The Gastrointestinal Microbiome and Probiotics: Effects on Intestinal Physiology and Mucosal Inflammation

James Versalovic, M.D., Ph.D.
Professor
Department of Pathology & Immunology
Baylor College of Medicine

Head, Department of Pathology
Chief, Pathology Service
Texas Children’s Hospital
Houston TX  USA
PROBIOTICS (1907)
Feeding Microbes to Humans

Ilya Ilyich Mechnikov

ANTIBIOTICS (1928)
Killing Microbes in Humans

Alexander Fleming
What are Probiotics?

“Probiotics are live micro-organisms which when administered in adequate amounts confer health benefit on the host” (FAO, 2001)

- Probiotics should be alive in the gastrointestinal tract.
- Mechanisms of probiosis should be clearly defined before making a health claim.
- The effect of one probiotic strain cannot be extrapolated to another strain from the same species.

Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods
Delphine MA Saulnier¹,4, Jennifer K Spinler¹,4, Glenn R Gibson³ and James Versalovic¹,2,4
Context:
The Human Microbiome
The Human Microbiome Project: Indigenous Microbiota and Microbiome


>60% novel bacteria
>80% nonculturable bacteria

Mixed Microbial Communities


*Firmicutes* include *Lactobacillus* spp.


Human Colon

>1000 species

*Bacteroidetes*
The Distal Gut Microbiomes of Healthy Children Differ from Healthy Adults

11 samples from healthy adults (18-40 yo) sequenced by HGSC at Baylor.

29 samples from healthy children (7-12 yo) sequenced by HGSC at Baylor

454 16S metagenomic sequencing

14-16K reads per sample

V1V3 regions only (1 replicate/sample)
454 sequencing reveals strain-specific changes and increased microbial diversity after probiotics

Untreated

Strain B
+4 hrs

Strain A
+4 hrs

Strain B
+24 hrs

Strain A
+24 hrs

% Genus Sequenced by 454
Indigenous *Lactobacillus* of the Human Gastrointestinal Tract

- *Lactobacillus gasseri*
- *Lactobacillus reuteri*
- *Lactobacillus ruminis*
- *Lactobacillus salivarius* *
- Autochthonous (Indigenous) versus Allochthonous (Transient) Microbiota
- Commensal-derived Probiotics
  - Derived from the autochthonous microbiota

Can we use indigenous microbes or probiotics to replenish missing elements or functions?

Replenish or increase microbial diversity in intestine. Increased diversity is beneficial.
Intestinal Physiology, Development and Maturation

• Probiotics may stimulate intestinal epithelial cell maturation and migration
• Probiotics may promote villus development
  – Increased absorptive surface area
• Probiotics may stimulate goblet cells and mucin production
• Probiotics may reduce intestinal permeability and promote tight junctions
• Probiotics enhance expression of anion exchangers (choride) on surfaces of IECs
Commensal Microbes and Mechanisms of Immunomodulation

- Stimulate proliferation of regulatory T cell or APC populations
- Stimulate expression of anti-inflammatory cytokines or factors in coordination with TLR ligation
- Suppress pro-inflammatory cytokine secretion by innate immune system
Affects barrier function, membrane permeability, mucin production, HSP induction, IgA and β defensin production

Influences signaling pathways

Macrophage

NF-κB MAPKs STATs

Probiotics

Proliferation/survival, changes in cytokine production

Enlarged IECs
Can commensal-derived probiotics block immune signaling in TNF-stimulated myeloid cells?
**L. reuteri** can inhibit TNF-dependent NF-κB activation

**TNF**

- **TNF** activates **TNFR**
- **TNFR** activates **IKK**
- **IKK** phosphorylates **IκBα**
- **IκBα** is ubiquitinated
- **Ubiquitination** leads to **IκBα** degradation
- **IκBα** degradation allows **NFκB** to translocate into the **nucleus**

**Nucleus**: Activate transcription of inflammatory and anti-apoptotic proteins

Chandra Iyer, Ph.D.
Pathways Affected by Probiotic *L. reuteri*

**Model System: THP-1 cells + TLR2 agonist (PCK)**
- **PCK**
- **TLR2**
- **MAPK Pathway**
- **c-Jun**
- **c-Fos**

Nucleus: block transcription of pro-inflammatory cytokines (TNF)

**Model System: KBM5 cells + TNFR agonist (TNF)**
- **TNF**
- **IKK**
- **NFκB**
- **IκBα**

Degradation

Nucleus: block transcription of pro-inflammatory and anti-apoptotic proteins
Carissa Thomas

Can probiotics regulate specific immune signaling pathways?
ATCC PTA 6475 Inhibits TNF via Downregulation of TAB1

- IL-1 Receptor Antagonist (IL1RN)
- Mitogen-activated protein kinase kinase kinase 7 interacting protein 1 (TAB1/MAP3K7IP1)
- Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase β (IKKB/IKBKB)
- Tumor necrosis factor (TNF)
Mechanisms of Immune Enhancement

• Probiotics stimulate Th1 immune responses
  – Interferon-gamma
• Intestinal bacteria may stimulate Th17 differentiation in the gut mucosa
• Probiotics enhance mucosal IgA and systemic IgA production
• Probiotics-derived CpG –containing DNA activate immune signaling pathways
• Probiotics stimulate epithelial cell proliferation and signaling pathways resulting in cytokine/chemokine production
Can probiotics reduce diarrheal disease burden in children?
**L. reuteri** reduces the duration of rotaviral diarrhea in infants

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% of Patients with Diarrhea

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Days After Therapy Initiation

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* p = .0005

* p = .004

Intestinal Bacteria in a Neonatal Mouse Model

FISH probe specific to *L. reuteri* 16S rRNA / TRITC probe, 200x. Strain DSM 17938 shown.

(Geoff Preidis)
Experimental Design: Testing *L. reuteri* with mouse rotavirus

- Daily gavage *L. reuteri* (10^8 cfu)
- Rotavirus 10^3 ID50
- 0-4 diarrhea scoring (0-4, blinded)
- Intestine, Mesenteric Lymph Node, Spleen
- FISH, Fragment Culture, Histology, IHC
- Antibody ELISA, Serum

5 days old

15 days old

Score <2 No Diarrhea

Score 2+ Diarrhea
*L. reuteri* does not enhance mucosal IgA in uninfected mice

**Total Non-Specific IgA**

**Mesenteric Lymph Node**

**Ileum**
L. reuteri enhances the earliest RV-specific mucosal antibody response

Rotavirus-Specific Antibody

Mesenteric Lymph Node

Ileum

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Could *L. reuteri* enhance virus-specific antibody production?

- Virus-specific antibody clears rotavirus and protects humans and mice from subsequent infection

- Some probiotics increase fecal IgA in children

Franco et al., Vaccine 2006;24(15):2718-31.
Using BrdU to measure epithelial turnover

- Daily gavage *L. reuteri* (10^8 cfu)

- 3 DPI, 4 HPI, 1 DPI, 2 DPI, 4 DPI, 6 DPI

- Begin probiotics and BrdU

- 30 mg/kg i.p.

5 days old → 14 days old
Probiotics increase intestinal epithelial cell proliferation

No Treatment

Strain A

Strain B

n = 15 mice/group

***p < 0.001, *p < 0.05 vs untreated

ileum shown
Strain-specific differences in IEC migration are seen at 2 DPI

No Treatment  Strain A  Strain B

n = 7 mice/group

**p < 0.01, *p < 0.05 vs untreated

#p < 0.01 between strains

Ileum shown
Intestinal Bacterial and Immunological Balance

**PATHOGEN**

- *H. hepaticus*
- *Salmonella sp.*

**BENEFICIAL**

- *L. reuteri*
- *L. paracasei*

Future Directions

Understand traits that underlie probiotic function

Identify genes responsible

Derive robust mutants

“Designer strains” or genetically modified organisms (GMOs)

Compare strains at gene level and in vitro

Select natural strains for specific genes/gene products to improve probiotic function

Optimal natural strains

Clinical documentation

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WE ARE NOT THE SAME....