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REVIEW

Utility of in Vivo and Ex-vivo Models in Food Allergy Research: Lessons for Shrimp Allergy

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Abstract
We are living in an era where food allergy is increasingly prevalent, and shrimp allergy (SA) is among the most common and potentially severe reactions worldwide. Significant efforts have been made to improve diagnostic accuracy and treatment options. However, diagnosing SA remains challenging due to its diverse clinical presentations and the complexity of shrimp allergens. The pathogenesis of SA is complicated by various allergens sensitization, epithelial barrier disruption and gut microbiota dysbiosis. To date, at least ten shrimp allergens have been identified, but sensitization profiles vary significantly across populations. Promising novel immunotherapy approaches have been explored, showing potential in murine models. The use of physiologically relevant experimental systems, from in vivo models (e.g., murine studies) to advanced ex vivo human-based techniques (including organoids, organ-on-a-chip platforms, and tissue slice cultures), offers promising alternative for studying biological processes that are difficult to investigate directly in humans. These models have emerged as a valuable tool in food allergy research by providing critical insights into food-gut interactions, gut microbiota-host dynamics epithelial barrier integrity and cross-talk between epithelia and immune cells, and signaling and inflammatory markers. This review aims to summarize recent advancements in the diagnosis and treatment of SA, and the potential in vivo/ex-vivo models in SA research.

Keywords Shrimp allergy · Food allergy · Tropomyosin · Organoid · Enteroid

Introduction

The prevalence of food allergies (FAs) is increasing in Western and Asian countries, with a reported prevalence of up to 10% [1, 2]. FA mostly affects children, some of which can outgrow the allergies. Milk and egg allergies are commonly outgrown, whereas seafood allergy, such as shellfish and fish, tends to persist throughout life [3]. FA in early life is a risk factor for secondary allergies, including type 2 asthma [4]. Notably, the prevalence of shrimp allergy (SA) appears to be higher in Asian countries than that in Western countries, with the highest prevalence of up to 40.3% in Hebei, China [3]. Our previous work demonstrated that allergy to ≥3 seafood species and above increased the risk of severe reactions [5]. Therefore, efforts should be made to understand and improve tolerance and promote novel therapies for seafood allergies, especially SA.

Although several preclinical mouse models of SA have been developed [6–8], their translational value is limited because of interspecies differences [9–11]. Novel ex-vivo models, such as 3D cell cultures, organoids, and organ-on-a-chip (OoC) models, have been recently explored. These models are becoming valuable in food research because they provide a link between basic cell cultures and complex in vivo models [12]. This can also lead to the development of safer and more efficient food items and more focused nutritional therapies [9, 12]. Herein, we extensively review the current understanding of SA, focusing on pathogenic mechanism, current research models and their potential applications in SA.

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Current Understanding of SA

Clinical Phenotype

The clinical phenotypes of SA vary widely in terms of symptoms, severity, and onset.

*Immediate-type reactions*are the most common phenotype, triggered by IgE-mediated responses to shrimp allergens. Symptoms typically develop within minutes to two hours after ingestion and frequently include urticaria, angioedema, respiratory distress, and laryngeal edema. Cardiovascular involvement such as hypotension or syncope may occur, occasionally progressing to anaphylaxis. In pediatric cohorts, cutaneous manifestations predominate (≈70%), with anaphylaxis rates of 10–12% [13]. Severe systemic reactions, including respiratory compromise and cardiovascular collapse, have been documented, and fatal cases have been reported [13–15].

Delayed reactions, are less frequent and usually non-IgE-mediated. In a population-based survey of food allergies (FAs), the frequency of self-reported intestinal symptoms ranged from 7.7 to 21.2% [16]. These delayed reactions can occur hours to days after exposure and are characterized by symptoms such as eosinophilic esophagitis/eosinophilic gastritis (dysphagia and chest discomfort), food protein-induced enterocolitis, and food protein-induced allergic protocolitis [17]. Crustaceans can also trigger delayed reactions in adults [18]. Similarly, gastrointestinal distress can occur hours after shrimp ingestion without any evidence of IgE sensitization [19].

Atopic dermatitis (AD) and *contact dermatitis*(eczema or rash upon direct contact with shrimp or shrimp products) have been reported to be associated with shrimp. A study found that 6% of patients with AD had a SA, which could exacerbate skin symptoms [20–23]. This mechanism is attributed to mixed IgE and non-IgE-mediated reactions [24].

*Oral allergy syndrome (OAS)*occurs because of cross-reactivity between shrimp allergens and other allergens, especially house dust mites – a condition termed "mite-shellfish OAS" [16, 25]. Symptoms are typically mild and localized to the oropharyngeal region but can occasionally progress to systemic reactions [13, 26].

*Food-dependent exercise-induced anaphylaxis (FDEIA)*is a rare SA phenotype. Anaphylaxis is triggered by the combination of shrimp intake and subsequent physical activity [27]. A study from Japan discovered that the P75 homolog (myosin heavy chain) and fructose 1,6-bisphosphate aldolase (FBPA) were new potential allergens for shrimp-induced FDEIA [26].

Molecular Components of Shrimp

Currently, the World Health Organization/International Union of Immunological Societies (WHO/IUIS) Allergen Nomenclature Subcommittee has documented 10 shrimp allergens, including the major allergen TM, arginine kinase (AK), myosin light chain 1 (MLC1) and 2 (MLC2), sarcoplasmic calcium-binding protein (SCP), troponin C, hemocyanin, triosephosphate isomerase (TIM), fatty-acid-binding protein (FABP), glycogen phosphorylase (GP) [28]. Updates on shrimp allergens are summarized in TableS1. TM, an alpha-helical coiled-coil protein, is a major pan-allergen found in more than 13 crustacean species. Its high amino acid sequence homology with allergens from mollusks, arthropods (e.g., house dust mites [Der p 10] and cockroaches [Bla g 7]), and fish parasites (e.g., Anisakis simplex [Ani s 3]) explains its significant cross-reactivity [3, 28–30].

Several minor allergens also contribute to the heterogeneity of SA. AK, a heat-labile protein, partially retains its IgE-binding activity after heating because of the preserved linear epitope [31–33]. MLC1 shows cross-reactivity with cockroach allergens (Bla g 8) and contributes to allergic symptoms including respiratory reactions to aerosolized shrimp proteins [34, 35]. SCP, a heat-stable and highly conserved protein among crustaceans, shows high homology across shrimp species, but differs significantly from mollusks, making it a specific marker for shrimp allergen diagnostics [34, 36]. Hemocyanin, primarily located in the cephalothorax of shrimp, is another thermostable allergen that exhibits cross-reactivity with house dust mites and cockroaches [37]. Other less studied allergens, including enolase, GP, and FABP, and emerging candidates (e.g. titin, enolase, myosin heavy chain, glyceraldehyde 3-phosphate dehydrogenase, vitellogenin, ovarian peritrophin 1 precursor, β-actin, and pyruvate kinase) have been identified in SA, although their clinical implications remain underexplored [37–40].

Diagnostic Value of Tropomyosin and Other Shrimp Allergens

Conventional diagnostic methods, such as skin prick tests (SPT) and allergen-specific IgE (sIgE) assay, are commonly used for SA diagnosis. However, their performance is limited by variable sensitivity and specificity, particularly in cross-sensitized patients [25, 41]. Although oral food challenges (OFC) remain the gold standard, their risk and resource-intensive nature emphasize the need for more reliable in vitro diagnostics. TM-specific IgE testing has demonstrated improved diagnostic performance compared with whole shrimp extract-based assays, with

sensitivity and specificity as high as 82.8% and 56.3%, respectively, in Western populations [32, 34, 35]. However, current evidence demonstrates that TM alone cannot precisely predict SA in Central Europe [42]. In Asia, SCP (Pen m 4) demonstrate superior diagnostic accuracy [43]and strongly correlated with systemic allergic reactions, including anaphylaxis [31, 34, 34, 44]. Combining TM with other markers, such as Pen m 2 and Pen m 4, enhances the sensitivity and specificity [34, 44, 45].

Immunotherapy in SA

Despite the widespread prevalence of SA, no effective treatment options are currently available. Avoidance remains the primary management strategy. Nonetheless, several investigational approaches have shown promise.

*Sublingual immunotherapy (SLIT)*is safe and effective, mostly for peanut allergies [46]. A retrospective study included 66 patients demonstrated that SLIT for SA was safe and effective, with 61% achieving tolerance to oral challenges with up to 3 g of shrimp protein [47].

Oral immunotherapy (OIT)may be more effective than SLIT but carries a higher risk of allergic reactions[48]. Previous studies indicated that 53% of children tolerated a low-dose shrimp challenge without a reaction [49, 50]. In a phase 2 trial, 58.3% of participants with SA achieved desensitization after 52 weeks of OIT, with mild adverse events being the most common [51].

Recently, comprehensive research on shrimp allergens has led to the development of innovative immunotherapeutic strategies. Glycation of shrimp TM can produce hypoallergenic variants such as glycated TM (GTM), which exhibit reduced allergenicity. The combination of GTM and aluminum hydroxide is a promising candidate for desensitization in preclinical models [42, 52]. The identification of immunodominant T cell epitopes has led to the development of a T-cell epitope-based immunotherapy approach, which has demonstrated efficacy in reducing allergic symptoms in a murine model of SA [53]. Finally, hypoallergenic derivatives of TM, such as MEM49 and MED171, showed a marked reduction in IgE reactivity and the ability to block IgG antibodies [54]. Intradermal administration of pMED171, a DNA vaccine encoding the hypoallergenic shrimp tropomyosin variant MED171, triggers priming, activation, and migration of dermal dendritic cells, which promote the induction of regulatory T cells (Tregs), both locally and systemically, to suppress allergic responses to TM [55]. In addition to shrimp TM, a combination of the T cell epitope of shrimp AK with the TLR9 agonist CpG-ODN in nanoparticles (CpG-AKp NPs) was tested in a shrimp allergen-induced FA mouse model. Treatment with CpG-AKp NPs suppressed the anaphylactic responses, lowered specific IgE levels, and modulated T helper 2 (Th2) cytokine levels in response to Th1 cells. In addition, CpG-AKp NPs increased Foxp3 and IL-10 expression while reducing the activation of STAT6 and GATA3 [56].

Despite rapid advancements, there is still an unmet need for SA immunotherapy, with moderate tolerance rates (50–60%). Given these challenges, developing physiologically relevant in vivo and ex vivo models has become a research priority, providing critical platforms for studying allergen-specific immune responses and testing desensitization approaches.

Other Factors Involved in SA

The development of food allergies arises from complex interactions between the immune, epithelial, and neuroimmune systems, which transform harmless food antigens into potent allergens.

Epithelial Barrier Disruption

Sensitization to food allergens may occur when the epithelial barrier is disrupted, both in the gastrointestinal tract and in the skin [57]. Structural and functional defects have been observed in the terminal ileum and colorectal regions of patients with FAs [57–59]. In parallel, elevated levels of epithelial-derived cytokines have been detected in these patients [57, 60]. Shrimp TM directly impairs the intestinal epithelial barrier and initiates type 2 immune activation, considered a central event in sensitization. In vitro studies using human intestinal epithelial cell lines (e.g., Caco-2, and HT-29) demonstrated that shrimp TM – but not its non-allergenic chicken homolog – causes marked disruption of tight junctions (TJs), increased paracellular permeability, and epithelial activation [61]. In murine models, TM-sensitized animals exhibited increased inflammatory cell infiltration in the small intestine and duodenal epithelial apoptosis [62]. Recent reviews have emphasized that multiple factors within the modern "exposome," including dietary advanced glycation end-products (dAGEs) commonly found in processed foods, microbial proteases, and environmental pollutants, can degrade tight junction proteins (occludin, claudin-1, ZO-1), increase intestinal permeability, and thereby promote food allergy [54, 63–65].

Gut Microbiota Dysbiosis

Among the downstream consequences of epithelial barrier disruption, epithelial-derived cytokines such as thymic stromal lymphopoietin (TSLP), IL-25, and IL-33 act as pivotal alarmins that orchestrate type 2 immunity [57]. These molecules serve as early danger signals, bridging

epithelial injury to immune activation and shaping the allergic response. They promote dendritic cell activation, upregulation of costimulatory molecules, and priming of naïve T cells toward Th2 differentiation [46, 57, 60, 61]. Activated Th2 cells subsequently secrete IL-4, IL-5, and IL-13, which play central roles in allergic inflammation: IL-4 and IL-13 induce immunoglobulin class switching in B cells to produce allergen-specific IgE, while IL-5 recruits and activates eosinophils [65, 66].

Notably, a recent clinical study demonstrated significantly elevated serum thymic stromal lymphopoietin (TSLP) and IL-25 in patients with SA, whereas IL-33 remained unchanged; importantly, these increases occurred independently of allergen-specific IgE [49, 67]. Immunological reviews have reinforced that the TSLP–IL-33–IL-25 axis functions as an "epithelial alarm system," determining the shift from tolerance to type 2 inflammation [66]. Among these, experimental evidence highlights TSLP as the dominant factor driving allergic responses following epicutaneous sensitization, while IL-33 plays a subordinate role [50].

Crosstalk between epithelial-neuronal-immune Cell Interactions

By rupturing the epithelial barrier, allergens can penetrate the intestinal mucosa. This allows them to activate innate immune sensors, such as the NLRP3 inflammasome, which facilitates the release of alarmins (e.g., IL-25, IL-33, and TSLP) [49]. Following epithelial damage and alarmin release, dendritic cells (DCs) are activated and migrate to draining lymph nodes, where they prime naïve T cells toward a Th2 phenotype [66]. Th2 cytokines help maintain IgE production and recruit eosinophils, strengthening the allergic response. Allergen-specific IgE binds to FcεRI on mast cells and basophils, sensitizing them and leading to the release of histamine and other mediators upon re-exposure to the allergen, thereby driving acute allergic symptoms and anaphylaxis [50]. Recently, the imbalance between RORγt+antigen presenting cells and conventional dendritic cells in modulating immune responses have been noted. The RORγt+antigen presenting cells promote Treg induction and tolerance [51], and cDC1s are also involved in initiating dietary CD8αβ T cell responses [48], whereas inflammatory cDC2 drive Th2 polarization and allergic responses [68]. In summary, the epithelia–DC–Th2–IgE–mast cell/eosinophil axis forms a self-amplifying loop that sustains Th2-skewed inflammation in the intestinal mucosa [50, 66, 68].

Numerous studies advocate for the bidirectional communication between neuronal mediators and immune cells in initiating and amplifying type 2 immune response. Immune cells possess receptors for neuropeptides and neurotransmitters, while neurons have receptors for cytokines [69]. Cytokines such as IL-4, IL-13, IL-33, and TSLP, released from epithelial or immune cells, can sensitize sensory neurons by lowering their activation threshold, even without directly eliciting sensations such as itch or pain [70]. The acetylcholine (ACh) is released by neurons from enteric nervous system and involved in type 2 responses from ACh receptor on DC and mast cells [71]. In addition, ACh contributes to maintaining integrity of the epithelial barrier through muscarinic and nicotinic receptors [71]. Mast cell–derived mediators, including histamine, proteases (e.g., chymase), and leukotrienes, further stimulate neuronal receptors such as TRPV1 and TRPA1, contributing to pruritus, discomfort, and potentially visceral pain [70]. In turn, activated neurons release neuropeptides and neurotransmitters that modulate immune cell activity—including mast cells and type 2 innate lymphoid cells (ILC2s)—thereby sustaining or amplifying the allergic inflammatory [15]. This neuro-immune axis emphasizes that food allergy is more than just an immunological problem; it is also a disorder in which allergic sensitization influences host behavior.Together, these pathways show how neuro-immune crosstalk, innate immune activation, maladaptive adaptive responses, and epithelial barrier failure interact to create food allergies, opening up new options for targeted treatment [53].

Gut Microbiota Dysbiosis

Gut microbiota dysbiosis have been shown to be closely associated with allergic diseases [72, 73]. Dysbiosis has been strongly associated with allergic diseases [72, 73], characterized by reduced microbial diversity, a decrease in*Clostridiales*, and the expansion of mucin-degrading bacteria such as *Akkermansia muciniphila*, which thins the mucus layer and increases permeability, thereby facilitating allergen translocation [58, 72, 74]. The altered gut microbiota may initiate innate and adaptive immune responses, such as increased specific IgE, imbalance of pro-inflammatory cytokines/anti-inflammatory cytokines, and Treg suppression [75, 76]. Early infancy of the gut microbiota may precede FA outcomes [76]. Cohort studies in infants have shown that early-life microbiota signatures can predict the course of food allergy: enrichment of*Clostridia* and *Firmicutes*in the first months of life was associated with resolution of milk allergy by school age [76].

Some probiotic strains can alleviate allergic reactions by promoting immune tolerance and altering the gut microbiota composition. Microbial metabolites, particularly short-chain fatty acids (SCFAs) such as butyrate and acetate, protect against allergy by inducing Tregs and promoting tolerance [46]. Lactic acid-producing bacteria suppress TM-induced intestinal mucosal disorders, Th2 responses, and dysbiosis [76]. Similarly,*Bifidobacterium infantis*14.518

decreases TM-induced allergic responses by stimulating tolerogenic dendritic cell-dependent induction of Treg cells and restoring gut microbiota diversity [77].*Lactobacillus casei Zhang*reduces TM-induced food allergy by altering the development and function of immune cells, which are dependent on the NF-kB signaling pathway [78]. The aforementioned studies were based on TM-induced SA murine models; however, they highlight the superior role of probiotics in the induction of SA tolerance. These findings underscore the dual role of the microbiota: as both a maintainer of immune tolerance and, when dysregulated, as a driver of allergy.

In Vivo and Ex-vivo Models in SA Research

Given the complexity of FA pathogenesis, researchers require models that can closely mimic the complex interactions between allergens, epithelial barrier, and immune system [41, 58]. Physiologically relevant models, such as in vivo models (e.g., traditional murine systems) to advance ex vivo human-based approaches (e.g., organoids, organ-on-a-chip devices, biopsy-based tissue models, and precision-cut organ slices) offer powerful ways to study these processes in controlled settings [79–81]. Such models enable investigation of immune mechanisms, from allergen entry and antigen presentation to Th2 cytokine production, mast cell activation, and mediator release, all of which play a role in shrimp-induced reactions [79–81]. These models also serve as therapeutic strategies, such as allergen-specific immunotherapy or barrier-protecting interventions, before clinical trials [79, 82]. They more closely reflect human biology, therefore, ensuring that the findings are relevant to patients, while also reducing the need for animal testing [82, 83].

Murine Models in SA Research

Murine models are widely used in FA research, demonstrating substantial advantages for understanding disease mechanisms, evaluating allergenicity, and developing treatment strategies [82, 84]. Owing to their ability to mimic systemic immune responses, including sensitization, antigen-specific IgE production, and anaphylaxis, they allow the investigation of complex, multi-organ interactions involving the gastrointestinal tract, respiratory system, skin, and immune system [84, 85]. These models are especially helpful for understanding the sequential immunological events in FAs from allergen exposure and antigen presentation to Th2 cytokine release, IgE production, mast cell activation, and regulatory T cells in FA responses [86]. They can also trigger mast cell degranulation and hypothermic reactions upon exposure to an allergen, closely mirroring the anaphylaxis observed in patients with allergies [87]. Murine models are also useful for testing novel treatments such as oral, skin-based, or allergen-specific immunotherapies, showing promising results in models of FAs [82].

However, murine models have several limitations. Mice differ from humans in several important aspects of the immune system function, such as receptor expression, immune cell distribution, immunoglobulin subclasses, cytokine responses, and microbiota composition, which can limit the accuracy of applying these findings to humans [84, 85]. Additionally, gut microbiota composition, diet, and environmental factors in laboratory mice may differ from those in humans [86]. In murine FA research, ethical concerns arise from the triggering of severe allergic reactions, including anaphylaxis, in mice when studying disease mechanisms and treatment efficacy [82, 85]. These procedures can cause considerable stress or harm in animals, requiring strict humane guidelines and justification that research cannot be conducted using alternative in vitro or computer models [82, 85].

In shellfish allergy, particularly SA, murine models have been used to study immunological mechanisms. The major allergen tropomyosin has been widely used to induce SA mouse models [88, 89]. BALB/c and C3H/HeJ mice sensitized with shrimp TM via epicutaneous or oral routes exhibit elevated TM-specific IgE levels and Th2-biased immune responses, closely resembling human allergy phenotypes [90–92]. Shrimp extracts induced changes in lipid metabolism and immune inflammation in BALB/c mice, which were restored by exogenous glyceryl trioleate [93]. These models also make it possible to study the detailed mechanisms underlying allergy development and treatment, such as changes in immune cell activity, shifts in cytokine profiles, and regulation of IgE production, in ways that would not be ethical or feasible to attempt in humans [87, 92]. In addition, models such as CC027 mice have shown strong IgE responses and allergic cross-reactions to shrimp, crabs, and lobsters, as many people react to multiple shellfish [91]. In addition to TM, no other shrimp allergens have been evaluated in murine models. This represents a notable research gap, especially considering that sensitization profiles can vary significantly across populations [36, 43, 94, 95].

Overview of Intestinal ex-vivo Models

Various ex-vivo models have been introduced and used in FA research. The current ex vivo intestinal models are summarized in Fig. 1; Table 1. Generally, intestinal biopsies serve as a valuable source for generating ex-vivo tissue models, including precision-cut organ slices (PCOS), which involve using tissue samples directly from human or

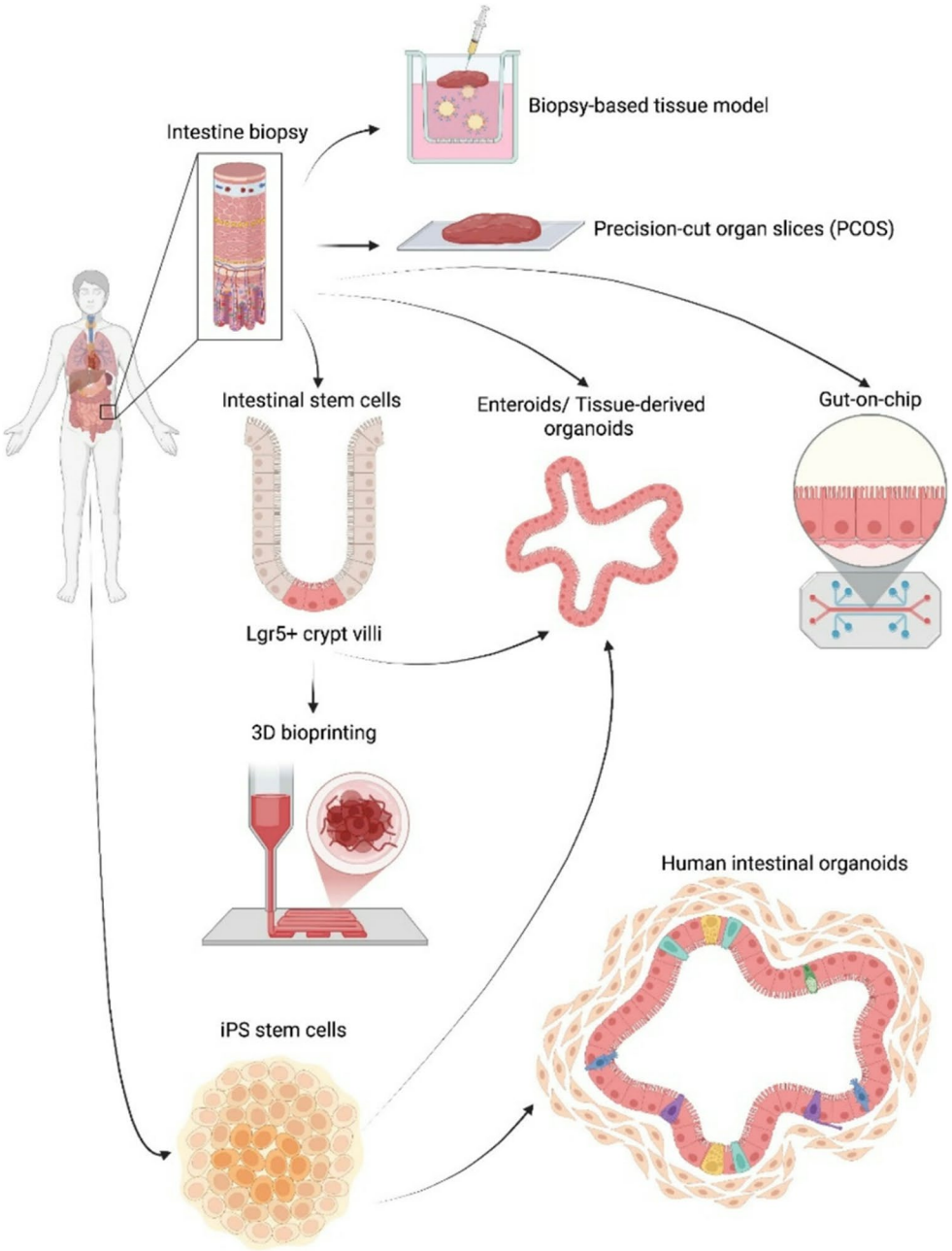

Fig. 1 An overview of intestinal ex-vivo models for FA research. Intestinal biopsies can be directly used to generate biopsy-based tissue models or precision-cut organ slices (PCOS), preserving the native multicellular architecture. From these biopsies, intestinal stem cells (Lgr5⁺ crypt villi) can be isolated and expanded to generate enteroids or tissue-derived organoids, which can model epithelial structure and function. Alternatively, induced pluripotent stem (iPS) cells can be harvested to differentiate into intestinal epithelial cells and form human intestinal organoids. Additionally, the intestine biopsy can be integrated into advanced platforms such as gut-on-chip systems for dynamic, physiologically relevant studies, or used in 3D bioprinting approaches to recreate complex tissue structures. *Created with BioRender.com*

animal sources, and are widely used in many studies [96–99]. From these samples, Lgr5⁺ stem cells located the base of intestinal crypts – can be differentiated into enteroids or tissue-derived organoids [100, 101]. Alternatively, human intestinal organoids (IOs) can be derived from induced pluripotent stem (iPSCs) via cells through direct differentiation into intestinal epithelial lineages [100, 102]. Furthermore, intestinal biopsy can be integrated into advanced platforms such as gut-on-chip systems, or 3D bioprinting [4, 103].

However, biopsy-based models have limitations such as loss of tissue complexity and short-term viability, whereas PCOS may not fully replicate the dynamic interactions and differentiation [97, 99]. Organoids provide a unique model for examining tissue-specific biology, and can replicate both the epithelial and mesenchymal components of tissues.

Organoids and Enteroids

Organoids and enteroids, derived from human intestinal stem cells (ISCs), replicate the 3D structure and cellular diversity of the gut epithelium, and a variety of cell types including enterocytes, goblet cells, and enteroendocrine cells [104, 105]. They preserve unique genetic and epigenetic profile, showing individual variability in

Table 1 Comparison the in vivo and ex vivo models in FA research

Model	Advantages	Limitations	Use in Food allergy and/or shrimp allergy
Murine Models	Systemic immunity, whole-body in vivo relevance, established protocols	Species differences, ethical concerns, interspecies immune variation	Extensively used
Organoids and Enteroids	Human-derived, preserve epithelial diversity and donor genetics, controlled mechanistic studies	Lack systemic context, no immune cells unless co-cultured, limited lifespan	Emerging use
Organ-on-a-Chip (OoC)	Dynamic flow, multicellular integration, can model multi-organ interactions (gut–immune, gut–lung)	Technical complexity, high cost, still under development for allergy	Potential only
Biopsy-based Tissue Models	Preserve native architecture, resident immune cells, donor-specific traits	Limited availability, short ex vivo lifespan, variability between donors	Very limited use
Precision-cut Organ Slices (PCOS)	Maintain 3D tissue structure, diverse cell types, and functional networks	Short viability outside body, limited tissue access, low throughput	Not yet applied
Intestinal Organoids	Replicate gut epithelium, site-specific modeling, donor variability, useful for allergen uptake and barrier studies	No systemic immune responses, lack vasculature and neural input	Emerging use

allergic responses that animal models cannot [104, 106]. This makes them valuable tools for studying epithelial-immune interactions, allergen uptake, and epithelial barrier integrity under controlled laboratory conditions [107, 108]. Organoids can be grown in systems, such as Transwells, which separate the top and bottom layers of cells, making it easier to study how allergens cross the gut lining and the signals they trigger [109, 110].

Intestinal organoids (IOs) represent the first successful establishment of a human organoid *in vitro*.The IO comprises a cystic epithelium, a progenitor reservoir that includes crypt-like cavities, Lgr5 + intestinal stem cells, Paneth cells, and mature enterocytes [83]. From the stem cells, such as adult ISCs, embryonic stem cells (ESCs), and iPSCs, IO can be generated by enzymatic digestion of small-intestinal organoids or isolation of Lgr5 + villi-crypt ISCs into small IOs [84]. ISCs are multipotent tissue-resident cells capable of generating adult-like organoids known as enteroids. In contrast, ESCs and iPSCs, which are pluripotent and capable of differentiating into all three germ layers, require complex differentiation protocols and typically produce organoids that resemble the fetal intestinal tissue. A variety of IO models have been delineated, and a nomenclature has been proposed by the National Institute of Health Intestinal Stem Cell Consortium [85]. Generally, small human IO can be generated from human PSCs*or*-derived from isolated multipotent stem cells and progenitor cells in the intestinal crypts, which are termed enteroids [9, 85]. The IO effectively recapitulates the developmental stages of the fetal small intestine and exhibits the capacity to differentiate into epithelial and mesenchymal cell types [86–89]. The latter is characterized as "reconstituted IO," which constitutes a multicellular cluster structure comprising both small intestinal or colonic epithelial and mesenchymal cellular components [85].

In the context of food allergy or food related disorders, organoid and enteroid applications are still limited compared to murine models. To date, no studies have established organoid models for shrimp allergy. Accumulating evidence indicates that these systems are capable of modeling key pathophysiological processes triggered by food proteins, including allergen uptake, epithelial barrier disruption, immune activation, and cytokine-mediated inflammation. In a model conducted by Noah et al. (2019), the transplantation of human intestinal organoids into immunodeficient NOD-SCID IL-2Rγnull (NSG) mice determined that IL-13/CD38/cADPR-dependent secretory antigen passages (SAPs) were essential for food allergen translocation, and that blocking this pathway reduced allergen passage and anaphylactic response [111].

Regarding studies on epithelial barrier disruption, a study by Freire et al. (2019), which modeled celiac disease by exposing human gut organoid monolayers to gliadin and gluten, which triggered increased epithelial permeability and elevated pro-inflammatory cytokine release [112]. Nesice et al. examined the effect of Act d 1, a primary kiwifruit allergen, on intestinal epithelial cells using mouse-derived 2D intestinal organoids [113]. Act d 1 treatment of mouse 2D organoids up-regulated tight junction (TJ) genes (occludin, claudins 1–4, ZO-1) but simultaneously induced structural disruption of Claudin-3, E-cadherin, and ZO-1 [113]. This protease activity also triggered strong secretion of the pro-inflammatory cytokines TNF-α, IL-1β, and IL-33 [113]. Besides, in the RNA-seq study of shrimp tropomyosin sensitization of Li et al. (2025), tropomyosin exposure was associated with downregulation of genes linked to tight junction structure and barrier function, suggesting that TM may impair epithelial integrity at the gene expression level [114]. In addition, Masuda et al. employed 3D esophageal organoids derived from transgenic mice overexpressing

epithelial IL-33 to model type 2 inflammation [115]. Upon stimulation, these organoids exhibited reactive epithelial remodeling and increased IL-13 expression, mirroring features of eosinophilic esophagitis and highlighting the utility of organoid systems for studying cytokine-mediated epithelial–immune crosstalk in allergic disease [115]. Bouffi et al. demonstrated that human IOs transplanted into humanized mice and exposed to microbial antigens upregulated M cell markers (e.g. GP2), that promoted antigen translocation, and activated downstream immune responses, leading to IgA secretion into the organoid lumen [116]. This model highlights the potential of human IOs to recapitulate functional epithelial–immune crosstalk and mucosal immunoglobulin signaling [116].

Organoids have also been used to study the interplay between the gut microbiota and nutritional elements [96–98], which can interfere with food allergy tolerance. Co-culture of gut organoids with immune cells facilitates the understanding of how allergens penetrate the intestinal barrier, elicit immunological responses, and contribute to allergic inflammation [100, 101]. Numerous studies have advocated a relationship between gut barrier dysfunction and the increased prevalence of many chronic diseases, including FA [102, 103]. Environmental factors, lifestyle, industrialization, and urbanization affect the epithelial barrier of the skin, respiratory tract, and intestinal tract. This leads to microbial dysregulation, bacterial translocation, and tissue microinflammation, which are prone to the development of allergic diseases [103, 104]. Food emulsifiers such as polysorbate 20 (P20) and polysorbate (P80) in bread, ice cream, confectionery, beverages, and dressings are associated with gut barrier dysfunction. P20 and P80 were incubated with iPSC-derived human IO, colon organoid OoC, and liquid-liquid interface cells. P20 and P80 significantly decreased transepithelial electrical resistance, disrupted barrier integrity, and induced irregular tight junction expression in both organoid models. RNA sequencing of the transcriptome and targeted proteomic analyses have shown increased cell proliferation, apoptosis and inflammatory responses [117].

Besides, the gut-immune-skin axis has become a crucial research area in FA studies, particularly in relation to conditions, such as eczema. The development of IO models that can be co-cultured with microbiota enables investigation of the effects of specific bacterial strains on gut cell health, food processing, and immunological regulation [97, 98, 106]. These systems enable the study of how gut-derived factors, such as cytokines or allergens, sensitize the skin and contribute to allergic conditions.

Moreover, organoid models help to observe the effects of dietary fibers and other prebiotic components on beneficial bacteria [106–108]. Exposure of IO to gliadin enhanced intestinal permeability and pro-inflammatory cytokine levels. Barrier function and inflammation are restored by the microbiota-derived bioproducts butyrate, lactate, and polysaccharide-A [112]. These approaches can be applied in SA research to examine the effects of shrimp allergens, genetic modifications, and treatments.

Although organoids and enteroids offer many advantages, they also have notable limitations. Organoids and enteroids lack immune cells, blood vessels, and neural components unless these elements are added to co-culture systems, limiting their ability to fully mimic how the body responds in real life [104, 118, 119]. As a result, they cannot reproduce whole-body allergic reactions, such as anaphylaxis or multi-organ mediator release [118, 119]. They also have limited lifespans in advanced differentiated states, batch variability, and scalability in high-throughput screening [120, 121]. Thus, 3D-bioprinted organoids have been developed as adaptable models that can replicate human tissues, such as the immune and gut systems, allowing for a more precise and controlled study of interactions between food allergens [103]. Despite, bioprinted organoids still have difficulty obtaining sufficient tissue vascularization and complexity for extensive research and clinical applications [103].

Organ-on a Chip

Organ-on-a-chip (OoC) systems are innovative tools that offer a unique method to study FAs by combining dynamic fluid flow, mechanical forces, and multi-organ interactions in highly controlled environments [122]. These microengineered devices can integrate multiple human cell types, such as intestinal epithelial cells, endothelial cells, and immune cells, to rebuild tissue connections that function and interact as they do in the human body [123]. By mimicking natural processes such as muscle contractions in the gut, fluid flow, and the movement of nutrients or allergens, OoC platforms offer a more realistic representation of human organ function than traditional static cultures [124]. They can also be designed to connect multiple organ systems, termed multi-organ-on-a-chip, enabling researchers to model the gut-immune or gut-lung axis, both of which play roles in allergic disease progression [125, 126]. OoC systems use human cells, therefore, they avoid species differences that limit animal models, and improve translational relevance [127]. Gut-on-chip technologies enable dynamic interactions among gut cells, immune cells, and microorganisms by integrating microfluidics to replicate the gut environment [128]. Incorporating compartmentalized immune cells into the gut and skin-on-a-chip has the potential to is replicate the effects of food allergens on the gut-immune-skin axis, which could significantly improve the assessment of food allergen sensitization [4]. Various commercial OoC models have been introduced, including the 3-Lane OrganoPlate

(MIMETAS), MOTiF biochips, and HUMIMIC Chip3plus (TissUse GmbH) [4].

Despite these strengths, OOC systems are complex to design, operate, and maintain; as well as high cost in building and operating, which can be a barrier for large-scale studies [129]. Moreover, current designs for FA modeling are still under development and there is limited standardization across platforms [122, 129]. Most current OoC studies have focused on drug toxicity, infections, and cancer, with relatively few studies on allergic disorders [130–132].

Biopsy-based Tissue Models in FA Research

Biopsy-based tissue models in FA research use freshly obtained human intestinal or skin biopsies to study the allergic responses in an ex vivo setting [79]. These models preserve the native structure, cell diversity, and resident immune cell populations of the samples, allowing direct observation of how allergens affect barrier function, trigger cytokine release, and activate immune pathways [133]. They originate from human donor tissue, therefore, they reflect each person's unique genetics, microbiota composition, and prior immune exposure, showing a high level of physiological relevance compared to animal models [134]. They are particularly useful for mechanitic studies, allergen validation, and therapeutic intervention tests under realistic conditions [133, 135]. However, their use is limited by the availability of fresh biopsies, short lifespan of tissues outside the body, and differences between donors, which can make interpretation of results difficult [136]. Biopsies require informed consent and involve invasive procedures, further restrict their application [137]. Despite these limitations, biopsy-based models remain a valuable link between in vitro assays and in vivo studies to advance FA research [133]. In the study by Lam et al. (2015), a murine model of shrimp tropomyosin hypersensitivity was established through oral sensitization and challenge, and small intestinal tissues from these mice exhibited increased eosinophils, mast cells, goblet cells, elevated Th2 cytokine mRNAs, and epithelial apoptosis, demonstrating intestinal allergic changes in vivo [62]. Currently, there is limited application of biopsy-based tissue models in SA research, with only a few studies exploring ex vivo responses to shrimp allergens.

Precision-cut Organ Slices in FA Research

Precision-cut organ slices (PCOS) are thin, uniformly cut sections of tissues such as the lung, intestine, or liver that maintain their native 3D structure, mixed cell types, and functional signaling networks [138]. In FA research, PCOS makes it possible to study tissue reactions to allergens outside the body while preserving the natural arrangement of epithelial cells, immune cells, and supporting structures [139]. These models enable real-time measurements of allergen effect on barrier strength, trigger the release of signaling molecules, cause muscle contractions, and activate immune cells [139]. Additionally, they are derived from either human or animal tissues, these slices can reflect species-specific traits or the unique biology of individual patients, making them useful for both basic research and applied studies [140, 141]. They are particularly suited for studying localized allergic responses and tissue-specific effects of food allergens [139]. However, their lifespan outside the body is short, and obtaining fresh tissue samples can be challenging [79]. In addition, most PCOS applications have focused on respiratory infectious diseases, lung transplantation research, liver fibrosis and cirrhosis, and there are few or no published studies specifically applying this model to FAs, especially shrimp allergens [142–144]. In a study by Ren et al. (2023), precision-cut intestinal slices (PCIS) have been passively sensitized ex vivo with plasma from peanut-allergic patients and from the same donors after oral immunotherapy (OIT) to model tissue-level effector responses [145]. Upon allergen challenge, PCIS sensitized with allergic plasma displayed robust, allergen-specific smooth muscle contractions, whereas slices sensitized with post-OIT plasma exhibited markedly reduced responses, illustrating the model's ability to distinguish between allergic and desensitized states in human gut tissue [145]. Despite these constraints, PCOS provides a valuable intermediate tool between simple cell culture and animal models, offering a practical way to investigate FA mechanisms [139].

Currently, there are limited data on organoids in SA research. However, extensive studies have elucidated the mechanism of action of FAs using organoid models. Organoids have also been used to study the interplay between the gut microbiota and nutritional elements [96–98], which can interfere with SA tolerance. The co-culture of gut organoids with immune cells facilitates the understanding of how allergens penetrate the intestinal barrier, elicit immunological responses, and contribute to allergic inflammation [100, 101]. Numerous studies have advocated a relationship between gut barrier dysfunction and the increased prevalence of many chronic diseases, including FA [102, 103]. Environmental factors, lifestyle, industrialization, and urbanization affect the epithelial barrier of the skin, respiratory tract, and intestinal tract. This leads to microbial dysregulation, bacterial translocation, and tissue microinflammation, which are prone to the development of allergic diseases [103, 104]. Food emulsifiers such as polysorbate 20 (P20) and polysorbate (P80) in bread, ice cream, confectionery, beverages, and dressings are associated with gut barrier dysfunction. P20 and P80 were incubated with iPSC-derived human IO, colon

organoid OoC, and liquid-liquid interface cells. P20 and P80 significantly decreased transepithelial electrical resistance, disrupted barrier integrity, and induced irregular tight junction expression in both organoid models. RNA sequencing of the transcriptome and targeted proteomic analyses have shown increased cell proliferation, apoptosis and inflammatory responses [117].

Conclusion

In conclusion, the advancement of molecular techniques holds great promise for unraveling the complexity of SA. While multiple shrimp allergens contribute to the allergic mechanism, significant variations in sensitization profiles exist across different populations. The pathogenesis is complicated and integrated by crosstalks between epithelial-neuronal-immune cells, with the effects of probiotics.Unmet needs remain for clinical diagnosis and treatment options. With the rapid development of technology, the in vivo and ex vivo models offer immense potential for SA research, elucidating the underlying mechanisms and facilitating the development of treatment strategy.

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Declarations

Competing interests The authors declare no competing interests.

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