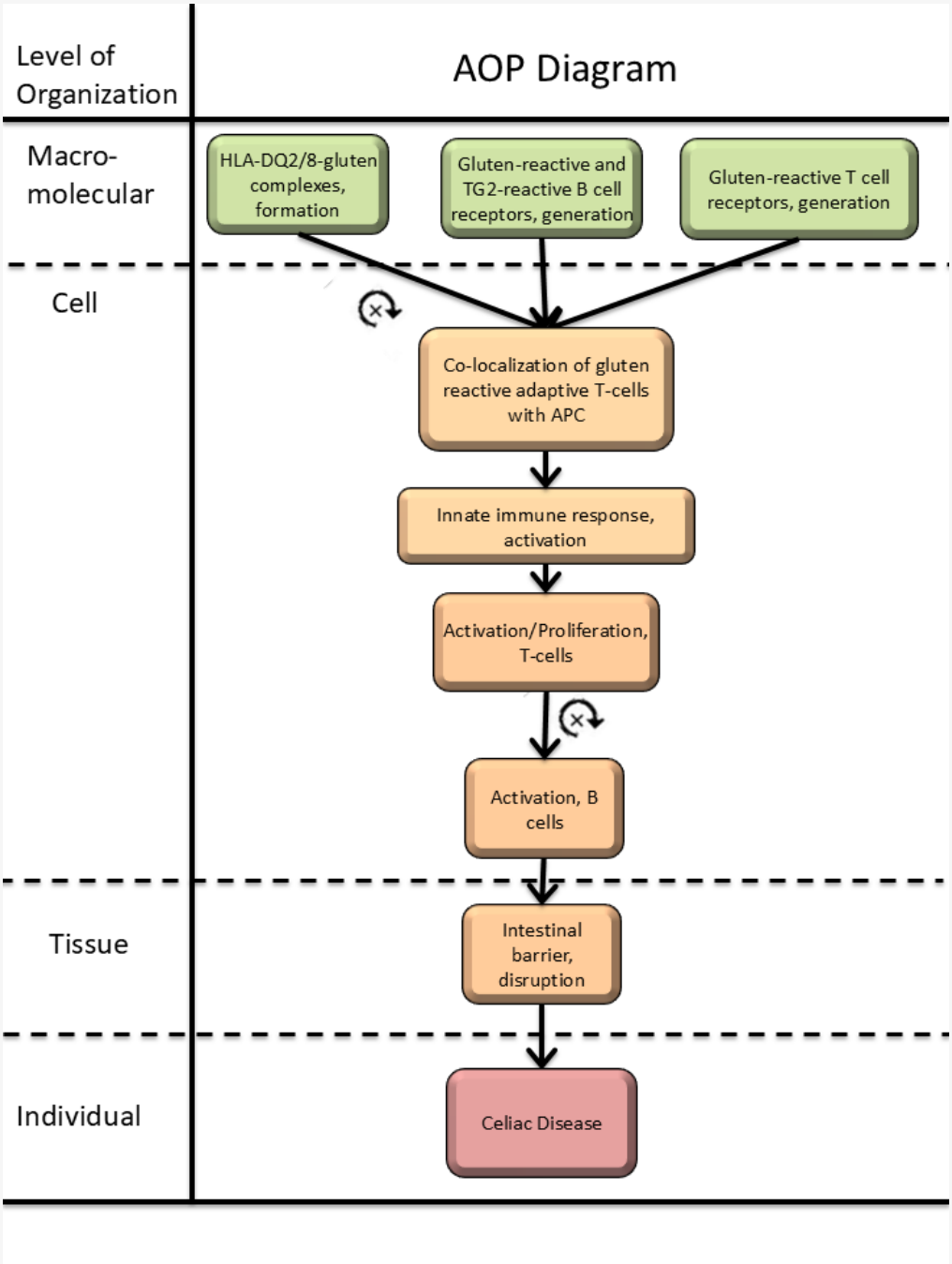
AOP ID and Title:

AOP 524: Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals
Short Title: Gluten-driven immune activation leading to celiac disease

Graphical Representation



Authors

Rodríguez-Fernández, Pablo

Koning, Frits

Gil González, Aina

Moreno Andújar, Javier

Noriega Fernández, Estefanía

Fernandez Dumont, Antonio

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite			

Abstract

Celiac disease is an immune-mediated disorder triggered by the ingestion of gluten in genetically susceptible individuals carrying human leukocyte antigen (HLA)-DQ2 or HLA-DQ8 molecules. This Adverse Outcome Pathway (AOP) describes the sequence of molecular and cellular events leading to celiac disease, beginning with key molecular initiating events (MIEs) and culminating in intestinal damage and disease manifestation.

The pathway is initiated by the formation of HLA-DQ2/8-gluten complexes, the generation of gluten-reactive T cell receptors, and the production of gluten- and transglutaminase 2 (TG2)-reactive B cell receptors. These MIEs facilitate the co-localization of gluten-reactive adaptive T-cells with antigen-presenting cells (APCs), an essential step in the immune response. This interaction triggers the activation of the innate immune response and subsequently leads to the activation of gluten-reactive CD4+ T cells. The cascade continues with the activation of gluten- and TG2-reactive B cells, which further amplifies the immune response and contributes to the disruption of the intestinal barrier. The final adverse outcome (AO) is the development of celiac disease, characterized by chronic intestinal inflammation, villous atrophy, and malabsorption.

The relationships between key events (KEs) in this AOP are supported by moderate levels of evidence, reflecting a well-characterized yet complex immunopathological process. Understanding this AOP provides valuable insights for risk assessment, the development of targeted therapies, and the refinement of strategies for gluten-related disorder management.

Background

In 2017, the EFSA GMO Panel published a guidance document (EFSA, 2017) that, for the first time, outlined a specific risk assessment strategy to predict the capacity of innovative or novel proteins to trigger celiac disease. This strategy, characterized by an integrated, stepwise, case-by-case approach, was made possible due to the well-documented pathogenesis of celiac disease and the known proteins involved. Specifically, gluten peptides presented by the disease-predisposing Human Leukocyte Antigen (HLA) class II molecules, HLA-DQ2 or HLA-DQ8, activate pro-inflammatory T-cells in the inflamed intestines of patients.

Ongoing efforts to refine risk assessment methodologies in this area are driven by new findings that suggest proteins from sources other than cereals may pose a hazard to individuals with celiac disease (Peterson et al., 2019) . This AOP is created to integrate the scientific knowledge into a conceptual framework in the regulatory context.

The risk assessment strategy developed for evaluating the potential of innovative or novel proteins to induce celiac disease is regarded as a benchmark, serving as an inspiration for the broader food safety assessment of novel proteins in the food sector.

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
	MIE	2252	Human leukocyte antigen DQ2/8-gluten complexes, formation	Formation of HLA-DQ2/8-gluten complexes
	MIE	2253	Gluten-reactive T cell receptors, generation	Generation of gluten-reactive T cell receptors
	MIE	2254	Gluten-reactive and transglutaminase 2 reactive B cell receptors, generation	Generation of gluten-reactive and TG2-reactive B cell receptors
	KE	2275	Gluten reactive adaptive T-cells with antigen presenting cells, co-localization	Co-localization of gluten reactive adaptive T-cells with APC
	KE	2255	Innate immune response, activation	Activation of the innate immune response
	KE	2260	Gluten-reactive CD4+ T cells, activation	Activation of gluten-reactive CD4+ T cells

Sequence	Type	Event ID	Title	Short name
	KE	2256	Gluten-reactive B cells and transglutaminase 2-reactive B cells, activation	Activation of gluten- and TG2-reactive B cells
	KE	1931	Intestinal barrier, disruption	Disruption of the intestinal barrier
	AO	2257	Celiac disease	Celiac disease

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Human leukocyte antigen DQ2/8-gluten complexes, formation	adjacent	Gluten reactive adaptive T-cells with antigen presenting cells, co-localization	Moderate	
Gluten-reactive and transglutaminase 2 reactive B cell receptors, generation	adjacent	Gluten reactive adaptive T-cells with antigen presenting cells, co-localization	Moderate	
Gluten-reactive T cell receptors, generation	adjacent	Gluten reactive adaptive T-cells with antigen presenting cells, co-localization	Moderate	
Gluten reactive adaptive T-cells with antigen presenting cells, co-localization	adjacent	Innate immune response, activation	Moderate	
Innate immune response, activation	adjacent	Gluten-reactive CD4+ T cells, activation	Moderate	
Gluten-reactive CD4+ T cells, activation	adjacent	Gluten-reactive B cells and transglutaminase 2-reactive B cells, activation	Moderate	
Gluten-reactive B cells and transglutaminase 2-reactive B cells, activation	adjacent	Intestinal barrier, disruption	Moderate	
Intestinal barrier, disruption	adjacent	Celiac disease	Moderate	

Stressors

Name	Evidence
Gluten	
Bacterial or viral Infections	
Tissue transglutaminase 2 (TG2) enzyme activity	

Overall Assessment of the AOP

KER1: Formation of HLA-DQ2/8-gluten complexes leads to Co-localization of gluten-reactive adaptive T-cells with APC

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The formation of the HLA-DQ2/8-gluten complex is a fundamental event for initiating the immune response in genetically predisposed individuals. The co-localization of gluten-reactive adaptive T cells with antigen-presenting cells (APCs) depends on the recognition of these complexes by the immune system. This relationship is essential for activating the adaptive immune system, a critical step in the development of celiac disease.
  Supporting Evidence: The interaction between gluten-HLA complexes and T cells is well-documented, and co-localization with APCs is required for T-cell activation (Sollid, 2002; van de Wal et al., 1999).

KER2: Generation of gluten-reactive and TG2-reactive B cell receptors leads to Co-localization of gluten-reactive adaptive T-cells with APC

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The generation of gluten-reactive and TG2-reactive B cell receptors facilitates the production of antibodies that contribute to the autoimmune response in celiac disease. While the direct influence on T-cell co-localization is less clear, the B-cell receptor generation is part of the broader immune response, influencing the progression of celiac disease. The co-localization of T-cells with APCs is indirectly impacted by the production of antibodies and antigen presentation.
  Supporting Evidence: While there is strong evidence for the generation of gluten-reactive B cells, the direct relationship with T-cell co-localization has moderate support, but it is still considered relevant for the disease process (Kagnoff, 2007).

KER3: Generation of gluten-reactive T cell receptors leads to Co-localization of gluten-reactive adaptive T-cells with APC

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: Gluten-reactive TCR generation is a critical early event in the immune response to gluten. Once these TCRs are generated, the T-cells are able to recognize gluten peptides presented by APCs, facilitating their co-localization. This step is essential for initiating the adaptive immune response, a key event in the pathogenesis of celiac disease.
  Supporting Evidence: There is strong evidence for the role of gluten-reactive TCRs in initiating immune responses, and their interaction with APCs is fundamental for the disease process (Jabri & Sollid, 2017).

KER4: Co-localization of gluten-reactive adaptive T-cells with APC leads to Activation of the innate immune response

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The co-localization of gluten-reactive T-cells with APCs activates the adaptive immune system, which in turn triggers innate immune pathways. Activation of the innate immune response amplifies the overall immune reaction, driving inflammation and tissue damage seen in celiac disease. Without this co-localization, the full immune activation needed for disease progression would not occur.
  Supporting Evidence: Studies indicate that activation of adaptive T-cells by APCs is tightly linked to subsequent activation of innate immune pathways (Lundin et al., 1993; Anderson et al., 2011).

KER5: Activation of the innate immune response leads to Activation of gluten-reactive CD4+ T cells

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The innate immune response plays a pivotal role in amplifying the activation of gluten-reactive CD4+ T cells, which is essential for driving the adaptive immune response in celiac disease. This relationship is critical because it ensures that the immune system's inflammatory reaction is properly mediated and directed toward the intestines.
  Supporting Evidence: The innate immune system is known to activate CD4+ T cells in response to antigenic stimulation, further promoting the inflammatory cascade in celiac disease.

KER6: Activation of gluten-reactive CD4+ T cells leads to Activation of gluten- and TG2-reactive B cells

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The activation of gluten-reactive CD4+ T cells is necessary to help activate B cells that produce gluten- and TG2-specific antibodies. These antibodies are markers of disease and contribute to the autoimmune responses that drive the pathology of celiac disease. Without T-cell activation, B-cell activation cannot occur, and the autoimmune response would be incomplete.
  Supporting Evidence: The interaction between activated T cells and B cells is well-established in the context of autoimmune diseases like celiac disease, where T-helper cells provide necessary signals for B cell activation (Kagnoff, 2007)**.**

KER7: Activation of gluten- and TG2-reactive B cells leads to Disruption of the intestinal barrier

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The activation of gluten- and TG2-reactive B cells results in the production of antibodies, such as anti-TG2, which play a significant role in tissue damage. This damage contributes to the disruption of the intestinal barrier, a hallmark of celiac disease. Without B-cell activation, the autoimmune-mediated intestinal damage would be less pronounced, and the disease would not progress in the same way.
  Supporting Evidence: The presence of anti-TG2 antibodies and their involvement in intestinal injury is well-documented in celiac disease (Lundin et al., 1993; Green & Cellier, 2007).

KER8: Disruption of the intestinal barrier leads to Celiac Disease

- **Adjacency:** Adjacent
- **Evidence:** Moderate
- **Essentiality:** High
  Rationale: The disruption of the intestinal barrier is the key event that allows gluten peptides and other immune activators to enter the mucosa, triggering the immune response and leading to celiac disease. This barrier disruption is essential for disease progression, as it creates the conditions for subsequent inflammation, villous atrophy, and clinical symptoms.
  Supporting Evidence: The breakdown of the intestinal barrier is considered a critical step in the pathogenesis of celiac disease. Without this disruption, immune activation would be limited, and disease symptoms would not manifest (Anderson et al., 2011).

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Sex Applicability

Sex	Evidence
Unspecific	High

The AOP applies specifically to humans, as celiac disease is inherently linked to the HLA-DQ2/8 genotype, which is unique to humans. The described mechanisms are particularly relevant to individuals with genetic susceptibility.

Essentiality of the Key Events

MIE1: Formation of HLA-DQ2/8-gluten Complexes

Essentiality: High
Rationale: The presence of HLA-DQ2/8 is a critical requirement for the development of celiac disease. Without these alleles, individuals cannot form gluten-HLA complexes, and celiac disease does not occur. The formation of this complex is a fundamental step in initiating the immune response against gluten in genetically predisposed individuals. (Sollid, 2002; van de Wal et al., 1999)

MIE2: Generation of Gluten-Reactive T Cell Receptors

Essentiality: High
Rationale: The generation of gluten-reactive TCRs is essential for the immune system to recognize gluten peptides. This step triggers the adaptive immune response, and individuals who lack gluten-reactive TCRs are unable to develop the disease. Clinical data consistently shows the presence of these TCRs in celiac patients, which play a direct role in the disease process (Lundin et al., 1993; Dieterich et al., 1997).

MIE3: Generation of Gluten-Reactive and TG2-Reactive B Cell Receptors

Essentiality: High
Rationale: B cells with receptors for both gluten and transglutaminase 2 (TG2) play a role in the immune response of celiac disease. These B cells contribute to the production of antibodies such as anti-TG2, which are a hallmark of

celiac disease. The formation of these receptors is crucial for the onset of the disease as they facilitate the autoimmune response (Kagnoff, 2007).

KE1: Co-localization of Gluten Reactive Adaptive T-cells with APCs

Essentiality: High
Rationale: Co-localization of gluten-reactive T cells with antigen-presenting cells (APCs) is essential for the activation of T cells and the subsequent immune response. This interaction is necessary for the initiation of the adaptive immune response, which drives the inflammatory processes seen in celiac disease. Without this step, the disease cannot progress.

KE2: Activation of the Innate Immune Response

Essentiality: High
Rationale: Activation of the innate immune response is crucial for amplifying the immune response in celiac disease. This step helps recruit additional immune cells to the site of inflammation and promotes further activation of adaptive immune cells. Disruption of this pathway can prevent the development of disease (Lundin et al., 1993).

KE3: Activation of Gluten-Reactive CD4+ T Cells

Essentiality: High
Rationale: The activation of gluten-reactive CD4+ T cells is central to celiac disease pathology. These T cells recognize gluten peptides and drive the autoimmune response, leading to intestinal inflammation and damage. This step is directly linked to the development of disease symptoms and is essential for disease progression (Lundin et al., 1993).

KE4: Activation of Gluten- and TG2-Reactive B Cells

Essentiality: High
Rationale: The activation of gluten- and TG2-reactive B cells leads to the production of antibodies such as anti-TG2 and anti-gluten antibodies. These antibodies contribute to the pathological immune response in celiac disease and are markers of disease activity (Kagnoff, 2007).

KE5: Disruption of the Intestinal Barrier

Essentiality: High
Rationale: The disruption of the intestinal barrier is a key event in celiac disease and contributes to the leakage of antigens, including gluten peptides, into the intestinal mucosa. This leads to further activation of immune cells and is a critical step in disease pathogenesis. Without this disruption, the immune response would not be sufficiently activated to trigger celiac disease (Anderson et al., 2011).

AO: Celiac Disease

Essentiality: High
Rationale: Celiac disease is the adverse outcome of the AOP. It is characterized by chronic inflammation of the small intestine, leading to villous atrophy, malabsorption, and various systemic manifestations. Without the preceding key events, the disease cannot occur. Therefore, celiac disease as an outcome is directly dependent on the successful progression of the earlier KEs (Green & Cellier, 2007).

Considerations for Potential Applications of the AOP (optional)

This AOP holds significant translational value, particularly for:

- Diagnostic development: Insights into antigen presentation and immune responses support biomarker identification (e.g., TG2 autoantibodies).
- Therapeutic strategies: Potential interventions targeting gluten processing, HLA binding, or immune modulation.
- Regulatory applications: Could support safety assessments of gluten-derived products or alternative treatments.

References

- Anderson, R. P., Degano, P., Godkin, A. J., Jewell, D. P., & Hill, A. V. S. (2011). In vivo antigen challenge in celiac disease: A randomized controlled study comparing oat and wheat challenge. *Gut, 60*(3), 420–427. https://doi.org/10.1136/gut.2010.221762
- Bebi C, Urbani D, Evangelisti M, Grossi V, Russo F, Del Rio A. (2024).Outsourcing preparatory work based on a systematic literature review for the development of adverse outcome pathways (AOPs) relevant for the capacity of proteins to trigger celiac disease. EFSA Supporting publication 2024:EN-8570. doi: 10.2903/sp.efsa.2024.EN-8570.
- Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med*. 3:797-801.

- EFSA Panel on Genetically Modified Organisms (GMO), Naegeli H, Birch AN, Casacuberta J, De Schrijver A, Gralak MA, Guerche P, et al. (2017). Guidance on allergenicity assessment of genetically modified plants. *EFSA Journal*, 15(6):4862. doi: 10.2903/j.efsa.2017.4862.
- Green, P. H. R., & Cellier, C. (2007). Celiac disease. *New England Journal of Medicine, 357*(17), 1731–1743. https://doi.org/10.1056/NEJMra071600
- https://doi.org/10.3109/08916934.2012.665520
- Jabri B, Sollid LM. T Cells in Celiac Disease. J Immunol. 2017 Apr 15;198(8):3005-3014. doi: 10.4049/jimmunol.1601693. PMID: 28373482; PMCID: PMC5426360.
- Kagnoff, M. F. (2007). Celiac disease: Pathogenesis of a model immunogenetic disease. *Journal of Clinical Investigation, 117*(1), 41–49. https://doi.org/10.1172/JCI30253
- Lundin, K. E. A., Scott, H., Hansen, T., Paulsen, G., Halstensen, T. S., Fausa, O., ... & Sollid, L. M. (1993). Gliadin-specific, HLA-DQ2-restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *Journal of Experimental Medicine, 178*(1), 187–196. https://doi.org/10.1084/jem.178.1.18
- Petersen J, Ciacchi L, Tran MT, Loh KL, Kooy-Winkelaar Y, Croft NP, Hardy MY, Chen Z, McCluskey J, Anderson RP, Purcell AW, Tye-Din JA, Koning F, Reid HH, Rossjohn J. (2020). T cell receptor cross-reactivity between gliadin and bacterial peptides in celiac disease. *Nature Structural & Molecular Biology*, 27: 49–61. doi: 10.1038/s41594-019-0354-z
- Sollid, L. M. (2002). Coeliac disease: Dissecting a complex inflammatory disorder. *Nature Reviews Immunology, 2*(9), 647–655. https://doi.org/10.1038/nri885
- van de Wal, Y., Kooy, Y. M., van Veelen, P. A., Peña, S. A., Mearin, M. L., Molberg, Ø., ... & Koning, F. (1999). Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proceedings of the National Academy of Sciences, 96*(11), 12005–12010. https://doi.org/10.1073/pnas.96.22.12005

Appendix 1

List of MIEs in this AOP

Event: 2252: Human leukocyte antigen DQ2/8-gluten complexes, formation

Short Name: Formation of HLA-DQ2/8-gluten complexes

Key Event Component

Process	Object	Action
antigen presentation trait	Human leukocyte antigen complex	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

dendritic cell

Organ term

Organ term

duodenum

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Human individuals with celiac disease, particularly those expressing HLA-DQ2 (including HLA-DQ2.5 and HLA-DQ2.2) or HLA-DQ8 (Sollid, 1989; Lundin, 1993; Dieterich, 1997; Molberg, 1997; Dieterich, 1998; Molberg, 1998; Nilsen, 1998; Van de Wal, 1998; Van de Wal, 1998b; Van de Wal, 1999; Arentz-Hansen, 2000; Vader, 2002; Vader, 2002b; Vader, 2003; Vader, 2003b; Meresse, 2004; Meresse, 2006; Tollefsen, 2006; Fallang, 2009; Qiao, 2011; Broughton, 2012; Di Niro, 2012; Sollid, 2012; Petersen, 2014; Qiao, 2014; Steinsbo, 2014). Patients with a genetic predisposition to celiac disease, especially those with specific haplotypes like DR3/DQw2, are most affected (Sollid, 1989; Nilsen, 1998; Van de Wal, 1998; Sollid, 2012).

Key Event Description

Celiac disease is an intestinal disorder triggered by gluten ingestion. It exclusively occurs in individuals who are positive for HLA-DQ2, HLA-DQ8, or both (Sollid, 1989; Dieterich, 1997). In patients with celiac disease, $CD4^+$ T cells specifically recognize complexes formed between HLA-DQ2/8 molecules and modified gluten peptides (Molberg, 1997; Meresse, 2004; Sollid, 2012). The formation of these HLA-DQ2/8-gluten complexes is a prerequisite for T-cell activation (Molberg, 1998; Arentz-Hansen, 2000; Vader, 2002).

Upon gluten ingestion, proteolytic fragments are generated through enzymatic cleavage in the upper gastrointestinal tract. These fragments are subsequently modified by tissue transglutaminase (TG2), which converts specific glutamine residues into glutamic acid (Dieterich, 1997; Molberg, 1998). This modification introduces negatively charged residues, which are crucial for high-affinity binding of the modified peptides to HLA-DQ2 or HLA-DQ8, as these molecules preferentially bind peptides with such negatively charged residues (Vader, 2003; Sollid, 2012). This process underlies the immune response observed in celiac disease.

How it is Measured or Detected

- Peptide binding assays: To measure the direct binding of gluten peptides to HLA-DQ molecules (Sollid, 1989; Arentz-Hansen, 2000; Sollid, 2012).
- Mass spectrometry: To analyze deamidated gluten peptides and their interaction with HLA-DQ (Van de Wal, 1998b; Vader, 2002; Fallang, 2009).
- HLA-binding assays: To assess the stability and affinity of peptide-HLA complexes (Molberg, 1998; Vader, 2002; Sollid, 2012).
- T cell proliferation assays: To measure T cell response to gluten peptides (Lundin, 1993; Meresse, 2004; Tollefsen, 2006).
- Tetramer staining: To detect gluten-specific T cells bound to HLA-DQ (Qiao, 2011; Broughton, 2012).
- Flow cytometry: To analyze cell surface markers for gluten peptide presentation (Meresse, 2004; Di Niro, 2012; Steinsbo, 2014).
- Immunohistochemistry & Immunofluorescence: To visualize tTG-gluten complexes (Dieterich, 1997; Di Niro, 2012).
- ELISA: To detect IgA anti-tTG antibodies as a marker for gluten interaction (Dieterich, 1998; Di Niro, 2012).
- Surface plasmon resonance (SPR): To measure TCR affinity for HLA-DQ-gluten complexes (Vader, 2002b; Broughton, 2012; Petersen, 2014).
- HPLC: To purify gluten peptides for further analysis (Van de Wal, 1998b; Van de Wal, 1999; Vader, 2002).
- Serological typing & ASO probes: To identify HLA-DQ alleles in patients (Sollid, 1989; Vader, 2003).

References

- Arentz-Hansen H, Körner R, Molberg Ø, Quarsten H, Vader W, Kooy YMC, Lundin KEA, Koning F, Roepstorff P,

Sollid LM, McAdam S. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J Exp Med*. 191:603-612.

- Broughton SE, Petersen J, Theodossis A, Scally SW, Loh KL, Thompson A, van Bergen J, Kooy-Winkelaar Y, Henderson KN, Beddoe T, Tye-Din JA, Mannering SI, Purcell AW, McCluskey J, Anderson RP, Koning F, Reid HH, Rossjohn J. (2012). Biased T cell receptor usage directed against human leukocyte antigen DQ8-restricted gliadin peptides is associated with celiac disease. *Immunity*. 37:611-621.
- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KE, Wilson PC, Sollid LM. (2012). High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. *Nat Med*. 18:441-445.
- Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med*. 3:797-801.
- Dieterich W, Laag E, Schöpper H, Volta U, Ferguson A, Gillett H, Riecken EO, Schuppan D. (1998). Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology*. 115:1317-1321.
- Fallang LE, Bergseng E, Hotta K, Berg-Larsen A, Kim CY, Sollid LM. (2009). Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nat Immunol*. 10:1096-1101.
- Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. (1993). Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med*. 178:187-196.
- Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. (2004). Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity*. 21:357-366.
- Meresse B, Curran SA, Ciszewski C, Orbelyan G, Setty M, Bhagat G, Lee L, Tretiakova M, Semrad C, Kistner E, Winchester RJ, Braud V, Lanier LL, Geraghty DE, Green PH, Guandalini S, Jabri B. (2006). Reprogramming of CTLs into natural killer-like cells in celiac disease. *J Exp Med*. 203:1343-1355.
- Molberg Ø, Kett K, Scott H, Thorsby E, Sollid LM, Lundin KE. (1997). Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. *Scand J Immunol*. 46:103-109.
- Molberg Ø, McAdam SN, Körner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P, Lundin KE, Sjöström H, Sollid LM. (1998). Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med*. 4:713-717.
- Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, Jahnsen J, Scott H, Brandtzaeg P. (1998). Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. *Gastroenterology*. 115:551-563.
- Petersen J, Montserrat V, Mujico JR, Loh KL, Beringer DX, van Liempt M, Thompson A, Mearin ML, Schweizer J, Kooy-Winkelaar Y, van Bergen J, Drijfhout JW, Kan WT, La Gruta NL, Anderson RP, Reid HH, Koning F, Rossjohn J. (2014). T-cell receptor recognition of HLA-DQ2-gliadin complexes associated with celiac disease. *Nat Struct Mol Biol*. 21:480-488.
- Qiao SW, Christophersen A, Lundin KE, Sollid LM. (2014). Biased usage and preferred pairing of alpha- and beta-chains of TCRs specific for an immunodominant gluten epitope in coeliac disease. *Int Immunol*. 26:13-19.
- Qiao SW, Raki M, Gunnarsen KS, Loset GA, Lundin KE, Sandlie I, Sollid LM. (2011). Posttranslational modification of gluten shapes TCR usage in celiac disease. *J Immunol*. 187:3064-3071.
- Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. (1989). Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J Exp Med*. 169:345-350.
- Sollid LM, Qiao SW, Anderson RP, Gianfrani C, Koning F. (2012). Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. *Immunogenetics*. 64:455-460.
- Steinsbø Ø, Henry Dunand CJ, Huang M, Mesin L, Salgado-Ferrer M, Lundin KE, Jahnsen J, Wilson PC, Sollid LM. (2014). Restricted VH/VL usage and limited mutations in gluten-specific IgA of coeliac disease lesion plasma cells. *Nat Commun*. 5:4041.
- Tollefsen S, Arentz-Hansen H, Fleckenstein B, Molberg Ø, Raki M, Kwok WW, Jung G, Lundin KE, Sollid LM. (2006). HLA-DQ2 and -DQ8 signatures of gluten T cell epitopes in celiac disease. *J Clin Invest*. 116:2226-2236.
- Vader W, de Ru A, van der Wal Y, Kooy Y, Benckhuijsen W, Mearin L, Drijfhout JW, van Veelen P, Koning F. (2002). Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J Exp Med*. 195:643-649.
- Vader W, Stepniak D, Bunnik EM, Kooy Y, de Haan W, Drijfhout JW, van Veelen PA, Koning F. (2003). Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology*. 125:1105-1113.

- Vader W, Stepniak D, Kooy Y, Mearin ML, Thompson A, Spaenij L, Koning F. (2003). The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T-cell responses. *Proc Natl Acad Sci U S A*. 100:12390-12395.
- Vader W, Kooy Y, van Veelen P, de Ru A, Harris D, Benckhuijsen W, Pena S, Mearin L, Drijfhout JW, Koning F. (2002). The gluten response in children with recent onset celiac disease. A highly diverse response towards multiple gliadin and glutenin-derived peptides. *Gastroenterology*. 122:1729-1737.
- van de Wal Y, Kooy Y, van Veelen P, Pena S, Mearin L, Papadopoulos G, Koning F. (1998). Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 161:1585-1588.
- van de Wal Y, Kooy Y, van Veelen P, Pena S, Mearin L, Papadopoulos G, Koning F. (1998). Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc Natl Acad Sci U S A* 95:10050-10054.
- van de Wal, Y., Kooy, Y.M.C., Veelen, van P., August, S.A., Drijfhout, J.W. and Koning, F. (1999). Glutenin is involved in the gluten-driven mucosal T cell response. Eur. J. Immunol. 29, 3133-3139.

Event: 2253: Gluten-reactive T cell receptors, generation

Short Name: Generation of gluten-reactive T cell receptors

Key Event Component

Process	Object	Action
gene conversion	alpha-beta T cell receptor complex	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term

T cell

Organ term

Organ term

thymus

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
During development and at adulthood	

Sex Applicability

Sex	Evidence
Unspecific	

Individuals with celiac disease, especially those expressing HLA-DQ2 (such as HLA-DQ2.5) or HLA-DQ8 (Sollid et al., 1989; Lundin et al., 1993; Molberg et al., 1997; Vader et al., 2002). The generation of these T cell receptors is specific to patients with a genetic predisposition to celiac disease (Sollid et al., 1989; Molberg et al., 1997; Qiao et al., 2011; Di Niro et al., 2012). Celiac disease patients with HLA-DQ2.2 are still susceptible to generating gluten-reactive T cell receptors, but their risk of developing the disease is generally lower than for those carrying HLA-DQ2.5 (Sollid et al., 1989; Vader et al., 2003; Tollefsen et al., 2006; Qiao et al., 2014).

Key Event Description

For T cell recognition of the HLA-DQ2/8-gluten complexes, T cell receptors specifically tuned to recognize these complexes must be generated (Molberg et al., 1997; Arentz-Hansen et al., 2000; Vader et al., 2002; Broughton et al., 2012; Qiao et al., 2014). This occurs through gene rearrangement during T cell development (Sollid et al., 1989; Molberg et al., 1997; Molberg et al., 1998; Vader et al., 2002). Notably, T cell receptors specific for the immunodominant gluten epitopes exhibit distinct characteristics, which are consistently shared among patients with celiac disease (Lundin et al., 1993; Dieterich et al., 1997; Molberg et al., 1997; Molberg et al., 1998; Vader et al., 2002; Vader et al., 2002b; Meresse et al., 2004; Tollefsen et al., 2006; Fallang et al., 2009; Qiao et al., 2011; Broughton et al., 2012; Petersen et al., 2014).

How it is Measured or Detected

- TCR sequencing: To identify the specific gene sequences of gluten-reactive T cell receptors (Sollid et al., 1989; Vader et al., 2002; Qiao et al., 2011; Qiao et al., 2014).
- T cell proliferation assays: To measure the activation and proliferation of gluten-reactive T cells in response to gluten peptides (Lundin et al., 1993; Molberg et al., 1998; Meresse et al., 2006; Fallang et al., 2009).
- Flow cytometry: To detect TCR expression and cell surface markers on gluten-reactive T cells (Meresse et al., 2004; Broughton et al., 2012).
- Tetramer staining: To identify gluten-reactive T cells by binding HLA-peptide complexes to T cells (Molberg et al., 1997; Tollefsen et al., 2006).
- Cytokine production assays: To measure cytokine release (e.g., IFN-γ) to assess T cell activation (Nilsen et al., 1998; Meresse et al., 2004).
- Mass spectrometry: To analyze deamidated gluten peptides and their interactions with TCRs (van de Wal et al., 1998; Vader et al., 2003).
- Chromium release assays: To measure the cytotoxicity of gluten-specific CD8+ T cells (Molberg et al., 1998; Tollefsen et al., 2006).

References

- Arentz-Hansen H, Körner R, Molberg Ø, Quarsten H, Vader W, Kooy YMC, Lundin KEA, Koning F, Roepstorff P, Sollid LM, McAdam S. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J Exp Med*. 191:603-612.
- Broughton SE, Petersen J, Theodossis A, Scally SW, Loh KL, Thompson A, van Bergen J, Kooy-Winkelaar Y, Henderson KN, Beddoe T, Tye-Din JA, Mannering SI, Purcell AW, McCluskey J, Anderson RP, Koning F, Reid HH, Rossjohn J. (2012). Biased T cell receptor usage directed against human leukocyte antigen DQ8-restricted gliadin peptides is associated with celiac disease. *Immunity*. 37:611-621.
- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KE, Wilson PC, Sollid LM. (2012). High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. *Nat Med*. 18:441-445.
- Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med*. 3:797-801.
- Dieterich W, Laag E, Schöpper H, Volta U, Ferguson A, Gillett H, Riecken EO, Schuppan D. (1998). Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology*. 115:1317-1321.
- Fallang LE, Bergseng E, Hotta K, Berg-Larsen A, Kim CY, Sollid LM. (2009). Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nat Immunol*. 10:1096-1101.
- Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. (1993). Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med*. 178:187-196.
- Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. (2004). Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity*. 21:357-366.

- Meresse B, Curran SA, Ciszewski C, Orbelyan G, Setty M, Bhagat G, Lee L, Tretiakova M, Semrad C, Kistner E, Winchester RJ, Braud V, Lanier LL, Geraghty DE, Green PH, Guandalini S, Jabri B. (2006). Reprogramming of CTLs into natural killer-like cells in celiac disease. *J Exp Med*. 203:1343-1355.
- Molberg Ø, Kett K, Scott H, Thorsby E, Sollid LM, Lundin KE. (1997). Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. *Scand J Immunol*. 46:103-109.
- Molberg Ø, McAdam SN, Körner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P, Lundin KE, Sjöström H, Sollid LM. (1998). Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nat Med*. 4:713-717.
- Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, Jahnsen J, Scott H, Brandtzaeg P. (1998). Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. *Gastroenterology*. 115:551-563.
- Petersen J, Montserrat V, Mujico JR, Loh KL, Beringer DX, van Liempt M, Thompson A, Mearin ML, Schweizer J, Kooy-Winkelaar Y, van Bergen J, Drijfhout JW, Kan WT, La Gruta NL, Anderson RP, Reid HH, Koning F, Rossjohn J. (2014). T-cell receptor recognition of HLA-DQ2-gliadin complexes associated with celiac disease. *Nat Struct Mol Biol*. 21:480-488.
- Qiao SW, Christophersen A, Lundin KE, Sollid LM. (2014). Biased usage and preferred pairing of alpha- and beta-chains of TCRs specific for an immunodominant gluten epitope in coeliac disease. *Int Immunol*. 26:13-19.
- Qiao SW, Raki M, Gunnarsen KS, Loset GA, Lundin KE, Sandlie I, Sollid LM. (2011). Posttranslational modification of gluten shapes TCR usage in celiac disease. *J Immunol*. 187:3064-3071.
- Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. (1989). Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J Exp Med*. 169:345-350.
- Sollid LM, Qiao SW, Anderson RP, Gianfrani C, Koning F. (2012). Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. *Immunogenetics*. 64:455-460.
- Steinsbø Ø, Henry Dunand CJ, Huang M, Mesin L, Salgado-Ferrer M, Lundin KE, Jahnsen J, Wilson PC, Sollid LM. (2014). Restricted VH/VL usage and limited mutations in gluten-specific IgA of coeliac disease lesion plasma cells. *Nat Commun*. 5:4041.
- Tollefsen S, Arentz-Hansen H, Fleckenstein B, Molberg Ø, Raki M, Kwok WW, Jung G, Lundin KE, Sollid LM. (2006). HLA-DQ2 and -DQ8 signatures of gluten T cell epitopes in celiac disease. *J Clin Invest*. 116:2226-2236.
- Vader W, de Ru A, van der Wal Y, Kooy Y, Benckhuijsen W, Mearin L, Drijfhout JW, van Veelen P, Koning F. (2002). Specificity of tissue transglutaminase explains cereal toxicity in celiac disease. *J Exp Med*. 195:643-649.
- Vader W, Stepniak D, Bunnik EM, Kooy Y, de Haan W, Drijfhout JW, van Veelen PA, Koning F. (2003). Characterization of cereal toxicity for celiac disease patients based on protein homology in grains. *Gastroenterology*. 125:1105-1113.
- Vader W, Stepniak D, Kooy Y, Mearin ML, Thompson A, Spaenij L, Koning F. (2003). The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T-cell responses. *Proc Natl Acad Sci U S A*. 100:12390-12395.
- Vader W, Kooy Y, van Veelen P, de Ru A, Harris D, Benckhuijsen W, Pena S, Mearin L, Drijfhout JW, Koning F. (2002). The gluten response in children with recent onset celiac disease. A highly diverse response towards multiple gliadin and glutenin-derived peptides. *Gastroenterology*. 122:1729-1737.
- van de Wal Y, Kooy Y, van Veelen P, Pena S, Mearin L, Papadopoulos G, Koning F. (1998). Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 161:1585-1588.
- van de Wal Y, Kooy Y, van Veelen P, Pena S, Mearin L, Papadopoulos G, Koning F. (1998). Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. *Proc Natl Acad Sci U S A*. 95:10050-10054.
- van de Wal, Y., Kooy, Y.M.C., Veelen, van P., August, S.A., Drijfhout, J.W. and Koning, F. (1999). Glutenin is involved in the gluten-driven mucosal T cell response. Eur. J. Immunol. 29, 3133-3139.

Event: 2254: Gluten-reactive and transglutaminase 2 reactive B cell receptors, generation

Short Name: Generation of gluten-reactive and TG2-reactive B cell receptors

Key Event Component

Process	Object	Action
gene conversion of immunoglobulin genes	B cell receptor complex	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	MolecularInitiatingEvent

Biological Context

Level of Biological Organization

Molecular

Cell term

Cell term
B cell

Organ term

Organ term
bone marrow

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Humans, with a female to male proportion of approximately 2 to 1

Key Event Description

The presence of TG2-specific antibodies is a hallmark of celiac disease and is commonly used in its diagnosis of celiac disease (Fleur du Pre et al., 2020). Additionally, antibodies targeting deamidated gluten peptides are frequently detected in patients with celiac disease. The persistent production of these deamidated gluten- and TG2-reactive antibodies contributes to chronic inflammation and tissue damage in the small intestine.

For the antibody-mediated recognition of deamidated gluten and TG2, B cell receptors must be generated during B cell development. Similar to T cell receptors, this process occurs through gene rearrangement. During this process, constant and variable gene segments are joined, encoding distinct light and heavy chains. Antibodies consist of two light and two heavy chains, with a structure that includes two antigen-binding sites formed by the variable regions and a single constant region. Notably, specific variable gene segments encoding TG2-specific antibodies are consistently shared among patients with celiac disease.

How it is Measured or Detected

Gene rearrangement itself can be detected through molecular biological techniques. In practice, however, it is much more common to detect antibodies specific for TG2 and deamidated gluten with enzyme-linked immunosorbent assay (ELISA) or rapid test kits.

References

- Arentz-Hansen H, Körner R, Molberg Ø, Quarsten H, Vader W, Kooy YMC, Lundin KEA, Koning F, Roepstorff P, Sollid LM, McAdam S. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J Exp Med*. 191:603-612.
- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KE, Wilson PC, Sollid LM. (2012). High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. *Nat Med*. 18:441-445.
- du Pré MF, Blazevski J, Dewan AE, Stamnaes J, Kanduri C, Sandve GK, Johannesen MK, Lindstad CB, Hnida K, Fugger L, Melino G, Qiao SW, Sollid LM. (2020). B cell tolerance and antibody production to the celiac disease autoantigen transglutaminase 2. J Exp Med. Feb 3;217(2):e20190860. doi: 10.1084/jem.20190860.
- Fallang LE, Bergseng E, Hotta K, Berg-Larsen A, Kim CY, Sollid LM. (2009). Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nat Immunol*. 10:1096-1101.

- Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. (1993). Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med*. 178:187-196.
- Vader W, Kooy Y, van Veelen P, de Ru A, Harris D, Benckhuijsen W, Pena S, Mearin L, Drijfhout JW, Koning F. (2002). The gluten response in children with recent onset celiac disease. A highly diverse response towards multiple gliadin and glutenin-derived peptides. *Gastroenterology*. 122:1729-1737.

List of Key Events in the AOP

Event: 2275: Gluten reactive adaptive T-cells with antigen presenting cells, co-localization

Short Name: Co-localization of gluten reactive adaptive T-cells with APC

Key Event Component

Process	Object	Action
T cell migration	T cell	occurrence
dendritic cell migration	professional antigen presenting cell	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

T cell

Organ term

Organ term

duodenum lamina propria

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence

Sex	Evidence
Unspecific	

Taxonomic: Homo sapiens
Life-stage: any
Sex: Female to Male ration: approximately 2 to 1

Key Event Description

For the initiation of adaptive responses, the cellular components required need to be simultaneously present in lymphoid structures present in the target tissue/organ. In the case of the gastrointestinal system these lymphoid structures are the Peyer's patches and the mesenteric lymph nodes. The relevant cellular components are professional antigen presenting cells like dendritic cells, antigen specific B cells and T cells expressing gluten-reactive T cell receptors. Both dendritic cells and antigen specific B cells can endocytose and degrade complex antigens, resulting in peptides of variable length. Upon intracellular binding of such peptide antigens to HLA-class II molecules, like HLA-DQ2.5 and HLA-DQ8, such HLA-peptide complexes will be transported to the cell surface of the antigen presenting cells, where they can be recognized by T cells expressing T cell receptors specific for the HLA-peptide complex. Thus, once all relevant cellular components are present in the lymphoid structures, the stage is set for the induction of a gluten-specific T cell response.

How it is Measured or Detected

Detection methods could include the measureing of T-cell activation by T cell stimulation assays (e.g. cytokine secretion assays, cytotoxicity assay), or could also include quantification of peptide-HLA-DQ complexes by ELISA (e.g. against HLA-peptide complex) or mass spectrometry (e.g. peptide mass fingerprinting).

References

Parham P. The Immune System, Fifth Edition. ISBN: 978-0-393-53334-7

Event: 2255: Innate immune response, activation

Short Name: Activation of the innate immune response

Key Event Component

Process	Object	Action
immune system process		occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	KeyEvent

Biological Context

Level of Biological Organization
Cellular

Organ term

Organ term
small intestine

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	High

Homo sapiens

Key Event Description

An adaptive T and B cell response is only initiated after innate immune activation. While the exact nature of the agent causing innate immune activation remains unknown, it could involve exposure to viruses or bacteria, particularly when this occurs in the gastrointestinal tract, or exposure to environmental factors, such as gluten.

Certain infections may induce inflammation that promotes the activation of self-reactive B cells. Preexposure to IL-15, a cytokine upregulated in inflammatory and infectious conditions (Meresse et al., 2006; Fehniger et al., 2001), plays a key role in this process. IL-15 activates NK cells and intraepithelial lymphocytes (IELs) (CD8+ T cells), which become cytotoxic, damaging epithelial cells in the intestine. This epithelial damage allows gluten peptides to cross into the lamina propria, further perpetuating the immune response and inflammation.
Nilsen et al. (1998) hypothesized that a Th1-like profile, characterized by predominantly high levels of IFNγ, results from various types of intestinal immune responses. This was recently observed in studies following intestinal astrovirus infection (Molberg et al., 1998) and in cases of cow's milk-sensitive enteropathy.
Additionally, infections can cause molecular mimicry, where pathogen-derived antigens resemble self-antigens. This can trigger an immune response that cross-reacts with the body's own tissue, contributing to the development of autoimmune conditions such as celiac disease (Petersen et al., 2020).

How it is Measured or Detected

Measured by biomarkers or functional tests. The assessment would focus on immune cell activation, cytokine production and pathogen recognition. Methods could include, flow cytometry, microscopy and staining, ELISA, RT-PC, oxidative burst assays, Toll-like receptor simuation assays.

References

- Fehniger, T.A., and M.A. Caligiuri. (2001). Interleukin 15: biology and relevance to human disease. Blood. 97:14–32.
- Meresse B, Curran SA, Ciszewski C, Orbelyan G, Setty M, Bhagat G, Lee L, Tretiakova M, Semrad C, Kistner E, Winchester RJ, Braud V, Lanier LL, Geraghty DE, Green PH, Guandalini S, Jabri B. (2006). Reprogramming of CTLs into natural killer-like cells in celiac disease. J Exp Med. 203:1343-1355.
- Molberg Ø, Nilsen EM, Sollid LM, Scott H, Brandtzaeg P, Thorsby E, Lundin KEA. (1998). CD41 T-cells with specific reactivity against astrovirus isolated from normal human small intestine. Gastroenterology 114:115–122.
- Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, Jahnsen J, Scott H, Brandtzaeg P. (1998). Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. Gastroenterology. 115:551-563.
- Petersen J, Ciacchi L, Tran MT, Loh KL, Kooy-Winkelaar Y, Croft NP, Hardy MY, Chen Z, McCluskey J, Anderson RP, Purcell AW, Tye-Din JA, Koning F, Reid HH, Rossjohn J. (2020). T cell receptor cross-reactivity between gliadin and bacterial peptides in celiac disease. Nat Struct Mol Biol. Jan;27(1):49-61. doi: 10.1038/s41594-019-0353-4.

Event: 2260: Gluten-reactive CD4+ T cells, activation

Short Name: Activation of gluten-reactive CD4+ T cells

Key Event Component

Process	Object	Action
T cell activation involved in immune response	T cell	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

T cell

Organ term

Organ term

duodenum

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Homo sapiens

Key Event Description

In the intestinal mucosa of celiac disease patients, gluten-specific CD4+ T cells recognize gliadin antigens, particularly those deamidated by the tissue transglutaminase enzyme (TG2) (Lundin et al., 1993; Arentz-Hansen et al., 2000). These antigens are presented by antigen-presenting cells (APCs) expressing HLA-DQ2 or HLA-DQ8 molecules (Arentz-Hansen et al., 2000; Broughton et al., 2012). Antigen presentation activates the gluten-specific CD4+ T cells, initiating a cascade of immune responses.

Upon activation, gluten-specific CD4+ T cells undergo rapid clonal expansion and differentiation into a pro-inflammatory population that secretes cytokines such as interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) (Nilsen et al., 1998). Additionally, B cells with B cell receptors (BCRs) specific for TG2-gliadin complexes can present these complexes to gluten-specific CD4+ T cells, further stimulating their activation (Di Niro et al., 2012).

The release of IFNγ upregulates the expression of HLA class II molecules on APCs, enhancing the efficiency of gluten peptide presentation (Meresse et al., 2006). This creates a feedback loop that amplifies antigen presentation and

intensifies the T cell-mediated immune response to gluten.

How it is Measured or Detected

- Isolation from Intestinal Biopsies: Gluten-reactive T cells can be isolated from patients with celiac disease but not from healthy controls.
- T Cell Activation Measured by Phenotyping: Activated T cells, specifically CD25+ (expressing the IL-2 receptor α-chain), can be identified through phenotyping. When small intestinal biopsies from celiac disease patients on a gluten-free diet are challenged ex vivo with gluten, CD4+ T cells in the lamina propria become activated and express CD25 (Lundin et al., 1993).
- Proliferation Assays: Proliferation assays are performed using antigen-presenting cells (APCs), specific peptides or digested gliadin, and T cells labeled with a marker such as tritiated thymidine (^{3}H). After incubation, plates are harvested, and the incorporation of ^{3}H is measured to assess T cell proliferation (Arentz-Hansen et al., 2000, Broughton et al., 2012, Fallang et al., 2009).
- In Vitro T/B-Cell Cooperation Assay: T/B-cell cooperation is assessed by culturing A20 B-cells with TCR transfectants in the presence of various complexes and conditions. Murine IL-2 secretion, a marker of T cell activation, is measured by ELISA as the readout (Di Niro et al., 2012).
- Additional Applications of ELISA: ELISA is also used to quantify cytokines in biopsies, providing insights into immune responses (Nilsen et al., 1998).
- Quantification of Cytokine mRNA Expression: Competitive PCR is employed to compare cytokine production at the mRNA level. mRNA is reverse-transcribed, amplified by PCR, and visualized on agarose gels. Band intensities are analyzed to determine the ratios of cytokine expression (Nilsen et al., 1998).

References

- Arentz-Hansen H, Körner R, Molberg Ø, Quarsten H, Vader W, Kooy YMC, Lundin KEA, Koning F, Roepstorff P, Sollid LM, McAdam S. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med. 191:603-612.
- Broughton SE, Petersen J, Theodossis A, Scally SW, Loh KL, Thompson A, van Bergen J, Kooy-Winkelaar Y, Henderson KN, Beddoe T, Tye-Din JA, Mannering SI, Purcell AW, McCluskey J, Anderson RP, Koning F, Reid HH, Rossjohn J. (2012). Biased T cell receptor usage directed against human leukocyte antigen DQ8-restricted gliadin peptides is associated with celiac disease. Immunity. 37:611-621.
- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KE, Wilson PC, Sollid LM. (2012). High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. Nat Med. 18:441-445.
- Fallang LE, Bergseng E, Hotta K, Berg-Larsen A, Kim CY, Sollid LM. (2009). Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. Nat Immunol. 10:1096-1101.
- Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. (1993). Gliadin-specific, HLA-DQ(alpha 10501,beta 10201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med. 178:187-196.
- Meresse B, Curran SA, Ciszewski C, Orbelyan G, Setty M, Bhagat G, Lee L, Tretiakova M, Semrad C, Kistner E, Winchester RJ, Braud V, Lanier LL, Geraghty DE, Green PH, Guandalini S, Jabri B. Reprogramming of CTLs into natural killer-like cells in celiac disease. J Exp Med 2006;203:1343-1355.
- Molberg Ø, Kett K, Scott H, Thorsby E, Sollid LM, Lundin KE. (1997). Gliadin specific, HLA DQ2-restricted T cells are commonly found in small intestinal biopsies from coeliac disease patients, but not from controls. Scand J Immunol. 46:103-109.
- Molberg Ø, McAdam SN, Körner R, Quarsten H, Kristiansen C, Madsen L, Fugger L, Scott H, Noren O, Roepstorff P, Lundin KE, Sjöström H, Sollid LM. (1998). Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. Nat Med. 4:713-717.
- Nilsen EM, Jahnsen FL, Lundin KE, Johansen FE, Fausa O, Sollid LM, Jahnsen J, Scott H, Brandtzaeg P. (1998). Gluten induces an intestinal cytokine response strongly dominated by interferon gamma in patients with celiac disease. Gastroenterology. 115:551-563.

Event: 2256: Gluten-reactive B cells and transglutaminase 2-reactive B cells, activation

Short Name: Activation of gluten- and TG2-reactive B cells

Key Event Component

Process	Object	Action

Process	Object	Action
B cell activation involved in immune response	B cell	occurrence

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	KeyEvent

Biological Context

Level of Biological Organization

Cellular

Cell term

Cell term

B cell

Organ term

Organ term

duodenum

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Activation of B cells specific to tissue transglutaminase 2 (TG2) are well documented in humans. Although Irish setter can develop partial lymphocyte infiltration in response to wheat diet, it is not a CD4 T cell mediated disease. Monkeys can produce anti gliadins IgA and IgG but the levels of these antibodies do not change with removal or reintroduction of dietary gluten. In mice there is a clear antibody response to TG, but not in a gluten dependent way (Marietta et al., 2011).

Key Event Description

B cells specific to tissue transglutaminase 2 (TG2) are activated in a CD4+ T-cell-dependent manner. Gluten-specific T cells, once activated, provide the necessary "help" to TG2-reactive B cells, facilitating their activation and differentiation into plasma cells that produce anti-TG2 antibodies in the lamina propria (Di Niro et al., 2012). TG2-specific B cells appear to undergo limited affinity maturation, even under chronic antigen exposure, suggesting that their activation relies on naive B cells and is sustained by ongoing gluten exposure (Di Niro et al., 2012; Steinsbo et al., 2014). The crosslinking of TG2 with B-cell receptors (BCRs) may lower the activation threshold for naive TG2-specific B cells, enhancing their activation and subsequent proliferation. This contributes to the high abundance of plasma cells secreting anti-TG2 antibodies, creating a feedback loop that further amplifies antigen presentation to T cells (Di Niro et al., 2012).

Mechanistic Insights

The selection of high-affinity B cells during affinity maturation depends on peptide presentation to T cells. Evidence from Di Niro et al. (2012) supports the necessity of T-cell help, as mutations reducing affinity were linked to a loss of function. B cells engineered to express anti-TG2 BCRs were shown to process and present TG2-gliadin complexes, activating gluten-specific T cells derived from celiac patients, confirming a T-cell-dependent model for antibody generation (Sollid et al., 1997; Sollid et al., 2002; Di Niro et al., 2012).

Further insights by Fleur du Pré et al. (2020) reveal that while most autoreactive B cells are typically removed or silenced through central and peripheral tolerance mechanisms, in celiac disease these controls fail. Anti-TG2 B cells survive and produce autoantibodies when T-cell help is available. These autoreactive B cells act as antigen-presenting cells (APCs), driving the anti-gluten T-cell response, creating an amplification loop central to disease pathogenesis.

Failure of Tolerance Mechanisms

In healthy individuals, autoreactive B cells are controlled through receptor editing, apoptosis, or anergy (Gay et al., 1993; Tiegs et al., 1993; Nemazee and Bürki, 1989; Goodnow et al., 1988). However, in celiac disease, TG2-reactive B cells escape these mechanisms. Some autoreactive B cells may remain "ignorant" in the absence of sufficient antigenic stimulation but become pathogenic when gluten-derived peptides and TG2 form complexes. This failure enables both TG2- and gluten-reactive B cells to survive and contribute to the disease state (Fleur du Pre et al 2020).

How it is Measured or Detected

B cell activation can be evaluated by measuring the generation of monoclonal antibodies. Single plasma cells can be isolated from intestinal biopsies and cultured or sorted with gluten peptide tetramers. The resulting monoclonal antibodies can be analyzed for their reactivity to gluten and TG2 antigens, by ELISA or AlphaLISA (Di Niro et al., 2012). Alternatively, fluorescently labeled peptides (e.g., biotinylated gliadin peptides) can be used in flow cytometry to sort and analyze gluten-specific IgA+ plasma cells, allowing for the detection and characterization of B cell activation in celiac lesions (Di Niro et al., 2012).

References

- Arentz-Hansen H, Körner R, Molberg Ø, Quarsten H, Vader W, Kooy YMC, Lundin KEA, Koning F, Roepstorff P, Sollid LM, McAdam S. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. *J Exp Med*. 191:603-612.
- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KE, Wilson PC, Sollid LM. (2012). High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. *Nat Med*. 18:441-445.
- du Pré MF, Blazevski J, Dewan AE, Stamnaes J, Kanduri C, Sandve GK, Johannesen MK, Lindstad CB, Hnida K, Fugger L, Melino G, Qiao SW, Sollid LM. (2020). B cell tolerance and antibody production to the celiac disease autoantigen transglutaminase 2. J Exp Med. Feb 3;217(2):e20190860. doi: 10.1084/jem.20190860.
- Fallang LE, Bergseng E, Hotta K, Berg-Larsen A, Kim CY, Sollid LM. (2009). Differences in the risk of celiac disease associated with HLA-DQ2.5 or HLA-DQ2.2 are related to sustained gluten antigen presentation. *Nat Immunol*. 10:1096-1101.
- Gay D, Saunders T, Camper S, and Weigert M. (1993). Receptor editing: an approach by autoreactive B cells to escape tolerance. J. Exp. Med. 177:999–1008. 10.1084/jem.177.4.999
- Goodnow CC, Crosbie J, Adelstein S, Lavoie TB, Smith-Gill SJ, Brink RA, Pritchard-Briscoe H, Wotherspoon JS, Loblay RH, Raphael K. (1988). Altered immunoglobulin expression and functional silencing of self-reactive B lymphocytes in transgenic mice. Nature. 334:676–682. 10.1038/334676a0
- Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. (1993). Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *J Exp Med*. 178:187-196.
- Marietta EV, David CS, Murray JA. (2011). Important lessons derived from animal models of celiac disease. Int Rev Immunol. Aug;30(4):197-206. doi: 10.3109/08830185.2011.598978.
- Nemazee DA, and Bürki K. (1989). Clonal deletion of B lymphocytes in a transgenic mouse bearing anti-MHC class I antibody genes. Nature. 337:562–566. 10.1038/337562a0
- Sollid LM, Molberg O, McAdam S, Lundin KE. (1997). Autoantibodies in coeliac disease: tissue transglutaminase--guilt by association?. Gut Dec;41(6):851-2. doi: 10.1136/gut.41.6.851.
- Sollid LM. (2002). Coeliac disease: dissecting a complex inflammatory disorder. Nat Rev Immunol. 2:647–655.
- Steinsbø Ø, Henry Dunand CJ, Huang M, Mesin L, Salgado-Ferrer M, Lundin KE, Jahnsen J, Wilson PC, Sollid LM. (2014). Restricted VH/VL usage and limited mutations in gluten-specific IgA of coeliac disease lesion plasma cells. Nat Commun. 5:4041.
- Tiegs SL, Russell DM, and Nemazee D. (1993). Receptor editing in self-reactive bone marrow B cells. J. Exp. Med.

177:1009–1020. 10.1084/jem.177.4.1009

- Vader W, Kooy Y, van Veelen P, de Ru A, Harris D, Benckhuijsen W, Pena S, Mearin L, Drijfhout JW, Koning F. (2002). The gluten response in children with recent onset celiac disease. A highly diverse response towards multiple gliadin and glutenin-derived peptides. Gastroenterology. 122:1729-1737.
- van de Wal Y, Kooy Y, van Veelen P, Pena S, Mearin L, Papadopoulos G, Koning F. (1998). Small intestinal T cells of celiac disease patients recognize a natural pepsin fragment of gliadin. Proc Natl Acad Sci U S A. 95:10050-10054.

Event: 1931: Intestinal barrier, disruption

Short Name: Disruption of the intestinal barrier

Key Event Component

Process	Object	Action
	barrier epithelial cell	disrupted

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:422 - Binding of SARS-CoV-2 to ACE2 in enterocytes leads to intestinal barrier disruption	KeyEvent
Aop:530 - Endocytotic lysosomal uptake leads to intestinal barrier disruption	AdverseOutcome
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	KeyEvent

Stressors

Name
Sars-CoV-2

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
intestine

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Male	

Sex	Evidence
Female	

Human

Key Event Description

A proper definition (and related ontology) of the intestinal barrier and permeability would benefit the understanding of this biological event central in many diseases. However, it is generally accepted that the intestinal barrier is a multilayer system encompassing :

- a chemical barrier able to detoxify bacterial endotoxins,

- a mucus layer providing a physical barrier against bacteria,

- an one-cell-thick epithelial layer which physical barrier function is ensured by epithelial cell integrity and by tight junction proteins (occludins, claudins and zonulins), adherence junctions and desmosomes [2,4,5]

- the cellular immune system present in the lamina propria underlying the epithelial cell layer

- the antibacterial proteins secreted by the specialized intestinal epithelial cells or the Paneth cells.

Together with the chemical barrier of the mucosal layer and the cellular immune system, the intestinal epithelial cell layer has actually two **barrier** functions:[1–3]

i. It acts as a **physical** barrier against external factors (pathogens, toxins),
ii. It acts as a **selective** barrier by regulating the absorption of essential dietary nutrients and ions, meaning their transport from the lumen into the blood.

Intestinal permeability[6] describes the movement of molecules across the intestinal barrier from the lumen to the blood (Figure 1), and as such, is the measurable feature of the intestinal barrier.

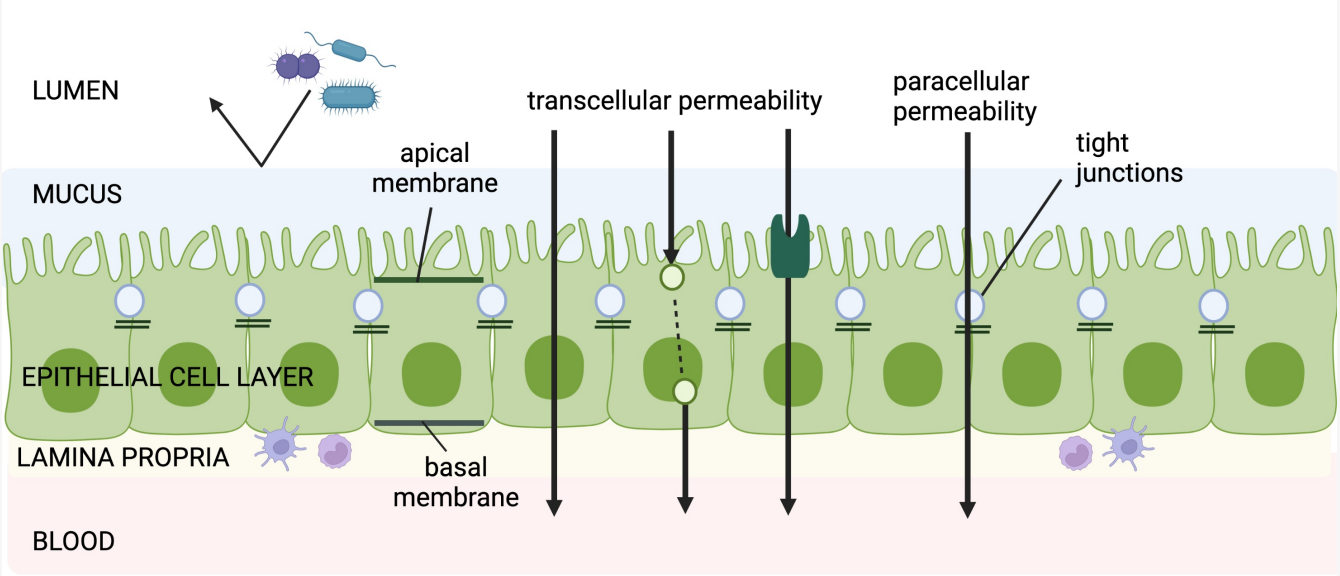


Figure 1. Created with Biorender.com

Molecules can cross the epithelium via paracellular or transcellular route. Transcellular permeability encompass passive diffusion from the apical to the basal side (from the lumen to the blood), vesicle-mediated transcytosis and uptake mediated by a membrane receptor. Paracellular permeability is regulated by the tight junctions between adjacent cells and by the integrity of the epithelium.

Alteration or disruption of one or more layers of the intestinal barrier leads to increased intestinal permeability, also called intestinal hyperpermeability or “leaky gut”, enhancing the transport of pathogens, toxins (such as lipopolysaccharides), undigested nutrients and the translocation of bacteria of the gut microbiota from the intestinal lumen into the systemic circulation[3].

How it is Measured or Detected

The definition of intestinal permeability being relatively broad includes altered paracellular route, regulated by TJ proteins, transcellular routes involving membrane transporters and channels, and endocytic mechanisms. Paracellular intestinal permeability can be assessed *in vivo* via different molecules and via putatiive blood biomarkers and *ex vivo* in Ussing chambers combining electrophysiology and probes of different molecular sizes. The latter is still the gold standard technique for assessing the epithelial barrier function, whereas *in vivo* techniques are also broadly used despite limitations (doi: 10.3389/fnut.2021.717925).

In humans.

Virtually all *in vivo* methods to assess paracellular intestinal permeability rely on the urinary excretion of orally ingested probes. Several markers, including different sizes of PEG, ^{51}CrEDTA, and especially sugars have been used, each with advantages and disadvantages (doi:

10.3389/fnut.2021.717925). **Intestinal Permeability Assessment** (IPA) directly measures the ability of two non-metabolized sugar molecules (lactulose and mannitol) to permeate the small intestinal barrier by paracellular passage (sign of perturbed TJ-lactulose) or by transcellular passage (giving information of the whole epithelial absorptive area-mannitol), respectively. The patient drinks a premeasured amount of those sugars and 6h after, the ratio of Lactulose/Mannitol levels is measured in the urine [11].

Levels in plasma/serum or in feces of:

- Markers of epithelial cell damage, such as intestinal fatty acid binding protein (FABP)
- Markers of tight junction alterations, such as zonulin levels (doi:10.1080/21688370.2016.1251384)
- Microbial translocation, such as peptidoglycans and lipopolysaccharides (LPS) and gut microbiota alteration.

In vitro systems[12]

Transepithelial electrical resistance (**TEER**) or the Lucifer Yellow (**LY**) leakage assay are techniques to measure barrier integrity and permeability of a cell layer[13]. Caco-2 cells are human epithelial colorectal adenocarcinoma cells with a structure and function similar to the differentiated small intestinal epithelial cells (e.g. exhibit microvilli). Caco-2 cells can be plated in wells as monolayers[14,11]. Other cell lines can be used, such as intestinal epithelial cells (IEC) or primary epithelial cells from human intestinal biopsies[12]. Co-culturing of enterocyte-like cells with immune cells in three-dimensional structure and within a microfluidic gut-on-chip has been shown to reflect better the physiology of the gut epithelium. Epi-Intestinal™ is an example of 3D human primary cell-based organotypic small intestinal model which allows evaluation of TEER and LY leakage assay (doi: 10.1007/s11095-018-2362-0).

In vivo system

In mice, one way to study intestinal paracellular permeability is by measuring the ability of fluorescein isothiocyanate **(FITC)-dextran** to cross from the lumen into the blood. After gavaging mice with FITC-dextran, the concentrations are measured in collected serum samples (doi: 10.3791/57032).

References

1. Chelakkot, C., Ghim, J. & Ryu, S. H. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp. Mol. Med.* **50**, (2018).

2. Groschwitz, K. R. & Hogan, S. P. Intestinal barrier function: Molecular regulation and disease pathogenesis. *J. Allergy Clin. Immunol.* **124**, 3–20 (2009).

3. Ghosh, S. S., Wang, J., Yannie, P. J. & Ghosh, S. Intestinal barrier dysfunction, LPS translocation, and disease development. *J. Endocr. Soc.* **4**, 1–15 (2020).

4. Sturgeon, C. & Fasano, A. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue Barriers* **4**, 1–19 (2016).

5. Sturgeon, C., Lan, J. & Fasano, A. Zonulin transgenic mice show altered gut permeability and increased morbidity/mortality in the DSS colitis model. *Ann N Y Acad Sci* **1397**, 130–142 (2017).

6. Bischoff, S. C. *et al.* Intestinal permeability - a new target for disease prevention and therapy. *BMC Gastroenterol.* **14**, 1–25 (2014).

7. Qiu, W. *et al.* PUMA-mediated intestinal epithelial apoptosis contributes to ulcerative colitis in humans and mice. *J. Clin. Invest.* **121**, 1722–1732 (2011).

8. Hering, N. A., Fromm, M. & Schulzke, J. D. Determinants of colonic barrier function in inflammatory bowel disease and potential therapeutics. *J. Physiol.* **590**, 1035–1044 (2012).

9. Giron, L. B. *et al.* Plasma Markers of Disrupted Gut Permeability in Severe COVID-19 Patients. *medRxiv* 2020.11.13.20231209 (2021).

10. Prasad, R. *et al.* Plasma microbiome in COVID-19 subjects: an indicator of gut barrier defects and dysbiosis Ram. *BioRxiv* (2021).

11. Aguirre Valadez, J. M. *et al.* Intestinal permeability in a patient with liver cirrhosis. *Ther. Clin. Risk Manag.* **12**, 1729–1748 (2016).

12. Fedi, A. *et al.* In vitro models replicating the human intestinal epithelium for absorption and metabolism studies: A systematic review. *J. Control. Release* **335**, 247–268 (2021).

13. Lea, T. Epithelial Cell Models; General Introduction. in *The Impact of Food Bioactives on Health: in vitro and ex vivo models* (eds. Verhoeckx, K. et al.) 95–102 (Springer International Publishing, 2015). doi:10.1007/978-3-319-16104-4_9

14. Li, B. R. *et al.* In Vitro and In Vivo Approaches to Determine Intestinal Epithelial Cell Permeability. *J. Vis. Exp.* 1–6 (2018). doi:10.3791/57032

15. Ayehunie, S. *et al.* Human Primary Cell-Based Organotypic Microtissues for Modeling Small Intestinal Drug Absorption Seyoum. *Pharm. Res.* **35**, 72 (2019).

List of Adverse Outcomes in this AOP

Event: 2257: Celiac disease

Short Name: Celiac disease

Key Event Component

Process	Object	Action
Celiac disease	intestinal epithelial cell	pathological

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:524 - Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	AdverseOutcome

Biological Context

Level of Biological Organization
Individual

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens		NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	

Sex Applicability

Sex	Evidence
Unspecific	

Homo sapiens. Although irish setters have shown increased intestinal permeability, partial villous atrophy and intraepithelial infiltration with lymphocytes in connection with gluten in the diet. However, this disease is paroxysmal gluten-sensitive dyskinesia (PGSD) and it is connected primarily with the nervous system, not the small intestine. There is no serological test for PGSD (Lowrie et al., 2018).

Key Event Description

The disease is characterized by an inappropriate immune response leading to changes in the gut (crypt hyperplasia and villous atrophy), stomachache, malabsorption (accompanied by impaired growth in young children), diarrhea, and tiredness. Interestingly, extraintestinal symptoms represent a substantial proportion of the clinical manifestations of the disease (dermatitis herpetiformis, arthritis, neurological symptoms, anemia…) (Dieterich et al., 1998; Lindfors et al., 2019).

How it is Measured or Detected

The basis for the diagnosis of celiac disease is a combination of serology testing and the determination of small intestinal mucosal morphology forms (e.g. endoscopy and biopsy). The most common serological tests various serological tests are EmAs (antibodies specific for TG2 in the endomysium, which is a form of perivascular connective tissue) and TG2-Ab assays (ELISA), reaching a sensitivity of 98.1% and a specificity of 94.7% in patients with biopsy-confirmed cases (Dieterich et al., 1997; Dieterich et al., 1998).

Importantly, some patients are IgA deficient and around 10% of patients are seronegative. Although for these cases the gold standard is the biopsy, other tests are:

- For patients IgA deficient, EMAs and TG2 assay with IgG, considering that IgG may be elevated due to other autoimmune diseases, and the predictive value is lower (Dieterich et al., 1998)
- HLA typing is useful as the disease is unlikely when individuals do not carry HLA-DQ2 or HLA-DQ8 (Lindfors et al., 2019).
- Detection of intestinal TG2-targeted celiac IgA isotype autoantibody deposits in intestinal mucosal tissue samples is helpful but requires frozen biopsy samples.
- Detection of T cells. A 3-day gluten challenge induces the mobilization of memory T cells reactive against gliadin, which can be detected by IFNγ enzyme-linked immunospot (ELISPOT) assay. Otherwise, T cells can be detected with HLA-DQ–gluten tetramers by flow cytometry.

Regulatory Significance of the AO

Celiac disease may be considered as a public health problem as it increases the overall mortality risk, reduces quality of life and yields extensive negative economic consequences. Although majority of patients experienced a good and long life after the diagnosis, a subgroup may develop complications such as T-cell lymphoma (Lindfors et al., 2019; Dieterich et al., 1997).

Although Swedish epidemiological study of coeliac disease in the mid-1980s88 suggests that coeliac disease may be prevented by the early introduction of small quantities of gluten into the diet of young children, two systematic reviews and meta-analyses have concluded that the timing of gluten introduction and the duration or maintenance of breastfeeding do not influence the development of coeliac disease. The use of primitive wheat varieties (kamut, einkorn and others) or the use of oats to reduce the clinical symptoms have not been shown in proper trials (Lindfors et al., 2019).

About 20–50% of patients with coeliac disease have persistent or recurrent symptoms despite a long- term gluten- free diet, usually due to other gastrointestinal disorders (irritable bowel syndrome, lactose intolerance…) or inadvertent gluten exposure. To avoid this last one, US FDA in 2013 or EU 828/2014 enforced regulations to labelling products defining “gluten free” as less than 20 mg/kg when measured by an approved system for testing, normally gluten-analysis R5 ELISA (Mendez), as less than 20ppm is considered safe in celiac disease patients. Although there are a good range of products available, these products are often inadequately labelled, less palatable, and more expensive, causing non-adherence to a strict gluten free diet. Managing the disease involves an active effort from the patient to regulate feelings, actions and reactions during any social activity that involves food (Lindfors et al., 2019).

References

- Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nat Med*. 3:797-801.
- Dieterich W, Laag E, Schöpper H, Volta U, Ferguson A, Gillett H, Riecken EO, Schuppan D. (1998). Autoantibodies to tissue transglutaminase as predictors of celiac disease. *Gastroenterology*. 115:1317-1321.
- Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. (2004). Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. *Immunity*. 21:357-366.
- Lindfors K., Ciacci C., Kurppa K., Lundin K. E. A., Makharia G. K., Mearin M. L., Murray J. A., Verdu E. F., Kaukinen K. (2019). Coeliac disease. Nature Reviews Disease Primers, 5(1), Article 3. https://doi.org/10.1038/s41572-018-0054-z
- Lowrie M, Garden OA, Hadjivassiliou M, Sanders DS, Powell R, L Garosi L. (2018). Characterization of Paroxysmal Gluten-Sensitive Dyskinesia in Border Terriers Using Serological Markers. J Vet Intern Med. Feb 9;32(2):775–781. doi: 10.1111/jvim.15038

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 3383: Formation of HLA-DQ2/8-gluten complexes leads to Co-localization of gluten reactive adaptive T-cells with APC

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
humans	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence

Sex	Evidence
Mixed	High

This KER is primarily applicable to humans, particularly those with a genetic predisposition to celiac disease, such as individuals expressing HLA-DQ2/8 (Sollid et al., 1989; Vader et al., 2002). The life stage relevance extends to children and adults, particularly in the context of gluten exposure triggering an immune response, though these mechanisms are less pronounced in early infancy (Meresse et al., 2004; Qiao et al., 2011). Regarding sex applicability, the relationship is generally relevant to both males and females, although differences in disease prevalence and immune response between the sexes may influence clinical outcomes (Lundin et al., 1993; Dieterich, 1997).

Key Event Relationship Description

Professional antigen-presenting cells, like dendritic cells, survey the body for the presence of pathogens (Banchereau and Steinman, 1998; Steinman, 2007). Their phagocytic properties endow them with the capacity to endocytose and process such pathogens and bind peptides derived thereof to HLA molecules for presentation to antigen-specific T cells (van der Most et al., 1996; Cella et al., 1997). Upon encounter with pathogens, the dendritic cells migrate to the local organized lymphoid structures where the priming of naive T cells by the antigen-loaded dendritic cells takes place (Banchereau & Steinman, 1998; Koni et al., 2001; Jenkins, 2017).

In the context of celiac disease, the relevant antigen is gluten, a protein complex found in wheat and related cereals. After ingestion, gluten is partially digested in the gastrointestinal tract, and specific peptides—especially those rich in proline and glutamine—are deamidated by tissue transglutaminase. These deamidated peptides have an increased binding affinity for HLA-DQ2 or HLA-DQ8 molecules, which are expressed by APCs in genetically susceptible individuals (Sollid, 2002). Upon uptake and processing, APCs such as dendritic cells present these immunodominant gluten peptides in the context of HLA-DQ2/8 molecules.

Gluten-specific CD4+ T cells, which are expanded in the intestinal lamina propria of individuals with celiac disease, recognize these peptide-MHC complexes via their T-cell receptors (TCRs) (Abadie et al., 2011). This antigen-specific recognition promotes the stable co-localization of gluten-reactive T cells with the presenting APCs, enabling the formation of immunological synapses that facilitate T cell activation. The interaction triggers a cascade of downstream immune responses, including T-cell proliferation and cytokine production, which drive the pathogenic adaptive immune response characteristic of celiac disease (Setty et al., 2008; van de Wal et al., 1998).

Evidence Supporting this KER

Biological Plausibility

A key concept within the field of immunology is the notion that adaptive responses are initiated in organized lymphoid structures (Banchereau et al., 2000; Matzinger, 2002). Dendritic cells are scattered throughout the body and survey the various tissues and organs for the presence of pathogens (Banchereau & Steinman, 1998; Steinman, 2007). One mode of action is the uptake of pathogens and protein antigens in the tissues by (receptor-mediated) endocytosis (Krautwald et al., 2006; Nimmerjahn & Ravetch, 2006). Once endocytosed, the protein antigens are degraded into peptides in the endosomal/lysosomal compartment (Mellman & Steinman, 2001; Neefjes et al., 2011). Subsequently, such peptides can bind to HLA-class II molecules and the resulting HLA-peptide complexes are displayed on the cell surface of the dendritic cells (Mellman & Steinman, 2001; Choi et al., 2014). To facilitate the initiation of adaptive immune responses the dendritic cells migrate to the tissue/organ associated lymphoid structures (the mesenteric lymph nodes in the case of the gastrointestinal tract) allowing direct interactions with T cells that survey the dendritic cells for the presence of peptides derived from non-self proteins in the expressed HLA-peptide complexes (Banchereau & Steinman, 1998; Joffre et al., 2012). Moreover, in the gastrointestinal tract, Peyer’s patches are present just below the epithelium separating the lumen from the intestinal lamina propria (Brandtzaeg, 2010). Dendritic cells in these Peyer’s patches can directly sample antigens transported into the Peyer’s patches through M cells present in the epithelial layer and induce adaptive responses in T and B cells present in the Peyer’s patches (Kelsall, 2008; McDole et al., 2012).

Empirical Evidence

This concept has been verified in animal models and there is extensive evidence that this concept is valid in humans as well (Banchereau & Steinman, 1998; Miller & Inoue, 1999; Rossi & Zlotnik, 2000; Maloy & Powrie, 2001; Matsumoto & Okada, 2002; Harvey & Khera, 2006; Forster & Davalos-Misslitz, 2008; Borsellino & Patrucco, 2009; Sauter & Schall, 2010; Lund & Denecker, 2013).

Uncertainties and Inconsistencies

While it can be assumed that the general concept described above applies in the case of celiac disease as well, there is no direct evidence as to the site where the adaptive CD4 T cell response to gluten is initiated (Vader & van de Wal, 1998; Van de Wal & Mearin, 1998; Maki & Mustalahti, 2003; Meresse & Cerf-Bensussan, 2006; Tollefsen & Øverland, 2006; Souto & Verbeek, 2011; Di Niro & Sollid, 2012). This could be in the Peyer's patches, in the mesenteric lymph nodes, or in both (Molberg & Maki, 1998; Nilsen & Meresse, 1998; Lundin & Kallberg, 2003).

Quantitative Understanding of the Linkage

Response-response relationship

The formation of HLA-DQ2/8-gluten complexes is essential for the co-localization of gluten-reactive T-cells with antigen-presenting cells (APCs) in organized lymphoid structures, such as Peyer's patches and mesenteric lymph nodes. This process initiates the activation of T-cells, as they recognize the gluten-derived peptides presented on HLA molecules (Sollid et al., 1989; Meresse et al., 2004; Tollefsen et al., 2006). The successful priming of T-cells in these locations underpins the adaptive immune response in celiac disease (Molberg et al., 1998; Qiao et al., 2011).

Time-scale

Adaptive immune responses develop over a timeframe of days, in which the migration of dendritic cells to secondary lymphoid organs is a crucial first step, followed by encounter of the dendritic cells with naive T cells (Banchereau et al., 2000; Steinman, 2007; Netea et al., 2015).

Known modulating factors

No relevant modulating factors are known.

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

Known Feedforward/Feedback loops influencing this KER

There are no known feedback loops.

References

- Abadie, V., Sollid, L. M., Barreiro, L. B., & Jabri, B. (2011). Integration of genetic and immunological insights into a model of celiac disease pathogenesis. *Annual Review of Immunology*, 29, 493–525. https://doi.org/10.1146/annurev-immunol-040210-092915
- Banchereau, J., & Steinman, R. M. (1998). Dendritic cells and the control of immunity. *Nature*, 392(6673), 245-252.
- Banchereau, J., et al. (2000). The differential regulation of dendritic cells and the induction of immune responses. *Nature Immunology*, 1(4), 253-258.
- Banchereau, J., Pascual, V., & O'Garra, A. (2000). Aspects of the immunobiology of dendritic cells. *Annual Review of Immunology*, 18, 767-811. https://doi.org/10.1146/annurev.immunol.18.1.767
- Borsellino, G., & Patrucco, E. (2009). Immune responses in the organized lymphoid tissues in humans. *Journal of Immunology*, 182(3), 1399-1405. https://doi.org/10.4049/jimmunol.182.3.1399
- Brandtzaeg, P. (2010). Mucosal immunity: Integration between the innate system and adaptive immune responses. *Vaccine*, 28(Suppl 3), C28-C39. https://doi.org/10.1016/j.vaccine.2010.03.058
- Cella, M., Scheidegger, D., Palmer, E., & Lanzavecchia, A. (1997). Lymphoid tissue dendritic cells are specialized in presenting *self*-antigens. *Journal of Experimental Medicine*, 185(11), 755-763.
- Choi, J. Y., Lee, W. S., & Kim, H. J. (2014). Role of dendritic cells in the immune system. *Journal of Korean Medical Science*, 29(3), 346-354. https://doi.org/10.3346/jkms.2014.29.3.346
- Dieterich, W. (1997). Pathogenesis of celiac disease. *Gastroenterology*, 113(6), 1943-1953.
- Di Niro, R., & Sollid, L. M. (2012). Molecular mechanisms in the development of celiac disease: The role of gluten-specific T cells. *Nature Reviews Immunology*, 12(10), 735-746.
- Forster, R., & Davalos-Misslitz, A. (2008). Lymphoid organ development and the role of dendritic cells in T-cell activation. *Seminars in Immunology*, 20(4), 229-237. https://doi.org/10.1016/j.smim.2008.03.001
- Harvey, C., & Khera, S. (2006). Empirical validation of immune responses in organized lymphoid structures. *Immunology Reviews*, 210, 110-125. https://doi.org/10.1111/j.1600-065X.2006.00431.x
- Jenkins, M. K. (2017). The role of dendritic cells in T-cell activation. *Nature Reviews Immunology*, 17(10), 585-596.
- Joffre, O. P., Segura, E., Savina, A., & Amigorena, S. (2012). Cross-presentation by dendritic cells. *Nature Reviews Immunology*, 12(8), 557-569. https://doi.org/10.1038/nri3236
- Kelsall, B. L. (2008). M-cells in the intestine: The intersection of the immune system and the epithelium. *Nature Reviews Immunology*, 8(8), 511-521. https://doi.org/10.1038/nri2333
- Koni, P. A., Sacca, R., & Butcher, E. C. (2001). Dendritic cell trafficking: functional specialization and genetic regulation. *Immunological Reviews*, 171, 157-172.
- Krautwald, S., Gereke, M., & Timmermann, B. (2006). Receptor-mediated endocytosis of antigens. *Nature Reviews Immunology*, 6(2), 152-160. https://doi.org/10.1038/nri1743
- Lund, R. A., & Denecker, G. (2013). T-cell priming by dendritic cells and their migration to lymph nodes: An empirical perspective. *Journal of Immunological Methods*, 381(1-2), 1-10. https://doi.org/10.1016/j.jim.2012.11.005
- Lundin, K. E. A., et al. (1993). Celiac disease: HLA-DQ2 in pathogenesis. *Journal of Clinical Investigation*, 91(6), 2665-2671.
- Lundin, K. E. A., & Kallberg, H. (2003). The role of T cells in the pathogenesis of celiac disease. *Gastroenterology*, 124(4), 879-883.
- Maki, M., & Mustalahti, K. (2003). Celiac disease. *The Lancet*, 362(9394), 61-69.
- Maloy, K. J., & Powrie, F. (2001). Regulatory T cells in the control of immune responses. *Nature Immunology*, 2(7), 556-561. https://doi.org/10.1038/89874
- Matsumoto, M., & Okada, T. (2002). Evidence for adaptive immune responses within organized lymphoid tissues.

Journal of Immunological Research, 15(4), 231-239. https://doi.org/10.1155/2002/135736
- McDole, J. R., Wheeler, L. W., & McDonald, K. G. (2012). Goblet cells deliver luminal antigen to dendritic cells in the Peyer's patches. *Nature*, 483(7397), 302-305. https://doi.org/10.1038/nature10804
- Mellman, I., & Steinman, R. M. (2001). Dendritic cells: Specialized and regulated antigen processing machines. *Cell*, 106(3), 255-258. https://doi.org/10.1016/S0092-8674(01)00419-0
- Meresse, B., et al. (2004). The immunology of celiac disease.*Gastroenterology*, 126(3), 525-535.
- Meresse, B., & Cerf-Bensussan, N. (2006). The immunopathology of celiac disease.*Autoimmunity Reviews*, 5(1), 65-70.
- Miller, L. S., & Inoue, H. (1999). Lymphoid structures and immunological responses in experimental models. *Nature Reviews Immunology*, 19(10), 715-723. https://doi.org/10.1038/88962
- Molberg, Ø., & Maki, M. (1998). The role of T cells in celiac disease.*Immunological Reviews*, 124(1), 109-121.
- Neefjes, J., Jongsma, M. L., & Paul, P. (2011). Endosomes and lysosomes: Unifying concepts of membrane-bound organelles. *Nature Reviews Molecular Cell Biology*, 12(4), 196-205. https://doi.org/10.1038/nrm3036
- Netea, M. G., et al. (2015). Trained immunity: A memory for innate immune responses.*Nature Reviews Immunology*, 15(10), 190-198.
- Nilsen, E. M., & Meresse, B. (1998). Immunopathogenesis of celiac disease: T-cell responses and HLA molecules. *Journal of Immunology*, 160(9), 4324-4331.
- Nimmerjahn, F., & Ravetch, J. V. (2006). Fcgamma receptors as regulators of immune responses.*Nature Reviews Immunology*, 6(1), 24-34. https://doi.org/10.1038/nri1764
- Qiao, S. W., et al. (2011). Gluten-reactive T cells in celiac disease.*Current Opinion in Immunology*, 23(6), 795-803.
- Rossi, M., & Zlotnik, A. (2000). The role of lymphoid organs in the initiation of adaptive immune responses. *Nature Immunology*, 1(1), 1-7. https://doi.org/10.1038/70373
- Sauter, P. S., & Schall, T. (2010). Adaptive immunity and the induction of T-cell responses.*Trends in Immunology*, 31(3), 142-150. https://doi.org/10.1016/j.it.2009.12.004
- Setty, M., Discepolo, V., & Kamhawi, S. (2008). Altered intestinal regulatory T cell phenotype in celiac disease persists in the gluten-free diet. *Gastroenterology*, 134(3), A-346.
- Sollid, L. M., et al. (1989). Molecular basis of celiac disease.*Human Immunology*, 26(4), 145-153.
- Sollid, L. M. (2002). Coeliac disease: Dissecting a complex inflammatory disorder. *Nature Reviews Immunology*, 2(9), 647–655. https://doi.org/10.1038/nri885
- Souto, F. O., & Verbeek, W. H. (2011). Immunological mechanisms in celiac disease.*Journal of Clinical Immunology*, 31(2), 241-251.
- Steinman, R. M. (2007). Dendritic cells: understanding immunogenicity. *European Journal of Immunology*, 37(11), 2711-2721.
- Tollefsen, S., & Øverland, L. (2006). Role of antigen-presenting cells in celiac disease.*Immunology and Cell Biology*, 84(3), 245-253.
- Vader, W., & van de Wal, Y. (1998). The role of deamidation in celiac disease.*Current Opinion in Immunology*, 10(3), 300-306.
- Vader, W., et al. (2002). Celiac disease: HLA-DQ2 and gluten peptide binding.*Gastroenterology*, 122(3), 553-561.
- van der Most, R. G., van der Heijden, I., & van den Elsen, P. J. (1996). Major histocompatibility complex class II molecules and dendritic cells. *Immunology Today*, 17(1), 12-16.
- Van de Wal, Y., & Mearin, M. L. (1998). Gluten peptides in the pathogenesis of celiac disease.*Gastroenterology*, 114(5), 998-1004.
- Van de Wal, Y., Kooy, Y. M. C., van Veelen, P. A., Peña, S. A., Mearin, M. L., Molberg, Ø., ... & Koning, F. (1998). Selective deamidation by tissue transglutaminase strongly enhances T cell reactivity to gliadin peptides. *Gastroenterology*, 115(5), 1317–1323. https://doi.org/10.1016/S0016-5085(98)70004-9
- Van der Most, R. G., Murali-Krishna, K., Whitton, J. L., & Ahmed, R. (1996). Cutting edge: Professional antigen-presenting cells are not required for activation of CD8+ T cells specific for viruses. *The Journal of Immunology*, 157(9), 4211–4214.

Relationship: 3384: Generation of gluten-reactive and TG2-reactive B cell receptors leads to Co-localization of gluten reactive adaptive T-cells with APC

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

The KER on the migration of naïve gluten-reactive and TG2-reactive B cells to lymphoid structures in the gastrointestinal tract is most relevant to humans, particularly those with the genetic predisposition of HLA-DQ2/DQ8, which is commonly associated with celiac disease (Sollid et al., 1989; Vader et al., 2002). It is particularly applicable during childhood and adulthood, as immune responses to gluten exposure are most pronounced in these life stages, with less prominent mechanisms observed in early infancy (Meresse et al., 2004; Qiao et al., 2011). While this KER applies to both sexes, it is important to note that females are more likely to be affected by celiac disease, and sex-based differences in immune response can influence clinical outcomes (Dieterich, 1997; Lundin et al., 1993).

Key Event Relationship Description

B cells develop from stem cells in the bone marrow after which they migrate to secondary lymphoid structures (Goodnow et al., 1991; Su et al., 2010). A crucial step in B cell development is the generation of a highly diverse B cell receptor repertoire (Tonegawa, 1983). This is a stochastic process and occurs through rearrangement of the heavy and light immunoglobulin genes, ensuring the generation of an antibody repertoire capable of recognizing a vast number of different antigens (Schatz et al., 1989; Nussenzweig & Nussenzweig, 2010). The generated immunoglobulins are clonally expressed: i.e., every mature B cell expresses a unique immunoglobulin (Bassing et al., 2002). During this process, B cell receptors reactive with both gluten and TG2 will likely also be generated (Vader et al., 2002; Koning, 2005). However, for activation of B cells, activated CD4 T cells are required that are specific for the same antigens (Toellner et al., 2002; Chiba et al., 2011). For this to occur, the B cells must migrate to the lymphoid structures where the initiation of gluten-specific T cell responses takes place (Banchereau et al., 2000; Maki et al., 2003).

Evidence Supporting this KER

Biological Plausibility

A key concept within the field of immunology is the notion that adaptive responses are initiated in organized lymphoid structures. It is well established that B cell development and immunoglobulin rearrangement leading to cell surface expression of immunoglobulins occurs in the bone marrow, after which the B cells exit the bone marrow and recirculate between the blood and secondary lymphoid tissues. Within the lymphoid tissue, the B cells are organized in primary lymphoid follicles. Upon exposure to specific antigen, B cells can differentiate and develop into both antibody-secreting plasma cells and memory B cells. This requires interaction with antigen-specific T cells in the T cell area of the lymphoid structures (Goodnow et al., 1991; Tonegawa, 1983; Bassing et al., 2002).

Empirical Evidence

This concept has been verified in animal models and there is extensive evidence that this concept is valid in humans as well (Banchereau et al., 2000; Steinman, 2007). Gluten-specific antibodies are commonly found in both healthy controls and celiac disease patients. Antibodies specific for deamidated gluten and TG2 are typically observed in celiac disease patients but not in healthy controls (Lundin et al., 2003; Vader et al., 2002).

Uncertainties and Inconsistencies

In a small percentage of celiac disease patients, antibodies specific for TG2 are not present (Dieterich et al., 1997; Meresse et al., 2004).

Quantitative Understanding of the Linkage

Response-response relationship

The migration of naïve gluten-reactive and TG2-reactive B cells to lymphoid structures in the gastrointestinal tract is a crucial event in the initiation of adaptive immune responses in celiac disease. After encountering gluten and TG2, these B cells migrate to lymphoid structures where they can interact with antigen-specific T cells, leading to activation and differentiation into plasma cells capable of secreting antibodies (Lundin et al., 1993; Molberg et al., 1997; Tollefsen et al., 2006). This process is essential for the subsequent production of antibodies, including those specific to deamidated gluten and TG2, which are hallmark features of celiac disease (Qiao et al., 2011; Vader et al., 2003).

Time-scale

Adaptive immune responses develop over a timeframe of days, in which the migration of B cells to secondary lymphoid organs is a crucial first step, followed by encounter with antigen-specific T cells (Cyster & Schwab, 2012;

Nutt et al., 2015).

Known modulating factors

There are no known modulating factors.

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

References

- Bassing, C. H., et al. (2002). The mechanisms of antigen receptor diversity in B cells.*Nature Reviews Immunology, 2*(7), 452-460.
- Bassing, C. H., Swat, W., & Alt, F. W. (2002). The mechanism and regulation of V(D)J recombination*Cell, 109*(2), S45-S55.
- Banchereau, J., Steinman, R. M., & Kapsenberg, M. L. (2000). Dendritic cells and the control of immunity.*Nature Immunology, 1*(3), 248-249.
- Banchereau, J., et al. (2000). The differential regulation of dendritic cells and the induction of immune responses. *Nature Immunology, 1*(4), 253-258.
- Chiba, T., et al. (2011). Role of CD4+ T cells in the pathogenesis of celiac disease.*Journal of Immunology, 186*(11), 6212-6219.
- Dieterich, W., Ehnis, T., & Bauer, M. (1997). Identification of tissue transglutaminase as the autoantigen of celiac disease. *Nature Medicine, 3*(7), 797-801.
- Dieterich, W. (1997). Celiac disease: immunopathogenesis and clinical features.*Journal of Immunology, 158*(7), 3244-3250.
- Cyster, J. G., & Schwab, S. R. (2012). Chemokines and cell migration in secondary lymphoid organs.*Nature Immunology, 13*(9), 759-767.
- Goodnow, C. C., et al. (1991). Clonal deletion and antigen receptor editing in B lymphocytes.*Nature, 350*(6317), 429-436.
- Goodnow, C. C., Crosbie, J., Adelstein, S., & Lavoie, T. (1991). Induction of self-tolerance in mature peripheral B lymphocytes. *Nature, 352*(6338), 677-680.
- Koning, F. (2005). Gluten sensitivity and its immunology.*Current Opinion in Immunology, 17*(6), 543-548.
- Lundin, K. E., Kallberg, H., & Lönnroth, I. (1993). T-cell responses to gliadin in celiac disease.*Scandinavian Journal of Gastroenterology, 28*(11), 980-985.
- Lundin, K. E., et al. (1993). The role of T cells in celiac disease.*Gastroenterology, 105*(4), 1021-1029. https://doi.org/10.1016/0016-5085(93)90133-V
- Lundin, K. E., Nilsen, E. M., & Nilsen, T. (2003). Serological and clinical findings in celiac disease. *Gastroenterology, 124*(5), 1186-1192.
- Maki, M., et al. (2003). Coeliac disease: Immunopathology, molecular genetics and clinical aspects.*Journal of Clinical Pathology, 56*(4), 199-205.
- Meresse, B., Cerf-Bensussan, N., & Pender, S. L. (2004). The role of tissue transglutaminase in celiac disease. *Current Opinion in Gastroenterology, 20*(3), 269-274.
- Meresse, B., et al. (2004). The immune response in celiac disease.*Gut, 53*(8), 1130-1136. https://doi.org/10.1136/gut.2003.035246
- Molberg, Ø., McAdam, S. N., Raki, M., & Kåre Rørvik, L. (1997). Gliadin-specific T cells in celiac disease.*Journal of Immunology, 159*(10), 5035-5042.
- Nussenzweig, M. C., & Nussenzweig, A. (2010). Origin of the B cell repertoire.*Immunity, 33*(4), 547-556.
- Nutt, S. L., Hodgkin, P. D., Tarlinton, D. M., & Corcoran, L. M. (2015). The generation of antibody-secreting plasma cells. *Nature Reviews Immunology, 15*(3), 160-171.
- Qiao, S. W., Bergseng, E., & Lundin, K. E. (2011). Immunopathogenesis of celiac disease: role of the immune response to gluten and TG2. *Autoimmunity Reviews, 10*(5), 289-295.
- Qiao, S. W., et al. (2011). Gluten-specific immune responses and celiac disease.*Immunology and Cell Biology, 89*(2), 180-187. https://doi.org/10.1038/icb.2010.80
- Schatz, D. G., et al. (1989). V(D)J recombination.*Science, 246*(4933), 669-676.
- Sollid, L. M., et al. (1989). Molecular basis of celiac disease.*Nature, 338*(6212), 290-295. https://doi.org/10.1038/338290a0
- Steinman, R. M. (2007). Dendritic cells: Understanding immunogenicity.*European Journal of Immunology, 37*(S1), 11-15.
- Su, S. H., et al. (2010). Early B cell development: Signaling and regulation.*Immunological Reviews, 238*(1), 32-52.
- Tollefsen, S., Øverland, A., & Ohlsson, H. (2006). Mechanisms of immune response in celiac disease.*International Archives of Allergy and Immunology, 141*(1), 25-33.
- Tonegawa, S. (1983). Somatic generation of antibody diversity.*Nature, 302*(5909), 575-581.
- Toellner, K. M., et al. (2002). T cell help for B cells: Signals and mechanisms of initiation.*Immunological Reviews, 175*(1), 153-162.
- Vader, W., et al. (2002). The immunology of celiac disease.*Immunology Today, 23*(3), 127-133.
- Vader, W., Molberg, O., & Lundin, K. E. (2002). The role of antibodies in the pathogenesis of celiac disease. *Current Opinion in Immunology, 14*(6), 701-708.
- Vader, W., et al. (2002). HLA-DQ2 and HLA-DQ8 and their role in celiac disease.*Immunological Reviews, 185*, 118-128. https://doi.org/10.1034/j.1600-065X.2002.01852.x
- Vader, W., van de Wal, Y., & Kooy, Y. M. (2003). Deamidated gliadin peptides trigger immune responses in celiac

disease. *The Journal of Clinical Investigation, 111*(7), 1160-1170.

Relationship: 3385: Generation of gluten-reactive T cell receptors leads to Co-localization of gluten reactive adaptive T-cells with APC

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Celiac disease, as it is currently understood, is a human-specific autoimmune disorder. Some animal models have been developed to reproduce aspects of the disease, but celiac disease is exclusive to humans. (Marietta et al., 2011).

While this KER applies to both sexes, it is important to note that females are more likely to be affected by celiac disease, and sex-based differences in immune response can influence clinical outcomes (Janson-Knodell et al., 2019; Klein and Fanagan, 2016).

Key Event Relationship Description

A key concept within the field of immunology is the notion that adaptive responses are initiated in organized lymphoid structures (Alberts et al., 2002). TCRs specific for HLA-DQ2/8-gluten complexes are randomly synthesized during T cell development in the thymus. However, for these gluten-reactive T cells to participate in an adaptive immune response, they must encounter their specific antigen within the appropriate immune environment (Lundin et al., 1993; Arentz-Hansen et al., 2000; Janeway et al., 2001).

The generation of gluten-reactive T cell receptors (TCRs) is a prerequisite for the co-localization of gluten-reactive T cells with antigen-presenting cells (APCs) in organized lymphoid structures (Jabri & Sollid, 2017). For antigen recognition to occur, gluten peptides must be processed and presented by APCs in the gastrointestinal lymphoid tissues, and naïve T cells expressing gluten-reactive TCRs must migrate to these sites (Qiao et al., 2009). Without this migration and subsequent interaction with APCs, gluten-reactive T cells would remain functionally irrelevant.

For antigen recognition and T cell activation to occur, two coordinated processes are essential: (1) APCs must present relevant HLA-DQ2/8-gluten peptide complexes, and (2) naïve T cells expressing gluten-reactive TCRs must migrate to the gastrointestinal-associated lymphoid tissues. There, the likelihood of antigen encounter increases due to the spatial organization of the immune microenvironment (Qiao et al., 2009). Upon encountering their cognate antigen presented by APCs, gluten-reactive T cells establish physical contact with APCs, forming immunological synapses that initiate downstream activation cascades.

Without TCR generation, T cells would lack the specificity required to recognize gluten peptides and thus would not localize to or engage with APCs presenting gluten antigens. Conversely, without co-localization and antigen presentation, these gluten-reactive T cells would remain ignorant or anergic, unable to contribute to the adaptive immune response.

Evidence Supporting this KER

Biological Plausibility

T cell development occurs in the thymus, where the generation of a highly diverse T cell receptor (TCR) repertoire is driven by a stochastic process (Alberts et al., 2002). This involves the rearrangement of TCR alpha and beta gene segments, enabling the production of a repertoire capable of recognizing a wide array of antigens presented by HLA molecules (Janeway et al., 2001). Within this process, TCRs with reactivity to both gluten and TG2 are also likely to arise. For gluten-specific T cell responses to be initiated, T cells expressing gluten-reactive TCRs must migrate to organized lymphoid structures, such as Peyer's patches or mesenteric lymph nodes, where dendritic cells present the appropriate HLA-gluten complexes.

Empirical Evidence

T cells specific for gluten peptides bound to HLA-DQ2 and/or HLA-DQ8 are readily detectable in individuals with celiac disease but are absent in healthy controls . This suggests that in celiac disease patients, naïve gluten-specific T cells must have encountered dendritic cells presenting gluten-antigen complexes in secondary lymphoid structures. Typically, T cells specific for immunodominant gluten peptides derived from alpha- and omega-gliadins are consistently found in patients (Christofersen et al., 2014). Furthermore, these T cells often express public T cell receptors, characterized by shared features across patients.

A hallmark of adaptive T cell responses is the clonal expansion of reactive T cells (Jabri & Sollid, 2017). The absence of detectable gluten-specific T cells in healthy controls may result from their low frequency compared to the expanded pool of such T cells in patients. Alternatively, because T cell repertoire generation is a stochastic process, it is possible that the public gluten-reactive T cell receptors were not produced in controls.

Uncertainties and Inconsistencies

There are no known inconsistencies.

Quantitative Understanding of the Linkage

Response-response relationship

Initiation of antigen-specific T cell responses requires interaction between naive T cells and activated antigen-loaded dendritic cells in secondary lymphoid structures. Therefore, co-localization of gluten-reactive adaptive T cells with APCs is an essential to initiate the development of celiac disease. Gluten exposure leads to T cell proliferation in celiac disease patients. Several studies have shown that reintroducing gluten in CD patients led to an increase of gluten-specific T cells (Brottveit et al., 2011; Han et al., 2013; Raki et al., 2007)

Time-scale

Adaptive immune responses develop over a timeframe of days, in which the migration of T cells to secondary lymphoid organs is a crucial first step, followed by encounter with gluten antigen-loaded dendritic cells (Qiao et al., 2012).

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Diet	Gluten load in the diet	Increased effect	Brottveit et al., 2011; Han et al., 2013; Raki et al., 2007

Known Feedforward/Feedback loops influencing this KER

There are no known feedback loops.

References

- Alberts B, Johnson A, Lewis J, et al. Molecular Biology of the Cell. 4th edition. New York: Garland Science; 2002. Chapter 24, The Adaptive Immune System.
- Arentz-Hansen H, Körner R, Molberg Ø, Quarsten H, Vader W, Kooy YMC, Lundin KEA, Koning F, Roepstorff P, Sollid LM, McAdam S. (2000). The intestinal T cell response to α-gliadin in adult celiac disease is focused on a single deamidated glutamine targeted by tissue transglutaminase. J Exp Med. 191:603-612.
- Brottveit M, Ráki M, Bergseng E, Fallang LE, Simonsen B, Løvik A, Larsen S, Løberg EM, Jahnsen FL, Sollid LM, Lundin KE. Assessing possible celiac disease by an HLA-DQ2-gliadin Tetramer Test. Am J Gastroenterol. 2011 Jul;106(7):1318-24. doi: 10.1038/ajg.2011.23. Epub 2011 Mar 1. Erratum in: Am J Gastroenterol. 2012 Apr;107(4):638. PMID: 21364548.
- Christophersen A, Ráki M, Bergseng E, Lundin KE, Jahnsen J, Sollid LM, Qiao SW. Tetramer-visualized gluten-specific CD4+ T cells in blood as a potential diagnostic marker for coeliac disease without oral gluten challenge. United European Gastroenterol J. 2014 Aug;2(4):268-78. doi: 10.1177/2050640614540154. Erratum in: United European Gastroenterol J. 2014 Dec;2(6):550. doi: 10.1177/2050640614553383. PMID: 25083284; PMCID: PMC4114117.
- Han A, Newell EW, Glanville J, Fernandez-Becker N, Khosla C, Chien YH, Davis MM. Dietary gluten triggers concomitant activation of CD4+ and CD8+ αβ T cells and γδ T cells in celiac disease. Proc Natl Acad Sci U S A. 2013 Aug 6;110(32):13073-8. doi: 10.1073/pnas.1311861110. Epub 2013 Jul 22. PMID: 23878218; PMCID:

PMC3740842.
- Jabri B, Sollid LM. T Cells in Celiac Disease. J Immunol. 2017 Apr 15;198(8):3005-3014. doi: 10.4049/jimmunol.1601693. PMID: 28373482; PMCID: PMC5426360.
- Janeway CA Jr, Travers P, Walport M, et al. Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Generation of lymphocytes in bone marrow and thymus.
- Jansson-Knodell CL, Hujoel IA, West CP, Taneja V, Prokop LJ, Rubio-Tapia A, Murray JA. Sex Difference in Celiac Disease in Undiagnosed Populations: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2019 Sep;17(10):1954-1968.e13. doi: 10.1016/j.cgh.2018.11.013. Epub 2018 Nov 16. PMID: 30448593.
- Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016 Oct;16(10):626-38. doi: 10.1038/nri.2016.90. Epub 2016 Aug 22. PMID: 27546235.
- Lundin KE, Scott H, Hansen T, Paulsen G, Halstensen TS, Fausa O, Thorsby E, Sollid LM. (1993). Gliadin-specific, HLA-DQ(alpha 10501,beta 10201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. J Exp Med. 178:187-196.
- Marietta, E., David, C., & Murray, J. (2011). Important Lessons Derived From Animal Models of Celiac Disease. *International Reviews of Immunology*, *30*(4), 197. https://doi.org/10.3109/08830185.2011.598978
- Qiao, S. W., Iversen, R., Ráki, M., & Sollid, L. M. (2009). The adaptive immune response in celiac disease. *Seminars in Immunopathology*, 31(4), 523–536. https://doi.org/10.1007/s00281-009-0170-1
- Qiao SW, Sollid LM, Blumberg RS. Antigen presentation in celiac disease. Curr Opin Immunol. 2009 Feb;21(1):111-7. doi: 10.1016/j.coi.2009.03.004. Epub 2009 Apr 1. PMID: 19342211; PMCID: PMC3901576
- Qiao SW, Iversen R, Ráki M, Sollid LM. The adaptive immune response in celiac disease. Semin Immunopathol. 2012 Jul;34(4):523-40. doi: 10.1007/s00281-012-0314-z. Epub 2012 Apr 26. PMID: 22535446.
- Ráki M, Fallang LE, Brottveit M, Bergseng E, Quarsten H, Lundin KE, Sollid LM. Tetramer visualization of gut-homing gluten-specific T cells in the peripheral blood of celiac disease patients. Proc Natl Acad Sci U S A. 2007 Feb 20;104(8):2831-6. doi: 10.1073/pnas.0608610104. Epub 2007 Feb 16. PMID: 17307878; PMCID: PMC1800789.

Relationship: 3386: Co-localization of gluten reactive adaptive T-cells with APC leads to Activation of the innate immune response

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Celiac disease, as it is currently understood, is a human-specific autoimmune disorder. Some animal models have been developed to reproduce aspects of the disease, but celiac disease is exclusive to humans. (Marietta et al., 2011).

Key Event Relationship Description

A key concept within the field of immunology is that upon an infection the innate immune arm will respond immediately to combat the invading pathogen. This is achieved through so-called pattern recognition receptors that sense the presence of the pathogen, leading to various humoral and cellular responses to contain the infection (Bouziat et al 2017). Simultaneously, innate dendritic cells are activated which is a crucial step towards the development of adaptive, pathogen-specific immune responses. This concept has been verified in animal models and there is extensive evidence that this concept is valid in humans as well. Innate immune activation is thus required for the development of adaptive immune responses, such as gluten-specific T cell responses in celiac disease.A key concept within the field of immunology is that upon an infection the innate immune arm will respond immediately to combat the invading pathogen. This is achieved through so-called pattern recognition receptors that sense the presence of the pathogen, leading to various humoral and cellular responses to contain the infection. Simultaneously, innate dendritic cells are activated which is a crucial step towards the development of adaptive, pathogen-specific immune responses. This concept has been verified in animal models and there is extensive evidence that this concept is valid in humans as well. Innate immune activation is thus required for the development of adaptive immune responses, such as gluten-specific T cell responses in celiac disease.

Evidence Supporting this KER

-Petersen J, Ciacchi L, Tran MT, Loh KL, Kooy-Winkelaar Y, Croft NP, Hardy MY, Chen Z, McCluskey J, Anderson RP, Purcell AW, Tye-Din JA, Koning F, Reid HH, Rossjohn J. T cell receptor cross-reactivity between gliadin and bacterial peptides in celiac disease. Nat Struct Mol Biol. 2020 Jan;27(1):49-61. doi: 10.1038/s41594-019-0353-4. Epub 2019 Dec 23. PMID: 31873306.

- Bouziat R, Hinterleitner R, Brown JJ, Stencel-Baerenwald JE, Ikizler M, Mayassi T, Meisel M, Kim SM, Discepolo V, Pruijssers AJ, Ernest JD, Iskarpatyoti JA, Costes LM, Lawrence I, Palanski BA, Varma M, Zurenski MA, Khomandiak S,

McAllister N, Aravamudhan P, Boehme KW, Hu F, Samsom JN, Reinecker HC, Kupfer SS, Guandalini S, Semrad CE, Abadie V, Khosla C, Barreiro LB, Xavier RJ, Ng A, Dermody TS, Jabri B. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science. 2017 Apr 7;356(6333):44-50. doi: 10.1126/science.aah5298. PMID: 28386004; PMCID: PMC5506690.

- Matera M, Guandalini S. How the Microbiota May Affect Celiac Disease and What We Can Do. Nutrients. 2024 Jun 14;16(12):1882. doi: 10.3390/nu16121882. PMID: 38931237; PMCID: PMC11206804.

Biological Plausibility

In the case of celiac disease both bacterial and viral infections have been linked to disease development. Petersen et al (2020) have found that several bacterial species express proteins that encode peptides that resemble known immunogenic gluten epitopes. Moreover, they demonstrated that gluten-specific T cells isolated from celiac disease patients cross-react with such bacterial peptides. This is compatible with a model where a bacterial infection leads to innate immune activation, followed by the development of a pathogen-specific adaptive T cell response that cross-reacts with gluten, and consequently development of celiac disease. In addition, Bouziat et al (2017) observed in an animal model that reovirus infection can lead to adaptive T cell responses to dietary antigens by promoting Th1 immunity. In addition, they presented evidence supporting this concept in patients with celiac disease as well.

Empirical Evidence

This is a well known concept of immunology and also described in celiac disease (Petersen et al 2020; Bouziat et al 2017; Voisin and Abadie 2021; Matera et al 2024).

Uncertainties and Inconsistencies

Despite the evidence linking bacterial and viral infections to celiac disease development, it is extremely difficult to establish a causal relationship between these events in humans. As an animal model is lacking to confirm such relationships there remains a certain level of uncertainty. In addition, various papers have suggested that gluten itself may have innate stimulatory properties. However, a molecular mechanism through which gluten would exert such an effect has not been established. Moreover, it is unclear why such effects would manifest in only a minority of individuals.

Quantitative Understanding of the Linkage

Response-response relationship

Co-localization of gluten reactive adaptive T cells with APC in secondary lymphoid structures does not lead to the initiation of T cell responses unless the APC are activated and loaded with the appropriate antigen which requires innate immune activation.

Time-scale

Innate immune activation is immediate upon encounter with pathogens.

References

-Petersen J, Ciacchi L, Tran MT, Loh KL, Kooy-Winkelaar Y, Croft NP, Hardy MY, Chen Z, McCluskey J, Anderson RP, Purcell AW, Tye-Din JA, Koning F, Reid HH, Rossjohn J. T cell receptor cross-reactivity between gliadin and bacterial peptides in celiac disease. Nat Struct Mol Biol. 2020 Jan;27(1):49-61. doi: 10.1038/s41594-019-0353-4. Epub 2019 Dec 23. PMID: 31873306.

- Bouziat R, Hinterleitner R, Brown JJ, Stencel-Baerenwald JE, Ikizler M, Mayassi T, Meisel M, Kim SM, Discepolo V, Pruijssers AJ, Ernest JD, Iskarpatyoti JA, Costes LM, Lawrence I, Palanski BA, Varma M, Zurenski MA, Khomandiak S, McAllister N, Aravamudhan P, Boehme KW, Hu F, Samsom JN, Reinecker HC, Kupfer SS, Guandalini S, Semrad CE, Abadie V, Khosla C, Barreiro LB, Xavier RJ, Ng A, Dermody TS, Jabri B. Reovirus infection triggers inflammatory responses to dietary antigens and development of celiac disease. Science. 2017 Apr 7;356(6333):44-50. doi: 10.1126/science.aah5298. PMID: 28386004; PMCID: PMC5506690.

- Matera M, Guandalini S. How the Microbiota May Affect Celiac Disease and What We Can Do. Nutrients. 2024 Jun 14;16(12):1882. doi: 10.3390/nu16121882. PMID: 38931237; PMCID: PMC11206804.

-Voisine J, Abadie V. Interplay Between Gluten, HLA, Innate and Adaptive Immunity Orchestrates the Development of Coeliac Disease. Front Immunol. 2021 Jun 2;12:674313. doi: 10.3389/fimmu.2021.674313. PMID: 34149709; PMCID: PMC8206552.

Relationship: 3341: Activation of the innate immune response leads to Activation of gluten-reactive CD4+ T cells

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Celiac disease, as it is currently understood, is a human-specific autoimmune disorder. Some animal models have been developed to reproduce aspects of the disease, but celiac disease is exclusive to humans. (Marietta et al., 2011).

Key Event Relationship Description

Innate immune activation in the gastrointestinal tract will activate dendritic cells resulting in the processing and expression of specific antigens followed by migration to the draining lymphnodes. Alternatively, the antigens are captured by dendritic cells present in the Peyer's patches. Subsequently interaction between these activated, antigen-loaded dendritic cells with naive CD4 T cells expressing antigen specific T cell receptors will result in activation of these CD4 T cells, followed by proliferation and secretion of cytokines.

Evidence Supporting this KER

Essential textbook knowledge

Biological Plausibility

Celiac disease is caused by an intolerance to gluten food proteins. There is an exceptionally strong association between the occurrence of celiac disease and the presence of HLA-DQ2 and/or HLA-DQ8 molecules. This association is explained by the observation that CD4 T cells specific for modified gluten peptides bound to either HLA-DQ2 or HLA-DQ8 are typically found in patients but not in healthy individuals. CD4 T cells belong to the adaptive immune system. The initiation of adaptive immune responses depends on activation of the innate immune system, dendritic cells in particular. Dendritic cells can be activated through pattern recognition receptors (PRRs) that bind pathogen associated molecular patterns (PAMPs) like bacterial cell wall components (LPS) and viral double-stranded ribonucleic acid (dsRNA). Upon activation of dendritic cells, they process antigen derived from such pathogens and present them to adaptive T cells, resulting in the initiation of long-lasting T cell responses to eradicate the pathogens. Thus, innate immune activation is required for the initiation of disease-causing gluten-specific CD4 T cells.

Importantly, activated gluten-specific CD4 T cells typically produce cytokines, including IFN gamma, a cytokine known to enhance the expression of HLA-molecules, like HLA-DQ2 and HLA-DQ8. This thus feeds back into MEI, formation of HLA-DQ-gluten complexes, and constitutes an amplification loop enhancing the adaptive CD4 T cell response to gluten.

Empirical Evidence

The fact that innate immune activation precedes adaptive immune response is essential textbook knowledge.

Uncertainties and Inconsistencies

There are no known uncertainties or inconsistencies.

Quantitative Understanding of the Linkage

Time-scale

Innate immune responses are immediate upon exposure to pathogens followed by adaptive immune responses developing over a period of 1 to 2 weeks.

Known modulating factors

Gender is a strong modulator as females have an approximately 2 times higher chance of developing celiac disease. Other potential modulating factors are the composition of the intestinal microbiota. IgA deficiency is known to increase the risk of development of celiac disease.

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

Known Feedforward/Feedback loops influencing this KER

Mucosal tolerance maintains homeostasis in the gastrointestinal tract by suppressing immune responses to harmless food derived antigens. In part this is achieved by the activity of T regulatory cells that can suppress the

activity of effector T cells, like the gluten-specific CD4 T cells typically found in patients with celiac disease.

References

- Essential textbook knowledge

Relationship: 3342: Activation of gluten-reactive CD4+ T cells leads to Activation of gluten- and TG2-reactive B cells

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Celiac disease, as it is currently understood, is a human-specific autoimmune disorder. Some animal models have been developed to reproduce aspects of the disease, but celiac disease is exclusive to humans. (Marietta et al., 2011).

While this KER applies to both sexes, it is important to note that females are more likely to be affected by celiac disease, and sex-based differences in immune response can influence clinical outcomes (Janson-Knodell et al., 2019; Klein and Fanagan, 2016).

Key Event Relationship Description

The activation of gluten-reactive CD4+ T cells is a prerequisite for the activation of gluten- and TG2-reactive B cells. Gluten-reactive CD4+ T cells interact with B cells in the gastrointestinal lymphoid structures, providing the necessary signals for B cell activation and immunoglobulin class switching (Di Niro et al., 2012; Lindstad et al., 2021). This interaction produces plasma cells secreting IgA autoantibodies specific for transglutaminase 2 (TG2) and IgG antibodies specific for deamidated gliadin (Sollid et al., 1997). The production of these antibodies is a hallmark of celiac disease and is absent in healthy individuals (Dieterich et al., 1997).

Evidence Supporting this KER

Evidence was collected through a combination of literature searches and expert consultations. Experts contributed by reviewing drafted material asynchronously and participating in online discussions to refine the evidence base. Additionally, they provided key articles relevant to the topic, which served as a foundation for further literature searches in Scopus, PubMed, and Google Scholar. Keywords were tailored to each key event (KE) and key event relationship (KER) to ensure comprehensive coverage of relevant studies. The collected literature was systematically categorized in an Excel spreadsheet based on its relevance to specific KEs and KERs within the AOP. This approach facilitated the organization of data supporting different aspects of the pathway.

Biological Plausibility

B cells require direct interaction with antigen-specific helper T cells for activation (Lanzavecchia, 1985). This interaction is facilitated by the antigen serving as a bridge, allowing the T cell receptor (TCR) on T cells to engage with the peptide-MHC complex on B cells. Such antigen-specific T-B cell cooperation is crucial for initiating and sustaining adaptive immune responses, including those observed in celiac disease.

In celiac disease, gluten-reactive CD4+ T cells recognize gluten peptides presented by HLA-DQ2/8 molecules on antigen-presenting cells (APCs) (Koning et al., 2015). These activated T cells then provide essential help to B cells that have internalized and processed gluten or TG2-gluten complexes. The T-B cell interaction leads to B cell activation, proliferation, and differentiation into plasma cells secreting IgA autoantibodies against TG2 and IgG antibodies against deamidated gliadin.

Empirical Evidence

- Du Pre et al., (2019) studied B cell tolerance and autoantibody formation to TG2 with immunoglobulin knock-in mice. They demonstrated that gluten-reactive T cells assist autoreactive TG2-specific B cells through gluten-TG2 complex involvement.
- Lindstad et al., (2021) demonstrated that naive TG2-specific B cells and gluten-specific T cells can collaborate in an antigen-specific manner *in vitro.* Additionally, they show that TG2-gluten complexes are efficient antigens for TG2- and gluten-specific B cells and allow both types of B cells to receive help from gluten-specific T cells.

Uncertainties and Inconsistencies

There are no known inconsistencies.

Quantitative Understanding of the Linkage

Response-response relationship

- Lindstad et al., (2021) showed strong T-cell proliferation when exposed to TG2-specific B cells at concentrations as low as 0.01 µg/mL.

Time-scale

- In the study carried out by du Pre et al., (2019) it is demonstrated that antibody production occurs after exposure to gluten-specific T cell epitope, among other components, in contrast to not exposing the mice model to the T cell epitope. The time scale is unclear in the study, as the IgG measurement is done 6 days after administration, however, it indicates a sequential process.

Known Feedforward/Feedback loops influencing this KER

Deamidated gliadin—and TG2-specific B cells act as highly efficient antigen-presenting cells for the gluten-specific CD4 T cells, amplifying the gluten-specific T cell response, which, in turn, enhances B cell activation. (Di Niro et al., 2012).

References

- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KE, Wilson PC, Sollid LM. High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. Nat Med. 2012 Feb 26;18(3):441-5. doi: 10.1038/nm.2656. PMID: 22366952; PMCID: PMC4533878.
- du Pré MF, Blazevski J, Dewan AE, Stamnaes J, Kanduri C, Sandve GK, Johannesen MK, Lindstad CB, Hnida K, Fugger L, Melino G, Qiao SW, Sollid LM. B cell tolerance and antibody production to the celiac disease autoantigen transglutaminase 2. J Exp Med. 2020 Feb 3;217(2):e20190860. doi: 10.1084/jem.20190860. PMID: 31727780; PMCID: PMC7041703.
- Dieterich W, Ehnis T, Bauer M, Donner P, Volta U, Riecken EO, Schuppan D. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med. 1997 Jul;3(7):797-801. doi: 10.1038/nm0797-797. PMID: 9212111.
- Jansson-Knodell CL, Hujoel IA, West CP, Taneja V, Prokop LJ, Rubio-Tapia A, Murray JA. Sex Difference in Celiac Disease in Undiagnosed Populations: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2019 Sep;17(10):1954-1968.e13. doi: 10.1016/j.cgh.2018.11.013. Epub 2018 Nov 16. PMID: 30448593.
- Klein SL, Flanagan KL. Sex differences in immune responses. Nat Rev Immunol. 2016 Oct;16(10):626-38. doi: 10.1038/nri.2016.90. Epub 2016 Aug 22. PMID: 27546235.
- Koning F, Thomas R, Rossjohn J, Toes RE. Coeliac disease and rheumatoid arthritis: similar mechanisms, different antigens. Nat Rev Rheumatol. 2015 Aug;11(8):450-61. doi: 10.1038/nrrheum.2015.59. Epub 2015 May 19. PMID: 25986717.
- Lanzavecchia A. Antigen-specific interaction between T and B cells. Nature. 1985 Apr 11-17;314(6011):537-9. doi: 10.1038/314537a0. PMID: 3157869.
- Lindstad CB, Dewan AE, Stamnaes J, Sollid LM, du Pré MF. TG2-gluten complexes as antigens for gluten-specific and transglutaminase-2 specific B cells in celiac disease. PLoS One. 2021 Nov 3;16(11):e0259082. doi: 10.1371/journal.pone.0259082. PMID: 34731200; PMCID: PMC8565743.
- Marietta EV, David CS, Murray JA. Important lessons derived from animal models of celiac disease. Int Rev Immunol. 2011 Aug;30(4):197-206. doi: 10.3109/08830185.2011.598978. PMID: 21787225; PMCID: PMC3480308.
- Sollid LM, Molberg O, McAdam S, Lundin KE. Autoantibodies in coeliac disease: tissue transglutaminase--guilt by association? Gut. 1997 Dec;41(6):851-2. doi: 10.1136/gut.41.6.851. PMID: 9462222; PMCID: PMC1891617.

Relationship: 3333: Activation of gluten- and TG2-reactive B cells leads to Disruption of the intestinal barrier

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Human beings

Key Event Relationship Description

The activation of adaptive T and B cell responses to gluten due to loss of mucosal tolerance results in inflammation in the lamina propria of the upper small intestine characterized by a highly significant increase in the presence of both innate and adaptive immune cells. This is accompanied by a highly significant upregulation of the expression of HLA-class II molecules, the production of immune stimulatory and pro-inflammatory cytokines, including IFN-γ, TNF-α, IL-2, IL-7, and IL-21, by the gluten-reactive CD4+ T cells (Santos et al., 2024;De Nitto et al., 2009; Garrote et al., 2008). In addition, there is a massive increase in the number and activation status of intraepithelial lymphocytes (IEL) in the intestinal epithelium, likely driven by a combination of the pro-inflammatory cytokines produced in the lamina propria and local production of IL-15 by the enterocytes (Abadie et al., 2020). Moreover, the expression of non-classical MHC molecules is upregulated in the epithelium. These activated IELs mediate epithelial cell destruction, contributing to the flattening of the intestinal villi and leading to loss of barrier function (Abadie et al., 2012).

Evidence Supporting this KER

- Intestinal epithelial cells in active celiac disease express elevated levels of MHC class II molecules (HLA-DQ2/DQ8), enabling direct presentation of deamidated gluten peptides to CD4+ T cells. Organoid models expressing HLA-DQ2.5 demonstrate gluten-dependent activation of CD4+ T cells, leading to IL-2, IFN-γ, and IL-15 release (Rahmani et al., 2024).
- IL-15 induces IEL survival, IFN-γ production, and epithelial killing in refractory celiac sprue (Mention et al., 2003)
- There is a correlation between IEL activation, villous atrophy, and cytokine levels (IFN-γ, IL-15) in active celiac disease (Abadie et al., 2012)

Biological Plausibility

While the disease underlying gluten-specific CD4+ T cell response is located in the lamina propria, celiac disease is also characterized by a pronounced increase in the presence of intraepithelial lymphocytes (IEL) that express Natural Killer-receptors in the epithelium (Setty et al., 2015). In addition, while IEL normally reside in the basal portion of the intestinal villi, in celiac disease they spread all over the epithelium, including the tip of the villi. These IEL are activated, express Natural Killer-receptors, and can mediate epithelial cell destruction, contributing to the flattening of the villi and loss of barrier function. IL-15 expression by epithelial cells is a key cytokine involved in the activation of the IEL (James et al., 2021). Also, the cytokines produced by the gluten-specific T cells in the lamina propria, including IFN-γ, TNF-α, IL-2, IL-7, and IL-21 create a proinflammatory environment that is likely crucial for the increased presence and sustained activation of the IEL compartment as intestinal morphology normalizes upon the introduction of a gluten-free diet and is accompanied by a reduction of the numbers of IEL. Thus, a cascade where activation of gluten-specific T cell leads to a pro-inflammatory environment, which eventually results in activation of IEL, epithelial cell destruction and a loss of barrier function, and absorptive capacity in the upper intestine.

Empirical Evidence

The formation of HLA-DQ2/8-gluten complexes drives gluten-specific CD4+ T-cell activation by presenting deamidated peptides with high affinity, particularly in HLA-DQ2.5 homozygous individuals who exhibit stronger T-cell responses due to broader peptide presentation (Okura et al., 2023). Gluten-reactive T-cell receptor generation enables clonal expansion of T cells with focused repertoires, licensing B-cell help via CD40L and IL-21 (Zou et al., 2022). Gluten/TG2-reactive B-cell receptor allow B cells to internalize TG2-gluten complexes, process gluten peptides, and present them to T cells, facilitating epitope spreading and autoantibody production (Zou et al., 2022). T-cell/APC co-localization ensures direct collaboration, with TG2-specific B cells acting as APCs to amplify T-cell help. Innate immune activation via protease-resistant gluten fragments enhances APC maturation and antigen presentation (Voisine et al., 2021), while CD4+ T-cell activation provides critical cytokines (e.g., IL-21) that drive B-cell differentiation into plasma cells. Together, these upstream KEs create a feedforward loop where HLA-DQ2.5-mediated antigen presentation, T-B cell collaboration, and cytokine signaling converge to activate gluten- and TG2-reactive B cells, producing pathogenic autoantibodies (Zou et al., 2022).

Uncertainties and Inconsistencies

While the evidence supporting the role of the gluten-specific T and B cell response in disease pathogenesis is very strong, it is less clear what drives the upregulation of IL-15 and non-classical MHC-molecules in the epithelium. It has been suggested that gluten itself has the capacity to induce innate immune activation and could be responsible for the upregulation of IL-15 (Abadie et Jabri, 2014; Abadie et al., 2020). However, it is entirely unclear why this innate effect of gluten would only manifest itself in certain individuals, nor is there clarity about the molecular mechanism involved. Alternatively, viral and bacterial infections play a role in this as these can induce the expression of type I interferons (McNab et al., 2015; Mancuso et al., 2007).

Quantitative Understanding of the Linkage

Response-response relationship

B cell activation is closely linked to changes in the intestinal barrier in celiac disease. Patients with active celiac disease produce autoantibodies, predominantly targeting transglutaminase 2 (TG2). Recent studies have visualized plasma cells producing TG2-specific antibodies within celiac disease lesions, achieved through the use of labeled TG2 antigens. On average, approximately 10% of the plasma cells in a disease lesion are TG2-specific, with the majority producing immunoglobulin A (IgA). These TG2-specific plasma cells diminish once patients adopt a gluten-free diet (Di Niro et al., 2012; Sollid et Jabri, 2013).

The strict association of TG2-specific antibodies with individuals carrying specific HLA types, combined with the observation that antibody avidity decreases when reverted to their presumed germline configuration, suggests that these antibodies undergo affinity maturation. This process indicates that their development is T cell-dependent (Sollid et Jabri, 2013; Björck et al., 2010).

The levels of FABP2 (fatty acid binding protein), a marker of intestinal epithelial cell damage, are significantly elevated in celiac disease patients, correlating with the levels of IgA antibodies to TG2 (Uhde et al., 2016).

Time-scale

The exact time-scale of the development of celiac disease is unknown as patients are usually only identified when disease symptoms are manifest. However, based on the knowledge about the development of innate and adaptive immune responses one may assume the gluten-specific T cell response could develop within a period of weeks to months. It is also noteworthy that long-term exposure to gluten contributes to cumulative barrier disruption, suggesting a progressive timeline of damage rather than a specific time point (Schumann et al., 2012).

Strict compliance with a gluten free diet in most CD patients leads to the disappearance or significant decrease of antibodies within 12 months (18–24 months if the antibody titer is very high) together with regrowth of the intestinal villi (Caio et al., 2019).

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Gender	Female	Females have a higher chance (2:1) of developing celiac disease	Jansson-Knodell et al., 2017
Composition of the intestinal microbiota and metabolites of food derived compounds	For example, the vegetable-derived phytochemical indole-3-carbinol, a ligand for the aryl hydrocarbon receptor (AhR)	Higher chance of developing celiac disease	Abadie et al., 2012
IgA deficiency		It increases the risk of development of celiac disease	Leonard et al., 2017

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)

Known Feedforward/Feedback loops influencing this KER

There have been observations on the transient presence of TG2-specific antibodies in children predisposed to celiac disease development, suggesting that emerging gluten-specific adaptive immune responses may be controlled to maintain mucosal tolerance to gluten (Tosco et al., 2011).

In addition, cytokine release (e.g., IL-15) may promote further activation of B cells and perpetuate barrier dysfunction (Abadie et Jabri, 2014).

References

- Abadie V, Discepolo V, Jabri B. Intraepithelial lymphocytes in celiac disease immunopathology. Semin Immunopathol. 2012;34:551-566.
- Abadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. Immunol Rev. 2014 Jul;260(1):221-34.
- Abadie V, Kim SM, Lejeune T, Palanski BA, Ernest JD, Tastet O, Voisine J, Discepolo V, Marietta EV, Hawash MBF, Ciszewski C, Bouziat R, Panigrahi K, Horwath I, Zurenski MA, Lawrence I, Dumaine A, Yotova V, Grenier JC, Murray JA, Khosla C, Barreiro LB, Jabri B. IL-15, gluten, and HLA-DQ8 drive tissue destruction in celiac disease. Nature. 2020 Feb;578(7796):600-604.
- Björck S, Brundin C, Lörinc E, Lynch KF, Agardh D. Screening detects a high proportion of celiac disease in young HLA-genotyped children. J Pediatr Gastroenterol Nutr. 2010 Jan;50(1):49-53.
- Caio G, Volta U, Sapone A, Leffler DA, De Giorgio R, Catassi C, Fasano A. Celiac disease: a comprehensive current review. BMC Med. 2019 Jul 23;17(1):142.
- De Nitto D, Monteleone I, Franzè E, Pallone F, Monteleone G. Involvement of interleukin-15 and interleukin-21, two γ-chain-related cytokines, in celiac disease. World J Gastroenterol. 2009; 7;15(37):4609-4614.
- Di Niro R, Mesin L, Zheng NY, Stamnaes J, Morrissey M, Lee JH, Huang M, Iversen R, du Pré MF, Qiao SW, Lundin KEA, Wilson PC, Sollid LM. High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. Nat Med. 2012 Feb 26;18(3):441-5.
- Garrote JA, Gómez-González E, Bernardo D, Arranz E, Chirdo F. Celiac disease pathogenesis: the proinflammatory cytokine network. J Pediatr Gastroenterol Nutr. 2008;47 Suppl 1:S27-32.
- James OJ, Vandereyken M, Marchingo JM, Singh F, Bray SE, Wilson J, Love AG, Swamy M. IL-15 and PIM kinases direct the metabolic programming of intestinal intraepithelial lymphocytes. Nat Commun. 2021 Jul 13;12(1):4290.
- Jansson-Knodell CL, King KS, Larson JJ, Van Dyke CT, Murray JA, Rubio-Tapia A. Gender-Based Differences in a Population-Based Cohort with Celiac Disease: More Alike than Unalike. Dig Dis Sci. 2017 Nov 10;63(1):184-192.
- Leonard MM, Sapone A, Catassi C, Fasano A. Celiac disease and nonceliac gluten sensitivity: A review. JAMA. 2017 Aug 15;318(7):647-656.
- Mancuso G, Midiri A, Biondo C, Beninati C, Zummo S, Galbo R, Tomasello F, Gambuzza M, Macrì G, Ruggeri A, Leanderson T, Teti G. Type I IFN signaling is crucial for host resistance against different species of pathogenic bacteria. J Immunol. 2007 Mar 1;178(5):3126-33.
- McNab F, Mayer-Barber K, Sher A, Wack A, O'Garra A. Type I interferons in infectious disease. Nat Rev Immunol. 2015 Feb;15(2):87-103.
- Mention JJ, Ben Ahmed M, Bègue B, Barbe U, Verkarre V, Asnafi V, Colombel JF, Cugnenc PH, Ruemmele FM, McIntyre E, Brousse N, Cellier C, Cerf-Bensussan N. Interleukin 15: a key to disrupted intraepithelial lymphocyte homeostasis and lymphomagenesis in celiac disease. Gastroenterology. 2003 Sep;125(3):730-745.
- Meresse B, Chen Z, Ciszewski C, Tretiakova M, Bhagat G, Krausz TN, Raulet DH, Lanier LL, Groh V, Spies T, Ebert EC, Green PH, Jabri B. Coordinated induction by IL-15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease. Immunity. 2004 Sep;21(3):357-66.
- Okura Y, Ikawa-Teranishi Y, Mizoroki A, Takahashi N, Tsushima T, Irie M, Harfuddin Z, Miura-Okuda M, Ito S, Nakamura G, Takesue H, Ozono Y, Nishihara M, Yamada K, Gan SW, Hayasaka A, Ishii S, Wakabayashi T, Muraoka M, Nagaya N, Hino H, Nemoto T, Kuramochi T, Torizawa T, Shimada H, Kitazawa T, Okazaki M, Nezu J, Sollid LM. Characterizations of a neutralizing antibody broadly reactive to multiple gluten peptide:HLA-DQ2.5 complexes in the context of celiac disease. Nat Commun. 2023 Dec 22;14:8846.
- Rahmani S, Galipeau HJ, Clarizio AV, Wang X, Hann A, Rueda GH, Kirtikar UN, Constante M, Wulczynski M, Su HM, Burchett R, Bramson JL, Pinto-Sanchez MI, Stefanolo JP, Niveloni S, Surette MG, Murray JA, Anderson RP, Bercik P, Caminero A, Chirdo FG, Didar TF, Verdu EF. Gluten-dependent activation of $CD4^+$ T cells by MHC class II–expressing epithelium. Gastroenterology. 2024 Nov;167(6):1113-1128.
- Santos AJM, van Unen V, Lin Z, Chirieleison SM, Ha N, Batish A, Chan JE, Cedano J, Zhang ET, Mu Q, Guh-Siesel A, Tomaske M, Colburg D, Varma S, Choi SS, Christophersen A, Baghdasaryan A, Yost KE, Karlsson K, Ha A, Li J, Dai H, Sellers ZM, Chang HY, Dunn JCY, Zhang BM, Mellins ED, Sollid LM, Fernandez-Becker NQ, Davis MM, Kuo CJ. A human autoimmune organoid model reveals IL-7 function in coeliac disease. Nature. 2024; 632:401–410.
- Schumann M, Kamel S, Pahlitzsch M, Lebenheim L, May C, Krauss M, Hummel M, Daum S, Fromm M, Schulzke JD. Defective tight junctions in refractory celiac disease. Ann N Y Acad Sci. 2012 Jul;1258:43-51.
- Setty M, Discepolo V, Abadie V, Kamhawi S, Mayassi T, Kent A, Ciszewski C, Maglio M, Kistner E, Bhagat G, Semrad C,

Kupfer SS, Green PH, Guandalini S, Troncone R, Murray JA, Turner JR, Jabri B. Distinct and synergistic contributions of epithelial stress and adaptive immunity to functions of intraepithelial killer cells and active celiac disease. Gastroenterology. 2015 Sep;149(3):681-91.e10.
- Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. Nat Rev Immunol. 2013 Apr;13(4):294-302.
- Tosco A, Salvati VM, Auricchio R, Maglio M, Borrelli M, Coruzzo A, Paparo F, Boffardi M, Esposito A, D'Adamo G, Malamisura B, Greco L, Troncone R. Natural history of potential celiac disease in children. Clin Gastroenterol Hepatol. 2011 Apr;9(4):320-5; quiz e36.
- Uhde M, Ajamian M, Caio G, De Giorgio R, Indart A, Green PH, Verna EC, Volta U, Alaedini A. Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. Gut. 2016 Dec;65(12):1930-1937.
- Voisine J, Abadie V. Interplay between gluten, HLA, innate and adaptive immunity orchestrates the development of coeliac disease. Front Immunol. 2021 Jun 2;12:674313.
- Zhou C, Østerbye T, Dahal-Koirala S, Steinsbø Ø, Jahnsen J, Lundin KEA, Buus S, Sollid LM, Iversen R. Antibodies to native gluten arise from cross-reactive B cells with implications for epitope spreading in celiac disease. bioRxiv. 2022 Jan 29.

Relationship: 3335: Disruption of the intestinal barrier leads to Celiac disease

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Gluten-driven immune activation leading to celiac disease in genetically predisposed individuals	adjacent	Moderate	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
human	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Celiac disease is a human-specific condition linked to HLA-DQ2/DQ8, so most studies have been conducted in humans

Key Event Relationship Description

Inflammation in the upper gastrointestinal tract is a hallmark of celiac disease (CD). This inflammation is characterized by a massive infiltration of lymphocytes in both the lamina propria and the epithelial layer of the small intestine (Sollid & Jabri, 2013; Abadie & Jabri, 2014). The density of antigen-presenting cells (APCs), including dendritic cells and macrophages, is significantly increased in the intestinal mucosa of CD patients, facilitating the activation of gluten-specific T cells (Di Niro et al., 2012; Jabri & Sollid, 2009).

T cells specific for gluten peptides are readily detectable in the lamina propria. Upon activation, these T cells produce pro-inflammatory cytokines such as interferon-gamma (IFN-γ), which perpetuate the inflammatory response (Sollid & Jabri, 2013). In addition, B and plasma cells are abundant in the lamina propria and secrete autoantibodies targeting tissue transglutaminase 2 (TG2) and deamidated gluten peptides, which are hallmark features of CD pathogenesis (Di Niro et al., 2012; Schumann et al., 2012).

IL-15, a pro-inflammatory cytokine, is overexpressed in the intestinal epithelium of CD patients, contributing to the activation of intraepithelial lymphocytes (IELs). These IELs acquire cytolytic properties, leading to the destruction of enterocytes and disruption of the epithelial barrier (Abadie & Jabri, 2014; McNab et al., 2015). This process culminates in villous atrophy, characterized by flattening of the intestinal villi, loss of barrier function, and reduced intestinal absorptive surface area (Abadie & Jabri, 2014; Schumann et al., 2012). The resulting epithelial damage contributes to common symptoms such as diarrhea, abdominal pain, malabsorption, failure to thrive in children, and fatigue (Leonard et al., 2017).

The withdrawal of gluten from the diet typically results in the resolution of symptoms and normalization of intestinal morphology. Upon reintroduction of gluten, patients quickly experience a resurgence of symptoms and intestinal damage (Leonard et al., 2017; Sollid & Jabri, 2013). Currently, a lifelong gluten-free diet (GFD) remains the only

effective treatment for CD (Leonard et al., 2017; Fasano et al., 2012).

Evidence Supporting this KER

Biological Plausibility

The biological plausibility of this KER is well established. Dr. Willem-Karel Dicke first demonstrated in the 1940s that the consumption of gluten is the primary trigger for the symptoms of celiac disease (CD). Gluten ingestion induces inflammation in the small intestine, characterized by lymphocyte infiltration, villous atrophy, and loss of epithelial barrier integrity, all of which are hallmark features of the disease (Sollid & Jabri, 2013; Abadie & Jabri, 2014). The disruption of the intestinal barrier allows gluten peptides to interact with the immune system, triggering an autoimmune response involving T cells and the production of antibodies against tissue transglutaminase (TG2) and gluten peptides (Di Niro et al., 2012; Leonard et al., 2017).

Moreover, IL-15 overexpression in the intestinal epithelium contributes to the activation of cytotoxic intraepithelial lymphocytes (IELs), which directly destroy epithelial cells and exacerbate intestinal damage (Abadie & Jabri, 2014). This sequence of events explains how gluten ingestion disrupts the intestinal barrier, ultimately leading to the pathology of CD.

Empirical Evidence

A large body of empirical evidence supports the link between gluten consumption and the onset of CD symptoms. The therapeutic effect of a gluten-free diet (GFD) is well-documented, with studies showing rapid improvement in clinical symptoms and normalization of intestinal morphology upon gluten withdrawal (Fasano et al., 2012; Leonard et al., 2017). Conversely, the reintroduction of gluten into the diet quickly leads to the recurrence of symptoms, providing strong evidence for the causal role of gluten in disease pathogenesis (Sollid & Jabri, 2013).

Studies have consistently demonstrated that gluten-specific T cells are present in the intestinal mucosa of CD patients, and these cells produce pro-inflammatory cytokines upon gluten exposure (Sollid & Jabri, 2013; Di Niro et al., 2012). Additionally, the observation that antibodies against TG2 and gluten peptides are present in nearly all CD patients further substantiates the role of gluten in driving intestinal barrier dysfunction and subsequent autoimmune responses (Leonard et al., 2017).

Uncertainties and Inconsistencies

Symptoms associated with celiac disease are highly variable. Also, not all patients are equally sensitive to gluten exposure. It is at present unclear what causes these differences. There are:

Variability in Gluten Sensitivity Thresholds: The amount of gluten necessary to trigger symptoms and intestinal damage varies significantly among patients. While some individuals react to minute quantities of gluten, others tolerate small amounts without noticeable symptoms. This variability complicates efforts to establish uniform thresholds for gluten exposure in dietary guidelines (Fasano et al., 2012).

Silent and Potential CD: A subset of patients with CD remains asymptomatic or presents with "silent" disease, where characteristic intestinal damage is evident but symptoms are absent. Additionally, individuals with potential CD exhibit positive serology but lack intestinal damage, raising questions about the progression and triggers of disease activation (Tosco et al., 2011).

Overlap with Non-Celiac Gluten Sensitivity (NCGS): The differentiation between CD and NCGS remains challenging due to overlapping symptoms. NCGS patients report gluten-related symptoms without the autoimmune or histological markers of CD, suggesting additional, poorly understood mechanisms (Uhde et al., 2016). Role of Environmental and Genetic Factors: Although HLA-DQ2/DQ8 is a necessary genetic factor, not all carriers develop CD. Environmental factors, such as infections or gut microbiota alterations, are thought to modulate disease onset but remain incompletely characterized (Abadie & Jabri, 2014).

Quantitative Understanding of the Linkage

Response-response relationship

Gliadin Dose and Immune Response:

Studies have shown a dose-dependent relationship between gluten exposure and immune activation. Even small amounts of gluten (as low as 10-50 mg/day) can induce detectable mucosal damage and T-cell activation in individuals with CD. Higher doses lead to more severe villous atrophy, increased intraepithelial lymphocyte infiltration, and elevated antibody levels (Fasano et al., 2012).

IL-15 Expression and Cytotoxicity:

Increased levels of IL-15 correlate with enhanced cytotoxic activity of intraepithelial lymphocytes (IELs), promoting epithelial cell death and barrier disruption. Animal models and human biopsy data have confirmed that IL-15 overexpression accelerates epithelial destruction in response to gluten exposure (Abadie & Jabri, 2014).

Time-scale

The exact time-scale of the development of celiac disease is unknown as patients are usually only identified when disease symptoms are manifest. Besides, the progression of CD following intestinal barrier disruption varies depending on individual factors:

Acute Response: In gluten challenge studies, symptoms can appear within hours to days of gluten reintroduction. This aligns with the rapid activation of gluten-specific T cells and the early release of pro-inflammatory cytokines.

Histological Changes: Structural changes, such as villous atrophy and crypt hyperplasia, typically develop within weeks of continuous gluten exposure, as observed in longitudinal biopsy studies of gluten reintroduction in CD patients (Tosco et al., 2011).

Recovery Timeline: Following the initiation of a gluten-free diet (GFD), most individuals show significant symptom improvement within weeks. However, full histological recovery of the intestinal mucosa may take months to years, especially in adults (Fasano et al., 2012).

Known modulating factors

Modulating Factor (MF)	MF Specification	Effect(s) on the KER	Reference(s)
Certain infections	e.g. rotavirus	may exacerbate celiac disease	Abadie & Jabri, 2014
Quantity and frequency of gluten intake		individuals consuming high amounts of gluten are at increased risk of symptomatic disease and more pronounced intestinal damage	Fasano et al., 2012
IL-15 in the intestinal epithelium	overexpression	exacerbates tissue destruction and modulates the severity of disease progression	Abadie & Jabri, 2014

Known Feedforward/Feedback loops influencing this KER

Not known

References

- Abadie V, Jabri B. IL-15: a central regulator of celiac disease immunopathology. *Immunol Rev.* 2014 Jul;260(1):221-34.
- Di Niro R, Mesin L, Zheng NY, et al. High abundance of plasma cells secreting transglutaminase 2-specific IgA autoantibodies with limited somatic hypermutation in celiac disease intestinal lesions. *Nat Med.* 2012 Mar;18(3):441-5.
- Fasano A, Catassi C. Clinical practice. Celiac disease. *N Engl J Med.* 2012 Dec 20;367(25):2419-26.
- Jabri B, Sollid LM. Tissue-mediated control of immunopathology in coeliac disease. *Nat Rev Immunol.* 2009 Dec;9(12):858-70.
- Leonard MM, Sapone A, Catassi C, Fasano A. Celiac Disease and Nonceliac Gluten Sensitivity: A Review. *JAMA.* 2017 Aug 15;318(7):647-656.
- McNab F, Mayer-Barber K, Sher A, et al. Type I interferons in infectious disease. *Nat Rev Immunol.* 2015 Feb;15(2):87-103.
- Schumann M, Kamel S, Pahlitzsch ML, et al. Defective tight junctions in refractory celiac disease. *Ann N Y Acad Sci.* 2012 Jul;1258:43-51.
- Sollid LM, Jabri B. Triggers and drivers of autoimmunity: lessons from coeliac disease. *Nat Rev Immunol.* 2013 Apr;13(4):294-302.
- Tosco A, Salvati VM, Auricchio R, et al. Natural history of potential celiac disease in children. *Clin Gastroenterol Hepatol.* 2011 Apr;9(4):320-5.
- Uhde M, Ajamian M, Caio G, et al. Intestinal cell damage and systemic immune activation in individuals reporting sensitivity to wheat in the absence of coeliac disease. *Gut.* 2016 Dec;65(12):1930-1937.