The Role of Nutrition, Gut Microbiome, and Gut Permeability in Immunomodulation: The Celiac Disease and Type 1 Diabetes Paradigms

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Meeting Overview On New Concepts of Autoimmunity: Excessive and Inappropriate Inflammatory Process Associated to a Dysfunction of Barriers
The Epidemics of Immune-Mediated Diseases In The Western Hemisphere: The Hygiene Hypothesis

![Graphs showing incidence of infectious diseases and autoimmune disorders incidence](image)

![Maps showing high, moderate, and low incidence of helminths infestation](image)

*Personal communication from Dr. Joel Weinstock*
We Are Not Born With The Destiny to Develop Chronic Inflammatory Diseases
The Yin and Yang Between Tolerance and Immune Response Leading To Chronic Inflammatory Diseases: Lesson Learned From Celiac Disease
Several Cells Play a Role in Maintaining The Immune Homeostasis

- Epithelial cells
- Intestinal DCs
- B cells
- T cells
Excessive and Inappropriate Inflammatory Process Associated to a Dysfunction of Barriers: Loss of Mucosal Immune Homeostasis

1. Normal/physiologically controlled permeability
2. Minor barrier defect dietary/microbial Ag influx
3. Increased permeability
4. Massive dietary and microbial antigen influx

Mucosal Tolerance
Homeostasis
Anergy

Regulatory DC’s
Macrophages
Tregs
IL-10/TGF-β

Defensins
Mucus Synthesis & Quality

Break of Tolerance
Aptoptosis resistant T cells
Tissue damage
Chronic inflammation
Allergy

Inflammation - Allergy
Vicious circle
Proinflammatory
Allergic cytokines

Adapted from P. Brandtzaeg, Beneficial Microbes 2010
The Paracellular Pathway

…Tight junctions are a ‘dark horse’ implicated in a host of disease states, ranging from acute injury to chronic inflammation and autoimmune diseases

Zonulin Characterization and Signaling

Zonulin signaling

Freeze-Fracture

Following Pathway Activation

Resting State

Identification of human zonulin, a physiological modulator of tight junctions, as prehaptoglobin-2

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[Diagram of primate evolution]
DSS Mouse Models to Establish The Link Between Distribution of Haptoglobin Alleles And Susceptibility to Inflammation

- C57Bl/6 wild type mouse
  - HP 1-1 (no zonulin gene)

- C57Bl/6 mouse transfected with human HP2 gene
  - HP 1-2 (1 zonulin gene)

- Transgenic C57Bl/6 mouse engineered by duplicating α chain (α1 → α2)
  - HP 2-2 (2 zonulin genes)

**WB**

- α2
- α1
How Zonulin-Mediated Increased Ag Trafficking Leads to Chronic Inflammation: Insights From The Zonulin Trangenic Mouse Model

Daily Body Weight after 7 days all mice are put on Normal drinking water

Zonulin transgenic Hp2 mice are phenotypically normal despite increased small intestinal permeability

*p<0.05

Sturgeon C et al. Ann NY Acad Sci 2017 Apr 19 (Epub ahead print)
Ztm have increased morbidity (colonic mucosal damage) and mortality (70%) following DSS induced colitis.

Sturgeon C et al. Ann NY Acad Sci 2017 Apr 19 (Epub ahead print)
The Zonulin Inhibitor AT1001 (Larazotide Acetate) Treatment Ameliorates Increased DSS Induced Morbidity and Mortality in Ztm

AT1001 (larazotide acetate) treatment ameliorates increased DSS induced morbidity and mortality in ztm

Sturgeon C et al. Ann NY Acad Sci 2017 Apr 19 (Epub ahead print)
Zonulin Gene Is Located on Chromosome 16

Chromosome 16 contains about 98 million bases, or some 3% of the human genome, encoding for ~1,300 genes.
<table>
<thead>
<tr>
<th>Disease</th>
<th>Model</th>
<th>Zonulin Shown to be Involved</th>
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<tbody>
<tr>
<td>Ankylosis spondylitis</td>
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<td>Autism</td>
<td>Human</td>
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<td>Celiac Disease</td>
<td>Human</td>
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<tr>
<td>Colitis/IBD (Crohn’s disease)</td>
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<td>YES</td>
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<td>Colitis</td>
<td>Mouse</td>
<td>YES</td>
</tr>
<tr>
<td>Fe metabolism in heart transplant</td>
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<td>NO</td>
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<tr>
<td>Glioma</td>
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<td>YES</td>
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<tr>
<td>Glioma</td>
<td>Cell</td>
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<tr>
<td>Multiple sclerosis</td>
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<tr>
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<tr>
<td>Nonalcoholic fatty liver disease</td>
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<tr>
<td>Non-Celiac Gluten Sensitivity</td>
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</tr>
<tr>
<td>Obesity/Insulin resistance</td>
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<tr>
<td>Post-surgery Sepsis</td>
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<tr>
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<tr>
<td>Type 1 diabetes</td>
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</tr>
<tr>
<td>Type 2 diabetes</td>
<td>Human</td>
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</tr>
</tbody>
</table>
Serum Zonulin Levels and Their Correlation With Intestinal Permeability In Celiac Disease and Type 1 Diabetes

Celiac Disease

Type 1 Diabetes

Zonulin-LA/MA

multiple R=0.36; intercept p=1.71E-10; X variable 1 p=0.0004
Type 1 Diabetes
Evidence for Zonulin-Dependent Increased Intestinal Permeability in the Pathogenesis of Type 1 Diabetes

Watts et al PNAS 2005;102:2916-21
Blocking the Zonulin-Dependent Increased Intestinal Permeability Aborts The Autoimmune Process

Islet Immunohistochemistry

Insulin staining
Glucagon staining

Untreated BBDP rats that developed T1D

Larazotide-treated BBDP rats that DID NOT develop T1D: No insulitis

Presented in ICoMi 2017
Celiac Disease
Depiction of the intestinal mucosa with emphasis on the factors involved in the development of celiac disease in individuals with HLA-DQ2/DQ8 positive...
Larazotide Acetate Consistently Reduced Gastro-Intestinal Symptoms in 3 Gluten-Challenge Clinical Trials

**CLIN1001-002 Study**
(Baseline to Day 3, 12 mg QD)

**CLIN1001-004 Study**
(Baseline to Day 14, pooled active)

**CLIN1001-006 Study**
(Baseline to LDBTP, 1 mg TID)

% of patients with symptoms

Change in GSRS domains

LDBTP, Last Double-Blind Treatment Period Visit

- **Placebo**
- **Active**
Environmental Triggers Causing Zonulin Release

- Gluten
- Gut Microbiome
Dysbiosis is one of the key factors causing zonulin release and subsequent loss of barrier function.

The Microbiome as Possible Transducer of All Environmental Factors Affecting Onset of CID in Genetically Susceptible Individuals

The changing face of gut microbes

- The human gut harbors $10^{11}$-$10^{12}$ bacteria per gram colonic content (>10$^{14}$ total bacteria);
- Total bacteria outnumber human cells 10:1;
- Total bacterial genes outnumber human genes >150:1;
- >10,000 different species of bacteria are resident in the human intestinal microbiota (400-500 per person)
• The human gut harbors $10^{11}$-10$^{12}$ bacteria per gram colonic content (>10$^{14}$ total bacteria)
• Total bacteria outnumber human cells 10:1
• Total bacterial genes outnumber human genes >150:1
• >10,000 different species of bacteria are resident in the human intestinal microbiota (400-500 per person)
Is Microbiome Science Ready For Primetime Clinical Applicability?

Classification of Organisms

- **Domain**: Bacteria
  - **Kingdom**: Firmicutes
    - **Phylum**: Clostridia
      - **Class**: Clostridiales
        - **Order**: Ruminococcaceae
          - **Family**: Anaerotruncus
            - **Genus**: Anaerotruncus_sp

Why The First 1000 Days Of Life Are Instrumental For Clinical Destiny

- Vaginal Delivery
- Proper Nutrition
- No infections
- No Antibiotic treatments

- C section
- Inappropriate Nutrition
- Multiple infections
- Antibiotic treatments

Microbiome Composition

Balanced Microbiome

Appropriate GALT Maturation

Tolerogenic Response to Food Antigens - State of Health

Dysbiosis

Genetic Predisposition

Inappropriate GALT Maturation

Pro-inflammatory Response to Food Antigens - CID

Probiotics Prebiotics Symbiotics
Infants who developed autoimmune diseases during the time of the study had high levels of lactate combined with low levels of butyrate before the onset of the disease;

Just before developing the disease, lactate decreased while butyrate increased;

These changes paralleled changes in microbiome composition, with decreased of Lactobacillaceae and increased of butyrate-producing Firmicutes.
T REGULATORY CELLS IN CD

• Minor subpopulation of CD4⁺ T cells (5-10%).
• Traditionally described as CD⁴⁺CD₂⁵⁺⁺FOXP₃⁺
  ➢ Maintenance of self-tolerance and immune homeostasis.
  ➢ Induction of oral tolerance.

• Low frequency of CD4⁺CD25⁺ Treg cells in
  1. Systemic Lupus Erythematosus patients
  2. Multiple Sclerosis patients
  3. in Type 1 diabetes
• Number of Treg cells is increased in active celiac patients.
• Suppressive function of Treg cells is significantly impaired in celiac patients.
FOXP3

- Important for differentiation and function of Treg cells.

Transcriptional repression

Homodimerization

DNA binding, nuclear location

Delta2

Th17

RORγt

RORαT

EOS

HDAC7/9

TIP60

RUNX1

NFAT

NFκB
Different Foxp3 FL/Δ2 Ratio Between Gut Mucosa and PBMC

Increased Expression of FOXP3 Δ2 is Correlated With Increased Expression of RORGT and IL17A in CD Patients

CDA VS HC

Gut pro-inflammatory microenvironment

Microbiome composition

IFNγ

IFNγ+Butyrate Combination Leads to FOXP3 Isoforms Equilibrium in PBMC From HC BUT NOT CD subjects

Working Hypothesis Linking Gut Microbiome Composition, Metabolomic Profile, and Immune Functions Leading To CD

Activated inflammatory cells release cytokines that cause local inflammation responsible for GI symptoms.

Non-self antigens, including food antigens and microorganisms components gain access into the lamina propria.

Activated inflammatory cells migrate to other districts where they cause local inflammation responsible for systemic symptoms.

Dysbiosis causes changes in metabolic pathways affecting key mucosal biology functions.

Diet shapes microbiota composition that can lead to dysbiosis.

Working Hypothesis: The Gut Microbiome/Permeability Link in the Pathogenesis of Autoimmune Diseases

Non-self antigens, including food antigens and microorganisms components gain access into the lamina propria.

Antigen presenting cells (APC) present the non-self antigens to other immune cells.
Celiac Disease Genomic Environmental Microbiome and Metabolomic Study

www.CDGEMM.org
Modeling the Effects of Early Childhood Microbiome Composition On Entire Lifespan

Potential mechanisms: methylation of genes, acetylation of histones?
other epigenetic changes

Adult Health Outcomes

CVD
T2DM

Obesity
Metabolic
syndrome

Adult Health
Risk factors

Malnutrition
Enteral infections
Inflammation
Poor weight gain
Stunting

Childhood factors

Birth

Prenatal factors

Maternal nutrition, placental insufficiency

Prenatal life

Early childhood

First 1000 days of life

Microbiome establishment and modification

Presented in ICoMI 2017
Acknowledgments

The MIBRC Crew