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

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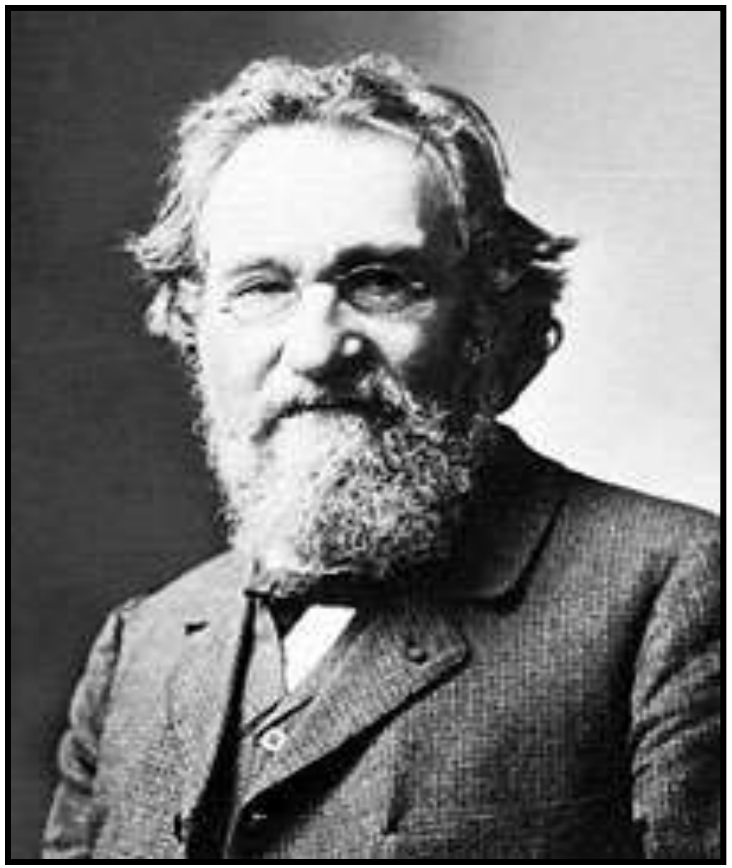
PROBIOTICS (1907)

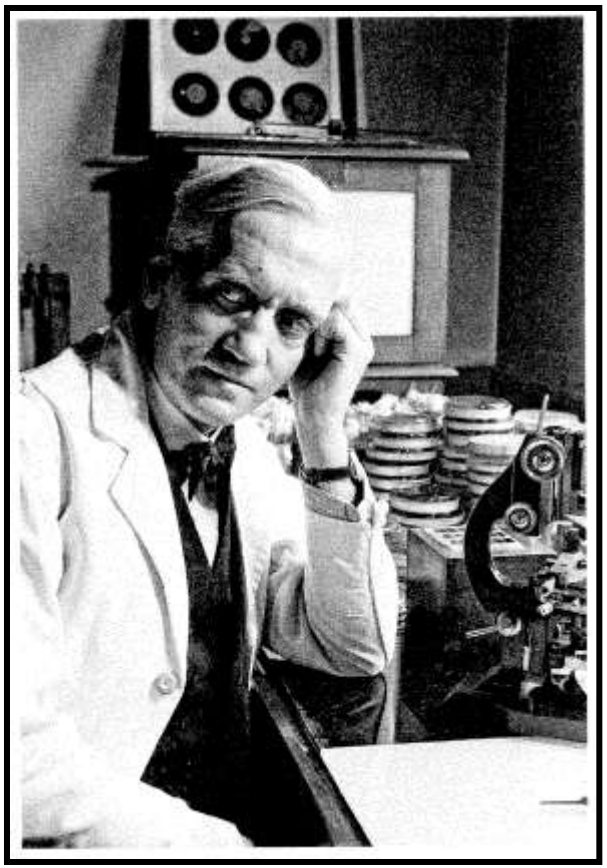
Feeding Microbes to Humans



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.

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Current Opinion in Biotechnology

Mechanisms of probiosis and prebiosis: considerations for enhanced functional foods

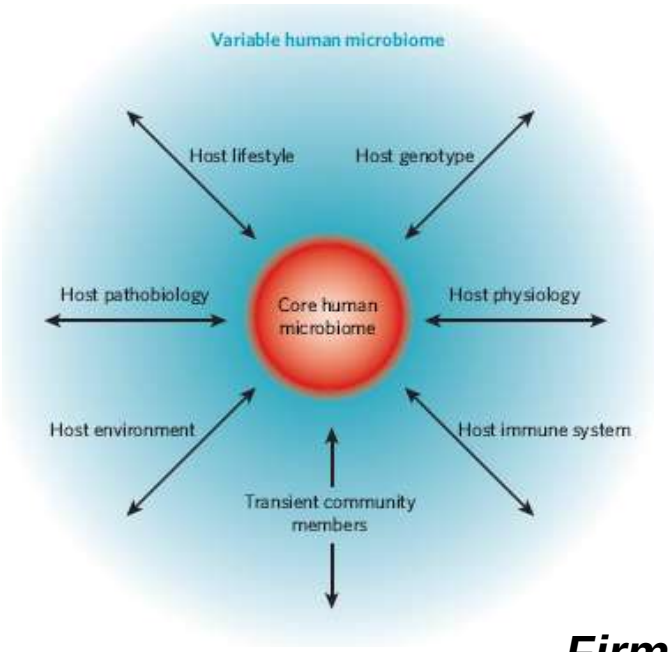
Delphine MA Saulnier[1,4], Jennifer K Spinler[1,4], Glenn R Gibson[3] and James Versalovic[1,2,4]

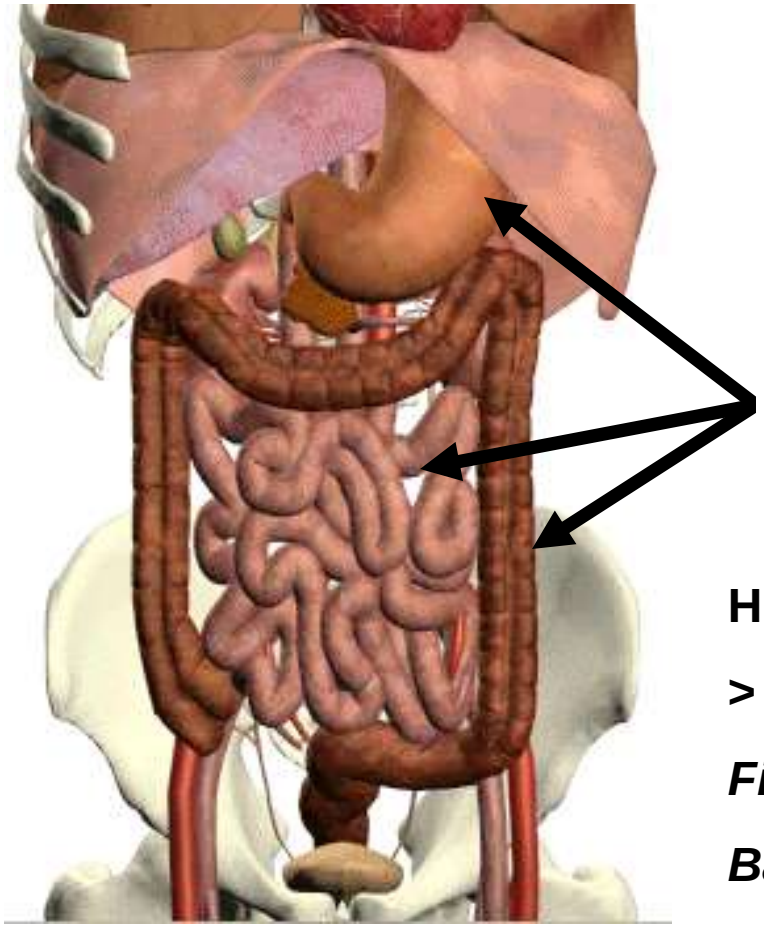
Context :
The Human Microbiome

The Human Microbiome Project: Indigenous Microbiota and Microbiome

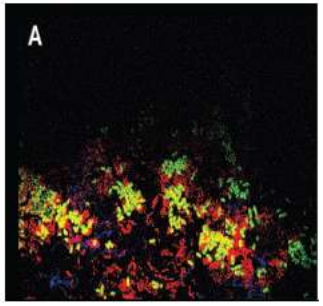
(P Eckburg et al. *Science* 2005;308:1635-38)

>60% novel bacteria
>80% nonculturable bacteria





Mixed Microbial Communities



Human Colon

> 1000 species

Firmicutes

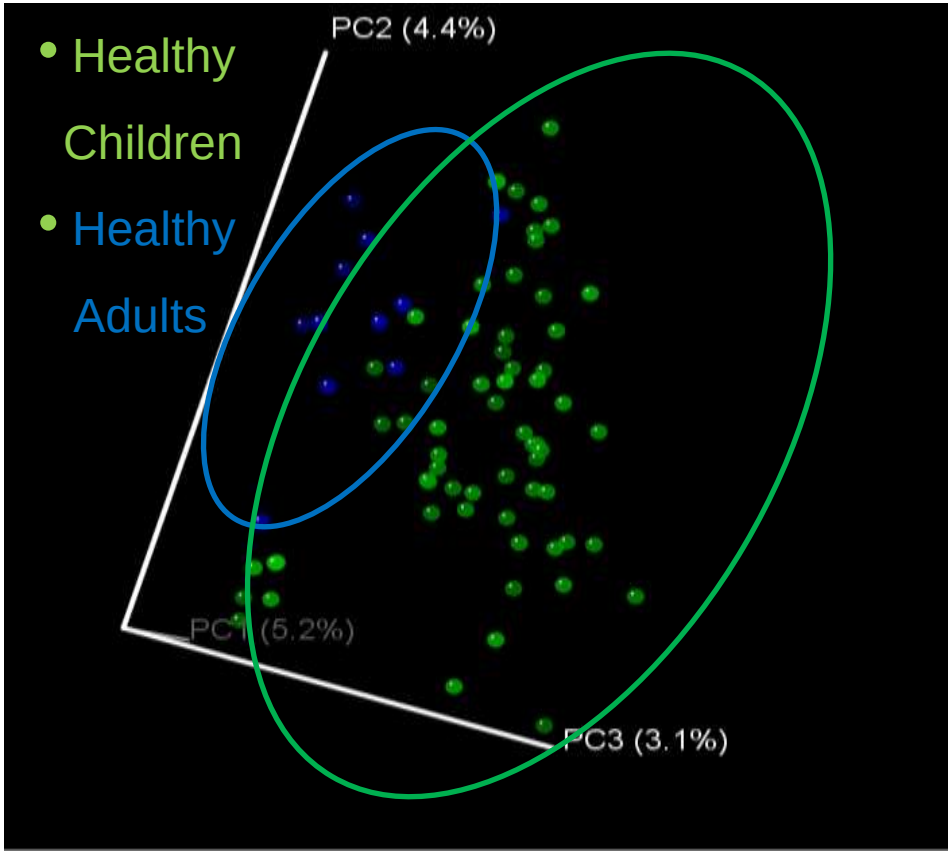
Bacteroidetes

***Firmicutes* include *Lactobacillus* spp.**

(PJ Turnbaugh et al. *Nature* 2007;449:804-10)

(S Macfarlane et al. *Appl Environ Microbiol* 2005;71:7483-92)

The Distal Gut Microbiomes of Healthy Children Differ from Healthy Adults



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

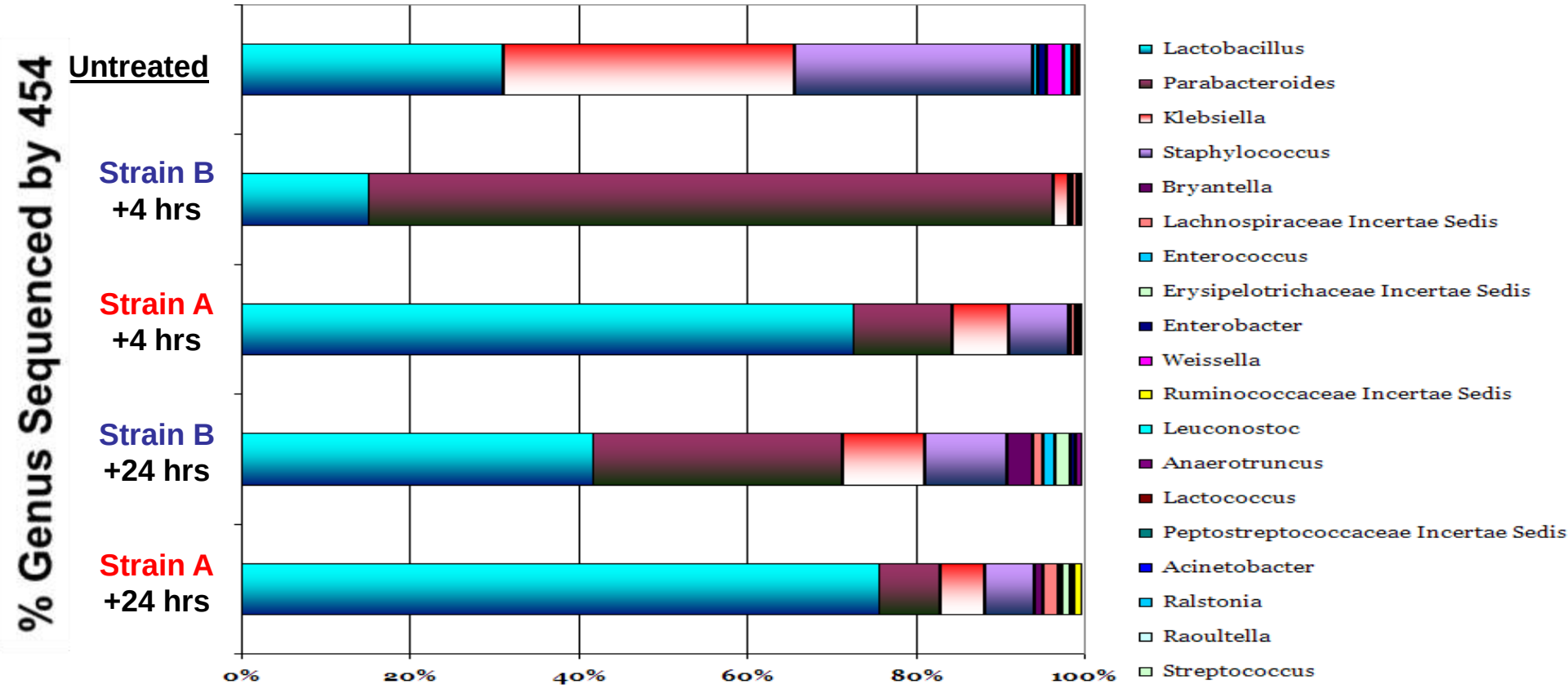
29 samples from healthy children (7-12 yo) sequenced by HGSC at Baylor

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



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

G. Reuter (2001) Curr. Issues Intest. *Microbiol.* 2:43-53

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

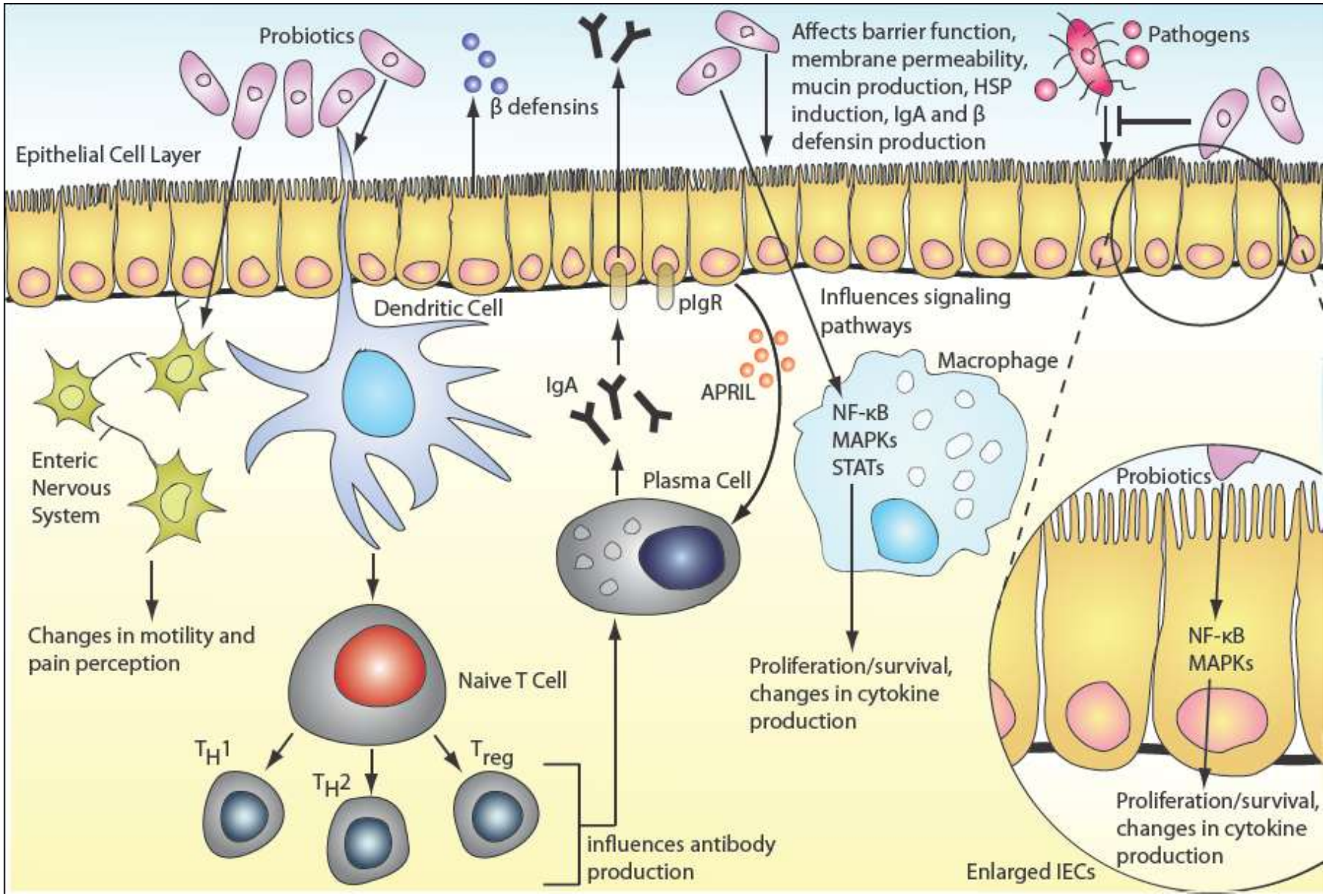
Replenish or increase microbial diversity in intestine. Increased diversity is beneficial.

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

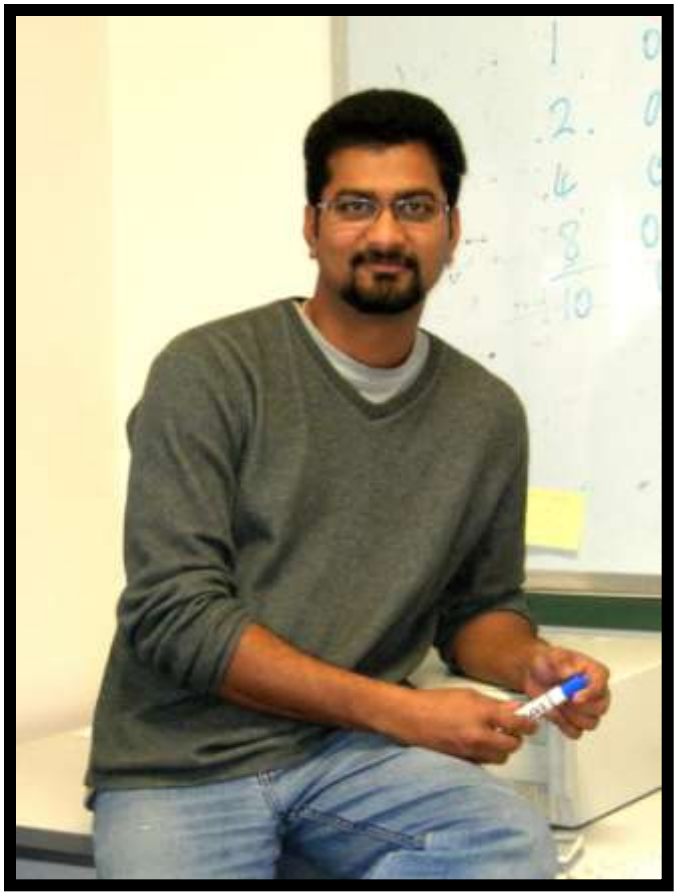
Commensal Microbes and Mechanisms of Immunomodulation

- 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



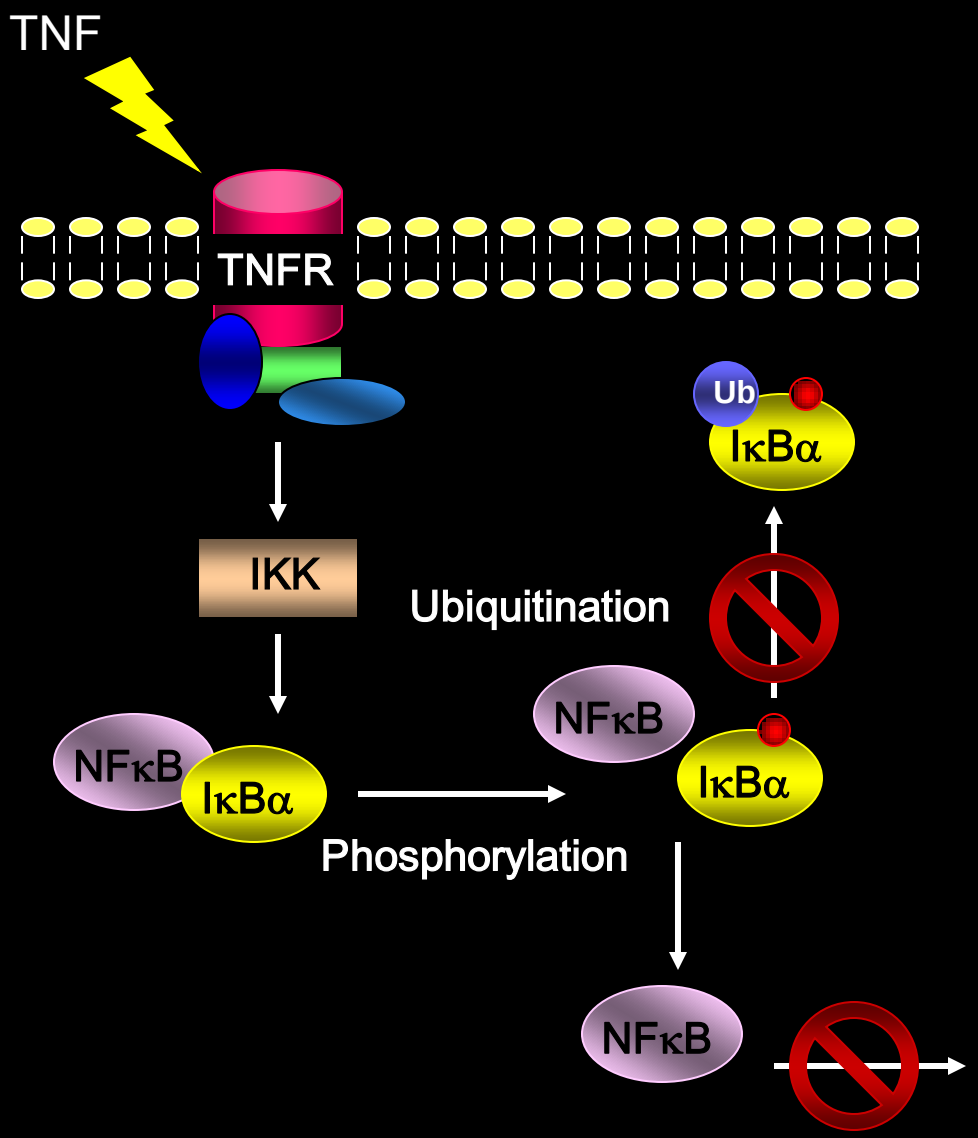
Thomas CM, Versalovic J. *Gut Microbes* 2010; 1:1-16.

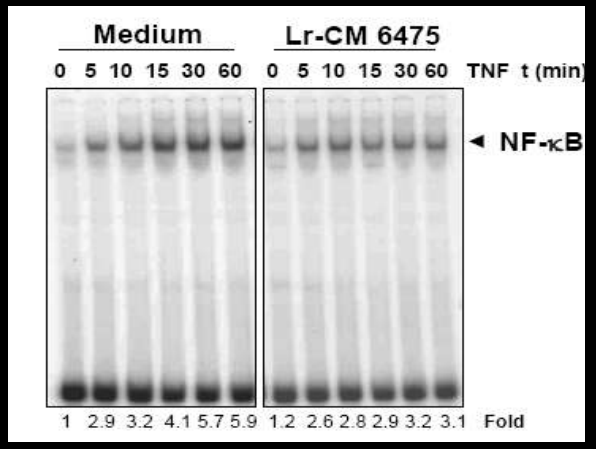
Chandra Iyer

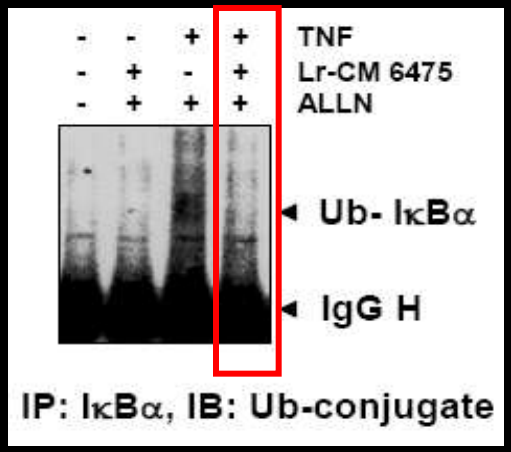


Can commensal-derived probiotics block immune signaling in TNF-stimulated myeloid cells?

L. reuteri can Inhibit TNF Dependent NF-κB Activation



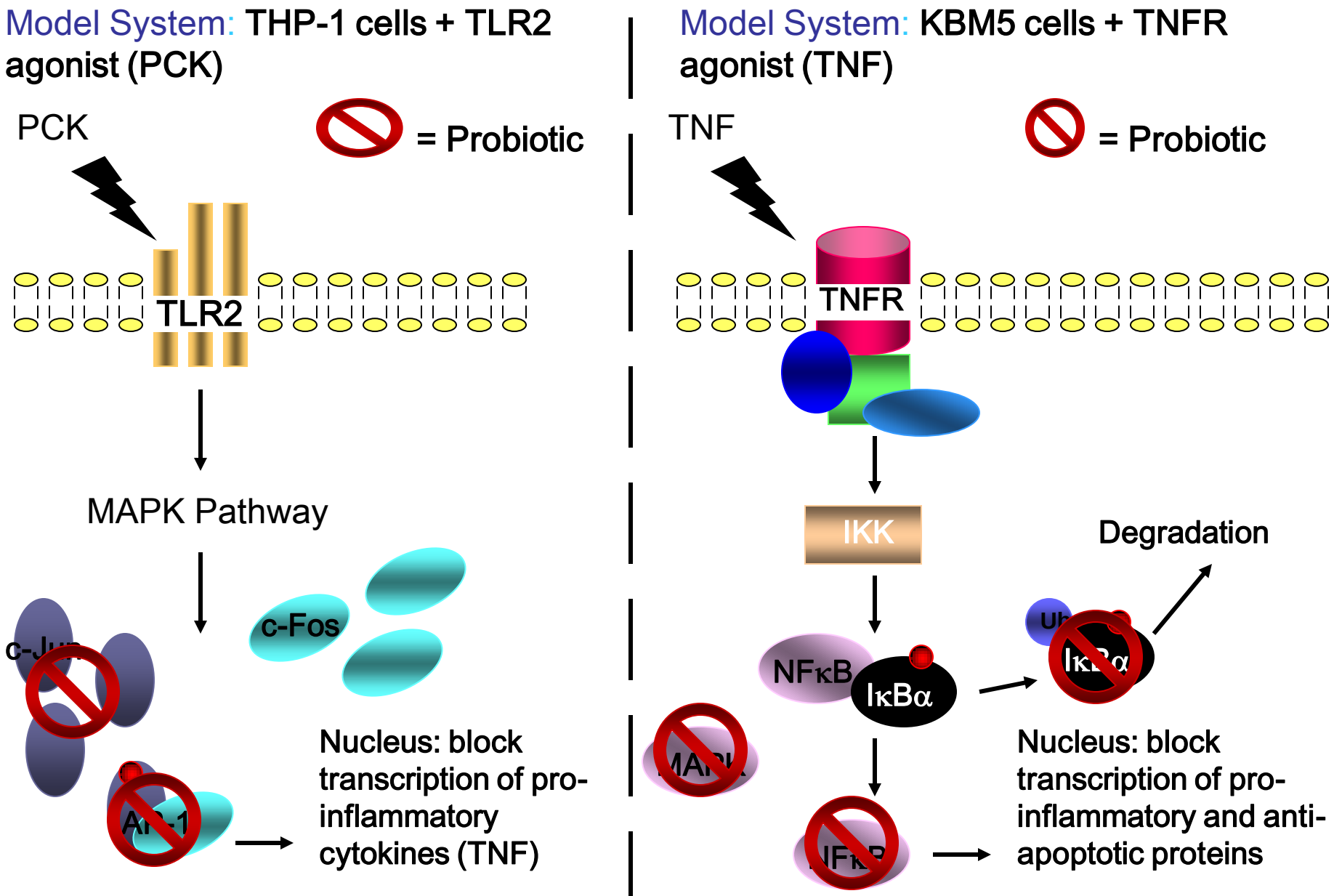




Nucleus: activate transcription of inflammatory and anti-apoptotic proteins

Chandra Iyer, Ph.D.

Pathways Affected by Probiotic *L. reuteri*

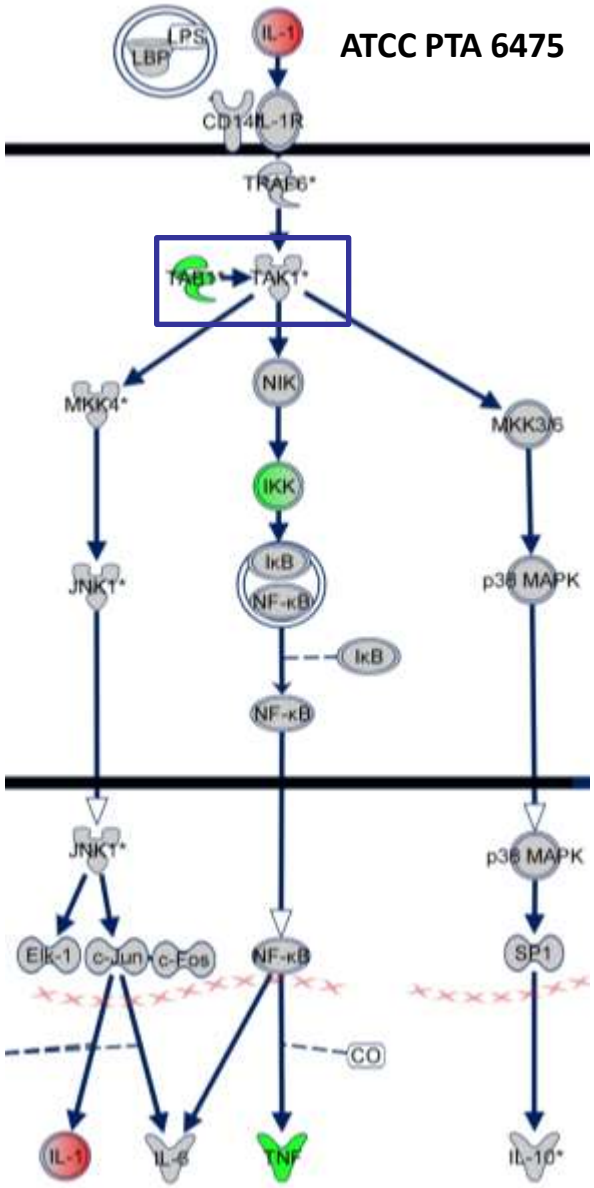


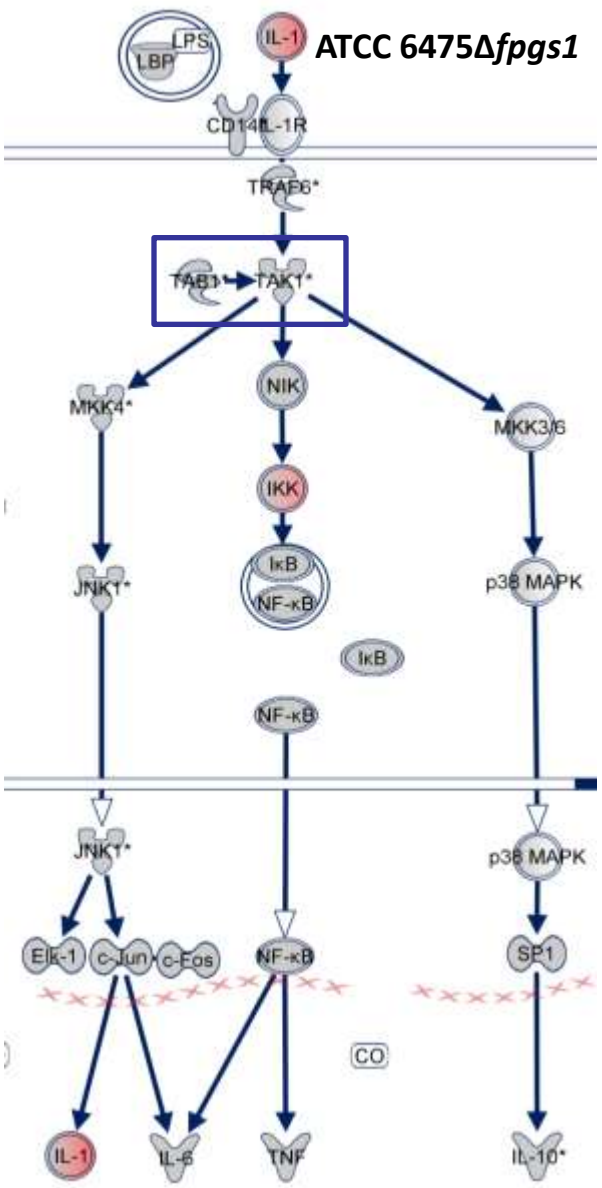
Carissa Thomas

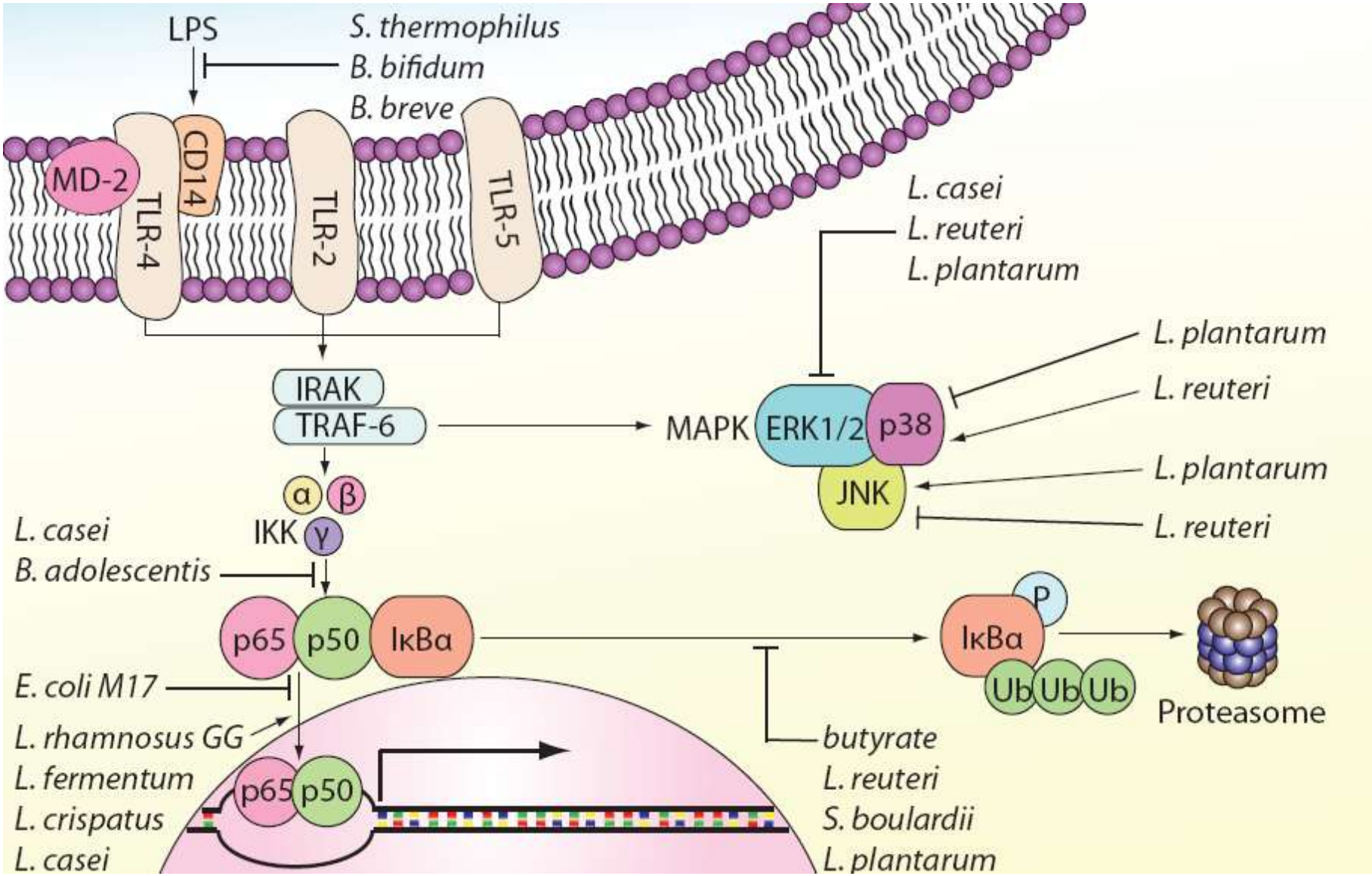
Can probiotics regulate specific immune signaling pathways?

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)







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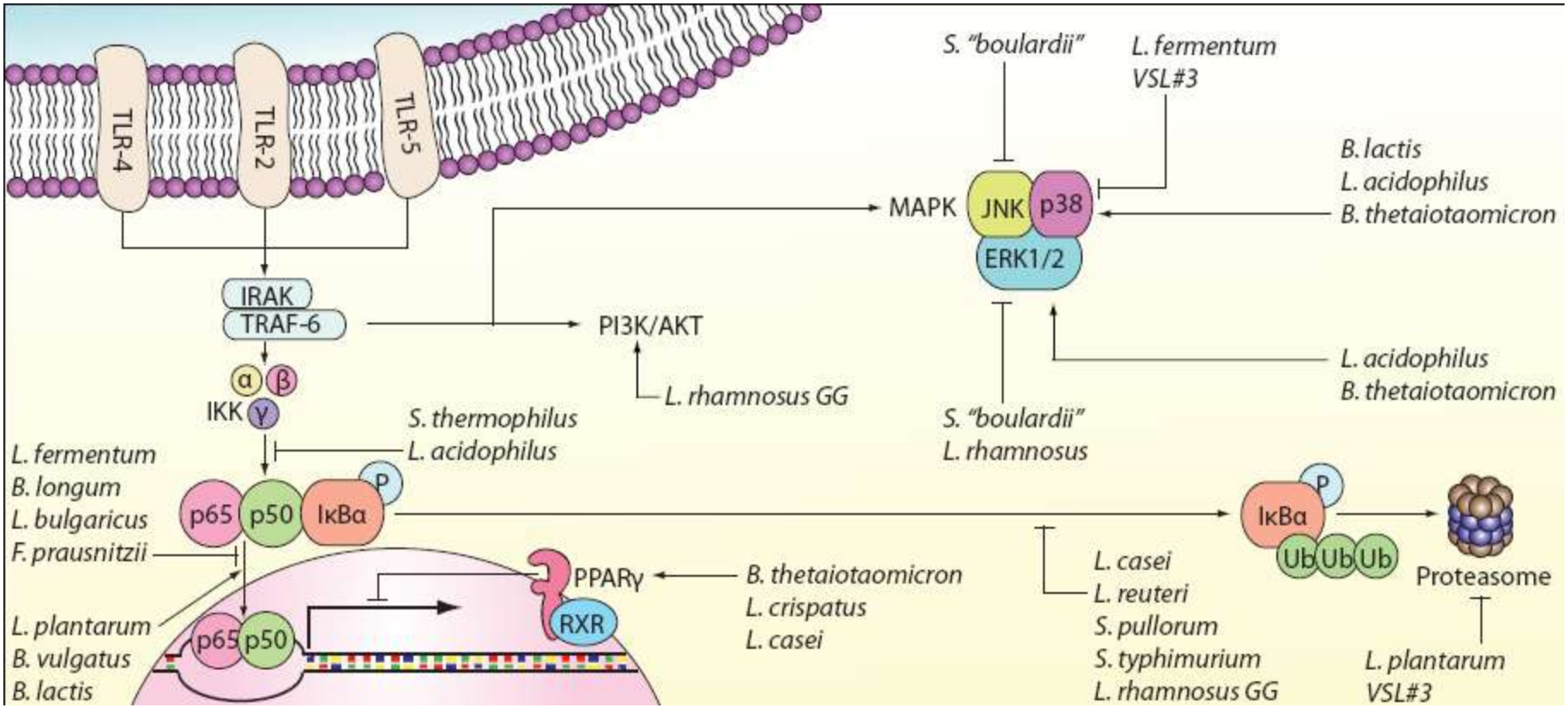

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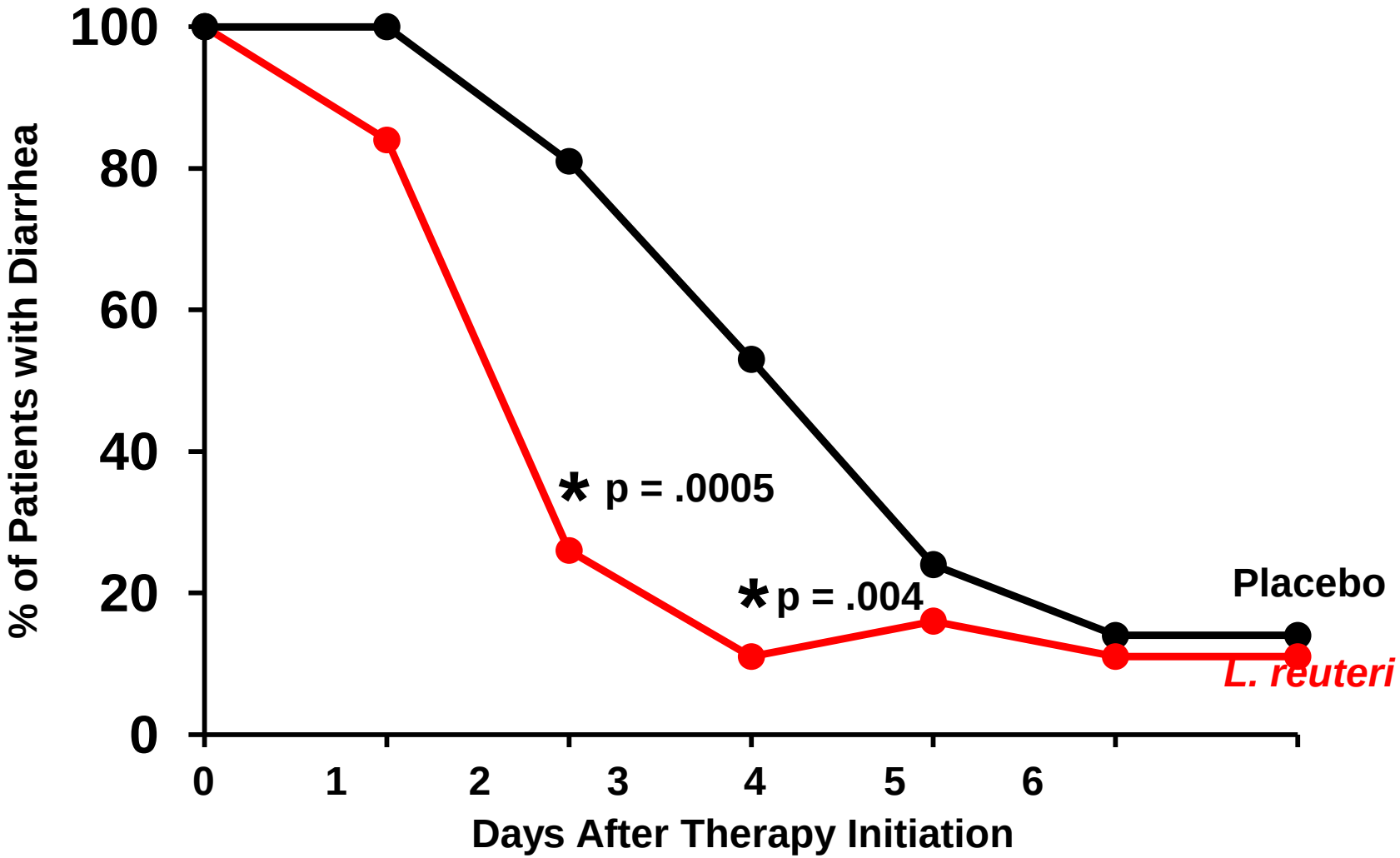
Mechanisms of Immune Enhancement

- 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

Geoff Preidis

Can probiotics reduce diarrheal disease burden in children?

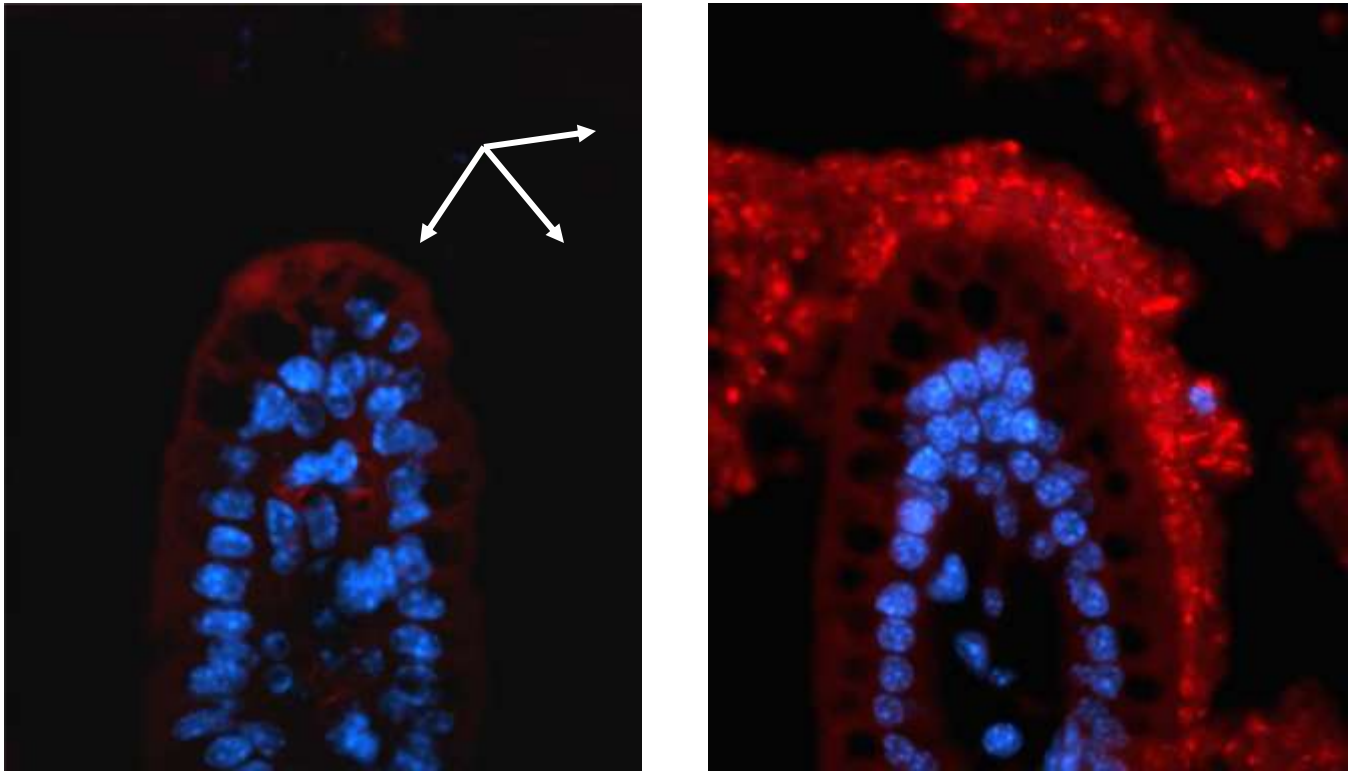
L. reuteri reduces the duration of rotaviral diarrhea in infants



n = 20

Shornikova et al. *J Pediatr Gastroenterol Nutr* 1997;24(4):399-404.

Intestinal Bacteria in a Neonatal Mouse Model



(Geoff Preidis)

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

Experimental Design: Testing *L. reuteri* with mouse rotavirus

5 days old

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

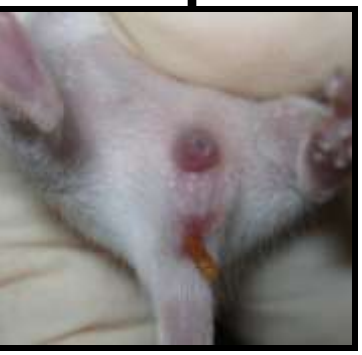
-3 -2 -1 0 1 2 3 4 5 6 7 dpi

Rotavirus
10^3 ID_{50}

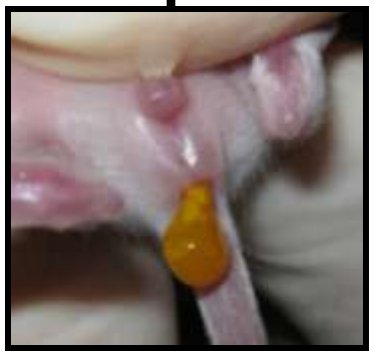
Diarrhea

15 days old

Clinical Scoring
(0-4, blinded)



Score <2
No Diarrhea



Score 2+
Diarrhea

Intestine
Mesenteric Lymph Node
Spleen

FISH

Fragment
Culture

Histology
IHC

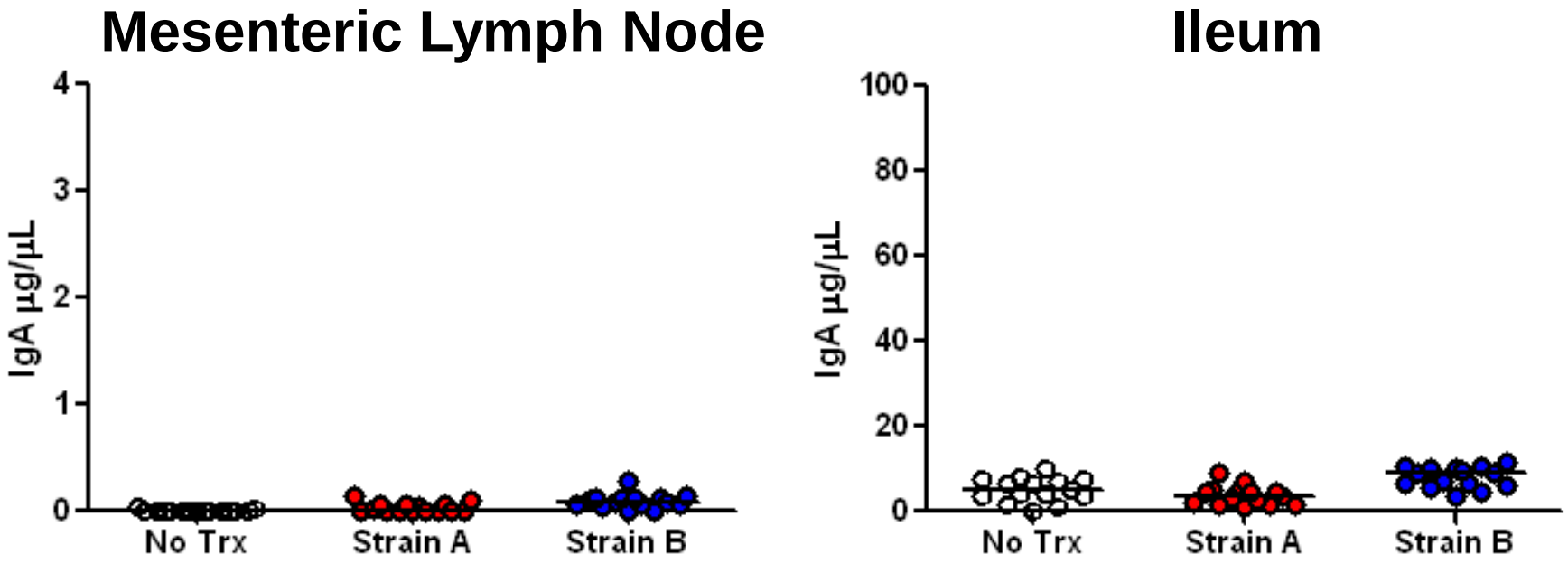
Antibody ELISA

Serum

Antibody ELISA

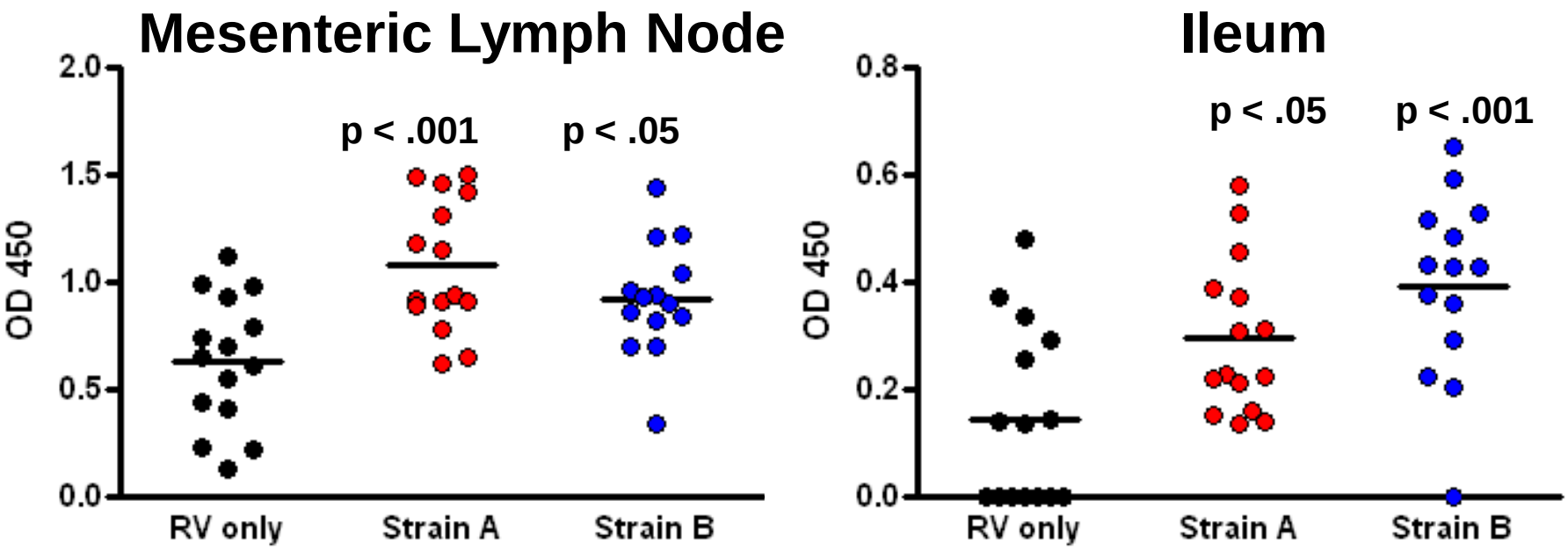
L. reuteri does not enhance mucosal IgA in uninfected mice

Total Non-Specific IgA



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

Rotavirus-Specific Antibody



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.
Fukushima et al., Int J Food Microbiol 1998;42(1-2):39-44.

Using BrdU to measure epithelial turnover

5 days old

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

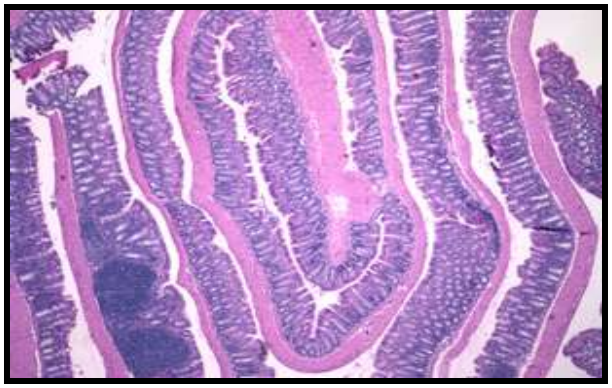
-3 DPI - - - 4 HPI 1DPI 2DPI - 4DPI - 6DPI

Begin probiotics

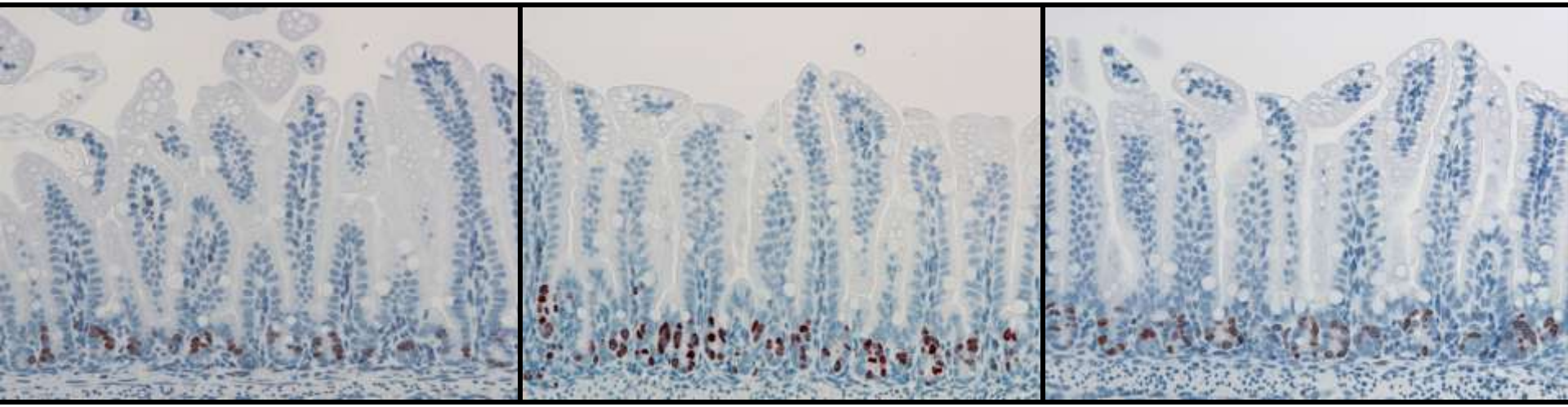
BrdU

30 mg/kg i.p.

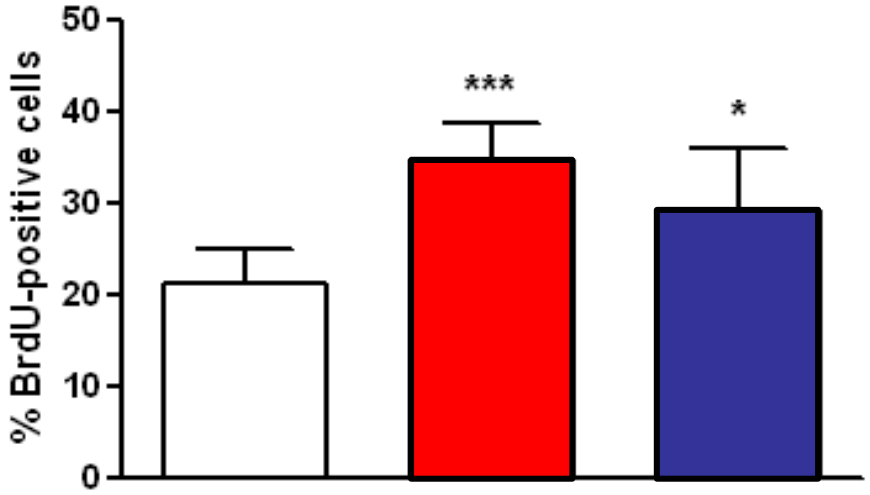
14 days old



Probiotics increase intestinal epithelial cell proliferation

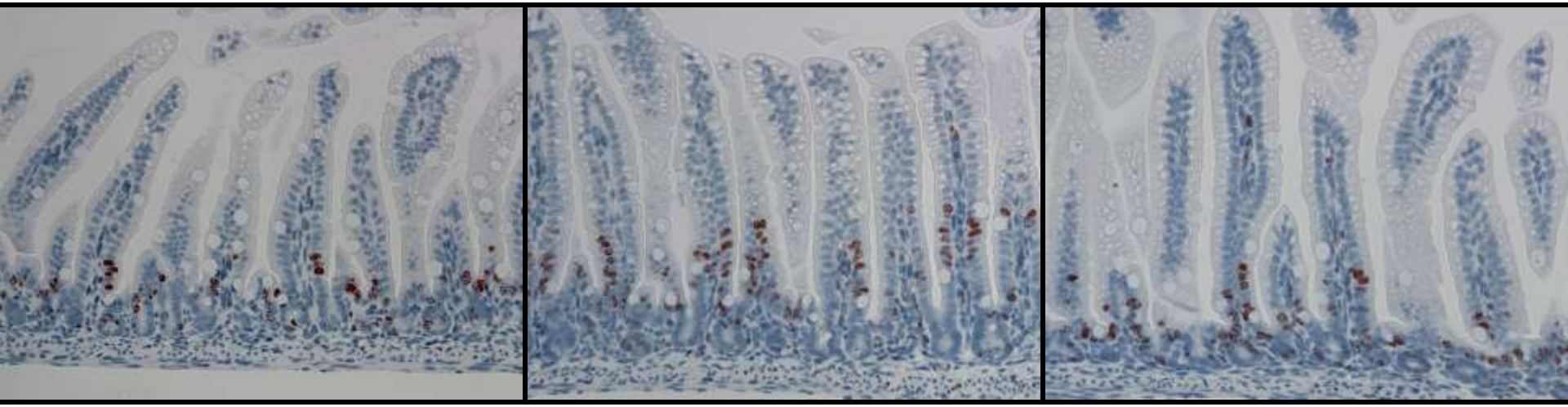


No Treatment **Strain A** **Strain B**

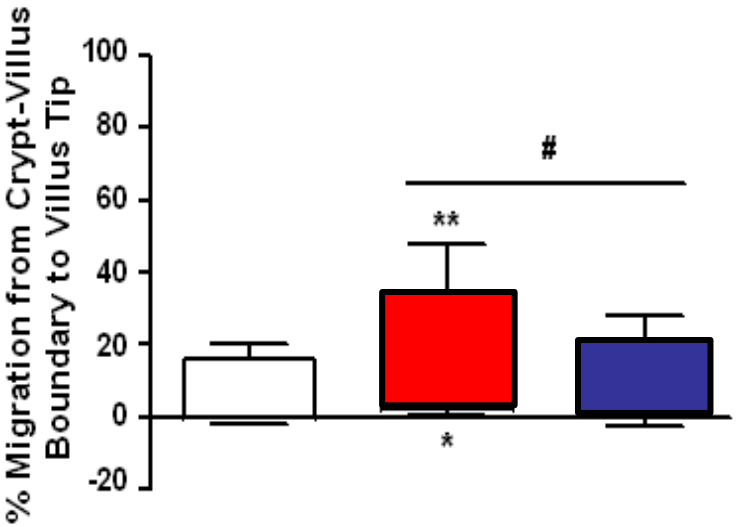


n = 15 mice/group
***p < 0.001, *p < 0.05 vs untreated
Ileum shown

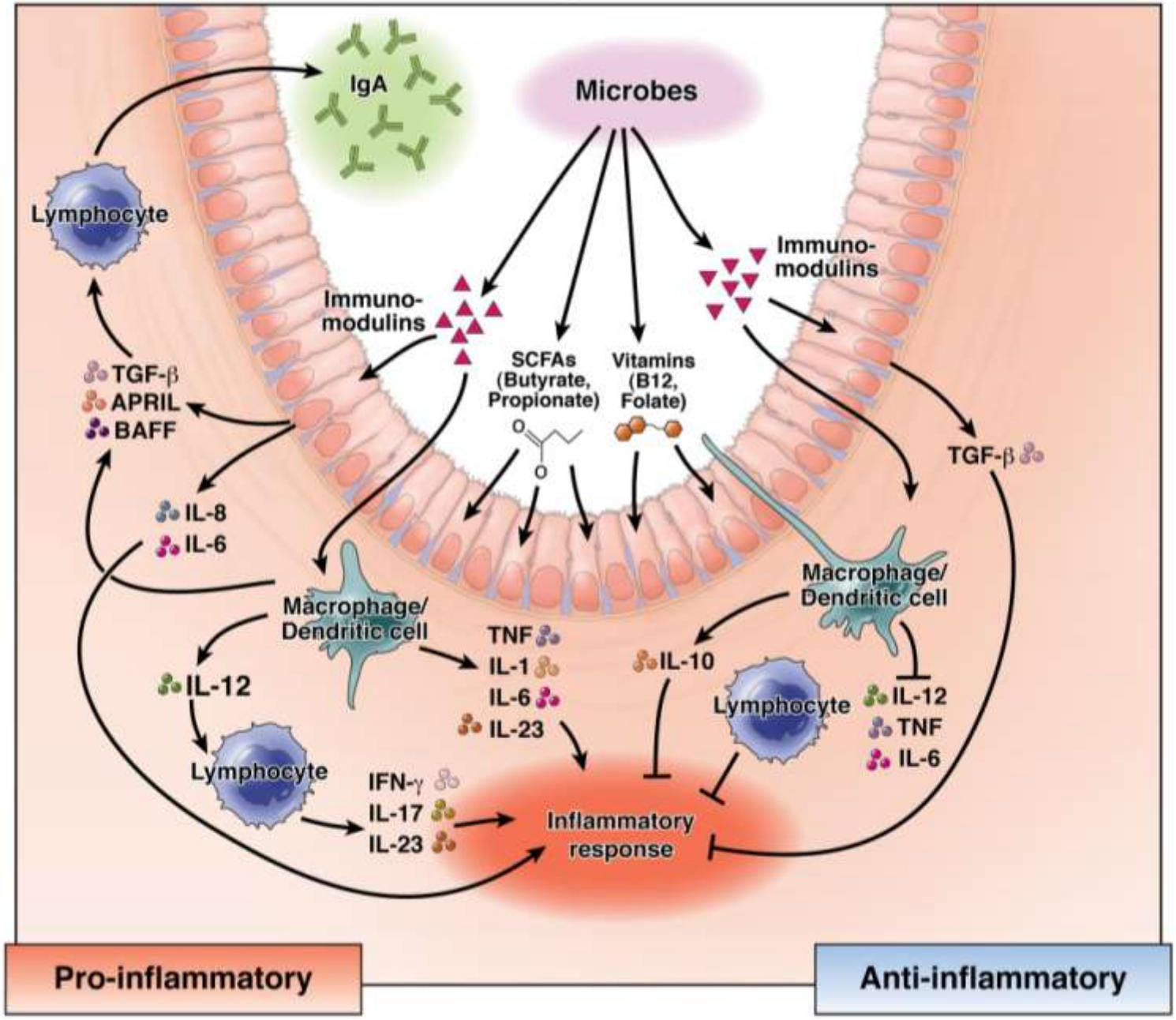
Strain-specific differences in IEC migration are seen at 2 DPI



No Treatment **Strain A** **Strain B**

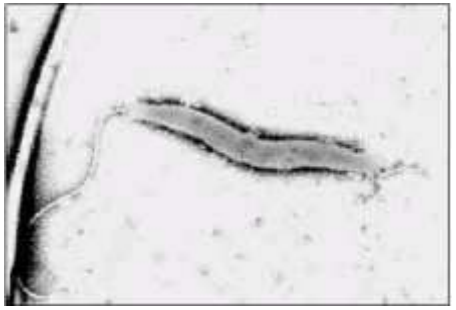


n = 7 mice/group
**p < 0.01, *p < 0.05 vs untreated
#p < 0.01 between strains
Ileum shown



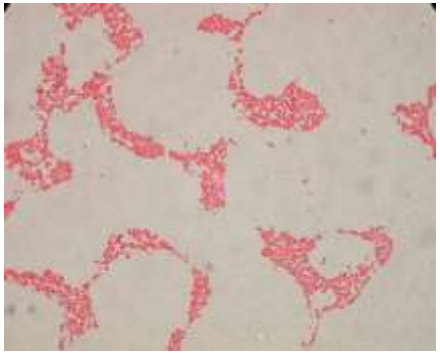
(GA Preidis and J Versalovic. *Gastroenterology* 2009;136:2015-31)

Intestinal Bacterial and Immunological Balance

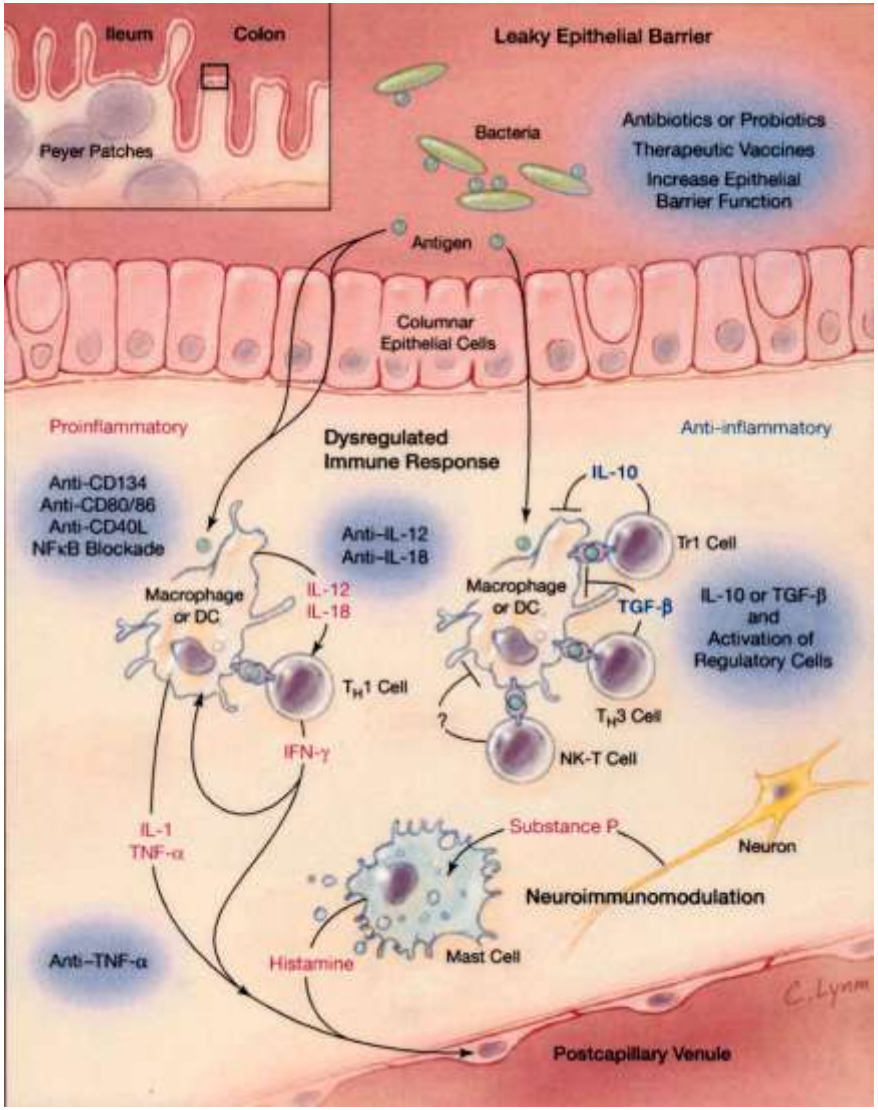


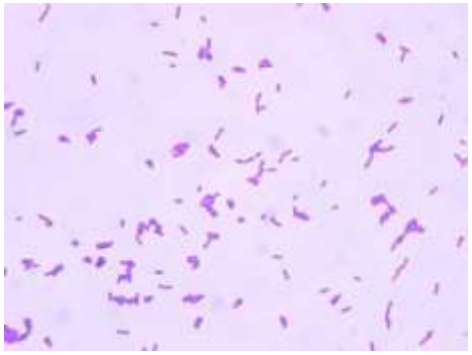
H. hepaticus

PATHOGEN



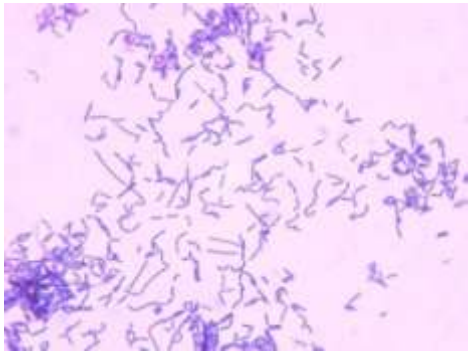
Salmonella sp.





L. reuteri

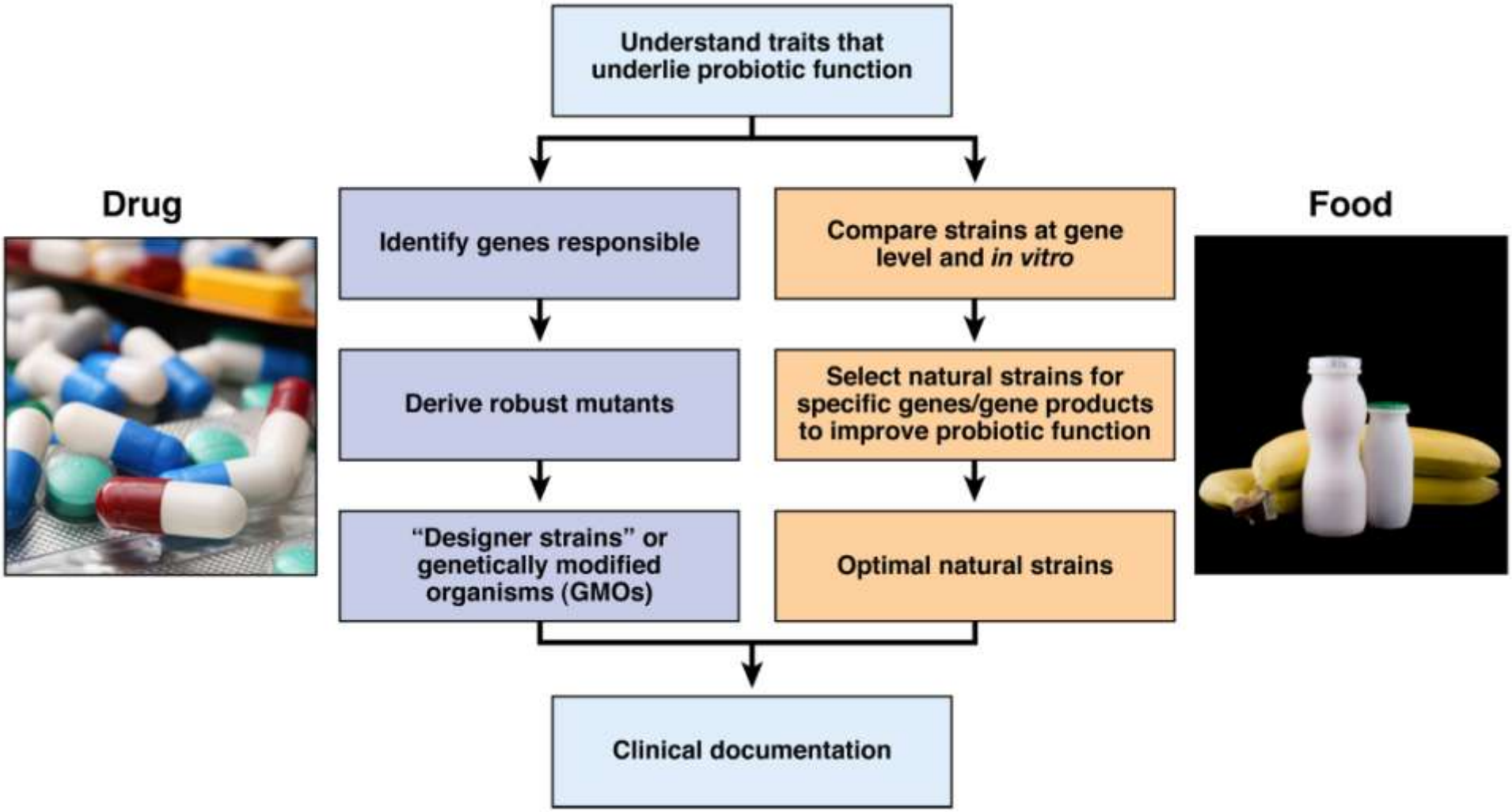
BENEFICIAL



L. paracasei

Blumberg RS and Strober W. 2001. *JAMA* 285: 645

Future Directions



(GA Preidis and J Versalovic. *Gastroenterology* 2009;136:2015-31)

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