Celiac disease: Beyond Gluten-free diet

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LSGE- Annual Meeting 2014
Pathogenesis

- Auto-immune disease, 1% western population
- 3 main pathways

Host Genetic background
- HLA-DQ2
- HLA-DQ8
- Non-HLA genes

Celiac disease

Trigger: Gluten
- Gliadin
- Hordeins
- Secalins

Unusual Gut permeability
- Tight junctions
Pathogenesis: Environmental triggers

- Exposure to gluten
- Major storage protein for wheat, barley and rye.
Pathogenesis: gut permeability

- Enterocytes connected by tight junctions
- In CD, loose TJ allow unusual permeability
- Gliadin binds to CXCR3 receptors \(\Rightarrow\) secretion of Zonulin by epithelial cells (induces TJ disassembly) \(\Rightarrow\) intestinal permeability and triggers inflammatory cascade
Pathogenesis: Genetics

- All patients must express HLA-DQ2 and/or DQ8
- Bind gluten peptides after deamidation by TG2
- Increased CD4+ T-cell response: cytokines
- Intestinal inflammation: villous atrophy, crypt hyperplasia, infiltration of IEL \(\rightarrow\) malabsorption
### Standard of care: Gluten-free diet

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>• Improves G.I symptoms in a few weeks</td>
<td>• Lifelong restrictive diet (compliance)</td>
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<td>• Histologic/serologic response in 1-2 yrs</td>
<td>• GF food not widely available, more expensive, palatability</td>
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<td>• Frequent small levels of contamination (up to 70%)</td>
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<td>• Lower health-related QoL</td>
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<td>• Poor response in 7-30%</td>
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<td>• Recurrent symptoms, inadequate cure and/or refractory disease 5%</td>
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Standard of care: Gluten-free diet

**Disadvantages**

- Patient perspective: GFD more effective than dialysis or insulin
- Burden of GFD rated as or greater than dialysis or insulin injections

- Overgrowth of opportunistic pathogens
- Weakens host defenses against infections

**Disadvantages**

- Higher glycemic index (CD in 4.4-11% of T1D)
- Poorer in fibers, richer in fat

![Diagram showing the impact of gluten-free diet on gut microbiota and immune response.](image)
Main targets of research

<table>
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<th>Therapeutic agent</th>
<th>Mechanism of action</th>
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<td>Genetically modified gluten</td>
<td>Decreases gluten exposure by transamidation of gliadin</td>
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<tr>
<td>Zonulin inhibitor</td>
<td>Decreases zonulin secretion and inhibits intestinal permeability</td>
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<td>Therapeutic vaccine</td>
<td>Creates immune tolerance to gluten fragments and desensitizes celiac disease patients to the toxic effects of gluten</td>
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<tr>
<td>Probiotics</td>
<td>Detoxify gliadin and promote intestinal healing</td>
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<tr>
<td>Tissue transglutaminase inhibitors</td>
<td>Stop t-TG from modifying gluten fragments, prevent trigger of immune response</td>
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Genetically Modified Gluten

- Bread: most commonly consumed food in the world (typically wheat)
- Gluten-free flour: corn, soya, brown rice, tapioca flours
- Good source of complex Carbohydrates, lack B vitamins + essential nutrients
- GMG with reduced immunogenicity

Bakshi et al., Gastroenterol & Hepatol, 2012
Genetically Modified Gluten
introduce detoxified wheat into diet

• Blocking deamidation with Lysine Methyl esters (Lys-CH3) strongly inhibited immune response → ▼ IFNγ-release from T-cells
  ➤ Treating wheat flour with microbial TG in the presence of Lys-CH3 neutralized immunotoxicity of digested products

• Selection of gluten that lacks 1 or more of known T-cell stimulatory sequences enzymatically engineered to inhibit immunotoxic effect

Bakshi et al., Gastroenterol & Hepatol, 2012
Modulation of permeability

**Zonulin Inhibitors**

- **Zonulin**: endogenous modulator of epithelial TJ
- **Gliadin**: ↑secretion of zonulin
  - Alters intestinal permeability
  - Facilitates transport of gluten
  - Triggers inflammatory process
- **AT-1001**: Larazotide Acetate
  - 1st in-class oral peptide that acts as TJ regulator
  - Prevents TJ opening
  - Maintains intestinal barrier function after gluten challenge

*Ludvigsson et al., Gut 2014*
Modulation of permeability

Zonulin Inhibitors

- 2009: Placebo controlled Phase IIa human trials
  - Prevents gluten-induced tight junction opening
  - ↓ Gluten uptake
  - ↓ production of inflammatory molecules
  - Blocks production of tG-Ab
  - ↓ GI symptoms in CD patients
Zonulin Inhibitors
Larazotide Acetate

- Wang et al., DDW 2014 abstract
- RDBPC multicenter trial, > 300 patients
- AT-1001 @ 0.5 mg/1 mg/2 mg TID for 12 weeks while on GFD
- 1ry EP: On-Treatment CeD GSRS
  - Diarrhea, abdominal pain and indigestion
  - Non-GI symptoms
Results:

- ↓ GI symptoms (p=0.022-ITT), total GSRS score
- ↓ headache/tiredness (p=0.01)
- 26% ↓ # of days of severe symptoms (p=0.017)
- 31% ↑ # days no/few symptoms (p=0.034)
- Safety profile comparable to placebo

1ˢᵗ trial that met 1ʳᵗEndpoints, sustained during active phase, dose 0.5 mg only

Larazotide Acetate: potential to be the 1ˢᵗ pharmacological Rx in CeD (Phase III trials)
Therapeutic vaccine

- Peptide-based therapeutic vaccine
  - Specifically modify pathogenic T-cell response (vs. ↓ amount of gluten presented to T-cells)
  - Effective only in HLA-DQ2 genotype (90%)
- Important step: identification of gluten peptides that trigger T-cell response:
  GLIADIN(gluten) / HORDEINS (Barley)/ SECALINS (rye)
- Nexvax2: combines 3 peptides into vaccine
- Once weekly
- Desensitizes patients to toxic effects of gluten

Science daily, 2011
Therapeutic vaccine

- Phase 1, Nexvax vs. placebo in CD pts on GFD
- Safety & tolerability comparable to placebo
- Mobilization of gluten specific T-cells similar to acute oral exposure
- Designed to:
  - Be given in multiple doses
  - Create immune tolerance
  - Lower toxicity
  - Prevent T cells from initiating immune cascade
  - Phase 2 trials expected

Keech et al., Gastroenterology 2009;136:A57
Endopeptidases

• Rationale: oral supplementation of prolyl endopeptidases (PEP) help degrade toxic gliadin peptides before they reach the mucosa

• Shan et al.
  – Unique 33-AA peptide containing T-cell stimulatory epitopes that trigger inflammatory cascade
  – Resistant to degradation in the GI tract
  – Degraded-lost Agenicity in vivo and in vitro, when exposed to bacterial PEP derived from Flavobacterium meningospeticum

\[ \Rightarrow \text{Oral bacterial peptidase could detoxify gliadin epitopes} \]

Shan et al., Science 2002
Endopeptidases

- Isolated from microbial sources
- Enzymatic cleavage of immunotoxic gliadin peptides ex-vivo
- RCT phase II, pts with proven CeD
  - ALV003, mixture of 2 recombinant gluten-specific proteases
  - PEP from *Sphingomonas capsulate* + germinating barley
  - ALV003 vs. placebo AND daily gluten challenge for 6 weeks
  - 1ry EP: villous height/crypt depth ratio, # IEL

*Lahdeaho et al., Gastroenterology 2014*
Endopeptidases: ALV003

• Results
  – small intestinal mucosal injury after gluten
  – GI symptoms significantly greater in placebo group

• If ALV003 makes it to the market
  – Availability
  – Cost
  – Who qualifies? Biopsy-proven CeD
  – Other forms of gluten sensitivity??
Probiotics with endopeptididases

- *Lactobacillus fermentum* + *Bifidobacterium Lactis* added to epithelial cells with Gliadin-induced injury
  - *BL* inhibited increased permeability
  - *BL* + *LF* protect against cell ruffling and TJ alterations

- probiotics + enzymes reduced damage caused by gluten (detoxification by PEP)
- accelerate intestinal healing after GFD

Potential treatment for celiac disease patients

Lindfors et al., Clin Exp Immunol, 2008
t-Transglutaminase inhibitors

- 3 classes:
  - Competitive amine inhibitors
    - Most common glutaminase inhibitor
    - Compete with natural amine substrates in the transamidation process
    - TG2 still active, transamidation continues
    - Link formed between natural amine substrate and competitive amine inhibitor

Gluten peptides → Glutamic acids ↗ affinity to HLA-DQ2-8 rec

Inflammatory cascade

TG inhibitors

TG2 deamidation
t-Transglutaminase inhibitors

• Reversible TG2 inhibitors
  – Prevent enzyme activity by blocking substrate access to active site without modifying the enzyme
  – Example of TG2 cofactors: G2P-G3P are reversible inhibitors

• Irreversible TG2 inhibitors
  – Prevent substrate binding
  – Covalently modify enzyme, block activity

Siegel et al., Pharmacol Ther 2007
t-Transglutaminase inhibitors

• Experience
  – Blocking endogenous TG2 activity in CD patients biopsies → > 50% resultant T-cells had reduced proliferative response
  – Incubation of intact CD small bowel bx with TG2 inhibitor prevented T-cell activation when exposed to gluten peptide
• Irreversible TG2 inhibition can prevent gluten peptide deamidation and ultimately ↓T-cell activation
• ↓induced response of gluten-reactive T cells
The Future
NEXT EXIT
Intestinal stem cells

- Epithelial cells: high proliferation rate
  - Replaced every 3-5 days
  - Renewal driven by common ISC in crypt base
- ISC give rise to TA cells
- Migrate upward
- Maturate to fully differentiated villous epithelial cells
- Each crypt $\rightarrow$ 5-15 ISC $\rightarrow$ 300 cells daily

Piscaglia AC, World J Stem Cells, 2014
Intestinal stem cells

- Under influence of genetically coded molecular pathways, ISC will give rise to TA cells → differentiate into enterocytes/EE cells/PC and GC
- Pathway alterations: colon cancers and IBD
- BM-derived SC: may be used in IBD-ulcers-achalasia-gastroparesis

Piscaglia AC, World J Stem Cells, 2014
ISCs and Celiac Disease

• ISC differentiation to PC and GC disturbed in active CD
  – Defective antimicrobial/mucus barrier → mucosa invaded by intest bacteria → inflammation
  – PC substituted by lysozyme-producing mucus cells
  – GC: reduced by exposure to Gliadin

• Weak knowledge about contribution of BM-derived ISCs in celiac disease

Piscaglia AC, World J Stem Cells, 2014
Potential therapy with ISCs

• Discovery of epithelial mitogens: R-spondin 1
  – Stimulate crypt growth
  – Accelerate mucosal regeneration
  – Restore intestinal architecture in experimental colitis in mice
  ⇒ infusion of mitogens could accelerate intestinal healing in CD

• BM-derived multipotent stem cell transplantation to promote intestinal repair
  • case reports of CD and AML, aplastic anemia, β-thalassemia major → allogeneic HSC transplantation
    → CD cured – no symptoms after reintroduction of gluten

Role of ISC in the development of CD not yet elucidated
Clinical application of SC-based treatment limited to case reports and uncontrolled trials
Modified Gluten

Probiotics: detoxify gliadin

Zonulin inhibitors

LTG2 inhibitors

Production of IgG, IgM, IgA antibodies to gluten, transglutaminase, tight junction proteins and other tissue proteins.

Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive