Manipulation of Gut Bacteria To Prevent The Onset Of Celiac Disease
A Paradigm of Multi-omics in Autoimmune Diseases

State Of The Art Research Lecture
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DISCLOSURES

• Alba Therapeutics: Co-founder and stock holder;
• Mead Johnson Nutrition: Sponsored research;
• Inova Diagnostics: Sponsored research;
• Regeneron: Sponsored research;
• Pfizer: Consultant
The Yin and Yang Between Tolerance and Immune Response Leading to Autoimmune Diseases

- Human Genome
- Environmental Factors
- Clinic Outcome
- Immune Response
- Microbiome

Increased Gut Permeability
Several Cells Play a Role in Maintaining The Immune Homeostasis

- Epithelial cells
- Intestinal DCs
- B cells
- T cells
Loss of Mucosal Immune Homeostasis

Chronic Inflammation-Autoimmunity

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

Break of Tolerance
Apoptosis resistant T cells
Tissue damage
Autoimmune Diseases

Inflammation
Vicious circle
Proinflammatory Allergic cytokines

Mucosal Tolerance
Homeostasis
Anergy

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

Defensins
Mucus Synthesis & Quality
SIgA
The Hygiene Hypothesis

Autoimmune disorders incidence

Helminths infestation incidence

Personal communication from Dr. Joel Weinstock
The Hygiene Hypothesis Has Been Recently Questioned

Improved Hygiene In Some Developing Countries Was Not Paralleled by Increased Autoimmune Diseases
Celiac Disease As A Unique Model of Autoimmunity

A TRIO OF CAUSES

Three factors underlie celiac disease: an environmental trigger, a genetic susceptibility and, according to the author’s research, an unusually permeable gut (below). The author suspects that the same basic triad contributes to other autoimmune diseases, although each disorder will have its own triggers and genetic components.

TRIGGER
The gluten protein, abundant in the endosperm of wheat kernels, sets off the aberrant immune response. Related proteins in barley and rye (hordein and secalin) do the same.

GENETIC PREDISPOSITION
Almost all patients harbor the genes HLA-DQ2 or HLA-DQ8, or both. These genes give rise to proteins of the same name that display gluten fragments to immune system cells, which then direct an attack on the intestinal lining. Other genes are likely to be involved as well, but these additional culprits may differ from person to person.

LEAKY SMALL INTESTINE
In most people, links known as tight junctions “glue” intestinal cells together. In those with celiac disease, the junctions come apart, allowing a large amount of indigestible gluten fragments to seep into the underlying tissue and incite immune system cells. Treatments that reduced leakiness could potentially ease not only celiac disease but also other autoimmune disorders involving unusually permeable intestines.
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.
Increased Prevalence Over Time in U.S.A. (in Line with Other Autoimmune Diseases)

During the past 35 years the true prevalence of CD in USA doubled every 15 years.

The Epidemics Of Celiac Disease: Which Additional Factors are Driving this Epidemics?

- Quality of gluten: GE grains
- Quantity of gluten;
- Breast Feeding;
- Timing of gluten introduction
- Maturity of gut functions influencing Ag trafficking and handling:
  - GALT
  - PRRs
  - Mucous production
  - Barrier function
- Changes in microbiome composition.
Introduction of Gluten, HLA Status, and the Risk of Celiac Disease in Children

Elena Lionetti, M.D., Stefania Castellaneta, M.D., Ruggiero Francavilla, M.D., Ph.D., Alfredo Pulvirenti, Ph.D., Elio Tonutti, M.D., Sergio Amarri, M.D., Maria Barbato, M.D., Cristiana Barbera, M.D., Graziano Barera, M.D., Antonella Bellantoni, M.D., Emanuela Castellano, M.D., Graziella Guariso, M.D., Maria Giovanna Limongelli, M.D., Salvatore Pellegrino, M.D., Carlo Polloni, M.D., Claudio Ughi, M.D., Giovanna Zuin, M.D., Alessio Fasano, M.D., Ph.D., and Carlo Catassi, M.D., Ph.D., for the SIGENP (Italian Society of Pediatric Gastroenterology, Hepatology, and Nutrition) Working Group on Weaning and CD Risk

Published on October 2, 2014
Home Take Messages

• Window of tolerance concept (4-7 months best period to introduce baby food) not supported anymore;

• Breast feeding in general or introduction of gluten while breast feeding showed no protective effect on CD onset in at-risk infants;

• Early introduction (16 weeks) of gluten traces to potentially induce tolerance did not protect against CD in at-risk infants;

• Delaying the introduction of gluten in at-risk infants does not prevent CD but merely postpones its onset by approximately 8 months (significant difference at 2 years FU that disappeared by 5 years FU);

• GI infections during the first year of life seems influential in increased the risk of CD onset;

• High-risk HLA profiles seems to be the most influential factor predictor of increased risk of CD onset;

• The high prevalence of CD among the study cohort suggests that the CD epidemics continues.
The Epidemics Of Celiac Disease: Which Additional Factors are Driving this Epidemics?

- Quality of gluten;
- Quantity of gluten;
- Breast Feeding;
- Timing of gluten introduction
- Maturity of gut functions influencing Ag trafficking and handling:
  - GALT
  - PRRs
  - Mucous production
  - Barrier function
- Changes in microbiome composition.
• 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)
Which Factors are Driving This Autoimmunity Epidemics?

Microbiome Composition

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

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

Pro-inflammatory Response to Food Antigens

Genetic Predisposition

Inappropriate GALT Maturation

Probiotics

Pre-, Pro-, And/or Symbiotics

Balanced Microbiome

Appropriate GALT Maturation

Tolerogenic Response to Food Antigens - State of Health

Dysbiosis

Pro-inflammatory Response to Food Antigens - CID
Role of Breastmilk

Maternal Milk:
- Antigen
  - Free
  - Complexed to IgA
  - Complexed to IgG
- Tolerogenic immune mediators
  - TGF-β, IL10, Vit A, ...
- Microbiota modulating factors
  - Prebiotics (oligosaccharides, glycoproteins)
  - Antimicrobial (lysosome, lactoferrine, IgA, ...)
- Gut growth factors (EGF, TGF-β, ...)

Food or environmental antigen

Antigen transferred across gut barrier

Antigen handling by maternal digestive system

Oral tolerance

http://www.nature.com
Impact of human milk glycobiome on the infant intestinal microbiota

Intestinal Flora Influences Postnatal Immune System Development

• The earliest colonizers were often organisms predicted to be aerobes (e.g., Staphylococcus, Streptococcus, and Enterobacteria), whereas the later colonizers tended to be strict anaerobes (Eubacteria and Clostridia).

• The Bacteroides varied greatly from baby to baby in the timing of their first appearance, but were consistently present in nearly all babies by 1 y.

• Several other taxa, including Prevotella, Acinetobacter, Desulfovibrio, Veillonella, and Clostridium perfringens, tended to appear only transiently, sometimes appearing and disappearing repeatedly within a baby’s first year of life.

• By the end of the first year of life, the microbial ecosystems in each baby had converged toward a profile characteristic of the adult GI tract.

• All these changes are mainly driven by nutritional variables
Infants genetically predisposed to CD were characterized by a low abundance of Bacteroidetes (undetectable to 1%) combined with abundance of Firmicutes.
The Real Story of Our Genetic Complexity: We Inherit two Parallel Genomes

**Human Genome:**
Inherited from both parents, stable, never change in its composition

**Microbiome:**
Inherited from the mother, extremely dynamic, changes from individual to individual and in the same individual over time
Higher Risk of Celiac Disease After Elective Cesarean Delivery

<table>
<thead>
<tr>
<th></th>
<th>Matched controls (%)</th>
<th>Celiac disease (%)</th>
<th>Odds ratio; 95% CI OR</th>
<th>P-value</th>
<th>Adjusted odds ratio; 95% CI AOR</th>
<th>P-value</th>
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<tbody>
<tr>
<td>Cesarean delivery</td>
<td>5,766/53,887 (10.7)</td>
<td>1,299/11,749 (11.1)</td>
<td>1.04; 0.98-1.10</td>
<td>0.232</td>
<td>1.06; 0.99-1.13</td>
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<td>Emergency cesarean</td>
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<td>444/8,827 (5.0)</td>
<td>0.99; 0.90-1.10</td>
<td>0.857</td>
<td>1.02; 0.92-1.13</td>
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<tr>
<td>Elective cesarean</td>
<td>2,125/41,688 (5.1)</td>
<td>508/8,891 (5.7)</td>
<td>1.11; 1.01-1.22</td>
<td>0.027</td>
<td>1.15; 1.04-1.26</td>
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</tbody>
</table>

Mårild et al Gastroenterology 2012;142(1):39
Infants' intestinal microbiome is influenced by mode of delivery

Dominguez-Bello et al. PNAS 2010;107(26):11971-5
Bacterial dysbiosis as possible mechanism responsible of increased risk for celiac disease in children born by C-Section

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.

During the active state of the disease (24 months) the same subjects showed an increase in butyrate production and a decrease in lactate, therefore suggesting that the acute phase of CD is characterized by a different metabolomic profile.
Intestinal Organoids

Organoids differentiate lysozyme positive cells (Paneth’s cells)

DAPI MUC2 ZO1
Volcano Plot Representing 80 Stem Cell Related Genes Expression Profile Macro Array Expressed In Crypts of Acute CD vs. Healthy Subjects.

<table>
<thead>
<tr>
<th>GENE</th>
<th>Fold Regulation</th>
<th>P-Value</th>
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<tbody>
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<td>BCL9</td>
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How Mechanistically Link Microbiome/Metametabolome Profiles to Clinical Outcome

Stem Cell Niche

Distribution of LGR5 positive cells in the intestinal crypt.

Gene Expression In Stem Cell Niche Using Gut Organoids

Muc2 expression in HuORG

Distribution of LGR5 positive cells in the intestinal crypt.
How Mechanistically Link Microbiome/Metametabolome Profiles to Clinical Outcome
Mucosal and Systemic Immune Functions

PROTEIN LEVEL IN Treg cells STIMULATED WITH METABOLITES
Hypothesis

Combination of introduction of gluten into the diet and particular microbiota composition of infants genetically at risk for CD activates specific metabolic pathways that can contribute to the loss of tolerance to gluten and to the onset of autoimmunity, as reflected by specific metabolomic phenotypes.
Activated inflammatory cells release cytokines that cause local inflammation responsible for the GI symptoms.
NMR Analysis

Systemic inflammation
CD Extraintestinal Symptoms
CD Onset (at any age)
Acknowledgments

The MIBRC Crew