

### Cellular and molecular induction of immune tolerance to dietary proteins (gliadin).

Although HLA-DQ2 or HLA-DQ8 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, as shown in this figure.

In the absence of major mechanical and chemical stress or infection (1), no damage is done to fibroblasts and endothelial cells, and only small quantities of tissue transglutaminase are released into the environment (2).

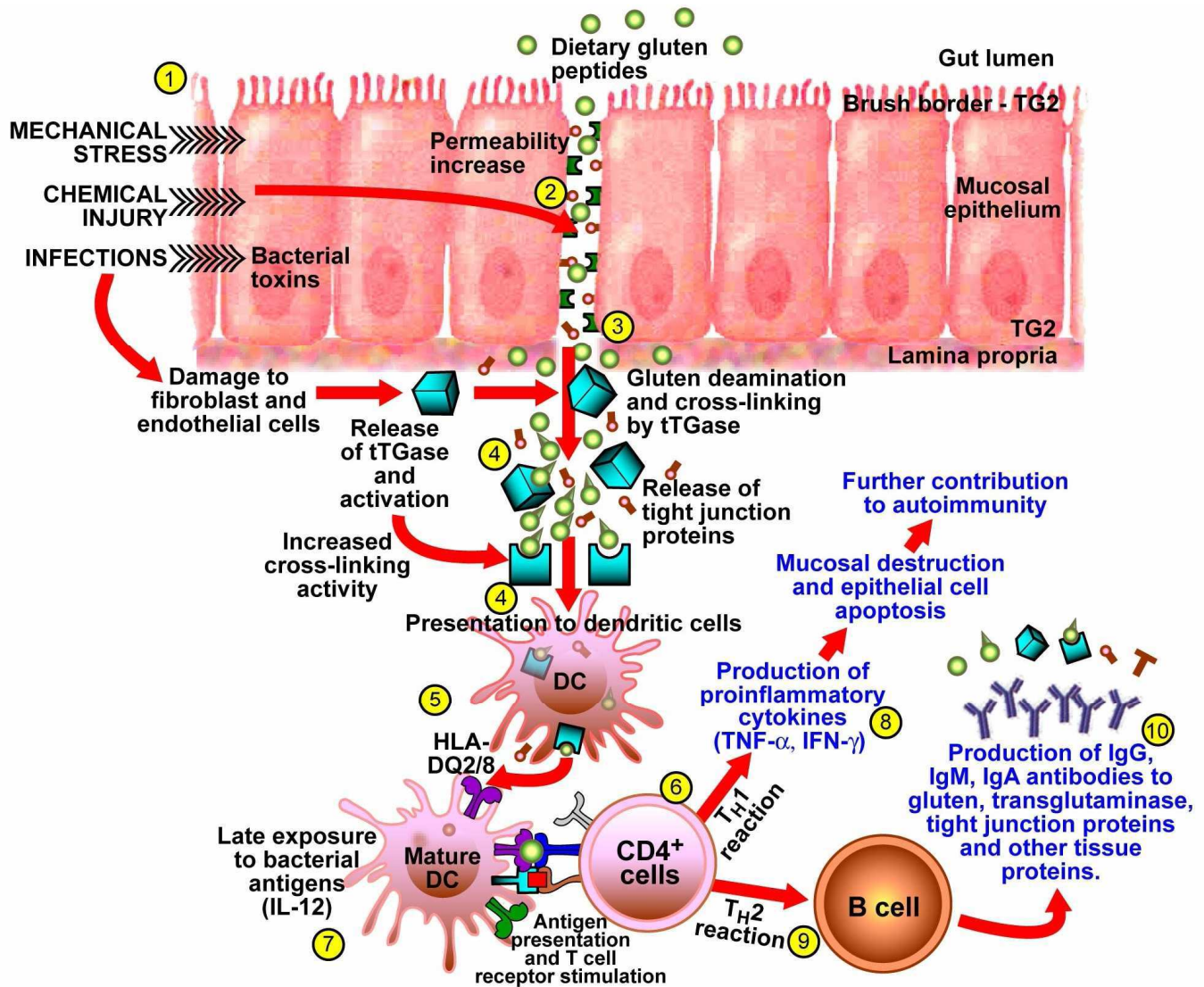
Since under these conditions the tight junctions are in perfect shape (3), only a few gliadin molecules may survive digestion and be transported across the mucosal epithelium (4).

If these molecules of gliadin are deamidated by transglutaminase (5), the key regulator of the immune system called dendritic cells or antigen-presenting cells (6) prime T cells for anergy or tolerance.

Early exposure to dietary proteins and bacterial antigens such as LPs (7) can activate regulatory T cells to produce TGF- $\beta$  and IL-10, inducing activation of tolerogenic DCs (8) to control immune response to dietary proteins (gliadin). Further activation of TR<sub>1</sub>, TH<sub>3</sub> and natural Treg (9) by IL-10 results in induction of central or peripheral tolerance (10).

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 factor FOXP<sup>3</sup>;
- b - Adoptive Treg Type1 cells (TR<sub>1</sub>), which function in a contact-independent manner and may or may not express CD25 and FOXP<sup>3</sup>. The TR<sub>1</sub> and TH<sub>3</sub> cells preferentially synthesize immunosuppressive cytokines IL-10 and TGF- $\beta$  respectively in order to maintain homeostasis of responses to foreign antigens, including gliadin.






**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 (1) can impair mucosal integrity (2).

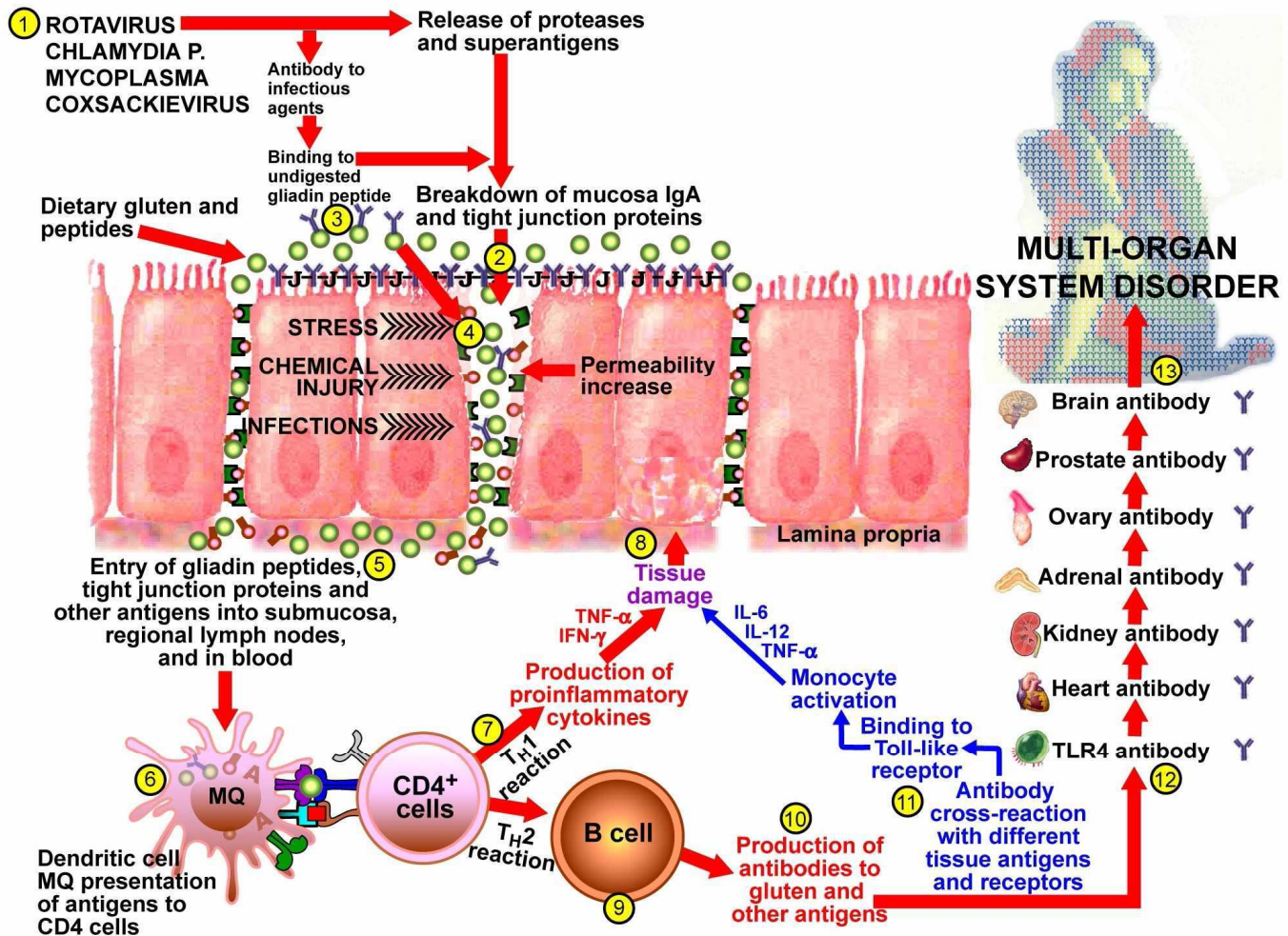
The parts of gluten that are resistant to brush-border enzymes will survive digestion and can be transported across the epithelial barrier as polypeptides (3).

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 TG (4).

Deamidated gliadin peptide by itself , deamidated gliadin peptide cross-linked to TG , and released tight junction proteins  are presented by dendritic cells or antigen-presenting cells as well as B cells (5) which carry HLA-DQ2 or DQ8 molecules to the CD4+ T cells in the lamina propria (6).

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 [IL-12] (7).

This antigenic presentation results in driving the CD4+ cell response either towards TH1 reaction, production of inflammatory cytokines (8), mucosal cell destruction and autoimmunity, or, toward TH2 response B-cell activation (9), and antibody production against deamidated gluten , transglutaminase , gliadin cross-linked to transglutaminase  and different tissue antigens **T** (10).







## Depiction of immunological mechanisms underlying gluten intolerance and its immunopathological consequences.

Precipitation of gluten intolerance appears to be preceded by acute gastroenteritis symptoms induced by infections such as rotavirus and others(1).

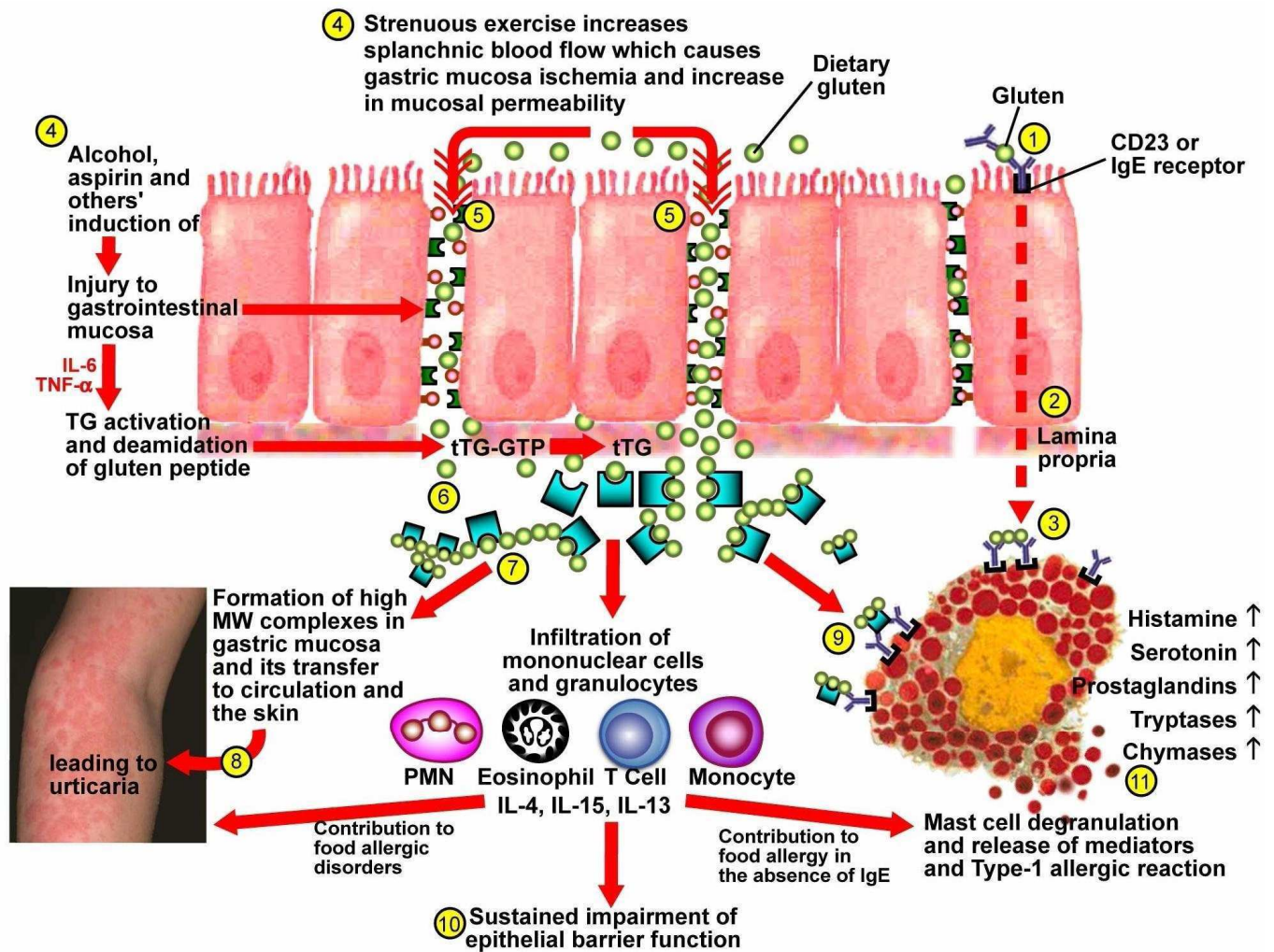
Rotavirus and its super-antigens can break down mucosal IgA directly (2) or indirectly by the local production of anti-rotavirus antibody. Due to partial linear homology or cross-reactivity between rotavirus protein and  $\alpha$ -gliadin, the anti-rotavirus antibody binds to gliadin and forms complexes with it (3).

The combination of infection antibody cross-reactivity with gliadin and additional stressors can severely impair mucosal integrity (4) and the entry of gliadin peptides, tight junction proteins and other antigens into the submucosa, regional lymph nodes, and the blood (5).

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<sup>+</sup> cells (6).

This antigenic presentation results in driving the cell CD4<sup>+</sup> response either towards TH<sub>1</sub> reaction (7), production of proinflammatory cytokines, mucosal cell destruction and autoimmunity (8); or towards TH<sub>2</sub> response B-cell activation (9) and antibody production against gluten, rotavirus, and tight junction proteins (10).

Cross-reaction of these antibodies with cell receptors such as toll-like receptor (11) and tissue antigens such as heart kidney, adrenal gland, ovary, prostate, brain and others (12) results in further tissue damage and multi-organ system disorders (13).



### Schematic presentation of the pathophysiology of the immediate hypersensitivity reactions (Type 1 allergy) of the intestine.

This hypersensitivity reaction may occur by the binding of dietary peptides (gluten) to low affinity IgE receptor CD23, which is expressed on the epithelium of the small intestine (1), facilitating uptake of antigen in an IgE-independent manner (2).

Gluten cross-links to IgE on the surface of MAST cells to induce degranulation (3). This MAST cell degranulation could be induced by strenuous exercise, alcohol and medication [aspirin] (4), causing injury to gastrointestinal mucosa and an increase in mucosal permeability (5).

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 (6), many gliadin peptides form high molecular weight complexes with transglutaminase (7) which can be transferred into the circulation and the skin, leading to urticaria (8).

These complexes can also bind to IgE receptors on MAST cells and induce further degranulation (9). Finally, infiltration of granulocytes, mononuclear cells and their cytokines can contribute to late phase responses, which results in the impairment of epithelial barrier function (10).

Also, products released from MAST cells, including histamine, serotonin, prostaglandins, tryptases and chymases (11) have been shown to have direct and indirect effects (via activation of the enteric nerve) on epithelial ion secretion, barrier function, and intestinal motility.



## TEST REQUEST FORM

### Celiac Neuroautoimmune Panel

PATIENT'S NAME (LAST) (FIRST)	
DATE OF BIRTH	DATE & TIME SPECIMEN WAS COLLECTED
STREET ADDRESS	
CITY	STATE ZIP CODE
PHONE (INCLUDE AREA CODE)	GENDER

DOCTOR'S NAME (LAST) (FIRST)	
ADDRESS	
CITY	STATE ZIP CODE
PHONE (INCLUDE AREA CODE)	DIAGNOSIS
Doctor's Signature	

<input type="checkbox"/> Celiac Neuroautoimmune Panel	Catalog Price
- Gliadin (IgG, IgA)	\$100.00
- Gluteomorphin (IgG)	50.00
- Casomorphin (IgG)	50.00
- Milk Butyrophilin (IgG)	50.00
- Transglutaminase (IgG, IgA)	100.00
- Glutamic Acid Decarboxylase (IgG)	50.00
- Heat Shock Protein (IgG)	50.00
- Parietal Cell (IgG)	50.00
- Myelin Basic Protein (IgG, IgM)	100.00
- Neurofilament Protein (IgG, IgM)	100.00
- Thyroglobulin (IgG)	50.00
- Thyroid Peroxidase (IgG)	50.00
- Corn (IgG)	50.00
- Soy (IgG)	50.00
- Egg (IgG)	50.00
- Immunoglobulin (IgA)	25.00
- Saliva Secretory IgA	50.00
<b>Total</b>	<b>\$1025.00</b>

<p><b>Specimen Requirements:</b>          10 ml Red top tube          4 ml Saliva collected over          a <u>24</u> hour period</p> <p style="color: red;"><b>Send by Overnight Carrier to:</b>  <b>Immunosciences Lab., Inc.</b>  <b>8693 Wilshire Blvd, Ste 200</b>  <b>Beverly Hills, CA 90211</b></p>	<p><b>Pricing:</b>          The discounted panel price is <span style="color: red;">\$512.50</span>. Payment required at time of service. Physician Billing option available at physician's request only (net 30 days). Please indicate method of payment:</p> <p style="text-align: center;"> <input type="checkbox"/> <b>Physician Billing</b>                      <input type="checkbox"/> <b>Check</b>  <input type="checkbox"/> <b>Visa</b>    <input type="checkbox"/> <b>MasterCard</b> </p>
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CREDIT CARD NUMBER	EXPIRATION DATE	DATE	TOTAL \$
CARD HOLDER'S NAME		CARD HOLDER SIGNATURE	