Postnatal Factors Influencing Tolerance vs. Immune Response: Lessons Learned From Food Allergies, Asthma, and Other Immune-Mediated Diseases

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Lecture Objectives
Genes, Environment and the Gut Mucosa Functions: How to Connect the Dots
The intestine is a long tube with a clean open on the top and a dirty open at the bottom.
Several Cells Play a Role in Maintaining The Immune Homeostasis

- Epithelial cells
- Intestinal DCs
- B cells
- T cells
All disease begins in the gut - Hippocrates 460 BC

The gut is not like Las Vegas: what happens in the gut does not stay in the gut – A.F. 2010 AC

The intestinal mucosa is the battlefield on which friends and foes need to be recognized and properly managed to find the ideal balance between tolerance and immune response.
The Yin and Yang Between Tolerance and Immune Response Leading To Immune-Mediated Diseases

- Human Genome
- Environmental Factors
- Clinic Outcome
- Increased Gut Permeability
- Immune Response
- Microbiome

Environmental Factors

Clinic Outcome

Increased Gut Permeability

Immune Response

Microbiome
Loss of Mucosal Immune Homeostasis

Chronic Inflammation-Allergy

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

- Defensins
- Mucus Synthesis & Quality
- SIgA

Mucosal Tolerance Homeostasis
Anergy

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

Innate or immuno-regulatory defect

Vicious circle

Proinflammatory Allergic cytokines

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

Adapted from P. Brandtzaeg, Beneficial Microbes 2010
The Epidemics of Immune-Mediated Diseases In The Western Hemisphere: The Hygiene Hypothesis

[Graphs and maps showing incidence of infectious diseases, autoimmune disorders, and helminths infestation incidence across different regions.]
Autoimmunity Epidemics: Autism
During the past 35 years the true prevalence of CD in USA doubled every 15 years.

Food anaphylaxis in Italy

Ministry of Health, Health planning, essential levels of care and ethical principles of the system - Office VI.

The Hygiene Hypothesis Has Been Recently Questioned

Improved Hygiene In Some Developing Countries Was Not Paralleled by Increased Immune-Mediated Diseases
Microbiome Composition

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

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

Probiotics

Balanced Microbiome

Appropriate GALT Maturation

Tolerogenic Response to Food Antigens - State of Health

Genetic Predisposition

Pro-inflammatory Response to Food Antigens - CID

Inappropriate GALT Maturation

Dysbiosis

Appropriate GALT Maturation

State of Health

Inappropriate Nutrition

Multiple infections

Antibiotic treatments
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 handling by maternal digestive system

Antigen transferred across gut barrier

Oral tolerance

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

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 “Omics” Revolution

• **Microbiome (Who is there):** A powerful tool used to analyze microbial communities (commensal, symbiotic, and pathogenic microorganisms) regardless of the ability of member organisms to be cultured in the laboratory.

• **Metagenomics (What language do they speak):** Genomic analysis of microbial DNA that is extracted directly from communities in environmental samples. This technology — genomics on a huge scale — enables a survey of the different microorganisms present in a specific environment, for example the human gut.

• **Metatrascriptomics (What are they talking about):** A branch of transcriptomics that studies and correlates the transcriptomes of a group of interacting organisms or species. This technique enables to identify the actively transcribed ribosomal and messenger RNA from a community.
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.
Gut Dysbiosis Correlates With Allergy Development

AT RISK NEONATES

ALLERGIC INFANTS

HEALTHY INFANTS

Microflora

Bacteroides

Clostridium

Months

0 1 2 24

**Lactobacillus casei** Abundance Is Associated with Profound Shifts in the Infant Gut Microbiome

Michael J. Cox¹, Yvonne J. Huang², Kei E. Fujimura¹, Jane T. Liu², Michelle McKean³, Homer A. Boushey², Mark R. Segal⁴, Eoin L. Brodie⁵, Michael D. Cabana²,³, Susan V. Lynch¹*  

16S rRNA PhyloChip  
*High-density, culture-independent microarray that can identify ~8,500 bacterial taxa*

Restoring Microbial Health

**Lactobacillus GG** (LGG) restores the normal microflora composition in infants with CMA

January 2010 | Volume 5 | Issue 1 | e8745
Proof of Concept of Microbiome-Metabolome Analysis and Delayed Gluten Exposure on Celiac Disease Autoimmunity in Genetically At-Risk Infants

Maria Sellitto¹, Guoyun Bai², Gloria Serena¹, W. Florian Fricke², Craig Sturgeon¹, Pawel Gajer², James R. White², Sara S. K. Koenig², Joyce Sakamoto², Dustin Boothe¹, Rachel Gicquelais¹, Deborah Kryszak¹, Elaine Puppa¹, Carlo Catassi¹, Jacques Ravel²*, Alessio Fasano¹*

¹ Mucosal Biology Research Center, Center for Celiac Research and Departments of Pediatrics, Medicine and Physiology, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, ² Institute for Genome Sciences and Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, ³ Department of Pediatrics, Università Politecnica delle Marche, Ancona, Italy

Abstract

Celiac disease (CD) is a unique autoimmune disorder in which the genetic factors (DQ2/DQ8) and the environmental trigger (gluten) are known and necessary but not sufficient for its development. Other environmental components contributing to CD are poorly understood. Studies suggest that aspects of gluten intake might influence the risk of CD occurrence and timing of its onset, i.e., the amount and quality of ingested gluten, together with the pattern of infant feeding and the age at which gluten is introduced in the diet. In this study, we hypothesize that the intestinal microbiota as a whole rather than specific infections dictates the switch from tolerance to immune response in genetically susceptible individuals. Using a sample of infants genetically at risk of CD, we characterized the longitudinal changes in the microbial communities that colonize infants from birth to 24 months and the impact of two patterns of gluten introduction (early vs. late) on the gut microbiota and metabolome, and the switch from gluten tolerance to immune response, including onset of CD autoimmunity. We show that infants genetically susceptible to CD who are exposed to gluten early mount an immune
Heatmap of relative abundance of bacterial phylum of longitudinal samples from DQ2+/DQ8+ infants analyzed in this study and those of Palmer et al.

A. Complete linkage clustering based on the phylum composition and abundance of GI microbiota. B. Color depicts the study and intervention group of the samples. C. Colors depict the time point at which the samples were collected. Time points D and E were omitted as no corresponding samples were collected in the Palmer et al. study [28].
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>
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<th>Matched controls (%) Celiac disease (%)</th>
<th>Odds ratio; 95% CI OR</th>
<th>P-value</th>
<th>Adjusted odds ratio*, P-value</th>
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<tbody>
<tr>
<td>Cesarean delivery</td>
<td>5,766/53,887 (10.7)</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>Number of participants</td>
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<td>65,636</td>
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<td>Emergency cesarean delivery</td>
<td>2,136/41,699 (5.1)</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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<td>50,526</td>
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<tr>
<td>Elective cesarean delivery</td>
<td>2,125/41,688 (5.1)</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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<td>50,579</td>
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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

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