Review / *Derleme*

DOI: 10.5578/ced.20250408 • *J Pediatr Inf 2025;19(4):e267-e277*

Residual Ovalbumin in Human Vaccines: A Comprehensive Review of Immunogenic Risk, Regulatory Gaps, and Allergen-Free Strategies

İnsan Aşılarında Kalıntı Ovalbumin: İmmünojenik Risk, Düzenleyici Boşluklar ve Alerjen İçermeyen Stratejilerin Kapsamlı Bir Değerlendirmesi

Ayşegül Bülbül[1,3](İD), Ateş Kara[2,3](İD)

[1] *Department of Biochemistry, Hacettepe University Faculty of Science, Ankara, Türkiye*
[2] *Department of Pediatrics, Hacettepe University Faculty of Medicine, Ankara, Türkiye*
[3] *Turkish Vaccine Institute, Presidency of Turkish Health Institutes, Ankara, Türkiye*

Cite this article as: Bülbül A, Kara A. Residual ovalbumin in human vaccines: A comprehensive review of immunogenic risk, regulatory gaps, and allergen-free strategies. J Pediatr Inf 2025;19(4):e267-e277.

Abstract

Ovalbumin (OVA), a structurally complex and immunologically potent glycoprotein, is the predominant egg white protein and a widely used model allergen in immunological research. Despite its value in experimental allergy models, OVA also represents a clinical concern due to its residual presence in vaccines produced using embryonated hen eggs. Trace amounts of OVA can trigger IgE-mediated hypersensitivity reactions in sensitized individuals, raising critical questions about vaccine safety, labeling consistency, and risk communication. This review presents a comprehensive analysis of OVA from molecular, clinical, and regulatory perspectives. We explore the structural determinants of its allergenicity, its persistence across vaccine production pipelines, and the analytical challenges in detecting residual levels. Furthermore, we assess emerging allergen-reduction strategies, including advanced processing methods, recombinant vaccine platforms, and epitope-masking technologies. Alternative production systems such as cell-culture and mRNA-based vaccines are also evaluated in terms of their potential to eliminate OVA exposure entirely. By integrating data from structural biology, clinical immunology, and public health policy, this review highlights both the translational risks posed by residual OVA and the innovations that may mitigate them. The findings support a shift toward precision-based vaccine design and a redefinition of allergen management strategies in global immunization programs.

Keywords: Ovalbumin, allergy risk, vaccine safety, regulatory gaps, allergen-free vaccines

Öz

Ovalbümin (OVA), yapısal olarak karmaşık ve immünolojik açıdan güçlü bir glikoprotein olup, yumurta akı proteinlerinin baskın olanıdır ve immünoloji araştırmalarında yaygın olarak kullanılan bir model alerjendir. Deneysel alerji modellerinde değerli bir yere sahip olmasına rağmen, embriyolu tavuk yumurtaları kullanılarak üretilen aşılarda artık olarak bulunması nedeniyle klinik açıdan da bir endişe kaynağıdır. İz miktardaki OVA, duyarlı bireylerde IgE aracılı aşırı duyarlılık reaksiyonlarını tetikleyebilir ve bu durum aşı güvenliği, etiketleme tutarlılığı ve risk iletişimi gibi konularda önemli soruları gündeme getirmektedir. Bu derleme, OVA'yı moleküler, klinik ve düzenleyici bakış açılarından kapsamlı şekilde analiz etmektedir. Alerjenitesine katkıda bulunan yapısal belirleyicileri, aşı üretim süreçleri boyunca kalıcılığını ve artık düzeylerin tespiti konusundaki analitik zorlukları inceliyoruz. Ayrıca, gelişmiş işleme yöntemleri, rekombinant aşı platformları ve epitop-maskeleme teknolojileri gibi ortaya çıkan alerjen azaltma stratejilerini değerlendiriyoruz. Hücre kültürü ve mRNA tabanlı aşılar gibi alternatif üretim sistemleri de OVA maruziyetini tamamen ortadan kaldırma potansiyelleri açısından değerlendirilmektedir. Yapısal biyoloji, klinik immünoloji ve halk sağlığı politikalarından elde edilen verileri entegre ederek, bu derleme artık OVA'nın oluşturduğu çevirimsel riskleri ve bu riskleri azaltabilecek yenilikçi yaklaşımları vurgulamaktadır. Bulgular, hassasiyete dayalı aşı tasarımına geçişi ve küresel bağışıklama programlarında alerjen yönetim stratejilerinin yeniden tanımlanmasını desteklemektedir.

Anahtar Kelimeler: Ovalbümin, alerji riski, aşı güvenliği, regülasyon eksiklikleri, alerjen içermeyen aşılar

Correspondence Address/Yazışma Adresi

Ayşegül Bülbül

Department of Biochemistry,
Hacettepe University Faculty of Science
Ankara, Türkiye

E-mail: aysegul_bulbul@hacettepe.edu.tr

Received: 21.05.2025 **Accepted:** 06.09.2025
Available Online Date: 25.12.2025

Introduction

Among food-derived allergens, ovalbumin (OVA) emerges as one of the most structurally complex and immunologically potent glycoproteins. With a molecular weight of approximately 45 kilodaltons and an isoelectric point (pI) ranging from 4.5 to 4.9, OVA exhibits high solubility under physiological conditions and strong binding affinity to immune receptors features that underpin its long-standing use as a model antigen in allergy and immunology research (1). Its combination of conformational and linear IgE-binding epitopes enables robust activation of Th2-skewed immune responses, including mast cell degranulation, eosinophilia, and elevated IgE titers (2). Beyond its experimental utility, OVA has clinical relevance due to its inadvertent presence in vaccines manufactured using embryonated hen eggs. Although purification methods substantially reduce egg protein content, trace levels often in the nanogram range may remain. Clinical evidence suggests that these low-level residues are generally well tolerated, even in individuals with severe egg allergy, including those with a history of anaphylaxis (3). However, the residual presence of OVA, despite being minimal, remains a subject of clinical scrutiny due to its theoretical potential to trigger IgE-mediated hypersensitivity in highly sensitized individuals. Recent evaluations also suggest that immunogenic risk cannot be entirely excluded, particularly in the context of inconsistent labeling and the absence of universally accepted thresholds for residual OVA in inactivated egg-based vaccines (4). While international consensus reports, such as that by Dreskin et al. indicate that live attenuated vaccines like measles and mumps, which are produced using chick embryo fibroblasts, contain only picogram quantities of residual egg protein levels considered clinically insignificant even for highly sensitized individuals a universally accepted threshold for OVA content across all vaccine platforms, particularly inactivated egg-based vaccines, remains undefined. Inconsistent labeling further complicates clinical guidance for egg-allergic individuals (5). Adding to this, recent studies highlight how environmental and dietary co-factors can amplify or attenuate OVA-triggered immune responses. Notably, aflatoxin B1, a common foodborne mycotoxin, has been shown to exacerbate OVA-induced allergic sensitization in BALB/c mice via activation of the TSLP-IL-33 axis (6). Conversely, fructooligosaccharides (FOS) may dampen allergic inflammation by enhancing oral tolerance through modulation of the gut microbiota (7). Collectively, these findings underscore the dual role of OVA as both a valuable scientific model and a potential immunological hazard in modern vaccine platforms. The convergence of trace exposure, individual sensitivity, and inconsistent regulatory thresholds reveals a critical gap in vaccine safety frameworks. This review integrates current insights into OVA's biochemical profile, clinical relevance, and regulatory implications, aiming to expose unresolved challenges and inform future strategies for allergen-free vaccine production.

Ovalbumin: Structure and Immune Response

Immunodominant Epitopes and Allergenicity

OVA exhibits potent immunogenicity due to its structurally complex tertiary architecture, which displays both linear and conformational IgE-binding epitopes on its globular surface. These epitopes are predominantly surface-exposed and exhibit resistance to gastrointestinal degradation, allowing sustained immune recognition even after food processing. Structural and immunochemical analyses have demonstrated that these epitopes bind with high affinity to FcεRI receptors on mast cells and basophils, facilitating crosslinking and initiating rapid degranulation characteristic of type I hypersensitivity reactions (8). In murine models, oral administration of OVA consistently induces hallmark features of Th2-driven allergic responses, including elevated serum histamine, OVA-specific IgE, murine mast cell protease-1, and Th2 cytokines such as IL-4, IL-5, and IL-13. These responses are further accompanied by mucosal mast cell infiltration and epithelial inflammation (9). Beyond immune interaction, the allergenic potential of OVA is modulated by its biophysical properties namely, thermal stability, aggregation propensity, and resistance to proteolysis. While heating can disrupt conformational epitopes, partial denaturation and enzymatic digestion may paradoxically expose cryptic IgE-binding sites, thereby enhancing immunogenicity under certain conditions (10). Crystallographic studies have identified a stabilized β-barrel core and glycosylated surface residues that contribute to the preservation of epitope conformation, even during processing (11). Furthermore, exposure to protease-activated epithelial environments such as those triggered by environmental co-allergens like house dust mite extract has been shown to increase epithelial permeability and facilitate enhanced epitope presentation, exacerbating allergic responses even at trace OVA concentrations (12).

Antigen Presentation and Th2 Polarization

Following mucosal entry, OVA is rapidly taken up by antigen-presenting cells (APCs), predominantly dendritic cells. These cells process OVA and present its derived peptides via MHC class II complexes to naïve $CD4^+$ T cells in the regional lymph nodes, initiating antigen-specific adaptive immunity (13). This interaction is a key driver of Th2 polarization, a central mechanism in allergic sensitization. The resulting Th2 effector cells secrete a characteristic cytokine profile, including IL-4, IL-5, and IL-13, which collectively promote IgE class switching, eosinophilic infiltration, and mucus hypersecretion, particularly in mucosal tissues such as the lungs and gut (14). Crucially, the quality of antigen presentation, not only antigen load but also

costimulatory signaling and cytokine environment, shapes the intensity and direction of the immune response. In vitro studies using human monocyte-derived dendritic cells have demonstrated that OVA exposure increases expression of key costimulatory molecules such as CD86 and OX40L, thereby enhancing Th2 polarization through secondary signaling pathways (15). In vivo murine models have further revealed that the route of OVA administration significantly influences immune outcomes. Intranasal delivery induces pronounced pulmonary eosinophilia and airway remodeling, whereas oral administration results in gastrointestinal Th2 responses marked by epithelial barrier dysfunction and localized inflammation (16). Recent findings suggest that environmental co-factors such as diesel exhaust particles or protease-rich allergens can synergize with OVA to amplify allergic responses. These co-exposures activate epithelial stress pathways, increasing the release of cytokines like IL-25, IL-33, and thymic stromal lymphopoietin, which enhance Th2 stabilization and tissue-level inflammation (17). Taken together, these observations delineate a robust antigen-driven cascade that underlies Th2 polarization and IgE-mediated hypersensitivity, schematically summarized in Figure 1.

Mucosal and Systemic Immune Effects

Beyond localized immune responses, OVA induces widespread immunopathological effects across mucosal barriers, culminating in systemic hypersensitivity. Repeated exposure to OVA disrupts epithelial homeostasis, promotes leukocyte infiltration, and impairs barrier integrity in both respiratory and gastrointestinal tissues.

In murine models of food allergy, oral administration of OVA has been shown to cause marked intestinal villus blunting, goblet cell hyperplasia, and a significant downregulation of tight junction proteins hallmarks of mucosal barrier dysfunction (18). These structural alterations coincide with elevated levels of IL-4 and IL-13 and increased circulating OVA-specific IgE, indicative of systemic Th2 sensitization. Respiratory models complement these findings. Inhalational or intranasal exposure to OVA provokes hallmark features of allergic asthma, including pulmonary eosinophilia, excessive mucus secretion, and bronchial wall remodeling (19). Clinically, elevated egg white-specific IgE which encompasses reactivity to OVA has been linked to persistent allergic phenotypes and a greater overall allergic disease burden in children,

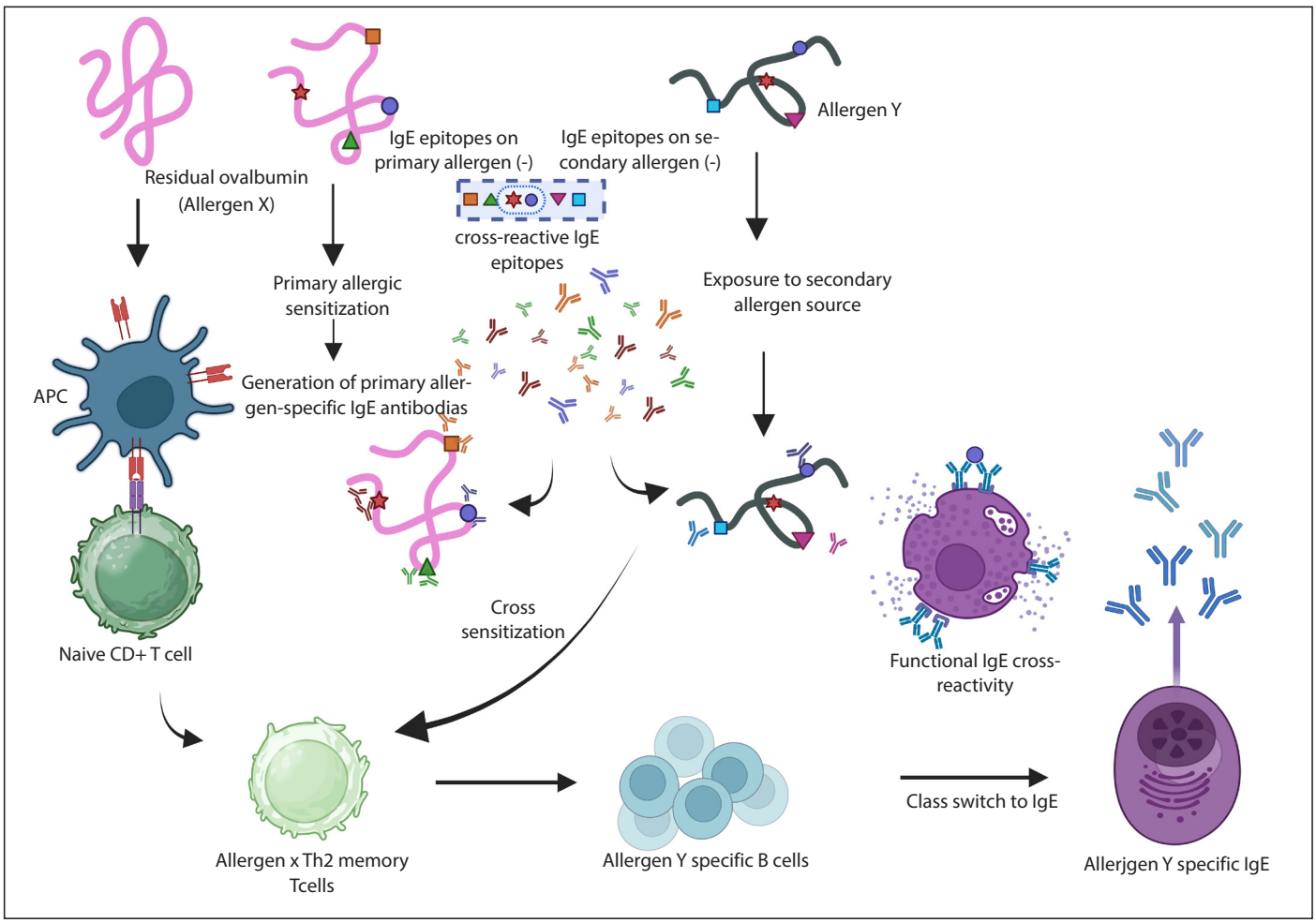

Figure 1. Immunological mechanism of ovalbumin-induced Th2 sensitization and IgE-mediated cross-reactivity (created by the author using BioRender).

indicating that sensitization to dietary antigens such as OVA may contribute to systemic immune activation that primes the airways (20). Emerging evidence highlights the role of the gut-lung axis in orchestrating compartmentalized immune responses to OVA. Probiotic-based interventions particularly with *Lactobacillus rhamnosus* have demonstrated dual-site efficacy: Reducing gut inflammation while concurrently mitigating airway hyperresponsiveness (21).

Immunomodulation by Nutrients and Bioactives

The allergenic potential of OVA is not fixed, but dynamically shaped by physicochemical and dietary interventions that alter its structural conformation and epitope accessibility. One well-characterized example is chitosan a biocompatible polysaccharide derived from crustacean exoskeletons which has been shown to stabilize OVA fibrils under alkaline conditions. This conformational stabilization mitigates aggregation and preserves native-like structure, thereby reducing in vitro IgE-binding affinity (22). Similarly, selective partial unfolding through gamma irradiation has been demonstrated to enhance the functional properties of OVA, such as foaming and emulsification, while simultaneously decreasing its immunogenicity. This controlled denaturation exposes non-allergenic regions and disrupts conformational epitopes, representing a promising strategy for hypoallergenic formulation design (23). High-pressure homogenization further supports this concept, as it has been shown to disrupt OVA's tertiary structure and conceal immunoreactive domains, thereby lowering IgE-binding potential (24). Emerging non-thermal processing techniques such as pulsed electric fields and high-intensity ultrasound offer scalable alternatives that modulate protein conformation without compromising structural integrity or nutritional value. These technologies preserve OVA's functional capacity while attenuating allergenicity, making them attractive candidates for industrial application (25). From a nutritional standpoint, naturally occurring polyphenols such as catechins and anthocyanins engage in non-covalent interactions with surface-exposed residues on OVA, effectively masking key epitopes and diminishing IgE reactivity (26). Furthermore, ionic strength modulation has been implicated as a key determinant of OVA's immunological profile. Exposure to monovalent (Na^+) and divalent (Mg^{2+}) ions alters protein surface hydrophobicity and aggregation dynamics, which in turn may affect epitope accessibility and immunogenic potential (27).

Ovalbumin in Egg-Based Vaccine Manufacturing

Egg-based vaccine production remains the predominant global platform for manufacturing influenza, measles, mumps, rubella (MMR), and yellow fever vaccines due to its scalability, cost-efficiency, and reliable viral yield (28). However, the use of embryonated chicken eggs inherently introduces trace amounts of egg proteins chiefly OVA into the final formulations. OVA, the most abundant protein in egg white, possesses well-characterized IgE-binding properties and has been implicated in hypersensitivity reactions among sensitized individuals. An overview of residual ovalbumin's origin, immunogenicity, and mitigation approaches is summarized in Figure 2.

Although purification procedures are implemented to reduce OVA content, studies report that licensed vaccines may retain residual OVA levels ranging from 0.1 to 1.0 µg per dose, depending on the manufacturer and production process (29). This variability underscores the importance of rigorous quantification methods and transparent labeling, especially for egg-allergic individuals who may react to even minimal exposures. Analytical methods for OVA detection differ in sensitivity and resolution. Enzyme-linked immunosorbent assay (ELISA) remains widely used for routine batch testing due to its cost-effectiveness and practicality, but it may underestimate low-level or structurally masked epitopes. In contrast, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) provides higher resolution and batch-to-batch consistency, particularly in highly purified vaccine preparations (30). Despite theoretical safety concerns, real-world clinical data remain reassuring. In a multicenter study involving 58 pediatric patients with confirmed egg allergy, yellow fever vaccines containing residual OVA were administered safely without requiring premedication or desensitization protocols (31). Similarly, MMR vaccines owing to the use of recombinant stabilizers and minimal egg protein content have been shown to be well tolerated in egg-allergic individuals (32). These findings are further corroborated by broader population studies. For instance, concurrent administration of COVID-19 and influenza vaccines in egg-allergic patients resulted in no serious allergic reactions, reinforcing the low anaphylaxis risk when OVA content remains under control (33). As a result, current Centers for Disease Control and Prevention and the Advisory Committee on Immunization Practices (ACIP) recommendations no longer classify egg allergy as a contraindication for influenza vaccination, provided that residual OVA levels remain below 1 µg per dose (34). In pediatric vaccination programs, maintaining clarity regarding vaccine composition is vital for fostering caregiver trust and enabling informed clinical decisions. From a biochemical perspective, OVA is considered less allergenic than ovomucoid (Gal d 1) due to its lower glycosylation, diminished thermal stability, and greater susceptibility to gastrointestinal digestion. These properties collectively contribute to a milder allergic profile though severe reactions may still occur in highly sensitized hosts (35). Allergy counseling plays a crucial role in vaccine acceptance, particularly within pediatric settings. Structured pre-vaccination risk assessments and open communication

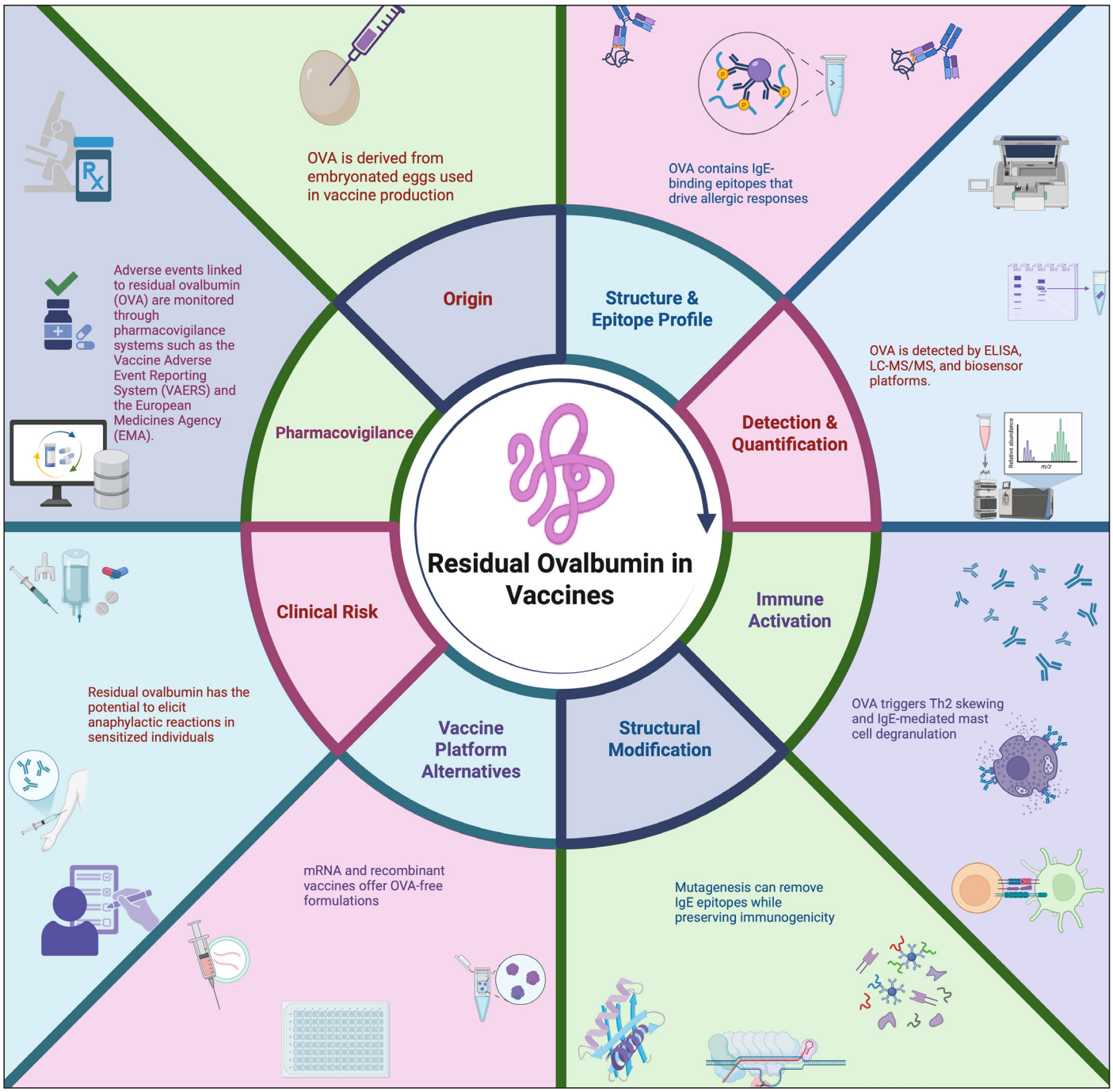

Figure 2. Graphical abstract illustrating the origin, immunogenicity, detection, and mitigation strategies of residual ovalbumin in egg-based vaccines (created by the author using BioRender).

with caregivers have been shown to significantly enhance confidence among egg-allergic families (36). Moreover, pharmacovigilance systems such as the VAERS continue to monitor rare adverse events. Current surveillance data confirm that severe OVA-induced anaphylaxis remains exceedingly rare and is often confounded by coexisting allergic conditions (37). Advances in vaccine manufacturing platforms further mitigate this risk. Cell-based expression systems (e.g., MDCK and Vero lines) and recombinant technologies (e.g., FluBlok) now enable the production of completely OVA-free vaccines. These approaches enhance both allergenic safety and responsiveness to antigenic shifts. However, economic and infrastructural considerations continue to support the dominance of egg-based systems in many low- and middle-income countries (38).

Detection and Quantification of Residual Ovalbumin in Vaccine Products

Accurate quantification of residual OVA in egg-based vaccines remains a critical component of immunization safety, particularly for individuals with confirmed IgE-mediated egg allergy. Recognizing this, regulatory bodies such as the U.S. Food and Drug Administration and the EMA have underscored the necessity of validated analytical methods in vaccine quality control systems, especially for trace-level allergens like OVA (39). Among currently employed assays, ELISA remains the most widely utilized method, favored for its operational simplicity, affordability, and adaptability to high-throughput batch testing. However, ELISA is subject to several limitations including matrix interference, thermal and enzymatic epitope alteration, and conformational masking which may result in underestimation of allergenic potential in processed formulations (40). To overcome these constraints, LC-MS/MS has emerged as a preferred technique, offering enhanced specificity and detection precision in complex vaccine matrices (41). The incorporation of isotopologue-labeled internal standards now enables quantification of OVA down to the nanogram-per-milliliter range. Importantly, clinical studies have demonstrated that residual OVA concentrations within this analytical range spanning from approximately 30 ng/mL to 1 µg/mL per dose are generally well tolerated, even in egg-allergic individuals with prior anaphylaxis, without provoking systemic reactions (42-44). These findings indicate that while ultra-sensitive detection improves analytical reliability, the clinical risk at such concentrations remains low. Additionally, chromatographic enrichment steps are now routinely used to reduce matrix-derived background noise, significantly enhancing the signal-to-noise ratio in low-abundance analyte detection (45). Beyond centralized laboratories, next-generation biosensing technologies are expanding the frontiers of decentralized allergen monitoring. Aptamer-based fluorescent biosensors, for instance, have shown exceptional selectivity for OVA, with stable performance under thermally or chemically denatured conditions (46). In parallel, epitope-imprinted polymeric electrochemical sensors have achieved detection limits below 1 ng/mL while maintaining minimal cross-reactivity (47). Colorimetric nanoparticle-based sensors, leveraging plasmonic signal shifts, also enable rapid visual detection of OVA without requiring sophisticated instrumentation (48). Spectroscopic methods, particularly surface-enhanced Raman spectroscopy, have gained attention for their multiplexing capability and ultrasensitive detection of allergens in heterogeneous matrices (49). A critical emerging focus involves the interplay between OVA's structural conformation and its immunoreactivity. Controlled unfolding through heat and pH modulation has been shown to selectively expose or shield IgE-binding epitopes, thereby modulating both allergenic potential and detection efficiency (50). Similarly, disruption of OVA's tertiary structure alters epitope accessibility, reducing mast cell activation and improving hypoallergenicity (51). These findings suggest that allergen detection protocols must evolve beyond protein quantification to integrate structural analysis. Importantly, recombinant and native forms of OVA exhibit distinct immunogenic profiles, complicating assay standardization and result interpretation. Hence, analytical platforms must select appropriate reference standards that reflect clinically relevant conformational states (52). In light of these complexities, regulatory frameworks must shift from simple mass-based thresholds to functionally informed criteria grounded in structural immunogenicity. Accordingly, hybrid workflows combining immunoaffinity enrichment with LC-MS are increasingly adopted in reference laboratories to provide both sensitivity and specificity for trace allergens (53). Nevertheless, analytical sensitivity alone does not equate to clinical safety. Detection results must be interpreted within the context of patient-level immune risk. Sensitivity thresholds should be aligned with real-world outcomes, such as symptom severity and sensitization profiles, and harmonized with current regulatory standards, including the EMA's ≤1 µg/dose limit for influenza vaccines and ACIP recommendations supporting the safety of vaccines within this range for egg-allergic individuals (54,55). Future advancements will likely incorporate real-time allergen quantification and stratified risk algorithms, paving the way for a new era of precision allergen analytics in vaccine safety evaluation (56).

Clinical Risk and Population-Based Guidelines on Ovalbumin Residues

Although residual OVA in vaccines typically exists at nanogram-to-microgram concentrations, robust clinical evidence continues to support their safety, including in individuals with documented IgE-mediated egg allergy. In a large multicenter cohort study involving 830 egg-allergic patients, Gagnon et al. demonstrated that trivalent influenza vaccines containing trace amounts of OVA were safely administered without prior skin testing, dose adjustment, or desensitization protocols, and no significant hypersensitivity reactions were reported (57). These findings reinforce the notion that, under proper clinical supervision, OVA-containing vaccines pose minimal immunologic risk even in sensitized populations. At the molecular level, the immunogenicity of OVA is closely linked to its structural features. Claude et al. reported that thermal aggregation of OVA reduces IgE-binding capacity and mast cell degranulation in murine models, indicating that physical denaturation may be leveraged to attenuate allergenicity (58). Complementing this, Cao et al. developed glycosylation-modified vaccine antigens that selectively mask IgE epitopes while preserving T-cell immunogenicity, demonstrating potential as a

platform for tolerance induction in high-risk individuals. Together, these mechanistic and clinical insights support a paradigm shift in vaccine design from passive avoidance of allergenic components toward precision-based modulation of immunogenicity. In this context, OVA serves not only as a residual impurity, but as a model antigen for translational innovation in allergen management and risk stratification strategies (59).

Alternative Technologies for Ovalbumin-Free Vaccine Manufacturing

The continued reliance on embryonated hen eggs for vaccine production inevitably introduces residual OVA into final formulations. Although typically present in trace amounts, this protein poses a theoretical risk to individuals with IgE-mediated egg allergy, particularly in pediatric and high-risk populations. As a result, this longstanding manufacturing model has catalyzed a global push toward OVA-free vaccine platforms that combine safety, scalability, and adaptability (60).

Among the earliest and most clinically validated alternatives are recombinant protein vaccines, especially those developed using the baculovirus expression vector system (BEVS). Baxter et al. demonstrated that FluBlok, a trivalent recombinant hemagglutinin influenza vaccine produced in insect cells, achieves high antigen purity and complete elimination of egg proteins, showing favorable tolerability even in egg-sensitized individuals (61). Similarly, cell-based vaccine platforms utilizing MDCK or Vero cells have gained prominence for producing both seasonal influenza and pandemic-response vaccines. These systems allow for enhanced batch consistency, faster production timelines, and better adaptation to antigenic drift compared to egg-based platforms (62). The most transformative advance, however, has been the widespread deployment of mRNA vaccine technologies, as exemplified by Comirnaty and Spikevax. These vaccines deliver lipid nanoparticle-encapsulated mRNA to host cells, enabling in vivo antigen expression without introducing any egg-derived proteins. Anderson et al. demonstrated that mRNA vaccines elicit robust immunogenicity and favorable safety profiles, even in recipients with allergic predispositions (63). Bordry et al. further confirmed durable humoral responses with minimal hypersensitivity reactions across demographically diverse cohorts (64). Beyond these major platforms, novel antigen delivery strategies are being actively explored. Park et al. demonstrated that multivalent nanoparticles functionalized with OVA could bias the immune response toward a Th1 phenotype, effectively minimizing IgE involvement and promoting a tolerance-oriented immunogenic profile (65). From a real-world safety perspective, post-marketing surveillance has reinforced these findings. Woo et al. analyzed population-level safety data from recombinant influenza vaccines and reported minimal allergic adverse events, supporting the practical utility of these platforms in allergy-vulnerable populations (66). These results are aligned with policy recommendations from the World Health Organization, which emphasizes continued investment in recombinant and cell-derived vaccine infrastructure to reduce global dependency on egg-based production especially in the context of future pandemic preparedness and allergen-sensitive populations (67).

Redesigning Ovalbumin Immunogenicity: Translational Pathways and Precision Strategies

Vaccine development is undergoing a strategic shift from passive allergen elimination toward active immunological reengineering in response to residual OVA concerns. Once considered an unavoidable contaminant in egg-based vaccines, OVA is now recognized as a modulable antigen with novel potential enabled by nanotechnology, immunology, and synthetic biology. A cornerstone of this innovation is the use of OVA-functionalized nanomaterials designed to dissociate allergenicity from immunogenicity. McCright et al. demonstrated that polymeric nanocarriers conjugated with OVA can reprogram dendritic cell activation, promoting Th1-skewed responses and suppressing IgE-mediated reactivity in murine models (68). Complementing this, dietary immunomodulators such as narirutin and FOS have been shown to reinforce gut epithelial barriers and downregulate Th2 cytokine responses, thereby facilitating oral tolerance induction in food allergy models (69). Similarly, microbiota-targeted strategies notably *L. rhamnosus* supplementation have been found to suppress IL-4 and IL-5 secretion, reduce eosinophil infiltration, and restore regulatory T cell activity in models of OVA-induced airway hypersensitivity, confirming their utility in respiratory allergy mitigation (70). At the antigenic level, epitope engineering has enabled the rational design of hypoallergenic OVA variants. Dona and Suphioglu illustrated that site-directed mutagenesis can effectively silence IgE-binding motifs while preserving T-cell immunogenicity, laying the groundwork for next-generation hypoallergenic vaccine formulations (71). Meanwhile, advances in computational immunology are enabling preclinical allergenicity prediction through machine learning and epitope scanning arrays. Grewal et al. proposed an integrative algorithm combining bioinformatics tools and curated allergen databases to pinpoint allergenic hotspots and stratify antigenic candidates for early-stage evaluation (72). In formulation science, injectable controlled-release platforms such as in situ–crosslinked hydrogels offer kinetic control of antigen delivery. Lee et al. reported that hyaluronic acid-based hydrogels loaded with OVA significantly attenuated allergic responses in murine models of allergic rhinitis by reducing serum IgE, Th2 cytokines, and

eosinophilic inflammation (73). These findings underscore the utility of physiochemically tuned antigen delivery systems in modulating immune response kinetics and improving safety profiles. Despite these innovations, real-world data reaffirm that trace OVA exposure in vaccines, when managed under clinical protocols, presents negligible risk to sensitized individuals. In a cohort study, Kim et al. found that even among patients with confirmed egg allergy, adverse reactions following influenza vaccination were rare and generally mild, with severe outcomes being both predictable and preventable through appropriate pre-screening and observation (74).

Discussion

This review has comprehensively examined OVA as a paradigmatic residual allergen in egg-based vaccine formulations, illustrating its far-reaching implications across immunology, vaccine production, and public health regulation. Historically considered a trace contaminant, OVA is increasingly recognized as a molecular indicator of broader challenges in allergen quantification, vaccine safety analytics, and precision immunoprophylaxis. The dual nature of OVA as both a clinically relevant allergen and a structurally informative model protein offers a unique vantage point for understanding the intersection of biopharmaceutical processing and immunological reactivity. From the evidence reviewed, it is clear that allergenicity is not a unidimensional function of protein concentration but emerges from complex, multiscale interactions involving epitope structure, glycosylation state, matrix context, and host-specific immune predisposition.

Technological advances have yielded new opportunities to manage this risk more precisely. Mass spectrometry, epitope-imprinting, and aptamer biosensing now allow for high-resolution detection at thresholds meaningful for patient safety. In parallel, recombinant and mRNA-based platforms demonstrate that OVA-free vaccine production is not only feasible but scalable, particularly critical for allergen-vulnerable populations and pandemic preparedness. A particularly compelling direction emerging from current research is the concept of immunological reengineering. Instead of focusing solely on the elimination of OVA, recent strategies such as epitope silencing, conformational masking, and immunomodulatory conjugation demonstrate that antigens can be structurally modified to retain immunogenic efficacy while significantly reducing hypersensitivity risks. These methods signal a shift from reactive allergen removal to proactive immunotolerance design a transition from threshold-based risk to systems-based immunological literacy. Regulatory frameworks must keep pace with these innovations. Labeling precision, batch-level disclosure, and harmonized detection standards remain variably implemented across jurisdictions. Integrating structural allergenicity metrics into regulatory pathways and aligning these with real-world clinical outcomes will be key to future progress. In essence, OVA is no longer just a residual concern; it is a lens through which the future of allergen-safe, structurally informed, and patient-centric vaccinology can be envisioned. Through molecular scrutiny, translational insight, and regulatory refinement, the trajectory of vaccine safety can be redirected from precautionary avoidance to intelligent integration.

Conclusion

OVA, long utilized as a model allergen in immunological research, has transitioned from a passive trace component in egg-based vaccines to a molecular fulcrum in the evolving discourse on vaccine safety and precision immunology. Despite being present only in residual quantities, its capacity to provoke IgE-mediated hypersensitivity in sensitized individuals positions it as both a scientific tool and a regulatory challenge. This review demonstrates that allergenicity is not governed by concentration alone, but by a constellation of factors including molecular structure, glycosylation status, epitope accessibility, host immune context, and formulation variables. These insights underscore the limitations of static safety thresholds and argue for structure-function-informed immunogenic risk assessments. Technological innovation is rapidly transforming the vaccine landscape. Recombinant, cell-culture, and mRNA-based platforms now provide egg-independent solutions, enabling OVA-free formulations that enhance safety while offering scalability and pandemic responsiveness. In parallel, analytical advances such as LC-MS/MS, immunoaffinity enrichment, and aptamer-based biosensors enable sensitive, reliable detection of trace allergens, strengthening quality assurance and risk communication. More progressively, the field is witnessing a paradigm shift: from allergen removal to immunoengineering. Epitope silencing, conformational masking, and computational allergenicity prediction have reframed OVA not as an inert residual, but as a modifiable antigen one that can be reprogrammed to preserve immune stimulation while minimizing allergenic risk. This positions OVA as a platform for testing broader strategies in precision vaccinology. Ultimately, the case of OVA serves as a compelling example of how residual proteins should not merely be eliminated but understood. A systems-level perspective integrating molecular immunology, analytical science, and regulatory policy is essential for future vaccine design. Rather than a contaminant, OVA becomes a paradigm: a molecular signal that urges us toward smarter, safer, and more transparent immunization strategies.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - All of authors; Design - All of authors; Supervision - AK; Resource - AK; Data Collection and/or processing - AB; Analysis and/or interpretation - All of authors; Literature search AB; Writing - AB; Critical review - AK.

Conflict of Interest: All authors declare that they have no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

References

1. *Takahashi H, Fujii T, Yamakawa S, Yamada T, Sato M, Watanabe T. Combined oral intake of short and long fructans attenuates ovalbumin-induced food allergy in mice. BMC Microbiol 2023;23:52. https://doi.org/10.1186/s12866-023-03021-6*
2. *Hou Y, Zheng S, Zou F, Wang D, Da H, Zhang S, et al. Lactobacillus rhamnosus 76 alleviates airway inflammation in ovalbumin-induced asthma model via Th2 cytokine inhibition. Immunobiology 2023;228(2):152712. https://doi.org/10.1016/j.imbio.2023.152712*
3. *Howe LE, Conlon AS, Greenhawt MJ, Sanders GM. Safe administration of seasonal influenza vaccine to children with egg allergy of all severities. Ann Allergy Asthma Immunol 2011;106(5):446-7. https://doi.org/10.1016/j.anai.2011.01.024*
4. *Gulani M, Arte T, Ferguson A, Pasupuleti D, Adediran E, Harsoda Y, et al. Recent advancements in non-invasive vaccination strategies. Vaccines (Basel) 2025;13(9):978. doi: 10.3390/vaccines13090978.*
5. *Dreskin SC, Halsey NA, Kelso JM, Wood RA, Hummell DS, Edwards KM, et al. International Consensus (ICON): allergic reactions to vaccines. World Allergy Organ J 2016;9:32. https://doi.org/10.1186/s40413-016-0120-5*
6. *Deng Y, Chen H, Wu Y, Yuan J, Shi Q, Chen L. Aflatoxin B1 can aggravate BALB/c mice allergy induced by ovalbumin via TSLP-IL-33 axis activation. Toxicon 2023;225:107121. https://doi.org/10.1016/j.toxicon.2023.107121*
7. *Yan X, Yan J, Xiang Q, Wang F, Dai H, Zhao L. Fructooligosaccharides protect against OVA-induced food allergy via promoting gut microbiota-mediated immune tolerance. Food Funct 2021;12:2915-26. https://doi.org/10.1039/D0FO03371E*
8. *Gieras A, Linhart B, Roux KH, Dutta M, Khodoun M, Badithe A, et al. IgE epitope proximity determines immune complex shape and effector cell activation capacity. J Allergy Clin Immunol 2016;137(5):1557-65. https://doi.org/10.1016/j.jaci.2015.08.055*
9. *Kim HI, Kim JK, Kim JY, Han MJ, Kim DH. Fermented red ginseng and ginsenoside Rd alleviate ovalbumin-induced allergic rhinitis in mice by suppressing IgE, interleukin-4, and interleukin-5. J Ginseng Res 2019;43(3):384-91. https://doi.org/10.1016/j.jgr.2019.02.006*
10. *Golias J, Schwarzer M, Wallner M, Kverka M, Kozakova H, Hrncir T. Heat-induced structural changes affect OVA-antigen processing and reduce allergic response in mouse model of food allergy. PLoS One 2012;7(5):e37156. https://doi.org/10.1371/journal.pone.0037156*
11. *Huntington JA, Stein PE. Structure and properties of ovalbumin. J Chromatogr B Biomed Sci Appl 2001;756(1-2):189-98. https://doi.org/10.1016/S0378-4347(01)00108-6*
12. *Jacquet A. Interactions of airway epithelium with protease allergens in the allergic response. Clin Exp Allergy 2011;41(3):305-13. https://doi.org/10.1111/j.1365-2222.2010.03661.x*
13. *Huang J, Zhang J, Wang X, Jin Z, Zhang P, Su H, et al. Effect of probiotics on respiratory tract allergic disease and gut microbiota. Front Nutr 2022;9:821900. https://doi.org/10.3389/fnut.2022.821900*
14. *Zuurveld M, Díaz CB, Redegeld FA, Folkerts G, Bruggeman RJ, Nijkamp FP, et al. An advanced in vitro human mucosal immune model to predict food sensitizing allergenicity risk: A proof of concept using ovalbumin as model allergen. Front Immunol 2023;13:1073034. https://doi.org/10.3389/fimmu.2022.1073034*
15. *Zuurveld M, Kiliaan PCJ, Van Grinsven SEL, Van Neerven RJJ, Garssen J, Knippels LMJ, et al. Ovalbumin-induced epithelial activation directs monocyte-derived dendritic cells to instruct type 2 inflammation in T cells. J Innate Immun 2023;15(1):222-36. https://doi.org/10.1159/000526528*
16. *Dunkin D, Berin MC, Mayer L. Allergic sensitization can be induced via multiple physiologic routes in an adjuvant-dependent manner. J Allergy Clin Immunol 2011;128(6):1251-8.e2. https://doi.org/10.1016/j.jaci.2011.06.007*
17. *Duchesne M, Okoye I, Lacy P. Epithelial cell alarmin cytokines: Frontline mediators of the asthma inflammatory response. Front Immunol 2022;13:975914. https://doi.org/10.3389/fimmu.2022.975914*
18. *Kim DI, Song MK, Kim SJ, Kim IS, Shin MY, Shin HS, et al. Comparison of asthma phenotypes in OVA-induced mice challenged via inhaled and intranasal routes. BMC Pulm Med 2019;19:177. https://doi.org/10.1186/s12890-019-1001-9*
19. *Flanagan TW, Jaeger C, Walsh MT, Harrington FJ, Carroll TP. 5-HT2 receptor activation alleviates airway inflammation and structural remodeling in a chronic mouse asthma model. Life Sci 2019;239:117018. https://doi.org/10.1016/j.lfs.2019.116790*
20. *Kim JD, Kim SY, Kwak EJ, Sol IS, Kim MJ, Kim YH, et al. Reduction rate of specific IgE level as a predictor of persistent egg allergy in children. Allergy Asthma Immunol Res 2019;11(4):498-507. https://doi.org/10.4168/aair.2019.11.4.498*
21. *Li S, Li M, Li G, Liu H, Zhu W, Sui D, et al. Lactobacillus rhamnosus RL-H3-005 ameliorates allergic airway inflammation by modulating the gut microbiota in asthmatic mice. Food Sci Hum Wellness 2024. https://doi.org/10.26599/FSHW.2024.9250282*
22. *Chen Q, Dong L, Li Y, Liu Y, Xia Q, Sang S. Research advance of non-thermal processing technologies on ovalbumin properties: The gelation, foaming, emulsification, allergenicity, immunoregulation and its delivery system application. Crit Rev Food Sci Nutr 2024;1-21.*
23. *Jiménez-Saiz R, Benedé S, Molina E, López-Expósito I. Effect of processing technologies on the allergenicity of food products. Crit Rev Food Sci Nutr 2015;55(13):1902-17. https://doi.org/10.1080/10408398.2012.736435*
24. *Dong X, Wang J, Raghavan V. Critical reviews and recent advances of novel non-thermal processing techniques on the modification of food allergens. Crit Rev Food Sci Nutr 2021;61(14):2203-23. https://doi.org/10.1080/10408398.2020.1722942*
25. *Hu X, Wang H, Hu Y, Tu Z. Insight into the effects of pulsed electric field on the structure, aggregation characteristics and functional properties of whey proteins. Food Hydrocolloids 2024;139:109230. https://doi.org/10.1016/j.foodhyd.2024.110111*
26. *Pi X, Sun Y, Cheng J, Fu G, Guo M. A review on polyphenols and their potential application to reduce food allergenicity. Crit Rev Food Sci Nutr 2023;63(21):2936-55. https://doi.org/10.1080/10408398.2022.2078273*
27. *Wu H, Chen B, Wu Y, Gao J, Li X, Tong P, et al. New perspectives on food matrix modulation of food allergies: immunomodulation and component interactions. J Agric Food Chem 2023;71(22):8570-85. https://doi.org/10.1021/acs.jafc.3c03192*
28. *Sampath V, Rabinowitz G, Shah M, Jain S, Diamant Z, Nadeau KC. Vaccines and allergic reactions: the past, the current COVID-19 pandemic, and future perspectives. Allergy 2021;76(6):1640-60. https://doi.org/10.1111/all.14840*
29. *Li F, Liu B, Xiong Y, Zhang Z, Zhang Q, Qiu R, et al. Enhanced downstream processing for a cell-based avian influenza (H5N1) vaccine. Vaccines (Basel) 2024;12(2):138. https://doi.org/10.3390/vaccines12020138*

30. Röder M, Wiacek C, Lankamp F, Kreyer J, Weber W. Improved sensitivity of allergen detection by immunoaffinity LC-MS/MS using ovalbumin as a case study. Foods 2021;10(12):2932. https://doi.org/10.3390/foods10122932
31. Cançado BLB, Aranda CS, Mallozi MC, Weckx LY, Solé D. Egg allergy and yellow fever vaccination. J Pediatr (Rio J) 2024;100(2):156-62. https://doi.org/10.1016/j.jped.2023.07.004
32. Clark AT, Skypala I, Leech SC, Ewan PW, Dugue P, Brathwaite N, et al. BSACI guideline for the diagnosis and management of egg allergy. Clin Exp Allergy 2010;40(8):1116-29. https://doi.org/10.1111/j.1365-2222.2010.03557.x
33. Gouma S, Zost SJ, Parkhouse K, Branche AR, Topham DJ, Cobey S, et al. Comparison of human H3N2 antibody responses elicited by egg-based, cell-based, and recombinant protein-based influenza vaccines during the 2017-2018 season. Clin Infect Dis 2020;71(6):1447-53. https://doi.org/10.1093/cid/ciz996
34. Centers for Disease Control and Prevention (CDC); Advisory Committee on Immunization Practices (ACIP). Prevention and control of seasonal influenza with vaccines: Recommendations of the ACIPUnited States, 2023-24 influenza season. MMWR Morb Mortal Wkly Rep 2023;72(29):822-8. https://doi.org/10.15585/mmwr.rr7202a1
35. Dhanapala P, De Silva C, Doran T, Suphioglu C. Cracking the egg: an insight into egg hypersensitivity. Mol Immunol 2015;63(2):76-85. https://doi.org/10.1016/j.molimm.2015.04.016
36. Turner PJ, Southern J, Andrews NJ, Miller E, Erlewyn-Lajeunesse M. Safety of live attenuated influenza vaccine in young people with egg allergy: multicentre prospective cohort study. BMJ 2015;351:h6291. https://doi.org/10.1136/bmj.h6291
37. Centers for Disease Control and Prevention (CDC). Update: Recommendations of the Advisory Committee on Immunization Practices (ACIP) regarding the use of influenza vaccine in egg-allergic individuals United States, 2023. MMWR Morb Mortal Wkly Rep 2023;72(40):1122-6.
38. Barr IG, Donis RO, Katz JM, McCauley JW, Odagiri T, Trusheim MR, et al. Cell culture-derived influenza vaccines in the severe 2017-2018 epidemic season: a step towards improved influenza vaccine effectiveness. NPJ Vaccines 2018;3:44. https://doi.org/10.1038/s41541-018-0079-z
39. Do Minh A, Kamen AA. Critical Assessment of Purification and Analytical Technologies for Enveloped Viral Vector and Vaccine Processing and Their Current Limitations in Resolving Co-Purified Contaminants. Vaccines (Basel) 2021;9(8):823. https://doi.org/10.3390/vaccines9080823
40. Hu W, Zhang X, Shen Y, Meng X, Wu Y, He J, et al. Quantifying allergenic proteins using antibody-based methods or liquid chromatography-mass spectrometry/mass spectrometry: A review about the influence of food matrices and processing. Compr Rev Food Sci Food Saf 2024;23(2):e70029. https://doi.org/10.1111/1541-4337.70029
41. Torkamannejad S, Chang G, Aroge FA, Sun B. Single isotopologue for in-sample calibration and absolute quantitation by LC-MS/MS. J Proteome Res 2024;23(4):1351-9. https://doi.org/10.1021/acs.jproteome.3c00848
42. Mahler V, Junker AC. Anaphylaxis to additives in vaccines: current insights and implications for clinical practice. Allergo J Int. 2022;31(6):159-168. doi:10.1007/s40629-022-00215-8. https://doi.org/10.1007/s40629-022-00215-8
43. Des Roches A, Paradis L, Gagnon R, Lemire C, Bégin P, Carr S, et al. Safe vaccination of patients with egg allergy with an adjuvanted pandemic H1N1 vaccine. J Allergy Clin Immunol 2010;126(2):317-23. https://doi.org/10.1016/j.jaci.2010.05.037
44. Greenhawt MJ, Chernin AS, Howe L, Li JT, Sanders GM. The safety of H1N1 influenza A vaccine in egg-allergic individuals. Ann Allergy Asthma Immunol 2010;105(5):387-93. https://doi.org/10.1016/j.anai.2010.08.015
45. Blom L, Verwer BJ, de Jonge J, Hendriksen CF. Reducing protein impurities in vaccine formulations. Vaccine 2014;32(23):2707-13.
46. Hong L, Pan M, Xie X, Liu K, Yang J, Wang S, et al. Aptamer-based fluorescent biosensor for the rapid and sensitive detection of allergens in food matrices. Foods 2021;10(11):2598. https://doi.org/10.3390/foods10112598
47. Khumsap S, Chanlek N, Pinyosopon T, Chailapakul O, Numnuam A. Label-free electrochemical immunosensor for sensitive detection of ovalbumin. Bioelectrochemistry 2021;139:107805. https://doi.org/10.1016/j.bioelechem.2021.107805
48. Xu L, Liu Y, He Y, Zhang M, Yu Q. Colorimetric detection of ovalbumin using gold nanoparticle-based plasmonic sensors. Microchem J 2023;186:109349. https://doi.org/10.1016/j.microc.2023.109349
49. Fu Y, Wang C, Zhang H, Zhang D, Huang D. Multiplex detection of allergens using SERS. Spectrochim Acta A Mol Biomol Spectrosc 2021;259:119673.
50. Yang H, Zhang L, Wu T, Li J, Chen Z. Heat/pH modification of OVA structure and detection. Food Biosci 2024;54:104148.
51. Liu R, Zhou Y, Sun Y, Huang X, Chen W. Unfolding pathways and allergenicity reduction of ovalbumin. J Agric Food Chem 2023;71(45):16582-91. https://doi.org/10.1021/acs.jafc.3c04613
52. Rupa P, Mine Y. Recombinant versus native ovalbumin: Comparative allergenicity. Clin Exp Allergy 2003;33(9):1267-73.
53. Sharma VK, Sharma I, Glick J. The expanding role of mass spectrometry in the field of vaccine development. Mass Spectrom Rev 2020;39(5):453-72. https://doi.org/10.1002/mas.21571
54. European Directorate for the Quality of Medicines & HealthCare (EDQM). European Pharmacopoeia. Monograph 01/2023:0159 - Influenza vaccine (inactivated). Strasbourg (France): Council of Europe; 2023. Ovalbumin limit ≤1 µg per dose.
55. Grohskopf LA, Shay DK, Shimabukuro TT, Sokolow LZ, Keitel WA, Bresee JS, et al. Prevention and control of seasonal influenza with vaccines: recommendations of the Advisory Committee on Immunization Practices (ACIP), United States, 2013-2014. MMWR Recomm Rep 2014;62(RR-07):1-43.
56. Taylor SL, Baumert JL, Kruizinga AG, Remington BC, Blom WM, Vlieg-Boerstra BJ, et al. Establishment of reference doses for residues of allergenic foods: report of the VITAL Expert Panel. J Allergy Clin Immunol 2014;133(1):156-64. https://doi.org/10.1016/j.fct.2013.10.032
57. Gagnon R, Primeau MN, Des Roches A, Lemire C, Kagan R, Clarke AE, et al. Safe vaccination of patients with egg allergy against influenza: a prospective cohort study. J Allergy Clin Immunol 2010;126(2):317-23.
58. Claude M, Lupi R, Bouchaud G, Bodinier M, Brossard C, Denery-Papini S. The thermal aggregation of ovalbumin as large particles decreases its allergenicity for egg allergic patients and in a murine model. Food Chem 2016;203:136-44. https://doi.org/10.1016/j.foodchem.2016.02.054
59. Cao H, Zhang C, Li Y, Wang Z, Zhao L, Sun X, et al. Glycosylation-modified antigens as a tolerance platform in allergic individuals. Cell Rep Med 2023;4(4):101346. https://doi.org/10.1016/j.xcrm.2023.101346
60. Arunachalam AB, Post P, Rudin D. Unique features of a recombinant haemagglutinin influenza vaccine that influence vaccine performance. NPJ Vaccines 2021;6(1):144. https://doi.org/10.1038/s41541-021-00403-7
61. Baxter R, Patriarca PA, Ensor K, Izikson R, Goldenthal KL. Safety, reactogenicity and immunogenicity of FluBlok® trivalent recombinant baculovirus-expressed hemagglutinin influenza vaccine administered intramuscularly to healthy adults. Vaccine 2011;29(17):2891-8. https://doi.org/10.1016/j.vaccine.2011.01.039

62. *Rando HM, Lordan R, Lee AJ, Naik A, Wellhausen N, Sell E, et al. Application of traditional vaccine development strategies to SARS-CoV-2. mSystems 2023;8(2):e0092722. https://doi.org/10.1128/msystems.00927-22*

63. *Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, et al. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med 2020;383(25):2427-38. https://doi.org/10.1056/NEJMoa2028436*

64. *Bordry N, Mamez AC, Fedeli C, Cantero C, Jaksic C, Alonso PU, et al. SARS-CoV-2 m-RNA vaccine response in immunocompromised patients: A monocentric study comparing cancer, people living with HIV, hematopoietic stem cell transplant patients and lung transplant recipients.Vaccines (Basel) 2023;11(8):1284. https://doi.org/10.3390/vaccines11081284*

65. *Park J, Pho T, Champion JA. Chemical and biological conjugation strategies for the development of multivalent protein vaccine nanoparticles. Biopolymers 2023;114(3):e23563. https://doi.org/10.1002/bip.23563*

66. *Woo EJ, Moro PL, Cano M, Jankosky C. Postmarketing safety surveillance of trivalent recombinant influenza vaccine: Reports to the vaccine adverse event reporting system. Vaccine 2017;35(30):3745-50.*

67. *World Health Organization (WHO). Global vaccine safety blueprint 2.0 (2021-2030): Enhancing vaccine safety. Available from: https://www.who.int/publications/i/item/9789240061465*

68. *McCright J, Ramirez A, Amosu M, Sinha A, Bogseth A, et al. Targeting the gut mucosal immune system using nanomaterials. Pharmaceutics 2021;13(11):1755. https://doi.org/10.3390/pharmaceutics13111755*

69. *Shi X, Zhao L, Niu L, Yan Y, Chen Q, Liu L. Oral intervention of narirutin ameliorates the allergic response of ovalbumin allergy. J Agric Food Chem 2022;70(6):1762-73. https://doi.org/10.1021/acs.jafc.2c05383*

70. *Jang SO, Kim HJ, Kim YJ, Kang MJ, Kwon JW, Seo JH, et al. Asthma prevention by Lactobacillus rhamnosus in a mouse model is associated with $CD4^+CD25^+Foxp3^+$ T cells. Allergy Asthma Immunol Res 2012;4(3):150-6. https://doi.org/10.4168/aair.2012.4.3.150*

71. *Dona DW, Suphioglu C. Egg allergy: Diagnosis and immunotherapy. Int J Mol Sci 2020;21(14):5010. https://doi.org/10.3390/ijms21145010*

72. *Grewal S, Hegde N, Yanow SK. Integrating machine learning to advance epitope mapping. Front Immunol 2024;15:1463931. https://doi.org/10.3389/fimmu.2024.1463931*

73. *Lee HJ, Kim JA, Lee Y, Lim S, Kim JH, Kim JH, et al. Allergen-specific immunotherapy using injectable in situ crosslinked hyaluronic acid hydrogels ameliorates allergic response in murine allergic rhinitis models. Allergy Asthma Immunol Res 2024;16(3):245-57.*

74. *Kim JS, Kim JH, Lee SY, Lee E, Lee S, Kang MJ, et al. Delayed-Onset Anaphylaxis Caused by IgE Response to Influenza Vaccination. Allergy Asthma Immunol Res. 2020 Mar;12(2):359-63. https://doi.org/10.4168/aair.2020.12.2.359*