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REVIEW

WILEY

New insights into the mechanisms of childhood food allergies

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Abstract

IgE-mediated food allergies are common and can be life-threatening, especially for children. With increasingly rapid advances in immunological technologies, including the ability to profile highly complex immune features from small sample volumes, our understanding of the immune mechanisms that underpin the development of food allergies continues to grow. This also extends to the immune mechanisms associated with the outcomes of oral immunotherapy (OIT). This review focuses on studies within the past 5 years related to immune signatures associated with food allergy in childhood, immune responses that determine reaction severities to offending allergens, immune alterations that occur during OIT in children, and immune effects of adjunct therapies including omalizumab, dupilumab, and abrocitinib. We conclude by providing a perspective on current evidence and directions for future research that will enable new prediction and screening tools and facilitate the development of effective curative strategies.

KEYWORDS
childhood, food allergies, immune mechanisms, immunotherapy

1 | INTRODUCTION

IgE-mediated food allergies are common and can be life-threatening, especially for children. Oral immunotherapy (OIT) has shown encouraging results in both research and clinical settings. However, at present, OIT requires specialized facilities and expertise where the treatment of adverse events can be rapidly and correctly managed, and as such, current management for the majority of children remains strict dietary avoidance and timely treatment of allergic reactions upon exposure. With increasingly rapid advances in immunological technologies, including the ability to profile highly complex immune features from small sample volumes, our understanding of the immune mechanisms that underpin the development of food allergies continues to grow. Likewise, the emergence of OIT and extensive high-resolution immunological studies nested within clinical trials have resulted in a much greater understanding of the mechanisms underlying short- and long-term treatment responses.

Recent advances in our understanding of the mechanisms of childhood food allergy are built upon foundational discoveries in allergy, immunology, and immunotherapy, which are beyond the scope of this review. These historical concepts, both in the context of food allergy and oral immunotherapy, have recently been expertly reviewed in Locke et al.[1] This review focuses on studies within the past 5 years related to (1) immune signatures associated with food allergy in childhood, (2) immune responses that determine reaction severities to offending allergens, (3) immune alterations that occur during OIT in children, and (4) immune effects of biologicals (omalizumab, dupilumab, and abrocitinib) when used as adjunct therapy (Figure 1). We conclude by providing a perspective on current evidence and directions for future research that will enable new prediction and

screening tools and facilitate the development of effective curative strategies.

2 | ALTERATIONS IN INNATE AND Th2 IMMUNE RESPONSES IN CHILDREN WITH IgE-MEDIATED FOOD ALLERGIES

Several recent studies have explored differences in immune cell abundancy, levels of soluble mediators of immunity, as well as in vitro cell function in children with IgE-mediated food allergies, with particular focus on egg, cow's milk, and peanut allergy (Table 1). A study by Song et al.[2] observed that children with egg allergy aged 10–15 years ($n=20$) have higher circulating levels of Th2 cytokines (IL-4, IL-5, and IL-13) when compared to age-matched non-allergic children ($n=20$). They showed that children with egg allergy also have lower levels of the immune regulatory protein soluble CD83 (sCD83) in their serum compared to healthy children, and that this negatively correlated with Th2 cytokine and sIgE levels. In in vitro assays, sCD83 was shown to reduce IL-4+ CD4 T cell proportions and reduce secretion of IL-4, IL-5, and IL-13. sCD83 also reduced the expression of GATA3 in ovalbumin (OVA)-specific Th2 T cells in a T-bet dependent manner. In a murine model of egg allergy, this study also showed that treatment with sCD83 reduced the frequency of OVA-specific CD4 T cells, increased the levels of IRF1 and T-bet in CD4 T cells, reduced the levels of GATA3 in CD4 T cells, and increased the frequency of mucosal Tregs, suggesting that sCD83 has suppressive effects on experimental food allergy. A study by Neeland et al.[3] identified an altered inflammatory immune profile in 1-year-old infants with egg allergy

Key message

This review summarizes our current understanding of the immune signatures that govern the development of IgE-mediated food allergies in childhood as well as those associated with treatment response to oral immunotherapy. Recent advances in immunological technologies have confirmed the key pathways (type 2 immunity, inflammation), revealed novel cell types (type 2 memory B cells), and elucidated new targets (type 17 immunity, unconventional T cells) that play essential roles in the development of food allergy and allergic responses. Despite these advances, many aspects of food allergy pathogenesis remain unclear, warranting ongoing and collaborative investigation.

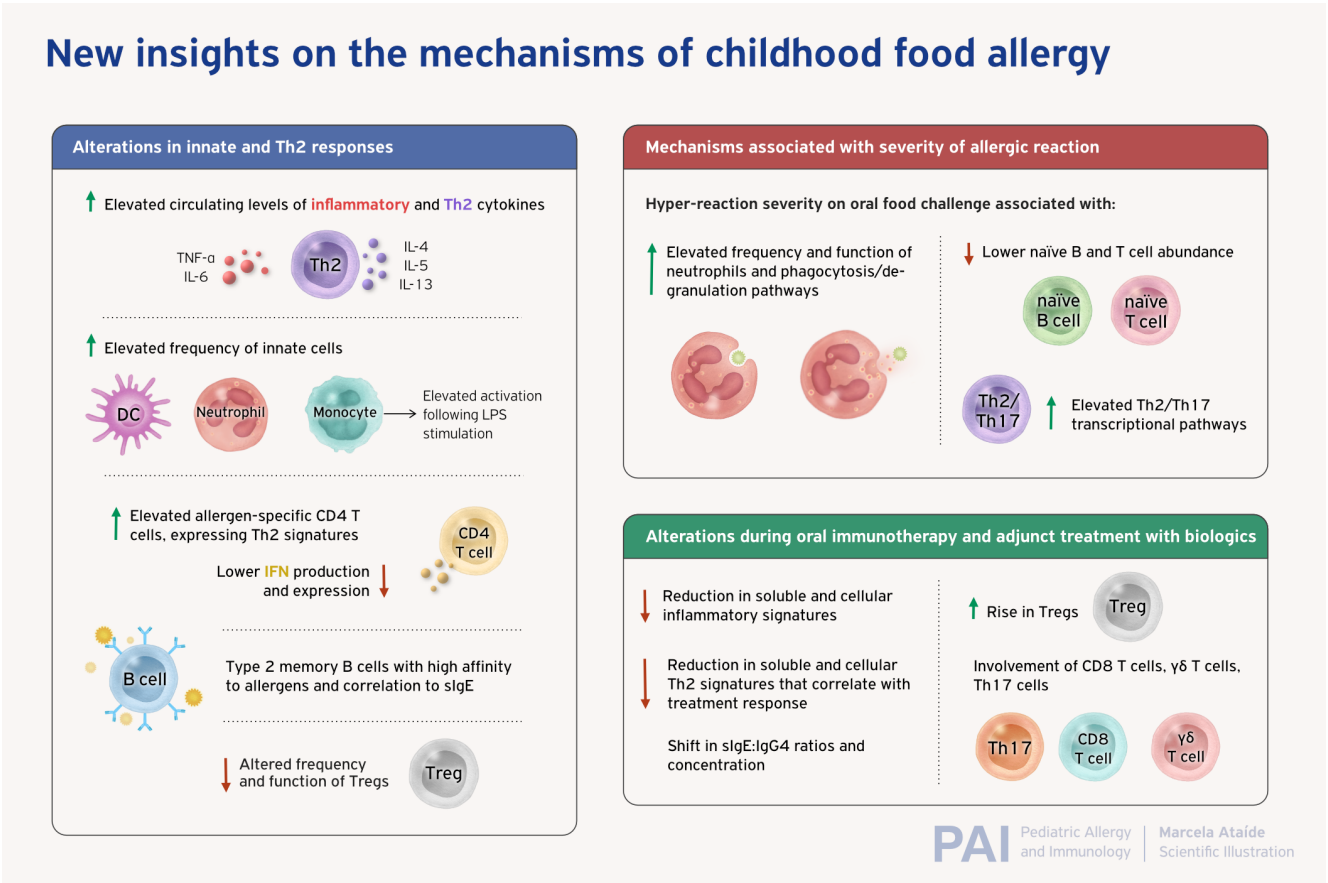

FIGURE 1 Summary of recent evidence on the immune mechanisms of childhood food allergy.

TABLE 1 Summary of findings from "Alterations in innate and Th2 immune responses in children with IgE-mediated food allergies."

Focus	Immune mechanism	Citation	Allergen
Cytokines /soluble factors	Egg allergic children have ↑Th2 cytokines (IL-4, IL5, and IL-13) in circulation compared to non-allergic controls	[2]	Ovalbumin
	↓ sCD83 observed in egg allergic children. Negatively correlated with sIgE and Th2 cytokines. Replenishment of sCD83 in vitro shown to reduce Th2 cytokines		
	Significantly ↑IL-6 in circulation and ↑TNFα (not statistically significant) in egg/cow's milk-allergic children versus non-allergic. Therapeutic elimination diet significantly reduces both IL-6 and TNFα in egg/cow's milk-allergic children	[6]	Cow's milk/egg
	↑IL-4, IL-12β, IL-2, IL-1β, IL-6, CXCL12, BDNF, and SERPINE1 observed in PA children's PBMCs when stimulated with peanut. Coding genes for said genes also shown to be less methylated in PA children's PBMCs when compared to non-allergic controls	[10]	Peanut
	Amish children have lower levels of circulating IgE compared to Hutterite children	[17]	N/A
	PMA/Ionomycin stimulated PBMCs from PA infants have higher frequencies of TNFα-producing cells compared to healthy infants	[7]	Peanut
	Peanut stimulated PBMCs from children with PA produce ↑ MCP1, MIP1α, MIP1β, IL1RA, IL-1β, IL-6, TNFα, IL-10, IL12P70, and IL12P40 versus non-peanut allergic children	[9]	
Monocytes	Monocytes from OVA allergic infants when stimulated with LPS produce ↑ TNFα, IL-6, IL1β, IL-8, and MIP1α when compared to monocytes from non-allergic infants	[3]	Ovalbumin
	Monocytes from PA adolescents produce ↑IL-1β, IL-1α, IL-6, IL-8, and TNF-α compared to non-allergic controls when stimulated with endotoxin (none reached statistical significance)	[8]	Peanut
	Monocytes from peanut-allergic children, when exposed to peanut protein, differentiate into CD11c+ CD209+ DCs that promote a Th2 environment in vitro	[9]	
	Hutterite children's monocytes have high HLA-DR expression (primed to sample antigen), while Amish children's monocytes express inhibitory ILT3 and ILT5 (suppressive phenotype)	[17]	N/A
DCs	↑ pDCs in peanut sensitized infants at baseline compared to non-allergic children	[7]	Peanut
	Peanut- and multi-food-allergic adolescents have more CD11c+ cDCs in circulation than non-allergic adolescents	[8]	
	CD11c+ CD209+ and CD11c+ CD23+ DCs promote a Th2 environment in peanut-allergic children, with both subtypes relying on IL-4 signaling. Inhibiting CD209 results in a decrease in ps-IL-4 and IL-13 expressing CD4 T cells in vitro	[9]	
	Peanut OIT reduces the frequency of both CD11c+CD209+ DCs and ps-CD4 T cells in PA children	[9]	
Neutrophils	Cow's milk allergic children have ↑ neutrophils in circulation compared to healthy children	[6]	Cow's milk
	Proportion of neutrophils positively correlated with gene modules involved in type I interferon production, cytokine production, and humoral immune responses in children with nut allergy	[13]	Nut allergy (tree nuts and peanuts)
T helper cells	↑ Pathogenic Th2 and T follicular helper cells identified in cow's milk allergic individuals	[5]	Cow's milk
	↓ naïve CD4 T cells at baseline in peanut sensitized infants	[7]	Peanut
	↑ IL-2 in naïve CD4 T cells from peanut sensitized infants post-PMA/Ionomycin stimulation	[7]	
	Peanut sensitized and & PA infants have ↓ IFN-γ in their CD4 HLA-DR+ T cells versus non-allergic controls	[7]	
	PA infants: ↑ Memory-phenotype peanut-reactive CD4 T cells post-peanut stimulation versus peanut sensitized and controls	[7]	
	CD3/CD28 stimulated CD4 T cells from PA adolescents produce ↓ IL-6, TNF-α, IFN-γ versus non-allergic controls	[8]	
	Food-allergic adolescents naïve CD4 T cells show distinct methylation at Th1/Th2 genes (RUNX3, RXRA, NFKB1A, IL4R) and TNFRSF6B promoter	[12]	
	Activated (CD3/CD28) naïve CD4 T cells: ↓ Interferon genes (e.g., IFN-γ) and BST2 (DMR at a key downstream gene) in food-allergic adolescents. Reduced IFN-γ gene expression was confirmed by lower IFN-γ protein levels in cell culture supernatants from the same food-allergic participants	[12]	
	CD3/CD28-stimulated PBMCs from peanut-allergic individuals exhibit high CYP11A1, IL-13 and IL-4 mRNA expression, along with increased frequencies of CYP11A1+ CD4+ cells compared to controls. Aminoglutethimide (anti-CYP11A1) ↓ IL-13 and CD4+ IL-13+ T cells in CD3/CD28-stimulated PBMC cultures from these individuals	[14]	
	Proportion of CD4 T cells negatively correlated with gene modules involved in type I interferon production, cytokine production, and humoral immune responses in children with nut allergy	[13]	Nut allergy (tree nuts and peanuts)
	Amish children have ↑ proportions of PD1 expressing CD4 T cells compared to Hutterite children	[17]	N/A

(Continues)

TABLE 1 (Continued)

Focus	Immune mechanism	Citation	Allergen
Tregs	↓ Peripheral Tregs in egg allergic infants compared to non-allergic infants	[3]	Egg
	OVA-specific Tregs: ↓ in egg-allergic versus non-allergic infants	[4]	
	sCD83 in vivo: ↓ GATA3, ↑ mucosal Treg frequency (murine model)	[2]	
	↑ Dysfunctional allergen-specific Tregs with high IFN and dysregulated chemokines observed in children allergic to cow's milk	[5]	Cow's milk
	↑ Activated, memory-like Tregs observed in PA adolescents versus non-allergic adolescents	[8]	Peanut
	Proportion of Tregs negatively correlated with gene modules involved in type I interferon production, cytokine production, and humoral immune responses in children with nut allergy	[13]	Nut allergy (tree nuts and peanuts)
	Lower frequencies of ICOS+ activated Tregs observed in Hutterite children compared to Amish children	[17]	NA
	Amish children have ↑ proportions of PD1 expressing Treg cells compared to Hutterite children	[17]	
Cytotoxic T cells	Amish children have ↑ CD28-null CD8 T cells, correlating with high T-cell IFN-γ production and low serum IgE	[17]	NA
B cells	↓$CD19^{hi}$ $HLADR^{hi}$ B cells at baseline in peanut-sensitized infants. ↑$CD19^{hi}$ $HLADR^{hi}$ B cells observed at baseline in peanut-allergic infants	[7]	Peanut
	PM20D1 (atopic disease), S100A1, S100A13, S100A14 (inflammation) differentially expressed in B cells from peanut-allergic versus non-allergic children	[11]	Peanut
	Enrichment of myeloid cell activation genes in B cells from multi-food allergic children	[11]	
	Enrichment of B/T-cell development and TGF-β signaling motifs in multi-food allergic versus single-food allergic children	[11]	
	CD23+ IgG1+ memory B cells in peanut-allergic children harbor high-affinity Ara h 2-specific clones, promote Th2 immunity, and drive IgE production, potentially contributing to the long-term persistence of peanut allergy	[15]	
Bregs	Egg allergic infants have ↓ OVA specific Bregs compared to non-allergic infants	[4]	Egg

(egg allergic: $n=16$, non-allergic: $n=11$), comprising lower frequencies of circulating Tregs (CD4+ CD25+) and a higher frequency of monocytes. In vitro stimulation of purified CD14+ monocytes with LPS resulted in a heightened inflammatory phenotype (increased production of TNFα, IL-6, IL1β, IL-8, and MIP1α) compared to monocytes from non-allergic infants. Another study exploring immune signatures of egg allergy in infancy[4] showed that infants with egg allergy ($n=21$) have a blunted development of OVA-specific Tregs and lower frequency of OVA-specific Bregs when compared to non-allergic infants ($n=92$). Additionally, a significant correlation was found between OVA-specific CD137+ IL-10+ Tregs and egg-specific IgG4 in infants introduced to egg between 5 and 10 months and who did not develop egg allergy by 12 months, indicating that early exposure to egg protein may promote tolerance.

Two studies have also examined immune responses in children with cow's milk allergy. The first study, by Lewis et al.,[5] showed that cow's milk epitope stimulated PBMC from children with cow's milk allergy (cow's milk allergy: $n=89$, non-allergic controls: $n=66$) exhibited higher frequencies of cow's milk-reactive clonally expanded FOXP3+ Tregs. These Tregs also had increased expression of interferon responsive genes and dysregulated chemokine expression compared to children without cow's milk allergy, indicative of a dysfunctional Treg phenotype. This study also identified a small population of clonally expanded cow's milk specific CD4 T cells expressing genes associated with pathogenic Th2 and T-follicular helper responses in cow's milk allergic subjects. The second study by Kara et al.,[6] showed that children aged 1 to 33 months ($n=37$) with cow's milk and/or egg allergy have higher circulating IL-6 and TNFα, as well as elevated neutrophils compared to age-matched controls ($n=24$). Moreover, eliminating egg and milk from the diet was found to reduce elevated IL-6 and TNFα in allergic children.

Nine recent studies have examined the impact of peanut allergy on the pediatric immune system. The first study by Neeland et al.[7] found distinct immune profiles in 1-year-old peanut allergic (PA: $n=12$), peanut-sensitized but tolerant (PST: $n=12$), and non-allergic infants (NA: $n=12$) PBMCs at rest and following PMA/ionomycin stimulation. PST infants had lower naïve CD4+ T cells and $CD19^{high}HLADR^{high}$ B cells but more plasmacytoid dendritic cells (pDCs) at baseline, while PA infants had higher frequencies of $CD19^{high}HLADR^{high}$ B cells at baseline and produced high levels of TNF-α following stimulation. PST infants had higher levels of IL-2 in naïve CD4 T cells following stimulation, and both PA and PST infants had lower IFN-γ expression in effector memory (EM) CD4 HLA-DR+ T cells compared to controls. When stimulated with peanut protein, PA infants had significantly more peanut-reactive CD4 T cells with a memory phenotype compared to both PST and controls. A subsequent study by the same authors[8] found that single PA ($n=20$) and multi-food allergic ($n=20$) adolescents (ages 10–14 years) have a higher proportion of conventional CD11c+ DCs (cDCs) and memory-like activated Tregs compared to non-allergic ($n=19$) adolescents of the same age. When stimulated in culture (via anti-CD3/CD28), purified CD4 T cells from adolescents with

both single-peanut and multi-food allergy produced lower levels of IL-6, TNF-α, and IFN-γ compared to controls. Additionally, purified CD14+ monocytes from adolescents with food allergy tended to produce more IL-1β, IL-1α, IL-6, IL-8, and TNF-α when stimulated with endotoxin, although these findings did not reach statistical significance.

A study by Zhou et al.,[9] found that the PBMC of children aged 5–10 years with peanut allergy ($n=22$) when stimulated with peanut protein had higher levels of MCP1, MIP1α, MIP1β, IL-1RA, IL-1β, IL-6, TNFα, IL-10, IL-12P70, and IL-12P40 relative to controls (age 4–13: $n=26$). Exposure to purified peanut protein in culture also reduced the frequency of monocytes in PA PBMC as they differentiated into CD11c+ CD209+ DCs, which sample peanut antigen. Other subsets of DCs were also increased in PA participants, including CD11c+ DCs co-expressing the low-affinity IgE receptor CD23. These DC subtypes were shown to be dependent on (1) signaling through the IL-4 receptor, as blocking IL-4RA reduced their differentiation, and (2) the presence of IL-4 and IL-13 producing CD4 T cells in the PBMC fraction. A feedback loop was also identified, where blocking CD209 resulted in a decrease in CD4 T cells expressing IL-4 and IL-13, suggesting that CD11c+ CD209+ DCs and allergen-specific T cells act reciprocally to establish food allergy. This was further assessed using samples collected during peanut OIT, where a significant reduction in both CD11c+ CD209+ DCs and peanut-specific (ps) −CD4 T cells was observed.

Another study by Zhou et al.,[10] identified reduced DNA methylation in genomic regions of IL4, IL2, IL17F, IL1B, IL6, BDNF, CCR7, CD3E, and SERPINE1, and higher methylation in genomic regions of IL12B, CXCL12, and RUNX1 in the PBMCs of children aged 4–10 years with peanut allergy ($n=10$) compared to non-allergic ($n=10$), age-matched twins. To explore whether these DNA methylation signatures were associated with changes in the expression of their cognate proteins, PBMCs from non-allergic and PA children were incubated with peanut protein, resulting in increased production of IL-4, IL-12β, IL-2, IL-1β, IL-6, CXCL12, BDNF, and SERPINE1 proteins in PBMCs from children with peanut allergy. This suggests that epigenetic variation (DNA methylation) differences may drive altered immune responses to allergens in food allergic individuals. This study also explored the diagnostic potential of these identified DNA methylation signatures, showing that the combination of 3 DNA methylation signatures (CXCL12+BDNF+SERPINE1) have superior diagnostic performance against serum ps-IgE for discriminating peanut allergy from no allergy, although the authors note the small number of participants in this exploratory work.

Two additional studies have also explored the epigenetic and transcriptional landscape of immune cells in children aged 10–15 years with single peanut allergy, multi-food allergy, and no food allergy. The first study[11] compared the DNA methylation and transcriptomic profile of the total B cell fraction from PBMC of these adolescents ($n=10$ single peanut allergy, $n=7$ multi-food allergy, $n=9$ non-allergic controls). The authors identified 17 differentially methylated regions (DMRs) that distinguished the food allergy and no food allergy groups, as well as 34 DMRs that distinguished the single-peanut and multi-food allergy groups. Key genes associated with these DMRs included PM20D1, previously associated with atopic disease, and S100A1, S100A13, and S100A14, associated with inflammation. RNA sequencing analysis of these B cells showed enrichment of genes associated with myeloid cell activation in the multi-food allergic group, and motif enrichment analysis showed differential enrichment for motifs recognized by transcription factors regulating B- and T-cell development and TGF-β signaling between the multi- and single-food allergic groups. The second study[12] compared the DNA methylation and transcriptome profile of purified naïve CD4 T cells either unstimulated or following anti CD3/CD28 stimulation in this cohort ($n=29$ adolescents with single or multi-food allergy, and $n=18$ controls). This work showed that adolescents with food allergy exhibit unique DNA methylation signatures at quiescence and post-activation at key genes involved in Th1/Th2 differentiation (RUNX3, RXRA, NFKB1A, IL4R), including a DMR at the TNFRSF6B promoter, linked to Th1 proliferation. Combined analysis of DNA methylation and transcriptomic data from the same samples identified reduced IFN responses in naïve CD4 T cells from food allergic adolescents following activation, with decreased expression of interferon genes, including IFN-γ and a DMR at a key downstream gene, BST2. The reduced expression of IFN-γ gene in naïve CD4 T cells from food allergic individuals was additionally confirmed by assessing protein levels in cell culture supernatant, which showed the same reduced production of IFN-γ in food allergic adolescents.

Lee et al.,[13] conducted RNA sequencing on whole blood samples from children aged 1–16 years with nut allergies ($n=23$, including peanut and tree nut allergies) and age-matched healthy controls ($n=7$). In children with nut allergies, 184 genes were upregulated, and 490 genes were downregulated compared to healthy controls (although not significant after adjustment for multiple corrections). Gene co-expression network analysis revealed two upregulated gene modules (type I interferon production and cytokine production) and one downregulated module (humoral immune responses) in nut allergic children. These changes were positively correlated with neutrophil frequency and negatively correlated with CD4 T cell/Treg frequencies. The upregulated modules were driven by two genes, *IFIH1* and *DRAM1*, while the downregulated module was linked to *ZNF512B* expression.

A study by Wang et al.,[14] found that anti-CD3/CD28 stimulated PBMCs from individuals between the ages of 2–20 years ($n=33$) with peanut allergies highly express the steroidogenic enzyme CYP11A1, have elevated levels of IL-13 and IL-4 mRNA expression, and display higher frequencies of CD4+ IL-13+ T cells when compared to healthy controls ($n=11$). Inhibiting CYP11A1 protein with aminoglutethimide reduced IL-13 levels and the number of CD4+ IL-13+ T cells in anti-CD3/CD28 activated PBMC cultures. This link between CYP11A1 and Th2 induction was further explored using CRISPR knockout in a human T cell line (SUP-T1), where CYP11A1 deletion lowered the frequency of CD4+ IL-13+ T cells post stimulation with PMA/ionomycin.

Memory B cells have also recently shown to modulate the allergic environment by promoting Th2 immunity in PA children aged

5–14 years old (peanut-allergic: $n=45$, non-allergic: $n=13$).[15] A memory B cell population, characterized by its expression of CD23 and IgG1 and originally identified by Aranda et al.,[16] was shown to harbor high-affinity ps-B cell clones, poised to switch to IgE production upon activation. These cells are highly correlated with circulating IgE in PA children. scRNA-Seq and paired B cell receptor (BCR)-Seq of this B cell population revealed high IL-4 and IL-13 regulatory gene expression (FCER2/CD23+, IL4R, and germline IGHE) and high-affinity BCRs against the predominant peanut allergenic protein Ara h 2. This highlights that a subset of B cells appears primed to activate and class switch antibody production upon exposure to peanut antigen, potentially contributing to the long-term persistence of peanut allergy.

Recent work has also explored the interaction between the environment and the development of allergic diseases. Of note, a study by Hrusch et al.,[17] found that Hutterite children (ages 6–14, $n=30$), raised in a modern farming environment and with a higher risk for atopy and asthma than Amish children living in more traditional farming environments, have a more reactive and primed immune system. This reactive phenotype is characterized by monocytes ready to sample antigen (high HLA-DR expression), activated T cell populations (CD4+ expressing CD127, CD28, or ICOS), and lower frequencies of activated ICOS+ Tregs. In contrast, Amish children of a similar age ($n=30$) exhibited lower levels of circulating IgE, have more "suppressive" monocytes characterized by high expression of the inhibitory receptors ILT3 and ILT5, higher proportions of CD4 T and Treg cells expressing the inhibitory molecule PD1 and elevated CD28null CD8 T cells that correlate with high T cell IFNγ production and low serum IgE. This indicates a less reactive immune phenotype, which may contribute to their lower incidence of allergy.

3 | IMMUNE MECHANISMS ASSOCIATED WITH THE SEVERITY OF ALLERGIC REACTION

Three studies have recently assessed associations between childhood food allergies and reaction severity to peanut allergens (Table 2). One study by Do et al.[18] assessed reaction severity in children undergoing oral peanut challenge using a grading system that evaluates respiratory, gastrointestinal, cutaneous, cardiovascular, and conjunctival symptoms during challenge to define reaction severity to peanut. Transcriptomic analysis of longitudinal whole blood samples collected during oral food challenge from children aged 7–17 years (discovery cohort $n=21$, replication cohort $n=19$) showed 318 genes associated with reaction severity in both discovery and replication cohorts. Pathway analysis of genes upregulated with peanut severity was associated with neutrophil-related functions including phagocytosis, neutrophil activation, and neutrophil degranulation. Cellular deconvolution analysis of the transcriptomic data revealed that reaction severity positively correlated with a higher frequency of neutrophils and fewer naïve B cells and naïve CD4 T cells. Furthermore, DNA methylation analysis of CD4+ T cells as baseline revealed 203 CpG sites (mapping to 197 unique genes) associated with reaction severity in both discovery and replication cohorts. Integrated analyses of peanut severity genes and peanut severity CpGs identified four interconnected CpG-gene

TABLE 2 Summary of findings from "Immune mechanisms associated with severity of allergic reaction."

Focus	Immune mechanism	Citation	Allergen
Cytokines/soluble factors	↑ IL-4, IL-5, IL-9, IL-13 in peanut-stimulated PBMCs from reactive versus hyporeactive participants; positively correlated with ps-IgE	[20]	Peanut
Neutrophils	↑ Neutrophil frequency associated with reaction severity	[18,19]	
	↑ phagocytosis, neutrophil activation, and neutrophil degranulation pathways, positively correlated with reaction severity	[18]	
	Neutrophil abundance is linked to enriched gene modules for FCγR-mediated phagocytosis and TLR signaling, driven by the expression of the genes AP5B1, KLHL21, VASP, TPD52L2, and IGF2R	[19]	
T helper cells	Severe reactions to peanut associated with ↓ frequencies of naïve CD4 T cells	[18]	
	CpG-gene groups linked to immune response [cg06769918 (PHACTR1)], chemotaxis [cg17545300 (CHST15)], and macroautophagy regulation [cg12084124 (ZNF121 are involved in reaction severity	[18]	
	↑ ps-CD154+ CD4 T cells and ps-CDR3 clones in reactive patients	[20]	
	Ps-CD154+ CD4 T cells are enriched for Th2 (IL-5, IL-9) and Th17 (IL-22, IL-26) genes in reactive patients	[20]	
	Reactive patients' ps-CDR3s were skewed toward T-effector rather than T-regulatory phenotype	[20]	
Tregs	Ps-T cells from hyporeactive patients show increased expression of genes associated with T-regulatory function (TNFRSF9, CD137) and immune regulation (NFKBID, IL1RN, BDR)	[20]	
B cells	Severe reactions to peanut associated with ↓ frequencies of naïve B cells	[18]	

groups enriched for immune response, chemotaxis, and regulation of macroautophagy.

A study by Zhang et al.,[19] found a strong correlation between neutrophil abundance in whole blood samples of children aged 4–14 years ($n = 105$) taken during oral peanut challenge and the severity of reactions to peanut allergens. The severity of reactions was determined by the cumulative amount of peanut protein tolerated during the challenge, with lower tolerance indicating higher severity. Analysis of the whole blood transcriptome from these samples revealed that neutrophil abundance was associated with enrichment of modules for FCγR-mediated phagocytosis and TLR signaling, and identified five specific genes as key drivers of these modules: AP5B1, KLHL21, VASP, TPD52L2, and IGF2R.

A third study by Ruiter et al.,[20] in adults and children ($n = 62$, median age: 17 years) also assessed reaction severity based on tolerance to increasing doses of peanut during oral food challenge. The authors showed that peanut protein stimulation of PBMC resulted in increased production of Th2 cytokines (IL-4, IL-5, IL-9, IL-13), higher proportions of ps-CD154+ CD4 T cells, and an increase in ps-complementarity-determining region 3 (CDR3) clones in reactive participants compared to hyporeactive participants. RNAseq analysis of these ps-CD154+ CD4 T cells revealed that genes associated with Th2 cells (IL-5, IL-9) and Th17 cells (IL-22 and IL-26) were higher in reactive than hyporeactive patients, and that genes associated with T-regulatory function (TNFRSF9, CD137) and immune regulation (NFKBID, IL1RN, BDR) were increased in hyporeactive participants. Finally, they explored the distribution of ps-CDR3s in CD25 + CD127+ T-effector and CD25 + CD127- T-regulatory cells from reactive and hyporeactive participants and showed that the proportion of ps-CDR3s was skewed to the T-effector, and not T-regulatory, population in reactive patients.

4 | IMMUNE ALTERATIONS DURING OIT

Seven recent studies have highlighted immune system changes associated with OIT (Table 3). Five of these[21–25] have recently been comprehensively described by Ashley et al. in a review that focuses on key transcriptomic changes associated with OIT.[26]

Three of these studies used scRNA-Seq to characterize the cellular changes during peanut OIT. The first study by Anvari et al.,[21] performed scRNA-Seq on PBMCs from a single child undergoing peanut OIT with PA, and a single healthy non-peanut allergic control, and observed distinct fluctuations in immune cell populations over the first 24 weeks of treatment. Monocytes, B cells, and NK cells decreased transiently at 6 weeks on peanut OIT, with B cells later surpassing levels seen in the healthy child at 24 weeks. In contrast, both naïve and memory γδ Treg cells temporarily increased after 6 weeks in the PA participant receiving peanut OIT. Analysis of the γδ Treg cells transcriptome during treatment showed a shift in their programming, with naïve γδ Tregs beginning to resemble those of healthy children by week 24. Memory γδ Tregs upregulated Th2-promoting genes, such as OX40R (Th2 polarization) and GITR (inhibits Treg activity), while expressing suppressive genes (*TGFB1*, *CTLA4*, *ISG20*, *CD69*) and downregulating IL7R and SELL. In the context of peanut OIT, this suggests these cells promote tolerance by enhancing Treg suppressive activity, shifting away from a Th2 phenotype. Wang et al.,[23] found that individuals (subset of POISED study: 27 children: 8–16 years old, and 3 adults: 26–53 years old) who did not respond to peanut OIT had a higher frequencies of anergic (75% anergic) ps-CD4+ T cells pre commencing treatment compared to the success group (50% anergic). In the success group, there was also a transient increase in activated ps-Th2 cells with high STAT expression at 24 weeks, a response absent in the failure and placebo groups. Additionally, a transient rise in *TGFβ*-expressing cells at 52 weeks of treatment was observed in CD4+ T cell cultures from individuals who successfully achieved peanut desensitization. Monian et al.,[22] using scRNA-Seq and paired scTCR-Seq also observed substantial T cell modulation in children and adults (6 children: 8–16 years old, 6 adults: 22–36 years old) receiving peanut OIT. The authors found a gradual reduction in peanut-reactive CD4+ T cells in peanut protein-stimulated PBMC cultures when compared to cultures from children receiving a placebo. Throughout treatment, Th2 subsets were suppressed in peanut-reactive CD4+ T cells from children receiving peanut OIT, but no significant changes in the TCR repertoire were observed, regardless of clinical outcome. This suggests that peanut OIT modulates functional T cell types, not T cell clonotypes. Peanut-reactive CD4+ T cells from children who successfully tolerated the final peanut challenge showed greater suppression of pathogenic effector Th2A cells, dampened Th2 and Th1 effector signatures, and had lower baseline inflammation from Th1-conventional and Th17 cells compared to those who failed treatment. Peanut-reactive Tregs remained relatively unchanged throughout treatment and between outcome groups.

Ashley et al.,[25] found that children (aged 1–10 years, $n = 62$) undergoing probiotic and peanut OIT who achieved remission/sustained unresponsiveness (SU), defined as passing a 3950 mg cumulative peanut protein food challenge 2–6 weeks after ceasing treatment, have a distinct rewiring in Th2/IFN gene modules. This included a loss of Th2 gene expression and upregulation of type I interferon genes in purified CD4+ T cells when exposed to crude peanut protein in vitro, while CD4+ T cells from children who received a placebo for both OIT and probiotic treatment (remained allergic) retained a Th2 profile (IL-4, IL-9, IL-13, IL-31).

Kaushik et al.[27] examined PBMCs from participants in the POISED study (median participant age: 5 years, $n = 120$) at several time points throughout peanut OIT. They found a consistent reduction in Th2-polarized peanut-reactive CD4+ T cells throughout treatment, and corresponding reductions in the expression of type 2 proteins IL-4, IL-5, IL-9, and IL-13. When assessing baseline characteristics that may distinguish participants who develop SU as opposed to desensitization (DS), the authors showed that lower frequencies of naïve CD8 T cells and terminally differentiated

TABLE 3 Summary of findings from "Immune alterations during oral immunotherapy (OIT)."

Focus	Immune mechanism	Citation	Allergen
Cytokines/soluble factors	↓Th2 cytokines produced by peanut stimulated PBMCs from children receiving peanut OIT	[25,27,28]	Peanut
	Gal d 2-specific IgG4 and IgA ↑ and IgG4:IgE ratio ↑ over first 8 months of egg OIT	[24]	Egg
	↓ Inflammatory mediators over time (IL-1RA, IL-6, IL-17, TNF, IL-12p70, IL-8) in plasma samples from egg-allergic children receiving egg OIT	[24]	
T helper cells	Overall ↓ ps-CD4+ T cells with Th2 profile over the course of peanut OIT	[22,27,28]	Peanut
	Transient increase in peanut reactive CD4+ T cells at 4 months peanut OIT, followed by a gradual decline	[28]	
	↑ Anergic ps-CD4+ T cells before peanut OIT linked to treatment failure	[23]	
	Expansion of activated ps-Th2 (high STAT expression) cells at 24 weeks peanut OIT associated with desensitization to peanut	23]	
	Transient increase of TGFβ-expressing cells at 52 weeks peanut OIT associated with desensitization to peanut	[23]	
	Suppression of peanut-reactive pathogenic Th2A cells during peanut OIT associated with tolerance to peanut.	[22]	
	↓Th2 and Th1 effector signatures in peanut reactive cells during peanut OIT associated with tolerance to peanut.	[22]	
	↓Inflammation from peanut reactive Th1-conventional and Th17 cells prior to starting peanut OIT associated with tolerance to peanut.	[22]	
	↓ IL-4+ and IFNγ+ expressing CD4+ T effector memory cells at week 104 peanut OIT associated with SU (PBMCs stimulated with PMA/Ionomycin).	[27]	
	↓ Th2 genes and ↑ type 1 IFN expression by CD4+ T cells from children who achieve SU post probiotic and peanut OIT	[25]	
	↓ CD4+ cell frequency after 3 months egg OIT, returning to baseline by 8 months OIT	[24]	Egg
Cytotoxic T cells	↓ Baseline frequency of naïve CD8+ T cells and terminally differentiated CD57+ CD8+ T cells linked to SU post-peanut OIT. Frequency of naïve CD8 T cells positively correlated with sIgE levels at baseline	[27]	Peanut
	↑ CD8+ cell frequency after 3 months egg OIT, returning to baseline levels by 8 months OIT	[24]	Egg
Tregs	Minimal modulation of peanut-reactive Treg populations by peanut OIT	[22]	Peanut
	↑ peanut-reactive Tregs at 4 months peanut OIT, return to baseline by 8 months peanut OIT	[28]	
γδ T cells	Transient ↑ γδ Tregs (naïve & memory phenotypes) at 6 weeks peanut OIT	[21]	
	Naïve γδ Tregs after 24 weeks peanut OIT are transcriptionally similar to those found in healthy children	[21]	
	Memory γδ Tregs retain Th2 genes (OX40R, GITR) and have upregulated immunosuppressive genes (CD69, ISG20, CTLA4, TGFB). Indicates early modulation of Th2 gene expression in memory γδ Tregs due to peanut OIT	[21]	
	↓ IFNγ expression in memory γδ T cells at week 104 peanut OIT (PBMCs stimulated with PMA/Ionomycin) linked to SU	[27]	
	Continuous increase in γδ T during first 8 months of egg OIT	[24]	Egg

TABLE 3 (Continued)

Focus	Immune mechanism	Citation	Allergen
B cells	B cells ↓ at 6 weeks peanut OIT, ↑ above healthy levels by 24 weeks peanut OIT	[21]	Peanut
	↑ Memory B cell frequency after 3 months egg OIT, remain elevated at 8 months egg OIT	[24]	Egg
	↑ Naïve B cell frequency after 3 months egg OIT, returns to baseline at 8 months	[24]	
DCs	↓ Frequency of activated DCs during egg OIT	[24]	
Monocytes	Transient ↓ Monocytes at 6 weeks peanut OIT	[21]	Peanut
NK cells	Transient ↓ NK cells at 6 weeks peanut OIT (except one cluster)	[21]	
Basophils	↓ Basophil reactivity to peanut protein at 12, 24, and 36 months in low/high peanut OIT dose groups, coincided with ↑ ps-IgG4:IgE ratio	[28]	
Mast cells	Reduced frequency of activated mast cells over course of egg OIT	[24]	Egg
PBMCs (whole)	↓ Inflammatory mediators, Th2 pathways, DC maturation, and TREM1 signaling at 8 months egg OIT versus baseline	[24]	
	Desensitized patients: ↑ oxidative phosphorylation, glucocorticoid receptor, and IL-10 signaling pathways in PBMCs at 3 months OIT versus partially desensitized group	[24]	
	Desensitized patients: ↑ DC-NK crosstalk, NK signaling, Th1 pathways, and Th1/Th2 activation in PBMCs at 8 months OIT versus partially desensitized group	[24]	
	Partially desensitized patients: ↑ IL-4 signaling, B-cell development, and antigen presentation in PBMCs at 3 months OIT versus DS group	[24]	
	Partially desensitized patients: ↑ interferon signaling and antiviral responses in PBMCs at 8 months OIT versus DS group	[24]	

CD57+ CD8+ T cell subsets at baseline were associated with SU, and that the frequency of naïve CD8 T cells positively correlated with sIgE levels at baseline. Further, a lower frequency of IL-4+ CD4+ memory T effector cells, a lower frequency of IFNγ+CD4+ memory T effector cells, and lower expression levels of IFNγ by CD4+ memory T effector cells and memory γδ T cells were observed in SU compared with DS participants at week 104 post OIT commencement following non-specific PMA/ionomycin stimulation of PBMCs.

Kulis et al.[28] also observed a reduction in peanut-reactive CD4 T cells and Th2 cytokines in peanut-stimulated PBMC cultures from pre-school-aged children undergoing peanut OIT ($n=49$). Two dosing regimens were used during maintenance—one high (3000 mg peanut protein) and one low (300 mg). In both groups, children showed a transient increase in peanut-reactive CD4 T cells between 4 and 8 months, followed by a gradual decline. A similar temporary rise in peanut-reactive Tregs was noted at 4 months, suggesting early modulation of the Treg population by peanut OIT. There was a clear reduction in IL-13, IL-5, and IL-9 production in peanut-stimulated PBMCs throughout treatment at both doses. Additionally, basophil reactivity to peanut protein was significantly suppressed at 12, 24, and 36 months in both dose groups, which coincided with an increased peanut IgG4:IgE ratio, suggesting a reduced allergic response due to treatment.

In the past 5 years, only one study[24] has explored the effect of egg OIT in modulating Th2 immunity. Children receiving OIT (age 6–17 years, $n=50$) had distinct alterations in DCs, mast cells, CD4-T, and CD8-T cells in their PBMCs over the course of treatment. T cell dynamics showed a continuous increase in γδ T cells, with a transient decrease in CD4 and an increase in CD8 T cells at 3 months, returning close to baseline values by 8 months. At 3 months, memory B cells increased and remained elevated through to 8 months, while naïve B cells only increased transiently. The authors also observed a reduction in activated DCs, mast cells, and inflammatory mediators (plasma: IL-1RA, IL-6, IL-17, TNF, IL-12p70, and IL-8), along with an increased Gal d 2-specific IgG4:IgE ratio and higher Gal d 2 specific IgA titers over the course of treatment. Transcriptomic analysis of PBMCs revealed 145 and 331 differentially expressed genes (DEGs) at 3 and 8 months compared to baseline, respectively. DEGs at 8 months compared to 0 months showed downregulation in inflammatory mediators (including IL-6, IL-17), Th2 pathways, DC maturation, as well as *TREM1* signaling. Ingenuity Pathway Analysis linked these DEGs to reduced cell migration, especially of leukocytes, neutrophils, and mononuclear leukocytes. When stratified by desensitization status, desensitized children showed enriched pathways in oxidative phosphorylation, glucocorticoid receptor, and IL-10 signaling, while partially desensitized participants had upregulation of IL-4 signaling, B-cell development, and antigen presentation

TABLE 4 Summary of findings for "Use of biologicals to treat childhood food allergy (omalizumab, dupilumab, and abrocitinib)."

Focus	Immune mechanism	Citation	Biological used	Allergen
Cytokines/ soluble factors	↑ ps-IgG4:IgE ratio driven by IgG4 (not IgE decrease) in omalizumab-treated participants who continue OIT to 36 weeks and in those who discontinue treatment at 30 weeks. However, 1 g/0.3 g maintenance groups tolerated higher allergen doses from ≥2 foods at 36-week challenge versus participants that discontinued treatment at 30 weeks	[29]	Omalizumab	Multi FA (almond, cashew, egg, hazelnut, milk, peanut, pecan, sesame, shrimp, soy, walnut, and wheat)
	↓ Th2 cytokines in PBMCs post-therapy: IL-13, IL-9 (unstimulated) and IL-4 (PMA/Ionomycin-stimulated)	[30]		
	↑ in allergen specific IgG4 ratios by week 36 of therapy	[30]		
	↓ IL-17, IL-1β, MCP-1, IL-12p40, GM-CSF, and FLT3L in unstimulated PBMCs, and ↓ IL-17, IL-8, and MIP1α in stimulated PBMCs by week 36 with combined peanut OIT and OIT	[30]		
	Peanut-IgG4, Ara h 1-IgG4, and Ara h 2-IgG4 levels increase then decrease without returning to baseline	[31]		Peanut
	↓ peanut-IgE, Ara h 1-IgE, Ara h 2-IgE, SPT, and peanut IgE: total IgE ratios during peanut OIT	[31]		
	Participants who discontinue OIT have ↑ peanut-IgE and Ara h 2-IgE at 12 months versus participants who continue treatment	[31]		
	Participants who tolerated the final challenge post-omalizumab + OIT had lower Ara h 1–3 IgE and higher Ara h 2/6 IgG4 compared to the failure group	[32]		
	Over time, CD3/CD28-stimulated PBMCs showed a reduced Th2 cytokine profile (IL-5, IL-13, IL-9) and increased IFN-γ, indicating a shift in T cell polarity due to OIT	[33]		
	Dupilumab monotherapy ↓ ps-IgE and ↑ ps-IgG4/IgE ratio	[34]	Dupilumab	
	Combined OIT and dupilumab therapy ↓ total and ps-IgE (sustained post treatment), ↓ OIT-induced ps-IgG1, ps-IgG2, ps-IgG4. However, combination therapy had minimal effect on ps-IgG, ps-IgG3, ps-IgA. ↑ ps-IgG/ps-IgE ratio with dupilumab + OIT, especially in responders with low baseline ps-IgE	[35]		
	Abrocitinib ↓ Th2 cytokines in peanut protein-stimulated PBMC cultures from individuals with peanut allergies, when compared to cultures without abrocitinib	[36]	Abrocitinib	
T helper cells	Omalizumab alone ↓ IL-4 in peanut-reactive CD4+ T cells, IL-4+ peanut-reactive CD4+ T cells, and EM Th2 cells	[30]	Omalizumab	Multi FA (almond, cashew, egg, hazelnut, milk, peanut, pecan, sesame, shrimp, soy, walnut, and wheat)
	↓ peanut-reactive CD4+ T cells and EM Th2 cells persists throughout OIT treatment	[30]		
	↓ OX40 expression in EM Th2 cells post-omalizumab and OIT	[30]		
	Ps-Th2A cells decrease with the dupilumab monotherapy	[34]	Dupilumab	
	Ps-Th2A cells decrease with the combination of OIT and dupilumab therapy	[35]		
	Abrocitinib does not alter ps-T effector cell activation in vitro (PBMCs)	[36]	Abrocitinib	
Cytotoxic T cells	Omalizumab alone ↓ frequencies of peanut-reactive CD8+ T cells	[30]	Omalizumab	
	↓ frequencies of peanut-reactive CD8+ T cells persist throughout OIT treatment	[30]		
	Omalizumab alone ↓ CXCR3 expression in peanut-reactive CD8+ T cells and EM CD8+ T cells	[30]		
	OIT post-omalizumab ↓ PD1 expression in EM CD8+ T cells	[30]		
Tregs	Omalizumab alone ↑ frequency of non-peanut reactive Tregs	[30]		
	Abrocitinib does not alter ps-Treg activation in vitro (PBMCs)	[36]	Abrocitinib	

TABLE 4 (Continued)

Focus	Immune mechanism	Citation	Biological used	Allergen
γδ T cells	Omalizumab alone ↓ frequencies of naïve γδ T cells, and ↓CXCR3 expression memory γδ T	[30]	Omalizumab	
B cells	Omalizumab + OIT ↓ frequency of CD86+ memory B cells at week 36 of treatment compared to baseline	[30]		
	Omalizumab treatment did not affect B cells when appropriately dosed	[33]		Peanut
DCs	Omalizumab + OIT ↓ frequency of CD86+ mDC1 and mDC2 at week 36 of treatment compared to baseline	[30]		Multi FA (almond, cashew, egg, hazelnut, milk, peanut, pecan, sesame, shrimp, soy, walnut, and wheat)
Monocytes	Omalizumab + OIT ↓ frequency of CD86+ classical, intermediate, and non-classical monocytes at week 36 of treatment compared to baseline	[30]		
Basophils	Omalizumab + OIT ↓ basophil activation (indirect BAT) at Week 36 of treatment compared to baseline	[30]		
	Omalizumab alone ↓ basophil activation (BAT)	[32,33]		Peanut
	Basophil activation ↑ after omalizumab discontinuation with OIT, remaining below baseline: 85% for treatment failures, 30% for successes	[32]		
	Abrocitinib alone ↓ basophil activation (BAT)	[36]	Abrocitinib	
	Basophil activation (BAT) increased slightly with dupilumab monotherapy	[34]	Dupilumab	
	Dupilumab combined with OIT reduces basophil activation (BAT) more substantially than OIT alone	[35]		

at 3 months. By 8 months, partially desensitized participants showed upregulation in interferon signaling and antiviral responses, while desensitized participants exhibited gene enrichment pathways related to DC crosstalk with NK cells, NK cell signaling, Th1 pathways, and Th1/Th2 activation.

5 | USE OF BIOLOGICALS TO TREAT CHILDHOOD FOOD ALLERGY (OMALIZUMAB, DUPILUMAB, AND ABROCITINIB)

Eight recent papers have investigated the effects of using biologicals to treat food allergy in children (Table 4). Five of which focus on the use of omalizumab (an anti-IgE monoclonal antibody) in combination with OIT. In the first study by Andorf et al.,[29] participants aged 5–22 years ($n = 70$) with up to five food allergies (including almond, cashew, egg, hazelnut, milk, peanut, pecan, sesame, shrimp, soy, walnut, and wheat) underwent a two-stage trial. The first stage was an open-label phase that involved 16 weeks of omalizumab treatment (Weeks 0–16), followed by multi-OIT (including peanut), which consisted of rapid up-dosing of food allergens for 22 weeks (Weeks 8–30), reaching a maintenance dose of >1 g of each allergen. Participants were then randomized into three groups: maintaining a 1-g allergen dose, reducing to a 300-mg allergen dose, or discontinuing OIT for the final 6 weeks. At 36 weeks, reactivity to allergens was assessed through a 2-g allergen dose oral food challenge. Most participants tolerated the challenge at 36 weeks, but those who discontinued OIT were less likely to do so. Additionally, participants who continued OIT were more likely to tolerate a 4-g protein dose from at least two food allergens during the final challenge at 36 weeks. Across all groups, there was a significant increase in allergen-specific IgG4: IgE ratios, primarily driven by an increase in allergen-specific IgG rather than a decrease in ps-IgE.

The second study by Manohar et al.,[30] assessed cellular changes in the PBMCs of multi-food (peanut, walnut, hazelnut, and egg) allergic children (aged 4–15 years, $n = 15$) who were successfully desensitized to peanut following omalizumab and multi-OIT. Omalizumab treatment alone, administered during the first 8 weeks, increased the frequency of non-peanut reactive Tregs and significantly reduced the frequencies of IL-4+ peanut-reactive CD4+ T cells, peanut-reactive CD8+ T cells, EM Th2 cells, naïve γδ T cells, and decreased CXCR3 expression in memory γδ T cells, peanut-reactive CD8+ T cells, and EM CD8+ T cells. This reduction persisted for peanut-reactive CD8 T, peanut-reactive CD4 T, and EM Th2 cells throughout the combined OIT phase (Weeks 8–16) and the OIT alone phase (Weeks 16–36), though some participants' naïve γδ T and CD8+ EM cells returned to baseline levels. By week 36, peanut-reactive CD4+ T cells showed a marked increase in skin-homing receptor CCR4 and Th1 cytokine CXCR3, while peanut-reactive CD8 T cells and EM CD8 T cells showed an increase in expression of the skin homing marker CLA, and EM CD8+ T cells exhibited a marked decrease in the expression of the inhibitory receptor PD-1. Further, EM Th2 cells demonstrated reduced levels of Th2 marker OX40 at this time point. Antigen-presenting cells (classical, intermediate, and non-classical monocytes, myeloid DCs1 (CD123+), myeloid DCs2 (CD123−), and memory B cells) decreased in frequency and expressed lower levels of CD86 at week 36 compared to baseline. Basophil activation, assessed through basophil activation test (BAT), was also significantly reduced

(decreased % CD63+ basophils), with a marked increase in IgG4 ratios by week 36. This immune modulation was accompanied by a reduction in allergic cytokines, with IL-13 and IL-9 levels decreasing in unstimulated PBMC cultures and IL-4 levels decreasing in cultures following PMA/ionomycin stimulation. Additionally, shifts in the inflammatory response were observed, with reduced levels of IL-17, IL-1β, MCP1, IL12p40, GM-CSF, and FLT3L in unstimulated PBMCs, and reduced IL-17, IL-8, and MIP1α in PMA/ionomycin stimulated PBMCs by Week 36 compared to baseline.

Ye et al.[31] longitudinally assessed the outcomes of peanut-allergic children between the ages of 8 and 16 years ($n=13$) receiving a combination of omalizumab and peanut OIT over 72 months. Initially, participants underwent 12 weeks of omalizumab treatment, followed by gradually increasing doses of peanut protein until they reached a 2-g maintenance dose. Allergy status was assessed via oral food challenges. Only 54% of participants continued peanut OIT through to the 72-month mark, while 46% discontinued due to adverse reactions. Notably, all participants experienced at least one adverse event, with one developing eosinophilic esophagitis. Assessment of immune markers revealed that peanut-IgG4, Ara h 1-IgG4, and Ara h 2-IgG4 levels initially increased and then decreased, though they did not return to baseline levels. Additionally, peanut-IgE, Ara h 1-IgE, Ara h 2-IgE, SPT results, and peanut-IgE:total IgE ratios decreased during OIT. Participants who discontinued treatment had higher levels of peanut-IgE and Ara h 2-IgE at Month 12 compared to those who continued therapy.

The fourth study by Brandström et al.[32] focused on children aged 12–19 years ($n=23$) with peanut allergies undergoing combined omalizumab and peanut OIT. The peanut OIT dosage was gradually increased from 280 to 2800 mg over 8 weeks, after which omalizumab was gradually withdrawn while maintaining peanut OIT for an additional 12 weeks. An oral food challenge was then conducted to assess desensitization status. All participants reached the 2800 mg maintenance dose. Basophil reactivity (measured by BAT) was initially suppressed in 22 out of 23 participants before starting peanut OIT, and this suppression continued throughout the first 8 weeks of peanut OIT. However, basophil reactivity increased slightly between the maintenance phase and the final visit (12 weeks post-omalizumab withdrawal). In participants who failed the final challenge, this increase was more pronounced. Upon omalizumab reduction or discontinuation, basophil reactivity rose to 85% of baseline in the treatment failure group and to 30% in the treatment success group. Additionally, the treatment success group exhibited lower levels of IgE specific to Ara h 1–3 and higher levels of IgG4 to Ara h 2 and Ara h 6 compared to the failure group at the final challenge.

The final study using omalizumab by van der Heiden et al.,[33] using the same cohort, found that omalizumab treatment does not affect B cells when appropriately dosed. Additionally, the authors found a decrease in allergic activation in anti-CD3/CD28 stimulated PBMCs from children pre-OIT (omalizumab only), during peanut OIT with omalizumab, and during the maintenance phase (gradual reduction of omalizumab with continued peanut OIT). Over time, these PBMCs exhibited a reduced Th2 cytokine profile (IL-5, IL-13, and IL-9) and an increase in IFN-γ, indicating a shift in T cell polarity due to treatment.

Two recent studies have investigated the therapeutic potential of the anti-IL-4RA antibody dupilumab in treating childhood food allergy. The first study by Sindher et al.,[34] evaluated dupilumab as a monotherapy over 24 weeks in peanut-allergic children ($n=24$, ages 6–17). Treatment had little impact on peanut tolerance, with only one child passing a 1044 mg (cumulative) peanut protein food challenge 12 weeks post-treatment. Notably, no severe allergic reactions occurred during treatment. Dupilumab treatment reduced ps-IgE and ps-IgG while increasing the ps-IgG4/IgE ratio. Basophil activation slightly increased, and ps-Th2A cell frequency decreased post treatment. A consecutive study, led by Chinthrajah et al.,[35] evaluated 148 peanut-allergic children taking OIT with or without dupilumab. Participants received dupilumab for 40 weeks with adjunct OIT ($n=44$), placebo with OIT ($n=50$), or dupilumab for 24 weeks before switching to placebo ($n=44$). All completed an OIT up-dosing phase (12–300 mg peanut protein per day over 24–36 weeks) followed by 2 weeks of maintenance (300 mg peanut protein per day). During up-dosing, children on dupilumab had a 20% higher pass rate for a 2044 mg peanut protein (cumulative) food challenge compared to children on OIT alone, with better response rates in children ≤12 years. After maintenance, peanut tolerance was similar in children who stopped dupilumab and those continuously on placebo. dupilumab reduced ps-IgE and ps-IgG1 during up-dosing, decreased ps-IgG2 in maintenance, and increased the ps-IgG4/ps-IgE ratio post-treatment compared to placebo and dupilumab-discontinuation groups. Basophil activation (measured by BAT) was lower with dupilumab during up-dosing and decreased in the placebo group during maintenance. Th2A cells decreased more in the placebo group post–OIT up-dosing than in children receiving dupilumab.

The final study, by Ramsey et al.,[36] investigated abrocitinib, a Janus kinase 1 (JAK1) inhibitor, for treating food allergies in a cohort of 21 participants (ages 5–23, with 19 under 18). BATs on whole blood samples pretreated with abrocitinib or DMSO (vehicle control) showed consistently lower basophil activation in abrocitinib-treated samples compared to controls after peanut exposure. To assess peanut-induced Treg and T effector cell activation, PBMCs were stimulated with anti-CD3/CD28 or peanut protein, with or without abrocitinib. Abrocitinib suppressed T effector activation in anti-CD3/CD28 cultures without affecting Tregs but had no significant effect on Treg or T effector cell activation in peanut-stimulated cultures. Peanut-stimulated PBMC cultures had elevated IL-4, IL-13, IL-10, TNFα, and IL-17, but the addition of abrocitinib reduced these cytokine levels to those seen in non-peanut-stimulated cultures, indicating that abrocitinib suppresses Th2 signaling in vitro.

6 | CONCLUSIONS

There are common threads emerging from recent evidence, summarized in Figure 1. Type 2 immunity continues as an important player in the food allergy immune response, whether it is measured by

circulating cytokines (IL-4, IL-13, IL-5), via flow cytometry of cell surface markers (Th2 cells), molecular profiling (epigenetics and transcriptomics), or following in vitro stimulation with both specific and non-specific stimuli. It is now clear that this type 2 immunity extends beyond T cells with the identification of a novel memory B cell population poised to switch to IgE production, and it is also often associated with elevated markers of inflammation (for example increased levels of the soluble inflammatory mediator TNFα and increased innate cell activation). Other common signatures include altered T-regulatory cell frequency and function, as well as impaired interferon signaling, which may both contribute to the pathogenic allergic immune cycle. Regarding the mechanisms underlying OIT, a reduction in type 2 immunity, particularly Th2 cell frequency and concentration of type-2-related cytokines, has been reproducibly observed across studies, often correlated to treatment outcome. Data also implicates other cell types in the atypical immune response(s) of food allergy and changes following OIT, including Th17 cells, CD8 T cells, and unconventional T cells, that should not be ignored and warrant further investigation in future studies.

There are challenges in interpreting these data that make direct links across studies difficult, particularly for OIT where different time points and definitions are used to determine clinical outcomes. Further, a range of assays, stimulations, culture time points, and analytical strategies are used across all studies reviewed herein, and only rarely are findings validated in external cohorts. Many studies may be limited by type I and type II errors due to low sample size, mostly attributed to the expense of performing highly multiplex immunology experiments. A future goal as a community should be to standardize clinical definitions, laboratory assessments, and data reporting across studies and facilitate open access data repositories for replication and data integration. A limitation of current work is the reliance on peripheral blood, which may not reflect mucosal changes relevant to the development of food allergy. While difficult to access, mucosal responses in pediatric food allergy represent a significant knowledge gap that should be addressed.

Many other outstanding questions remain, including detailed assessment of the molecular and cellular mechanisms that account for differences between food sensitization and clinical food allergy in infancy and early childhood. Similarly, there is limited evidence on the mechanisms responsible for the acquisition of natural tolerance in childhood, and the immunological reasons underlying why some children have more severe multi-food allergies that persist through to adulthood. Understanding these factors in combination is critically important for the development of effective tools for prediction, diagnosis, and monitoring of disease. A key question at the forefront of this field with the emergence of OIT for children is whether OIT can induce the same shift in immune response as that observed in children who develop lifelong natural tolerance. A direct comparison is essential for informing effective curative treatments. Furthermore, research into therapies for childhood food allergy beyond OIT, omalizumab, dupilumab, and abrocitinib is limited. Monoclonal antibodies like omalizumab and dupilumab have been shown to enhance the safety and efficacy of OIT but are ineffective as standalone treatments. Therefore, exploring new treatments that target alternative allergic pathways, including those identified in the papers reviewed here, will improve quality of life for children with food allergies.

AUTHOR CONTRIBUTIONS

Liam Gubbels: Conceptualization; investigation; writing – original draft; methodology. **Richard Saffery:** Conceptualization; writing – review and editing; investigation. **Melanie R. Neeland:** Conceptualization; investigation; writing – review and editing; methodology.

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The authors have no conflicts of interest to declare.

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