“It is reasonable to propose that the composition of the microbiome and its activities are involved in most, if not all, of the biological processes that constitute human health and disease.

– Martin J. Blaser, PhD

= NEW PARADIGM

– Martin J. Blaser, PhD

PUBMED: “microbiome”
CHALLENGE:
Translating & applying this information clinically
1. Microbiome & Root Causes
2. Microbiome & Mucosal Interactions
3. Dysbiosis Triad & IBD Example
4. Implications for Gut Health
External (Environment)

Microbiome

Internal (Genes, etc.)
NEW PARADIGM

The microbiome is an important mediator of internal (gene) and environmental interactions that determine health vs. disease.
Microbiome & Root Causes

Microbiome & Mucosal Interactions

Dysbiosis Triad & IBD Example

Implications for Gut Health
Extremely complex, highly dynamic interactions
Details vary among niches along digestive tract
Details vary among individuals
Density

$10^1 - 10^3$
$10^4 - 10^7$
$10^{10} - 10^{13}$

Composition

Streptococcus
Lactobacillus

Streptococcus
Lactobacillus
Enterobacteriaceae

Bacteroides
Eubacterium
Clostridium
Ruminococcus
Bifidobacterium

Microbiome Functions (Eubiosis)

- Colonization resistance against pathogens
- Cell components & metabolic products act as signaling molecules
- Promote barrier maintenance & immune balance
- Metabolic products serve as energy source
- Modify bioactivity of food residues, drugs, etc.
- Modify gene expression & epigenetics
Cell components (LPS, flagella, DNA)
Metabolites (SCFAs, organic acids, bile acids)
$pH$, oxygen
Proximity to epithelial barrier

Microbiome

Intestinal Mucosa
LIPOPOLYSACCHARIDE

Cell wall component produced by gram-negative bacteria (e.g., Proteobacteria / E. coli)

Potent inducer of inflammation (lipid A core)

LPS varies among species & strains (O antigen)

Diet, Metabolites, and "Western-Lifestyle" Inflammatory Diseases

Immunity 40, June 19, 2014
Detecting the Microbiome

- **Pattern-recognition receptors (PRRs)**
  - Innate immune cells, epithelium, etc.
  - Recognize microbial cell components such as LPS, flagella, microbial DNA, etc. (MAMPs)

- **Metabolite / nutrient sensors**
  - Innate immune cells, epithelium, etc.
  - SCFA receptors
  - AhR receptor
  - Others
Gut Microbiome

Intestinal Mucosa
Mucosal Functions (Eubiosis)

- Manages microbiome composition & activity
- Provides chemical and structural barriers to prevent microbial attachment & invasion
- Immune cell activity and antimicrobial factors
- Provides substrates to promote commensals
- Regulates physiological conditions in lumen (pH, substrate & mineral availability, etc.)
Microbiome

Gut epithelium
Immune system
Secreted factors: Mucus, slgA, AMPs, IAP, miRNAs
IL-22, IL-17, IL-18
pH, oxygen
Motility & secretion

Intestinal Mucosa
IL-22

- Produced by innate immune cells in response to certain bacteria, their products, phytonutrients

- Regulates a variety of barrier functions:
  - Enhances mucus layer
  - Fortifies tight junctions
  - Increased expression of antimicrobial proteins (AMPs)
  - Promotes epithelial regeneration
  - Regulates microbiome composition
“Mucosal homeostasis depends on physical and molecular interactions between three components: the resident microbiota, the epithelial layer and the local immune system. The cytokine IL-22 helps to orchestrate this three-way interaction. IL-22 is produced by immune cells present beneath the epithelium and is induced by bacteria present in the intestine.”
Antimicrobial Peptides (AMPs)

- Secreted by epithelial (Paneth) & immune cells
- Inhibit /kill microbes by disrupting microbial membranes, binding LPS, etc.
- Immune signaling & regulation functions (cytokine-like functions)
- Expression influenced by microbiome & dietary nutrients (via IL-22, etc.)
Antimicrobial peptides (AMPs) are synthesized and secreted by immune and epithelial cells that are constantly exposed to environmental microbes. AMPs are essential for barrier defense, and deficiencies lead to increased susceptibility to infection. In addition to their ability to disrupt the integrity of bacterial, viral and fungal membranes, AMPs bind LPS, act as chemoattractants for immune cells and bind to cellular receptors and modulate the expression of cytokines and chemokines.”
“Vitamins A and D, dietary histone deacetylases [e.g., sulforaphane] and by-products of intestinal microbial metabolism (butyrate and secondary bile acids) have been found to regulate the expression of AMPs in humans. … Animal and human clinical studies with butyrate indicate that increasing expression of AMPs in the colon protects against infection.”
Here, we describe a metabolic pathway whereby Trp metabolites from the microbiota balance mucosal reactivity in mice. …The resulting IL-22-dependent balanced mucosal response allows for survival of mixed microbial communities yet provides colonization resistance to the fungus Candida albicans and mucosal protection from inflammation.”
“Li et al. (2011) uncover a link between diet and immunity, showing that specific dietary compounds found at high levels in cruciferous vegetables such as broccoli, cauliflower, and cabbage are essential for sustaining intestinal immune function. Moreover, they show that the molecular basis for this link involves the aryl hydrocarbon receptor (AhR)”
Intestinal Alkaline Phosphatase

- Produced by intestinal epithelial cells
- Two versions: secreted and attached
- Detoxifies LPS and other inflammatory microbial products
- Helps manage bacterial population
- Regulates tight junctions
- Deficiency implicated in IBD & others
Over the past few years, there is increasing evidence implicating a novel role for Intestinal Alkaline Phosphatase (IAP) in mitigating inflammatory mediated disorders ... Loss of IAP expression or function is associated with increased intestinal inflammation, dysbiosis, bacterial translocation and subsequently systemic inflammation.”
“The toxicity of LPS resides in the Lipid-A moiety, which permits it to bind to toll-like receptor-4 (TLR4). Removal of one of the two phosphate groups on the lipid-A moiety reduces LPS toxicity 100 fold”
“IAP’s role in the intestine is to dephosphorylate toxic microbial ligands such as lipopolysaccharides .... IAP’s ability to detoxify these ligands is essential in protecting the host from sepsis during acute inflammation and chronic inflammatory conditions such as inflammatory bowel disease.”
Diet influences the commensal bacteria in the gut while bacteria digest food as a food source for enterocytes.

Dietary factors and IAP:
- HFD ↑ IAP
- High ω-6 PUFA ↑ IAP
- ω-3 PUFA ↓ IAP
- Protein-free diets ↓ IAP
- Starvation ↓ IAP

IAP can alter microbial composition in the intestines.

Membrane bound IAP prevents transmigration of bacteria across the epithelium.

IAP is bidirectionally secreted by enterocytes into the lumen and circulation.

IAP in the blood can travel to distal regions.

IAP dephosphorylates circulating LPS inhibiting sepsis.

IAP dephosphorylates LPS derived from gram negative bacteria.

Intestinal lumen

Epithelium

Lamina propria

Blood
"Maintaining intestinal homeostasis is a key prerequisite for a healthy gut. Recent evidence points out that microRNAs (miRNAs) act at the epicenter of the signaling networks regulating this process. The fine balance in the interaction between gut microbiota, intestinal epithelial cells, and the host immune system is achieved by constant transmission of signals and their precise regulation."
Microbiota Small RNAs in Inflammatory Bowel Disease.
Beneficial probiotic bacteria
*Enterococcus faecium*
*Lactobacillus rhamnosus (LGG)*
*Lactobacillus delbrueckii (L.del)*

miR-423-5p
miR-155
miR-146a
miR-122a
miR-203, miR-483-3p, miR-595

↑
↓
↑
↓
↑
↓

IGLC
Immune response
NUMB
OCLN
ZO-2
PAR-3
PAR-6

Anti inflammatory response
Gut Microbiome

Intestinal Mucosa
Mucosal Barrier

Microbiome

Immune System
Dysbiosis

Barrier Dysfunction

Inflammation
<table>
<thead>
<tr>
<th>DYSBIOSIS FEATURE</th>
<th>CHANGE</th>
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<tbody>
<tr>
<td>Species Diversity</td>
<td>Lower</td>
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<tr>
<td>Colonization Resistance</td>
<td>Lower</td>
</tr>
<tr>
<td>Beneficial Microbes</td>
<td>Lower</td>
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<tr>
<td>Beneficial Metabolites</td>
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<tr>
<td>Protein Fermentation</td>
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<tr>
<td>Overall Population</td>
<td>Too High / Too Low</td>
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</table>
"Intestinal inflammation is frequently associated with an alteration of the gut microbiota, termed dysbiosis, which is characterized by a reduced abundance of obligate anaerobic bacteria and an expansion of facultative Proteobacteria such as commensal E. coli. "


Microbial Respiration and Formate Oxidation as Metabolic Signatures of Inflammation-Associated Dysbiosis.
Bacterial Dysbiosis

**BENEFICIAL**
- Akkermansia
- Bifidobacteria
- Lactobacilli
- **Clostridia:**
  - Faecalibacterium
  - Roseburia
  - Eubacterium

**INFLAMMATORY**
- Proteobacteria
- H. pylori
- Desulfovibrio
- **Enterobacteriaceae:**
  - E. coli (pathogenic)
  - Salmonella
  - Klebsiella
Several mechanisms may explain why dysbiosis is associated with CD. It is possible that dysbiosis contributes to CD because the loss of SCFA producing bacteria has multiple impacts on the gut environment, including impaired survival of enterocytes, increased production of inflammatory cytokines, and decreased suppression of potentially pathogenic Proteobacteria."
“It is also possible that dysbiosis is caused by the intestinal inflammation in CD. Bacterial species from *Proteobacteria* are facultative anaerobes that tend to have a higher resistance to reactive-oxygen species produced during inflammation, possibly giving them a selective advantage over the predominantly obligate anaerobes from *Firmicutes* and *Bacteroidetes*. These mechanisms are not mutually exclusive, and it is likely that microbial dysbiosis both contributes to and results from the intestinal inflammation seen in CD.”
A large body of evidence supports the view that bacteria in the gut participate in the pathophysiology of human bowel diseases. The unifying concept is chronic inflammation that is driven by microbial stimulation of the mucosal immune system. ... Adherent-invasive *E. coli* (AIEC) are Crohn’s disease (CD)-associated bacteria that are implicated in disease pathology.”
“AIEC are pro-inflammatory and may play a central role in maintaining chronic inflammation in response to other CD risk factors, such as acute infectious gastroenteritis … we show that indeed, acute infectious gastroenteritis creates an inflammatory environment in the gut that drives AIEC expansion and worsens disease severity … The long time period between recovery from acute gastroenteritis and new onset CD may allow for targeted interventions to mitigate the risk of CD in AIEC-positive individuals”
Dysbiosis

VICIOUS CYCLE

Barrier Dysfunction

Inflammation
“Paradoxically, antibiotic treatment can promote relapse of Salmonella gastroenteritis … antibiotic treatment lowers colonization resistance by depleting butyrate-producing Clostridia. Decreased butyrate availability increases epithelial oxygenation, thereby fueling aerobic pathogen expansion in the gut lumen.”
The impact of a HF/HS diet in mice was evaluated for the gut micro-inflammation, intestinal microbiota composition, function and selection of an E. coli population. The HF/HS diet created a specific inflammatory environment in the gut, correlated with intestinal mucosa dysbiosis characterized by an overgrowth of pro-inflammatory Proteobacteria such as E. coli, a decrease in protective bacteria, and a significant decrease in SCFA concentrations.

“Western diet induces a shift in microbiota composition enhancing susceptibility to Adherent-Invasive E. coli infection and intestinal inflammation.”
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Dysbiosis

Barrier Dysfunction

Inflammation

Arrows indicate the interconnected relationship between dysbiosis, barrier dysfunction, and inflammation.
Promoting Balance

- Avoid or reduce major disruptions to microbiome when possible (antibiotics, gut infections, diet)
- Gut healing /anti-inflammatory diet & protocols
- Emphasize the 4Ps: plants, prebiotics, probiotics, phytonutrients (polyphenols)
- Emphasize specific nutrients that influence specific microbial & mucosal factors
“... polyphenols modulate the composition of the gut microbial community mostly through the **inhibition of pathogenic bacteria and the stimulation of beneficial bacteria**. In the latter, they may act as a prebiotic metabolite and enrich the beneficial bacteria ...”
“Ten metabolic syndrome patients and ten healthy subjects were included in a randomized, crossover, controlled intervention study. … the subjects consumed red wine and de-alcoholized red wine over a 30 day period for each. In the metabolic syndrome patients, red wine polyphenols significantly increased the number of fecal bifidobacteria and Lactobacillus (intestinal barrier protectors) and butyrate-producing bacteria (Faecalibacterium and Roseburia) at the expense of less desirable groups of bacteria such as LPS producers (E. coli and Enterobacter cloacae).”
Mild, Balancing

Diverse, whole-plant foods

Strong, Disruptive

High potency, high-dose plant extracts
Mechanisms of Pediatric Inflammatory Bowel Disease.
Optimizing Mucosal Factors

- **AhR / IL-22**: Clostridia, Lactobacillus reuteri, cruciferous vegetables, quercetin, resveratrol
- **AMPs**: Vitamins A & D, sulforaphane, butyrate (Clostridia), curcumin
- **sIgA**: SCFAs, glutamine, vitamin D, omega-3s, FOS, S. Boulardii, intermittent fasting
- **IAP**: adequate protein & omega-6 fatty acids
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Barrier Dysfunction

Inflammation
- Microbiome Clinical Intensive
- Gut Pathogens MasterClass
- SIBO MasterClass
- Microbiome Certification Program
- NTA: 30-40% off through 3/15

MicrobiomeMastery.com/NTA