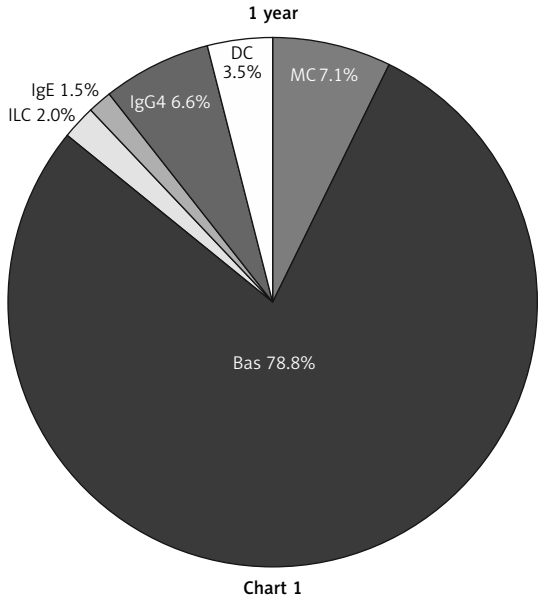
Cell type/antibody/ epigenetic changes	Change during successful immunotherapy
Mast cells	↓ Wheal diameter in skin prick test
Basophils	↓ Expression of CD63, CD203c, DAO
Innate lymphoid cells	↓ Number of CD117+ILC2s and IL-13+ILC2s
Dendritic cells	↑ Expression of C1Q, C1QA, FTL SLCO2B1, STAB1 ↓ Expression of CD141, GATA2, OX40, RIPK4
IgE	Initial growth of plasma concentration, then, after 6-12 months progressive decline ↓ sIgE/IgE ratio
IgG	↑ Serum inhibitory activity for IgE-FAB ↑ sIgG4 (weak correlation with clinical outcome)
IgA	↑ Concentration of sIgA
DNA methylation	↑ Methylation of IL-5 associated region of Th1 and Th2 ↓ Methylation of FOXP3 region Use of whole, complex methylation patterns seems to be able to predict allergic status of patients with the highest accuracy.
Others	↑ Number of iTregs ↑ FoxP3 expression

DAO – diamine oxidase, ILC2 – innate lymphoid cell 2, IL-13 – interleukin 13, C1Q – complement component 1, C1QA – complement component 1 subcomponent subunit A, FTL – ferritin light chain, SLCO2B1 – solute carrier organic anion transporter family member 2B1, STAB1 – stabilin-1, RIPK4 – receptor-interacting serine/threonine-protein kinase 4, sIgE – specific immunoglobulin E, IgE – immunoglobulin E, IgE-FAB – immunoglobulin E-facilitated allergen binding, sIgG4 – specific immunoglobulin G4, sIgA – specific immunoglobulin A, IL-5 – interleukin 5, Th1 – T helper cells 1, Th2 – T helper cells 2, FoxP3 – forkhead box P3, iTreg – induced regulatory T cell.

Table 2. Numbers of studies on potential biomarkers of immunotherapy in the last 1, 5, and 10 years

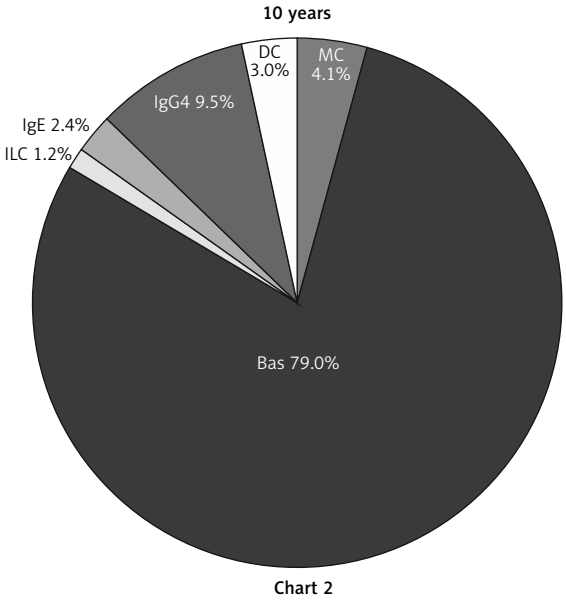
Biomarker	Article type	10 years	5 years	1 year
Mast cells	All	108	51	14
	Clinical trial	3	1	0
	Meta-analysis	0	0	0
	Randomised CT	2	1	1
	Systematic review	1	1	0
	Review	102	48	13
Basophils	All	2102	1095	156
	Clinical trial	71	69	5
	Meta-analysis	18	11	1
	Randomised CT	347	153	15
	Review	1571	812	125
	Systematic review	95	50	10
Innate lymphoid cells	All	33	19	4
	Clinical trial	2	1	0
	Meta-analysis	0	0	0
	Randomised CT	1	0	0
	Review	30	18	4
	Systematic review	0	0	0
IgE	All	63	27	3
	Clinical trial	11	4	1
	Meta-analysis	0	0	0
	Randomised CT	36	12	1
	Review	12	10	0
	Systematic review	4	1	1
IgG4	All	250	103	13
	Clinical trial	42	19	0
	Meta-analysis	2	0	0
	Randomised CT	112	41	2
	Review	88	40	9
	Systematic review	6	3	2
IgE-block activity	All	6	3	1
	Clinical Trial	6	3	1
	Meta-Analysis	0	0	0
	Randomised CT	0	0	0
	Review	0	0	0
	Systematic Rev	0	0	0
Dendritic cells	All	78	31	7
	Clinical trial	3	1	0
	Meta-analysis	0	0	0
	Randomised CT	4	1	0
	Review	71	29	7
	Systematic review	0	0	0
DNA-methylation	All	4	1	0
	Clinical trial	1	0	0
	Meta-analysis	1	0	0
	Randomised CT	0	0	0
	Review	2	1	0
	Systematic review	0	0	0

CT – clinical trial, IgE – immunoglobulin E, IgG4 – immunoglobulin G4.



DC – dendritic cells, IgG4 – immunoglobulin G4, IgE – immunoglobulin E, iLC – innate lymphoid cells, MC – mast cells, Bas – basophils.

Figure 1. Percent of studies on each discussed biomarker in the last year



DC – dendritic cells, IgG4 – immunoglobulin G4, IgE – immunoglobulin E, iLC – innate lymphoid cells, MC – mast cells, Bas – basophils.

Figure 2. Percent of studies on each discussed biomarker in the 10 last year

Analysis of trends in biomarker studies

To see the current direction of the latest studies, we performed a statistical analysis of the number of articles about each potential biomarker. Most (80%) of the found articles contained information about basophils. There were surprisingly few articles (4 in the last 10 years) about DNA methylation as an immunotherapy biomarker, considering the fact that those that we read seemed very promising. It is interesting that among all potential immunotherapy biomarkers only MCs were studied more frequently in the last year in comparison to the last 10 years (14/year vs. 10.8/year). All the data used to perform this analysis are attached as Table 2 and Figures 1 and 2.

Summary

In recent years there has been remarkable progress in our understanding of immunotherapy. However, we are still lacking substantial information that could help us to understand the reasons behind different outcomes of immunotherapy. We do not know why some patients achieve SU, some only desensitization, and some do not respond to currently used protocols at all. Advances in the fields of molecular and genetic sciences can be especially beneficial in explaining the precise mechanisms of persistent or recurrent responsiveness to allergens, thereby creating reliable biomarkers, which could discriminate between potential responders and non-responders, and indicate the moment of desensitization without the need for challenge tests. The key knowledge gap is the still unelucidated, precise cellular mechanism associated with both success and failure of AIT. We look forward to new research on these issues and to clinical trials of different AIT protocols, and hope that they will allow us to better understand the mechanisms of allergies and immunotherapy and to raise therapy safety and efficacy, thereby improving patients' health and quality of life.

Conflict of interest

The authors declare no conflict of interest.

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