The Microbiome and Genetics: The FUT2 Variant and its Role in GI disorders and Chronic Inflammation.

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Senior Functional Medicine Consultant

Michigan Osteopathic Association Spring Conference

May 17, 2019

Workshop Objectives:

- Define gastrointestinal hyperpermeability and its role in chronic inflammation
- Understand the role FUT2 variant and its relationship to multiple conditions
- Treatment considerations to influence gut bacteria

Gastrointestinal complaints are among the leading reasons that patients seek health care.

"Over 95 million Americans experience some kind of digestive problem…total health care costs exceed $40 billion annually."

American College of Gastroenterology

Common approaches may alleviate symptoms...

such as occasional indigestion, occasional heartburn, abdominal cramping, urgent bowel movements,
or occasional
constipation.

They may only temporarily mask the underlying issues and have potential adverse effects.

IBS, IBD, Crohn's, Ulcerative Colitis

- As many as 20 percent of the adult population, or one in five Americans, have symptoms of IBS, making it one of the most common disorders diagnosed by doctors.
- Irritable bowel syndrome may be a lifelong condition.
- For some people, symptoms are disabling and reduce the ability to work, travel, and attend social events.

http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0001292/#adam_000246.disease.prognosis

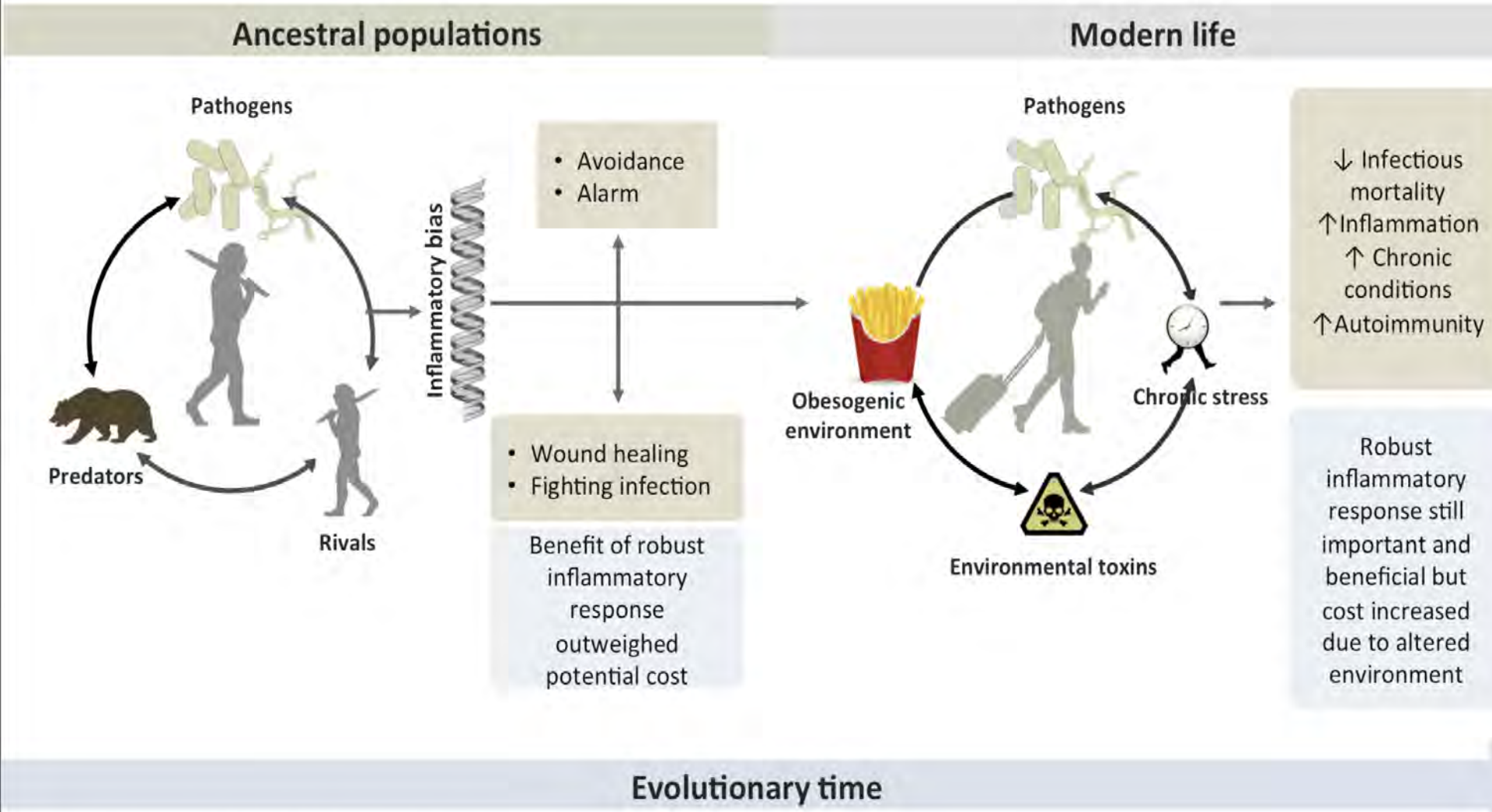
No Known Consensus....

- Crohn's disease and Ulcerative colitis are both major categories of inflammatory bowel diseases (IBD). IBD affects an estimated 1.6 million Americans.
- **What Causes Inflammatory Bowel Disease?**
  - IBD is a disease with an unknown cause. Some agent or a combination of agents -- bacteria, viruses, antigens -- triggers the body's immune system to produce an inflammatory reaction in the intestinal tract.

M'Koma, A. 2013 Aug; 6: 33-47. PMID: 24833941

Inflammation: Friend or Foe?

Inflammation is critical for survival, but excessive inflammation is linked with disease.



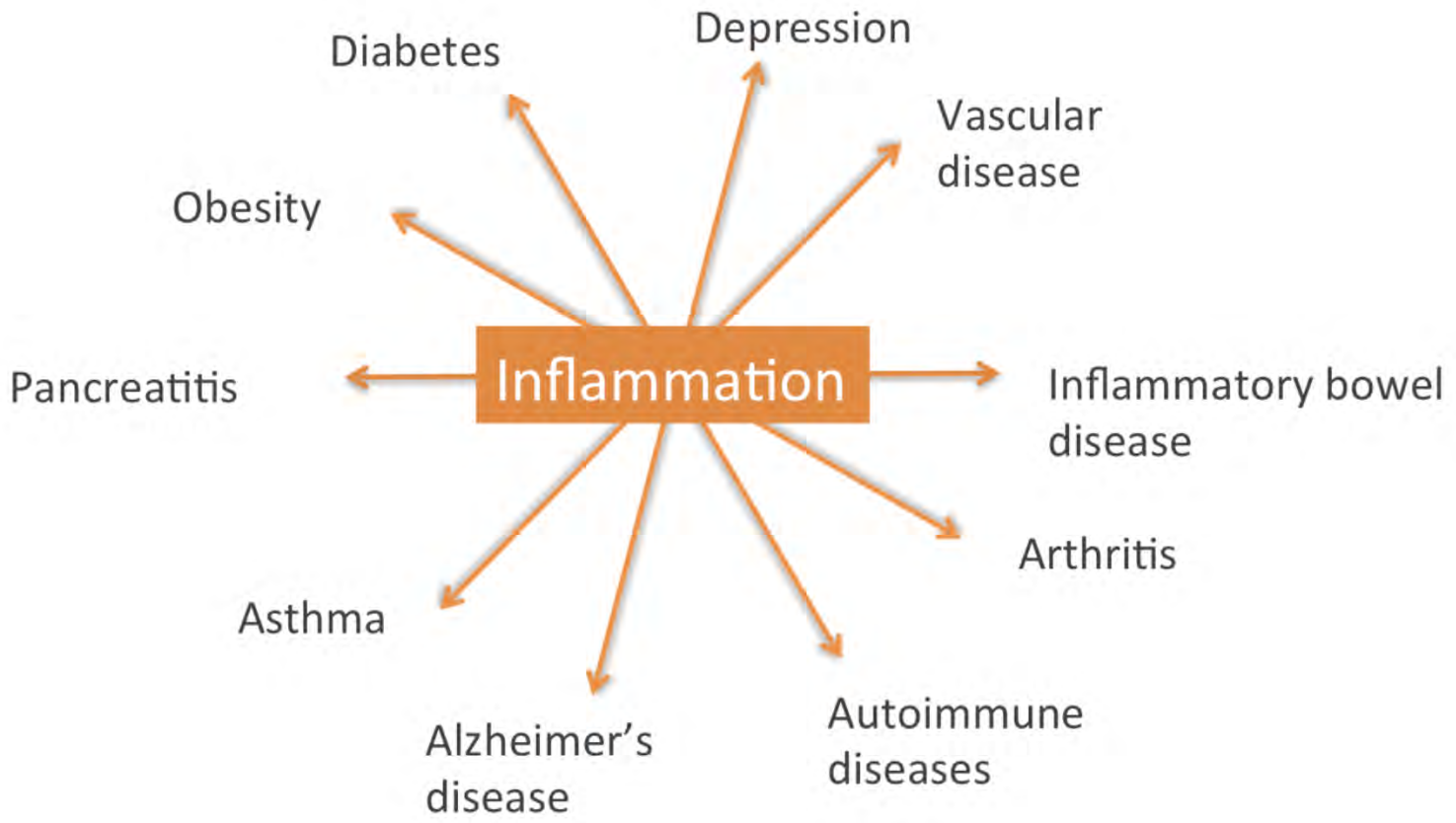
Adapted from: Miller AH, Raison CL. *Nat Rev Immunol*. 2016;16(1):22–34.

Inflammation & Pain: Key Regulator of Gene Expression

- Nuclear factor-kappa B (NF-κB)
  - Protein complex that controls DNA transcription/genetic expression
  - When activated, controls/regulates the expression of ~500 genes, including:
    - Pro-inflammatory enzymes: PLA_2, COX-1, COX-2, LOX
    - Pro-inflammatory cytokines: IL-1β, IL-6, IL-12, TNFα
    - Chemokines: CXCL8 (aka IL-8), MCP-1

Shih R et al. *Front Mol Neurosci.* 2015;8:77.

Uncontrolled Chronic Inflammation Is Linked to Many Chronic Diseases



Serhan CN et al. *Nat Immunol*. 2005;6(12):1191-1197.
Nathan C et al. *Cell*. 2010;140(6):871-882.

World Journal of Gastroenterology

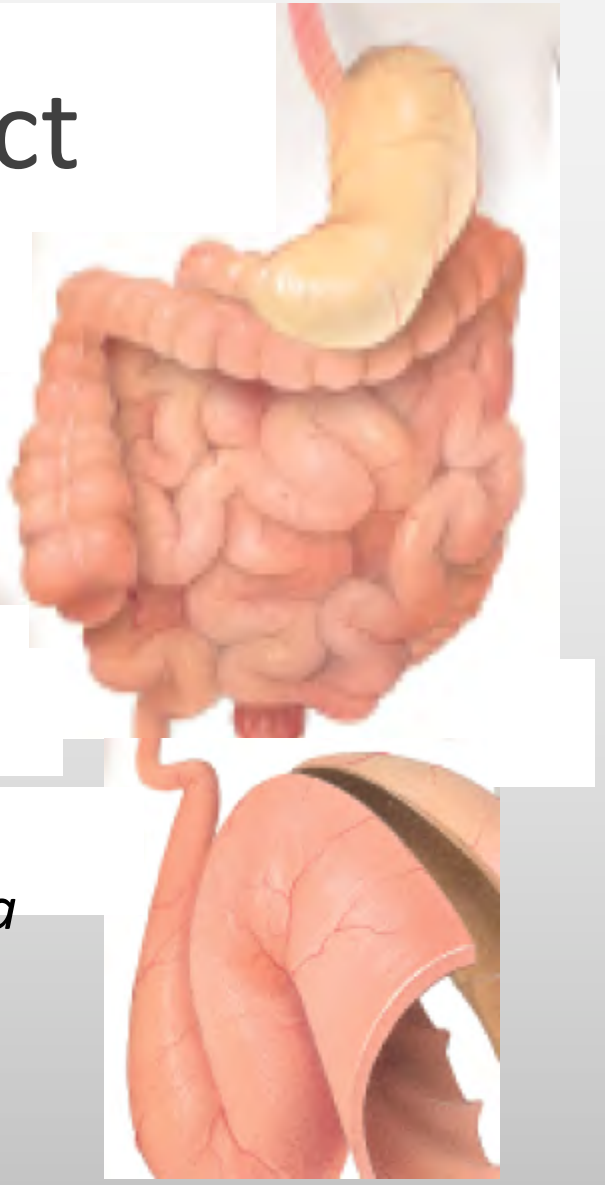
- Patients with inflammatory bowel disease have increased risk of autoimmune and inflammatory diseases.

- Chronic, noncommunicable, and inflammation-associated diseases remain the largest cause of morbidity and mortality globally and within the United States.

World J Gastroenterol. 2017 Sep 7; 23(33): 6137–6146.Published online 2017 Sep 7. doi: 10.3748/wjg.v23.i33.6137

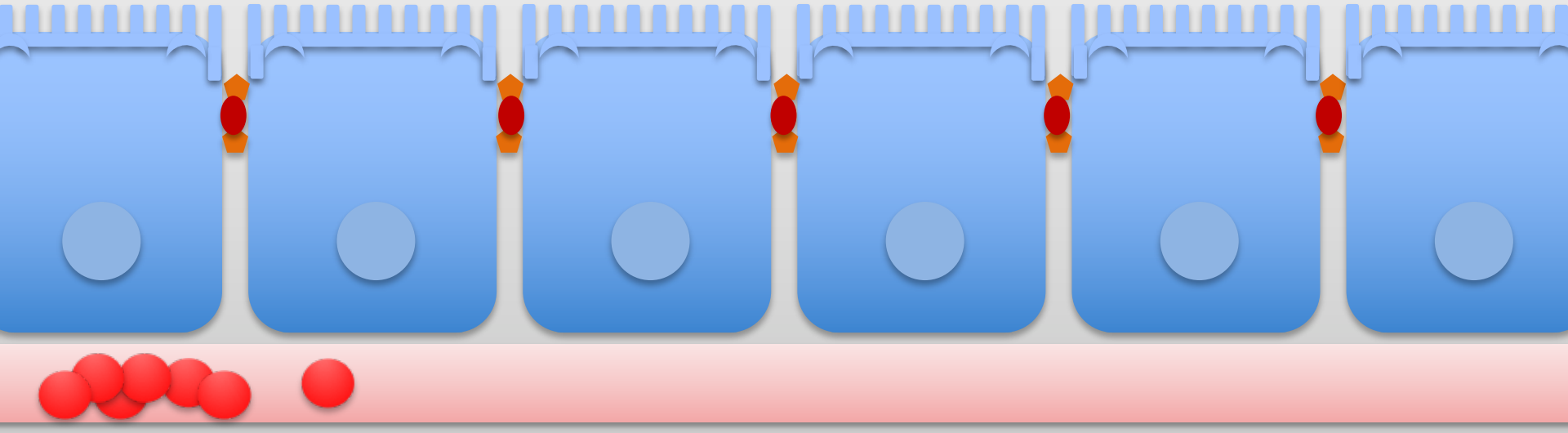
The Gastrointestinal Tract

- A 15 foot tube running through the body from mouth to anus where food from the stomach passes into the intestine for further digestion.
- Intestinal wall contains hundreds of different species of healthy and unhealthy bacteria numbering in the billions!
  - **Healthy:** *L. acidophilus* and *Bifidobacteria*
  - **Unhealthy:** Pathogenic bacteria and fungus
- **Normalization of gut function results in improved clinical outcomes across many diverse diseases.**



Normal Intestinal Permeability

Glucose



Transcellular Pathway

NSAIDs

Poor diet

Poor digestion

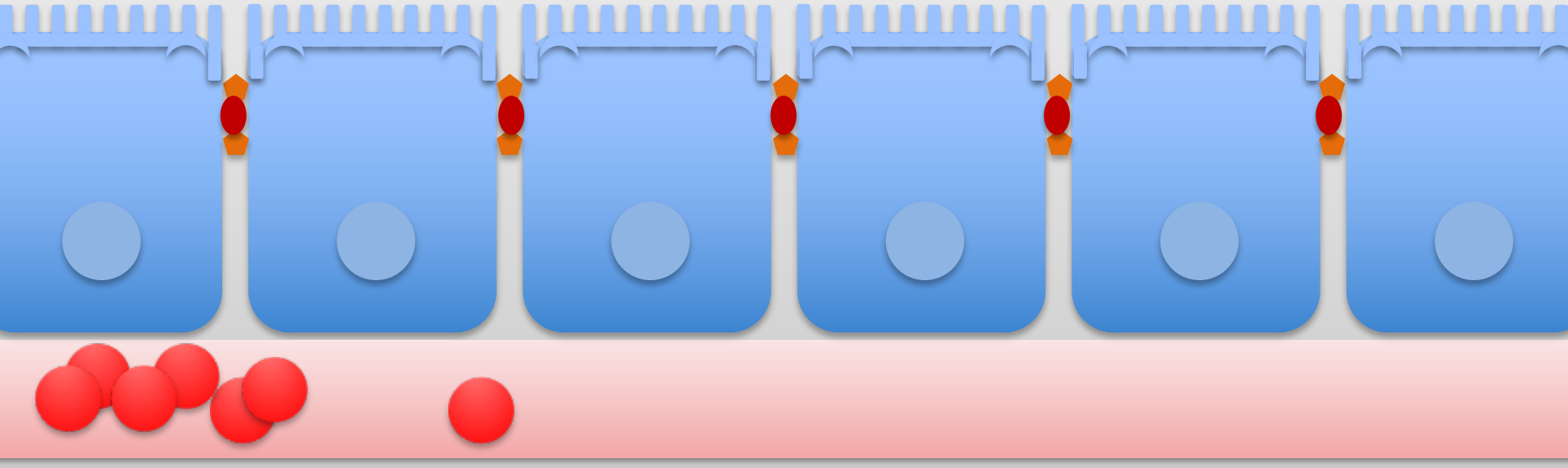
Alcohol

Infections

Dysbiosis

Antibiotics

Toxins



Inflammation
Food allergies & Intolerances
Immune system dysregulation

REVIEW

www.nature.com/clinicalpractice/gasthep

Mechanisms of Disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases

Alessio Fasano*

SUMMARY

The primary functi
perceived to be limit
electrolytes, and to v
and functional arra
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trafficking of macro
a barrier mechanism
neuroendocrine net
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REVIEW
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Correspon
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afasano@mbrc.umaryland.edu

Received 7 April 2005 Accepted 26 July
www.nature.com/clinicalpractice
doi:10.1038/ncpgasthep0259

Major Role of the Gastrointestinal Tract is
To act as a barrier to finely regulate the trafficking of macromolecules between the external (food/microbes) and internal environment (systemic, cells, tissues, etc).

When this complex **barrier is broken**, foreign macromolecules can enter, interact with the immune system, and result in an inflammatory response which **can lead to a multitude of local intestinal and systemic extraintestinal diseases.**

Fasano and Shea-Donohue, Nature Clinical Practice Gastroenterology & Hepatology September 2005 vol 2 no 9

What is *"Leaky Gut"?*

- **Leaky gut syndrome** is a condition that affects your digestive system.
- Your intestines have tight junctions, or small gaps, that allow nutrients and water to pass into your bloodstream.

What is *"Leaky Gut"?*

In **leaky gut syndrome**, these tight junctions loosen, potentially allowing harmful substances like bacteria, toxins, and undigested food particles to enter the bloodstream.

Cell. Mol. Life Sci.
DOI 10.1007/s00018-012-1070-x

Cellular and Molecular Life Sciences

REVIEW

Regulation of intestinal epithelial permeability by tight junctions

Takuya Suzuki

Received: 3 April 2012/Revised: 19 June 2012/

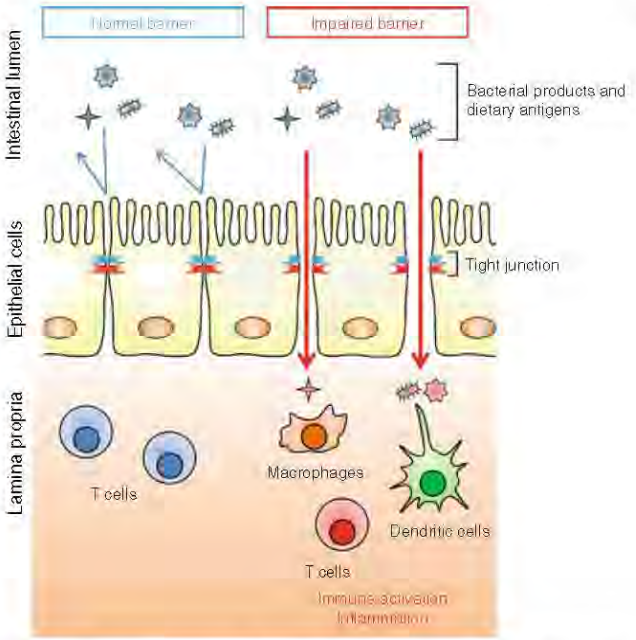
Abstract The gastrointestinal epitheli
boundary between the body and external
effectively provides a selective permeat
limits the permeation of luminal noxious
as pathogens, toxins, and antigens, whi
appropriate absorption of nutrients and w
tive permeable barrier is achieved by in
junction (TJ) structures, which regulate p
meability. Disruption of the intestinal TJ b
by permeation of luminal noxious molec
perturbation of the mucosal immune syst
mation, and can act as a trigger for the
intestinal and systemic diseases. In this
effort has been taken to understand the re
lular factors, including cytokines, patho
factors, for the regulation of the intestinal
I discuss the regulation of the intestinal TJ
with its implications for the pathogenesis

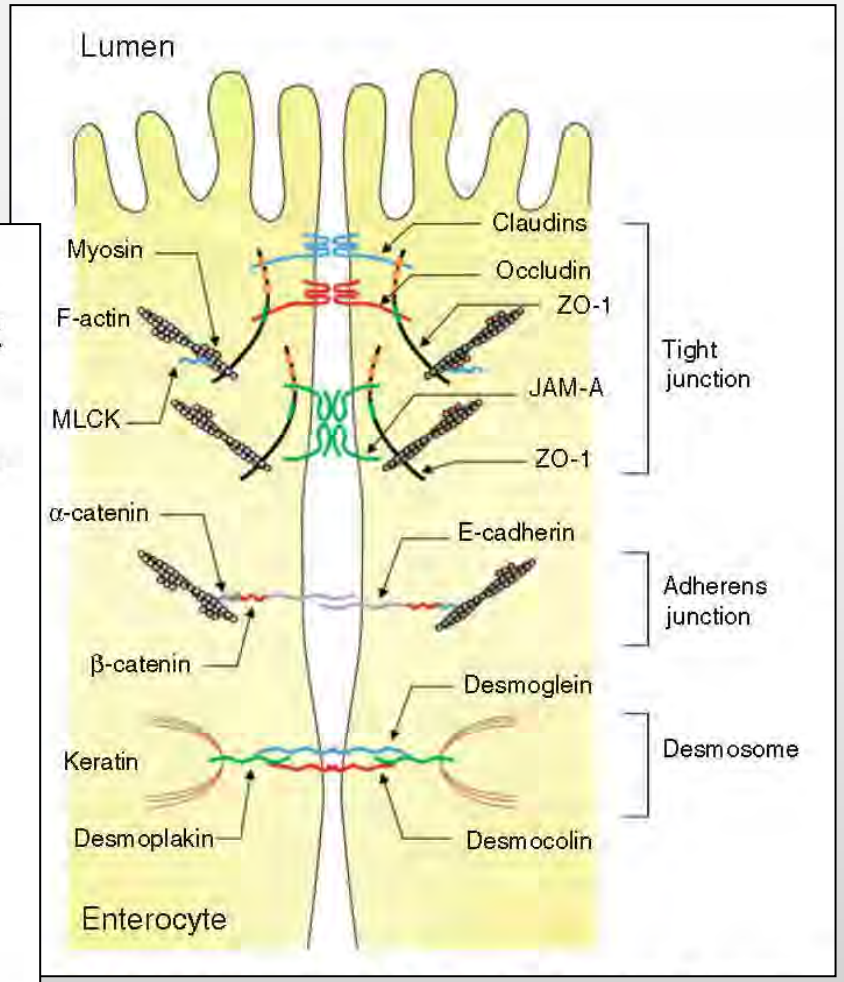
Keywords Tight junction · Intestinal epi
Cytokine · Pathogen · Nutrient

Abbreviations

AJ	Adherens junction
ALD	Alcoholic liver disease

T. Suzuki





"Disruption of the intestinal TJ barrier, followed by permeation of luminal noxious molecules, induces a perturbation of the mucosal immune system and inflammation, and can act as a trigger for the development of intestinal and systemic diseases."

Suzuki T Cell Mol Life Sci 2012; DOI 10.1007/s00018-012-1070-x

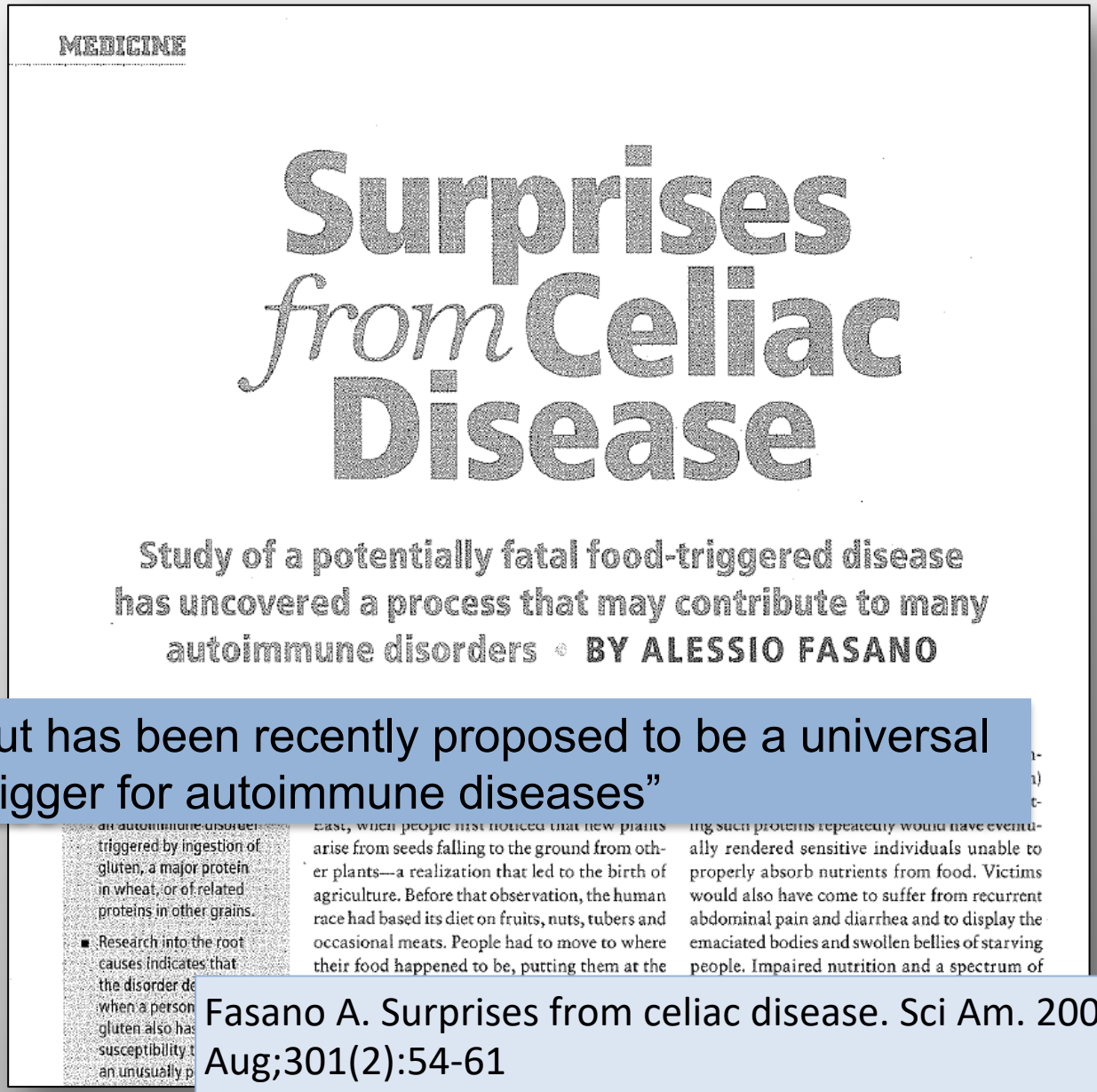
Why is *"Leaky Gut" a Concern?*

Leaky gut is associated with numerous acute and chronic diseases:

- Chronic inflammatory response syndrome (CIRS)
- Inflammatory bowel disease
- Type-1 diabetes
- Autoimmune disease
- Allergies
- Asthma
- Arthritis
- Liver disease
- Obesity
- Metabolic syndrome

Qinghui Mu, Jay Kirby, Christopher M. Reilly, Xin M. LuoFront Immunol. 2017; 8: 598. Published online 2017 May 23. doi

Gluten sensitivity may be an underlying factor as well..



MEDICINE

Surprises from Celiac Disease

Study of a potentially fatal food-triggered disease has uncovered a process that may contribute to many autoimmune disorders • BY ALESSIO FASANO

triggered by ingestion of gluten, a major protein in wheat, or of related proteins in other grains.

- Research into the root causes indicates that the disorder de... when a person... gluten also has... susceptibility t... an unusually p...

East, when people first noticed that new plants arise from seeds falling to the ground from other plants—a realization that led to the birth of agriculture. Before that observation, the human race had based its diet on fruits, nuts, tubers and occasional meats. People had to move to where their food happened to be, putting them at the

...ing such proteins repeatedly would have eventually rendered sensitive individuals unable to properly absorb nutrients from food. Victims would also have come to suffer from recurrent abdominal pain and diarrhea and to display the emaciated bodies and swollen bellies of starving people. Impaired nutrition and a spectrum of

"A leaky gut has been recently proposed to be a universal initiating trigger for autoimmune diseases"

Fasano A. Surprises from celiac disease. Sci Am. 2009 Aug;301(2):54-61

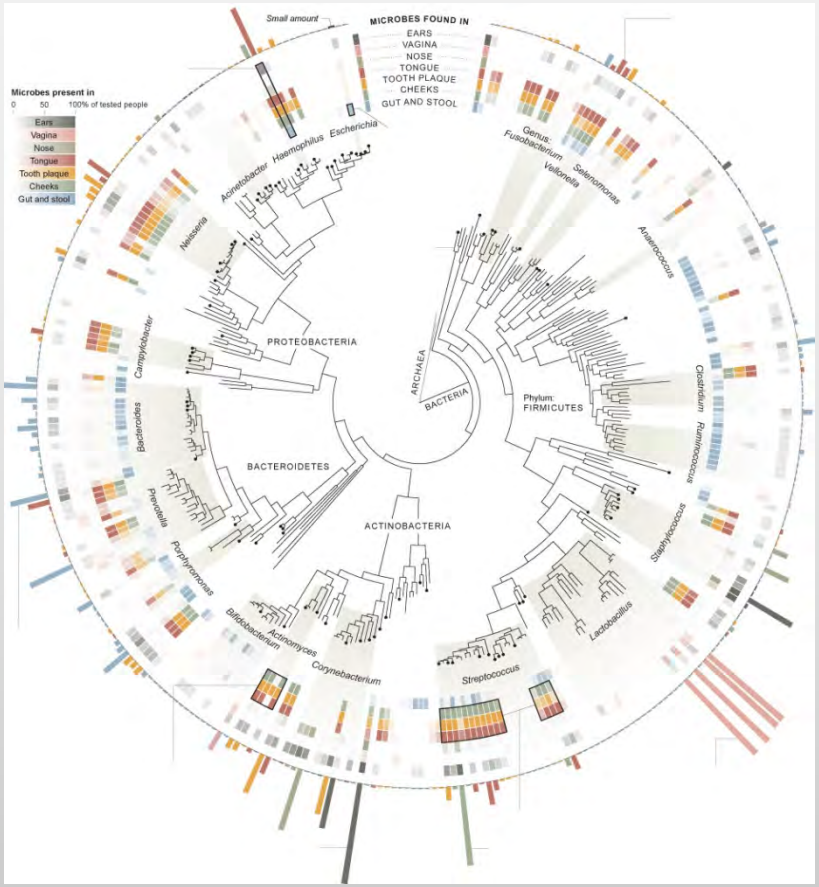
- **107,000 hospitalizations**
- **16,500 NSAID-related annual deaths**
- **15th most common cause of death in the United States**
- **80% of patients have no reliable warning signs**
- **Intestinal injury can begin within 72 hours of use**

Wolfe, M. MD, et al, The New England Journal of Medicine, June 17, 1999, Vol. 340, No. 24, pp. 1888-1889

Consumer Health Alert:

FDA Highlights the Dangers of Acetaminophen and Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

Human Microbiome Project



Microbes exist on all body surfaces: skin, oral cavity, vagina, gut.

The human microbiome is diverse (The gut contains thousands of species alone).

Dysbiosis or microbial imbalance may play a role in immunological, metabolic and neurological diseases.

The Human Microbiome Project has led to the finding that health conditions are in part defined and influenced by specific microbiota.

http://www.nytimes.com/interactive/2012/06/19/science/0619-microbiome.html?_r=0

http://www.nytimes.com/2013/05/19/magazine/say-hello-to-the-100-trillion-bacteria-that-make-up-your-microbiome.html?pagewanted=all

Establishment of the Gut Microbiota

At birth, the GI tract and immune system are immature

Colonization with microbes builds after birth

Once the microbiota are established around age 2-3, the community is relatively stable over time within an individual

- Palmer C, Bik EM, DiGiulio DB, et al. Development of the human infant intestinal microbiota. *PLoS Biol*. 2007;5(7):1556-1573.
- Faith JJ, Guruge JJL, Charbonneau M, et al. The long-term stability of the human gut microbiota. *Science*. 2013;341(6141):1237439.

Initial Colonization of the Gut

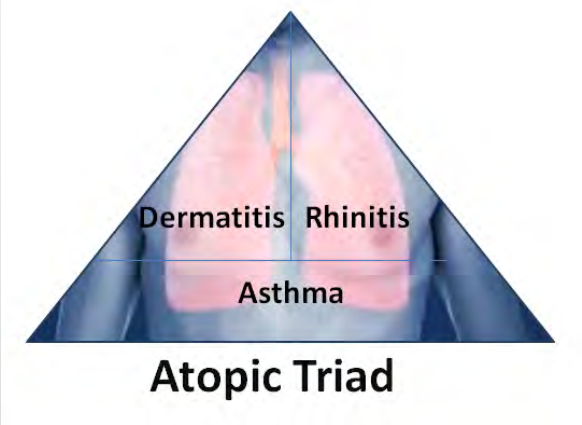
- Factors affecting initial colonization of the gut in infants:
  - Gestation length
  - Mode of delivery
  - Feeding practices
  - Antibiotic exposures
  - Birth order

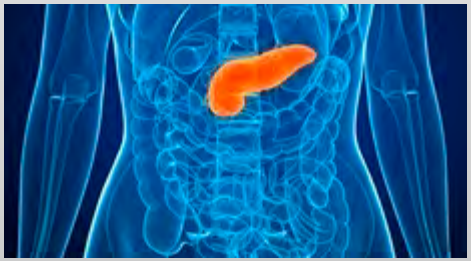
- Negative influences on the establishment of a healthy gut microbiota and factors that *decrease* microbial diversity in infants and young children can have lifelong detrimental health impacts.

- Matamoros S, Gras-Leguen C, Le Vacon F, et al. Development of intestinal microbiota in infants and its impact on health. *Trends Microbiol*. 2013;21:167-73.
- Adlerberth I, Wold AE. Establishment of the gut microbiota in Western infants. *Acta Paediatr*. 2009;98:229-38.

Mode of Delivery - Long Term Sequelae

- Children born by C-section are at increased risk of developing:
  - Conditions of the “Atopic Triad”
    - Atopic dermatitis
    - Allergic rhinitis
    - Asthma
    - Type 1 diabetes





- Penders J, Thijs C, van den Brandt PA, et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut*. 2007;56:661-7.
- Thavagnanam S, Fleming J, Bromley A, et al. A meta-analysis of the association between Caesarean section and childhood asthma. *Clin Exp Allergy*. 2008;38:629-33.
- Cardwell CR, Stene LC, Joner G, et al. Caesarean section is associated with an increased risk of childhood-onset type 1 diabetes mellitus: a meta-analysis of observational studies. *Diabetologia*. 2008;51:726-35.

Feeding Practices - Long Term Sequelae

- Formula fed infants are at higher risk of:
- Atopic dermatitis
- Pediatric inflammatory bowel disease (Ulcerative colitis, Crohn's disease).
- Type 2 diabetes later in life
- Obesity later in life

- Penders J, Thijs C, van den Brandt PA, et al. Gut microbiota composition and development of atopic manifestations in infancy: the KOALA Birth Cohort Study. *Gut*. 2007;56:661-7.
- Le Huërou-Luron I, Blat S, Boudry G. Breast- v. formula-feeding: impacts on the digestive tract and immediate and long-term health effects. *Nutr Res Rev*. 2010;23(1):23-36.

Research-Proven Benefits of Probiotics

- **Improved GI function**
  - **Better nutrient absorption**
  - **Reduced constipation, gas & bloating**
- **Reduce harmful bacteria**
- **Improved immune function: less sickness**
- **Reduced allergies**
- **Reduced urogenital infections**
- **Improved lactose digestion**

EMBO reports

reviews

The gut flora as a forgotten organ

Ann M. O'Hara[1] *& Fergus Shanahan*[1,2+]

Alimentary Pharmabiotic Centre, University College Cork, National University of Ireland, Cork, Ireland

The intestinal microflora
influences the normal str
the mucosal immune syst
dent intestinal microflo
immunosensory capacity
pathogenic bacteria. In g
components of the flora c
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matory bowel diseases. It
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to a virtual organ within a
the conditioning influence
and immune responses
improved understanding
that are relevant to humar
inflammatory and neoplastic

Keywords: commensal bacteri
EMBO reports (2006) 7, 688–693. doi:10

"Pharmabiotic is a generic term to encompass any form of therapeutic exploitation of the commensal flora, including the use of live probiotic bacteria, probiotic-derived biologically active metabolites, prebiotics, synbiotics or genetically modified commensal bacteria."

"Manipulation of the flora is becoming a realistic therapeutic and prophylactic strategy for many infectious, inflammatory and even neoplastic diseases within the gut."

O'Hara A et al. EMBO reports 2006 7(7) 688-692

Introduction

Host–microbe interactions
faces, and one of the larges
mucosa. The intestine is adapted to bi-directional host–flora exchange and harbours a diverse bacterial community that is separated from the internal milieu by only a single layer of epithelial cells. Resident bacteria outnumber human somatic and germ cells tenfold and represent a combined microbial genome well in

face-adherent and luminal microbial populations also differ (Eckburg *et al*, 2005), and the ratio of anaerobes to aerobes is lower at the mucosal surfaces than in the lumen.

The fetal gut is sterile but colonization begins immediately after birth and is influenced by the mode of delivery, the infant diet,

Quality assurance criteria for probiotic bacteria[1–4]

Elina Tuomola, Ross Crittenden, Martin Playne, Erika Isolauri, and Seppo Salminen

ABSTRACT Acid and bile
adhesion properties are among t
microbes. The quality control o
tionally has relied solely on test
ber of viable bacteria are preser
shelf lives. Viability is an impor
rion for quality assurance. To b
retain the functional health cha
originally selected. Such charac
vive transit through the stomacl
nize the human gastrointestinal
readily adopted to examine the
tolerate acidic conditions, surviv
and metabolize selective substra
available to examine strain sta
may be an important quality-cor
rier effects. Adhesion has been
of diarrhea, immunogenic effe
other health effects. Adhesion p
itored, including adhesion to
human intestinal mucus. This a
testing that can be used to ensur
biotic strains. *Am J Clin Nu*

KEY WORDS Probiotics,
stability, viability

"The initial screening and selection of probiotics includes testing of the following important criteria: phenotype and genotype stability, including plasmid stability; carbohydrate and protein utilization patterns; acid and bile tolerance and survival and growth; bile metabolism; intestinal epithelial adhesion properties; production of antimicrobial substances; antibiotic resistance patters; ability to inhibit know gut pathogens, spoilage organisms, or both; and immunogenicity."

Tuomola E et al AJCN 2001;73(suppl):393S-398S

INTRODUCTION

Probiotics are viable bacteria that beneficially influence the health of the host (1, 2). Probiotic bacteria selected for commercial use in foods and in therapeutics must retain the characteristics for which they were originally selected (1–3). These include characteristics for growth and survival during manufacture and, after consumption, during transit through the stomach and small intestine. Importantly, probiotics must retain the

(6). All of these in vitro systems provide valuable information on the ability of probiotics to adhere and colonize the intestine.

Adhesion to colonic or intestinal biopsy samples, if possible, should be considered as a final in vitro adhesion test that would be most like the in vivo situation. Not only would this be a better approximation of the in vivo situation, it would allow for the study of adhesion to different parts of the intestine. This is especially important regarding immune stimulation by oral administration of probiotics.

Adhesion is also considered important for stimulation of the immune system. Adhesion to M cells or Peyer's patches may

Adv Biochem Engin/Biotechnol (2008) 111: 1–66
DOI 10.1007/10_2008_097

Published online: 7 May 2008

Probiotics, Prebiotics, and Synbiotics

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Determining a probiotic

"defined viable microorganisms, sufficient amounts of which reach the intestine in an active state and thus exert positive health effects."

de Vrese *et al. Adv Biochem Engin/Biotech* 111:1-66, 2008

- Found in humans
- "defined"
- "viable"
- "sufficient amounts of which reach the intestine in an active state"
- "exert positive health effects."

L. Salivarius UCC118 produce bacteriocins

"Here we demonstrate that *Lactobacillus salivarius* UCC118, a recently sequenced and genetically tractable probiotic strain of human origin, produces a bacteriocin in vivo that can significantly protect mice against infection with the invasive foodborne pathogen Listeria monocytogenes."

Corr, *et al*. 2007 *PNAS* 104;18:7617-7621

Bacteriocin production as a mechanism for the antiinfective activity of *Lactobacillus salivarius* UCC118

Sinéad C. Corr*, Yin Li*†, Christian U. Riedel*, Paul W. O'Toole*, Colin Hill*‡, and Cormac G. M. Gahan*§

*Alimentary Pharmabiotic Centre and Department of Microbiology and §School of Pharmacy, University College Cork, Cork, Ireland

Edited by Todd R. Klaenhammer, North Carolina State University, Raleigh, NC, and approved March 1, 2007 (received for review January 17, 2007)

The mechanisms by which probiotic strains enhance the health of the host remain largely uncharacterized. Here we demonstrate that *Lactobacillus salivarius* UCC118, a recently sequenced and genetically tractable probiotic strain of human origin, produces a bacteriocin *in vivo* that can significantly protect mice against infection with the invasive foodborne pathogen *Listeria monocytogenes*. A stable mutant of *Lb. salivarius* UCC118 that is unable to produce the Abp118 bacteriocin also failed to protect mice against infection with two strains of *L. monocytogenes*, EGDe and LO28, confirming that bacteriocin production is the primary mediator of protection against this organism. Furthermore, *Lb. salivarius* UCC118 did not offer any protection when mice were infected with a strain of *L. monocytogenes* expressing the cognate Abp118 immunity protein AbpIM, confirming that the antimicrobial effect is a result of direct antagonism between *Lb. salivarius* and the pathogen, mediated by the bacteriocin Abp118.

infection | *Listeria* | probiotic

The gastrointestinal microbiota presents a significant barrier that must be overcome for a pathogen to initiate an infection. The concept of preventing or ameliorating intestinal infections through dietary interventions designed to manipulate commensal bacteria, or as a means of introducing transiently colonizing probiotic strains, has received much attention in recent years. Such strategies could potentially decrease antibiotic use and associated problems of antimicrobial resistance. Probiotic organisms (live bacteria that have a beneficial effect on the host when consumed in adequate amounts) have been proposed to play roles in improving digestive function, in the reduction of chronic inflammation, and in hastening recovery from intestinal disease (1–3). Studies using rodent models of infection have demonstrated a role for probiotics in the amelioration of infections caused by *Helicobacter pylori*, *Citrobacter rodentium*, and *Salmonella typhimurium*, and human trials have confirmed that the consumption of probiotic cultures can play a role in the eradication of *H. pylori* in infected patients (4–6). *In vitro* studies have indicated that the regulation of mucus production by probiotics can prevent colonization by enteropathogenic *Escherichia coli*, and there is an apparent correlation between probiotic-derived immunomodulation and the elimination of foodborne pathogens (7, 8). Although the health benefits associated with use of probiotic bacteria are well documented, their mechanistic basis remains largely unclear, and *in vivo* identification of the precise mechanistic basis of these beneficial effects remains a significant goal.

Bacteriocins are a heterogeneous family of small, heat-stable peptides with potent antimicrobial activity that are produced by many bacterial species, including many probiotic strains (9). Bacteriocins produced by Gram-positive bacteria have a bactericidal or bacteriostatic effect on other species and genera, but activity is usually limited to other Gram-positives (10). Bacteriocins have been used by the food industry to reduce the use of chemical preservatives in foods with limited shelf life, or those foodstuffs that present a high risk for pathogen contamination (11).

Lactobacillus salivarius UCC118 is a genetically well characterized strain that produces a potent broad-spectrum class II bacteriocin, Abp118, which is active against *Listeria monocytogenes* (12, 13). Abp118 is regulated by a quorum-sensing mechanism, with bacteriocin production peaking in early stationary phase cultures in response to the accumulation of an induction peptide, AbpIP. In this study we determined that *Lb. salivarius* UCC118 offers protection against *L. monocytogenes* infection in mice. Generation of a nonproducing mutant of *Lb. salivarius*, suggested that Abp118 is the basis of the antilisterial effect in this model system. This was confirmed by constructing an Abp118-immune strain of *L. monocytogenes*, which was able to overcome the protective effect conferred by UCC118.

Results

Probiotic Administration Can Enhance Resistance to Infection by *L. monocytogenes* in Mice. The number of bacteria in the murine liver and spleen 3 days after oral inoculation with *L. monocytogenes* is a well established metric of infection. When A/J mice were orally infected with *L. monocytogenes* EGDe at 2×10^9 CFU per mouse, numbers of $\approx 10^5$ reaching the liver and the spleen by day 3 is typical of a normal infection. When mice were fed a strain of *Lactococcus lactis* (a food fermentation organism not normally associated with the gastrointestinal tract, used as a control in this instance) or one of six probiotic strains (all strains administered at 1×10^9 CFU per mouse per day for 3 days) and subsequently infected with *L. monocytogenes*, *Lb. salivarius* UCC118 significantly reduced the numbers of *Listeria* in both liver and spleen on day 3 after infection (Fig. 1). *Bifidobacterium longum* JCM7050 also provided some protection in both organs, albeit not to the same level as that provided by UCC118. *Bifidobacterium breve* UCC2003 and *Bifidobacterium infantis* CCUG36569 afforded statistically significant protection against listerial splenic infection, but no significant reduction in the numbers infecting the liver was observed.

***Lb. salivarius* UCC118 Reduces *Listeria* Infection in Mice.** Recently, a luciferase-based reporter system was developed to track systemic *Listeria* infection in the murine model (14). We used a derivative of this system, in which the *hly* promoter was translationally fused

Author contributions: S.C.C., Y.L., C.U.R., P.W.O., C.H., and C.G.M.G. designed research; S.C.C. and Y.L. performed research; S.C.C., Y.L., C.U.R., P.W.O., C.H., and C.G.M.G. analyzed data; and S.C.C., Y.L., P.W.O., C.H., and C.G.M.G. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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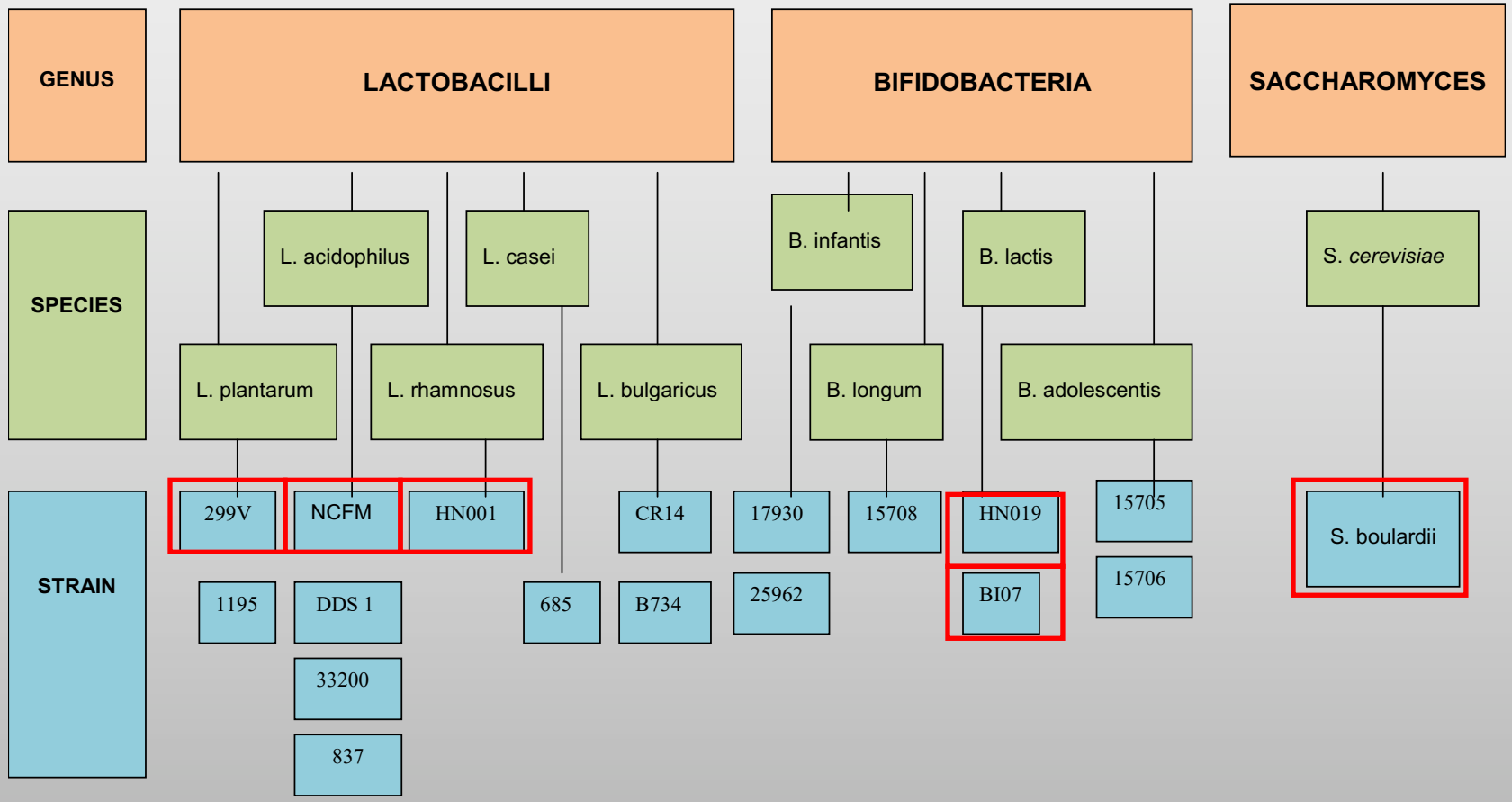
This article contains supporting information online at www.pnas.org/cgi/content/full/0700440104/DC1.

MICROBIOLOGY

Although many strains are commercially available, very few have supporting clinical data

Genus, Species and Strain Characterization



Am J Physiol Gastrointest Liver Physiol 303: G1029–G1041, 2012.
First published September 6, 2012; doi:10.1152/ajpgi.00003.2012.

Mechanism of protection of transepithelial barrier function by *Lactobacillus salivarius*: strain dependence and attenuation by bacteriocin production

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Submitted 4 January 2012; accepted in final form 4 September 2012

Miyauchi E, O'Callaghan J, Buttó LF, Hurley G, Melgar S, Tanabe S, Shanahan F, Nally K, O'Toole PW. Mechanism of protection of transepithelial barrier function by *Lactobacillus salivarius*: strain dependence and attenuation by bacteriocin production. *Am J Physiol Gastrointest Liver Physiol* 303: G1029–G1041, 2012. First published September 6, 2012; doi:10.1152/ajpgi.00003.2012.—Enhanced barrier function is one mechanism whereby commensals and probiotic bacteria limit translocation of foreign antigens or pathogens in the gut. However, barrier protection is not exhibited by all probiotic or commensals and the strain-specific molecules involved remain to be clarified. We evaluated the effects of 33 individual *Lactobacillus salivarius* strains on the hydrogen peroxide (H_2O_2)-induced barrier impairment in human epithelial Caco-2 cells. These strains showed markedly different effects on H_2O_2-induced reduction in transepithelial resistance (TER). The effective strains such as UCC118 and CCUG38008 attenuated H_2O_2-induced disassembly and relocalization of tight junction proteins, but the ineffective strain AH43324 did not. Strains UCC118 and CCUG38008 induced phosphorylation of extracellular signal-regulated kinase (ERK) in Caco-2 cells, and the ERK inhibitor U0126 attenuated the barrier-protecting effect of these strains. In contrast, the AH43324 strain induced phosphorylation of Akt and p38, which was associated with an absence of a protective effect. Global transcriptome analysis of UCC118 and AH43324 revealed that some genes in a bacteriocin gene cluster were upregulated in AH43324 under TER assay conditions. A bacteriocin-negative UCC118 mutant displayed significantly greater suppressive effect on H_2O_2-induced reduction in TER compared with wild-type UCC118. The wild-type strain augmented H_2O_2-induced phosphorylation of Akt and p38, whereas a bacteriocin-negative UCC118 mutant did not. These observations indicate that *L. salivarius* strains are widely divergent in their capacity for barrier protection, and this is underpinned by differences in the activation of intracellular signaling pathways. Furthermore, bacteriocin production appears to have an attenuating influence on lactobacillus-mediated barrier protection.

lactobacilli; barrier integrity; ERK; transepithelial resistance; tight junction

THE INTESTINAL EPITHELIUM consists of a single layer of cells separating the internal milieu from the external microenvironment of the gut lumen. Paracellular transepithelial ingress of antigenic and microbial contents from the lumen is strictly regulated by the apical junctional complexes composed of the tight junction and the adherens junction (62). The tight junction, at the most apical region of the junctional complexes, is a multiprotein assembly composed of transmembrane and cytoplasmic linker proteins. occludin (15), junctional claudin family members proteins such as zonula oc the underlying actin cyto critical role in maintainin

Increased intestinal perm integrity have been obser ders including inflammato Although the pathogenes recent genomewide associ els have suggested that tig tribute to the pathogenes creased intestinal permea their disease activity and i cological and surgical relie production of proinflamm tive species (50) and cytok patients, the latter impairi 47, 60). These factors m expression of tight junct intracellular signaling pat sitide 3-kinase, or NF-κB disturbance in the intesti creased antigen penetratic sponse, the protection of factors is a potential thera

Accumulating evidence ria ameliorate inflammatio effect is currently attribut modulation of the mucosa tivity, and inhibition of ep certain probiotic and com tion dysfunction in vitro a the precise mechanisms b junction integrity, and the terial metabolite(s) respon not been fully clarified.

The properties of diff species vary, and import recognized as being strain specific (4). For example, Meijerink and colleagues (32) demonstrated that different strains of *Lactobacillus plantarum* differentially induced proinflammatory and anti-inflammatory cytokines from dendritic cells, in a manner related to bacteriocin production and bile salt hydrolase gene expression. The interaction and effects of *L. casei* on macrophages and monocytes was modulated by varying pro-

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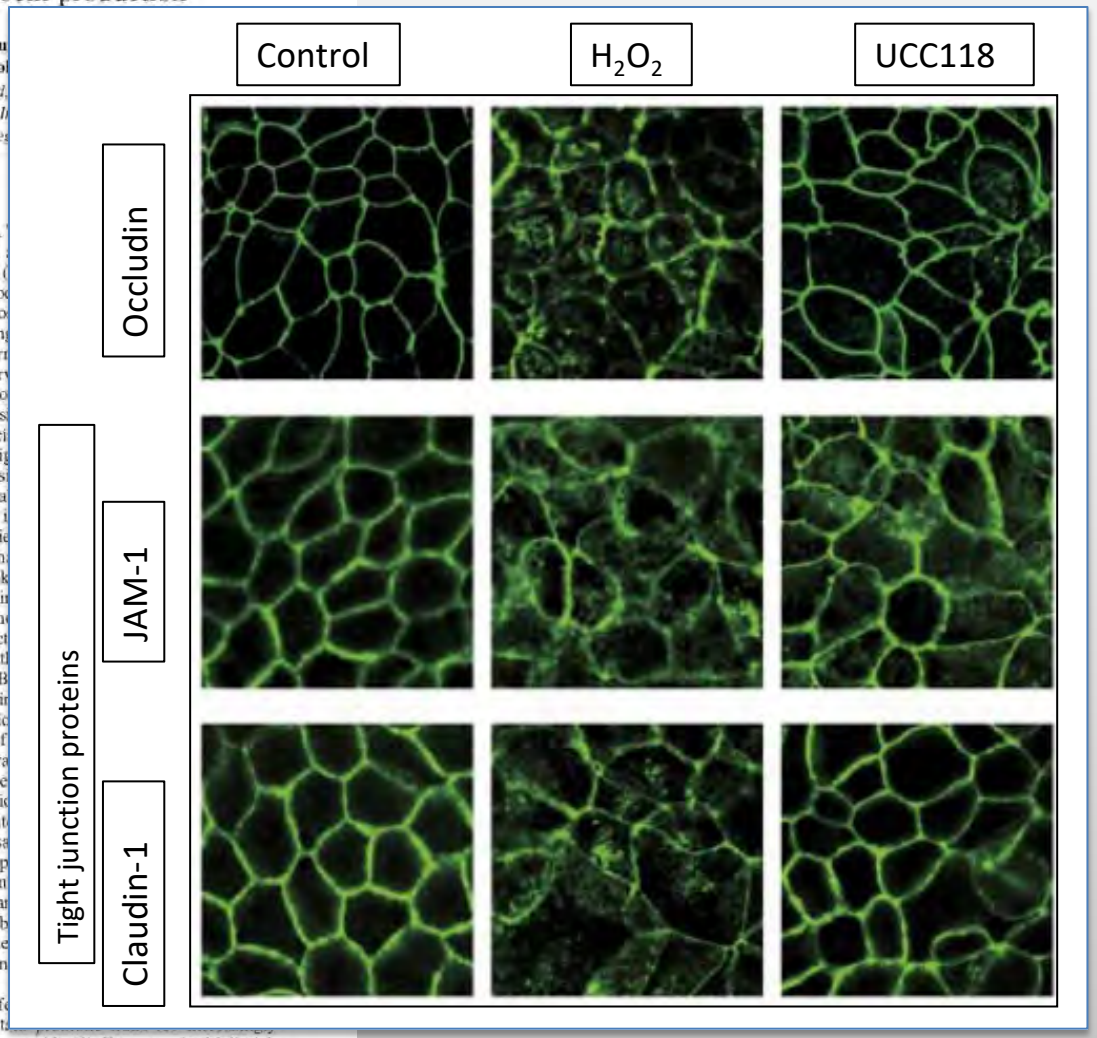


Miyauchi *et al. Am J Physiol Gastro Liver* 2012 303:1029-1041

L. salvarius UCC118

In an *in vitro*, validated model of human intestinal epithelial cells, researchers assessed the effects of L. *salivarius* exposure on localization of tight junction proteins by confocal microscopy.[1]

- Hydrogen peroxide (H2O2) exposure disrupted and redistributed the tight junction proteins occludin, JAM-1, and claudin-1.[1]
- Pretreatment with L. salvarius UCC118 helped maintain tight junction protein integrity (Figure1).*[1]
- In separate preclinical research, L. salivarius UCC118 has been shown to produce a bacteriocin, a beneficial protein that may influence intestinal microbial composition.*[2,5,6,7]

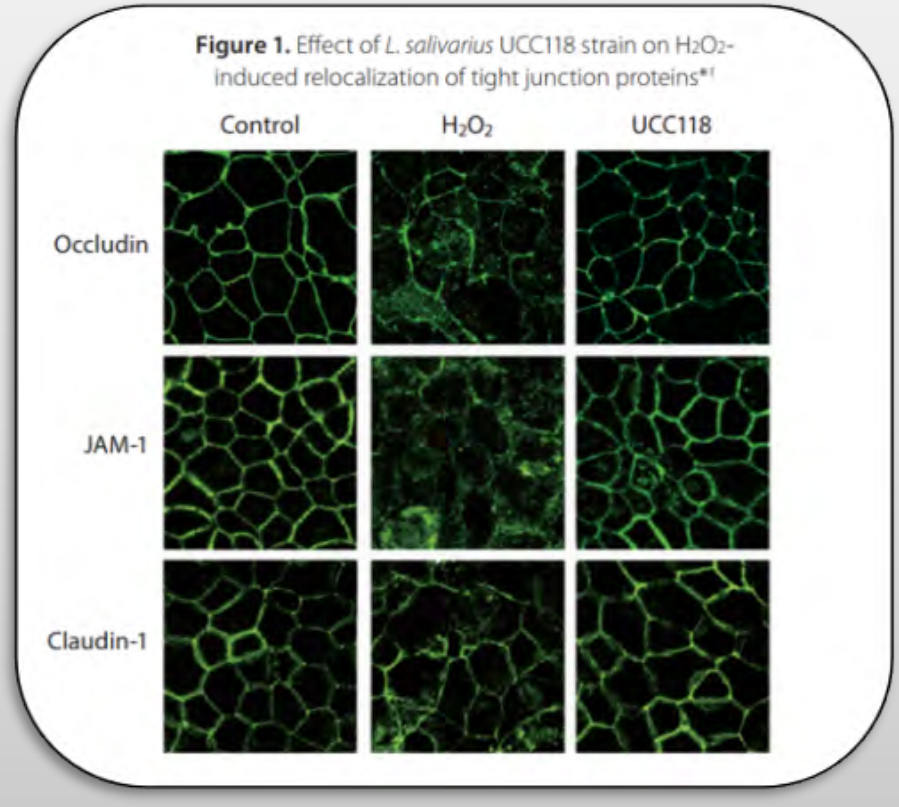
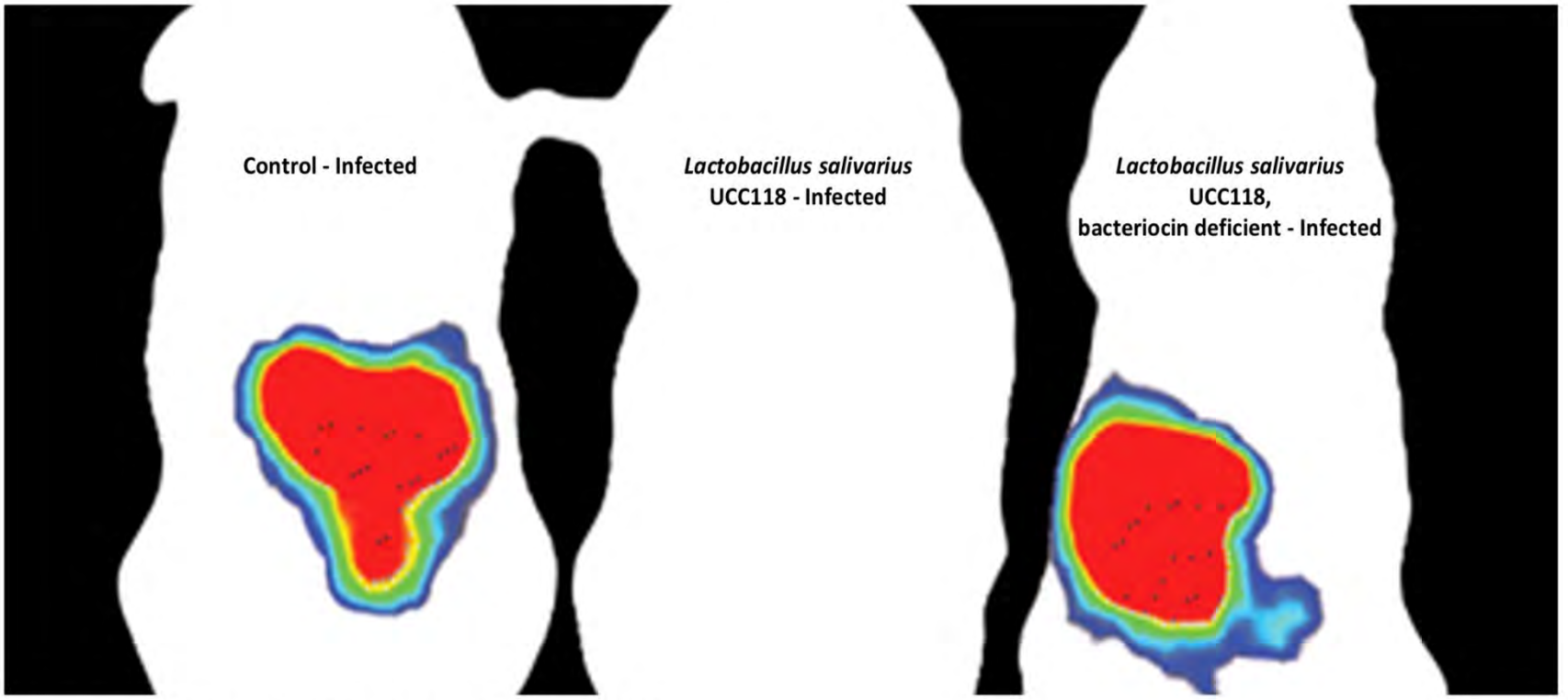


Figure 1. Effect of *L. salivarius* UCC118 strain on H_2O_2-induced relocalization of tight junction proteins*†

* These statements have not been evaluated by the Food and Drug Administration. This product is not intended to diagnose, treat, cure, or prevent any disease.

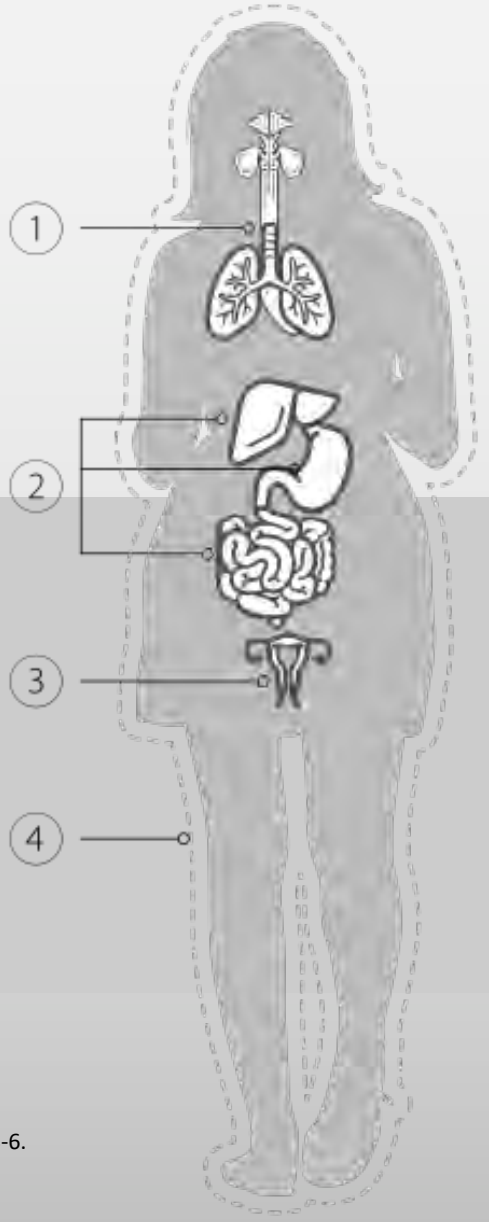
UCC118 prevents *Listeria* infection, via a bacteriocin dependent mechanism in mice



Corr, et al. *Proc Natl Acad Sci* U S A. 2007 May 1;104(18):7617-21.

Bacteriocins are peptides produced by bacteria that kill or inhibit other bacterial strains.

Probiotics Influence More than Just Gut Health*



1. Nasal, sinus, and respiratory health*
2. Gastrointestinal health, increased nutrient absorption, and digestive comfort*
3. Urogenital health*
4. Immune health*

Leaky Gut-Leaky Brain
Anxiety, Depression

Reference:
1. Vighi et al. Allergy and the gastrointestinal system. *Clin Exp Immunol* 2008; 153 (Suppl 1):3-6.

*** These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.**

Prebiotic Fiber Supports Healthy Probiotic Bacteria

Prebiotics are food for beneficial microbes that live on or in us. They are metabolized by the “good” gut microbes, positively impacting the gut environment with an overall health benefit.*

Source: International Scientific Association for Probiotics and Prebiotics

*** These statements have not been evaluated by the Food and Drug Administration. These products are not intended to diagnose, treat, cure, or prevent any disease.**

Prebiotic Foods

Asparagus
Banana
Dandelion greens
Eggplant
Endive
Garlic
Honey
Jerusalem artichokes (sunchokes)
Jicama
Leeks
Legumes
Onions
Peas
Radicchio
Whole grains

Gut Health and Your Genes – FUT2 Genetic Variants

Genetic variant : An alteration in the most common DNA nucleotide sequence. The term variant can be used to describe an alteration that may be benign, pathogenic, or of unknown significance. The term variant is increasingly being used in place of the term mutation.

Gene Coding

- The gene coding for your blood type lies on chromosome *9q34.* However, a separate gene (called *FUT2*) actually interacts with your blood type gene, and determines your ability to *secrete* your blood type antigens into body fluids and tissues, such as saliva, mucus (in the digestive tract and the respiratory cavities), tears, and sweat.

- Whether you secrete your blood type plays a significant role in the type of bacteria that dwell in our gut microbiome

Secretor or Non-Secretor

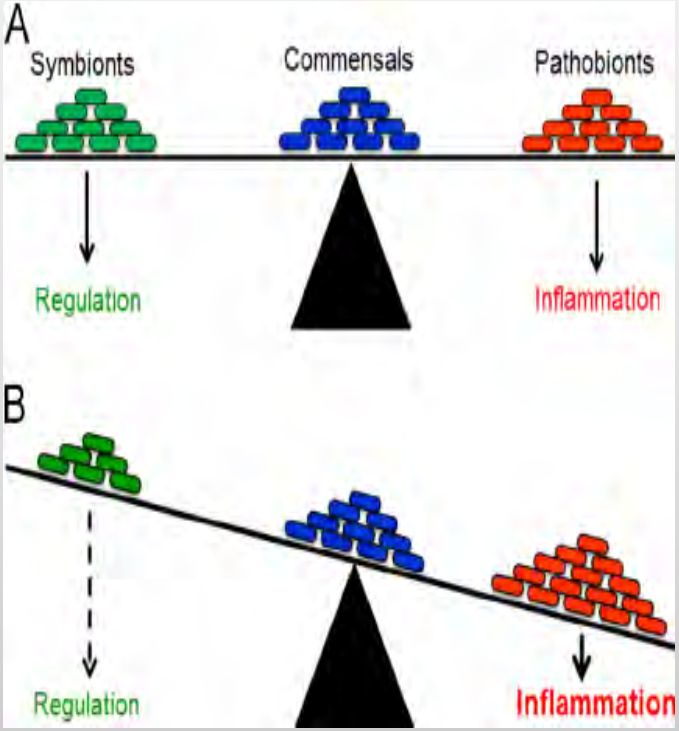
- The determination of secretor status is important because secretor status is associated with a wide variety of diseases (like urinary tract infections, diabetes, digestive disorders, etc.)

- Frequency of ABH **secretor** status in the world population is about 80% **secretors** and 20% **non-secretors** with some geographic and racial differences.

- Pak J Med Sci. 2014 Jan-Feb; 30(1): 189–193.

FUT2 influence of Gut Bacteria

- When FUT2 SNPs are present, we are unable to feed healthy bacteria due to the gut lacking its pre-biotic.
- This changes the balance between the good and bad bacteria, allowing the bad bacteria to dominate over the gut.
- Diseases derive from this such as SIBO, IBS, Chronic Constipation and diarrhea, mood issues, sleep issues, and focus issues.



ISME J. 2014 Nov;8(11):2193-206. PMID: 24781901

The Secretor Status and infection risk

- Several studies show that secretor status affects susceptibility to infection.
  - Because our ABO blood group antigens are a carbohydrate food source for many bacteria, the presence or absences of these antigens effect the population of the gut bacteria and provide protection against certain pathogenic bacteria.
  - Non-secretors are at an increased risk for development of celiac disease (up to 48% of patients with celiac disease have been reported to be Non-secretors).

Nash, G ND, 2019 “FUT2 Secretor Status: Effects on Gut Health”

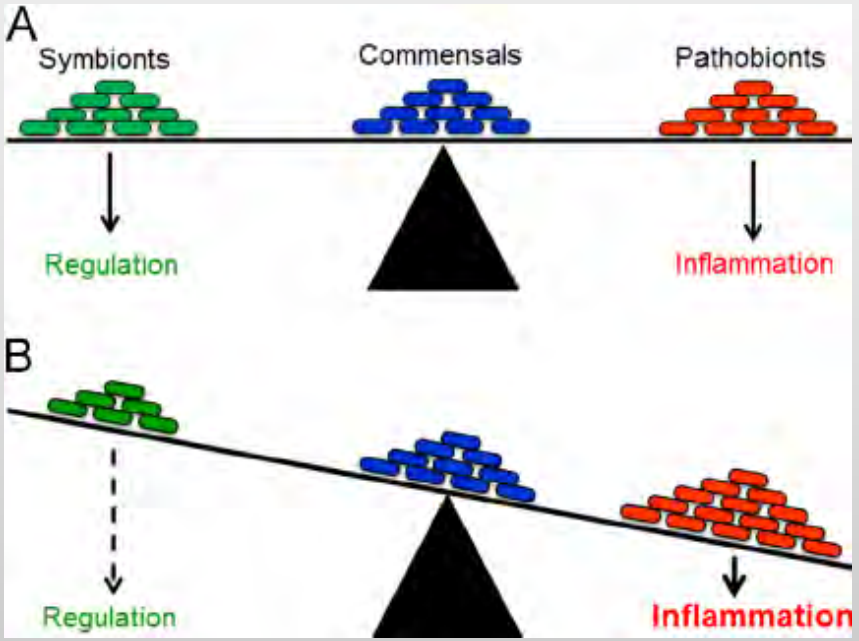
Less dental caries among secretors than among non-secretors

As a general rule, a higher intensity of oral disease is found among Non-secretors. This includes dysplasia (precancerous changes to the tissue) and an increase in cavities.

Scand J Dent Res. 1976 Nov;84(6):362-6.

FUT2 might be a target of natural selection due to evolving pathogens

Being homozygous for the inactive "non-secretor" rs601338(A) allele confers resistance to certain infections (e.g. *Norovirus*, *Rotavirus*) and susceptibility to others (e.g. *Haemophilus influenza*, *Streptococcus pneumonia*).



Microbiome. 2015 Apr 10;3:13. PMID: 25922665

Azad MB, Wade KH, Timpson NJ. *FUT2* secretor genotype and susceptibility to infections and chronic conditions in the ALSPAC cohort. *Wellcome Open Res*. 2018;3:65. Published 2018 Sep 25. doi:10.12688/wellcomeopenres.14636.2

FUT2 Enzyme and IBD

- Crohn's Disease (CD)
  - Non-Secretors, who are homozygous for the loss-of-function alleles of FUT2 gene, have increased susceptibility to Crohn's Disease.
  - However, the molecular mechanism of the association between non-secretor status and CD remains unknown.
- Ulcerative Colitis (UC)
  - Dysbiosis of intestinal microbiota has been shown in ulcerative colitis.

ISME J. 2014 Nov;8(11):2193-206. PMID: 24781901

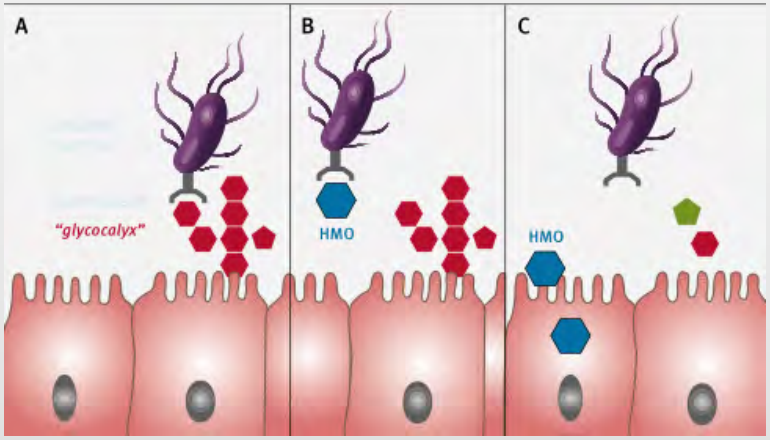
PLoS One. 2016 Jan 14;11(1):e0146557. PMID: 26766790

What are Human Milk Oligosaccharides (HMOs)?

- **2'-Fucosyllactose***
  - A human milk oligosaccharide (HMO), similar to that found in breast milk*
  - Targeted prebiotic to promote healthy intestinal mucosal health and nourish beneficial microorganisms
  - Encourages the production of short-chain fatty acids (SCFA)

*The 2'FL in UGIR is not derived from human breast milk

What are Human Milk Oligosaccharides (HMOs)?



- HMOs are a group of **carbohydrate compounds** unique to human milk. To date over **150 unique carbohydrate structures** have been identified. **2'Fucosyllatctose* is the most abundant** HMO.
- HMOs have **prebiotic effects, selectively serving as a source of energy and nutrients for desired bacteria to colonize the intestine.**
- HMOs have been **shown to be anti-adhesive, mimicking the attachment sites for certain pathogens and blocking their adhesion, colonization, and invasion.**
- HMOs might also have intestinal epithelial cell surface glycome-modifying effects, **changing the glycosylation machinery of intestinal epithelial cells, altering the expression profiles of pathogen** attachment sites, and reducing infectious diseases.

1. Gibson GR, Probert HM, Loo JV, et al. *Nutr Res Rev.* 2004;17(2):259-275.
2. Yu ZT, Chen C. *Glycobiol.* 2013;23(11):1281-1292.
3. Chen C, Kling DE, et al. *Glycobiol.* 2013;23(2):169-177.
4. Bode L. *Glycobiol.* 2012;22(9):1147-1162.
5. Sharon N. *Adv Exp Med Biol.* 1996;408:1-8.

*The 2'FL in UGIR is not derived from human breast milk

What are Human Milk Oligosaccharides (HMOs)?

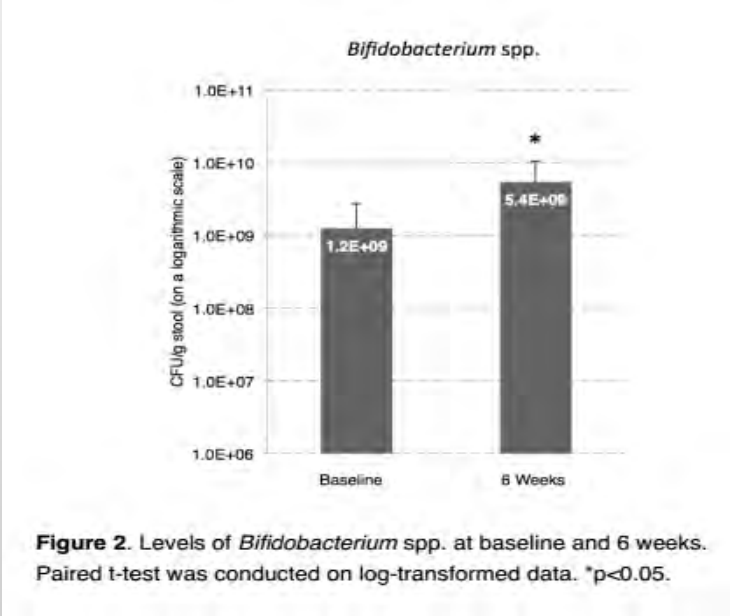


Figure 2. Levels of *Bifidobacterium* spp. at baseline and 6 weeks. Paired t-test was conducted on log-transformed data. *p<0.05.

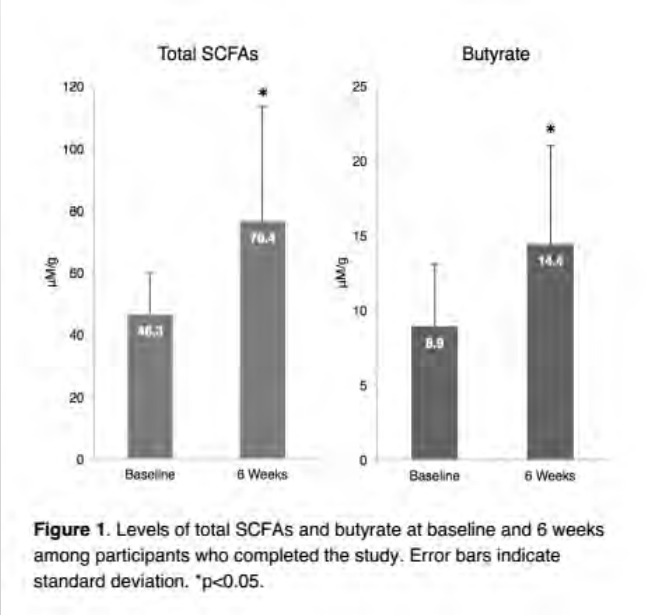


Figure 1. Levels of total SCFAs and butyrate at baseline and 6 weeks among participants who completed the study. Error bars indicate standard deviation. *p<0.05.

2'-FL also supports GI health by blocking certain potentially harmful bacterial strains from adhering to their host cell receptors; 2'-FL mimics host cell surface receptors and acts as a decoy.27 2'-FL has been shown to act as an anti-adhesive antimicrobial to *Campylobacter jejuni*, *Vibrio cholera, Escherichia coli.* and Norovirus.

A Medical Food (UGIR) Reduces Gastrointestinal Symptoms and Beneficially Alters Gut Microbiota in Adults with IBS and IBD

Functional Medicine Considerations for IBD

- Genetics, Environmental triggers, and an Inflammatory response
- Leaky Gut contributors, diet, gluten, stress, medication
- Calm gut inflammation (probiotics)
- Prebiotics through diet and supplementation
- Nutritional Kinase modulation (NF-kB)
- Consider genetic variants: FUT2

"… but if you remove the environmental pressure, the epigenetic marks will eventually fade, and the DNA code will — over time — begin to revert to its original programming."

GENETIC TESTING

MTHFR SUPPORT

MTHFR SUPPORT

MTHFRSupport Variant Report v2.5
Based on: AncestryDNA.zip
http://www.mthfrsupport.com

Liver Detox - Phase I (Figure 1)				
SNP ID	SNP Name	Risk Allele	Your Alleles	Your Results
rs1048943	CYP1A1*2C A4889G	C	TT	-/-
rs2472304	CYP1A2*1F	A	AA	+/+
rs2069526	CYP1A2*1K -739T>G	G	TT	-/-
rs28399424	CYP1A2*6 R431W	T	CC	-/-
rs9341266	CYP1B1 C1871T	A	GG	-/-
rs1800440	CYP1B1 N453S	T	TT	+/+
rs8192719	CYP2B6 C26570T	T	CC	-/-
rs7260329	CYP2B6 G29435A	G	GG	+/+
rs28399499	CYP2B6 I328T	C	TT	-/-
rs2279345	CYP2B6 T23499C	T	CC	-/-
rs12767583	CYP2C19 C5709T	T	CC	-/-
rs4917623	CYP2C19 T106C	C	TT	-/-
rs4986894	CYP2C19 T98C	T	TT	+/+
rs4244285	CYP2C19*2 G681A	A	GG	-/-
rs4986893	CYP2C19*3 G636A	A	GG	-/-
rs28399504	CYP2C19*4 A5001G	G	AA	-/-
rs1057909	CYP2C9 42612A>G	G	00	-/-
rs4917639	CYP2C9 A6326C	C	AA	-/-
rs4086116	CYP2C9 C334T	T	CC	-/-
rs10509680	CYP2C9 G2337T	T	GG	-/-
rs9332146	CYP2C9 G9617A	A	GG	-/-

https://www.metagenicsinstitute.com/video/enhancing-digestive-health-hmos-gut-protection/

Thank you