CHARACTERIZATION OF IMMUNE BIOMARKERS IN A NOVEL MOUSE MODEL OF WHEAT GLUTENIN-INDUCED LIFE-THREATENING SYSTEMIC ANAPHYLAXIS

By

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ABSTRACT

Glutenin-induced life-threatening systemic anaphylaxis (GILTSA) is a serious food safety and public health issue growing at an alarming rate in the United States of America. A novel adjuvant-free mouse model of GILTSA was developed by Dr. Gangur and colleagues at Michigan State University. This model was used to test the following hypothesis in this thesis work: GILTSA will be associated with substantial elevation of a selective set of organ-specific immune biomarkers in the small intestine, liver, heart and lungs. Organs collected from mice with GILTSA (n = 10) and from 3 groups of healthy mice (n = 10 per group) were used in the analysis. Tissue protein extracts were used in protein microarray analysis of 640 immune biomarkers. Differentially expressed immune biomarkers were identified. The immune biomarkers markers that were increased by 25-fold or higher in mice with GILTSA compared to the control groups were identified as the most critical signature immune biomarkers of GILTSA as follows: In the small intestine, 2 signature immune biomarkers were identified; in the liver, 6 signature immune biomarkers were identified; in the heart, 12 signature immune biomarkers were identified; and in the lungs, 5 signature immune biomarkers were identified. These signatures were associated with the following immune pathways: Th2 immune activation pathways, cytotoxic immune activation pathway, tissue repair/remodeling pathway, metabolic regulation pathway, vascular integrity pathway, and immune homeostasis pathways. Some of the signature immune biomarkers identified may have therapeutic potential in GILTSA. Findings from this study have advanced the molecular mechanisms of GILTSA. This approach may also be used to identify signature immune biomarkers in other types of food anaphylaxis.

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LIST OF ABBREVIATIONS

WDEIA	Wheat-dependent exercise-induced anaphylaxis
WAO	Wheat allergy organization
TLR4	Toll-like receptor 4
LPS	Lipopolysaccharide
IL-4	Interleukin 4
IL-4R	Interleukin-4 receptor
HWP	Hydrolyzed wheat protein
HLA	Human leukocyte antigen
IL-18	Interleukin-18
MBL	Mannose-binding lectin
RBFOX1	RNA-binding fox-1 homolog 1
aOR	Adjusted odds ratio
PPI	Proton pump inhibitor
TNP-ova	Trinitrophenyl-ovalbumin
Th2, Th17	T helper2, T helper17
CXCR3	C-X-C motif chemokines receptor 3
PAF	Platelet-activating factor
AAPs	Anaphylaxis activation pathways
ATI	Anti-trypsin inhibitor
NSAIDs	Nonsteroidal anti-inflammatory drugs
PAMP	Pathogen-associated molecular pattern
PRR	Pattern recognition receptors

LOAELs	Lowest observable adverse effect levels
NOAELs	No observable adverse effect levels
WIA	Wheat-induced anaphylaxis
IP	Intraperitoneal
HSR	Hypothermic shock response
TDE	Transdermal exposure
mMCP-1	Murine mucosal mast cell protease-1
SSP	Salt-soluble protein
IV	Intravenous
SC	Subcutaneous
V/P	Vehicle application over the skin/protein injection IP
P/P	Protein application over the skin/protein injection IP
SIgE	Specific IgE antibodies
TIgE	Total IgE antibodies

Chapter 1: Introduction

Background and Significance

Food allergies are one of the growing food safety concerns for the food industry and for public health in many countries including United States of America (USA) (Fong et al., 2022; Gupta et al., 2018). They cause adverse reactions which are caused by the immune system to food proteins known as food allergens (Renz et al., 2018; Sicherer & Sampson, 2018; Wong & Santos, 2024). The prevalence of food allergies has been increasing in many countries over the past few decades. In the USA approximately 10.8 percent adults and 8 percent children have at least one food allergy (Gupta et al., 2018., 2019, Warren et al., 2019., 2020).

Food allergy is one of the most challenging food safety concerns because of life-threatening systemic anaphylaxis (LTSA) that allergenic foods are capable of eliciting in food allergic subjects; such LTSA reactions are the reason why food allergens are regulated in the USA and many other countries including Canada, European Union, United Kingdom, Japan, Australia and New Zealand (U.S. Food and Drug Administration, 2025; European Food Safety Authority, 2025; Food Standards Australia New Zealand, 2025; Health Canada, 2017). Even a trace amount of food allergens can cause LTSA among food allergic subjects; therefore, food allergens are regulated as zero tolerance contaminants by the US FDA (Marinho et al., 2025; U.S. Food and Drug Administration, 2025).

According to US FDA, there are 9 major allergenic foods (commonly called as food allergens) in the USA: milk, eggs, fish, crustacean shellfish, tree nuts, peanuts, wheat, soybeans, and sesame (U.S. Food and Drug Administration, 2025). These 9 food allergens are responsible for at least 90 percent of food allergic reactions across the states in the US (NIAID 2010; U.S. Food and Drug Administration, 2025).

Globally, apart from the 9 major food allergens, lupin, celery, molluscs, sulphites, and mustard are other food allergens that officially need food allergen labelling in other countries but not in the USA (European Food Safety Authority, 2025; Food Standards Australia New Zealand, 2025; Health Canada, 2017). As evident, wheat is therefore considered as a major food allergen in many countries in addition to USA due to its prevalence and the safety concern due to its potential to cause deadly anaphylactic shock (Liu et al., 2023).

Approximately 2.4 million people including children in the US have wheat allergy (Gupta et al., 2018., 2019; Warren et al., 2019). Wheat has two groups of proteins, namely glutens (glutenins and gliadins) and non-gluten proteins (albumin and globulin). Both groups of wheat proteins can cause food allergic and anaphylactic reactions in humans (Pastorello et al., 2007; Ricci et al., 2019). In this work, immune biomarker was conducted using a novel mouse model of wheat glutenin-induced (GI) LTSA that was published previously from our research group (Jorgensen et al., 2023).

Food allergies including wheat allergy develop in two stages as follows: Stage 1) when there is a exposure to wheat allergens via oral, skin, eyes and airways, immune system responds by producing food allergen specific IgE antibodies; These antibodies bind to high affinity IgE receptor present on two types of immune cells (mast cells and basophils); Once this happens, the subject is considered as sensitized to the food allergen; at this stage there are no clinical symptoms of disease (Bao et al., 2023; Watson et al., 2017). Stage 2) Subsequent exposure to food allergen results in clinical symptoms of disease such as vomiting, diarrhea, hives, rhinitis, conjunctivitis, asthma, and sometimes LTSA (Jorgensen et al., 2024; Sampson, 2004). In this work, a mouse model of wheat GILTSA was used to study immune biomarkers.

To understand the mechanisms of wheat allergy and anaphylaxis, various animal (dogs, rats, guinea pigs and mice) models have been used in the literature (Arul Arasan et al., 2025; Gao et al., 2022; Jin et al., 2020; Jorgensen et al., 2023; Kohno et al., 2016; Morita et al., 2009; Tanaka et al., 2011; Yamada et al., 2019, 2022). The most common wheat allergy animal model is based on laboratory inbred strain of mice such Balb/c (Arul Arasan et al., 2025). This study used Balb/c mouse model of GILTSA to identify immune biomarkers.

Mouse models of wheat allergy and anaphylaxis are of two types: Adjuvant-based mouse models and adjuvant-free mouse models. Both models are used for studying wheat anaphylaxis. The strength of adjuvant-based model is that it is quick to produce efficient sensitization readouts (such as elevated IgE antibodies). The drawback of this model is that the reaction specificity is sacrificed for sensitivity, making it difficult to determine intrinsic protein allergenicity (Dearman & Kimber., 2009). Furthermore, the mechanism underlying the development of wheat allergy is also different in adjuvant-based model compared to adjuvant-free model (Jin et al., 2020).

To eliminate the limitations of adjuvant-based wheat allergy models, our research group developed an adjuvant-free mouse model of wheat allergy and anaphylaxis (Jin et al., 2020; Gao et al., 2022,2023; Jorgensen et al., 2023, 2024). Using the adjuvant-free mouse model of GILTSA, our research group recently reported spleen immune biomarkers and a methodology to classify immune biomarkers for further research (Jorgensen et al., 2023; Jorgensen et al., 2024).

This study was conducted using the same model and published methodology to identify organ specific immune biomarkers associated with GILTSA in small intestine, liver, heart, and lungs.

Problem statement

Mechanisms of GILTSA are incompletely understood at present. For example, organ specific immune biomarker signatures of small intestine, liver, heart and lungs linked to GILTSA are largely unknown.

Hypothesis

In this study, we tested the following hypothesis using a novel mouse model of wheat glutenin allergy and anaphylaxis: GILTSA will be associated with substantial elevation of a selective set of organ-specific immune markers in small intestine, liver, heart and lungs.

Rationale

Immune markers that are substantially elevated during GILTSA may be targeted for novel therapeutics/ diagnostics/management of GILTSA.

Aims

There were four aims for this study:

1) To conduct a proteomic analysis of 640 immune markers using protein extracts of small intestine obtained from control vs GILTSA mice.
2) To conduct a proteomic analysis of 640 immune markers using protein extracts of liver obtained from control vs GILTSA mice.
3) To conduct a proteomic analysis of 640 immune markers using protein extracts of heart obtained from control vs GILTSA mice.
4) To conduct a proteomic analysis of 640 immune markers using protein of lungs obtained from control vs GILTSA mice.

To identify organ-specific signatures linked to GILTSA, protein array analysis was conducted in four groups of mice (n = 10 per group) as follows: vehicle sensitized/vehicle

challenged (v/v), vehicle sensitized/protein challenged (v\p), protein sensitized/vehicle challenged (p\v), protein sensitized/protein challenged (p\p). Immune response analysis of IgE antibody, mucosal mast cell degranulation response, hypothermic shock response, and clinical symptom scoring of GILTSA were conducted by other students working on this project. Those data have been published (Jorgensen et al., 2023). Organs collected from these mice were used in this thesis work. Using an optimized published method, tissue protein extracts were prepared and used in microarray analysis of 640 immune biomarkers (Goa et al., 2022; Jorgensen et al., 2023). Differentially expressed immune biomarkers were identified based on heat map analysis and fold change in protein expression. The markers that were increased by 25-fold or higher in v/v vs. p/p but not in v/p or p/v groups were identified as the most critical signature markers of GILTSA.

Findings from this work

Chapter 2: Review of literature (Published as Arul Arasan et al., 2025)

Chapter 3: Organ-specific immune biomarker signatures of small intestine, liver, lungs and heart in a novel mouse model of glutenin-induced life-threatening systemic anaphylaxis (Arul Arasan et al 2025 Manuscript in preparation for submission to journal)

Chapter 4: Conclusions and future directions

Scope of this work

This work was conducted using a Balb/c mouse model of GILTSA as reported before (Jorgensen et al., 2023). IgE responses were elicited upon skin exposure to glutenin extract without the use of adjuvants and without causing any skin wounds. GILTSA was elicited by intraperitoneal injections with glutenin extract without the use of adjuvants. The following wheat species was used to obtain glutenin extract in this study: *T. aestivum* (common wheat, Ambassador variety) was obtained from the MSU Wheat Breeding Program. Breeder pairs of

Balb/c mice strain were obtained from The Jackson Laboratories (Bar Harbor, ME) and housed in the MSU animal facilities where mouse breeding was conducted to produce all animals used in this study. All animals were maintained on a plant-protein-free diet throughout this study (AIN-93G, Envigo). Adult female mice were used in the experiments described in this thesis.

Expected benefit and outcome from this work

Findings from this study are expected to advance the molecular mechanisms of wheat-induced life-threatening systemic anaphylaxis in this model. Identification of a panel of organ-specific signature immune markers linked to GILTSA demonstrates the broader utility of this approach for identification of similar biomarker signatures for other types of systemic anaphylaxis caused by other allergenic foods as well as other agents. Findings from this study may inform the development of novel targets for therapy, diagnosis and management of GILTSA.

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Chapter 2: Review of Literature

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Abstract

Wheat is a staple and nutritious food that is consumed globally. However, it is identified as a major allergenic food because of its capacity to trigger life-threatening systemic anaphylaxis. The specific mechanisms that underlie this systemic anaphylaxis in wheat allergy are incompletely understood. As a result, several rodent models have been developed to study anaphylaxis in wheat allergies. In this paper, we have conducted a comprehensive review of wheat-induced anaphylaxis using Google Scholar and PubMed databases with relevant keywords. The following objectives were addressed: (1) to determine the complexity of wheat-induced anaphylaxis; (2) to summarize the role of genetic susceptibility in wheat anaphylaxis; (3) to identify the environmental factors involved in the development of wheat anaphylaxis; (4) to map the current status of mechanisms involved in wheat anaphylaxis; (5) to identify the approaches, strengths, and limitations of rodent models of wheat anaphylaxis; and (6) to identify challenges and opportunities in this area of science. Our findings provide a comprehensive updated critical resource for the future research agenda in wheat allergy-associated anaphylaxis, particularly using rodent models as attractive pre-clinical tools.

Keywords: wheat; wheat gluten; wheat non-gluten; anaphylaxis; animal models; IgE; exercise; genetics; environment

Introduction

Wheat is one of the most prominent foods globally that has been in the human diet for a long period of time (at least 10,000 years) (Levy & Feldman, 2022). Although wheat serves as a staple food in different parts of the world, allergy caused by wheat proteins such as gluten is a growing concern among people in developed countries, as well as in some of the developing countries in Asia, such as China, Hong Kong, Singapore, Malaysia, and India (Sampath et al., 2021). Consequently, food safety regulations are enforced in many countries, including the USA, Canada, European Union countries, and Australia, to prevent and manage potential wheat allergic reactions (Cianferoni, 2016; European Food Safety Authority, 2025; Food Standards Australia New Zealand, 20254; Health Canada, 2017; U.S. Food and Drug Administration, 2025). Wheat is regulated as a major food allergen in these countries (Jorgensen et al., 2024). In the USA, between 0.4% and 4.3% of the population have wheat allergies (Venter et al., 2006, 2008; Vierk et al., 2007). There is ample evidence that the prevalence of food allergies in general and wheat allergies in particular has increased dramatically over the past three decades (J. Savage et al., 2016). A major concern for public safety from wheat allergies is the potential of deadly reactions known as systemic anaphylaxes, where multiple vital organs (heart, lungs, brain), in addition to gut and skin, are involved in the disease elicitation (Cianferoni, 2016). However, the underlying deadly reactions of the mechanism are incompletely understood at present.

The general mechanism of food allergy development involves two phases: It begins with sensitization to the food concerned, where subjects induce IgE antibodies, followed by disease elicitation upon subsequent exposure to the concerned food. Before sensitization, there are no clinical reactions to the food. Therefore, sensitization is a required factor for the subsequent

expression of clinical disease symptoms. When allergic reactions are limited to the gut (for example, diarrhea, vomiting), it is not considered as systemic anaphylaxis (Bao et al., 2023; Watson et al., 2017). However, when symptoms expand to vital organs (heart, lungs, brain) in addition to the gut, the reaction is regarded as systemic anaphylaxis that is potentially fatal (Cabanillas, 2020; Finkelman et al., 2005; Sicherer & Leung, 2015). There has been marked progress recently in the classification of anaphylaxis as reported in recent papers (Cardona et al., 2020; Chinthrajah et al., 2022; Turner et al., 2024). Turner et al. (2024) proposed a modification of the previous WAO grading system for systemic anaphylactic reactions, which aligns closely with version 3.0 of the Consortium for Food Allergy Research (COFAR) grading scale (Rutkowski et al., 2012; Wang et al., 2024). According to this proposal, allergic reactions are classified into five grades based on objective clinical parameters of disease manifestation. Reactions in grades 1 and 2 involve skin, gut, or other systems with mild symptoms. Grades 3 to 5 involve additional reactions affecting the cardiovascular and neurological systems. So, the definition of anaphylaxis per this proposal requires clinical reactions from grade 3 to grade 5. The present study was conducted to develop a comprehensive review on the subject and identify specific topics for future research.

In this study, the following objectives were addressed: (1) to determine the complexity of wheat anaphylaxis; (2) to summarize the role of genetic susceptibility in wheat anaphylaxis; (3) to identify the environmental factors involved in the development of wheat anaphylaxis; (4) to map the current status of mechanisms involved in wheat anaphylaxis; (5) to identify the approaches, strengths, and limitations of rodent models of wheat anaphylaxis; and (6) to identify challenges and opportunities to advance science in wheat anaphylaxis. In order to address the above-mentioned objectives, we used keywords such as “wheat,” “anaphylaxis,” “animal

model," "human," and "gluten" in various combinations in PubMed and Google Scholar for this research. The output was evaluated, and objective-relevant information was obtained. The results were synthesized into concepts, figures, and tables as presented.

This review has identified the complexity associated with wheat-induced anaphylaxis in humans as well as rodent models. We have identified specific gaps and opportunities for future research to enable the development of improved methods for the prevention, treatment, and management of wheat-induced anaphylaxis. Our findings provide a comprehensive updated critical resource for developing the future research agenda in wheat allergy-associated anaphylaxis using rodent models as very attractive pre-clinical tools.

Complexity of Anaphylaxis in Wheat Allergy: Current Status

Anaphylaxis is clinically defined as a potentially deadly reaction of the immune system to certain environmental agents including insect stings, drugs, radiological contrast media, and allergenic foods (Rutkowski et al., 2012). In the USA, the following nine foods are regulated for their capacity to trigger such deadly reactions: wheat, egg, milk, soy, peanut, tree nut, fish, shellfish, and sesame. The scope of this article is limited to wheat allergy-associated anaphylaxis.

Overall Classification of Types of Anaphylaxis in Wheat Allergy

Anaphylaxis is triggered by different types of agents, and the clinical characteristics leading to the deadly condition are largely similar. However, based on the mechanisms involved, it is classified into three major groups: (i) IgE antibody-mediated anaphylaxis; ii) non-IgE-mediated anaphylaxis; and (ii) idiopathic anaphylaxis with unknown mechanisms (Wang et al., 2024). It is noteworthy that all reported systemic anaphylaxes in wheat allergy belong to the first group since they are all mediated by the wheat-specific IgE antibodies (Jorgensen et al., 2024). Furthermore, based on whether cofactors such as exercise, etc., are required in addition to wheat

consumption, anaphylaxis in wheat allergy is classified into two groups: (i) classical systemic anaphylaxis upon wheat exposure and (ii) cofactor-dependent systemic anaphylaxis in wheat allergy. In the first group, wheat-sensitized patients react within minutes upon re-exposure to wheat. In the second group, wheat-sensitized subjects react to wheat only when they are exposed to other factors simultaneously (alcohol, infection) or within a short time (exercise) after exposure to wheat. The latter condition is known as wheat-dependent exercise-induced anaphylaxis (WDEIA) (Shin, 2021).

Role of Host Genetic Susceptibility in the Development of Wheat Allergy and Anaphylaxis

Wheat is a staple food consumed by billions of people around the world. However, only a small but significant number of individuals who are exposed to wheat allergens develop a wheat allergy. It is therefore clear that the genetic susceptibility to developing a wheat allergy is different among different people. There are eight studies reporting specific genetic factors associated with wheat allergies (Table 1). These studies and their significance are reviewed below.

The first study to identify the genetic risk of developing a wheat allergy was conducted by Cho et al. (2011) in a South Korean adult population. They investigated the relationship between polymorphism at the TLR4 locus and the risk of developing respiratory wheat allergy (Cho et al., 2011). They found that the homozygous variant of two TLR4 alleles was associated with significantly reduced risk of developing respiratory wheat allergy. TLR4 encodes for the toll-like receptor, which is an innate receptor with a critical function in immune responses to both microorganisms and allergens (El-Zayat et al., 2019). It serves as a receptor for endotoxin lipopolysaccharide (LPS) present in the outer membrane of gram-negative bacteria according to hygiene hypothesis. Exposure to LPS may be protective against the development of allergic

diseases (Lin et al., 2017). Therefore, results from this study support the hygiene hypothesis related to respiratory wheat allergy development.

Table 1. Genetic susceptibility to developing wheat allergy in humans: current evidence.

Genetic Factors	Evidence from the Study	References
TLR4	Lower risk of respiratory wheat allergy was associated with *TLR4* polymorphism as follows: homozygotes for the −2027 G and −1608 C alleles (*n* = 381, adults, South Korean bakers study).	(Cho et al., 2011)
IL-4	Single nucleotide polymorphism at the *IL-4* locus (IL-4-C590T) was associated with WDEIA; Chinese study, *n* = 51, Age 5–77 years.	(Cai & Yin, 2013)
IL-4R	Single nucleotide polymorphism at *IL-4R* alpha locus (*IL-4RA* A1727G) was not associated with WDEIA; Chinese study, *n* = 51, Age 5–77 years.	(Cai & Yin, 2013)
	Increased positive skin-prick test to wheat flour in bakery workers (*n* = 373, South Korean study, adults) was associated with polymorphic variant of *IL-4Rα* (Ile375Val and Gln576Arg polymorphisms).	(Hur et al., 2013)
Filaggrin gene	A patient (woman age 51) had developed WDEIA upon using detergents containing HWP (Glupearl); however, she had no mutation in filaggrin gene that had been implicated for skin sensitization in Japanese subjects.	(Iga et al., 2013)
	In a Japanese family, a mother–daughter pair with the same filaggrin loss-of-function mutation developed WDEIA; the daughter was compound heterozygous for c.441_442delAG (p.Gly149Glufs*4) and c.5368C > T (p.Gln1790Ter), and the mother was heterozygous for c.441_442delAG.	(Mizuno et al., 2015)

Table 1 (cont'd)

	In a Denmark population (*n* = 7931, age: 18–69), filaggrin gene loss-of-function mutation was associated with self-reported food allergy, including wheat allergy, but not oral allergy syndrome (OR for wheat allergy 3.59; 95% CI 1.61–8.02).	(Linneberg et al., 2013)
	HLA class II DPB1*02:01:02 allele was associated with increased risk of WDEIA; Japanese population study, *n* = 77, adults.	(Fukunaga et al., 2021)
HLA-class II variants	HLA class II (HLA DQ) locus on chromosome 6p21 was associated with wheat allergy (skin, eye, airways symptoms when used soap containing hydrolyzed wheat protein and/or skin, eye, airways, gut, and shock symptoms upon eating wheat products/SPT, IgE, basophil activation positive); Japanese population study, *n* = 452, adults.	(Noguchi et al., 2019)
RBFOX1	RBFOX1 locus on chromosome 16p 13 locus was associated with wheat allergy; same population as above.	(Noguchi et al., 2019)
IL-18	Increased risk of WDEIA was associated with *IL-18* gene locus (haplotype AGG); (*n* = 130, Han Chinese study, adults).	(X. Gao et al., 2020)
	Increased risk of sensitization to wheat among South Korean bakery workers was associated with IL-18 polymorphism (373 adults; South Korean study Genotype 137G/C (GC or CC) and haplotype *ht3* [ACC].	(S. H. Kim et al., 2012)

Table 1 (cont'd)

MBL	Higher levels of blood MBL are associated with increased risk of baker's asthma in Korean population (*n* = 273); MBL levels were associated in the *MBL2* gene haplotypes.	(M. A. Kim et al., 2017)
Family genetics aggregation study	IgE-mediated food allergy trait (including wheat allergy) was associated with estimated heritability of 0.15–0.35; American nuclear family study (*n* = 581).	(Tsai et al., 2009)

It is well established that allergic diseases in general, including wheat allergies, are mediated by the T helper 2 immune response to allergens that help allergen-specific B cells to produce IgE antibodies (Vecchione et al., 2024). Cytokine IL-4 is the prototypic T helper 2-derived cytokine implicated in IgE class switching (Finkelman et al., 1988; Katona et al., 1988). Cai et al. studied genetic polymorphism at the IL-4 and IL-4R alpha locus in relation to susceptibility to WDEIA in Chinese subjects (children and adults) (Table 1). They found a significant association of SNP at the IL-4 locus but not the IL-4R alpha locus with WDEIA (Table 1). In contrast, Hur et al. (2013) reported the association of IL-4 receptor alpha locus with sensitization to wheat among South Korean bakery workers (Table 1). These results suggest that genetic control at the IL-4 and IL-4R plays (or may play) a critical role in the development of wheat allergies.

Filaggrin is an important protein required for skin barrier function (Sandilands et al., 2009). There are several studies implicating that mutation of the filaggrin gene results in the development of food allergies, such as peanut allergy (Linneberg et al., 2013). There are,

however, only two studies reporting the relationship between filaggrin loss-of-function mutation in three Japanese subjects with WDEIA condition (Table 1). In the first study, researchers found that a Japanese woman had developed WDEIA after skin exposure to a facial soap containing Glupearl, which is a hydrolyzed wheat gluten. However, this person did not carry a loss-of-function mutation in the filaggrin gene, suggesting that such mutation is not necessary to develop WDEIA upon skin exposure to wheat allergens. In the second study, the researchers found that a mother–daughter pair both carrying loss-of-function mutation polymorphism in the filaggrin gene developed WDEIA. Together, these studies suggest that WDEIA development does not necessarily require filaggrin gene mutation.

Linneberg et al. (2013) studied the role of filaggrin gene loss-of-function mutation in self-reported food allergies, including wheat allergy. The study population was adult Danish subjects (Linneberg et al., 2013). Interestingly, individuals with this mutation exhibited an increased tendency to have food allergies in general, with 3.59 times more risk of developing wheat allergies (Table 1).

Noguchi et al. (2019) studied genetic polymorphism in HLA class II variants (HLA DQ) and RBFOX1 in a Japanese cohort of wheat-allergic subjects with symptoms of airways, skin, gut allergy, and anaphylaxis (Noguchi et al., 2019). They found a positive association for both genes. HLA class II is critical for allergen presentation by antigen-presenting cells. There is a report associating the RBFOX1 gene with the development of pediatric food allergies in general (Li et al., 2015).

Fukunaga et al. (2021) studied the genetic contribution of HLA class II to increasing the risk of developing WDEIA in Japanese adults (Fukunaga et al., 2021) (Table 1). They identified a specific HLA class II DPB1 allele associated with increased risk of developing WDEIA.

Interleukin 18 plays a key role in immune responses and inflammation (Ihim et al., 2022). There are two studies investigating IL-18 polymorphisms in wheat allergy. The first study reported increased risk of developing WDEIA among Han Chinese adults associated with IL-18 Haplotype AGG. Another study examined the role of IL-18 genetics in developing wheat allergy among South Korean bakers. They identified two genetic variants linked with wheat allergy (S. H. Kim et al., 2012).

Kim et al. (2017) reported a relationship between the innate immune system marker, mannose-binding lectin (MBL) levels, gene polymorphisms, and baker's asthma in South Korean bakers (M. A. Kim et al., 2017). Higher levels of circulating MBL protein were related to baker's asthma, as well as specific polymorphisms in *MBL2* gene.

Tsai et al. (2009) reported a genetic study on food allergies in American nuclear families (Tsai et al., 2009). They found that both genetics and environmental factors are critical in food allergies among the population under study. Using IgE-mediated food allergy as a genetic trait, they estimated that sensitization to food, including wheat, was controlled genetically with a heritability of 0.15–0.35 (Table 1). They did not study any specific type of gene polymorphisms.

In summary, there is growing evidence for a genetic basis underlying the development of both WDEIA and wheat allergy. However, the specific genetic polymorphisms underlying these two conditions appear to be different. Genetic polymorphisms associated with increased risk of developing WDEIA are as follows: IL-18, IL-4, and HLA class II DPB. In contrast, increased risk of developing wheat allergy sensitization or disease is associated with polymorphism of the following genes: TLR4, RBFOX1, IL-4 alpha receptor, and HLA class II DQ. The filaggrin gene's loss-of-function mutation with relation to WDEIA is therefore controversial.

Role of Environment Factors in the Development of Wheat Allergy and Anaphylaxis

There are a limited number of studies examining the role of environmental factors in wheat allergy and anaphylaxis. The role of exposure to cats, gut microbiome diversity, Vitamin D deficiency, and the use of antacids have been studied as potential environmental factors associated with wheat allergy (Table 2).

Table 2. Environmental factors in wheat allergy.

Environmental Factors	Evidence from the Study	References
Exposure to cats	Exposure to cats during pregnancy reduced the risk of wheat allergy in children until the age of 3 years (aORs [95% CIs] 0.54 [0.34–0.85]); Exposure to cats during early infancy reduced the risk of wheat allergy until the age of 3 years (0.63 [0.42–0.92]); Japanese study	(Okabe et al., 2023)
Gut microbiome	Food (milk, egg, peanut, soy, wheat, and walnut) sensitization in adults was associated with reduction in the following genera: *Haemophilus*, *Dialister*, *Dorea*, and *Clostridium* sensitization (n = 85 total food sensitized, wheat sensitization n = 33); Food (including wheat) allergy in children was associated with a reduction in the following genera *Citrobacter*, *Oscillospira*, *Lactococcus*, and *Dorea*; wheat allergic children (n = 3) among food allergic subjects (n = 14); United States study, adults, pregnant women, infants.	(J. H. Savage et al., 2018)

Table 2 (cont'd)

Vitamin D deficiency	Vitamin D deficiency during childhood increased the risk of sensitization (specific IgE antibody) to wheat (OR 4.2; 95% CI 1.1–15.8); South Korean study.	(Baek et al., 2014)
Use of antacids/antiulcer medications	Antacids (H2R blocker or PPI) treatment for 3 months increased sensitization (IgE) to food allergens, including wheat (Total *n* = 152, Hungarian study, adults)	(Untersmayr et al., 2005)

In a Japanese study, exposure to cats during pregnancy and early infancy were studied. The researchers reported that such exposure to cats may prevent the development of wheat allergies. However, the duration of protection and whether such protection influences anaphylaxis are unknown (Okabe et al., 2023).

The role of gut microbiome diversity in food allergy is a highly researched topic. However, there is only one study associated with wheat allergy. Savage et al. (2018) reported gut microbiome variation in general food allergy that included wheat allergic sensitization. They found a reduction in four genera of bacteria in subjects that had developed sensitization to food allergens, including wheat. However, the number of children with wheat sensitization was very low (*n* = 3–14), preventing the generalization of findings (J. H. Savage et al., 2018).

There is a significant deficiency of vitamin D in human populations living in colder areas of the world, such as North America. There is also growing evidence that Vitamin D deficiency may be an important factor in the development of allergic diseases in general (Zhang et al., 2024). However, there is only one study that reported on the association of vitamin D with wheat allergy. Baek et al. (2014) studied the prevalence of sensitization to wheat among South Korean

children with vitamin D deficiency and reported an increased risk of sensitization to wheat by four folds in this population (Baek et al., 2014).

Stomach acidity plays an important role in the breakdown of food allergens (Pali-Schöll et al., 2018) . Therefore, the chronic usage of antacids such as (HIIR) blockers or proton pump inhibitors (PPI) may prevent the breakdown of allergens, thus facilitating allergic sensitization. There is one study associating antacids with sensitization to food allergens, including wheat (Untersmayr et al., 2005) (Table 2).

We thoroughly reviewed the relationship between the environmental factors (farming environment, drinking unpasteurized milk, exposure to bacterial endotoxins, and contact with livestock at fetal stage and early childhood) and the development of airways allergies, food allergies (including wheat allergies), asthma, and atopic dermatitis. There are several reports in the literature suggesting significant relationships between these environmental factors and the development of airways allergies, asthma, and atopic dermatitis. Notably, these studies did not include food allergies or wheat allergies in their research (Downs et al., 2001; Illi et al., 2012). Furthermore, in another independent study, no relationship was found between farming factors and food allergies, including wheat allergies (Orivuori et al., 2014). Thus, the implications of hygiene hypothesis on wheat allergies remain to be fully investigated in the future (Table 2).

In summary, very limited information is available on the protective versus promotive effects of environmental factors in the development of wheat allergies. There is therefore an ample opportunity to investigate the protective versus promotive role of environmental factors in the development of wheat allergies and anaphylaxis using validated animal models for the effects of environmental factors in wheat allergy development.

Mechanisms of Anaphylaxis in Wheat Allergy

In this section, we review the current understanding of the mechanisms involved in systemic anaphylaxis associated with wheat allergies. In the first part, we discuss this problem in humans and information from mouse models of classical anaphylaxis caused by wheat exposure. In the second part, we discuss wheat-dependent cofactor-induced/enhanced systemic anaphylaxis in humans and animal models.

Mechanism of Classical Anaphylaxis in Wheat Allergy

An overview of the current status of scientific knowledge on the classical wheat-induced anaphylaxis is illustrated in Figure 1. The first requirement for systemic anaphylaxis is the prior sensitization of the host to wheat-derived proteins. Both gluten proteins (gliadins and glutenin) and non-gluten proteins (salt-soluble proteins such as alpha amylase inhibitor) have been identified as sensitization-causing allergens. The mechanism of sensitization to wheat proteins is thought to occur as follows: The exposure of a genetically susceptible host to wheat allergens in the context of yet-to-be-identified abnormal environmental conditions results in immune responses characterized by T helper (Th)-2/Th17 dominance. The B lymphocytes specific to wheat allergens, under the influence of Th2/Th17 lymphocytes, start producing the IgE class of antibodies. The allergen-specific IgE antibodies then bind to FcεRI present on mast cells in the tissues and basophils in the blood. When the host reaches this stage of immune response, they are considered as sensitized to wheat allergens. In humans as well as animals, such sensitization is determined by measuring the allergen-specific IgE antibodies present in the blood using immune assays. In humans, clinical sensitization is established by performing a skin-prick test by administering a small quantity of allergens intradermally. A positive reaction occurs within a few minutes by the presentation of a wheal and flare reaction that is quantified by measuring the

diameter of the reaction at 20–30 min. A positive control injection with histamine is commonly used to compare the reactions (Sampson, 2004).

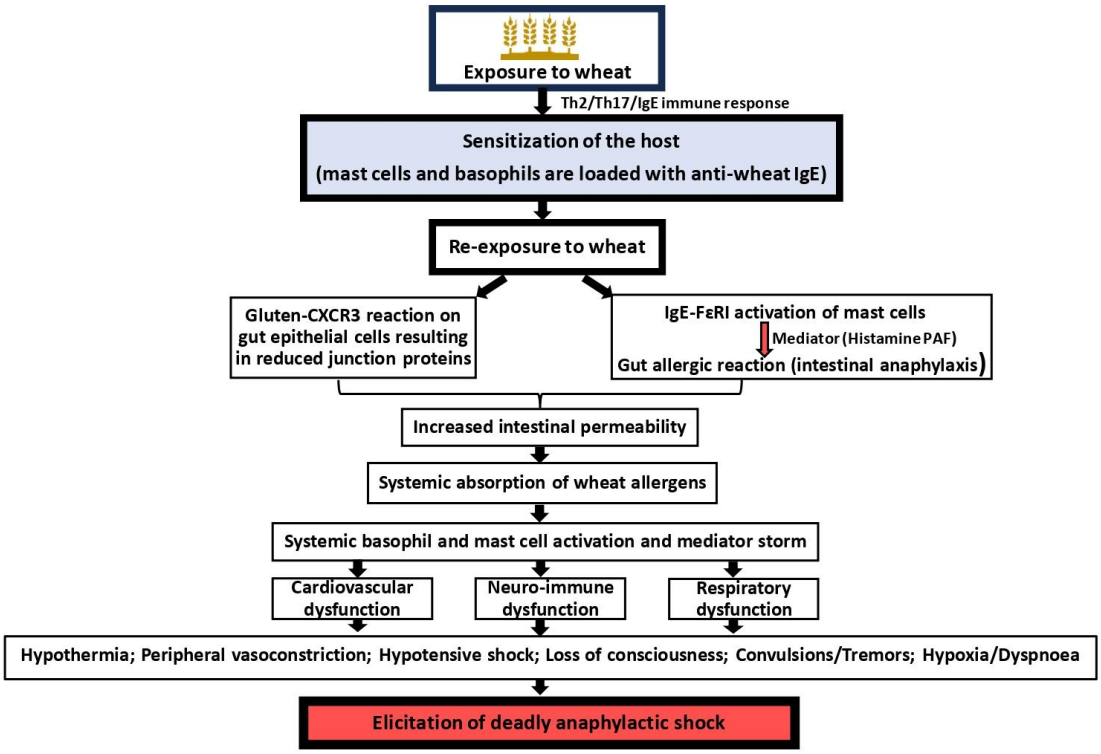

Figure 1: Mechanisms of elicitation of classical wheat-induced anaphylaxis. Exposure of genetically susceptible host to wheat proteins via mouth, skin, eyes, and airways can activate the immune system to produce wheat-specific IgE antibodies. Tissue mast cells and blood basophils bind to IgE via a high-affinity receptor (FcεRI), resulting in sensitization. Subsequent exposure to wheat elicits potentially deadly systemic anaphylaxis via the pathways illustrated in the figure (Jin et al., 2019, 2020; Tanabe, 2008).

Re-exposure of a sensitized host to the same wheat allergen results in the clinical expression of allergic symptoms that can include local intestinal reactions resulting in vomiting and diarrhea; allergic reactions are also usually manifested by skin rashes. If the sensitized host is re-exposed to the allergen via the eyes and nose (as happens in baking/wheat flour industry

workers), allergic reactions are manifested in the respective organs as allergic conjunctivitis and allergic rhinitis. In severe cases, asthma attacks are known as baker's asthma. If the reaction is restricted to this type of symptoms, it is not considered as systemic anaphylaxis. However, if the allergic reaction expands to other body systems besides the gastro-intestinal tract upon consumption of wheat-containing foods, it is regarded as systemic anaphylaxis. For example, simultaneous reactions occurring in the gut as well as the skin, airways, cardiovascular system, and neurological reactions upon consumption of wheat are all considered as systemic anaphylaxis. The mechanism of how a wheat allergic reaction progresses to become a systemic anaphylactic reaction is unclear at present. The current hypothesis is that wheat allergens that survive gastric-intestinal digestion leak into the blood and make their way to different organs of the body, causing allergic reactions systemically (Juhász et al., 2022). There is some evidence that gluten interactions with CXCR3 receptors on the gut epithelial cells can decrease the expression of zonulin proteins, resulting in increased permeability in the gut (Lammers et al., 2008). Additionally, the local allergic reaction in the intestine mediated by gut mast cell degranulation is amplified by unknown factors, resulting in extremely high histamine and PAF production. Intestinal permeability, as well as vascular permeability, will increase facilitating systemic absorption of wheat allergens. These processes are likely dependent on the dose of the allergen consumed, as well as the extent of IgE sensitization and the total number of IgE-sensitized mast cells in the gut. So, the higher the sensitization and mast cell density, the more intense the allergic reaction is expected to be in the intestine. Such a severe reaction is sometimes referred to as intestinal anaphylaxis (Ahrens et al., 2012).

Once the wheat allergens enter the blood stream from the gut, they bind to the IgE-sensitized basophils circulating in the blood, triggering a histamine reaction in the cardiovascular

system. Furthermore, allergens reaching different vital organs via the blood can also cause IgE/FcεR1/mast cell degranulation in different organs, such as the heart, brain, and skin, and the airways. The resulting mediator immune storm is thought to contribute to the clinical symptoms of systemic anaphylaxis. The commonly reported symptoms of wheat-induced systemic anaphylaxis are hypothermia and tachycardia, followed by bradycardia, hypotensive shock, and loss of consciousness. The failure of cardiovascular as well as respiratory functions is attributed to deadly outcome from wheat-induced anaphylaxis. It is noteworthy that specific mediators of systemic anaphylaxis in different vital organs have not been well studied. The most studied mediators of anaphylaxis include histamine, tryptase, and PAF (Nguyen et al., 2021).

Mechanism of Wheat-Dependent Exercise-Induced Anaphylaxis

There is sufficient evidence in the literature regarding the existence of food-dependent exercise-induced anaphylaxis in humans (Srisuwatchari et al., 2023). Wheat is among the implicated foods associated with this condition. Systemic anaphylaxis occurs in some wheat-allergic subjects upon consumption of wheat followed by high-strain physical activity, such as exercising, marathon running, etc. In these individuals, no such reaction is noted after consumption of wheat-containing food products. Mechanisms on how exercise induces anaphylaxis in wheat-sensitized subjects are reported as follows in Figure 2.

Sensitization to wheat allergy in these subjects is the requirement for this condition. The immunological mechanism, genetic predisposition, and the role of environmental factors associated with sensitization to wheat allergens appear to be like those individuals with classical systemic anaphylaxis, as discussed in the previous section. It is unclear as to how and why WDEIA patients can tolerate wheat when they do not perform high-strain physical activity. Upon exercise, three anaphylaxis activation pathways (AAPs) are identified, as discussed below.

AAP-1: Exercise has been shown to increase the expression of pro-inflammatory/allergic cytokine IL-6 (Steensberg et al., 2000). Exercise has also been shown to increase the expression of tissue transglutaminase enzyme activity, leading to increased breakdown of gluten protein into small gliadin peptides that can activate mast cells via FcεR1 signaling.

AAP-2: Exercise causes blood flow redistribution, resulting in transient hyper-osmolality (Miyazaki et al., 2007). Under these conditions, mast cells exhibit enhanced histamine production.

AAP-3: Exercise causes the release of beta endorphins (Schwarz & Kindermann, 1990). Under the influence of these hormones, mast cells exhibit hyperactivity, causing excessive histamine release.

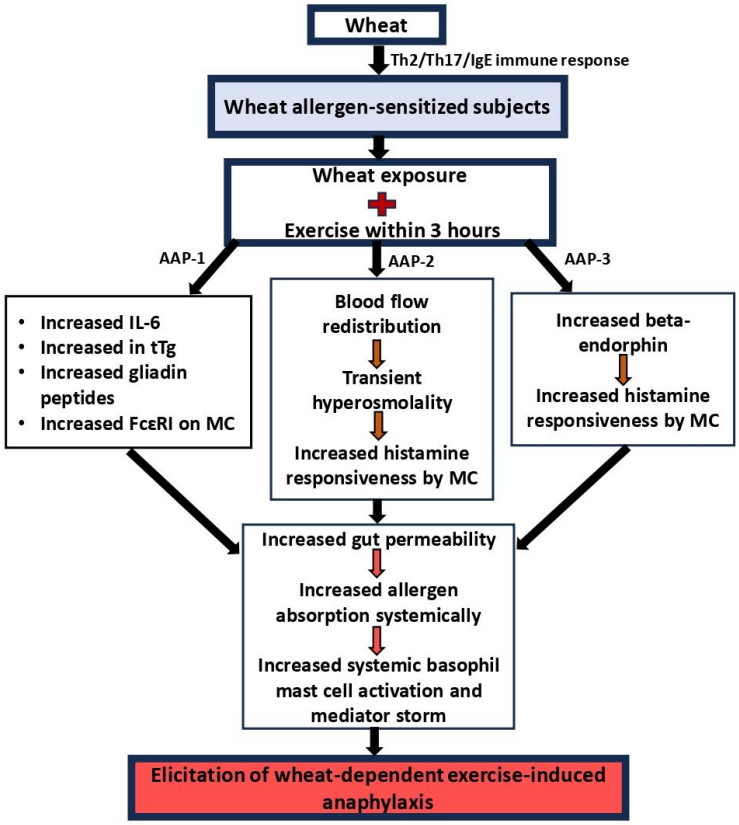

Figure 2: Mechanisms of elicitation of wheat-dependent exercise-induced anaphylaxis. Host sensitization to wheat proteins occurs in a similar fashion as described in Figure 1. However, subsequent exposure to wheat does not elicit systemic anaphylaxis unless the subject undergoes high-strain physical activity such as working out, marathon running, etc., that acts as the inducer of anaphylaxis rather than wheat per se. Although the mechanisms underlying this condition are not completely clear, at least three anaphylaxis activation pathways (AAPs) have been proposed to explain the underlying pathophysiology, as illustrated in the figure (Asaumi et al., 2017).

One or more of the above AAPs results in the same consequence: exaggerated gut permeability leading to leakage of wheat allergens from the intestine into the systemic circulation. Bioactive allergenic peptides can activate basophils in the blood and mast cells in other organs, such as heart and lungs. Thus, it is hypothesized that WDEIA patients can tolerate

wheat when they are not active but develop systemic anaphylaxis upon increased physical activity due to the combination of the above mechanisms.

Defining clearly the pathophysiological mechanisms of WDEIA is an ongoing challenge facing the field. Regarding the mechanisms underlying EIA, the European Academy of Allergy and Clinical Immunology has published a position statement previously (Ansley et al., 2015). According to these experts, the mechanisms proposed in the literature up until 2015 lacked validity. They also recommended that a global research network be formed to address this issue and facilitate improved diagnoses and treatment of EIA. Since then, scientific knowledge in this area has advanced (Srisuwatchari et al., 2024).

Gabler et al. (2022) reported novel findings in WDEIA (Gabler et al., 2021). It is generally assumed that only gluten proteins (ω5 gliadins) cause WDEIA. They studied skin-prick test and basophil activation in 12 patients with WDEIA and 10 control patients. Based on the results, they concluded that non-gluten proteins with unidentified epitopes are relevant in WDEIA and suggested the role of nutritional anti-trypsin inhibitor (ATI) as a potential candidate explaining a novel hypothesis to be researched in the WDEIA mechanism.

Scherf et al. (2019) studied the mechanism of WDEIA with a focus on the absorption of gliadin in the gut (Scherf et al., 2016). They examined the influence of cofactors such as exercise (aerobic and anaerobic), acetyl salicylic acid, alcohol, and pantoprazole on absorption of gliadin, as measured by serum levels of gliadin up to 2 h after consumption. Interestingly, they found the cofactors do not influence the absorption of gliadin in healthy subjects. They suggested that, instead, patients with WDEIA may have a predisposition, such as damaged intestinal epithelial or hyperresponsive intestinal epithelial. Other possibilities suggested including cofactor-induced

blood flow redistribution, increased tissue transglutaminase activity, plasma osmolality, and acidosis, leading to excess histamine release basophils and mast cells.

The Potential Role of Emerging Immune Mediators or Pathways That Have Not Been Extensively Covered in Current Literature

Recent research has identified novel immune pathways in systemic anaphylaxis in peanut allergic subjects and in a mouse model of food allergy using peanut as a model food allergen. Watson et al. (2017) studied acute allergic reactions to peanuts in severely allergic children (n = 19) upon double-blind oral challenges in controlled clinical conditions (Watson et al., 2017). Their goal was to identify the key molecular drivers of severe acute allergic reactions to peanuts that may be similar to systemic anaphylaxis. They identified the following molecular drivers by conducting advanced gene profile analysis using blood samples collected at baseline and at 2 h and 4 h after oral peanut or placebo challenge: LTB4R, PADI4, IL1R2, PPP1R3D, KLHL2, and ECHDC3. These genes were identified as drivers of severe reactions associated with changes in neutrophil, naïve $CD4^+$ T cell, and macrophage. Thus, in contrast to the current paradigm that IgE, IgE^+ B cells, mast cells, and the basophils pathway are critical players in severe allergic reactions, this study proposes a new pathway involving phagocytes and T cells as drivers of severe reactions in peanut allergy. These cells, genes, and pathways may be targeted for severe peanut allergy therapy. Although they used peanut allergic subjects in this study, the results may be applicable to other types of severe food allergic reactions, including wheat allergy, that need to be confirmed. In this study, due to ethical reasons, anaphylaxis was not deliberately induced. However, the reactions were severe enough to treat with epinephrine; therefore, the molecular drivers and pathways of severe allergic reactions may reflect key mechanisms leading to systemic anaphylaxis if untreated with epinephrine.

Using a mouse model, Bao et al. (2023) reported for the first time the involvement of the central nervous system in causing life-threatening anaphylactic shock caused by food proteins (Bao et al., 2023). This study clearly demonstrated how mediators released by the mast cell upon allergen activation engage neurons and the brain to cause life-threatening anaphylactic shock. Hypothermic shock response as measured by a drop in body (rectal) temperature is a widely used objective measure of systemic anaphylaxis in rodent models of food allergy and other types of allergic reactions. It is generally assumed that it is caused by IgE/mast cell interaction. In this paper, authors identified the involvement of the body's thermoregulatory neural circuits in hypothermic shock reaction. They identified mast cell-derived chymase as an activator of $TRPV1^{+}$-sensitive neurons via protease-activated receptor-1. Because of this signal, the regulatory neural network stops brown adipose tissue-mediated thermal regulation, resulting in hypothermic reaction. This was an unexpected pathway in systemic anaphylaxis. Although this study used trinitrophenol (TNP)-specific IgE and TNP-ova albumin protein as a model allergen, these bindings may be relevant for wheat anaphylaxis, although this needs to be confirmed. Thus, a novel pathway and therapeutic target (chymase, mast cell chymase, and TRPV1) for anaphylaxis have been identified for further research.

Mechanism of Other Cofactor-Induced Anaphylaxis

It is reported that some wheat-allergic subject drugs, such as NSAIDs, alcohol, and infections can act as cofactors, resulting in a loss of tolerance to wheat, expressed as systemic anaphylaxis. The specific mechanisms of these conditions are little known. It is hypothesized that NSAIDs bind to membrane phospholipids and cause mitochondrial dysfunction, leading to gut damage (Lichtenberger et al., 2010). Such loss of tissues can facilitate allergen absorption

into the systemic compartment. Furthermore, NSAIDs can increase calcium channel activity and lipid-derived mediators of anaphylaxis that can increase mast cell responses.

Mechanisms on how alcohol and infections can act as cofactors for triggering systemic anaphylaxis are not well studied. Acetaldehyde produced upon metabolism of alcohol can act as a non-specific activator of mast cells (Kawano et al., 2004).

Some infections, such as bacterial and viral infections, can act via innate immune receptors (e.g., TLRs) via the PAMP/PRR pathway that can release pro-inflammatory mediators, thus facilitating the absorption of allergens, subsequently triggering systemic anaphylaxis (Shamim et al., 2024; Xia et al., 2021).

Utility of Rodent Models in Elucidating Mechanisms of Anaphylaxis in Wheat Allergy

Rodent Models of Classical Anaphylaxis in Wheat Allergy

Adjuvant-Based Models

There are several rodent models reported in the literature to study wheat-induced anaphylaxis in wheat-sensitized animals using adjuvants such as alum, salicylic acid, and detergents. Most rodent models have used adjuvants (alum, detergent, etc.,) to elicit robust sensitization to wheat proteins (Table 3). Although the role of detergents in causing sensitization to gluten in the context of cosmetic exposure is plausible, alum adjuvant is not expected to be involved in human sensitization to wheat proteins. The use of adjuvants in rodent models helps in creating robust readouts of sensitization (for example, high levels of wheat-specific IgE antibodies) very quickly by activating inflammatory conditions at the site of injection of a mixture of wheat allergen plus adjuvant. Although, technically, it is easy to do this, the underlying mechanism of sensitization for anaphylaxis is different compared to the sensitization method that does not involve adjuvants (Jin et al., 2019). Previously, researchers have expressed

concerns about using adjuvant-based models for determining the intrinsic allergenicity of proteins because this approach increases sensitivity at the loss of specificity of the reactions (Dearman & Kimber, 2009). Therefore, some researchers have proposed that adjuvant-free methods are ideal for establishing the intrinsic allergenicity of any protein, including conventional food proteins such as wheat allergens, as well as novel food proteins (for example, genetically engineered food proteins and chemically/physically altered food proteins) (H. Gao et al., 2021, 2022; Jorgensen et al., 2023; Jorgensen et al., 2023). Thus, adjuvants have the potential to influence sensitization as well as the underlying mechanisms. Therefore, their use can influence anaphylactic reactions indirectly.

With the exception of one report that used guinea pigs, all others used mice or rats. The major characteristics of these models are reviewed below.

Table 3. Adjuvant-based rodent models of wheat-induced anaphylaxis.

Model	Anaphylaxis Severity	Mechanism	Suggestions for Improvement
B10 female mice and ICR mice; Sensitization: IP injection of gliadin with alum adjuvant. Elicitation: oral high dose gliadin, high dose ω5-gliadin for 30 min to induce WIA (Tanaka et al., 2011)	Mild HSR (<2 °C drop in temperature in 30 min).	SIgE, elevation of proteins in the portal blood after oral challenge at 1 hr	Study cytokines, histamine, and other mediators

Table 3 (cont'd)

Female BALB/c mice; Sensitization: TDE (tape stripping); gluten + adjuvant Elicitation: IP challenge, gluten to induce HSR * (Adachi et al., 2012)	Severe; HSR: (more than 3 °C, 30 min); anaphylactic score: 3 vs. 0 in control;	Histamine, Th1/Th2 cytokines, IgE, IgG1	Study mMCP-1, chemokines, and other immune markers
Male Kud: Hartley guinea pigs; Sensitization: fasting 16 h + intragastric administration with salicylic acid and, 1 h later, gluten solution. Repeated for 9 days; Elicitation: IP injection of gliadin to elicit classical anaphylaxis reaction (Kohno et al., 2016)	Classical systemic anaphylaxis (Severe: 4–6 in IP group)	Unknown	Study: (1) time to exhaustion; (2) antibodies: SIgE/SIgG1; (3) mediators (histamine, cytokines, etc.)
Female Balb/c mice; Sensitization: IP; SSP+ Alum. Elicitation: IP, SSP to elicit HSR (Jin et al., 2017, 2020)	Modest severity; HSR: (3 °C, 30 min;)	SIgE, TIgE, mMCP-1, spleen cytokine, chemokine, adhesion molecule.	Study histamine

Table 3 (cont'd)

Male Bn rats; Sensitization: IP, ω5-gliadin + Alum. Elicitation: IV, ω5-gliadin or gluten extract to elicit HSR (Yamada et al., 2019)	Very mild; HSR: (0.4 °C, 30 min)	Unknown	Study antibodies, mediators (histamine, cytokines, etc.)
Female BN rat; Sensitization: TCI gluten ω5-gliadin + alum, SC. Elicitation: IV, TCI gluten or ω5-gliadin to elicit HSR (Yamada et al., 2022)	Very mild; HSR: (approx. 0.8–1.4 °C, 30 min)	SIgE and SIgG1	Study mediators (histamine, cytokines, etc.)
Female Balb/c mice; Sensitization: IP, peptin + trypsin digested gluten + alum. Elicitation: IP, gluten to elicit HSR ** (Fu et al., 2022)	Modest Severity; HSR: (2.5–3 °C, 30–60 min); Clinical scores: 2–3 by 60 min	SIgE, increased mast cell number in duodenum. Reduced Th1 cytokine (spleen)	Study other cytokines, histamine, and other mediators

* Allergy scores were calculated as follows: 0 = no symptoms; 1 = pilar erecti, scratching, and rubbing around the nose and head; 2 = redness and puffiness reaction, diarrhea; 3 = wheezing, labored respiration, and cyanosis around the mouth and tail; 4 = death (Adachi et al., 2012). **

Table 3 (cont'd)

Allergy scores were calculated as follows: 0 = no symptoms; 1 = repetitive mouth/ear scratching and ear canal digging with hind legs; 2 = decreased activity, self-isolation, and puffiness around the eyes and/or mouth; 3 = periods of motionlessness for more than 1 min, lying prone on stomach, and decreased activity; 4 = no response to whisker stimuli and reduced or no response to probing; 5 = tremor, convulsion, and death (Fu et al., 2022). Abbreviations used in the table: WIA—wheat-induced anaphylaxis; HSR—hypothermic shock response; SSP—salt-soluble protein; IP—intraperitoneal; SC—subcutaneous.

Tanaka et al. (2011) developed the first mouse model of wheat-induced anaphylaxis. Animals were sensitized to gliadin via an IP injection along with an alum adjuvant (Tanaka et al., 2011). Oral challenge with a high dose of ω-5 gliadin was conducted to induce anaphylaxis. Sensitization was confirmed by SIgE measurements, and anaphylaxis was quantified by HSR. Sensitized mice developed mild reactions within 30 minutes post-challenge. The strengths of the model are as follows: (i) the measurement of SIgE and (ii) the demonstration of appearance of allergens in the portal blood after oral challenge at 1 h. The limitations of the study are as follows: (i) mediators were not studied and (ii) non-gliadins were not studied.

Adachi et al. (2012) reported a transdermal sensitization mouse model using the tape stripping method followed by application of gluten + SDS detergent (Adachi et al., 2012). Anaphylaxis was elicited by IP challenge with gluten, and sensitization was measured using SIgE and SIgG1. It was noted that sensitized mice developed severe HSR (3 °C drop in rectal temperature at 30 min), as well as clinical symptom scores (3). The strengths of this study include the following: (i) the measurement of SIgE and IgG1; (ii) histamine measurement; and (iii) the study of spleen TH1 and TH2 cytokine. The limitations of the model are as follows: (i)

repeated tape stripping (10 times) of the top layer of skin raises animal well-fare issues; (ii) an oral challenge was not conducted; and (iii) mMCP-1 was not studied.

Jin et al. (2017) reported a mouse model of anaphylaxis using IP sensitization with SSP and alum (Jin et al., 2017). Disease elicitation was conducted by IP challenge with SSP. Mice exhibited modest clinical reaction of anaphylaxis, as evidenced by HSR (3 °C). The strengths of this model are as follows: (i) the measurement of SIgE, TIgE, mMCP-1, and spleen immune biomarkers. The limitations of this study include the following: (i) there was no study of glutens; (ii) there was a lack clinical symptoms scoring; and (iii) histamine measurements were not carried out.

Fu et al. (2022) reported a mouse model of anaphylaxis using enzyme digested in gluten (Fu et al., 2022). Mice were sensitized by IP injection of protein plus alum adjuvant. Disease was elicited by IP challenge with gluten through IP route. They reported modest anaphylaxis, as evidenced by HSR (2.5–3 °C drop) and clinical symptoms score (2–3). The strengths of the study include the following: (i) the SIgE measurement; (ii) the Th1 and Th2 cytokine measurement; and (iii) the measurement of mast cell numbers in the intestine. The limitations of the study include the following: (i) the lack of measurement of histamine and mMCP-1.

Kohno et al. (2016) reported a guinea pig model of anaphylaxis using intragastric sensitization of gluten with salicylic acid (Kohno et al., 2016). Disease elicitation was conducted by IP challenge with gliadin. They reported severe anaphylaxis (4–6 clinical symptom score). The strengths of the model include the following: (i) this is the first guinea pig model of wheat-induced anaphylaxis and (ii) oral sensitization was measured. The limitations of the model include the following: (i) no antibodies were studied and (ii) mediators were not studied. Therefore, the immune basis for the observed clinical symptoms of anaphylaxis is unclear.

Yamada et al. (2019) reported the first rat (male) model of gliadin/gluten-induced anaphylaxis (Yamada et al., 2019). The rats were sensitized by IP injections with gliadin and alum. Disease elicitation was conducted by IV challenge with ω-5 gliadin/gluten. They reported very mild HSR (0.4 °C in 30 min). The strengths of this model include the following: (i) this was the first rat model of ω-5-induced anaphylaxis. The limitations of the study include the following: (i) no antibodies were studied and (ii) mediators were not studied. Thus, the immune basis of the observed mild HSR is unclear.

Yamada et al. (2022) reported a rat model of ω-5 gliadin/gluten-induced anaphylaxis using female rats (Yamada et al., 2022). The animals were sensitized by SC with proteins and alum adjuvant, and disease elicitation was carried out by IV challenge with the respective proteins. They reported very mild anaphylaxis evidenced by HSR (approximately 0.8–1.4 °C). The strengths of the model are as follows: (i) measurement of SIgE and SIgG1 was performed and (ii) this was the first animal model to use the SC route for sensitization and IV routes for challenge. The limitations of this model include the following: (i) mediators were not studied and (ii) non-gluten proteins were not studied.

In summary, most adjuvant-based models have used mice, followed by rats. The mouse models produced anaphylaxis reactions varying from mild to severe. It was observed that rat models produced very mild reactions. Additionally, only one guinea pig model reported severe reactions. The mechanisms of anaphylaxis were studied in mice, to a limited extent in rats, and not at all in guinea pigs. Thus, there is a critical need to advance the development of improved models examining mechanisms of anaphylaxis in more detail. For example, whether the use of an adjuvant itself contributes to increasing the ability of wheat proteins to cause anaphylaxis remains to be addressed.

Adjuvant-Free Models

There are three studies reported to develop adjuvant-free mouse models of WIA using salt-soluble protein, alcohol-soluble gliadin, and acid-soluble glutenin (Table 4).

Table 4. Adjuvant-free rodent models of wheat-induced anaphylaxis.

Model	Anaphylaxis Severity	Mechanism	Suggestions for Improvement
Female Balb/c mice; sensitization: TDE, SSP; Challenge: Oral, SSP to elicit HSR (H. Gao et al., 2022, 2023)	Severe; HSR: (around 3.5°, 30 min)	TIgE, SIgE, SIgG1, mMCP-1, spleen immune biomarkers	Study histamine, IP challenge
Female Balb/c mice; Sensitization: TDE; Gliadin; Challenge: IP/oral Gliadin to elicit HSR * (Jorgensen et al., 2023)	Life-threatening; HSR: (8 °C, 30 min); Clinical symptoms scores 4	TIgE SIgE, mMCP-1, spleen immune biomarkers	Study histamine, oral challenge
Female Balb/c mice; Sensitization: TDE; Glutenin; Challenge: IP Glutenin to elicit HSR * (Jorgensen et al., 2023)	Life-threatening; HSR: (8 °C, 30 min); Clinical symptom score: 4 vs. 0 in control	TIgE SIgE, mMCP-1, spleen immune biomarkers	Study histamine, oral challenge

Table 4 (cont'd)

* Allergy scores were calculated as follows: 0 = no symptoms; 1 = scratching and rubbing around the nose and head; 2 = puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity and/or decreased activity with a noted increase of respiratory rate; 3 = wheezing, labored respiration, and cyanosis around the tail and the mouth; 4 = no activity after prodding, tremors, and convulsions; 5 = death (Jorgensen et al., 2023); Abbreviations used in the table: HSR—hypothermic shock response; SSP—salt-soluble protein; IP—intraperitoneal.

Gao et al. (2022, 2023) used a transdermal exposure (TDE) method to induce sensitization to SSP without using adjuvants such as alum, salicylic acids, or detergents (H. Gao et al., 2022, 2023). Anaphylaxis was elicited by oral challenge with SSP. They reported severe anaphylaxis, as evidenced by HSR (3.5 °C, 30 min). The strengths of the model include the following: (i) it achieved skin exposure without damaging the skin, such as through the tape stripping used in other models; (ii) it used the oral route for disease elicitation; (iii) it studied IgE and IgG1 antibodies; (iv) it studied mMCP-1; and (v) it studied spleen immune biomarkers. The limitations include of the model include the following: (i) they did not study histamine and (ii) IP challenges were not carried out to induce HSR.

Jorgensen et al. (2023) reported the first adjuvant-free mouse model of anaphylaxis induced by gliadin (Jorgensen et al., 2023). Sensitization was conducted by TDE with gliadin. Disease was elicited by IP challenge with gliadin. They reported a life-threatening dramatic reaction of anaphylaxis, as evidenced by HSR (8 °C in 30 min). The strengths of this model include the following: (i) it was the first model to develop life-threatening anaphylaxis reactions and (ii) it studied IgE, mMCP-1, and spleen immune biomarkers. The limitations of the study include the following: (i) it did not report histamine responses.

Jorgensen et al. (2023) reported the first adjuvant-free mouse model of glutenin-induced anaphylaxis (Jorgensen et al., 2023). Mice were sensitized by TDE with glutenin. Disease was elicited by IP challenge with glutenin. They reported life-threatening anaphylaxis, as evidenced by HSR (8 °C, 30 min) and a clinical symptom score of 4. The strengths of this model include the following: (i) it was the first adjuvant-free animal model of glutenin-induced life-threatening anaphylaxis and (ii) it studied IgE, mMCP-1, and spleen immune biomarkers. The limitations of the study are as follows: (i) it did not report histamine response and (ii) it did not report oral challenge.

In summary, adjuvant-free skin sensitization mouse models have been developed for all three types of wheat allergen-induced systemic anaphylaxis. These models provide a unique opportunity to dissect the mechanisms of severe and life-threatening anaphylaxis induced by wheat allergens. Furthermore, they also provide the opportunity to determine the impact of food processing on altering the intrinsic capacity of wheat allergens to elicit anaphylaxis.

Rodent Models of WDEIA

There are three papers published using mice (2) and guinea pigs (1) on this topic (Table 5). The first model was based on using mice (Kozai et al., 2006). In this model, the authors used SSP, gliadin, and glutenin with alum to sensitize by using IP injection. Later, mice were orally challenged with protein and subjected to exercise, with measurement of time used for exhaustion. Sensitized mice exhibited SIgE for gliadin and glutenin but not for salt-soluble protein. Gliadin and glutenin-sensitized mice were exhausted by about 30 and 50 mins, respectively. SSP-sensitized mice did not significantly differ from the control—unsensitized mice. The strengths of the study were as follows: (i) it used three types of proteins and (ii) it was the first published animal model study on WDEIA. The limitations of the study are as follows:

(i) it did not study clinical symptom scores and HSR; (ii) there are missing controls for V/P and P/P groups; and (iii) it did not study mediators.

Table 5. Rodent models of wheat-dependent exercise-induced anaphylaxis.

Model	Anaphylaxis Severity	Mechanism	Suggestions for Improvement
Female B10.A mice; Sensitization: IP, protein (salt-soluble protein, gliadin, and glutenin) + alum; Elicitation: Oral protein + treadmill to induce WDEIA (Kozai et al., 2006)	Treadmill exhaustion time: gliadin and glutenin 35–50 min vs. control 150 min vs. control (*v*/*v*) 200 min	SIgE; Poor response to SSP and good response to gliadin and glutenin. Mucosal lesions in small intestine, leakage of proteins into blood and liver after challenge	Controls missing for V/P and P/P without exercise; Therefore, unclear if this model is truly WDEIA or just WIA; Study mediators

Table 5 (cont'd)

B10 female mice and ICR mice; Sensitization: IP injection of gliadin with alum adjuvant. Elicitation: oral gliadin + treadmill for 30 min to induce WDEIA (Tanaka et al., 2011)	Mild HSR (1.5 degrees drop in temperature in 30 min). Treadmill exhaustion test: the mice were exhausted by 3 h and remained so up to 9 h (revolutions stay <400 up to 9 h post-challenge vs. around 1000 in control) control—unsensitized mice orally challenged with vehicle (acetic acid 0.1 M).	SIgE	Controls missing for V/P and P/P without exercise; Therefore, unclear if this model is truly WDEIA or just WIA; Study cytokines, histamine, and other mediators
Male Kud: Hartley guinea pigs; Sensitization: fasting 16 h + intragastric administration with salicylic acid and, 1 h later, gluten solution. Repeated for 9 days; Elicitation: oral gluten + treadmill for 30 min to elicit WDEIA (Kohno et al., 2016)	WDEIA clinical symptom scores * (Mild: 1–1.4 in oral + exercise group)	Unknown	Controls missing for V/P and P/P without exercise; Therefore, unclear if this model is truly WDEIA or just WIA; Study: (1) time to exhaustion: (2) antibodies: SIgE/SIgG1; (3) Mediators (histamine, cytokines, etc.)

Table 5 (cont'd)

* Allergy scores were calculated as follows: 0 = no symptoms; 1 = pilar erecti, scratching and rubbing around the nose and head; 2 = redness and puffiness reaction, diarrhea; 3 = wheezing, labored respiration, and cyanosis around the mouth and tail; 4 = death (Kohno et al., 2016). Abbreviations used in the table: WDEIA—wheat-dependent exercise-induced anaphylaxis; WIA—wheat-induced anaphylaxis; HSR—hypothermic shock response; SSP—salt soluble protein; IP—intraperitoneal; V/P—vehicle/protein; P/P—protein/protein.

Tanaka et al. (2011) reported a mouse model of WDEIA sensitization, which was conducted using gliadin and alum adjuvant via intraperitoneal injection (Tanaka et al., 2011). Mice were later orally challenged with gliadin and subjected to exercise for 30 min. Mice developed mild anaphylaxis. Upon treadmill exercise, sensitized mice were exhausted by 3 h as opposed to control mice, which were running for up to 9 h. The strengths of the study are as follows: (i) measurement of HSR and (ii) measurement of SIgE. The limitations of this study are as follows: (i) mediators such as histamine were not measured and (ii) control groups were missing for v/p and p/p without exercise group. Therefore, it is unclear if the model is truly WDEIA or simply WIA.

Kohno et al. (2016) developed a guinea pig model using gluten and salicylic acid as an adjuvant (Kohno et al., 2016). Sensitization was conducted by oral route. Disease elicitation was carried out after oral gluten challenge plus exercise for 30 min. Clinical symptoms scores were recorded; the studied reported mild symptoms upon exercise in sensitized guinea pigs.

In summary, there are limited efforts to develop rodent models of WDEIA. The reported models show mild symptoms of anaphylaxis. However, the absence of additional control groups prevents confirmation of the exercise dependency of observed anaphylaxis in these models.

Future studies are required to further develop and validate robust rodent models of WDEIA by including quantifiable readouts of anaphylaxis and analysis of mediators of anaphylaxis, such as histamine measurements.

Lessons Learnt from the Rodent Models and Potential Utility to Advance the Field

Extensive research using rodent models of wheat allergy-associated anaphylaxis has advanced science with the following critical knowledge and opportunities for future research agendas:

1. All three species of laboratory rodents (rats, guinea pigs, and mice) can be used to develop models simulating the two critical aspects of human disease—namely, sensitization, as demonstrated by induction of wheat-specific IgE antibodies, and elicitation of systemic anaphylaxis, as demonstrated by clinical disease and/or disease markers, such as hypothermic response.
2. Similar to humans, both gluten (gliadin and glutenin), as well as non-gluten proteins, elicit sensitization and anaphylaxis in rodents.
3. In the reported rodent models, the symptoms of anaphylaxis vary broadly from mild to moderate, severe, and life-threatening reactions; this spectrum of variation is also noted in humans.
4. In rodent models, sensitization is typically quantified by measuring wheat-specific IgE antibodies; there are no reports of developing skin testing in rodents, in contrast to the skin-prick test commonly carried out in humans to determine sensitization. However, in dog models of wheat food allergy, such tests are routinely performed (Buchanan & Frick, 2002). Therefore, it may be possible to develop such a test in rodents in the future.

5. In rodent models, identified immune mediators associated with anaphylaxis include not only a selected set of Th2/Th17 cytokines and chemokines but also histamine, PAF, and mMCP-1. There is ample scope to expand the mediator analysis to include novel targets for potential diagnosis and therapy.
6. There are two mouse models and one guinea pig model of WDEIA. In a mouse model, it was demonstrated that exercise leads to lesion formation in the intestine associated with increased gut permeability and leakage of glutenin allergen into portal circulation and appearance in the liver. However, in these studies, appropriate controls were not used. Therefore, it remains to be clarified whether leakage of allergens to the liver from the gut is caused by exercise or whether such leakage happens in classical wheat-induced anaphylaxis. The guinea pig mouse model provides another useful model to study mechanisms of WDEIA, for which, currently, there is very limited information in the literature (Kohno et al., 2016).
7. There is strong direct evidence from rodent models that exposure to wheat proteins (both glutens and non-glutens) via undamaged skin can clinically sensitize the host to subsequent life-threatening systemic anaphylaxis caused by wheat proteins. These findings have further bolstered the case for wheat anaphylaxis as an occupational public health issue in the food industry (e.g., baking) where such exposures must be closely monitored, prevented, and managed.
8. There are no rodent models reported for wheat-dependent alcohol, drug, or infection-induced systemic anaphylaxis at present; clearly, rodent models are needed in this area.

9. Rodent models provide ample opportunity to elucidate the role of genetic and environmental factors in determining anaphylaxis in wheat allergies; however, they have not been explored so far—they therefore constitute areas for further research.
10. There is growing evidence that the food and industrial processing of wheat proteins can influence its allergenic properties; therefore, rodent models can be employed to determine the impact of processing technology on the anaphylaxis-eliciting properties of wheat proteins.
11. There is growing interest in using rodent models to test novel genetically modified wheats for food safety assessment; currently, this has been carried out using rats and guinea pigs, but mouse models offer improved opportunities for this application (Jorgensen et al., 2024).
12. Currently, there are extensive efforts in the field of biomarker research to advance disease diagnosis, prevention, and management of anaphylaxis in general (Beck et al., 2019; Dass et al., 2020; Fernandez-Bravo et al., 2022; Pampura et al., 2024); rodent models of wheat anaphylaxis are expected to be critical tools to advance this area of research.

Limitations of Rodent Models and Challenges in Translating Findings from Rodent Models to Human Clinical Settings

There are several limitations that must be considered when translating findings from rodent models of anaphylaxis to human clinical settings, including the following:

1. In humans, specific mechanisms of sensitization to wheat are thought to occur upon oral ingestion of wheat-containing foods, although wheat dust inhaled in bakery settings and skin exposure to gluten via cosmetics (soaps, detergents, shampoos, etc.) is also reported (Chinuki & Morita, 2012; Cho et al., 2011; Hur et al., 2013; Iga et al., 2013; M. A. Kim et

al., 2017; S. H. Kim et al., 2012). In contrast, rodent models generally use sensitization methods that are artificial (for example, IP injections) (Tables 3–5).

2. In humans, sensitization to wheat proteins occurs after exposure to a complex mixture of proteins as they exist in the food matrix. In contrast, purified wheat proteins [glutens (gliadins, glutenin) and non-glutens (albumin and globulins)] are used in most rodent models (Tables 3–5).
3. Most rodent models have used adjuvants such as alum and detergents to elicit detectable sensitization to wheat proteins (Tables 3–5); although the role of detergents in causing sensitization to gluten in the context of cosmetic exposure is plausible, alum adjuvant is not expected to be involved in human sensitization to wheat proteins (Adachi et al., 2012; Iga et al., 2013; Noguchi et al., 2019).
4. Human wheat anaphylaxis is reported after oral exposure to wheat-containing foods (Cianferoni, 2016; Cianferoni & Spergel, 2009). In contrast, in rodent models, except for studies conducted by Gao et al. (2022, 2023), Tanaka et al. (2011), Jorgensen et al. (2023), Kohno et al. (2016), and Kozai et al. (2006), where oral wheat protein challenges were carried out to elicit systemic anaphylaxis, all other studies used intraperitoneal or intravenous challenge to elicit anaphylaxis (Tables 3–5) (H. Gao et al., 2022, 2023; Jorgensen et al., 2023; Kohno et al., 2016; Kozai et al., 2006; Tanaka et al., 2011). Unlike in humans, where IgE primarily causes wheat anaphylaxis upon oral exposure to wheat allergens, in mouse models, anaphylaxis upon IP challenge with wheat allergens involves both IgE- and IgG1-mediated activation mechanisms (Finkelman et al., 2005).

5. In humans, anaphylaxis is generally reported after exposure to wheat present in cooked food (Cianferoni, 2016). In contrast, all rodent models have used raw wheat proteins for eliciting anaphylaxis and inducing sensitization (Tables 3–5).
6. All rodent models used inbred strains of animals that are expected to be genetically identical for each type of strain. Therefore, results from such studies must be interpreted carefully for translation to humans, where the population is outbred in nature. There is ample opportunity to develop outbred rodent models to simulate human wheat anaphylaxis. Such efforts are already in place for other human diseases, including asthma, obesity, diabetes, and cardiovascular diseases (Anderson et al., 2023; Guo et al., 2024; Sweet et al., 2025; Tokuda et al., 2009; Williams et al., 2025).

Current Efforts and Future Directions to Refine the Rodent Models to Better Mimic the Full Spectrum of Human Anaphylactic Reactions and the Influence of Cofactors

Classical systemic anaphylaxis to wheat in humans is a complex immune-mediated reaction involving interaction between various wheat proteins (a mixture of gliadins, glutenin, albumins, and globulins in specific ratios as a component of the food matrix) and protein-specific IgE antibodies present on mast cells and basophils (Faihs et al., 2023; Scherf et al., 2016). Keeping this in mind, rodent models require further refinement to simulate human exposure conditions. Currently existing rodent models show variability and in full spectrum of anaphylaxis (ranging from very mild to mild, moderate, severe, and life-threatening reactions). However, dose responses in animal models have not been determined completely. For example, it is possible to determine the lowest observable adverse effect levels (LOAELs) and no observable adverse effect levels (NOAELs) in rodent models. Then, the validity of the rodent response in rodent models needs to be compared to human LOAELs and NOAELs.

Cofactor-dependent (exercise, alcohol, infections, drugs, etc.) wheat anaphylaxis in humans has been well established, with incompletely understood mechanisms (Ansley et al., 2015; Asaumi et al., 2017; Brockow et al., 2015; Chinuki & Morita, 2012; Faihs et al., 2023; Fukunaga et al., 2021; Gabler et al., 2021; Harada et al., 2001; Iga et al., 2013; Matsukura et al., 2010; Mizuno et al., 2015; Morita et al., 2023; Motomura et al., 2017; Schwarz & Kindermann, 1990; Steensberg et al., 2000) (Figure 2). This is the area where very limited and less optimal work has been carried out in rodent models, with reports only on WDEIA. Clearly there is ample opportunity to improve rodent models of WDEIA as well as to create novel models of alcohol-, infection-, and drug-dependent wheat anaphylaxis models in future. Such models will be valuable both for basic work on mechanisms and for translational work involving novel therapeutic development, as well as for vaccine development for wheat anaphylaxis in humans.

Conclusions and Future Directions

Wheat allergy associated with anaphylaxis is a major food safety problem at the global level. All four groups of wheat allergens (gliadins, glutenin, albumin, and globulin) exhibit intrinsic allergenicity and anaphylaxis-eliciting properties. Wheat-associated anaphylaxis is a complex clinical phenotype. Current knowledge identifies two broad groups of wheat anaphylaxis—namely, classical wheat-induced anaphylaxis and wheat-dependent exercise-induced anaphylaxis. However, additional cofactors such as alcohol, drugs, and infections can also precipitate anaphylaxis in wheat-allergic subjects who can otherwise tolerate wheat consumption. There is a growing critical need discussed in literature to advance the mechanisms underlying life-threatening versus non-life-threatening allergic reactions to wheat. The identification of specific immune biomarkers to distinguish various types of anaphylaxes in wheat allergies is a major research need at present. Several rodent models of wheat-induced

anaphylaxis and WDEIA with their own strengths and limitations are described in the literature. These models provide unique opportunities to study mechanisms and biomarker discovery in this field. This research has identified the complexity associated with wheat-induced anaphylaxis in humans as well as rodent models. We have identified specific gaps and opportunities for future research to enable the development of improved methods for prevention, treatment, and management of wheat-induced anaphylaxis. Overall, this study represents a comprehensive updated critical resource for the development of future research agendas in wheat-allergy-associated anaphylaxis using rodent models as very attractive pre-clinical tools for this endeavor.

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Chapter 3: Organ-specific immune biomarker signatures of the small intestine, liver, heart and lungs in a novel mouse model of glutenin-induced life-threatening systemic anaphylaxis

Abstract

Glutenin-induced life-threatening systemic anaphylaxis (GILTSA) is a serious food safety and public health issue growing at an alarming rate in the United States of America. A novel adjuvant-free mouse model of GILTSA was developed by Dr. Gangur and colleagues at Michigan State University. This model was used to test the following hypothesis in this thesis work: GILTSA will be associated with substantial elevation of a selective set of organ-specific immune biomarkers in the small intestine, liver, heart and lungs. Organs collected from mice with GILTSA (n = 10) and from 3 groups of healthy mice (n = 10 per group) were used in the analysis. Tissue protein extracts were used in protein microarray analysis of 640 immune biomarkers. Differentially expressed immune biomarkers were identified. The immune biomarkers markers that were increased by 25-fold or higher in mice with GILTSA compared to the control groups were identified as the most critical signature immune biomarkers of GILTSA as follows: In the small intestine, 2 signature immune biomarkers were identified; in the liver, 6 signature immune biomarkers were identified; in the heart, 12 signature immune biomarkers were identified; and in the lungs, 5 signature immune biomarkers were identified. These signatures were associated with the following immune pathways: Th2 immune activation pathways, cytotoxic immune activation pathway, tissue repair/remodeling pathway, metabolic regulation pathway, vascular integrity pathway, and immune homeostasis pathways. Some of the signature immune biomarkers identified may have therapeutic potential in GILTSA. Findings from this study have advanced the molecular mechanisms of GILTSA. This approach may also be used to identify signature immune biomarkers in other types of food anaphylaxis.

Introduction

Food allergy is caused due to hypersensitive immune reactions to food protein allergens (Renz et al., 2018; Sicherer & Sampson, 2018). Its prevalence and severity have been increasing in many countries including the United of America (USA); severe life-threatening reactions are known as systemic anaphylaxis (R. Gupta et al., 2013; R. S. Gupta et al., 2011; Renz et al., 2018). Many countries including the USA, Canada, the European Union, Australia and New Zealand, regulate 9-14 major foods as allergenic foods, including wheat, milk, soybean, egg, fish, tree nuts, sesame, peanut, shellfish, lupin, mustard and celery; the first 9 foods are regulated as major allergenic foods in the USA. (European Food Safety Authority, 2025; Food Standards Australia New Zealand, 20254; Health Canada, 2017; U.S. Food and Drug Administration, 2025). Thus, wheat is regulated as a major allergenic food in many countries.

Wheat is one of the three major staple foods that are consumed in the world along with rice and corn, with its consumption rate set to increase by 11 percent by 2031 (OECD-FAO Agricultural Outlook 2021-2030, 2021). Wheat allergy is highly prevalent in the USA among both adults (0.9-3.6%) and children (0.2-1.3%) (Poole et al., 2006; Venter et al., 2006, 2006, 2008; Verrill et al., 2015; Vierk et al., 2007). Furthermore, wheat glutenin-induced life-threatening systemic anaphylaxis (GILTSA) is also a major public health and food safety concern (Cabanillas, 2020; Cianferoni et al.,2016).

Food allergies including wheat allergies are thought to develop into two phases: phase i) Sensitization to food allergens upon initial exposure via oral, nasal, skin, conjunctival routes; during this phase, immune system produces IgE antibodies to food allergens. This process is called sensitization (Jin et al., 2019; Sicherer & Sampson, 2018); and phase ii) When sensitized subjects are re-exposed to wheat allergens, these allergens bind to the IgE antibodies present on

the mast cells and basophils, triggering the release of histamine and other mediators. This reaction causes various symptoms, including GILTSA (Cianferoni 2016; Gao et al., 2021; Jin et al., 2019; Reber et al., 2017; Renz et al., 2018). Although this basic mechanism leading to GILTSA is well established, specific molecular immune changes in different vital organs during GILTSA are not well studied. Improved definition of organ specific changes in immune pathways in vital organs is urgently needed to facilitate development of novel therapeutic and management methods (Arul Arasan et al., 2025, Faihs et al., 2023, Borque et al., 2022). In this thesis work, immune biomarkers in the small intestine, liver, heart and lungs were investigated. Wheat proteins can be broadly categorized into gluten and non-gluten proteins, both of which are linked to allergies and anaphylaxis in humans (Pastorello et al., 2007; Ricci et al., 2019). Non-gluten proteins are related to metabolic and structural functions, and can further be classified into albumins, which are water-soluble, and globulins, which are salt-soluble (de Sousa et al., 2021).

Gluten proteins are seed storage proteins and can be categorized into gliadins and glutenins. Gliadins are ethanol-soluble prolamin proteins, while glutenins are glutelin proteins that are soluble in weak acid solutions such as acetic acid solutions (Bao et al., 2023; Cabanillas., 2020; Jorgensen et al., 2024; Sicherer & Leung., 2015; Watson et al., 2017). Both gliadins and glutenins have been linked to LTSA in humans (Cianferoni 2016). Although glutenin extract contains multiple glutenin proteins, for the sake of simplicity the term glutenin has been used to represent glutenin extracts in this thesis. The work presented in this thesis was conducted in a mouse model of GILTSA; This mouse model has been published previously (Jorgensen et al., 2023).

Immune biomarker discovery is a cutting-edge scientific field that is expected to play a vital role in the prevention, management, and therapy of immune mediated chronic inflammatory

diseases including cardiovascular diseases, metabolic diseases, autoimmune diseases and allergic diseases in general (Jain et al., 2023; Powell, 2024; Qiao et al., 2025; Williams et al., 2024). Biomarker discovery in food allergy and anaphylaxis is necessary in addressing the gaps in prevention, diagnosis and treatment of food induced anaphylaxis (Devonshire et al., 2023). This biomarker discovery is also essential to study the molecular pathways responsible for food allergies and anaphylaxis (Patil et al., 2020). As mentioned, wheat is one of the prominent foods that is being consumed and thus discovery in anaphylaxis-specific immune biomarkers will thus reduce the growing concern about the wheat allergy and anaphylaxis by providing novel options of improved targets for therapy and management (Wang et al., 2023). In this thesis work immune biomarkers were studied in a mouse model of GILTSA.

Animal models used to study wheat anaphylaxis can be classified into adjuvant-based and adjuvant-free models (Arul Arasan et al., 2025). Both types of models have utilized mostly rats or mice, and rarely guinea pigs. Adjuvant-based models use adjuvants such as alum, salicylic acid and detergents, along with wheat allergens to induce sensitization. Adjuvants enhance immune and inflammatory response there by yielding robust sensitization readouts such as IgE antibodies very quickly. While it is easy to execute these methods, there is a stark difference between the underlying sensitization mechanisms for anaphylaxis in the models that use an adjuvant versus the models that do not (Jin et al., 2020). Furthermore, adjuvant-based models are not considered useful to determine the intrinsic allergenicity of proteins because they increase sensitivity at the cost of specificity of immune reaction (Dearman & Kimber, 2009). Therefore, adjuvant-free models are used when it is vital to investigate the intrinsic allergenicity of a given protein, including conventional (like wheat allergens) and novel (like genetically engineered or altered food proteins) food proteins (Gao et al., 2021 and 2022; Jorgensen et al., 2023 and 2024).

Recently, adjuvant-free models have been used by our research group to identify spleen immune biomarkers associated with anaphylaxis (Jin et al., 2020; Gao et al., 2022 and 2023; Jorgensen et al., 2023 and 2024). In this thesis work, a novel adjuvant-free mouse model of GILTSA that was reported before was used to identify organ-specific immune biomarkers (Jorgensen et al., 2023).

Here, the following hypothesis was tested using a novel mouse model of wheat GILTSA reported by our group recently (Jorgensen et al., 2023): GILTSA will be associated with substantial elevation of a selective set of organ-specific immune biomarkers in small intestine, liver, heart and lungs. Here, we report organ-specific immune biomarker signatures linked to GILTSA for the first time.

Results

The general experimental design for the present study is illustrated in Figure 1. Characterization of IgE antibody response, mucosal mast cell degranulation response, hypothermic shock response, and clinical symptom scoring of GILTSA were conducted by other students. And those data have been published (Jorgensen et al., 2023). A relevant summary of this data is provided in Table 1. Organs collected from these mice were used to conduct the immune biomarker analysis in this thesis work.

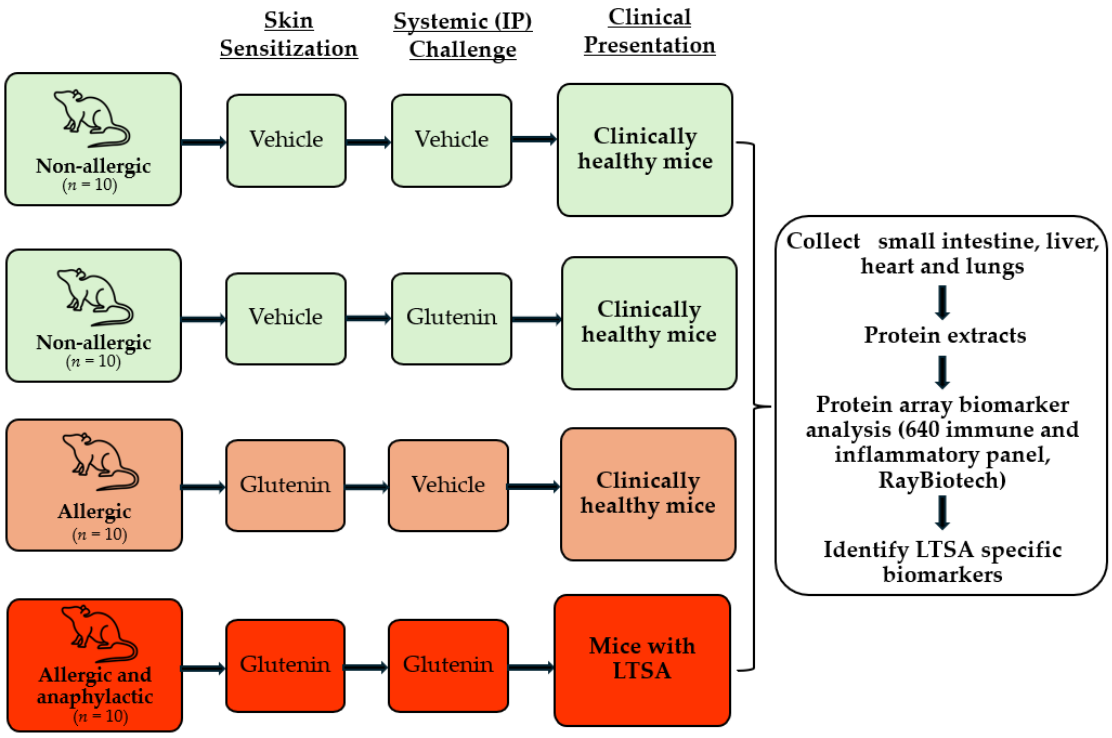

Figure 3: Experimental design and approach to identify anaphylaxis-specific immune biomarkers elevated in glutenin-induced life-threatening systemic anaphylaxis (GILTSA)

Table 6. Assessment of clinical symptom scores and hypothermic shock responses in this mouse model of glutenin-induced life-threatening systemic anaphylaxis[#].

Group*	**Glutenin specific IgE antibody ****	**Clinical symptom scores *****	**Average rectal Temperature ******
Non-allergic mice (vehicle/vehicle)	No	0 (10/10)	38 °C
Non-allergic mice (vehicle/glutenin)	No	0 (10/10)	37.8 °C
Allergic mice (glutenin/vehicle)	Yes	0 (10/10)	37.3 °C
Allergic & Anaphylactic mice (glutenin/glutenin)	Yes	4 (10/10)	29 °C

Table 6 (cont'd)

[#] These data have been published as Jorgensen et al., (2023) where Tamil Selvan Arul Arasan is a co-author on the paper.

*Vehicle/vehicle = vehicle sensitized/vehicle challenged; Vehicle/glutenin = vehicle sensitized/glutenin challenged; glutenin/vehicle = glutenin sensitized/vehicle challenged; glutenin/glutenin = glutenin sensitized/glutenin challenged.

**Glutenin specific IgE antibodies were measured before and after skin exposure as reported in.

***Systemic anaphylaxis symptom scores were calculated as follows: 0 = no symptoms; 1 = scratching and rubbing around the nose and head; 2 = puffiness around the eyes and mouth, diarrhea, pilar erecti, reduced activity and/or decreased activity with a noted increase of respiratory rate; 3 = wheezing, labored respiration, and cyanosis around the tail and the mouth; 4 = no activity after prodding, tremors, and convulsions; 5 = death.

****Rectal temperature was measured at 30 mins post challenge as reported (Jorgensen et al., 2023).

Proteomic analysis and identification of anaphylaxis-specific immune biomarkers in the small intestine of mice undergoing glutenin-induced life-threatening systemic anaphylaxis

The goal of this section of the study was to identify anaphylaxis-specific immune biomarkers elevated during glutenin-induced life-threatening anaphylaxis (GILTSA) in the small intestine. Tissues collected from four groups of mice (3 groups with normal clinical presentation and 1 group with GILTSA) were used in biomarker analysis as shown (Figure 1). Results of all 640 immune biomarkers studied in these four groups of mice were analyzed to identify those immune biomarkers that were unchanged, elevated or inhibited.

Heat map analysis showing anaphylaxis-specific biomarker profile of medium, high, critical and most critical immune biomarkers in all four groups of mice is presented in Figure 2.

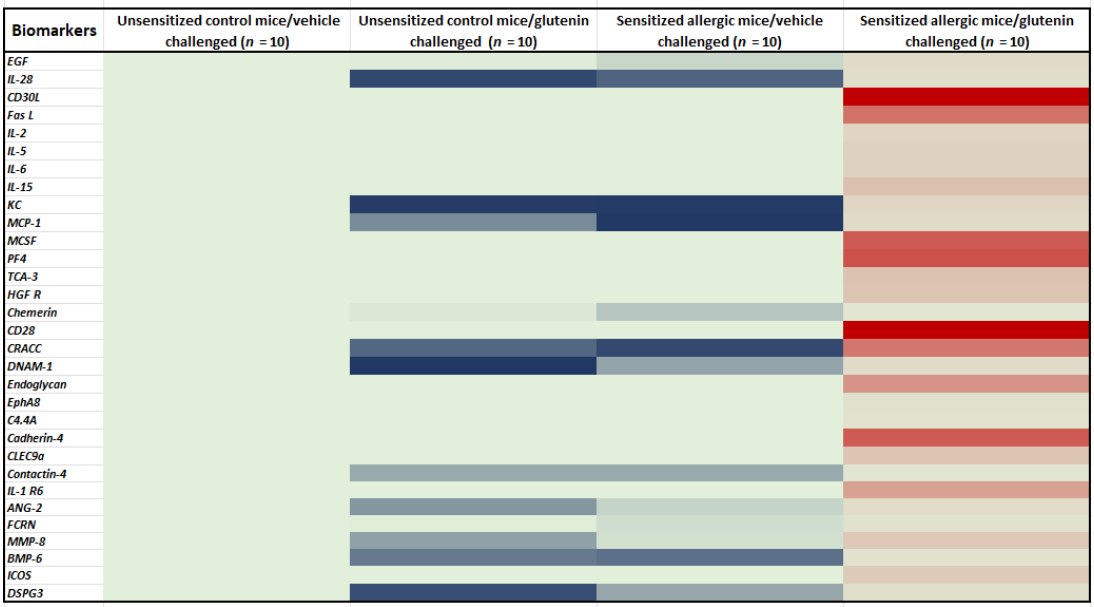

Figure 4: Heat map profile of important immune biomarkers in the small intestine of clinically healthy mice (first 3 panels) vs mice with glutenin-induced systemic anaphylaxis (last panel). Using small intestine extracts from control mice and anaphylactic mice, a proteomic microarray analysis was conducted for 640 immune biomarkers. Those immune biomarkers that were increased two-fold or higher in the last panel compared to the first panel are shown. Background levels of immune biomarkers are shown in green color. Upregulated immune biomarkers are shown in red color and down-regulated immune biomarkers are shown in blue color.

Using a modified published method as detailed in the method section we identified immune biomarkers associated specifically with GILTSA into 6 groups: Immune biomarkers of no, low, medium and high importance are presented in Table 2 (Jorgensen et al., 2023, 2024). Immune biomarkers of critical and most critical importance are presented in Figure 3.

Table 7. Identification of immune biomarkers of no, low, medium and high importance in the small intestine of mice with life-threatening systemic anaphylaxis.

Fold Increase in protein expression *	**Significance (number of immune biomarkers) ****	**Identified Immune biomarkers**
1.2 – 1.49	No Importance **(15)**	GITR, SCF, BAFF R, CCL6, Clusterin, IL-33, Progranulin, IL-22, Cathepsin H, IL-20 Rbeta, IL-1F8, GPV, LIGHT, GAPDH
1.5 – 1.9	Low Importance **(6)**	IL-17E, B7-H3, Ephrin-A2, FGF-4, IFN-beta, CAMK4
2 – 3.9	Medium Importance **(14)**	EGF, IL-28, Chemerin, EphA8, IL-2, MCP-1, KC, DNAM-1, C4.4A, contactin-4, ANG-2, FCRN, BMP-6, DSPG3
4 – 5.9	High Importance **(8)**	IL-5, IL-6, IL-15, TCA-3, HGF-R, CLEC9a, MMP-8, ICOS

*Fold changes shown are in anaphylactic mice relative to control mice.

**Classification of significance of fold change in protein expression is based on the previous published method (Jorgensen et al., 2023, 2024).

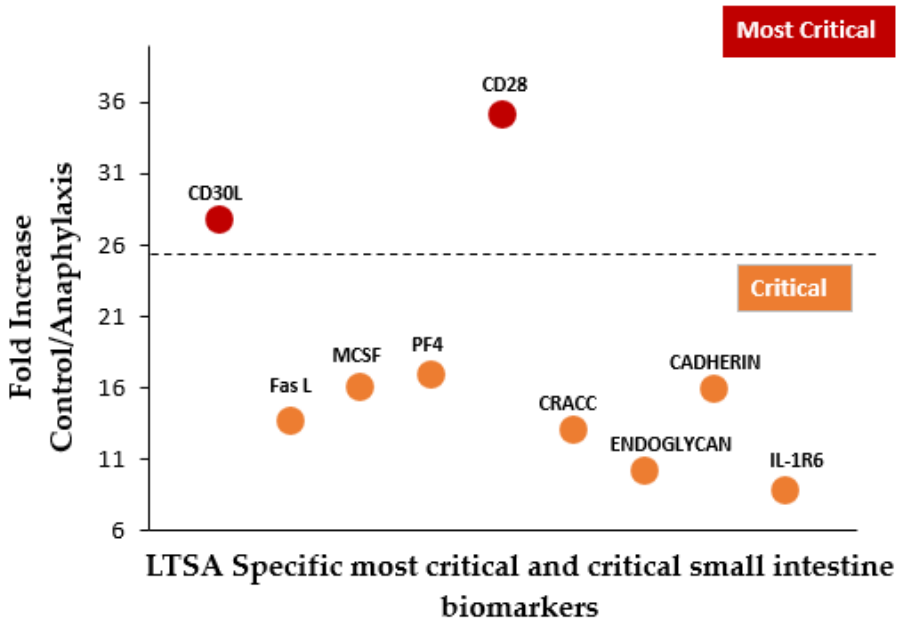

Figure 5. Identification of critical and most critical immune biomarkers in the small intestine associated with glutenin-induced life-threatening systemic anaphylaxis in this model. The Y axis Shows the relative protein expression of the immune biomarkers that are elevated significantly (6-fold to 24.9 = critical markers; 25-fold and higher = most critical markers) during glutenin-induced anaphylactic mice relative to the control healthy mice.

Anaphylaxis-specific immune biomarkers of critical and most critical importance are presented in figure 3. Thus, the following two anaphylaxis-specific immune biomarkers were identified as the most critical biomarker specifically linked to GILTSA in the small intestine in this model: CD28 and CD30L.

Thus, these results identify 2 most critical immune biomarkers and 7 critical immune biomarkers that are dramatically elevated in the small intestine of mice with GILTSA compared to the small intestine of healthy mice.

Proteomic analysis and identification of anaphylaxis-specific immune biomarkers in the liver of mice undergoing glutenin-induced life-threatening systemic anaphylaxis

The goal of this section of the study was to identify anaphylaxis-specific immune biomarkers elevated during GILTSA in the liver. Tissues collected from four groups of mice (3 groups with normal clinical presentation and 1 group with GILTSA) were used in biomarker analysis as shown (Figure 1).

Results of all 640 immune biomarkers studied in these four groups of mice were analyzed to identify those immune biomarkers that were unchanged, elevated or inhibited.

Heat map analysis showing anaphylaxis-specific immune biomarker profile of medium, high, critical and most critical immune biomarkers in all four groups of mice is presented in Figure 4.

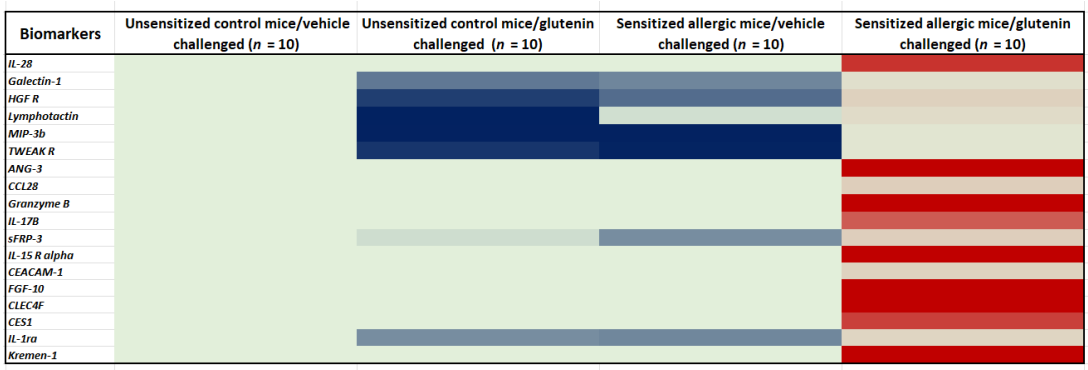

Figure 6. Heat map profile of important immune biomarkers in the liver of clinically healthy mice (first 3 panels) vs mice with glutenin-induced systemic anaphylaxis (last panel). Using liver extracts from control mice and anaphylactic mice, a proteomic microarray analysis was conducted for 640 immune biomarkers. Those immune biomarkers that were increased two-fold or higher in the last panel compared to the first panel are shown. Background levels of immune biomarkers are shown in green color. Upregulated immune biomarkers are shown in red color and down-regulated immune biomarkers are shown in blue color.

Using a modified published method as detailed in the method section we identified immune biomarkers associated specifically with GILTSA into 6 groups: Immune biomarkers of no, low, medium and high importance are presented in Table 3; Immune biomarkers of critical and most critical importance are presented in Figure 5.

Table 8. Identification of immune biomarkers of no, low, medium and high importance in the liver of mice with life-threatening systemic anaphylaxis.

Fold Increase	**Significance**	**Identified Immune biomarkers**
1.2 – 1.49	No Importance **(6)**	IL-2 Ra, ALK-1, CD45, CDNF, IL-1 R6, JNK1
1.5 – 1.9	Low Importance **(4)**	IGF-1, CD27, GPV, PTK6
2 – 3.9	Medium Importance **(6)**	Lymphotactin, CEACAM-1, IL-1ra, Galectin-1, MIP-3b, TWEAK R
4 – 5.9	High Importance **(1)**	HGF R

*Fold changes shown are in anaphylactic mice relative to control mice.

**Classification of significance of fold change in protein expression is based on the previous published method (Jorgensen et al., 2023, 2024).

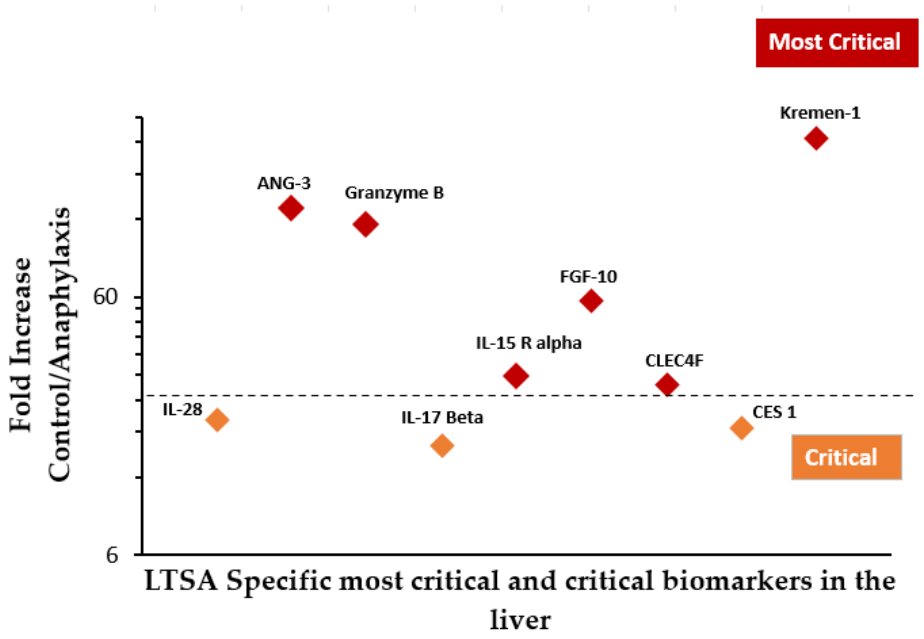

Figure 7. Identification of critical and most critical immune biomarkers in the liver associated with glutenin-induced life-threatening systemic anaphylaxis in this model. The Y axis shows the relative protein expression of the immune biomarkers that are elevated significantly (6-fold to 24.9 = critical markers; 25-fold and higher = most critical markers) during glutenin-induced anaphylactic mice relative to the control healthy mice.

Anaphylaxis-specific immune biomarkers of critical and most critical importance are presented in Figure 5. Thus, the following anaphylaxis-specific immune biomarkers were identified as the most critical biomarker specifically linked to GILTSA in the liver in this model: Kremen-1, ANG-3, Granzyme, FGF-10, IL-15 R alpha, and CLEC4F.

Thus, these results identify 6 most critical immune biomarkers and 3 critical immune biomarkers that are dramatically elevated in the liver of mice with GILTSA compared to the liver of healthy mice.

Proteomic analysis and identification of anaphylaxis-specific immune biomarkers in the heart of mice undergoing glutenin-induced life-threatening systemic anaphylaxis

The goal of this section of the study was to identify anaphylaxis-specific immune biomarkers elevated during GILTSA in the heart. Tissues collected from four groups of mice (3 groups with normal clinical presentation and 1 group with GILTSA) were used in immune biomarker analysis as shown (Figure 1).

Results of all 640 immune biomarkers studied in these four groups of mice were analyzed to identify those immune biomarkers that were unchanged, elevated or inhibited.

Heat map analysis showing anaphylaxis-specific biomarker profile of medium, high, critical and most critical immune biomarkers in all four groups of mice is presented in Figure 6.

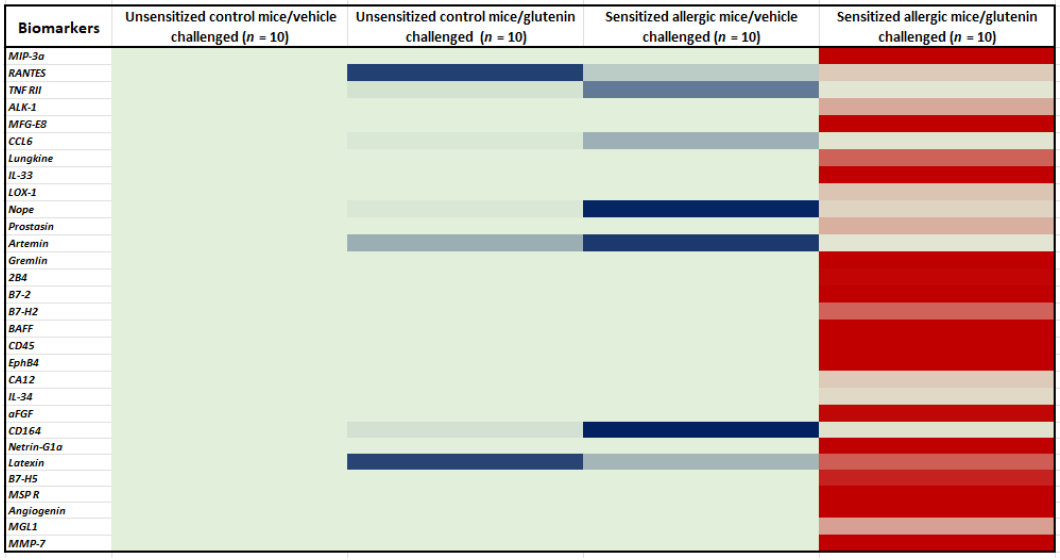

Figure 8: Heat map profile of important immune biomarkers in the heart of clinically healthy mice (first 3 panels) vs mice with glutenin-induced systemic anaphylaxis (last panel). Using heart extracts from control mice and anaphylactic mice, a proteomic microarray analysis was conducted for 640 immune biomarkers. Those immune biomarkers that were increased two-fold

Figure 8 (cont'd)

or higher in the last panel compared to the first panel are shown. Background levels of immune biomarkers are shown in green color. Upregulated immune biomarkers are shown in red color and down-regulated immune biomarkers are shown in blue color.

Using a modified published method as detailed in the method section we identified immune biomarkers associated specifically with LTSA into 6 groups: Immune biomarkers of no, low, medium and high importance are presented in Table 4; Immune biomarkers of critical and most critical importance are presented in Figure 7.

Table 9. Identification of immune biomarkers of no, low, medium and high importance in the heart of mice with life-threatening systemic anaphylaxis

Fold Increase	**Significance**	**Identified Immune biomarkers**
1.2 – 1.49	No Importance **(6)**	CD40, ICAM-1, Galectin-3, CA2, HAI-2, MGL2
1.5 – 1.9	Low Importance **(6)**	E-selectin, Gas 6, H60, CD53, HIN-1, IGFBP-1
2 – 3.9	Medium Importance **(6)**	IL-34, Nope, TNF RII, CCL6, Artemin, CD164
4 – 5.9	High Importance **(3)**	LOX-1, CA12, Rantes

*Fold changes shown are in anaphylactic mice relative to control mice

**Classification of significance of fold change in protein expression is based on the previous published method (Jorgensen et al., 2023, 2024).

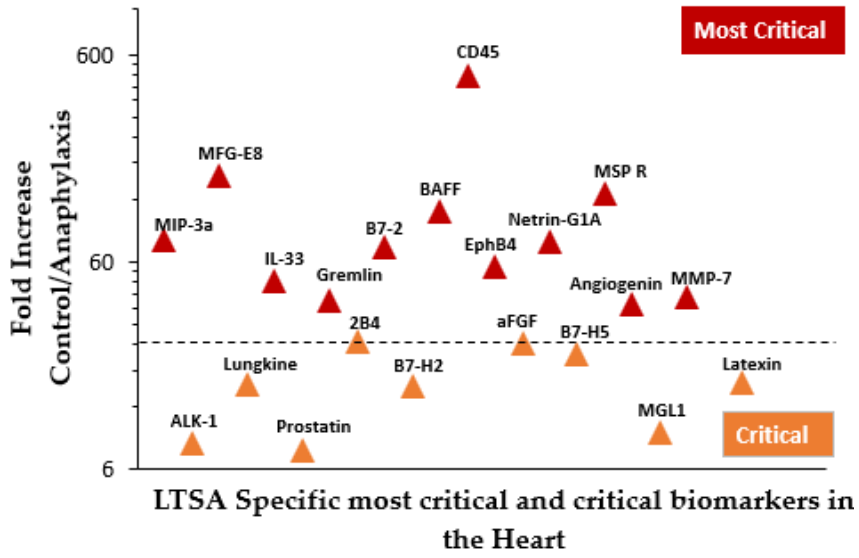

Figure 9. Identification of critical and most critical immune biomarkers in the heart associated with glutenin-induced life-threatening systemic anaphylaxis in this model. The Y axis Shows the relative protein expression of the immune biomarkers that are elevated significantly (6-fold to 24.9 = critical markers; 25-fold and higher = most critical markers) during glutenin-induced anaphylactic mice relative to the control healthy mice.

Anaphylaxis-specific immune biomarkers of critical and most critical importance are presented in Figure 7. Thus, the following anaphylaxis-specific immune biomarkers were identified as the most critical biomarker specifically linked to GILTSA in the heart in this model: CD45, MFG-E8, MSP R, BAFF, MIP-3a, Netrin-G1A, B7-2, EphB4, IL-33, MMP-7, Gremlin, Angiogenin and 2B4.

Thus, these results identify 12 most critical immune biomarkers and 9 critical immune biomarkers that are dramatically elevated in the heart of mice with GILTSA compared to the heart of healthy mice.

Proteomic analysis and identification of anaphylaxis-specific immune biomarkers in the lungs of mice undergoing glutenin-induced life-threatening systemic anaphylaxis

The goal of this section of the study was to identify anaphylaxis-specific immune biomarkers elevated during GILTSA in the lungs. Tissues collected from four groups of mice (3 groups with normal clinical presentation and 1 group with GILTSA) were used in biomarker analysis as shown (Figure 1).

Results of all 640 immune biomarkers studied in these four groups of mice were analyzed to identify those immune biomarkers that were unchanged, elevated or inhibited.

Heat map analysis showing anaphylaxis-specific biomarker profile of medium, high, critical and most critical immune biomarkers in all four groups of mice is presented in Figure 8.

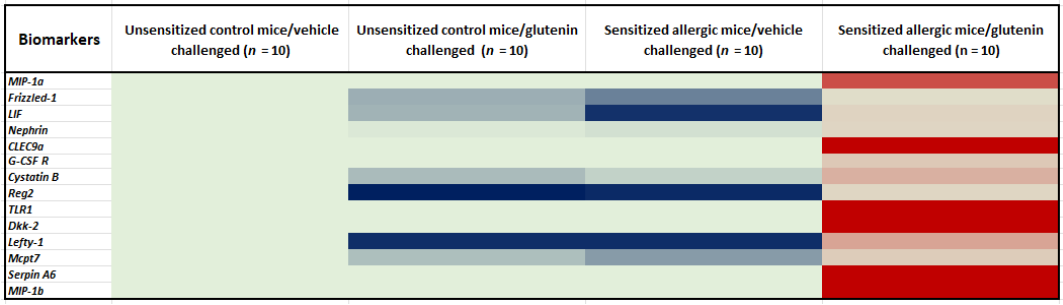

Figure 10. Heat map profile of important immune biomarkers in the lungs of clinically healthy mice (first 3 panels) vs mice with glutenin-induced systemic anaphylaxis (last panel). Using lung extracts from control mice and anaphylactic mice, a proteomic microarray analysis was conducted for 640 immune biomarkers. Those immune biomarkers that were increased two-fold or higher in the last panel compared to the first panel are shown. Background levels of immune biomarkers are shown in green color. Upregulated immune biomarkers are shown in red color and down-regulated immune biomarkers are shown in blue color.

Using a modified published method as detailed in the method section we identified immune biomarkers associated specifically with GILTSA into 6 groups: Immune biomarkers of no, low, medium and high importance are presented in Table 5; Immune biomarkers of critical and most critical importance are presented in Figure 9.

Table 10. Identification of immune biomarkers of no, low, medium and high importance in the lungs of mice with life-threatening systemic anaphylaxis

Fold Increase	**Significance**	**Identified Immune biomarkers**
1.2 – 1.49	No Importance **(7)**	FLt-3L, 6Ckine, Cathepsin H, Epimorphin, ASAHL, CRELD1, Arylsulfatase G
1.5 – 1.9	Low Importance **(5)**	IL-1F8, Syndecan, IL-22 R alpha 1, MSP R, PDGF R alpha, Laminin alpha 4
2 – 3.9	Medium Importance **(4)**	Lif, Nephrin, Reg 2, Frizzled-1
4 – 5.9	High Importance **(2)**	G-CSFR, Mcpt7

*Fold changes shown are in anaphylactic mice relative to control mice

**Classification of significance of fold change in protein expression is based on the previous published method (Jorgensen et al., 2023, 2024).

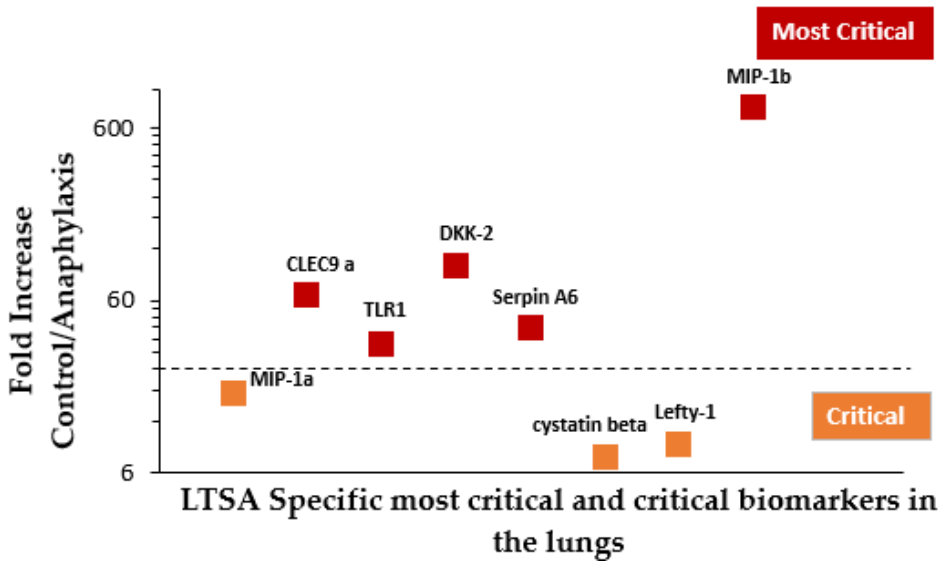

Figure 11: Identification of critical and most critical immune biomarkers in the lungs associated with glutenin-induced life-threatening systemic anaphylaxis in this model. The Y axis Shows the relative protein expression of the immune biomarkers that are elevated significantly (6-fold to 24.9 = critical markers; 25-fold and higher = most critical markers) during glutenin-induced anaphylactic mice relative to the control healthy mice.

Anaphylaxis-specific immune biomarkers of critical and most critical importance are presented in Figure 9. Thus, the following anaphylaxis-specific immune biomarkers were identified as the most critical biomarker specifically linked to GILTSA in the lungs in this model: DKK-2, CLEC9a, TLR1, Serpin A6 and MIP-1b.

Thus, these results identify 5 most critical immune biomarkers and 3 critical immune biomarkers that are dramatically elevated in the lungs of mice with GILTSA compared to the lungs of healthy mice.

Immune pathway analysis of organ-specific most critical immune biomarkers linked to GILTSA

Using an Artificial Intelligence program (Perplexity), immune pathway analysis of organ-specific most critical immune biomarkers was conducted. The results are presented in Table 6. These signatures were associated with the following immune pathways: Th2 immune activation pathways, cytotoxic immune activation pathway, tissue repair/remodeling pathway, metabolic regulation pathway, vascular integrity pathway, and immune homeostasis pathways.

Table 11. Immune pathway analysis of the most critical immune biomarkers linked to GILTSA in this model

Organ	Most critical immune biomarkers	Immune pathways
Small Intestine	CD30L	Th2 amplification pathway (Fuchiwaki et al., 2011)
	CD28	Th2 Priming, Costimulatory Pathways (Gogishvili et al., 2012)
Liver	Kremen-1	Viral Entry Receptor with Unexplored Roles in Allergic Sensitization (Jorgensen et al., 2023; Staring et al., 2018)
	ANG-3	Metabolic Dysregulation and Vascular Leakage (Li et al., 2021a; Liu et al., 2015; Quagliarini et al., 2012)
	Granzyme B	Cytotoxic Immune Activation in Hepatic Microenvironments (Reber et al., 2017; Tschopp et al., 2006)
	FGF-10	Hepatic Regeneration and Antioxidant Defense (Li et al., 2021b; Park et al., 2023)
	IL-15R alpha	Immune Homeostasis and Fibrosis Prevention (Jiao et al., 2016; Liu et al., 2015)

Table 11 (cont'd)

	CLEC4F	Kupffer Cell Activation and Immune Tolerance (Park et al., 2023) Convergent pathways: Immune activation pathway, tissue repair pathway, metabolic regulation pathway and vascular integrity pathway (Jiao et al., 2016; Li et al., 2021b; Park et al., 2023; Quagliarini et al., 2012; Reber et al., 2017b)
Heart	CD45	Immune Activation and Inflammation (Courtney et al., 2019**;** Hamaguchi et al., 2001)
	MFG-E8	Vascular Dysfunction and Leakage (Huang et al., 2020; Yi, 2016)
	MSP R	None identified so far
	BAFF	Immune Activation and Inflammation (Huang et al., 2020; Smulski & Eibel, 2018)
	MIP-3a	Immune Activation and Inflammation (Beutier et al., 2017)
	Netrin-G1A	Tissue Remodeling and Fibrosis (Reinhard et al., 2022)
	B7-2	Immune Activation and Inflammation (Greenwald et al., 1999; Yadav et., 2007)
	EphB4	Vascular Dysfunction and Leakage (Kounis et al., 2018; Luxán et al., 2019)
	IL-33	Immune Activation and Inflammation (Stolarski et al., 2010)
	MMP-7	Tissue Remodeling and Fibrosis (Drosatos et al., 2013; Kounis et al., 2018)
	Gremlin	Tissue Remodeling and Fibrosis (Nakashiba et al., 2000; Reinhard et al., 2022)

Table 11 (cont'd)

	Angiogenin	Vascular Dysfunction and Leakage (Drosatos et al., 2013)
Lungs	MIP-1b	Drives myeloid cell recruitment and amplifies Th2/Th17 inflammation (Alakhras et al., 2023; Reyfman et al., 2023)
	DKK-2	Suppresses Wnt-mediated tissue repair, exacerbating lung injury (Cui et al., 2021; Weckerle et al., 2023)
	CLEC9 a	Cross-present allergens and sustain adaptive immunity (Brown et al., 2021.; Caminschi et al., 2008; Hussain et al., 2025; Lahoud et al., 2011)
	Serpin A6	Modulates corticosteroid bioavailability, influencing inflammatory resolution (Rijavec et al., 2022)
	TLR1	Synergizes with FcεRI to enhance mast cell activation (Lee et al., 2011; Peavy & Metcalfe, 2008; Sandig & Bulfone-Paus, 2012)

Discussion

Wheat is a staple food linked to glutenin-induced life-threatening systemic anaphylaxis (GILTSA) in humans (Levy & Feldman, 2022). However, the underlying immune mechanisms of GILTSA by wheat or any other allergenic food is incompletely understood (Arul Arasan et al., 2025). Researchers have developed a variety of animal models to study mechanisms of anaphylaxis caused by wheat and other foods (Adachi et al., 2012; Fu et al., 2022; Kohno et al., 2016; Morita et al., 2009; Tanaka et al., 2011; Tanaka et al., 2011; Yamada et al., 2019). Our research group recently published a novel mouse model of GILTSA (Jorgensen et al., 2023). In this study, we tested the following hypothesis using this mouse model: GILTSA will be associated with substantial elevation of a selective set of organ specific immune markers in small

intestine, liver, heart and lungs. Overall, the result from this study collectively supports this hypothesis by providing organ specific immune marker signatures linked to GILTSA.

There are 7 novel findings from this study: i) Identification of small intestine specific biomarker signatures of GILTSA consisting of 2 most critical immune biomarkers (CD30L and CD28) and 7 critical immune biomarkers (Fas L, MCSF, PF4, CRACC, ENDOGLYCAN, CADHERIN, IL-1R6); ii) Identification of liver specific biomarker signatures of GILTSA consisting of 6 most critical immune biomarkers (ANG-3, Granzyme B, IL-15 R alpha, FGF-10, CLEC4F, Kremen-1) and 3 critical immune biomarkers (IL_28, IL-17 Beta, CES 1); iii) Identification of heart specific biomarker signatures of GILTSA consisting of 12 most critical immune biomarkers (MIP-3a, MFG-E8, IL-33, Gremlin, B7-2, BAFF, CD45, EphB4, Netrin-G1A, Angiogenin, MMP-7 and MSP R) and 9 critical immune biomarkers (ALK-1, Lungkine, Prostatin, 2B4, B7-H2, aFGF, B7-H5, MGL1, Latexin); iv) Identification of lungs specific biomarker signatures of GILTSA consisting of 5 most critical immune biomarkers (CLEC9a, TLR1, DKK-2, Serpin A6 and MIP-1b) and 3 critical immune biomarkers (MIP-1a, cystatin beta and Lefty-1); v) In this model heart tissue had the maximum changes in most critical/critical immune biomarkers combined (21) during GILTSA indicating that it is targeted most among the organs studied; vi) In this model, small intestine, liver and lungs showed comparable changes in most critical/critical immune biomarkers combined (9, 9, and 8 respectively); and vii) Collectively we demonstrate organ specific biomarker signatures in GILTSA using mouse model for the first time; No such findings are reported in the literature for any animal model of wheat allergy or any other food allergy (Arul Arasan et al., 2025).

We chose this particular animal model in this study because: i) Previously Dr. Gangur and colleagues published this novel mouse model of GILTSA where all the experimental conditions

and characteristic features of the model were optimized and validated (Jorgensen et al., 2023); However, mechanism of GILTSA in this model is incompletely understood and immune biomarker analysis of systemic organs other than spleen had not been studied before; ii) This model does not use adjuvants for sensitization to glutenin and therefore, exposure to glutenin during sensitization is similar to human skin exposure to glutenin; Therefore, mechanisms elucidated in this model are expected to be similar to human glutenin allergy; iii) Previous studies have shown that mechanisms of anaphylaxis can be different in adjuvant based models vs adjuvant-free models such as this one (Jin et al., 2020); and iv) This mouse model simulates life-threatening systemic anaphylaxis as opposed to any other model of wheat allergy in the literature that report anaphylaxis that are relatively milder in nature as reviewed recently (Arul Arasan et al., 2025).

In this study, we chose small intestine, liver, heart and lungs for biomarker analysis for the following reasons: i) Small intestine represented anaphylactic reaction in the gut; ii) Liver, heart and lungs represent vital organs; iii) Besides the gut, heart and lungs are the two major organs directly associated with food induced anaphylaxis; and iv) Liver has been implicated as a participant in anaphylactic reactions in wheat allergic humans and (Okano et al., 1999). Our findings show that a selective set of (9) immune biomarkers are dramatically elevated in the liver of mice with LTSA induced by wheat glutenin. Thus, our data provides the first molecular evidence for participation of liver in food induced systemic anaphylaxis in an animal model. Animal models have been developed using rats, guinea pigs, dogs and mice to study mechanisms of wheat allergy and anaphylaxis (Arul Arasan et al., 2025; Jorgensen et al., 2024). So far, focus of mechanism has been studying the following components involved in sensitization process and mast cell mediator release upon allergen challenge: Th2, Th1, Th17 cytokines, chemokines and

adhesion molecules expression in spleen tissue, IgE, IgG antibodies in the blood, mast cell derived mediators such as histamine and MMCP-1 in the blood, and histopathology of gut tissue for mast cell density and activation (Arul Arasan et al., 2025). So far only two studies have reported limited (120) biomarker analysis of spleen tissue in a mouse model of GILTSA (Jorgensen et al., 2024; Jorgensen et al., 2023). In contrast, in this study we conducted a large (640) biomarker analysis of small intestines, liver, heart and lungs tissues obtained from control mice vs mice with clinically confirmed GILTSA. No such study has been published before for wheat anaphylaxis. Therefore, our findings have advanced the mechanism of GILTSA by identifying organ specific biomarker signatures for 4 important organs.

There are no human studies reported in the literature focused on biomarker analysis for systemic anaphylaxis although there are recent suggestions identifying this approach as a critical need in the field (Arul Arasan et al., 2025; Preda et al., 2024).

McGrath et al (2021) reported identification of biomarkers associated with severe anaphylaxis among patients admitted to the emergency department in Australia (McGrath et al., 2021). They collected blood samples and analyzed nucleolar RNA transcripts by qRT-PCR to identify anaphylaxis-specific biomarkers. They identified seven genes (SNORD61, SNORD8, SCARNA21, SNORD69, SNORD110, SNORD119 and SNORD59A) that were elevated in severe anaphylaxis patients but not in healthy control patients. Notably, they did not specify whether food or other agents were responsible for anaphylaxis. They concluded that SnoRNAs that they identified may be useful biomarkers for diagnosis of severe anaphylaxis in general. However, it is unclear whether they might be useful for wheat anaphylaxis or food induced anaphylaxis.

Rijavec Matija et al (2022) studied blood transcriptome biomarkers in human peanut anaphylaxis and in a transgenic (IL9) mouse model of hapten (Tri nitro phenyl) induced anaphylaxis (Rijavec et al., 2022a). They report identification of many biomarkers associated with anaphylaxis in humans and mice. Notably, they did not study protein biomarkers.

We found that in the heart of mice with GILTSA, the highest (468) fold increase in protein expression was of the marker CD45 among all 640 markers tested. Previous study has shown that CD45 is a critical regulator of T cell signaling as well as IgE receptor signaling (Courtney et al., 2019; Hamaguchi et al., 2001); There is a previous report implicating CD45 as a critical component of the signaling pathway initiated by IgE and its high affinity receptor (Fcε receptor-1) (Hamaguchi et al., 2001). They demonstrated that H. TU-572 (TU-572, 2-[4-methylthiopyridin-2-yl) methyl sulfinyl]-5-isopropoxybenzimidazole), a potent and selective CD45 inhibitor, suppresses IgE-mediated anaphylaxis (Hamaguchi et al., 2001). There are few differences in the mouse models they used and food allergy mouse model that we used for our study. Their model used anti-DNP IgE for passive sensitization of mice followed by DNP-BSA as antigen for challenge. They did not conduct biomarker analysis. They tried TU-572 as a potential therapeutic agent based on invitro studies using peritoneal mast cells. Nevertheless, their study suggests that TU-572 may block or may suppress GILTSA in our mouse model by neutralizing CD45 signaling. One can test this in future studies as a potential novel drug to treat GILTSA.

We report for the first time that GILTSA is associated with specific Th2 immune activation pathways, cytotoxic immune activation pathway, tissue repair/remodeling pathway, metabolic regulation pathway, vascular integrity pathway, and immune homeostasis pathways (Drosatos et al., 2013; Fuchiwaki et al., 2011; Reber et al., 2017b; Reinhard et al., 2022;

Reyfman et al., 2023; Tschopp et al., 2006). Some of the immune biomarkers were identified to have therapeutic potential (particularly CD45 and IL-33) based on previous findings in other types of allergic and anaphylactic reactions (Beutier et al., 2017; Caminschi et al., 2008; Courtney et al., 2019; Cui et al., 2021; Drosatos et al., 2013; Fuchiwaki et al., 2011; Gogishvili et al., 2012; Greenwald et al., 1999; Huang et al., 2020; Hussain et al., 2025; Jiao et al., 2016; Kounis et al., 2018; Lahoud et al., 2011; Lee et al., 2011; Li et al., 2021b; Liu et al., 2015; Nakashiba et al., 2000; Park et al., 2023; Peavy & Metcalfe, 2008; Pushparaj et al., 2009; Quagliarini et al., 2012; Reber et al., 2017b; Reinhard et al., 2022; Rijavec et al., 2022a; Sandig & Bulfone-Paus, 2012; Smulski & Eibel, 2018; Staring et al., 2018; Tschopp et al., 2006; Weckerle et al., 2023).

In this study, we identified organ-specific immune biomarkers linked to GILTSA. Follow up work can be done to identify overlapping immune biomarkers common to more than one organ linked to GILTSA. Furthermore, identification of sensitization-specific immune biomarkers not linked to GILTSA and identification of glutenin-induced immune biomarker changes in unsensitized mice after a single injection are other important investigations that need to be conducted.

The limitation of our study includes the following that one may consider carefully before extrapolating findings to human wheat anaphylaxis: i) In this study mice were challenged systemically by intraperitoneal route of injection with glutenin to elicit LTSA. In humans wheat can cause GILTSA upon oral exposure to glutenin containing foods (Cianferoni et al., 2013). ii) Mouse model development involved using genetically identical inbred strain; In contrast human population is outbred and wheat anaphylaxis subjects are not necessarily and genetically identical (Arul Arasan et al., 2025). iii) In mouse we used wheat glutenin isolated from uncooked

flour; In contrast anaphylaxis is elicitated in humans after exposure glutenin present in cooked wheat foods (Arul Arasan et al., 2025; Cianferoni, 2016). iv) Systemic anaphylaxis in mice involves both IgE and IgG mediated immune activation; In contrast, in humans anaphylaxis is mainly mediated by IgE antibodies (Finkelman et al., 2016; Strait et al., 2006; Todorova et al., 2022).

We suggest the following future direction based on findings from this study: i) Study immune biomarkers in a further refined model where systemic anaphylaxis is induced by oral glutenin challenge; ii) Test whether other wheat allergens (gliadins, albumins and globulins) also elicit systemic anaphylaxis with the same profile of organ specific biomarker signature; iii) Future model can also be developed using outbred strains of mice and identify immune biomarkers of anaphylaxis; iv) To enable diagnostic application in human anaphylaxis, one can test whether critical and most critical immune biomarkers can be measured using blood, saliva and urine obtained anaphylactic mice; and v) Notably, the approach used in the study can also be used to identify immune biomarkers for LTSA elicitated by other types of allergenic foods, or insects, drugs and other agents.

Materials and Methods

Chemicals and reagents

The following chemicals and reagents were used from sources listed below:
A proprietary detergent – the Tissue Protein Extraction Reagent (T-PER^{TM}), having a composition of 25mM bicine and 150mM sodium chloride at pH 7.6, was procured from ThermoFisher Scientific (MA, USA). Sigma-Aldrich (MO, USA) provided a cocktail of serine, cysteine, acid proteases and aminopeptidases for protease inhibition. RayBiotech microarray cytokine panels were used (Norcross, Georgia, USA).

Mice breeding and establishment of a plant-protein-free mouse colony

This has been described in the previous publication (Jorgensen et al., 2023).

Preparation of Acid-Soluble Glutenin Protein Extract from Wheat Flour

This has been described in the previous publication (Jorgensen et al., 2023).

Skin sensitization, bleeding, and plasma sample preparation

This has been described in the previous publication (Jorgensen et al., 2023).

Elicitation of systemic anaphylaxis clinical symptom scoring

This has been described in the previous publication (Jorgensen et al., 2023).

Determination of hypothermic shock responses

This has been described in the previous publication (Jorgensen et al., 2023).

Clinical Scoring of Anaphylaxis

This has been described in the previous publication (Jorgensen et al., 2023).

Euthanasia, organ collection and storage

Mice were euthanized at one hour following the challenge, and their small intestine, liver, heart and lungs were collected and snap-frozen in liquid nitrogen and stored at -70°C.

Protein extraction and proteomic analysis of 640 immune markers

Pooled organs from each group were used in protein extract preparation using a published method developed in our laboratory (Gao et al., 2022 and 2023; Jin et al., 2020; Jorgensen et al., 2023 and 2024); Protein extracts were shipped on dry ice to RayBiotech to quantify immune biomarkers using a protein microarray based system; the following microarray panels were used: CYT- 4, 5, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, and 19; altogether they represented 640 protein immune biomarkers involved in inflammation, immune regulation, and hypersensitivity reactions. All analysis was conducted in quadruplicates using reference standards for each of 640

immune markers. Standard curves were generated to deduce biomarker concentrations in samples (Mouse Cytokine Array Q640 | RayBiotech).

Identification of anaphylaxis-specific immune biomarkers

To identify organ-specific signatures linked to GILTSA, protein array analysis was conducted in four groups of mice (n = 10 per group) as follows: vehicle sensitized/vehicle challenged (v/v), vehicle sensitized/protein challenged (v/p), protein sensitized/vehicle challenged (p/v), protein sensitized/protein challenged (p/p). Using a modified published method, we used the following strategy to identify anaphylaxis-specific markers that are elevated: we eliminated the immune biomarkers that were increased by systemic vehicle challenge in non-allergic mice, systemic glutenin challenge in non-allergic mice and those that were increased by systemic vehicle challenge in allergic mice (Jorgensen et al., 2023). The resulting markers were deemed anaphylaxis specific.

Statistical analysis and identification of most critical immune biomarkers

These analyses were done with an online software service (https://www.socscistatistics.com/tests/), which was accessed multiples times between March 1st and March 13th, 2025). A student's t-test was used for comparison of two groups and $p < 0.05$ was considered significant. Fold change analysis was conducted using average values for each biomarker as follows: v/v vs v/p, v/v vs p/v, v/v vs p/p. Previous analysis suggested that fold change of 2.5-fold or higher was generally significant between two groups (Jorgensen et al., 2023 and 2024). The biomarkers were classified into five groups of significance using a modified published method as follows: not important (up to 1.49 fold increase), low importance (1.5-1.9 fold increase), medium importance (2-3.9 fold increase), high importance (4-5.9 fold increase), critical importance (6 - 24.9 fold increase) and most critical importance (25 fold or

higher increase) (Jorgensen et al., 2023 and 2024). Those fold changes that were 25 or higher were used for pathway analysis.

Conclusion

In summary, we report identification of organ specific immune biomarkers linked to GILTSA for the first time. A similar approach may be used to identify organ specific immune biomarker signatures in other food induced LTSA.

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Chapter 4: Conclusions and Future directions

Conclusions

There is growing interest in the field of allergy and anaphylaxis to identify a novel biomarker to inform improved methods of diagnosis, of prevention and management of life-threatening systemic anaphylaxis (LTSA) in general (Arul Arasan et al., 2025; Jorgensen et al., 2024; 2023; Beck et al.; 2019). This study was conducted to identify organ-specific immune biomarkers linked to glutenin-induced life-threatening systemic anaphylaxis (GILTSA) using a novel adjuvant-free mouse model that our group had reported before (Jorgensen et al 2023). The identified organ-specific biomarker signatures linked to glutenin-induced (GI) LTSA in the small intestine, liver, heart and lungs are illustrated in figure (Figure 1). All biomarkers were at 1 hour post allergen challenge. Therefore, it is important to identify biomarkers that change at earlier points in future studies.

Thus, in this mouse model the most highly expressed protein immune biomarkers were noted in the heart followed by the liver, lungs and the small intestine. These data demonstrate the central role that heart plays in GILTSA in addition to the other three organs studied. The approach used in this study has not been reported in the literature before to identify organ-specific immune biomarkers in LTSA induced by wheat or any other food, thus making this study the first one in the field of food allergy and anaphylaxis. No such work has been published before in the literature before (Adachi et al., 2012; Arul Arasan et al., 2025; Fu et al., 2022; Gao et al., 2022, 2023; Jorgensen et al., 2023, 2024; Kohno et al., 2016; Morita et al., 2023; Tanaka, Nagano, Yano, Haruma, et al., 2011; Tanaka, Nagano, Yano, Matsuda, et al., 2011; Yamada et al., 2019)

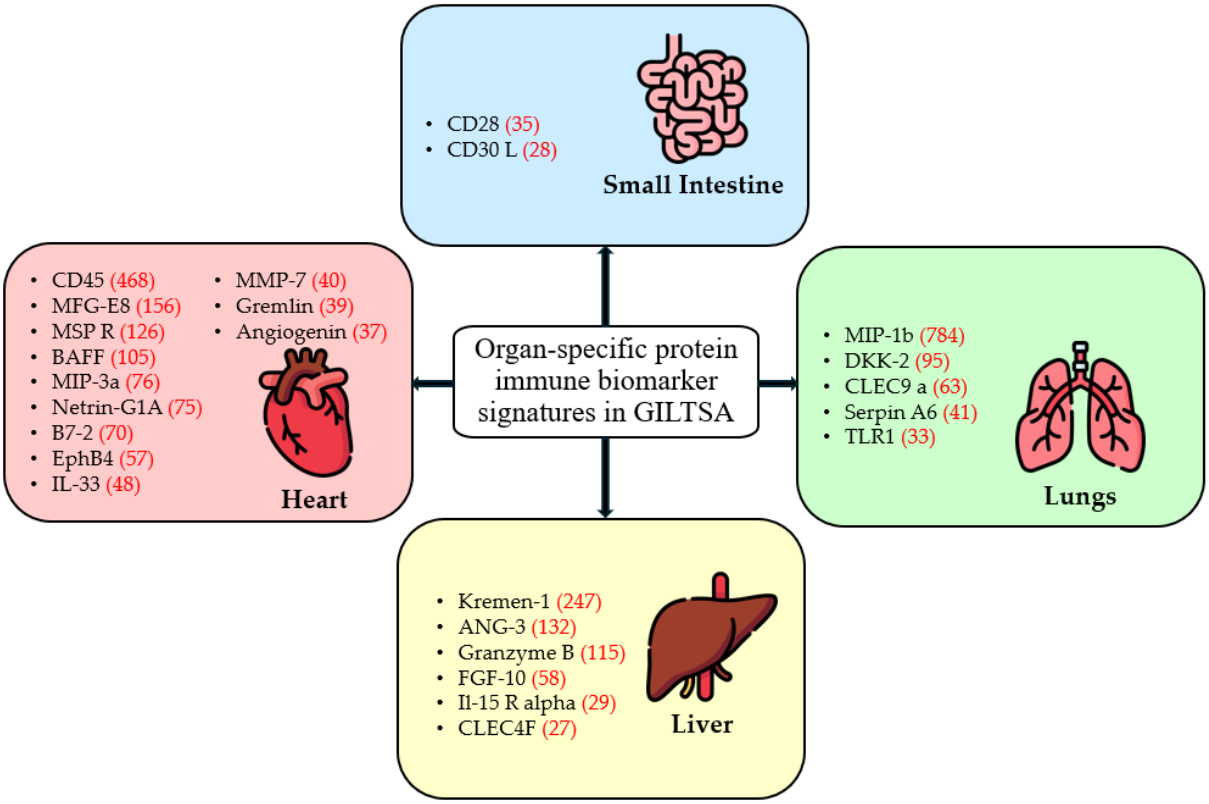

Figure 12. Identification of organ-specific protein biomarker signatures in GILTSA. Those immune biomarkers that are elevated by 25 folds or higher were regarded as the most critical immune biomarkers. Numbers in parentheses indicate the fold increase in protein expression in anaphylactic mice relative to healthy control mice.

Future Direction

The following future studies are suggested:

i) To identify the most critical signature immune biomarkers of GILTSA at earlier time points of clinical disease such as before challenge and at 5, 10, 15 and 20 minutes after challenge.

ii) To identify shared immune biomarkers linked to GILTSA among all four organs (small intestine, liver, heart and lungs)

iii) To confirm the most critical immune biomarkers identified in a further refined model where systemic anaphylaxis is induced by oral glutenin challenge

iv) To test whether wheat allergens when used in the same proportion as they are present in wheat flour (gliadins 30-40%, glutenins 45-50%, albumins 10-12% and globulins 5-8%) also elicit systemic anaphylaxis with the same profile of Organ-specific biomarker signatures.

v) To enable diagnostic application in human anaphylaxis, one can test whether most critical biomarker signatures can be replicated using blood, saliva and urine obtained mice with GILTSA.

vi) To analyze blood samples collected from healthy subjects and subjects with wheat anaphylaxis for critical and most critical immune biomarkers identified in this mouse model.

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