Chapter 7

Strategies to Study T Cells and T Cell Targets in Allergic Disease

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Abstract

Type I allergy is an immunoglobulin E (IgE)-mediated chronic disease. As such, disease diagnosis and identification of targeted allergens are primarily based on specific IgE reactivity. Over the past decades, the contribution of T cells in allergy pathogenesis has been extensively studied. T cells are not only significant for the onset and maintenance of allergic disease but likely also play a key role for the induction of tolerance by allergen-specific immunotherapy (AIT). Due to the complexity of allergic T cell responses, epitopes have only been thoroughly mapped for the most dominant and prevalent allergens. Recently developed laboratory approaches enable us to perform thorough peptide screens, identifying T cell epitopes in known and novel allergenic targets, irrespective of their IgE reactivity. Monitoring allergen-specific T cells and their phenotype will provide insights into disease manifestation and progression on a molecular level.

However, performing such experiments in the clinic is not feasible. The definition of dominant T cell epitopes will allow us to create a tool to assess allergen-specific T cells in the context of different disease severities, such as rhinitis, asthma, and/or immunotherapy which will likely hold the key for improved diagnostic, biomarkers, and even novel therapeutic approaches.

Keywords: allergy, T cells, Th2, IgE, epitope

1. Introduction

Type I allergy is an immunoglobulin E (IgE)-mediated chronic disease. As such, disease diagnosis and identification of targeted allergens are primarily based on specific IgE reactivity. Specifically, clinical practices for the diagnosis of allergic disease are most commonly based

on skin prick testing [1], which typically involves pricking the skin with a needle or pin containing a small amount of allergen [2]. A second diagnostic test is commonly performed in vitro for allergen-specific immunoglobulin E (IgE), which can accurately evaluate and quantify the presence or absence of IgE specific for the whole allergen extract or single protein components [3].

The importance of IgE in mediating allergic disease, especially immediate-type reactions occurring within minutes of exposure to the allergen, is evident. However, the involvement of allergen-specific T cells and their pathological role in mediating late-phase reactions [4, 5] is often underappreciated. Allergenic proteins are defined based on their ability to bind IgE and the frequency of allergic patients harboring specific IgE antibodies to a given allergen [6, 7]. The potential of an allergenic protein to induce T cell reactivity is mostly not taken into account when classifying a protein as an allergen. Over the past decades, however, the contribution of T cells, specifically T helper 2 (Th2) cells, in mediating the pathogenesis of allergy has been extensively studied [8]. Immunological studies have shown that T cells play a key role early on, before allergic disease is even established. Susceptible individuals initially exposed to allergen mount a dominant Th2 response, resulting in the production of type 2 cytokines, such as IL-4 and IL-13. These cytokines along with a direct physical interaction of T and B cells occurring between CD40L expressed on the surface of the activated T cell and CD40 constitutively expressed by B cells provide the signal for B cells to undergo antibody class switching and produce allergen-specific IgE [9, 10], a process referred to as allergic sensitization. Subsequently, IgE molecules now present in high abundance bind with high affinity to Fcε receptors expressed on granulocytes, where they are cross-linked by allergen molecules upon reexposure, leading to mediator release and immediate-type symptoms, such as urticarial, allergic rhinitis, and conjunctivitis. Immediate-type reactivity is followed by late-phase reactions, which typically occur several hours/days after exposure to allergen. During the late-phase reaction, the affected tissue is infiltrated by Th2 cells and other inflammatory cells including eosinophils and neutrophils, which secrete high levels of cytokines, such as IL-4 and IL-5 to promote inflammation [8].

T cells are not only significant for the onset and maintenance of allergic disease but likely also play a key role for the induction of tolerance, which can be achieved by allergen-specific immunotherapy (AIT) and is the only curative treatment for allergic disease to date. Due to the complexity of human T cell responses against allergens, epitopes have only been thoroughly mapped for the most dominant and prevalent allergens. Recently developed laboratory approaches enable us to perform thorough peptide screens, which achieve the identification and immunological characterization of T cell epitopes in known and novel allergenic targets, irrespective of their IgE reactivity [11, 12]. Mapping of T cell epitopes is of high importance: it greatly facilitates the detection, immunological analysis, and phenotypic characterization of allergen-specific T cells in patients suffering from allergic or asthmatic disease as well as providing a tool to monitor the efficacy of allergen-specific immunotherapy (AIT) treatment. While allergen extracts can also be used to stimulate allergen-specific T cell responses, extracts are not standardized resulting in great variability of allergen content between extract

batches [13–15], and endotoxin content is often not monitored [16]. Further, processing and presentation of a large number of peptides present in extract limit the abundance of peptides that represent dominant T cell epitopes. It has been reported that allergen-specific T cells in tissues and peripheral blood are of very low frequency [17, 18], ranging from approximately 10^{-5} to 10^{-3} CD4+ T cells, outside or within the pollen season, respectively. The rarity of these cells poses a great challenge for immune mechanistic studies designed to probe how allergic pathology or tolerance induction during AIT administration is orchestrated. The identification of dominant T cell epitopes can therefore be of great importance not only to understand the molecular entities targeted by allergen-specific T cells but also to use them as a tool to detect, isolate, and characterize allergen-specific T cells.

The frequency of patients harboring IgE responses against a specific allergen is most often known and used for classification of the allergen as a minor or major allergen in a respective population [19, 20]. In contrast, T cell epitope data is only available for a small subset of allergens listed by the International Union of Immunological Society (IUIS) database [12]. The relative lack of data on allergen T cell epitopes is likely due to the highly complex nature of T helper cell responses in allergic disease, which makes it a difficult system for immunological studies. Moreover, allergen-specific T cells occur at a very low frequency in the peripheral blood [18], making them hard to detect and isolate. Nevertheless, immunological studies on the allergic T cell response in humans have become of growing importance over the last years. Accordingly, new technologies and concepts have been developed to overcome the challenges of studying allergen-specific T cell responses, map single epitopes, and phenotypically characterize peptide-specific T cells to gain more insights into how T cells contribute to the pathology of allergy and asthma.

2. Challenges of T cell epitope mapping

The identification of T cell epitopes from major allergens is an important goal in allergy research. A critical step for inducing a T cell response against an allergen is the recognition of allergen-derived peptides. These peptides are presented to the T cell by antigen-presenting cells (APCs), such as dendritic cells or monocytes, in the context of major histocompatibility complex (MHC) class II molecules, which are constitutively expressed by APCs. MHC class II molecules are encoded by three different loci, designated HLA DR, DQ, and DP. Each of these three loci is extremely polymorphic adding a high degree of complexity, which has to be accounted for in the design of T cell epitope mapping strategies [21].

2.1. Overlapping versus predicted peptide

To identify T cell epitopes in allergy, the most diligent approach involves testing overlapping peptides that span the entire sequence of the allergen of interest. For this setup, the entire allergen sequence is broken down into short peptides, typically 12–20 amino acids in length, overlapping by 9–12 residues. These peptides are then tested for their ability to induce T cell

reactivity, using peripheral blood mononuclear cells (PBMCs) from allergic patients, often after in vitro expansion with allergen or allergen extract [22, 23]. Peptides that elicit T cell reactivity, as measured by cytokine production, proliferation, or upregulation of T cell activation markers, are reported as T cell epitopes. T cell epitope mapping using overlapping peptides is a very thorough approach, designed to identify any possible T cell-activating region within the allergen. However, mapping peptides for bigger allergens or even multiple allergens can add up to a very high number of peptides to test, also increasing the amount of blood needed for screening and the cost and effort associated. To make large-scale epitope identification more feasible, an approach was developed that involves preselection of peptides based on their ability to bind human MHC class II molecules. MHC molecules have a relatively broad specificity for peptide binding. The three-dimensional structure forms a binding cleft that can bind peptides of varying length, typically ranging from 15 to 25 amino acids [24, 25]. The capacity of a peptide ligand to bind MHC class II molecules can be quantitatively measured directly by assessing its ability to inhibit the binding of a radiolabeled probe peptide to purified MHC molecules [26]. However, such experiments are labor intensive and expensive; therefore, computational tools are continuously being developed to model and predict peptide-MHC binding [27, 28]. Using predicted peptide binding as a preselection criterion to decrease the number of peptides to screen for T cell epitope identification is less thorough than using overlapping peptides and may therefore increase the risk of missing T cell-reactive peptides. However, it has been reported that it is a reliable approach to identify the vast majority of T cell epitopes [28, 29], and it has been successfully used in several allergen systems, including Timothy grass [11], German cockroach [30], house dust mite [31], and others [32], to perform large-scale epitope identification studies. Therefore, the decision between using overlapping and predicted peptides is likely dictated by the size and number of allergens studied as well as the amount of cells available from the clinical cohort.

2.2. Allergen-specific T cell frequencies

Another challenging aspect of T cell epitope identification in allergy is the low frequency of allergen-specific T cells. A study that evaluated the ex vivo frequency of T cells specific for Fel d 1, the major cat allergen, reported that the percentage of CD4+ T cells specific for a single Fel d 1 epitope ranged from 0.014 to 0.0003% in allergic individuals [33]. Another study, focused on Mugwort allergy, reported an ex vivo frequency of peptide-specific T cells of 0–0.029% in allergic cohort [34]. In a third study, performed with cells from patients allergic to Timothy grass, the authors reported epitope-specific T cell frequencies of 0.6–0.75% of the total CD4+ T cell subset [35], with a modest increase in frequencies detected during grass pollen season. The rarity of allergen-specific T cells poses a great challenge for epitope identification, as it will require the T cell reactivity assay to reliably detect a few single cells that respond to the peptide among several thousands of CD4+ T cells. In addition, a large amount of blood volume would be required to screen a given number of peptides. To bypass this problem, in vitro expansion cultures are performed, in which lymphocytes from allergic individuals are cultured over a few days or weeks with allergen extract or recombinant allergen protein to which the donor is allergic. The allergen in the culture will activate and stimulate the few antigen-specific T cells

present in the culture, causing them to proliferate. Typically, recombinant human IL-2 is added in limiting dilution in regular intervals after the first few days of culture to increase proliferation of allergen-specific cells, which have upregulated their IL-2 receptor during cell activation. Over time, allergen-specific cells, which were rare in the starting culture, become highly enriched due to antigen-specific stimulation and proliferation. After several days, the cells can be harvested in screened for T cell reactivity in response to restimulation with single peptides. In the presence of allergen or whole allergen extract, allergen peptide-specific T cells will have expanded and are now present in high abundance, making them easily detectable after restimulation with single peptides. T cell reactivity I response to a peptide can be measured by a variety of assays, most commonly using proliferation, cytokine production, or upregulation of activation marker as a readout [22, 36, 37]. This method is extremely useful to expand very rare antigen-specific CD4+ populations. However, one major limitation associated with in vitro expansion culture is that it changes the original phenotype of the cells. Therefore, it cannot be performed if an immunological characterization of the phenotype of the antigen-specific cells is desired. Analyses designed to investigate the genetic expression profile have to be performed on cells isolated directly ex vivo, which is difficult due to their aforementioned rarity in the peripheral blood.

3. Immunological characterization of allergen-specific T cells

There are several approaches to isolate allergen-specific cells ex vivo for subsequent downstream immunological profiling using technologies, such as RNA or TCR sequencing. These technologies have become of increasing importance in areas, such as biomarker discovery or developing tools to monitor the efficacy of allergen-specific immunotherapy.

3.1. MHC tetramer assay

The use of MHC tetramer reagents to detect antigen-specific T cells is a well-established technique that allows detection and further downstream analysis of allergen-specific cells on a single cell level. The tetramer molecule is made up of a fluorescently labeled, centric streptavidin molecule bound to biotin-labeled MHC molecules, which are loaded with a peptide known to be a T cell epitope to form the peptide-MHC complex (**Figure 1A**) [38]. The resulting tetramer can then be used as a reagent to bind T cells that are specific for both, the MHC type and peptide used in the tetramer (**Figure 1B**). Cells that are specific and bind the tetramer are now fluorescently labeled and can be detected and isolated using a flow cytometer. There are several applications for tetramer staining all based on the premise that it allows the detection of single antigen-specific cells, even if they occur at low frequency. In vaccinology, tetramers are often used to track frequencies of peptide-specific T cells in the blood before and after vaccination or boost. Similarly, in allergy, tetramers have been used to quantify numbers of specific T cells as a variable of allergen season [35], allergen-specific immunotherapy [39], and disease status [34, 40]. In addition, tetramer staining can be combined with other methodologies to perform more detailed immunological characterization of allergen-specific T cells. Simultaneous assessment of cell proliferation, cytokine production, or activation can provide

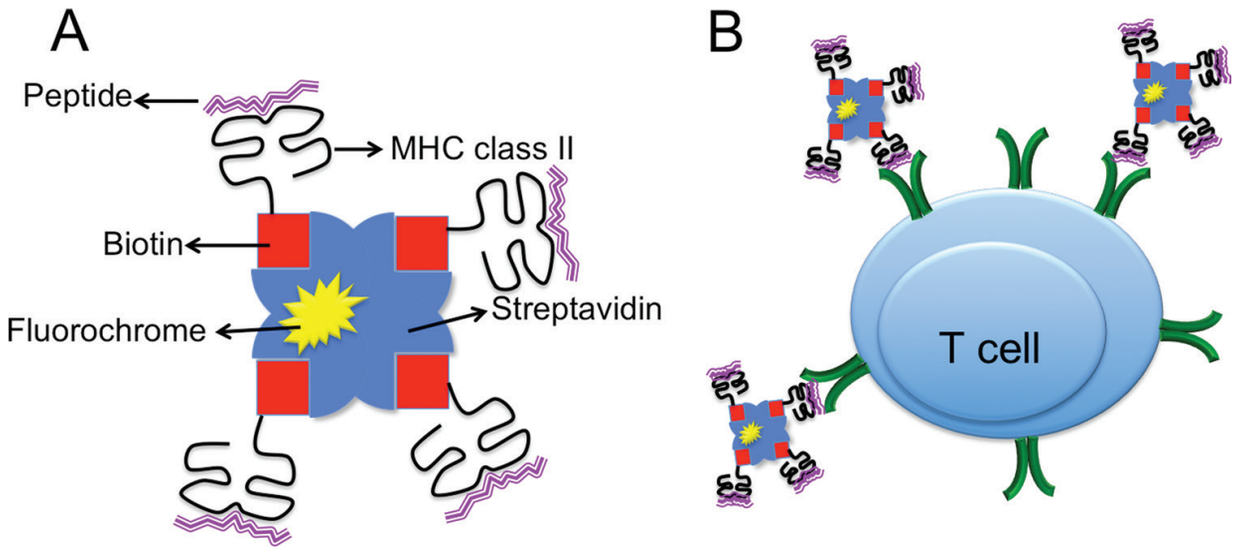

Figure 1. (A) Schematic representation of the structural complex of a MHC class II tetramer and (B) binding of tetramer molecules to the peptide-specific T cell via the T cell receptor (TCR).

functional information in addition to knowing the peptide specificity and MHC restriction of the cell. Tetramer reagents can even be used for T cell epitope mapping. This approach involves loading empty MHC molecules with pools of mixtures of overlapping peptides from the allergen of interest, each pool typically containing 5–10 peptides. These tetramers are then screened with PBMC that have been cultured with the allergen of interest. Pools that positively detect T cell populations are deconvoluted into single peptides, which are loaded onto MHC molecules individually and then analyzed to identify single epitopes. Tetramers that return positive stainings automatically provide a population of T cells with a known MHC restriction and antigen specificity, which can be sorted by fluorescence-activated cell sorting (FACS) allowing downstream analysis of phenotype and genotype. This approach has successfully been used in allergy to identify T cell epitopes [41].

MHC tetramer assays represent a revolution for the study of antigen-specific T cells, providing an efficient way to directly visualize, quantify, phenotype, and isolate T cells of interest. Yet, this technology is also associated with disadvantages and limitations. The construction of tetramer reagents is not trivial and requires an advanced level of expertise. Production and purification of high-quality MHC molecules are labor intensive, and only a subset of MHC alleles expressed by humans is available as tetramer. Further, the use of tetramers requires existing knowledge about the HLA restriction of the peptide of interest. In allergy, many dominant T cell epitopes are highly promiscuous, meaning they are restricted by multiple alleles, which makes finding their restriction more difficult. Determining the HLA restriction of given peptides can be done experimentally, for example, by inhibition with locus-specific antibodies [32]. This method, however, only identifies the restricting locus. Data from HLA-binding assays can also be a useful tool to narrow down the possible restriction [42]. Another approach designed to determine HLA restriction at the allele level involved the use of single HLA class II-transfected cell lines [43]; however, a large panel of cell lines is required to determine restrictions in multiple donors due to the heterogeneity of HLA types in a given population. As an alternative to the experimental

approaches, which are labor intensive and technically challenging, a bioinformatical prediction tool was developed. This tool uses T cell response data in an HLA-typed population to infer HLA restriction by genetic association [44]. Although this tool streamlines the prediction of HLA restriction, it still requires experimental T cell response data and an HLA-typed population large enough to make significant predictions possible.

The use of tetramer reagents requires preexisting knowledge about the HLA restrictions for a given peptide as well as the HLA type of the donor sample. Acquiring this information can be costly and labor intensive, making this approach less feasible for certain studies.

3.2. Cytokine capture assay

The isolation of antigen-specific cells based on cytokine production used to be complicated by the fact that T cells positive for cytokine production were detected by intracellular cytokine staining, which involved fixation and permeabilization of the cell. Fixed cells are no longer alive and can therefore not be used for downstream applications that require live cells, and even isolation of DNA or RNA from fixed cells is somewhat more complex than from live cells. A new approach that captures cytokines on the cell surface immediately after secretion was developed to allow detection and isolation of viable cells that secrete cytokines in response to antigen stimulation. In this protocol, cells are pre-labeled with a "catch reagent," a divalent complex consisting of a CD45-specific monoclonal antibody conjugated to monoclonal antibody directed against the cytokine of interest. The anti-CD45 antibody will bind to CD45 molecules expressed on the T cell surface and effectively coat the cell (**Figure 2**). Subsequently, cells are stimulated with antigen, and any cytokine produced will be bound to the cytokine-specific antibody conjugated to anti-CD45 immediately after secretion. Detection of cytokine-positive cells is achieved by using a fluorescent-labeled detection antibody with the same cytokine specificity but recognizing a different epitope from the catch reagent antibody (**Figure 2**). If the antigen-specific T cell population is extremely rare, which is often the case in allergy and asthma, an enrichment step can be performed. To further enrich antigen-specific cells before flow cytometric analysis or isolation, microbeads conjugated to monoclonal antibodies specific for the respective fluorophore used in the experiment can be used to label cells, followed by magnetic column enrichment [35]. After cells are labeled and the enrichment step has been performed if desired, viable cells can be analyzed and isolated by flow cytometry, facilitating downstream applications, such as further culture assays or DNA/RNA extraction for sequencing analysis. A potential limitation of this assay is the bias introduced by isolating cells based on production of a single cytokine. Often, cytokine production in response to allergens is heterogeneous, and cells produce different levels of different cytokines, such as IL-4, IL-5, IL-13, and sometimes IFNg. Detection of allergen-specific cells based on production of a single cytokine will likely lead to an underrepresentation of allergen-specific cells, since cells producing a different cytokine will not be detected.

3.3. Cell activation assays

Another hallmark of antigen-specific T cells is the upregulation of activation markers in response to antigen stimulation. Therefore, these activation markers can be targeted with

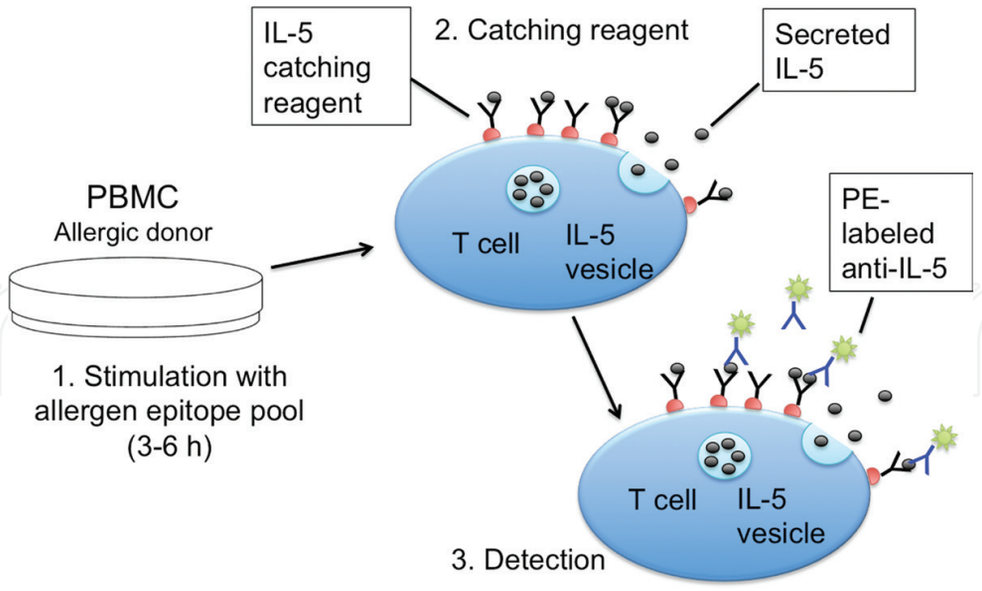

Figure 2. A schematic representation of the methodology involved for a cytokine capture assay, using IL-5 as representative cytokine.

fluorescent antibodies to detect antigen or allergen-specific cells. The challenge of this approach is to identify activation markers that are specific and highly expressed to allow reliable detection of allergen-specific T cells even at low frequency. One molecule that has become very popular for such an application is CD154, also known as CD40 ligand (CD40L). CD154 is a member of the tumor necrosis factor (TNF) superfamily and found to be primarily expressed on activated T cells, making it very specific. It acts as a co-stimulatory molecule, binding to CD40 on antigen-presenting cells, which can lead to several downstream events depending on the target cell type. Several studies designed to study allergen-specific effector cells in cohorts suffering from allergy, asthma, or who have been treated with AIT have successfully applied this methodology to immunologically characterize and isolate allergen-specific T cells ex vivo [37, 45]. The caveat of using CD154 as a selection marker for activated, allergen-specific T cells is that it is also typically stained intracellularly. In humans, CD154 molecules expressed on the cell surface quickly become unstable, making a large number of CD154 expressing cells undetectable. Therefore, this assay typically involves fixation and permeabilization to allow intracellular staining of CD154, making downstream applications less feasible.

As an alternative to CD154, other activation markers, such as Ox40 and CD25 have also been used to detect and isolate antigen-specific cells after short-term antigen stimulation [46]. The main advantage of this approach is that both Ox40 and CD25 are stably expressed on the cell surface and therefore cells can be detected and isolated in viable form without the need of fixation or permeabilization. However, CD25 is also strongly expressed by regulatory T cells, irrespective of activation; therefore, gating of Ox40 and CD25 double-positive cells has to be performed with great accuracy, and the inclusion of a third marker, such as PDL-1 may be considered to avoid contamination of nonspecific T cells.

3.4. Proliferation assays

The identification of antigen-specific cells based on the proliferative response to antigenic stimulation is perhaps the most classical approach and has been widely used for several applications including T cell epitope mapping, phenotypic characterization, T cell response kinetics, and others. In the past, the classic method to detect cell proliferation in response to allergen stimulation involved the addition of radioactive nucleoside, 3H-thymidine, to the culture, which would be automatically incorporated into new strands of chromosomal DNA during mitotic cell division. Subsequently, proliferation was assessed by measuring the radioactivity in DNA recovered from the cell sample using a scintillation beta-counter. Though this technology is still used in some laboratories, proliferation is now more commonly detected by flow cytometry. One common approach is the staining of cells with a special fluorescent dye, which is then diluted through each cell division. This decrease in the concentration of the dye can be visualized by flow cytometry. Another approach is to stain stimulated cells with antibodies targeting markers associated with proliferation, such as Ki67. The measure of proliferation in response to antigen stimulation is straightforward and inexpensive. The greatest caveat associated with using proliferation as a readout for antigen-specific reactivity is the relatively high rate of false positivity due to bystander activation. A study designed to directly compare the use of tetramer staining reagents versus allergen-induced proliferation for the detection of allergen-specific T cells found that while tetramers had a relatively low rate of sensitivity, cells identified based on proliferation contained extremely high fractions of bystander cells [34], making this approach more suitable if an enriched population is sufficient for the study rather than a desire for a pure antigen-specific population.

4. Targeting T cells in allergen-specific immunotherapy

Allergy and asthma are debilitating diseases that are most commonly treated using pharmacotherapy which are designed to improve the symptoms but not the cause of disease. To date, the only disease-modifying therapy available is allergen-specific immunotherapy (AIT). First administered over a century ago [47], AIT has been widely demonstrated to be a clinically effective treatment, inducing immunological tolerance and improvement of clinical symptoms beyond the time of treatment [48]. Despite its favorable duration of efficacy, a considerable effort is invested to improve current AIT protocols. Allergen-specific immunotherapy with whole extract can be associated with IgE-mediated adverse reactions that result from the patient's allergen-specific IgE molecules being cross-linked by the allergen present in the extract used for treatment, triggering degranulation and immediate-type reactions. The occurrence of such adverse events and the need for extended treatment periods that last several years can have a negative impact on treatment compliance. For this reason, researchers have strived to find a treatment that targets T cells and circumvents potential IgE reactivity. Removal of IgE epitopes, thereby eliminating the risk of IgE cross-linking, is one obvious approach. There are a variety of methods to achieve this goal, some of which have been evaluated in clinical trials.

4.1. Peptide immunotherapy

One extensively pursued approach for AIT focused on T cells while omitting IgE epitopes is called peptide immunotherapy, where instead of using whole allergen extract, allergic patients are treated with a mixture of short, synthetic peptides that constitute the major T cell epitopes of the allergen the patient is allergic to. The clinical efficacy of peptide immunotherapy has been demonstrated in several Phase IIb double-blind, placebo-controlled trials [49, 50]. A significant reduction in symptoms, measured as the total rhinoconjunctivitis symptom score (TRSS), was observed following the administration of only eight intradermal injections of the peptide formulation. In this study, TRSS levels remained suppressed both at the 1- and 2-year follow-up time point [51]. The immunological mechanisms by which peptide immunotherapy induces tolerance are not yet fully understood. However, studies have reported a downregulation of pathological type 2 cell responses and a concomitant increase in regulatory signals, such as the production of IL-10 in the periphery. Further, significant increases in IFNg-producing Th1 cells and CD25+ cells have been reported. The induction of IgG4-blocking antibodies, which are believed to contribute to clinical efficacy by occupying the allergen-binding sites, thereby preventing IgE-allergen binding, is a hallmark event during conventional AIT with allergen extract. Interestingly, increased levels of IgG4 are rarely observed, probably due to the lack of conformational B cell epitopes decreasing the likelihood of B cell stimulation and resulting IgG production. Therefore, though modulatory events on the cellular level appear to be broadly similar to those believed to occur during extract-based AIT, humoral responses may be more distinct. Although peptide immunotherapy has been shown to be clinically effective, it is also associated with challenges that need to be addressed. The route of administration has been debated, and the clinical effects seem to be very sensitive to dosing. Lower doses may not induce tolerance due to lack of potency for induction of regulatory T cells, while too high dose may stimulate and expand pathogenic Th2 cells. The selection of peptides is also a factor of consideration. Typically, mixtures used for peptide immunotherapy include between 5 and 10 peptides. However, epitope specificities can be very heterologous in a given population, and therefore the selection may not be straightforward. The consideration of these factors and others make the development of peptide immunotherapy challenging at times.

4.2. Fragmented allergens

Another approach of AIT that was designed to target T cells while bypassing IgE binding to avoid IgE-mediated side effects is the generation of fragmented allergens. This approach was tested using the major birch pollen allergen, Bet v 1, as a model. The fragmentation of the allergen involved its division into non-IgE-binding fragments, which retain their T cell reactivity. Birch pollen allergic patients were then vaccinated with these hypoallergenic derivatives in a double-blind, placebo-controlled study. This vaccination was found to reduce cutaneous sensitivity, improve symptoms, and significantly reduce rises in birch-specific IgE levels during season in the active group compared to placebo [52]. However, immunological mechanisms and long-term efficacy were not evaluated.

5. Concluding remarks

Allergic disease severity is very poorly understood. The degree of symptom manifestation, such as asthma versus rhinitis can often not be explained by allergen-specific IgE titers. There is a dire need for better diagnostics and biomarkers that will help us evaluate treatment options and disease prognosis. Gaining a better understanding of the immunological events on a cellular level may have a tremendous impact on how we treat patients in the clinic. Monitoring allergen-specific T cells and their phenotype will provide insights into disease manifestation and progression on a molecular level. However, performing such experiments in the clinic is not feasible. The definition of dominant T cell epitopes will allow us to create a tool to assess allergen-specific T cells in the context of different disease severities, such as rhinitis, asthma, and/or immunotherapy which will likely hold the key for improved diagnostic, biomarkers, and even novel therapeutic approaches.

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