

EDITORIAL

THE IMMUNOLOGY OF IMMEDIATE AND DELAYED HYPERSENSITIVITY REACTION TO GLUTENA. VOJDANI, T. O'BRYAN¹ and G.H. KELLERMANN²*Immunosciences Lab., Inc., Beverly Hills, CA; ¹Davis Parkway, Warrenville, IL;
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The immunology of gluten hypersensitivity and celiac disease has been pursued with significant interest in the past 20 years. For the prevention of systemic diseases, most pathogens that gain entry into our bodies must be met with an effective immune response, yet in the gastrointestinal tract it is equally important that commensal bacteria and a diverse collection of dietary proteins and peptides be recognized without eliciting an active immune response or constant activation of the inflammatory pathway. This phenomenon of hyporesponsiveness to food antigens is known as oral tolerance. This oral tolerance to dietary antigens is maintained by three different mechanisms: anergy, cell deletion and immune suppression. However, in the presence of mechanical/chemical stressors and infections, this tolerance may break down, and gut associated lymphoid tissues (GALT) will react to different luminal antigens. The reaction of GALT to these antigens may lead to the production of pro-inflammatory cytokines, opening of tight junctions, entry of undigested antigens into the circulation, and the subsequent production of IgA, IgG, IgM and IgE antibodies in blood and secretory components. Like any other food hypersensitivity reaction, gluten sensitivity can be divided into immediate and delayed hypersensitivities. In this review an attempt is made first to differentiate immediate hypersensitivity to gliadin, mediated by IgE, from delayed hypersensitivity, which is mediated by IgA and IgG. Furthermore, we attempt to differentiate between gluten hypersensitivity with enteropathy (celiac disease) and gluten hypersensitivity without enteropathy.

The mechanism of oral tolerance to dietary proteins

Although mucosal surfaces are exposed to many dietary proteins and infectious agents, the immune system normally will not react to these antigens (1-4). Unresponsiveness or tolerance to these antigens is maintained by three principal mechanisms: anergy or functional unresponsiveness; deletion through programmed cell death or apoptosis; and immune suppression by regulatory T cells (Tregs). This induction of immune suppression or anergy to gliadin is shown in Fig. 1.

Although HLA-DQ2 or HLA-DQ8, which are tissue types known to be associated with celiac disease, is found in roughly 30% of the western population, celiac sprue is encountered in 1 out of 50 carriers. Most carriers of these genes, like the rest of the population, harbor some form of immune protection through regulatory T-cells.

The regulatory T cells are divided into two major groups:

- a. Natural Tregs, which act in a contact-dependent fashion, and express CD25 and transcription

Key words: celiac disease, gluten hypersensitivity, delayed hypersensitivity, enteropathy

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1721-727 (2008)

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- factor FOXP3;
- b. Adoptive Treg Type 1 cells (TR₁), which function in a contact-independent manner and may or may not express CD25 and FOXP3. The TR₁ and TH₃ cells preferentially synthesize immunosuppressive cytokines IL-10 and TGF- β , respectively, in order to maintain homeostasis of responses to foreign antigens, including gliadin.

In the absence of tolerance, gut associated lymphoid tissues will react to luminal antigens, which may lead to the production of IgA and IgM antibodies, pro-inflammatory cytokines and subsequent inflammation and tissue damage or autoimmunity (5). Immediate and delayed hypersensitivity to gluten are characterized by IgE-mediated reaction or IgG, IgM, IgA plus T-cell reaction to gluten when tolerance to gluten is either not established properly or broken in these conditions (1-6).

A. Immediate type hypersensitivity to gluten

Like any other food hypersensitivity reaction, gluten hypersensitivity can be divided into immediate or delayed. The immediate hypersensitivity to gluten is IgE-mediated and may become life-threatening in severe cases when combined with exercise or some medication. This IgE-specific reaction may occur with IgE-specific epitopes of ω -5 gliadin, glutenins or allergenic epitopes of wheat formed after heat inactivation, hydrolyzation or chemical processes (6).

Food-dependent exercise or medication-induced anaphylaxis (FDEIA) is a distinct form of a common food allergy induced by a combination of causative food ingestion (wheat), physical exercise, and/or aspirin intake. Systemic allergic reactions such as anaphylactic shock and generalized urticaria are symptoms of FDEIA (7-8). This immediate hypersensitivity reaction is not limited solely to wheat antigen. Many kinds of foods such as shrimp, shellfish, hazelnut, buckwheat, corn, apple and orange have been reported to cause this type of food allergic reaction (9-14). The mechanism for food induction of IgE-mediated hypersensitivity is shown in Fig. 2.

Diagnosis of FDEIA is normally done by an exercise challenge test combined with ingestion of

food that is known to have given patients episodes of anaphylaxis after its intake. The challenge test is unsafe for patients since it can provoke anaphylactic shock during testing. Therefore, an *in vitro* diagnostic method predicting development of symptoms by food and exercise challenge is a safer option for testing. However, for accurate *in vitro* testing it is necessary to identify IgE-binding epitopes (8).

This identification of IgE-binding epitopes of gliadin and high molecular weight gluten subunit was completed using sera from patients with WDEIA and enzyme immunoassay. Twenty-nine of thirty patients with wheat-dependent-exercise-induced anaphylaxis had specific IgE antibodies to these epitope peptides. Conversely, none of the 25 sera from healthy subjects reacted to both gluten and gluten peptides. These results indicate that measurement of IgE levels specific to epitope peptides of ω -5 gliadin and high molecular weight gluten peptide is useful as an *in vitro* diagnostic method for the assessment of patients with wheat-induced exercise-induced anaphylaxis, baker's asthma and contact urticaria.

In addition, in many industries wheat isolates have been produced by means of chemical and enzymatic treatment. This treatment induces the solubilization of gliadins in aqueous buffers by means of deamidation (15). The high protein content and solubility of treated gliadin in water provide interesting technological properties for their use in the food industry. The wheat isolates are used as food emulsifiers, gelling agents, film formation aid, stretchability agents in meat products, sauces, soups, and as clarifying agents in red wines.

This extensive use of wheat isolates in the food industry may be the major cause of hidden food allergies, which can be extremely dangerous to individuals with IgE-mediated allergy to wheat. In fact, anaphylaxis to wheat isolates was recently reported and proved by means of double-blind, placebo-controlled food challenge. Interestingly, the subject individual did not react to native wheat flour, but had very severe reaction to wheat antigens isolated from meat products. It was therefore concluded that treatments used for gluten deamidation generate new allergenic epitopes. A case of contact urticaria was recently attributed to hydrolyzed wheat in cosmetics combined with a generalized urticaria induced with

the ingestion of sausages with lentils and a French cassoulet. This patient could also eat cereal-based products without any problem (15-17).

Because food isolates or deamidated gluten are new food ingredients, when allergy to wheat is suspected, immune reaction to wheat isolates should be tested for a final determination of allergy to wheat or its chemically modified antigens.

B. Delayed type hypersensitivity to gluten

Delayed type hypersensitivity to gliadin is IgG, IgA or T-cell mediated. This reaction to gluten develops because of the loss or failure of the tolerance mechanism, or intolerance to ingested gluten. When this immune reaction to gluten occurs with the involvement of tissue transglutaminase in genetically susceptible individuals who present chronic inflammation in the small intestine, villous atrophy and flattening of the mucosa, it is called celiac disease. However, this immune reaction to gliadin and glutenin peptides of gluten may also occur in an individual without the involvement of genetic makeup and tissue transglutaminase, being induced instead by a loss of immune tolerance to gluten peptides and by enhanced gut permeability (18-19). If this loss of tolerance to gluten peptides does not involve enteropathy and is accompanied by intestinal barrier dysfunction, followed by the entry of these peptides into the circulation and systemic IgG and IgA response to gluten, then for this delayed type hypersensitivity we suggest the terminology gluten sensitivity without enteropathy.

B1. Celiac disease or gluten sensitivity with enteropathy

Celiac disease (CD) is a typical complex inflammatory disorder in which crucial genetic and environmental factors have been identified. It is an acquired disorder occurring in both adults and children. The condition is characterized by sensitivity to gluten that results in inflammation and atrophy of the mucosa of the small intestine. Similar protein components of related grains such as barley, rye, oat, kamut and spelt also cause an immune response in patients with CD. The clinical presentation of CD is very non-specific, and may vary from patient to patient. Patients may complain of abdominal

cramps, bloating, diarrhea, and/or excessive gas production after meals. They may also note general malaise, lassitude, weakness, undesired weight loss, constipation, anemia (B₁₂ deficiency), osteoporosis/osteopenia, poor dentition, peripheral neuropathy, seizures/ataxia with cerebral calcifications, irritability or poor growth in children, birth defects in infants, small stature, and amenorrhea/infertility/recurrent miscarriage in females (18-21).

Diagnosis of celiac disease

Because CD presentation varies so greatly, many affected individuals do not suspect they have the disease and therefore do not seek medical attention. Even when medical attention is sought, if patients have atypical symptoms, CD may not be diagnosed unless the physician suspects and tests for it. Therefore, diagnosed celiac disease is most likely the 'tip of the iceberg' accounting for only approximately 12% of total cases. Characteristic villous atrophy and symptoms of intestinal malabsorption are present in the classic form of the disease (22); however, now many newly-diagnosed patients have milder, atypical symptoms often without diarrhea or malabsorption ("atypical CD") or have no symptoms at all ("silent CD").

Recently, serological testing has been increasingly used to test patients with suspected gluten-sensitive enteropathy as well as for monitoring dietary compliance. Both IgG and IgA antibodies are detected in sera of patients with gluten-sensitive enteropathy (5). IgA antibodies are less sensitive but more specific markers of the disease. IgG antibodies appear to be more sensitive but less specific markers of disease than IgA. It is recommended that both antibodies should be measured due to the high incidence of IgA deficiency among celiac patients, which may mask the disease. Antibody testing is also important in detecting individuals who are at risk of having celiac disease but have no symptomology, in individuals with atypical symptoms or extraintestinal manifestations of celiac disease (gluten sensitivity without enteropathy), and in individuals with presumed celiac disease who fail to respond to a gluten-free diet. Patients with positive antibody tests must undergo small intestine biopsy to confirm the diagnosis and assess the degree of mucosal involvement (23-25).

Immune mechanism in celiac disease

Gluten is composed of two proteins, gliadin and glutenin. Gliadin, the alcohol-soluble component, is the preferred substrate of tissue transglutaminase, an enzyme that deamidates or removes an amino group from gliadin and adds the remainder of the peptide into existing proteins as part of the normal repair process. Transglutaminase is present in the cytoplasm of most cells in an inactive state, but inflammation and mechanical injury activate and release it into the intracellular matrix. It is present in high concentrations in the connective tissue of the small intestinal wall, especially surrounding smooth muscle cells in the lamina propria. Transglutaminase complexes with gliadin to form a “neoantigen” recognized as immunogenic by patients with celiac disease. The neoantigen is processed by antigen-presenting cells such as macrophages, which then present it to CD4+ T-lymphocytes. The CD4+ T-lymphocytes then activate to produce interferon- γ and to proliferate. Interferon- γ , produced by T cells, is thought to be primarily responsible for injuring and killing mucosal epithelial cells (19-20). This immunological mechanism underlying celiac disease in individuals with specific HLA subtype is shown in Fig. 3.

In addition to mechanical stress, chemical injury, infectious agents, macrophages and CD4+ T-lymphocytes, other lymphocyte subsets are also involved in the immune response in CD. Early in celiac disease, certain “toxic” small gliadin peptides generated by transglutaminase activity stimulate secretion of IL-15 by epithelial cells and lamina propria macrophages. These gliadin peptides also increase mucosal permeability, enhancing lymphocyte infiltration. IL-15 is a key inflammatory mediator that stimulates intraepithelial lymphocytes. The humoral immune mechanism is activated when sensitized CD4+ T cells stimulate B cells to make anti-gliadin and anti-transglutaminase antibodies. B-lymphocytes mature into increased numbers of plasma cells in the intestinal submucosa where they produce the antibodies characteristic of CD. The presence of T cells that recognize deamidated gluten peptides in celiac disease might be relevant to autoimmunity and the implication of celiac disease in many autoimmune diseases (26-29).

C. Delayed hypersensitivity to gluten without

enteropathy or gluten sensitivity without enteropathy

The terms gluten sensitivity and celiac disease (also known as gluten-sensitive enteropathy) have thus far been used synonymously to refer to a disease process affecting the small bowel and characterized by malabsorption and gastrointestinal symptoms. Yet, gluten sensitivity can exist even in the absence of an enteropathy. The systemic nature of this disease, the overwhelming evidence of an immune pathogenesis and the accumulating evidence of diverse manifestations involving organs other than the gut, such as the skin, heart, bone, pancreas, joints, nervous system, liver, uterus and other organs necessitate a re-evaluation of the belief that gluten sensitivity is solely a disease of the gut (30). This involvement of multi-organ system disorder could be independent of HLA type and production of antibodies against tissue transglutaminase (26, 30). The immune reaction to gliadin peptide and its cross-reaction with different tissues might result from a breach in oral tolerance to gliadin and the induction of intestinal barrier dysfunction by environmental factors such as xenobiotics and infections (rotaviruses).

Indeed, human rotaviruses are the most frequent etiologic agents of gastroenteritis in infants and young children in most parts of the world. Anti-gliadin peptide antibodies from patients with gluten sensitivity recognize the viral product, suggesting a possible link between rotavirus infection and gluten sensitivity. It has also been demonstrated that purified rotavirus peptide antibodies are capable of cross-reacting with gliadin peptide, tight junction protein (desmoglein peptide) and monocytes toll-like receptor-4 peptide. These findings further implicate alteration of cell permeability in gluten sensitivity and autoimmunity (26, 31-32).

Therefore, since affinity-purified rotavirus peptide antibody not only binds to gliadin peptide but also recognizes endomysial structure, activates TLR4, and alters epithelial cell permeability, it suggests that the rotavirus epitope may be important in determining an anti-virus immune response's ability to cross-react with self-antigens. This cross-reaction between rotavirus peptide and human tissue antigens has functional consequences on TLR4, tight junction proteins and intestinal permeability. It

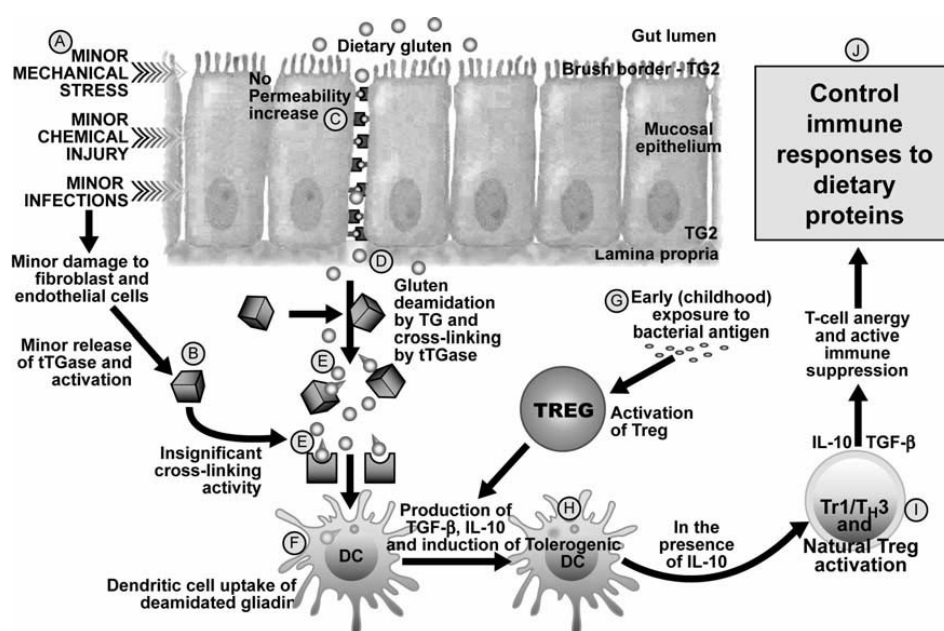


Fig. 1. Cellular and molecular induction of immune tolerance to dietary proteins (gliadin). In the absence of major mechanical and chemical stress or infection (A), no damage is done to fibroblasts and epithelial cells, and only small quantities of tissue transglutaminase are released into the environment (B). Since under these conditions the tight junctions are in perfect shape (C), only a few gliadin molecules may survive digestion and be transported across the mucosal epithelium (D). If these molecules of gliadin are deamidated by transglutaminase (E), the key regulator of the immune system called dendritic cells or antigen-presenting cells (F) prime T cells for anergy or tolerance. Early exposure to dietary proteins and bacterial antigens such as LPS (G) can activate regulatory T cells to produce TGF- β and IL-10, inducing activation of tolerogenic DCs (H) to control immune response to dietary proteins (gliadin). Further activation of TR₁, TH₃ and natural Treg (I) by IL-10 results in induction of central or peripheral tolerance (J).

is likely, then, that a molecular mimicry mechanism may be involved in the pathogenesis of gluten sensitivity with or without enteropathy (33-37).

The gliadin peptide also shares homology with other self-antigens such as heat shock protein-60 (HSP60), glutamic acid decarboxylase, myotubularin-related protein-2 and toll like receptors. Heat shock proteins are highly conserved proteins synthesized when cells are exposed to stress stimuli, such as infection and inflammation. Increased expression of HSPs has been observed in jejunal epithelial cells in patients with CD. Antibodies against the celiac peptide cross-react with HSP60 and may therefore induce epithelial cell cytotoxicity, thus amplifying the damage of the intestinal mucosa with increased intestinal permeability (37).

Matrix metalloproteinase-2 (MTMR2) belongs to the protein-tyrosine phosphatase family. Defects

in MTMR2 are the cause of Charcot-Marie-Tooth disease type 4, which is an autosomal recessive demyelinating neuropathy. A demyelinating nervous system disease can be observed in patients with CD.

Finally, TLRs are type I transmembrane proteins involved in innate immunity by recognition of conserved microbial structures. Activation of antigen presenting cells via innate immune receptors such as TLR4 can break self-tolerance and trigger the development of autoimmunity (38-40). The anti-gliadin peptide antibodies bind TLR4 on monocytes and induce both the expression of activation molecules such as CD83 and CD40 and the production of pro-inflammatory cytokines similar to the action of bacterial antigens. The mimicry mechanism by which rotaviruses or other environmental factors are involved in the pathogenesis of gluten sensitivity without enteropathy is shown in Fig. 4.

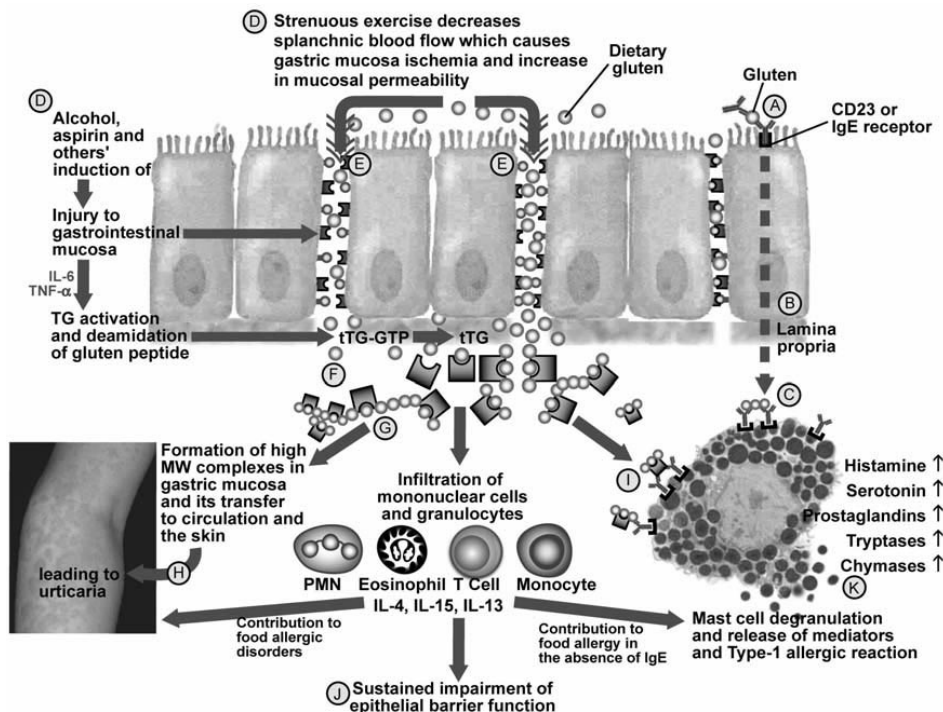


Fig. 2. Schematic presentation of the pathophysiology of the immediate hypersensitivity reactions (Type I allergy) of the intestine. Hypersensitivity reaction occurs by the binding of dietary peptides (gluten) to low affinity IgE receptor CD23, which is expressed on the epithelium of the small intestine (A), facilitating uptake of antigen in an IgE-independent manner (B). Gluten cross-links to IgE on the surface of MAST cells to induce degranulation (C). This MAST cell degranulation could be induced by strenuous exercise, alcohol and medication (aspirin) (D), causing injury to gastrointestinal mucosa and an increase in mucosal permeability (E). Under these conditions, parts of gluten that are resistant to processing by luminal and brush-border enzymes will survive digestion and be transported across the mucosal epithelium as polypeptides. Upon activation of transglutaminase in the subepithelial region (F), many gliadin peptides form high molecular weight complexes with transglutaminase (G) that can be transferred into the circulation and the skin, leading to urticaria (H). These complexes can also bind to IgE receptors on MAST cells and induce further degranulation (I). Finally, infiltration of granulocytes, mononuclear cells and their cytokines can contribute to late phase responses, which result in the impairment of epithelial barrier function (J). Also, products released from MAST cells, including histamine, serotonin, prostaglandins, tryptases and chymases (K), have been shown to have direct and indirect effects (via activation of the enteric nerve) on epithelial ion secretion, barrier function, and intestinal motility.

Based on this mechanism of action, we should think about the immunology of gluten sensitivity beyond the gut and emphasize laboratory testing for celiac disease and gluten sensitivity beyond gliadin and transglutaminase antibodies.

CONCLUSIONS

Immediate type hypersensitivity to gluten is IgE

mediated. This IgE-mediated reaction to gluten may become life-threatening if wheat ingestion is combined with exercise or with medication, such as aspirin.

Strenuous exercise, medications and xenobiotics, by decreasing splanchnic blood flow, may cause an increase in mucosal permeability and the entry of gliadin into the circulation, hence, antibody response against gliadin polypeptides.

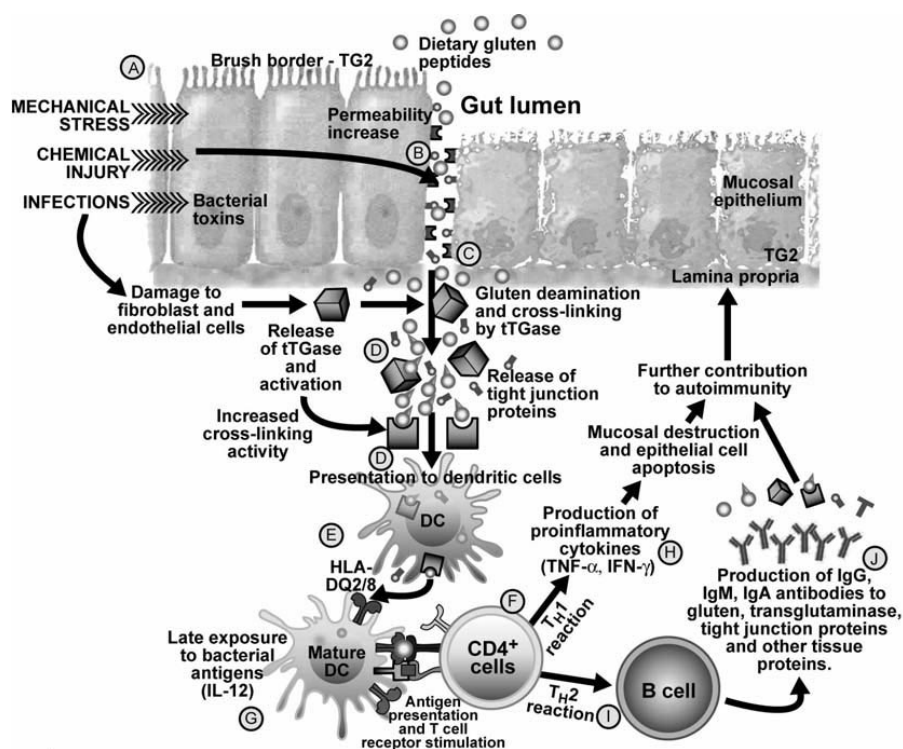







Fig. 3. Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive. Infection, mechanical and chemical stress (A) can impair mucosal integrity (B). The parts of gluten that are resistant to brush-border enzymes will survive digestion and can be transported across the epithelial barrier as polypeptides (C). Tissue transglutaminase in the intestinal mucosa (lamina propria) become activated and deamidate gluten peptides. Some of the deamidated gliadins may cross-link to transglutaminase and form complexes of gliadin with tTG (D). Deamidated gliadin peptide by itself, deamidated gliadin peptide cross-linked to tTG, and released tight junction proteins are presented by dendritic cells or antigen-presenting cells as well as B cells (E) which carry HLA-DQ2 or DQ8 molecules to the CD4+ T cells in the lamina propria (F). It is believed that this antigenic presentation is enhanced in an individual with later-in-life exposure to bacterial antigens whose mature dendritic cells produce significant amounts of interleukin-12 (G). This antigenic presentation results in driving the CD4+ cell response either towards T_H1 reaction, production of inflammatory cytokines (H), mucosal cell destruction and autoimmunity, or, toward T_H2 response B-cell activation (I), and antibody production against deamidated gluten, transglutaminase, gliadin cross-linked to transglutaminase, and different tissue antigens (J).  - Deamidated gliadin peptide;  - deamidated gliadin peptide cross-linked to tTG;  - tight junction proteins;  - transglutaminase;  - different tissue antigens.

Clinicians should be aware that during food processing many wheat isolates are produced by chemical and enzymatic treatment and used in many food products. Therefore, some patients may have immune reaction to treated gliadin used in sausage, but not to gluten or wheat itself.

Unlike immediate type hypersensitivity to gluten, which occurs within minutes, the delayed type hypersensitivity to gluten may occur hours or days

after ingestion of wheat.

Delayed type hypersensitivity to gluten is an antibody- (IgG, IgA) and T-cell-mediated reaction. Immune reaction to gluten occurs in genetically susceptible individuals with the involvement of tissue transglutaminase, resulting in chronic inflammation of the small intestine. This delayed type hypersensitivity to gluten is called celiac disease or gluten sensitivity with enteropathy.

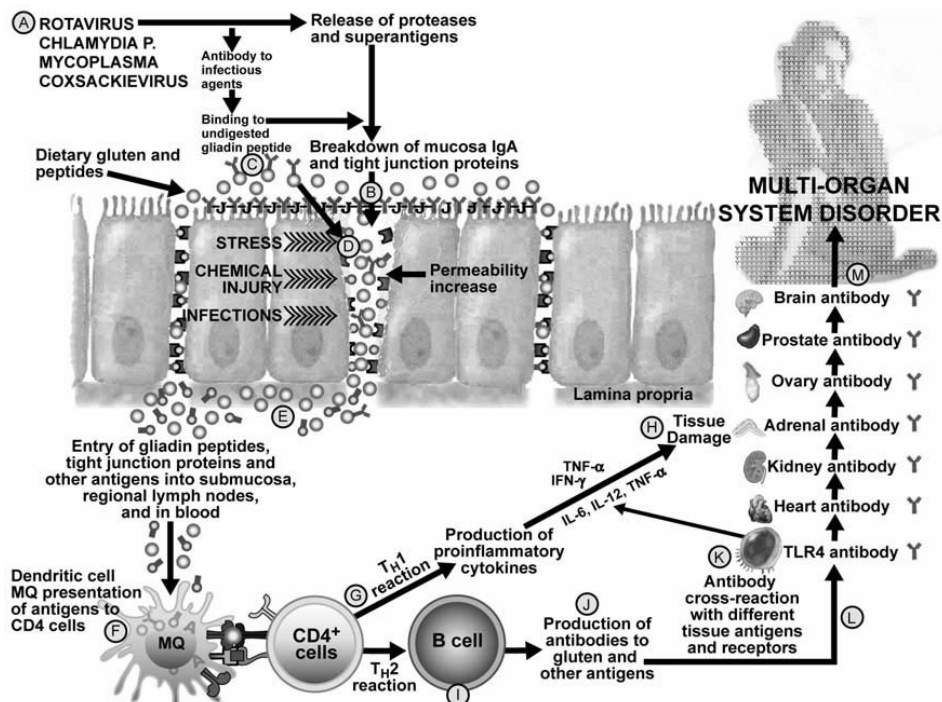





Fig. 4. Depiction of immunological mechanisms underlying gluten sensitivity and its immunopathological consequences. Precipitation of gluten sensitivity without enteropathy appears to be preceded by acute gastroenteritis symptoms induced by infections such as rotavirus and others (A). Rotavirus and its super-antigens can break down mucosal IgA directly (B) or indirectly by the local production of anti-rotavirus antibody. Due to partial linear homology or cross-reactivity between rotavirus protein and α -gliadin, the anti-rotavirus antibody binds to gliadin and forms complexes with it (C). The combination of infection antibody cross-reactivity with gliadin and additional stressors can severely impair mucosal integrity (D) and the entry of gliadin peptides, tight junction proteins and other antigens into the submucosa, regional lymph nodes, and the blood (E). Gliadin peptides, rotavirus antigens, rotavirus antibody bound to gliadin, and tight junction proteins are presented by dendritic cells with or without HLA-DQ2/DQ8 to CD4⁺ cells (F). This antigenic presentation results in driving the cell CD4⁺ response either towards TH1 reaction (G), the production of proinflammatory cytokines, which contributes to autoimmunity (H); or towards TH2 response B-cell activation (I) and antibody production against gluten, rotavirus, and tight junction proteins (J). Cross-reaction of these antibodies with cell receptors such as toll-like receptors on monocytes and the release of IL-6, IL-12 and TNF- γ (K), and tissue antigens such as heart, kidney, adrenal gland, ovary, prostate, brain and others (L) results in further tissue damage and multi-organ system disorders (M).  - Gliadin peptides;  - rotavirus antibody bound to gliadin;  - tight junction proteins.

Gluten sensitivity without enteropathy may occur in individuals without the involvement of genes, tissue transglutaminase and presence of inflammation in the small intestine. Gluten sensitivity without enteropathy is induced mainly by enhanced gut permeability due to infection (rotavirus), stress or chemical injuries.

Impaired mucosal integrity results in the entry of

gliadin peptides, tight junction proteins and others to the submucosa, regional lymph nodes, and the blood. The entry of gliadin peptides, tight junction proteins and infections in the blood results in the production of antibodies against them.

The cross-reaction of these antibodies with different tissue antigens such as heart, kidney, adrenal gland, ovary, thyroid, parathyroid, prostate,

brain and others results in multi-organ disorder, which will be discussed in a subsequent article.

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