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REVIEW ARTICLE

Advances in allergen-specific immune cell measurements for improved detection of allergic sensitization and immunotherapy responses

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Abstract

Over the past two decades, precision medicine has advanced diagnostics and treatment of allergic diseases. Component-resolved analysis of allergen sensitization facilitates stratification of patients. Furthermore, new formulations of allergen immunotherapy (AIT) products can more effectively deliver the relevant components. Molecular insights from the identification of allergen component sensitization and clinical outcomes of treatment with new AIT formulations can now be utilized for a deeper understanding of the nature of the pathogenic immune response in allergy and how this can be corrected by AIT. Fundamental in these processes are the allergen-specific B and T cells. Within the large B- and T-cell compartments, only those that specifically recognize the allergen with their immunoglobulin (Ig) or T-cell receptor (TCR), respectively, are of clinical relevance. With peripheral blood allergen-specific B- and T-cell frequencies below 1%, bulk cell analysis is typically insufficiently sensitive. We here review the latest technologies to detect allergen-specific B and T cells, as well as new developments in utilizing these tools for diagnostics and therapy monitoring to advance precision medicine for allergic diseases.

KEYWORDS
allergen, B cell, basophil, flowcytometry, T cell, tetramer

1 | INTRODUCTION—THE CHALLENGE: DETECTION OF ALLERGEN SPECIFICITY

The general understanding of the pathogenesis of allergic disease is high,[1] and there are multiple forms of treatment, including allergen immunotherapy (AIT), which has been practiced for >100 years.[2] Although the key question of why some individuals mount a hypersensitivity response to environmental proteins remains unanswered, the allergic response itself is well characterized. Due to what are presumed to be genetic and environmental triggers, an individual may mount an aberrant type 2 immune response and become allergen-sensitized.[3] This response is driven by type 2 T helper cells (Th2),[4] which subsequently skew the B-cell response toward the production of IgE type antibodies (Figure 1).[5] IgE binds with high affinity to Fcε receptors (FcεRI) on the immune effector cells: mast cells and basophils.[6] Upon subsequent allergen exposure, allergen molecules are recognized by cell-bound IgE, inducing FcεRI cross-linking and cellular degranulation to release effector molecules that elicit the allergic symptoms (Figure 1).[7]

Detection of soluble allergen-specific IgE in an individual with clinical disease is typically regarded as the gold-standard for identification of allergen sensitization (reviewed in Ref. 8). Even though serum IgE correlates well with responses to allergen challenges,[9] this has its limitations:

- Serum-specific IgE can be used to distinguish sensitized from non-sensitized individuals, but it is a marker that is only present after the development of a pathogenic response and can provide little if any information on the disease cause/origin;
- Serum-specific IgE persists following clinically successful treatment with AIT. Thus, it cannot be used to obtain understanding of how this treatment modifies the pathogenic response or to monitor clinical response.

Theoretically, detailed examination of the cells that form the basis of disease and those that mediate the effector response can provide a better understanding of the nature of the pathogenic response and its modification following treatment.[5,10,11] Specifically, these involve allergen-specific T and B cells, as well as mast cells and basophils that capture IgE (Figure 1).[4,12] The challenge in studying these cells lies in the technologies that enable allergen-specific detection, and in the case of B and T cells, the need for high-throughput analysis to facilitate detection of rare events.

We here review technological advancements that have enabled detailed examination of allergen-specific immune cells, how these have already provided new insights into disease pathology, treatment evaluation, and differential diagnosis, and what is to be expected in the near future.

Future Research Perspectives

- Allergen-specific B and T cells can provide highly specific biomarkers for predicting immunotherapy outcome.
- Flow cytometric detection of allergen sensitization on blood basophils (CytoBas) forms a rapid test for component-resolved and differential diagnosis of allergen sensitization.

Major Milestone Discoveries

- Detection of allergen-specific T cells with fluorescently tagged recombinantly produced peptide-MHC multimers.
- Sensitive detection of allergen-specific B cells using double discrimination with two fluorescent allergen tetramer conjugates.
- Multiplex flow cytometric interrogation of surface IgE bound to FcεRI on blood basophils using fluorescent allergen tetramers to better define allergen sensitization.

2 | THE TARGETS: SPECIFIC ANTIGEN RECEPTORS ON LYMPHOCYTES AND FCR-CAPTURED IG

T and B cells are defined by the expression of antigen receptors, that is, T-cell receptor (TCR) and B-cell receptor (BCR), the surface-expressed variant of immunoglobulin (Ig).[13] T helper (Th) cells are characterized by the expression of CD4, which acts as a co-receptor with the TCR to bind to major histocompatibility complex (MHC) class II molecules.[14] MHC class II expression is restricted to immune cells, in particular monocytes, dendritic cells, and B cells, and presents peptides derived from foreign proteins (Figure 1).[14] Human leukocyte antigens (HLA) corresponding to MHC class II are HLA-DP, HLA-DM, HLA-DOA, HLA-DOB, HLA-DQ, and HLA-DR. While for MHC class I, there is a dominant allele in the population worldwide (HLA-A*02; 29–46%), this is not the case for any MHC class II encoding genes.[15] As a result, there is a large inter-individual variation in HLA and the peptides that are presented. Each T cell expresses a TCR with distinct specificity, which results from genomic rearrangement of V, D, and J genes in their TCR loci to create a unique first exon that encodes the variable domain.[14] Typically, the TCR of Th cells is a heterodimer composed of a TCRα and TCRβ chain. Antigen-recognition via the TCR requires co-stimulation of the CD28 receptor to fully activate the T cell.[14] Additional cytokine exposure can then drive the T-cell fate, with thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 driving the Th2 fate.[16]

The BCR is composed of two identical Ig heavy (IgH) and two identical Ig light chains, and thus contains two identical antigen-binding sites. Similar to the TCR genes, the *IGH* as well as the *IGK* and *IGL* light chain loci undergo V(D)J recombination in progenitor B cells to create a functional receptor with unique specificity.[17] In contrast to the TCR, the BCR does not require antigen presentation and can directly recognize linear or conformational epitopes in 3D structures, either in solution or on cell surfaces.[18] In addition to the BCR signal, B cells require a co-signal for activation. This is typically provided by CD154 on Th cells that acts as a ligand for CD40 on B cells.[19] Cognate T-cell help is given by activated T cells that recognize processed antigen presented in MHC class II by the B cell. Activated Th2 cells secrete IL-4 and IL-13 that skew Ig class switching toward IgE.[20] The activated B cell will then differentiate into either IgE-expressing memory B(mem) cells or IgE-secreting plasma cells.[21]

Serum IgE has a relatively short half-life (2–3 days).[22] However, a large fraction of secreted IgE is captured by the high-affinity Fcε receptor (FcεRI) on mast cells and basophils where it can remain fixed for weeks.[23] On these cells, IgE acts as a sensor for antigen, and when cross-linked, FcεRI induces signaling to activate the cell and induce degranulation to release inflammatory factors. Whereas each B cell only expresses surface Ig with the same specificity, the IgE molecules bound to FcεRI on the surface of a mast cell or basophil have a diverse range of specificities.

3 | THE TECHNOLOGIES: IN VITRO ACTIVATION VERSUS LABELED MHC AND PROTEIN "BAITS"

With the high repertoire diversity of T- and B-cell antigen receptors (>10^{15}),[24] detection of those T and B cells directed against a

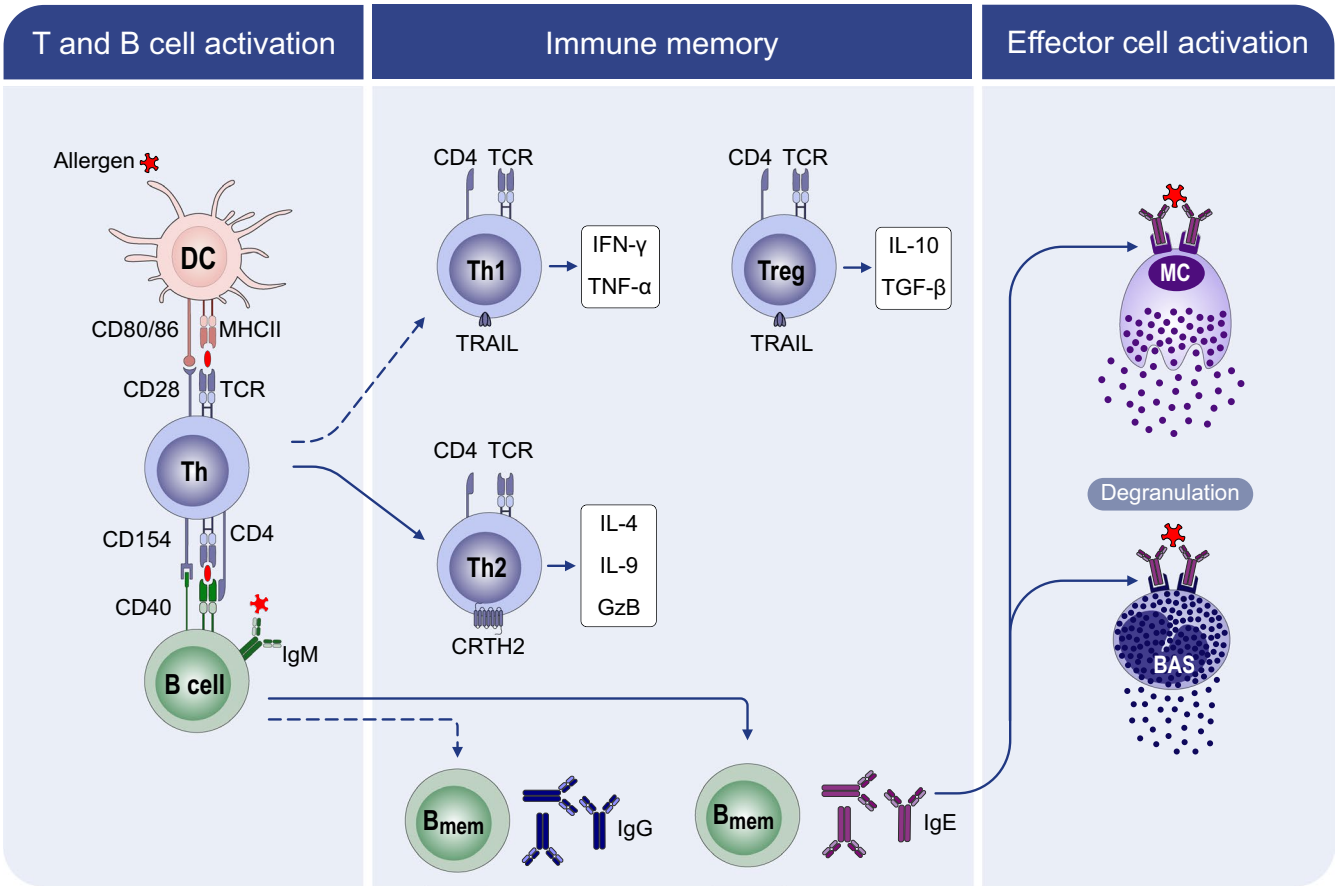

FIGURE 1 Specificity of immune cells and molecules in the allergic response. *T- and B-cell activation*: allergen presentation by dendritic cells (DC) to T cells via peptide-MHC class II – T-cell receptor (TCR) interactions with co-stimulation, esp. CD28 – CD80/86. *Immune memory*: Activated T helper (Th) cells provide cognate support for B-cell activation through CD40 – CD154. In an allergic response, Th cell differentiation is skewed toward Th2 with expression of CRTH2, IL-5, IL-9, and granzyme B (GzB). Furthermore, B cells are poised to IgE class switching. *Effector cell degranulation*: Soluble IgE binds to Fcε receptors on target cells: mast cells (MC) and basophils (BAS). Upon re-exposure to the same allergen, it will bind to this cell-bound IgE and induce receptor cross-linking, cell activation and degranulation, with the release of inflammatory mediators

single allergen can be challenging due to their low frequencies. This is also the case for the memory T- and B-cell compartments, which are already developed and diverse from early childhood.[25] Thus, detection of rare allergen-specific lymphocytes requires evaluation of large cell numbers and approaches to specifically label the cells of interest. In the case of T cells, there are two technological approaches: in vitro stimulation with antigen or peptide, or labeling with peptide-MHC (pMHC) multimers.

The first involves in vitro stimulation of peripheral blood mononuclear cells (PBMC) with either whole allergen or immunodominant peptides (Figure 2A). Limiting dilution has traditionally been used to identify and amplify antigen-specific cells over several days in culture.[26] ELISPOTs can then detect cytokine production for functional analysis.[27] Using flowcytometry, dilution of a labeling dye such as carboxyfluorescein succinimidyl ester (CFSE) in combination with an activation marker (eg, CD25) can improve sensitivity and specificity.[28] Identification of markers that are upregulated shortly after activation has enabled detection of antigen-specific T cells within 24 h. For this activation-induced markers (AIM) T-cell assay, a combination of surface markers can be used to detect antigen-specific CD4+ T cells (CD25, CD69, CD137, CD154, and/or OX40) and CD8+ T cells (CD25, CD69, CD107a, CD137, and/or OX40).[29] These markers can be readily combined with intracellular cytokine staining for functional analysis.[29] Detection following in vitro activation has the advantage of applicability to all subjects, irrespective of their HLA types, because the allergens are presented by their own antigen-presenting cells in the PBMC fraction. Furthermore, the activation enables detection of cytokine expression to delineate regulatory T cells (Treg) and various Th-cell subsets (Th1, Th2, Th17, and Th22).[28,30] However, it can be a disadvantage that the cells are manipulated in vitro, as this might change their phenotype and relative numbers within the examined population, limiting absolute cell number quantification.

The second approach omits the need for in vitro stimulation by direct staining of T cells ex vivo with recombinant pMHC complexes.[31,32] Typically, recombinant MHC proteins are produced with a biotin label, followed by tetramerization to fluorochrome-conjugated streptavidin for flowcytometric detection (Figure 2B).

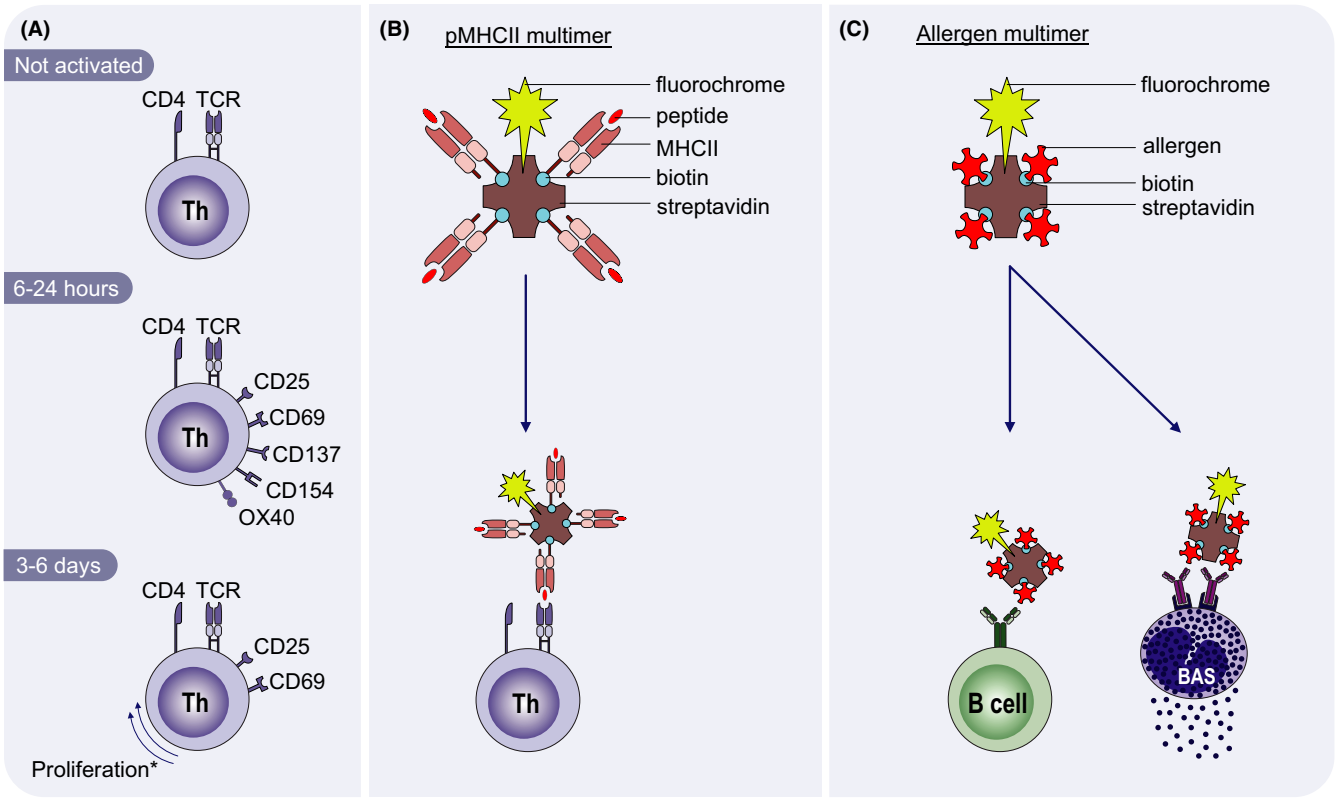

FIGURE 2 Detection of allergen specificity on immune cells. A. Activation-induced markers (AIM) on in vitro activated T cells. Following in vitro stimulation with allergen, several surface markers are specifically expressed on activated CD4+ Th cells. After 6–24 h, these include CD25, CD69, CD137, CD154, and OX40. After 3–6 days culture, CD25 and CD69 are still detectable, and allergen-specific cells can be identified on the basis of dilution of a cell-labeling dye. B. peptide-MHC and C. allergen tetramers for the detection of allergen-specific T cells, B cells, and allergen sensitization on basophils. Schematics are shown for pMHC and allergen tetramers formed by targeted biotinylation and coupling to a fluorescently-labeled streptavidin. Abbreviations: TCR, T-cell receptor; Th, T helper cell; pMHCII, peptide-MHC class II; and BAS, basophil

These MHC tetramers can then be loaded with the peptide of choice and used as "bait" to fluorescently stain those T cells that recognize the pMHC complex. Tetramerization is an important means to increase avidity, because TCR interaction with soluble pMHC has an inherently fast dissociation rate.[33] Higher-order multimers including five MHC molecules linked to a coiled-coil multimerization domain (pentamers), and six MHC molecules attached to a dextran backbone (dextramers) can provide more sensitive means to detect antigen-specific T cells with low-affinity TCRs.[34]

pMHC multimers have been widely utilized for the detection of antigen-specific CD8 T cells, with the major advantage being that a single allele, HLA-A*0201, is present in 29–46% of the population. This limits the variation in dominant CD8 T-cell epitopes between individuals. There is not such a prominent allele encoding an MHC II molecule.[35] Therefore, even though the platform for class II MHC tetramers for detection of antigen-specific CD4 T cells is available,[36] uptake has been more restricted than for class I MHC tetramers. HLA II genotyping is often required to select the appropriate MHC II reagent and then identify the dominant epitope(s).[37] In some cases, this can be addressed by selection of immunodominant epitopes that bind promiscuously to multiple HLA class II molecules (reviewed in Ref. 38). In addition to higher order multimers, engineered mutations in pMHC tetramers to enhance CD4 binding have been shown to outperform conventional tetramers for detection of antigen-specific T cells.[39,40] Translation of these findings to reagents for human T-cell analysis could provide an alternative for increased pMHC valency[40] or could be used in combination with higher valencies.

There are pros and cons for both the in vitro activation and the pMHC multimer approach for allergen-specific T-cell detection.[41] pMHC tetramer staining is highly specific, and on high-end instruments, multiple reagents can be included in a single analysis to detect and compare unique specificities. However, this approach is currently limited by sensitivity of detection,[42] and the relatively high costs for pMHC multimers, especially when multiple reagents are combined. In contrast, the in vitro proliferation assay is not restricted to certain HLA types, and through the use of peptide mixes or whole protein, the total pool of reactive CD4 and CD8 T cells can be quantified in a single assay.[29] As a result, the reagent cost will be substantially lower. However, the experimental procedure is more labor intensive and requires optimization to limit background signals from bystander T-cell activation.

In contrast to the TCR, the BCR on B cells recognizes antigen without the need for presentation, and in the case of proteins, there may be linear or structural/discontinuous epitopes. Hence, for

initial studies of fluorescent detection of antigen-specific B cells in experimental animal models, phycoerythrin (PE) was used: a large protein complex with bright red-orange fluorescence.[43] To achieve sensitive detection with smaller antigens, these were tetramerized: the influenza hemagglutinin protein, and mimetopes of dsDNA or phosphorylcholine.[44,45] Furthermore, the availability of pMHC tetramers resulted in early adoption of detection of pMHC-specific B cells to examine alloantigen recognition.[46] pMHC tetramers are recombinantly produced with a peptide tag for targeted biotinylation by enzymatic reaction (Figure 2B). This is advantageous as fluorescent labeling is facilitated through the peptide tag, which will avoid issues with epitope masking.[47] Recombinant proteins with such a tag would theoretically be the best approach for generating B-cell targets (Figure 2C).[48] However, as each target protein will be different, production of a recombinant protein with the same structure and post-translational modifications as the native protein can be a challenge. Alternatively, native proteins can be purified and then directly biotinylated with a chemical reaction.[49,50] As biotinylation can potentially result in epitope masking, this approach will require optimization through comparison of reactive moieties that target different specific amino acid functional groups (eg, amines on lysine, sulfhydryls on cysteines, or carboxyls on aspartic and glutamic acids).[51]

By nature, surface Ig on B cells is capable of binding large molecules.[43] This includes fluorochromes such as PE and allophycocyanin (APC) that are commonly used for pMHC multimer reagents. With the frequencies of B cells binding to the same antigen being as low as 0.01–0.3%,[48-50,52] it is critical to exclude fluorochrome-reactive from antigen-specific cells. A common approach is to incubate the cell mixture with two tetramer reagents of the target antigen coupled to distinct fluorochromes, followed by electronic gating on the double positive events: "double discrimination" (Figure 3A).[48-50,52] In practice, fluorochrome-specific events are less frequent for smaller chemical polymers, but not completely absent.[48]

4 | RECENT INSIGHTS INTO ALLERGIC DISEASE PATHOGENESIS

The allergic response is associated with a skewed Th2 fate,[53,54] and subsequent induction of B cells to Ig class switch to IgE (Figure 1). Long-lived T-cell and B-cell memory directed against the allergen[10,11] drives continued risk of hypersensitivity responses upon re-encounter with the same allergen.

Th2>Th1 skewing in house dust mite (HDM) allergy was recently confirmed through unbiased single-cell transcriptomics of CD154+ Th and CD154-CD137+ Treg cells following 6 h in vitro stimulation with HDM allergen peptides.[55] In addition, a subset of *IL-9*–expressing Th2 cells was observed, consistent with an independent study demonstrating that IL-9–producing Th cells are a subpopulation of Th2 cells.[56] Importantly, IL-9–producing Th2 cells express

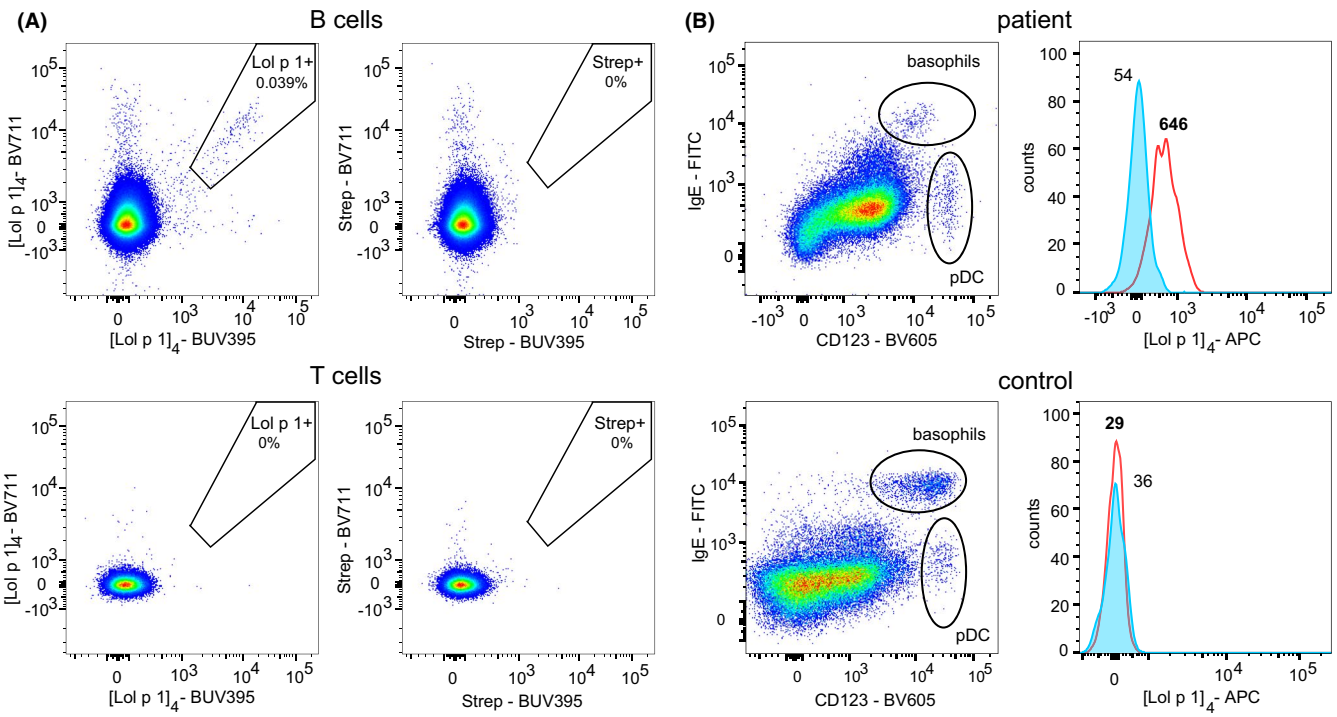

FIGURE 3 Applications of fluorescent allergen tetramers. A. Specific detection of allergen-specific B cells using the double discrimination approach. Staining of BUV395/BV711 Lol p 1 tetramers and BUV395/BV711 streptavidin on B cells (CD19+) and T cells (CD3+). Percentages indicate the proportions of double-positive events within total B or T cells. B. Detection of allergen sensitization on basophils with the CytoBas approach. Left plots depict CD45+SSCdim positive events with gates for CD123+IgE- plasmacytoid dendritic cells (pDC) and CD123+IgE+basophils. Right plots depict the expression levels of APC-conjugated Lol p 1 tetramers on pDC and basophils of a ryegrass pollen-allergic individual (top plot) and a non-allergic control (bottom plot)

increased IL-5 and cytotoxicity molecules, such as granzyme B, suggesting that their role in allergic asthma goes beyond the production of IL-9. Notably, in this study, there was no significant difference in expression of *PTGDR2* that encodes CRTH2, a G-protein coupled receptor found on pathogenic Th2 cells.[57] This could be due to in vitro stimulation affecting *PTGDR2* expression or causing selective depletion of CRTH2+ T cells that tend to have a terminally differentiated (CD27-) phenotype.[58] This could illustrate the need to analyze critical markers on allergen-specific T cells using MHC tetramers without their expression being affected by in vitro stimulation.[59,60]

The reduced proportions of HDM-specific Th1 and Treg found in HDM-allergic individuals were shown to particularly involve cells expressing interferon-response (IFNR) signatures.[55] These ThIFNR and TregIFNR subsets expressed *TNFSF10* encoding TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) that dampens Th cell activation.[61] TRAIL expression could be a functional mechanism through which Th1 and Treg actively inhibit inflammatory responses in an allergen-specific manner.

A distinct subset of CD4+ T cells function to support B-cell responses in germinal centers.[62] These follicular T helper cells (Tfh) are functionally diverse and can be detected in peripheral blood.[63] Recently, it was found that in peanut-allergic patients, peanut-specific Tfh cells were enriched for an IL-4-, IL-5-, and IL-13-producing subset.[64] Although it remains unclear how or why these are generated, they are functionally equipped to support IgE B-cell responses,[64] and will be a relevant target for disease identification and monitoring.

Similar to T cells, allergen-specific B cells can be detected in both allergic and non-allergic or tolerant subjects.[52,65] In blood, single protein-specific memory B cells (Bmem) are rare (well below 1% of total Bmem),[52] and predominantly express IgG or IgA.[65] IgE-expressing Bmem cells are extremely scarce, and their level of surface Ig is 10–100 fold lower than for IgG- and IgA-expressing Bmem.[21] Still, IgE-expressing Bmem are expanded in allergic disease[10] and show molecular signs of enhanced affinity.[21] Many IgE-switched Bmem and plasma cells show molecular signs of Ig class switching via an IgG intermediate, suggesting that pathogenic IgE arises from IgG^+ Bmem.[66] Due to the low levels of surface IgE expression,[21] IgE^+ Bmem are presumed to be short-lived, and as a result, their repertoire is mainly maintained in the IgG^+ Bmem compartment.[67] These IgG^+ Bmem show distinct Ig variable gene repertoires between allergic and tolerant individuals,[65] implying that the epitope specificity and potentially immunophenotypes of these cells can be used as biomarkers for clinical status.

5 | RECENT ADVANCES IN ALLERGEN IMMUNOTHERAPY

The repeated allergen exposure during AIT changes the immune profiles of patients with shifts toward Th1 and Treg cells,[28,68] which down-regulate the Th2 response,[69] and drive generation of allergen-specific IgG4.[50,70] These changes form the basis of immune tolerance.[71] Crucially, the increase in serum IgG4 following AIT is specific for the administered allergen and does not extend to other specificities,[72] demonstrating the need for identification of specificity of allergen sensitization for optimal selection of patients for immunotherapy.[73]

Allergen-specific immune cell measurements have provided multiple insights into the effects of immunotherapy. Following AIT for Alder pollen, Wambre et al. observed a preferential deletion of pathogenic CRTH2+CD27- allergen-specific CD4+ T cells.[58] This was accompanied by IL-10 induction in CD27+ allergen-specific CD4+ T cells.[58] These most likely include Treg cells, as more recently HDM AIT was shown to expand the proportions of Der p 1-specific Treg cells, with those expressing IL-10 remaining significantly increased after 3 years of AIT.[68] AIT for grass pollen allergy was shown to similarly induce Treg cells within 4 months, but required three 4-month courses over 3 seasons for shifting Th cytokine production from Th2 toward Th1 and Treg.[28] Furthermore, HDM AIT induced changes in the B-cell compartment with significantly higher frequencies of Der p 1-specific IgA+and IgG4+ Bmem cells, plasmablasts, and IL-10+ regulatory B cells (Breg).[50]

With AIT strategies for food allergens being less well-established than for aeroallergens and insect venoms, allergen-specific immune cell measurements are instrumental in obtaining early and objective markers for successful outcomes. Sublingual immunotherapy with recombinant Mal d 1 (the Bet v 1-homologue in apple) downregulated allergen-specific T-cell proliferation and IL-4 transcript expression.[30] Furthermore, oral AIT for peanut allergy was shown to induce dynamic changes in the immune system. Using Ara h 2 peptide-MHCII dextramers, Wang et al showed increased TGFβ production in allergen-specific Th cells.[74] Adjunctive anti-IgE (omalizumab) treatment enables rapid multifood oral immunotherapy and has been shown to reduce circulating IL-4+ peanut-specific Th cells.[75] In addition, the frequencies of peanut-specific CD8+ and TCRγδ+ T cells were reduced,[75] suggesting reduced capacity of these inflammatory cells to migrate to the gastrointestinal tract.

Using fluorescent Ara h 2 allergen tetramers, Patil et al. showed a transient increase in allergen-specific Bmem in peanut-allergic individuals after 7 weeks of oral AIT.[76] These Bmem predominantly expressed IgG and IgA with highly mutated variable regions. It will be of interest to identify whether their subsequent decline is important for tolerance and potentially the result of differentiation into IgA- and IgG-producing plasma cells.

In addition to variations in AIT administration, different formulations of AIT are being trialed. One approach is that of peptides representing T-cell epitopes without B-cell epitopes to minimize the risk of adverse reactions.[77-80] As intended, Bet v 1 peptide cocktails successfully adjusted the T-cell compartment of birch pollen-allergic individuals with increased proportions of Treg cells.[81] Furthermore, Fel d 1 peptide immunotherapy has been shown to adjust the CRTH2+ T-cell compartment in cat allergy.[59] The main question that remains is whether peptide AIT will lead to changes in the B-cell compartment and if these changes are needed for sustained effects. Another means to overcome adverse reactions to AIT is by chemical modification of

allergens. Such allergoids, generated by formaldehyde or glutaraldehyde treatment, can disrupt structural (B-cell) epitopes, while retaining linear T-cell epitopes.[82] Patients treated with glutaraldehyde-treated birch pollen extract showed clinical improvement and increased Treg cells with a transient increase in specific IgG4,[82] thus illustrating the potential of such modified AIT products.

6 | FUTURE PROSPECTS

As a complex laboratory approach, the applications of allergen-specific T- and B-cell detection were limited in the first 15 years of this millennium. With advances in multi-color flowcytometry and standardized protocols for T-cell activation and MHC and antigen tetramer preparation, the past few years have seen a rapid uptake. Importantly, there is a convergence in the methods for activation and detection of activated T cells, now commonly called an "AIM assay." Furthermore, sensitive detection of T cells with pMHC has been improved with commercially available pentamers and dextramers. Finally, the use of small chemical polymer fluorochromes and double discrimination strategies has improved the detection of antigen-specific B cells. These improvements and convergence in approaches will be critical to drive the generation of new insights. Importantly, this is needed to differentiate common and unique aspects of allergic disease pathogenesis, as well as detection of those effects of immunotherapy that confer durable clinical benefits. Only through highly specific detection of the pathogenic, allergen-specific cells, can these be optimally assessed for the expression of unique molecules.

The application of recombinant allergen tetramers transcends the detection of B cells. By virtue of polyclonal IgE binding with high-affinity FcεRI, blood basophils can be a target cell for evaluation of allergen sensitization (Figures 2C and 3B).[83] A recent study from our group demonstrated nearly 100% positive predictive values for the detection of sensitization to bee venom and ryegrass pollen using fluorescent allergen tetramers.[84] With clinical flowcytometers currently having 10–12 fluorescent parameters, multiplex detection is possible with 8–10 allergen components in combination with IgE and CD123 as basophil markers. Without the need for basophil activation,[85] cytometric detection of allergen sensitization on blood basophils (CytoBas) can be performed on fresh and frozen PBMC in a standard pathology laboratory.[84] Targeted panels of allergen components would be more cost-effective than large-scale array technologies. Furthermore, serum IgG can easily be washed away from cells, thereby not interfering with detection as seen for serum IgE tests.[86] Thus, the novel CytoBas assay[84] could bring precision medicine with differential and/or component-resolved diagnosis of allergen sensitization[87] one step closer to reality.

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CONFLICTS OF INTEREST

MCvZ and ROH are inventors on a patent application related to this work. All the other authors declare no conflict of interest.

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