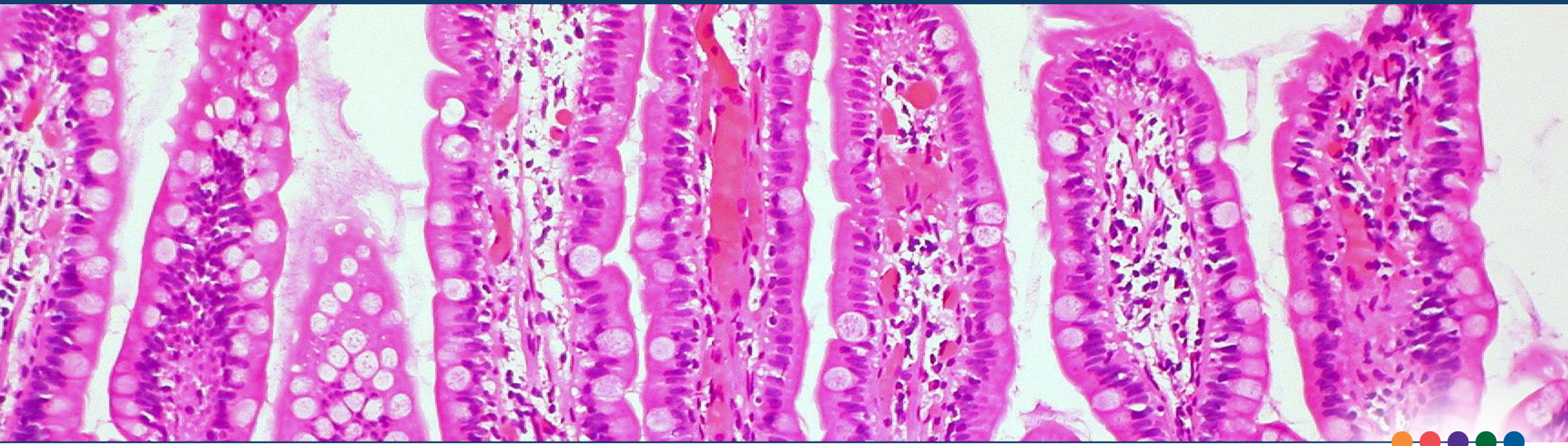

Supporting Gut Barrier Function

Thomas G. Guilliams, PhD

Point Institute- Stevens Point, WI (USA)

September 27th, 2017

Michael Chapman, ND

Medical Education Specialist - Asheville

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Thomas G. Guilliams, PhD

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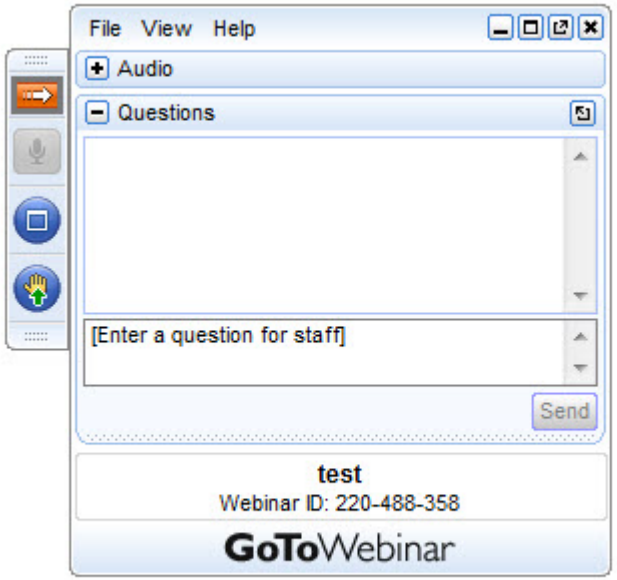

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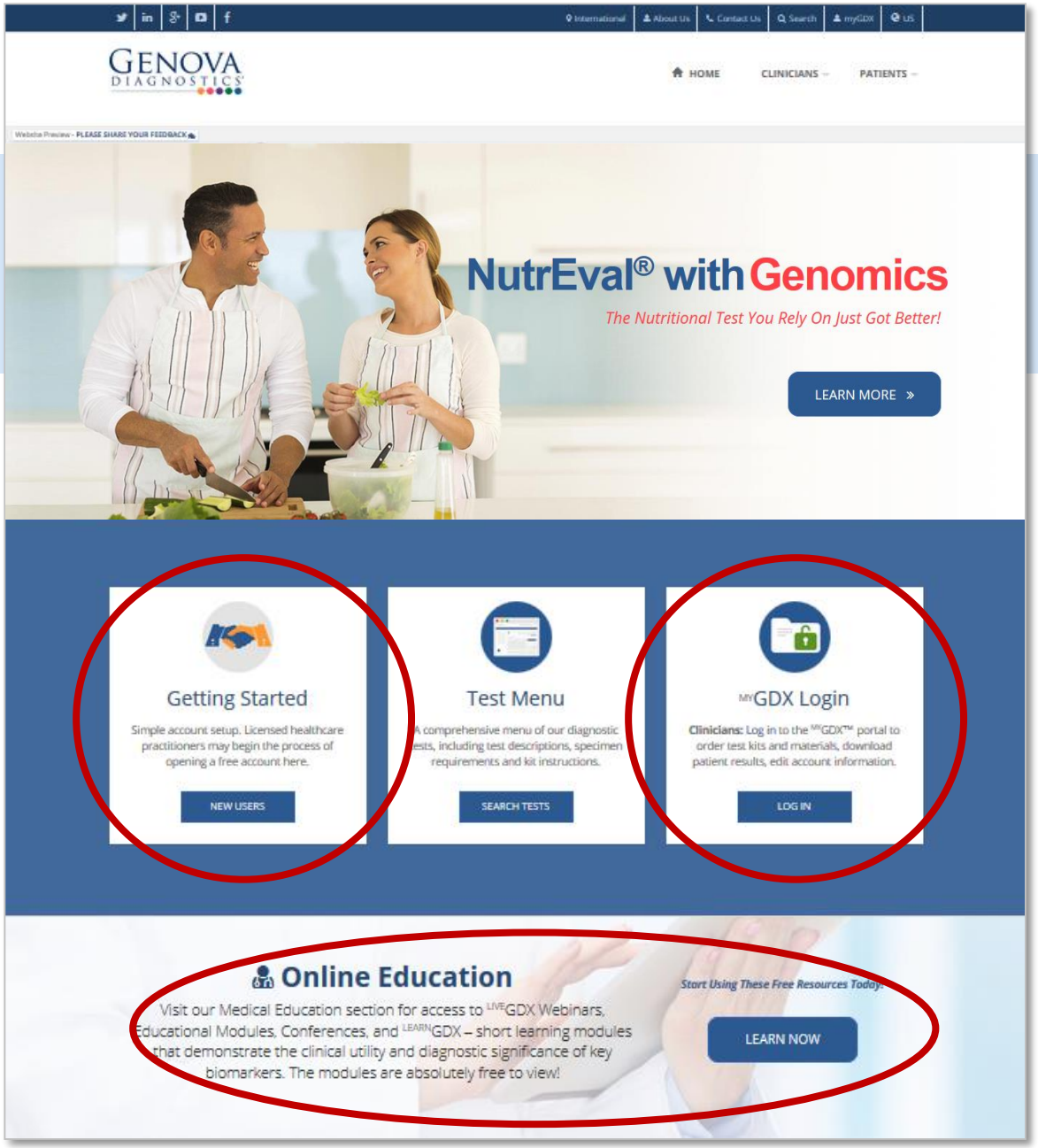

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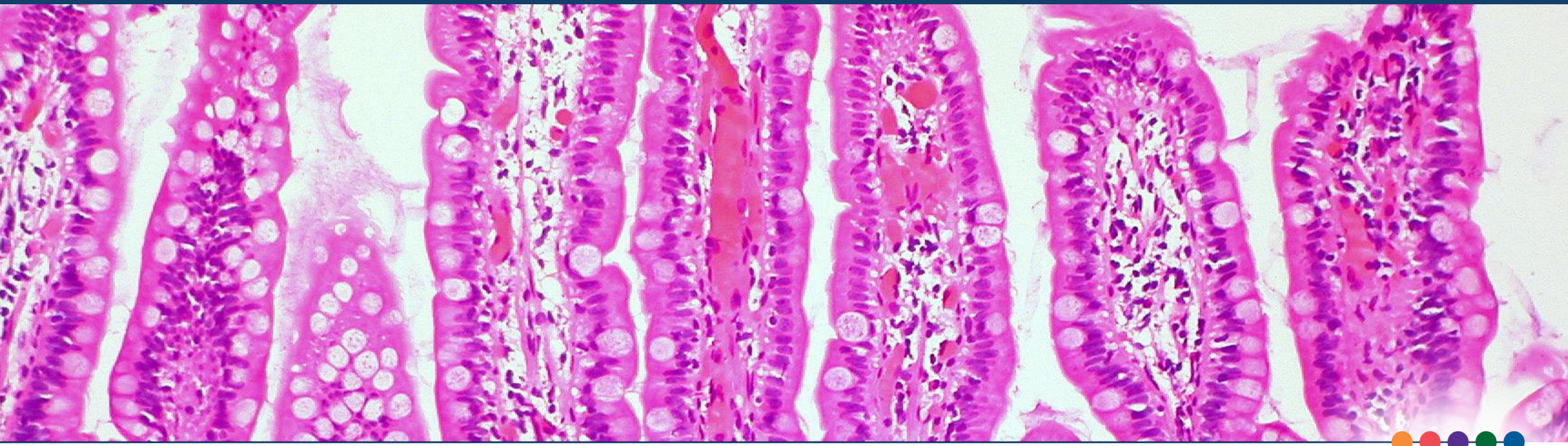

Supporting Gut Barrier Function

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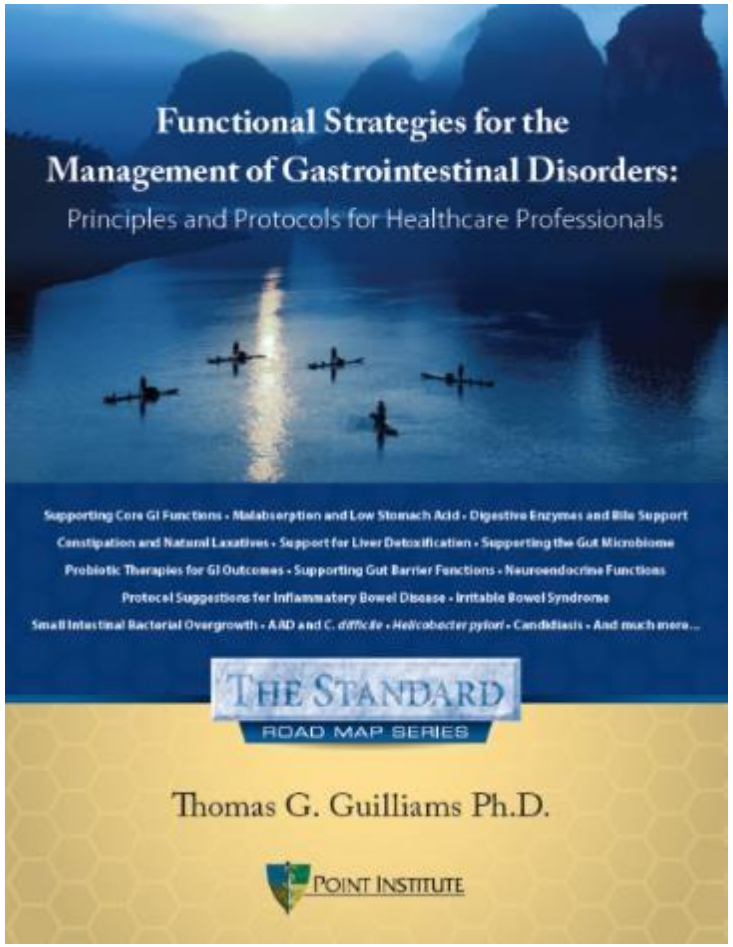
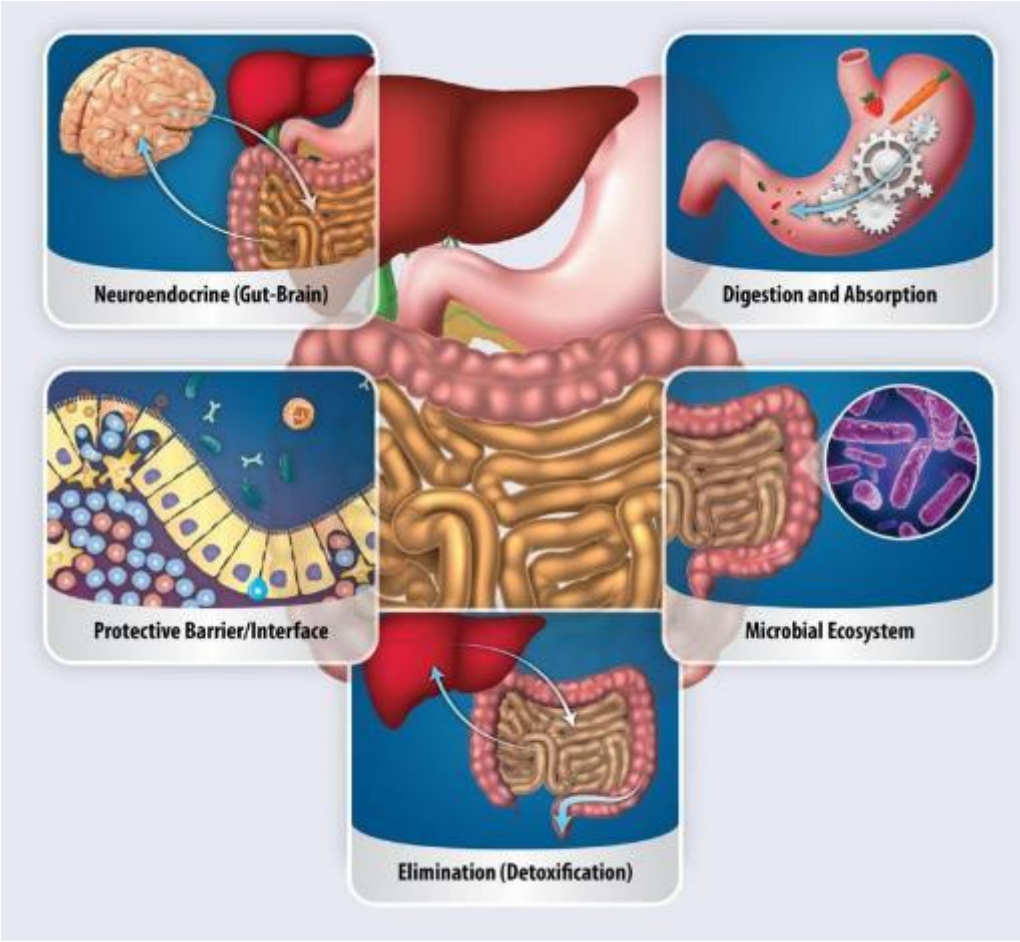
Core Functions of the GI





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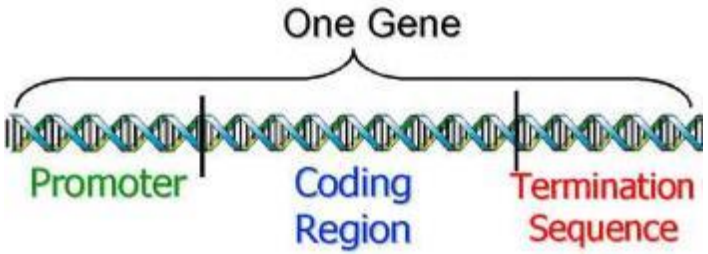
Everything Happens at the Interface!

- Biological systems are designed to create discrete functional units
  - Tissues
  - Cells
  - Organelles
  - Genes
- All of which are equipped to modulate each other by signals at their interfaces



Functional interfaces require intact barriers!

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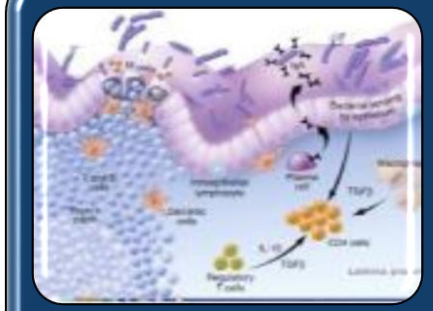
Coordinated Surveillance Systems: Protecting “Self” at the Interfaces

HPA Axis (Stress Response)

- Assessing threats from outside (interface with outside world)
- Compensating for internal imbalances

Immune System

- Surveillance of self vs. non-self
- Highly coordinated by GC signals, highly concentrated in the gut



Gastrointestinal Tract - GALT

- Maintaining barrier function (interface with outside world)
- Signal coordination to brain using direct and immune facilitated signals

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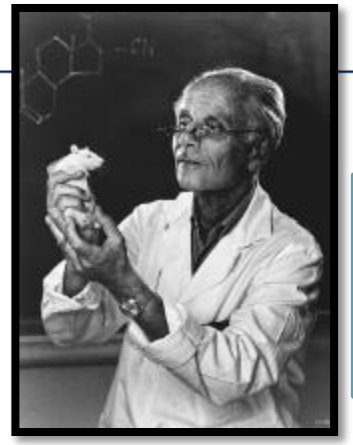
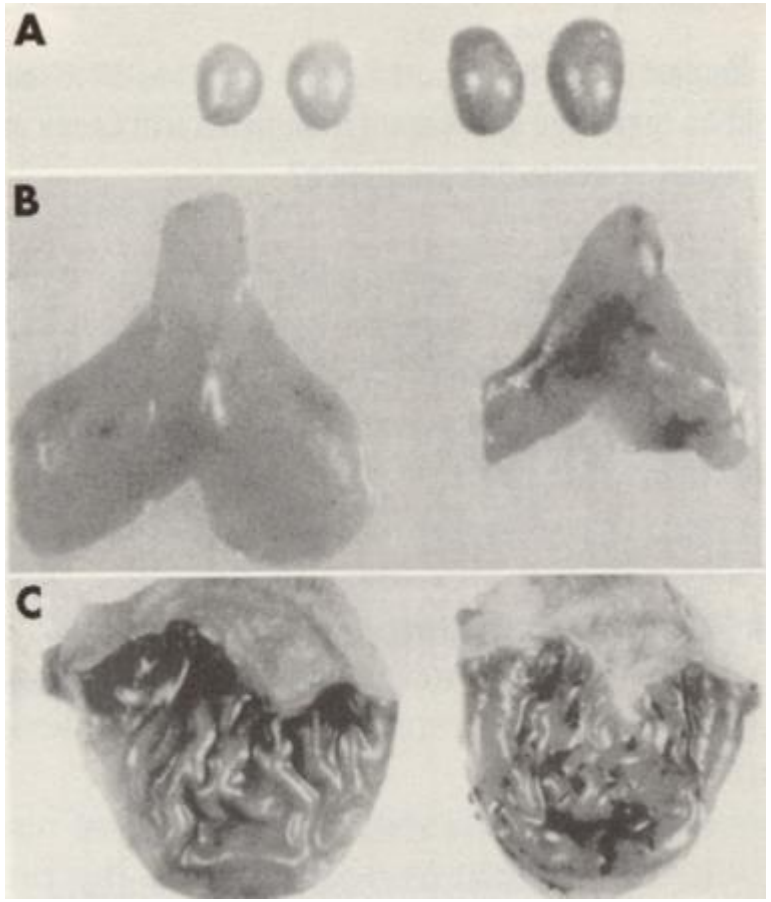


Selye and Surveillance System Stress

Control "Stress"



Hypertrophy of Adrenal Gland (HPA)

Atrophy of the thymus and other lymphatic glands (Immune system)

Erosions and ulcers in the duodenum (GI-system)

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Interface or Barrier?

"Therefore, the barrier/permeability functions of the gut represent one of the most important interfaces between a person and the external environment. However, we should not imagine this barrier function as simply a means to keep things out, but as a sophisticated system to communicate with, and allow selective entry of, certain contents from the gut lumen into the body. This requires a tightly controlled, but thin barrier of tissues and secretions ***intentionally designed for close proximity to the gut lumen****. This proximity permits the absorption of available nutrients and physiological interaction with trillions of non-human microbes and their metabolites and signals, but also creates a vulnerability to those same microbes, toxins and immunologically reactive components from the gut lumen."*

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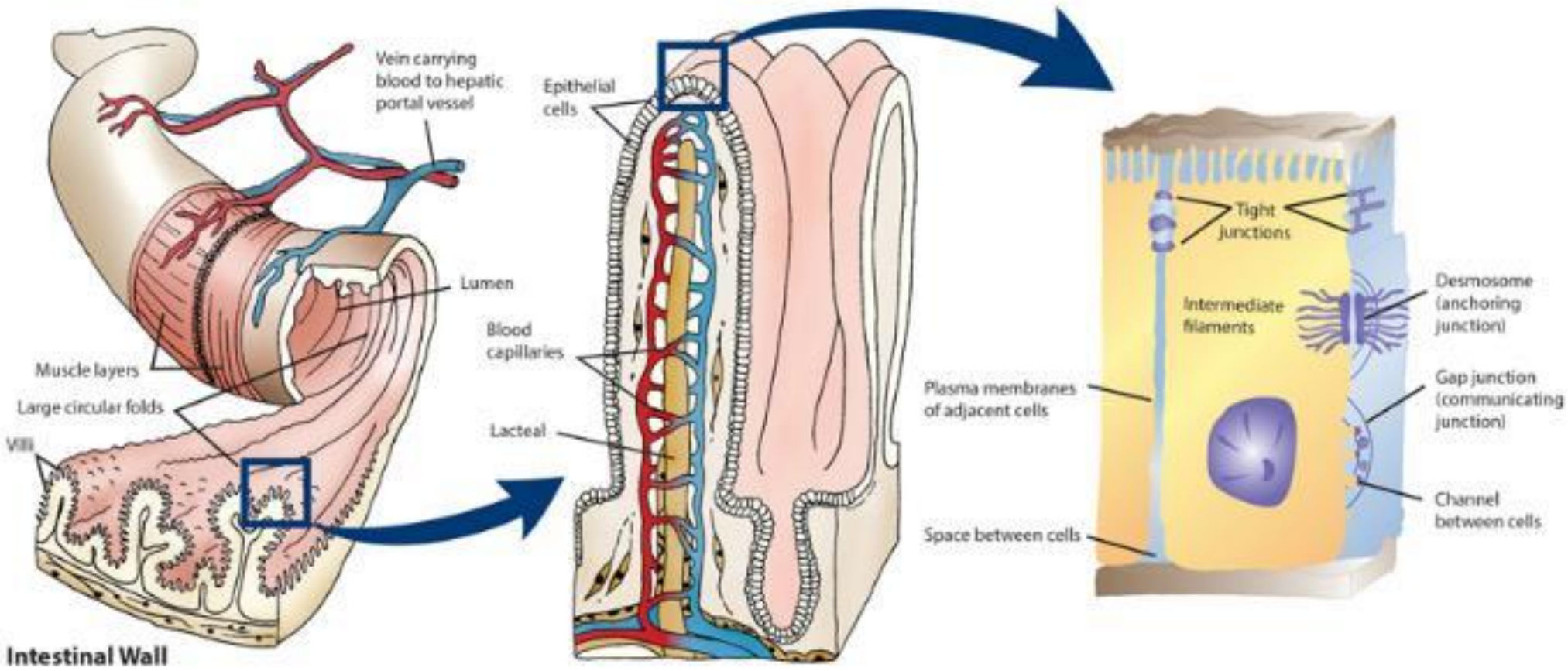
Expanding the Surface Area



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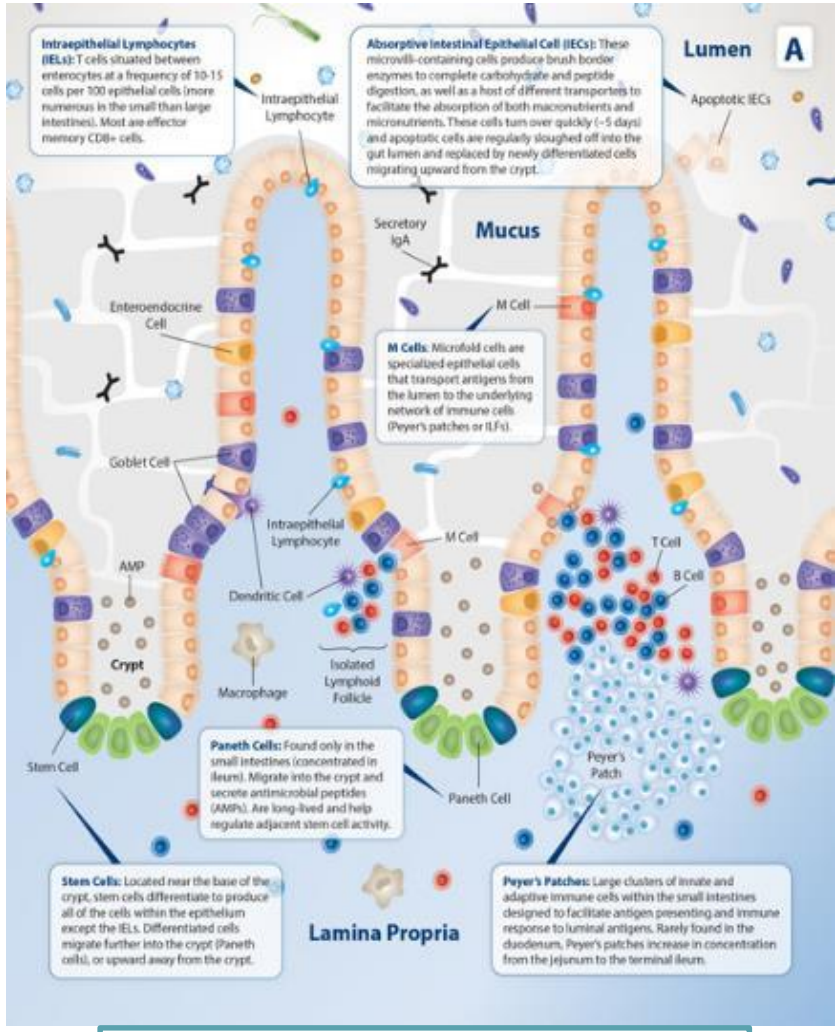
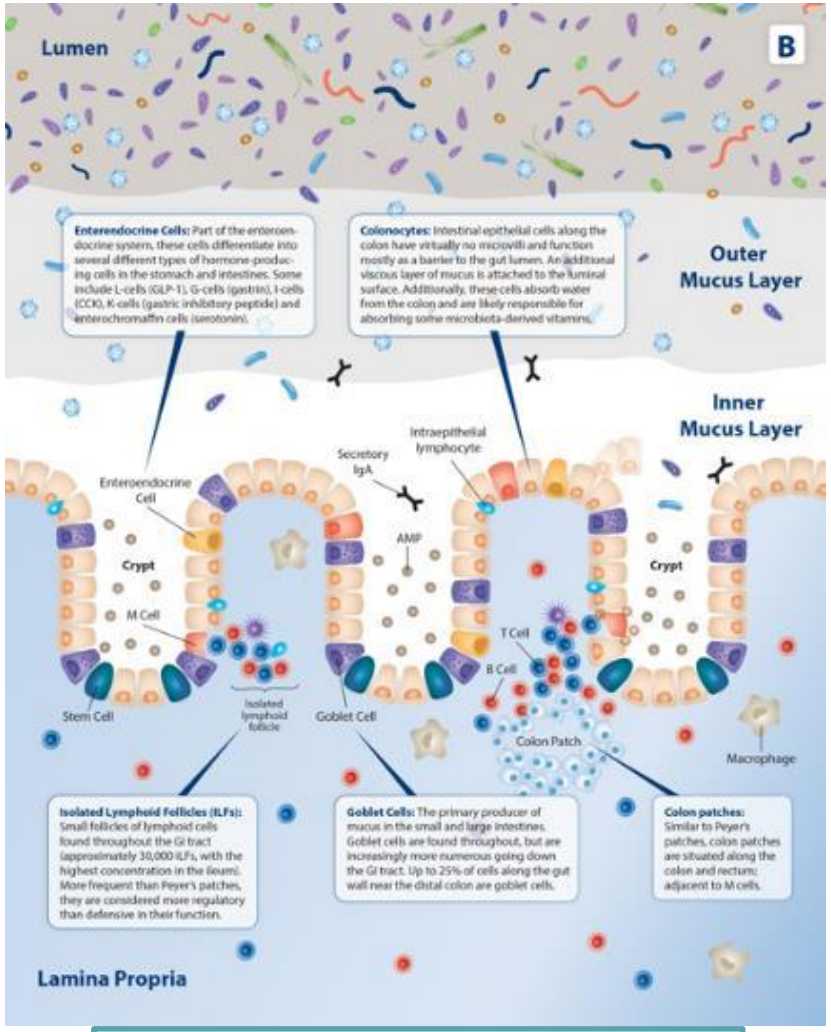
The Functional Components of the Gut Barrier

- Human GI cells that create the interface (enterocytes, colonocyte etc.)
- Human immune cells that line the inside or penetrate the interface
- Human neuroendocrine cells and neurons with synapses nearby
- Luminal excretions from human cells (mucus, sIgA, anti-microbial peptides, enzymes, acid, neurotransmitters, etc...)
- Non-human microbes in the lumen and mucus lining
  - Commensal, pathobiont, pathogenic bacteria
  - Viruses (free and bacteriophages)
  - Fungi
  - Non-human eukaryotic organisms (are any of these commensals?)

Basic Features of the Gut Barrier



Small Intestine- villi and crypt



Colon- 2 mucus layers, crypt

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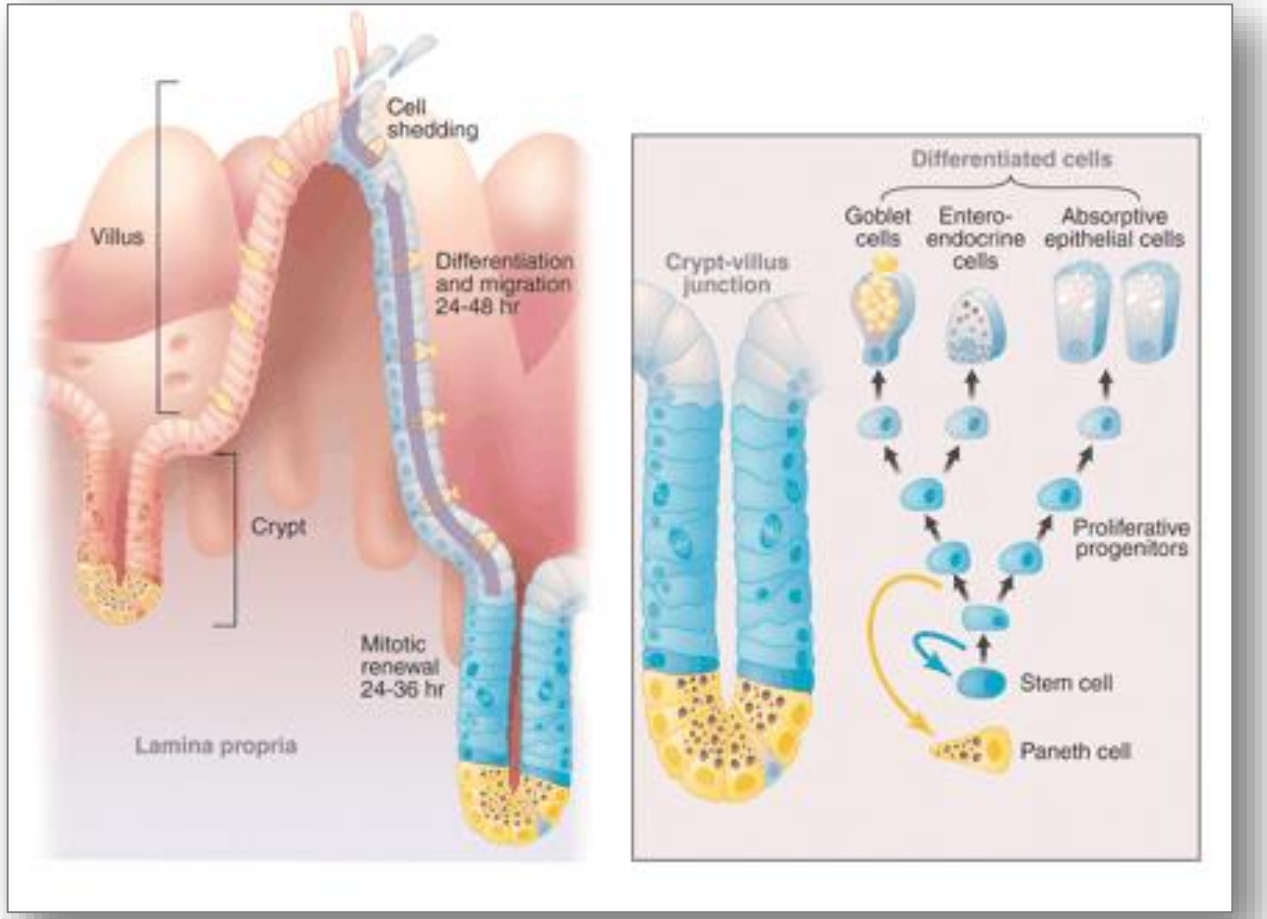
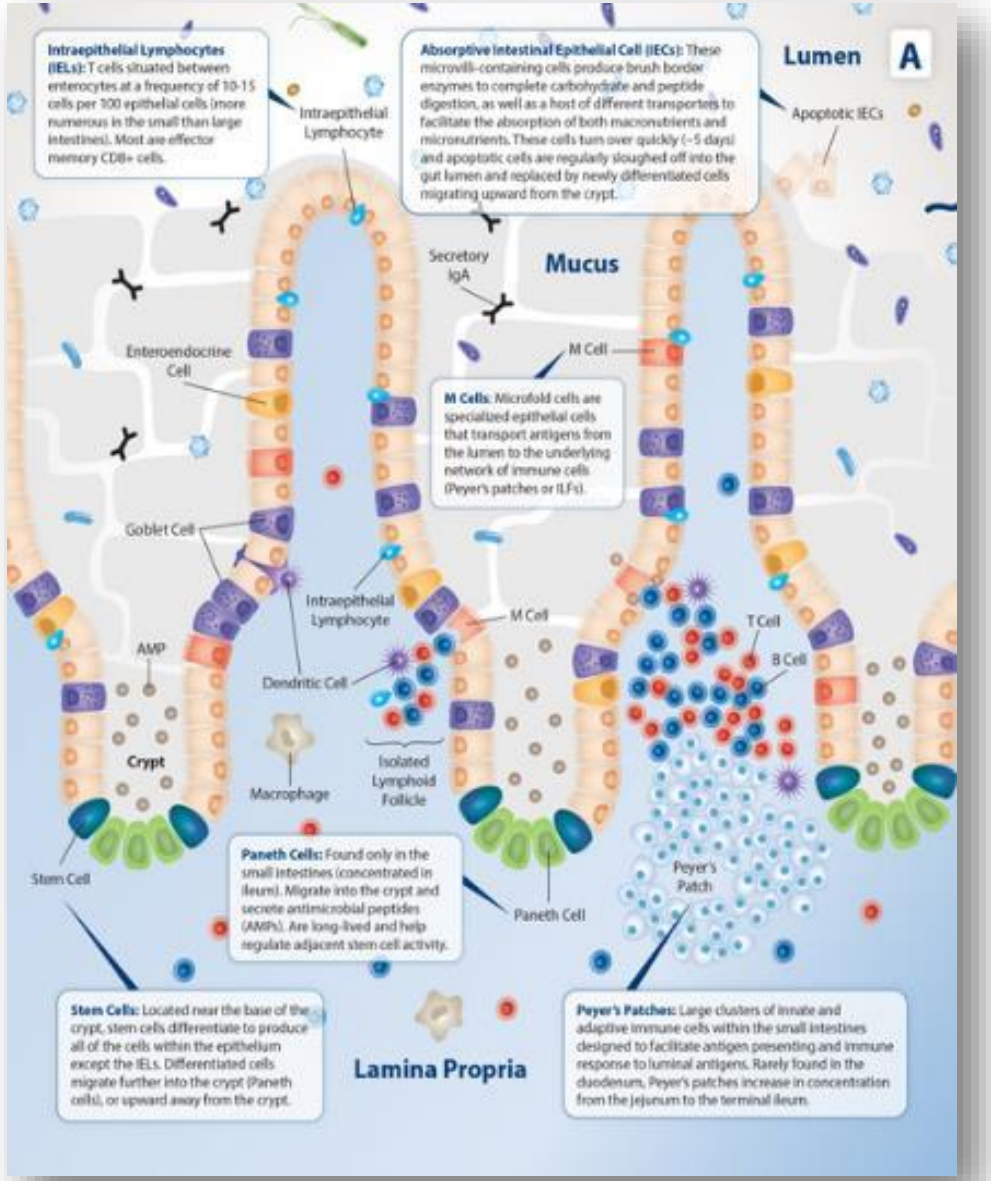
Stem Cells: Constant Turnover





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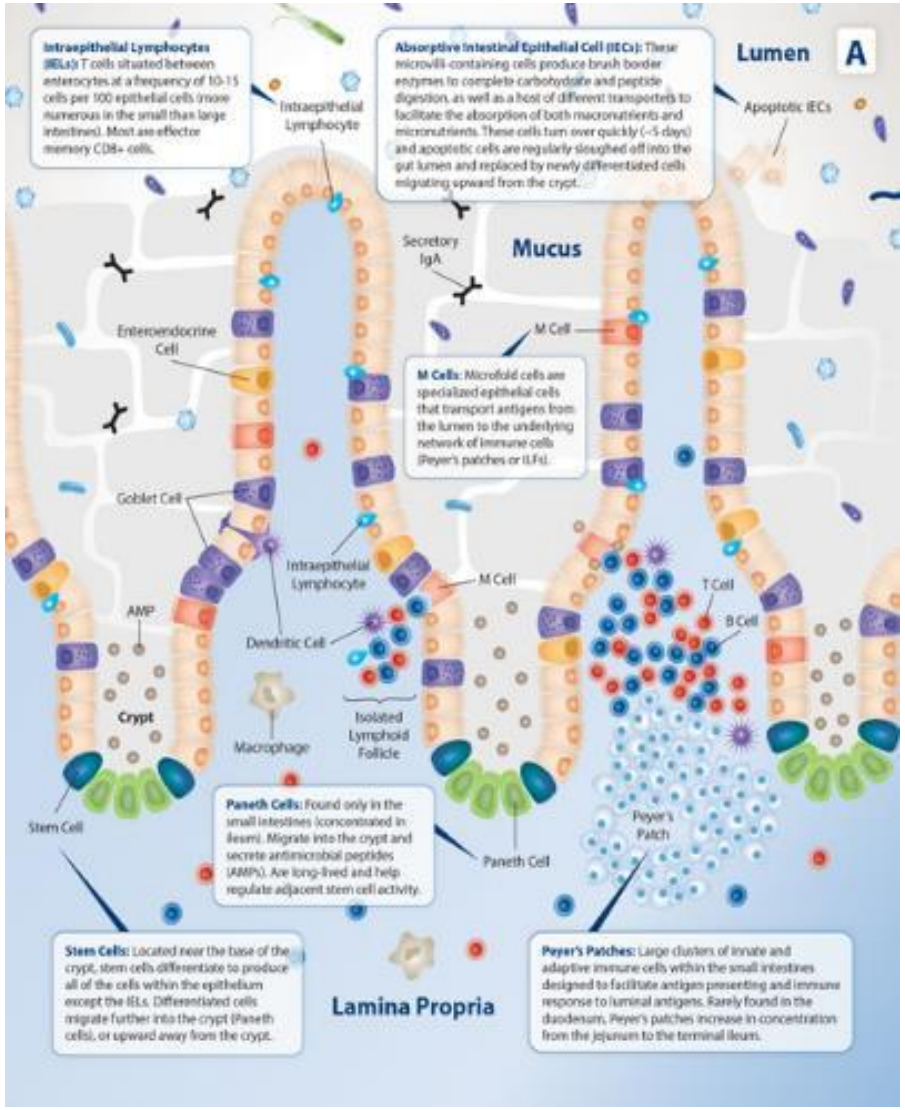
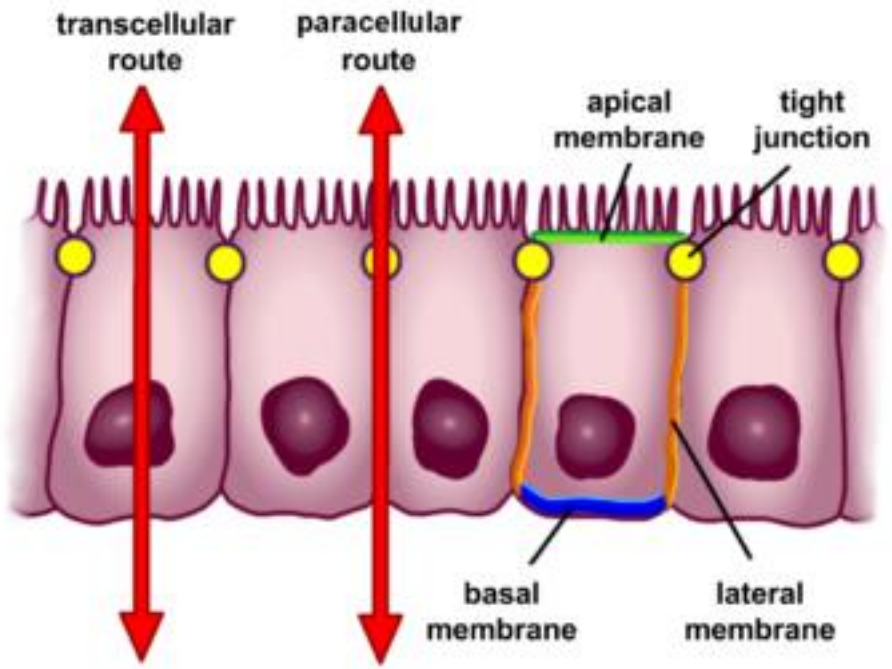
Absorptive Epithelial Cells





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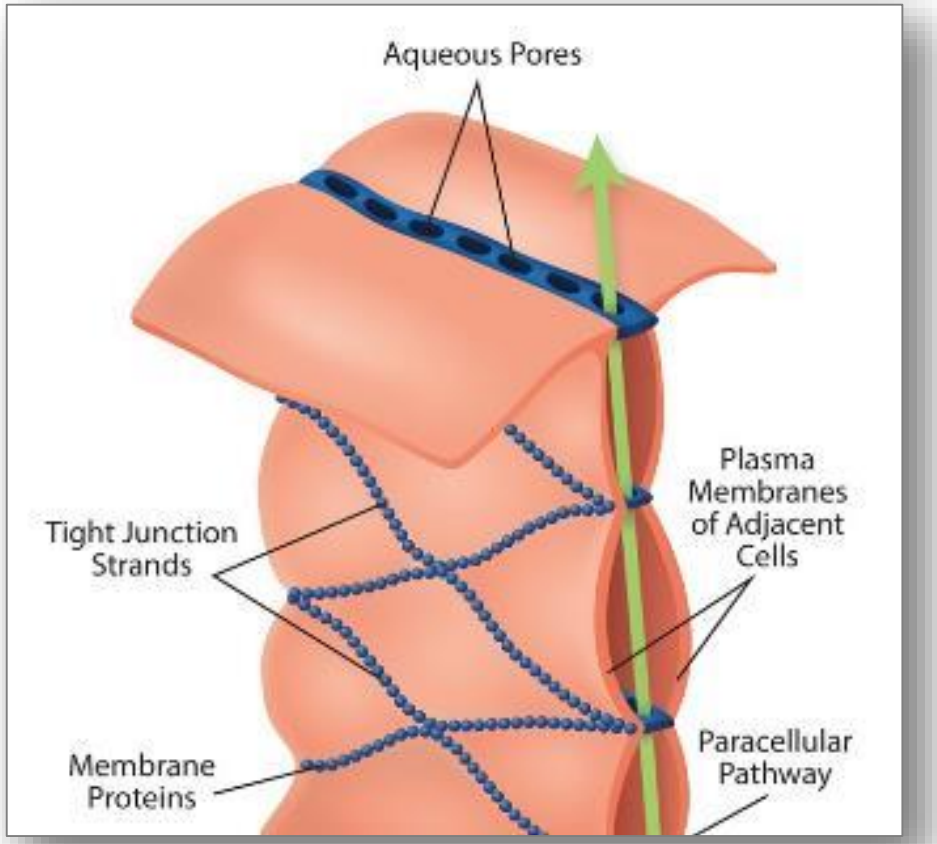
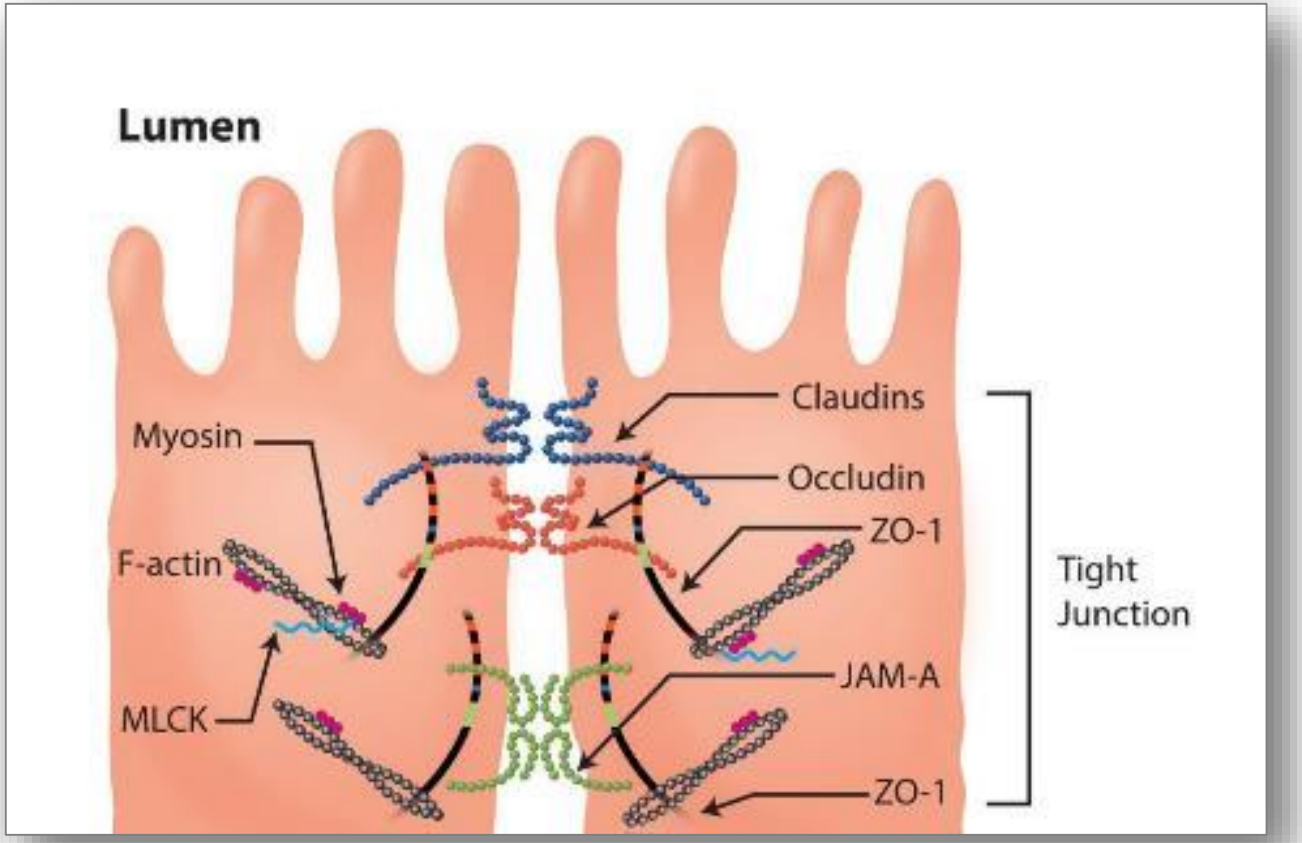
Tight Junctions





MLCK - myosin light chain kinase
ZO - zonula occludens

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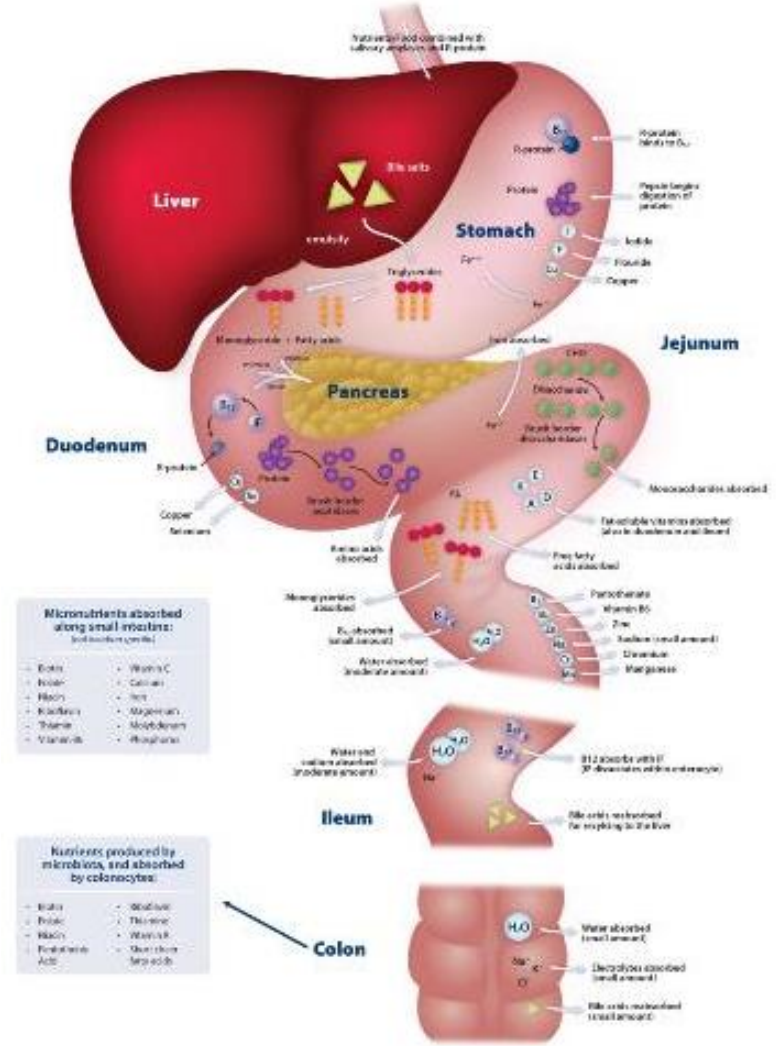
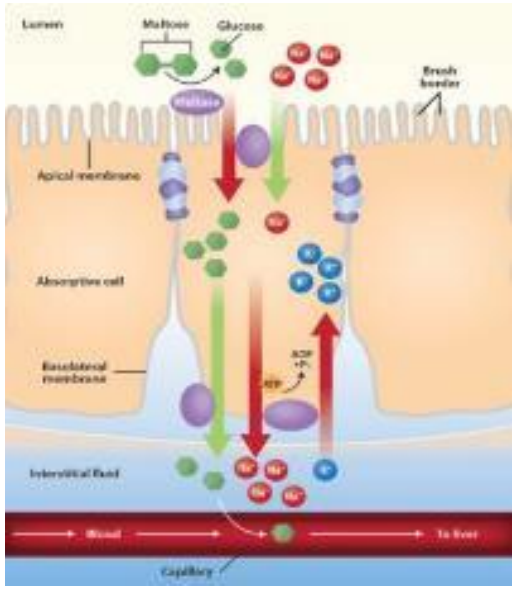
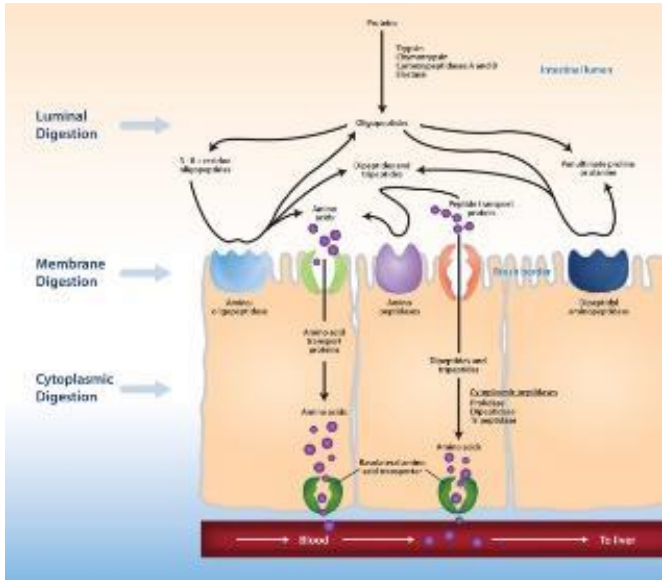
Digestion and Absorption







Transcellular transport of key nutrients coordinated by enzymes and transporters within absorptive cells in small intestine

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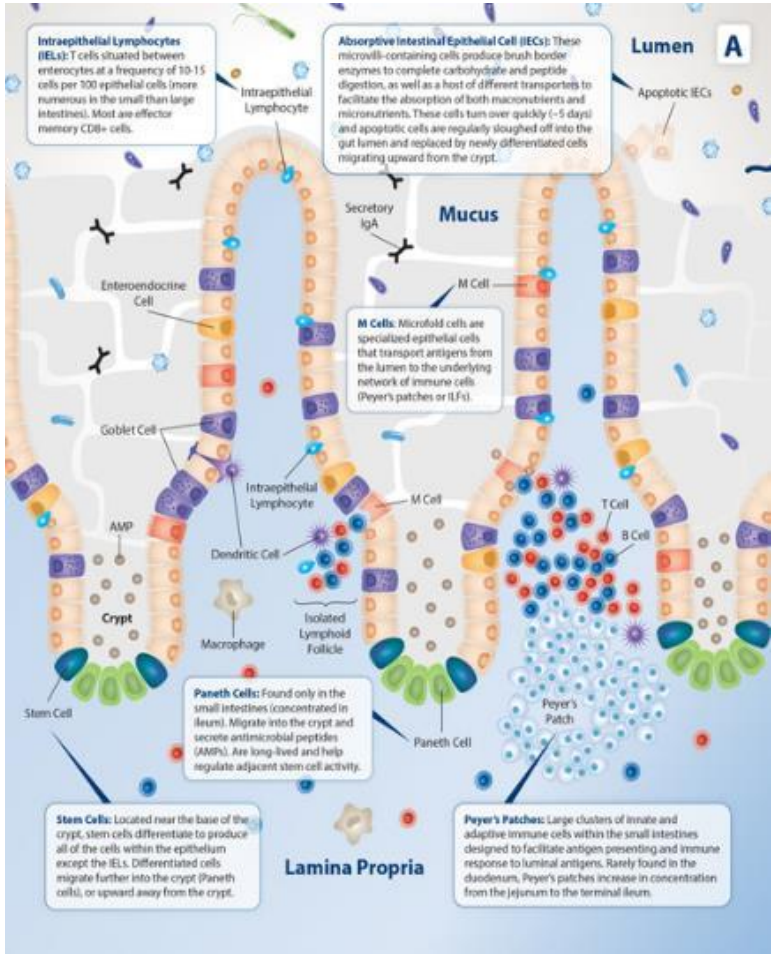
Paneth Cells: Managing Dysbiosis

- Found only in small intestine (primarily Ilium)
- Migrate into crypt after differentiation from stem cells
- Secrete antimicrobial peptides (AMPs) into gut lumen
- Are long-lived (months) compared to absorptive cells
- Help regulate stem cell activity



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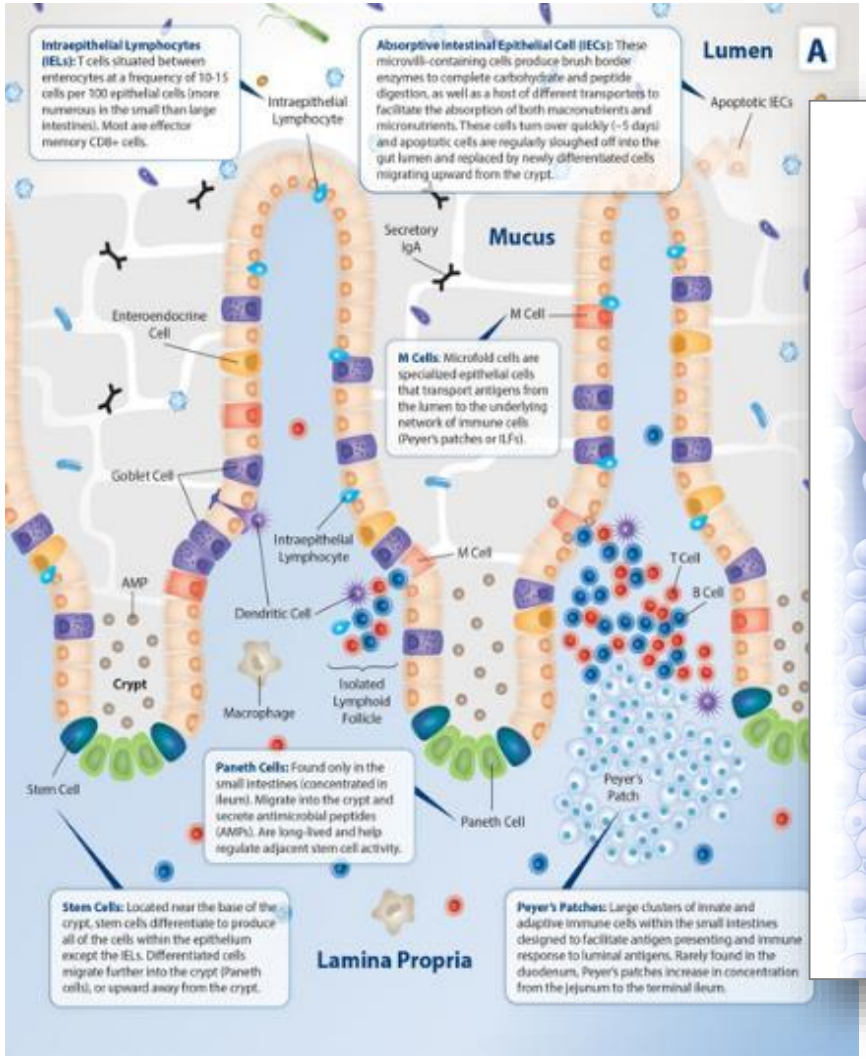
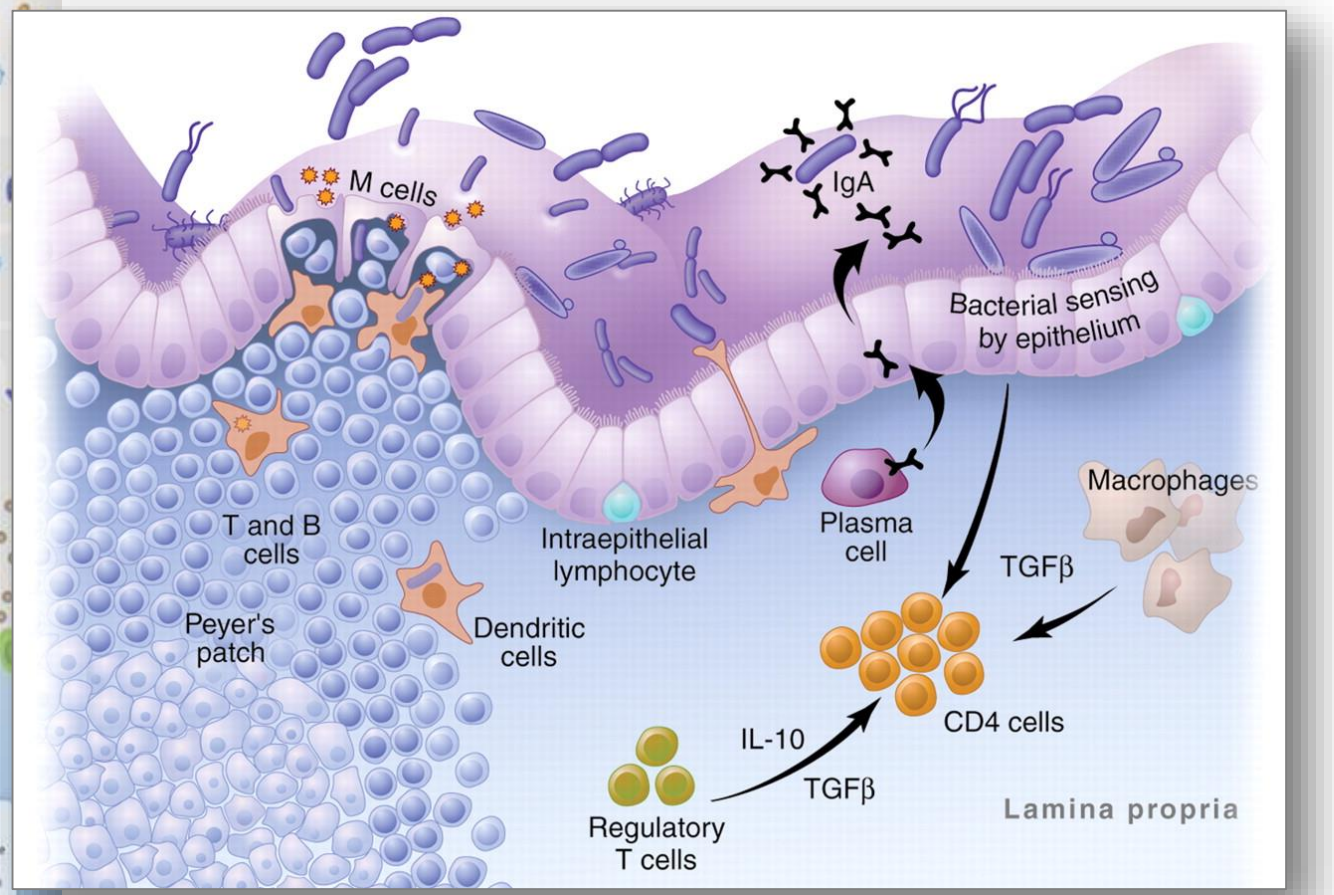
Immune System-Tightly Bound





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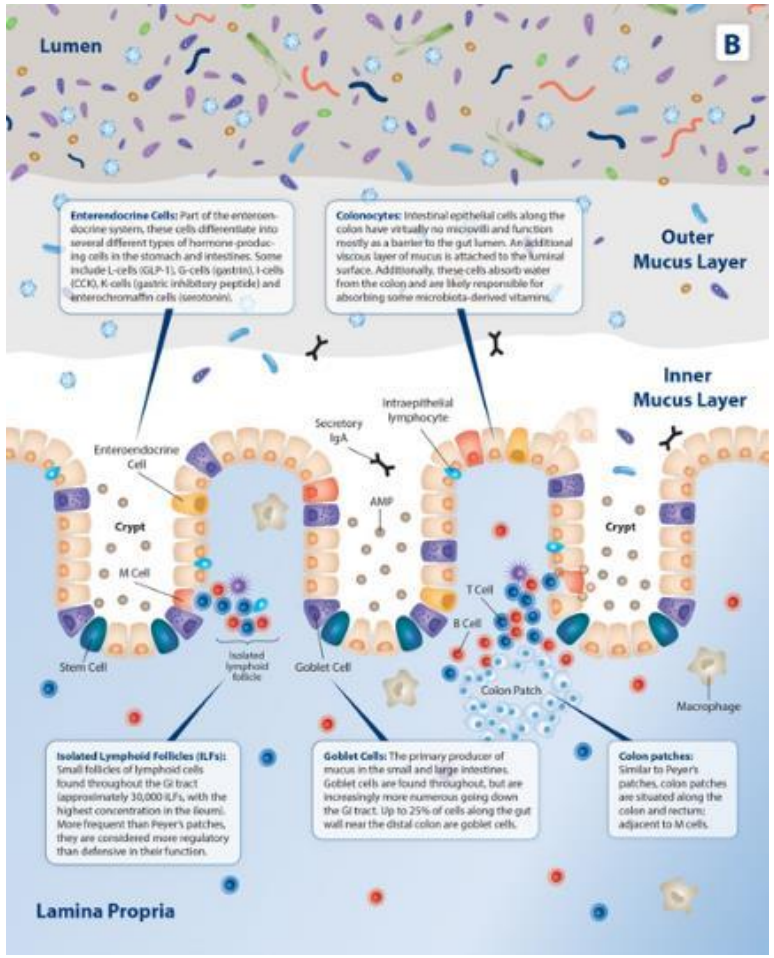
Basic Features of the Colon Barrier

- Two layers of mucus
- Increased number of goblet cells
- Less interface, more barrier
- Lower concentration of immune cells
- Fewer enteroendocrine cells
- Lumen acts as large fermenting vessel



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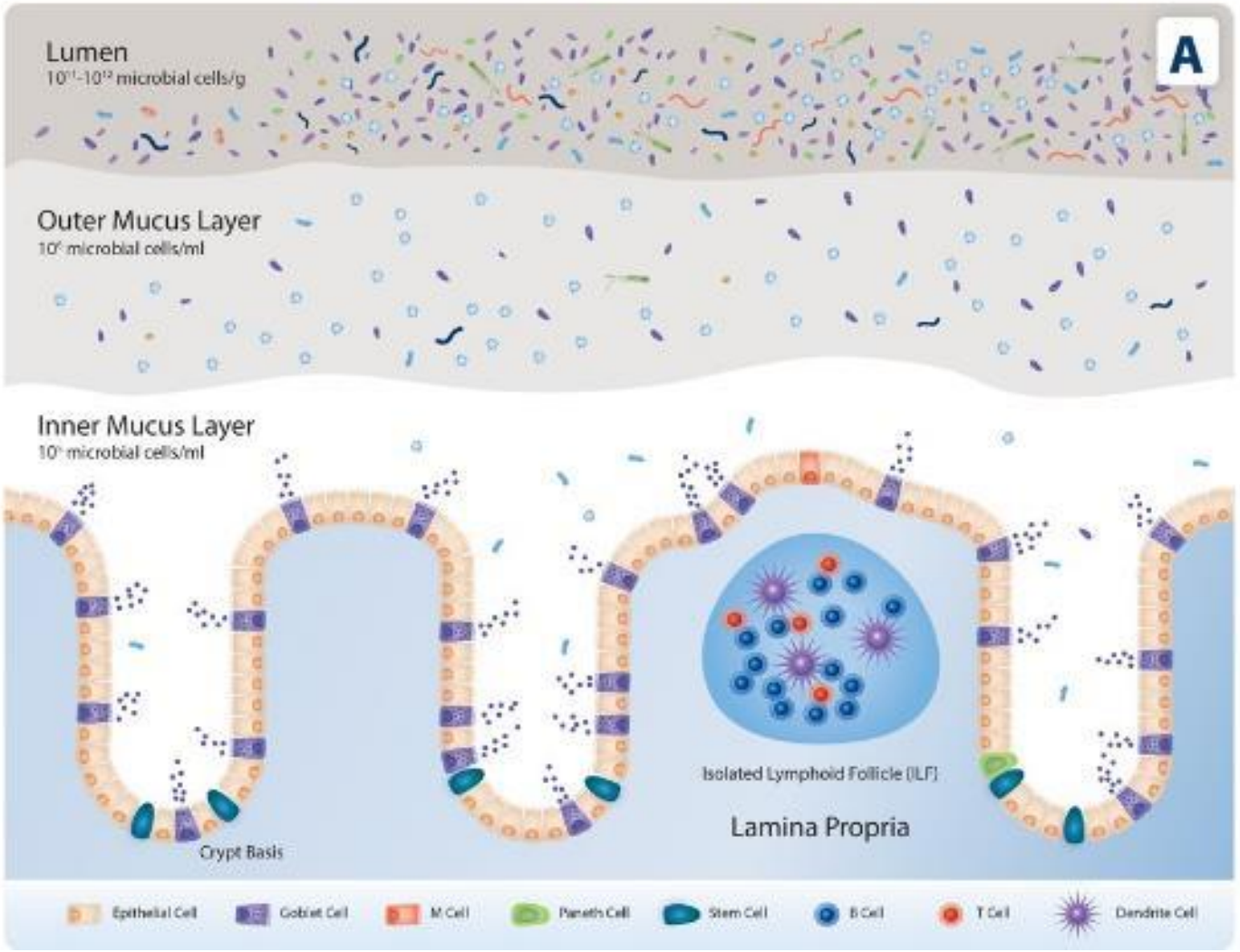
The Mucosal Micro-Environments



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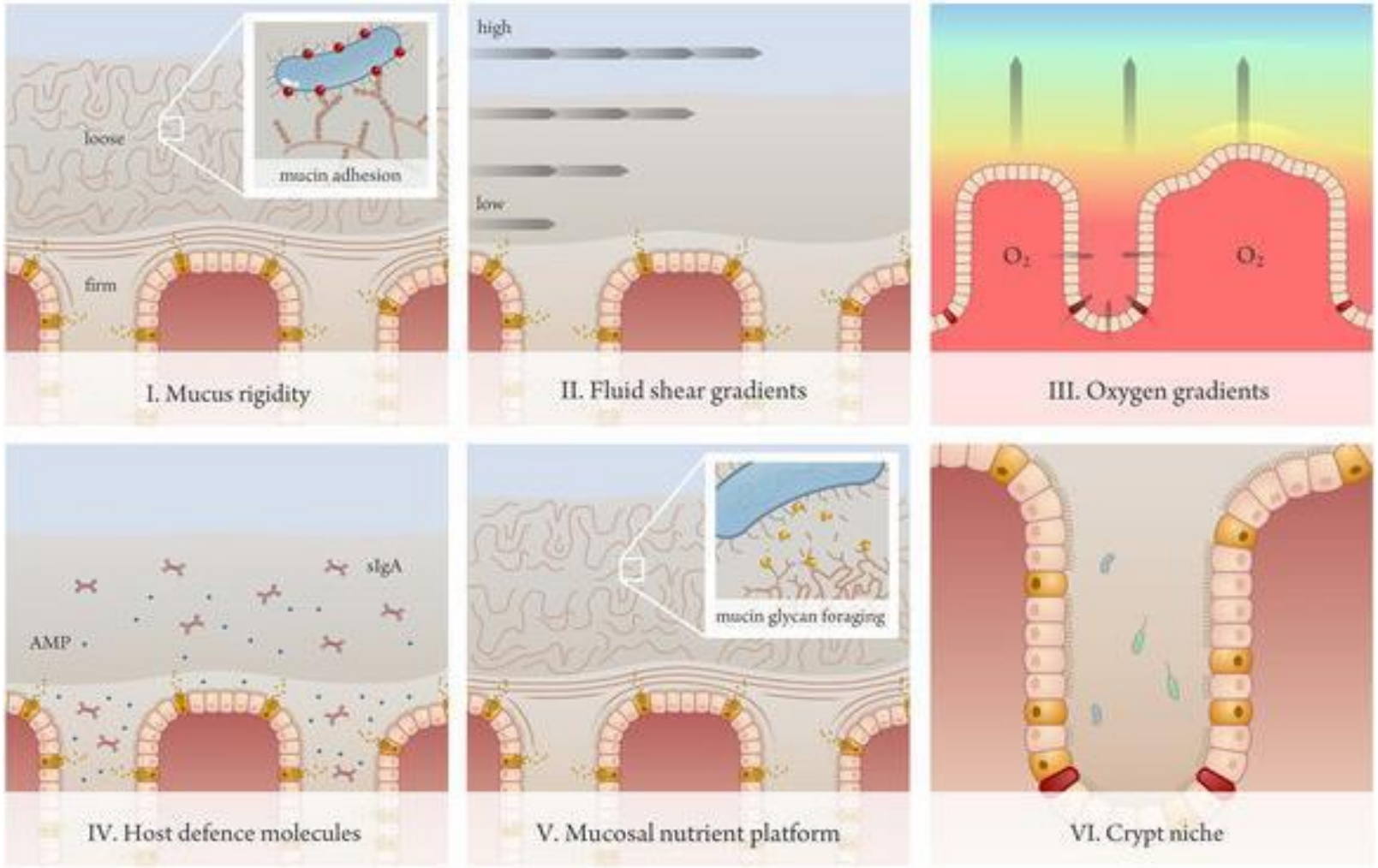
Mucus Factors That Influence Microenvironments



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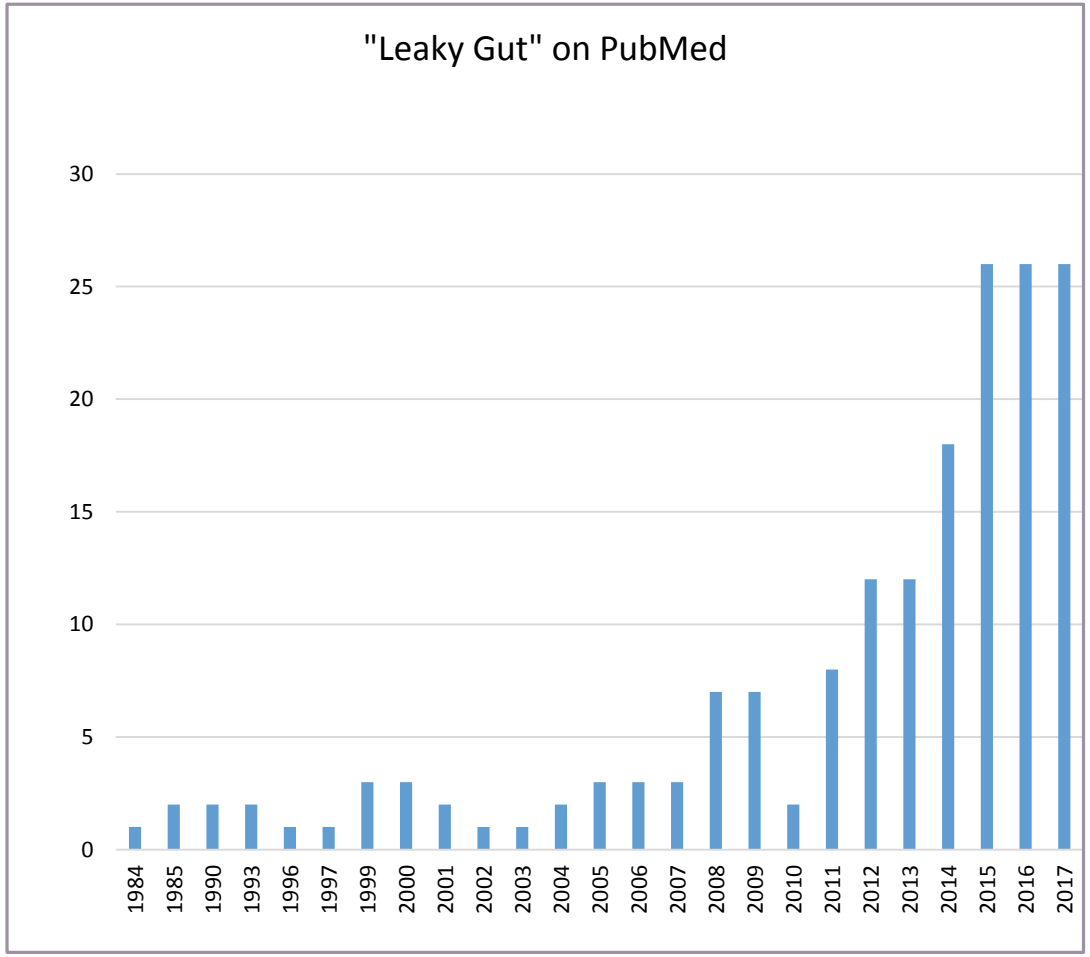
Is “Leaky Gut” a Legitimate Term?

- “From an MD’s standpoint, it’s a very gray area,” says gastroenterologist Donald Kirby, MD, director of the Center for Human Nutrition at the Cleveland Clinic. “Physicians don’t know enough about the gut, which is our biggest immune system organ.”
- "Leaky gut syndrome" isn't a diagnosis taught in medical school. Instead, "leaky gut really means you’ve got a diagnosis that still needs to be made,” Kirby says. “You hope that your doctor is a good-enough Sherlock Holmes, but sometimes it is very hard to make a diagnosis.”
- “We don’t know a lot but we know that it exists,” says Linda A. Lee, MD, a gastroenterologist and director of the Johns Hopkins Integrative Medicine and Digestive Center. “In the absence of evidence, we don’t know what it means or what therapies can directly address it.” - *WebMD*



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“Leaky gut”- More Commonly Used

frontiers in Immunology

REVIEW

Leaky Gut As a Danger Signal for Autoimmune Diseases

Qinghui Mu[1], Jay Kirby[1], Christopher M. Reilly[2] and Xin M. Luo[1]*

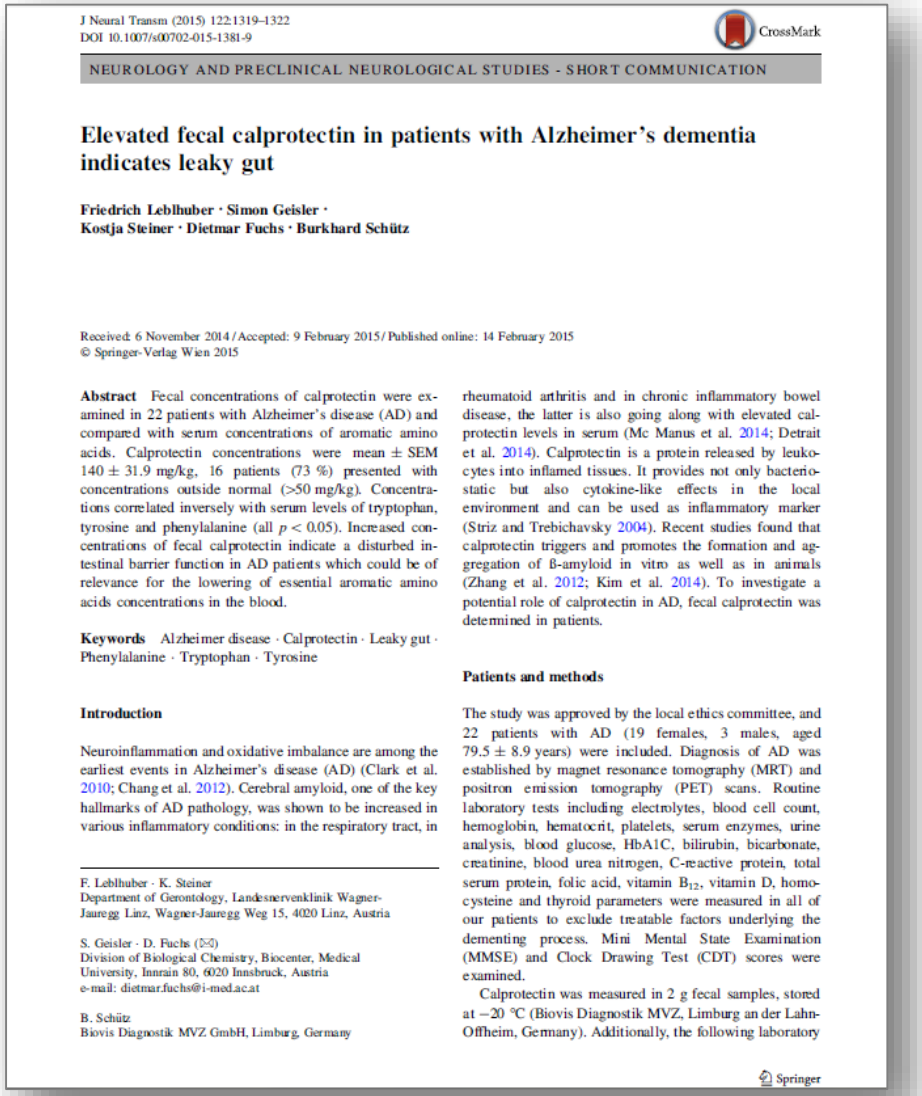
The intestinal epithelial lining, together with factors secreted from it, forms a barrier that separates the host from the environment. In pathologic conditions, the permeability of the epithelial lining may be compromised allowing the passage of toxins, antigens, and bacteria in the lumen to enter the blood stream creating a "leaky gut." In individuals with a genetic predisposition, a leaky gut may allow environmental factors to enter the body and trigger the initiation and development of autoimmune diseases. Growing evidence shows that the gut microbiota is important in supporting the epithelial barrier and therefore plays a key role in the regulation of environmental factors that enter the body. Several recent reports have shown that probiotics can reverse the leaky gut by enhancing the production of tight junction proteins; however, additional and longer term studies are still required. Conversely, pathogenic bacteria that can facilitate a leaky gut and induce autoimmune symptoms can be ameliorated with the use of antibiotic treatment. Therefore, it is hypothesized that modulating the gut microbiota can serve as a potential method for regulating intestinal permeability and may help to alter the course of autoimmune diseases in susceptible individuals.

Keywords: leaky gut, microbial translocation, gut microbiota, probiotics, autoimmunity

OPEN ACCESS

INTRODUCTION

For digestion and absorption purposes, mammals have developed a very complicated and highly specialized gastrointestinal system maintained by the mucosal barrier (1). However, apart from absorbable nutrients, the intestinal mucosa also faces tremendous exterior antigens, including food antigens, commensal bacteria, pathogens, and toxins. Thus, a specialized barrier function is required to block the entry of diverse exterior antigens while absorbing nutrients. Impressively, in the intestine, the front line of this barrier is maintained by only a single layer of specialized epithelial cells that are linked together by tight junction (TJ) proteins. Many other factors aid in support of this barrier including mucins, antimicrobial molecules, immunoglobulins, and cytokines. If any abnormalities occur among these factors, the intestinal permeability may increase, which is termed a "leaky gut." A leaky gut allows the entry of exterior antigens from the gut lumen into the host, which may promote both local and systemic immune responses. Multiple diseases may arise or be exacerbated due to a leaky gut, including autoimmune diseases such as inflammatory bowel disease, celiac disease, autoimmune hepatitis, type 1 diabetes (T1D), multiple sclerosis, and systemic lupus erythematosus (SLE) (2–6). Numerous factors can affect gut permeability, such as various diet-derived compounds, alcohol consumption, and gut microbiota dysbiosis. While this review is focused on chronic inflammation and gut barrier functions in mammals, it is worth noting that leaky gut is a phenomenon that is widespread in both mammalian and non-mammalian animals (7). Thus, studies in systems



J Neural Transm (2015) 122:1319–1322
DOI 10.1007/s00702-015-1381-9

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NEUROLOGY AND PRECLINICAL NEUROLOGICAL STUDIES - SHORT COMMUNICATION

Elevated fecal calprotectin in patients with Alzheimer’s dementia indicates leaky gut

Friedrich Leblhuber · Simon Geisler · Kostja Steiner · Dietmar Fuchs · Burkhard Schütz

Received: 6 November 2014 / Accepted: 9 February 2015 / Published online: 14 February 2015

Abstract Fecal concentrations of calprotectin were examined in 22 patients with Alzheimer’s disease (AD) and compared with serum concentrations of aromatic amino acids. Calprotectin concentrations were mean ± SEM 140 ± 31.9 mg/kg, 16 patients (73 %) presented with concentrations outside normal (>50 mg/kg). Concentrations correlated inversely with serum levels of tryptophan, tyrosine and phenylalanine (all $p < 0.05$). Increased concentrations of fecal calprotectin indicate a disturbed intestinal barrier function in AD patients which could be of relevance for the lowering of essential aromatic amino acids concentrations in the blood.

Keywords Alzheimer disease · Calprotectin · Leaky gut · Phenylalanine · Tryptophan · Tyrosine

Introduction

Neuroinflammation and oxidative imbalance are among the earliest events in Alzheimer’s disease (AD) (Clark et al. 2010; Chang et al. 2012). Cerebral amyloid, one of the key hallmarks of AD pathology, was shown to be increased in various inflammatory conditions: in the respiratory tract, in rheumatoid arthritis and in chronic inflammatory bowel disease, the latter is also going along with elevated calprotectin levels in serum (Mc Manus et al. 2014; Detrait et al. 2014). Calprotectin is a protein released by leukocytes into inflamed tissues. It provides not only bacteriostatic but also cytokine-like effects in the local environment and can be used as inflammatory marker (Striz and Trebichavsky 2004). Recent studies found that calprotectin triggers and promotes the formation and aggregation of ß-amyloid in vitro as well as in animals (Zhang et al. 2012; Kim et al. 2014). To investigate a potential role of calprotectin in AD, fecal calprotectin was determined in patients.

Patients and methods

The study was approved by the local ethics committee, and 22 patients with AD (19 females, 3 males, aged 79.5 ± 8.9 years) were included. Diagnosis of AD was established by magnet resonance tomography (MRT) and positron emission tomography (PET) scans. Routine laboratory tests including electrolytes, blood cell count, hemoglobin, hematocrit, platelets, serum enzymes, urine analysis, blood glucose, HbA1C, bilirubin, bicarbonate, creatinine, blood urea nitrogen, C-reactive protein, total serum protein, folic acid, vitamin B_{12}, vitamin D, homocysteine and thyroid parameters were measured in all of our patients to exclude treatable factors underlying the dementing process. Mini Mental State Examination (MMSE) and Clock Drawing Test (CDT) scores were examined.

Calprotectin was measured in 2 g fecal samples, stored at −20 °C (Biovis Diagnostik MVZ, Limburg an der Lahn-Offheim, Germany). Additionally, the following laboratory

F. Leblhuber · K. Steiner
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S. Geisler · D. Fuchs (✉)
Division of Biological Chemistry, Biocenter, Medical University, Innrain 80, 6020 Innsbruck, Austria
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B. Schütz
Biovis Diagnostik MVZ GmbH, Limburg, Germany

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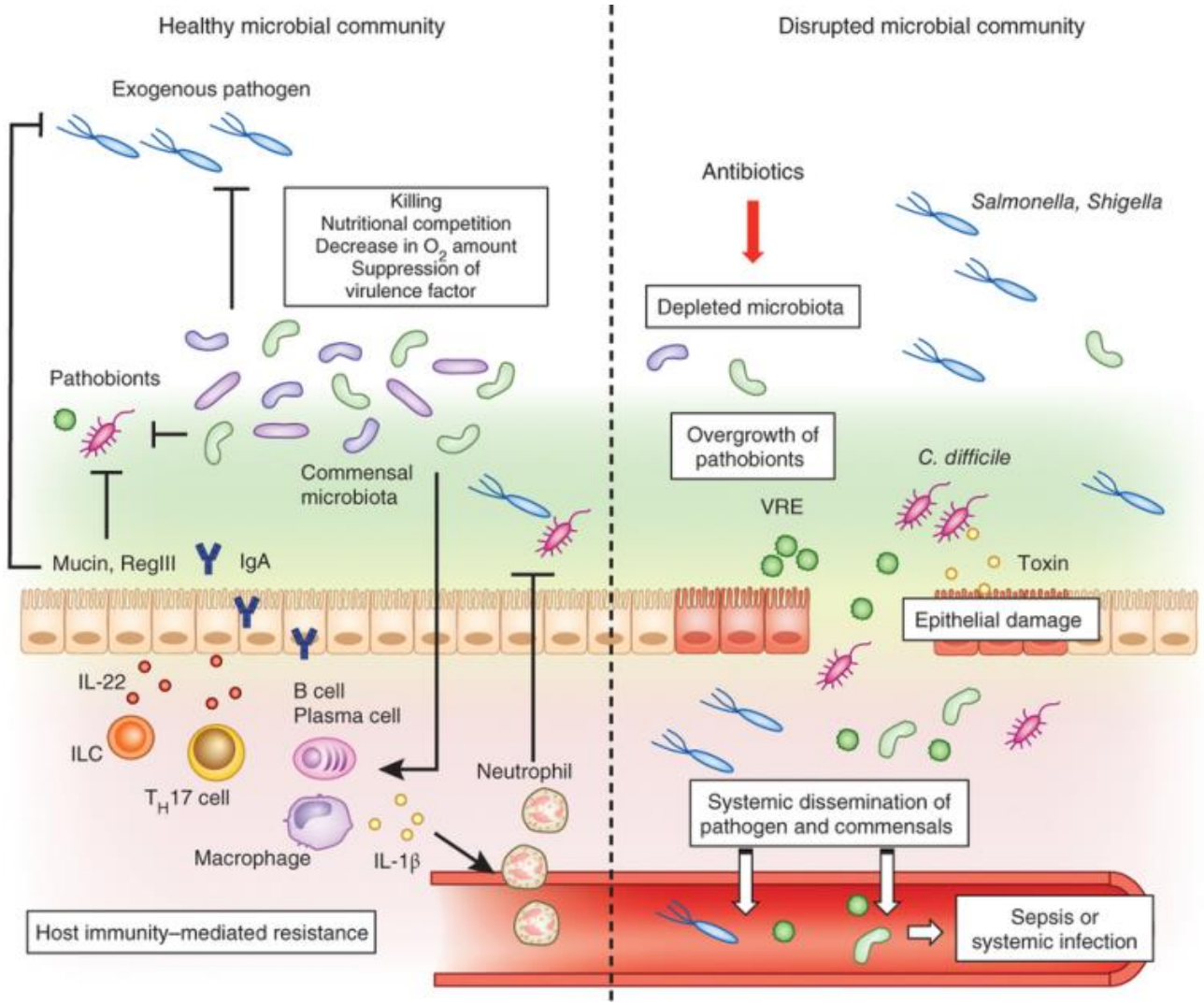
Leaky Gut: An Extreme View



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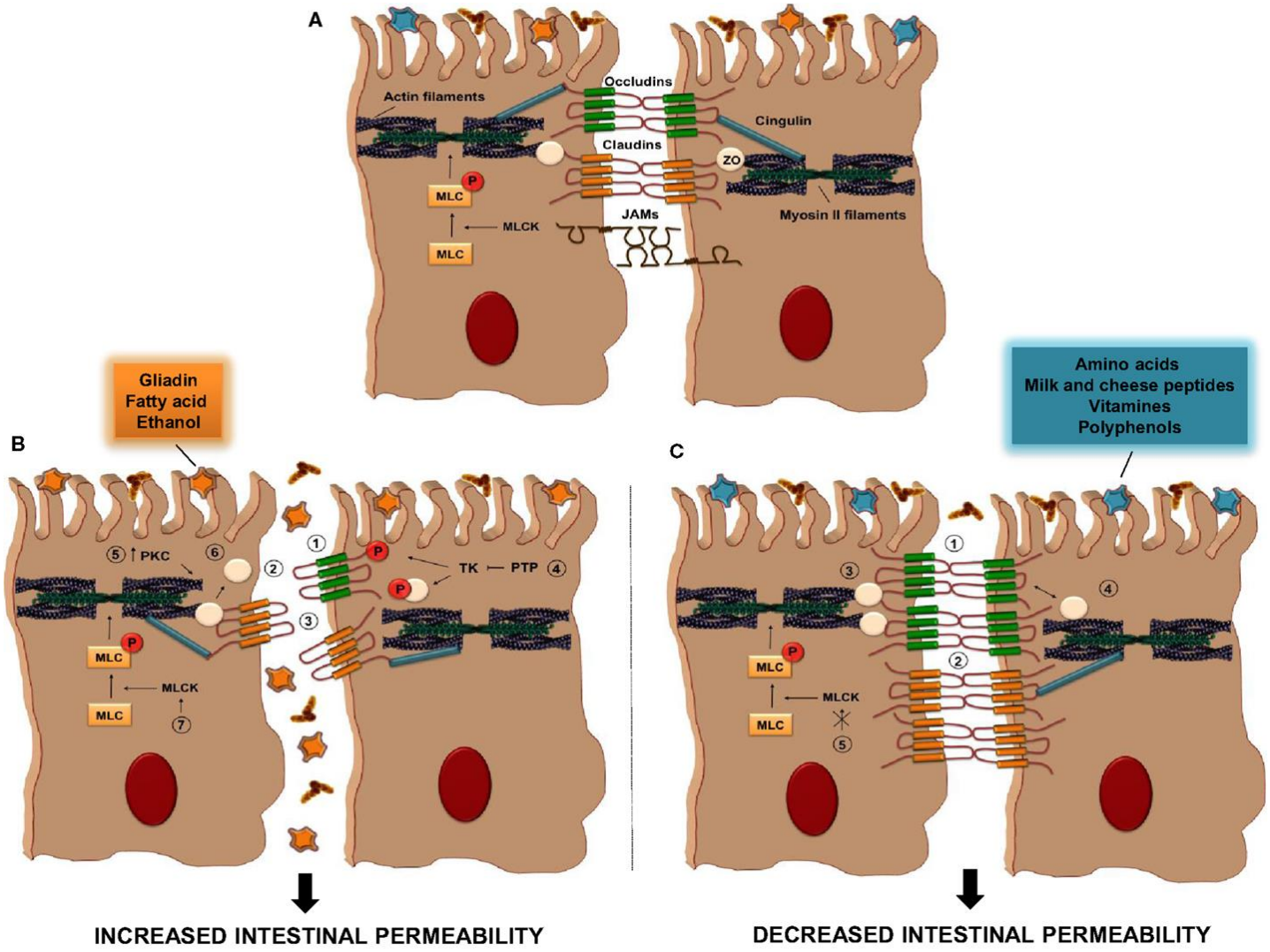
Kamada N, Chen GY, Inohiro, I, & Nunez, G. Control of pathogens and pathobionts by the gut microbiota. *Nature Immunology*. 2013; 14, 685-690.

More Common Scenario



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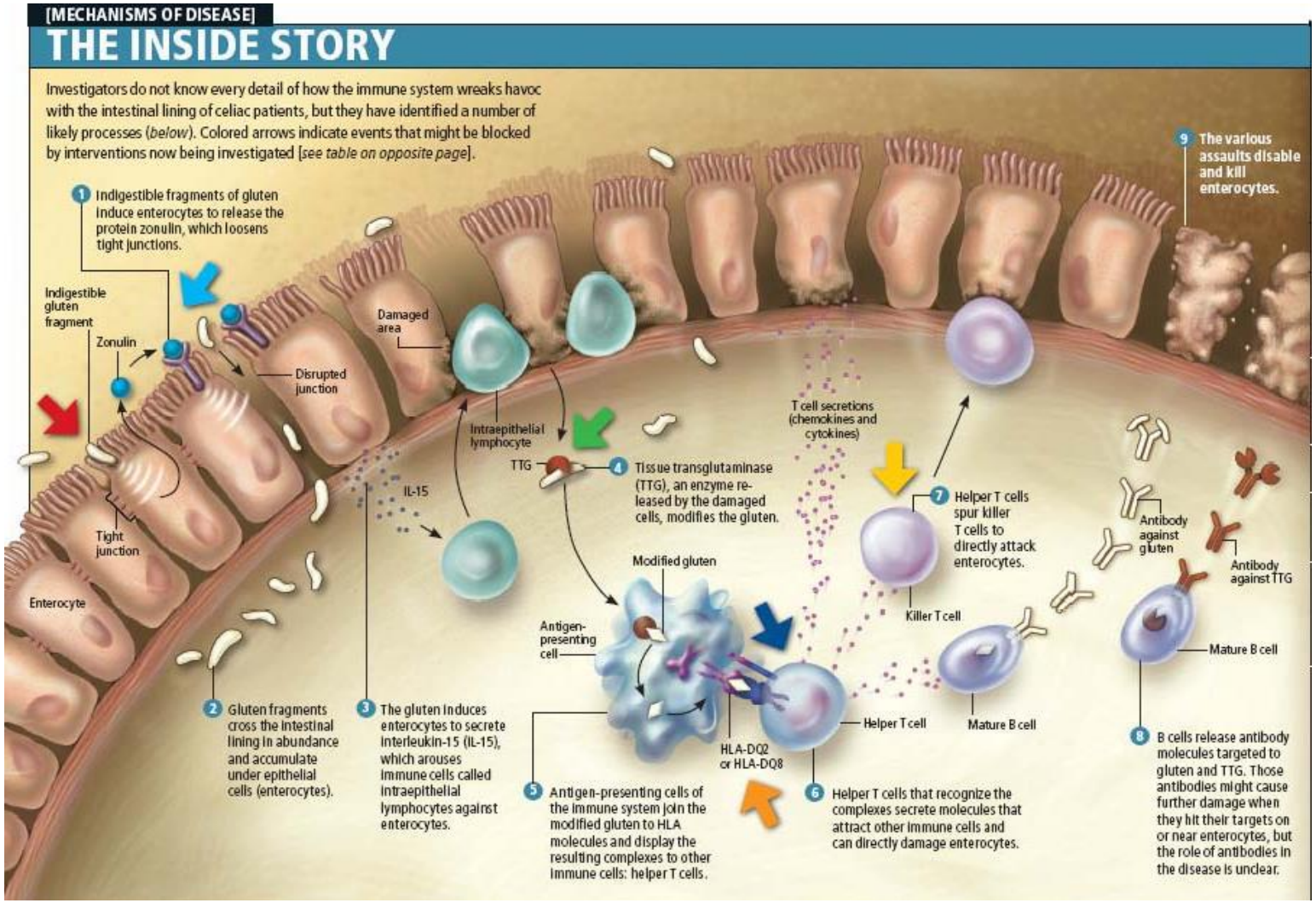
Santis SD, Cavalcanti E, Mastronardi M, Jirillo E, Chieppa M. Nutritional Keys for Intestinal Barrier Modulation. *Frontiers in Immunology*. 2015; 07(612), 1-15.

What We Can Learn From Celiac Disease



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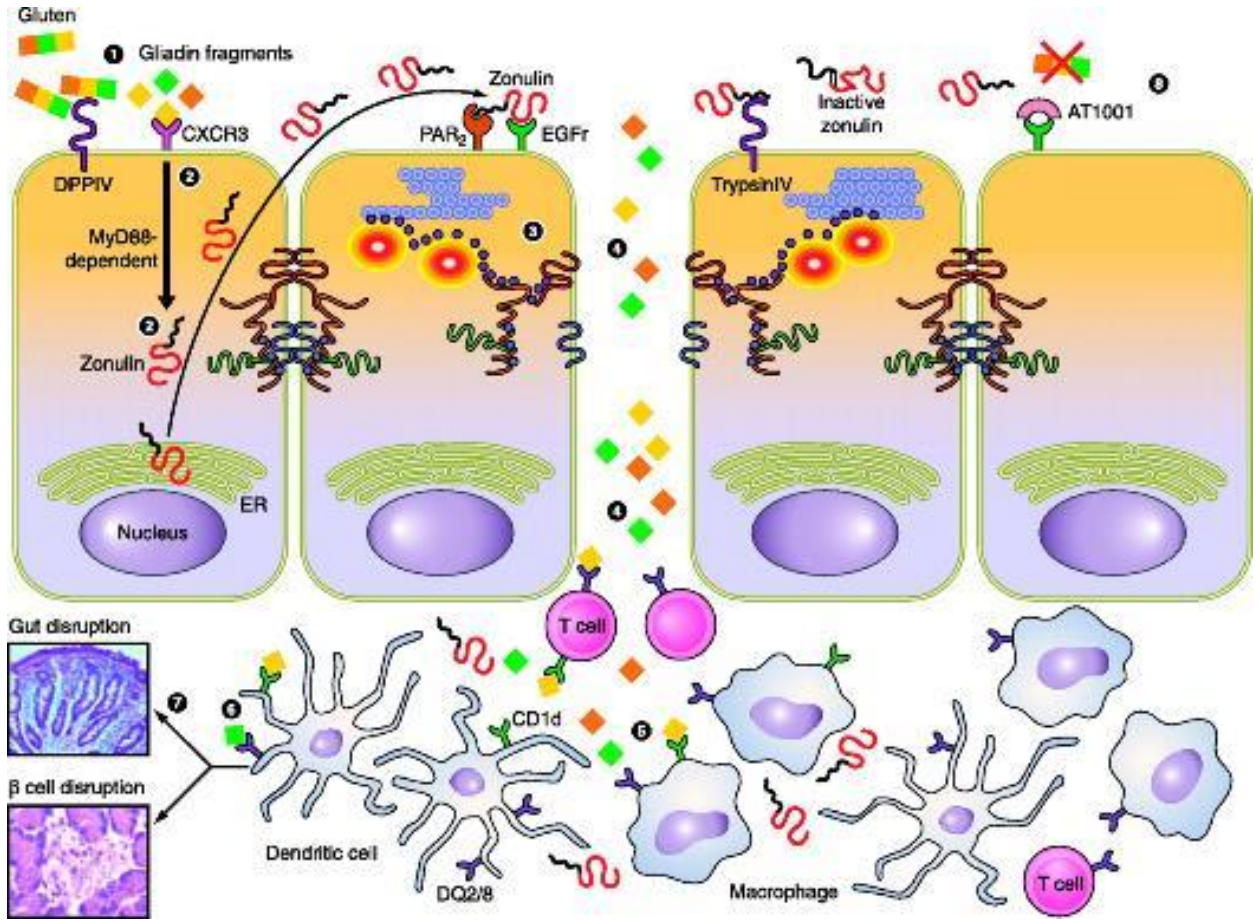
Fasano A. Surprises from Celiac Disease. *Scientific American.* 2009; 301, 54-61.

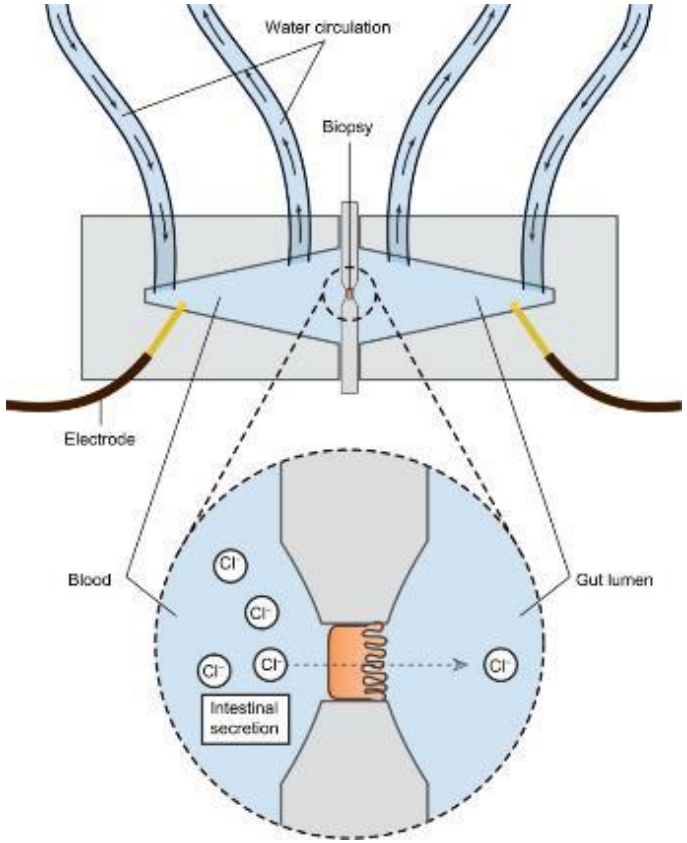
Mechanisms of Gliadin Induced Zonulin Release, Increased Intestinal Permeability, and Onset of Autoimmunity



Fassano A. Zonulin and its regulation of intestinal barrier function: The biological door to inflammation, autoimmunity, and cancer. *Physiological Reviews*. 2011; 91(1), 151-75

Measuring Gut Barrier function

Gold Standard: Ex-VIVO Ussing Chamber



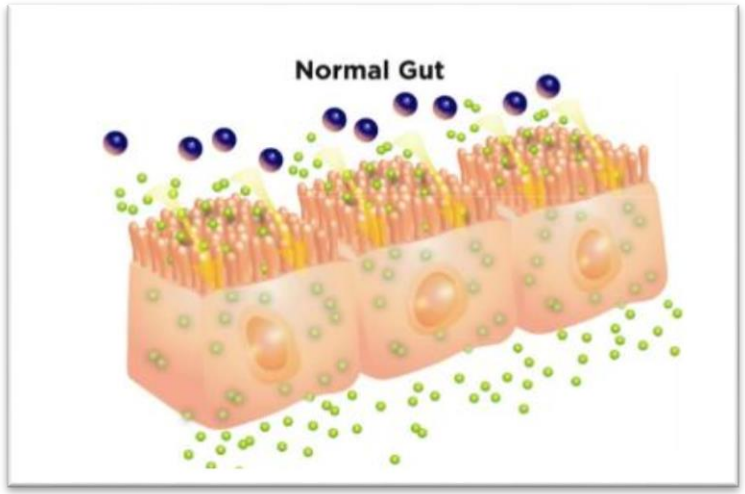
- Biopsied tissue (or experimental monolayer) oriented across membrane
- Can measure transepithelial electrical resistance (TEER)
- Model system for measuring insults to gut epithelium
- No support cell structures, no microbiome, etc.

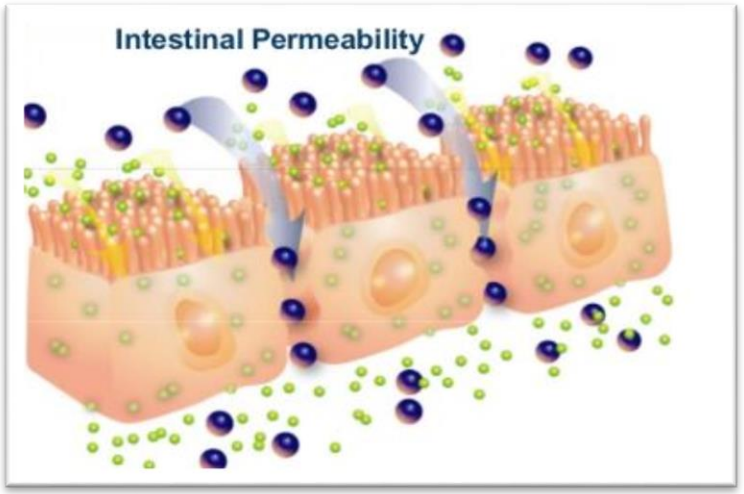
GENOVA DIAGNOSTICS

TG Guilliams (2016). *Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*. Stevens Point, WI: Point Institute

Measuring Gut Barrier Function

In Vivo: Size Exclusion Test (urine analysis)





- Lactulose/Mannitol test most common
- Mannitol is general measure of gut area, denominator can be altered (low) during atrophy (celiac, inflammation etc.) - ratio can rise even when lactulose levels do not increase due to low mannitol absorption
- Other test reagents: rhamnose, different size PEG molecules, etc.
- Be careful to follow dietary and timing instructions to prevent false interpretations

GENOVA DIAGNOSTICS

TG Guilliams (2016). *Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*. Stevens Point, WI: Point Institute

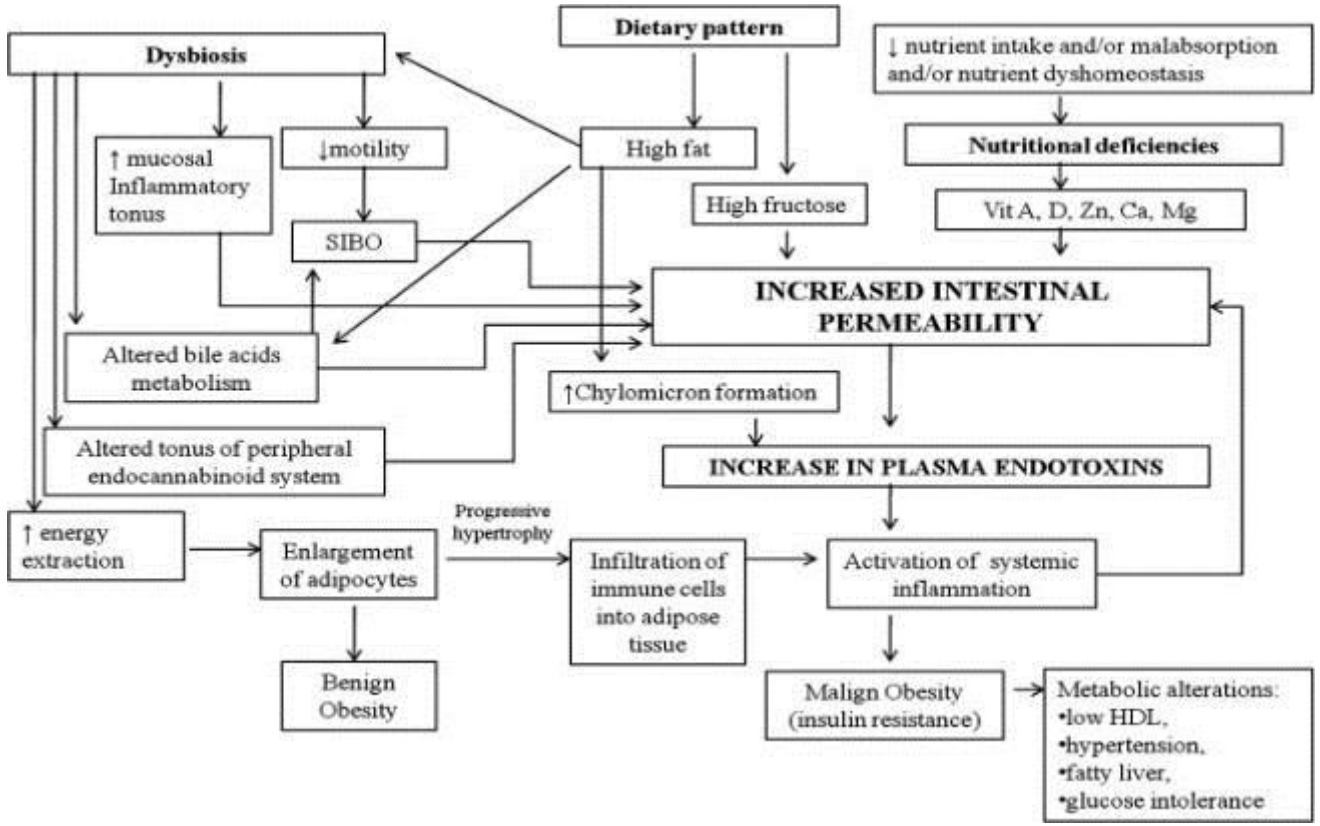
Other (Potential) Measures of Gut Permeability

- Urine/Serum levels of microbial metabolites: d-lactate, endotoxin, etc.
- Increased level of bacteria in dense mucus (biopsy)
- Reduced plasma citrulline (biomarker of glutamine)
- Fecal calprotectin (inflammation)
- Measures of TJ proteins (ZO, claudins, occluding, etc.)
- Serum (or fecal) zonulin

Conditions for Which Barrier Function is Compromised

- GI Infections (*V.cholera*, *EH E.coli*, *C.diff*, *H.pylori, etc…*)
- Gut inflammation of any kind likely triggers some gut permeability
- Celiac Disease and 30% of asymptomatic relatives
- Inflammatory Bowel Disease (both UC and Crohn's Disease)
- IBS-D (though not statistically significant in all studies)
- SIBO?

Gut Permeability Connected to Obesity, Insulin Resistance and the Western Dietary Pattern



GENOVA DIAGNOSTICS

Teixeira TF, Collado MC, Ferreira CL, Bressan J, Peluzio MC. Potential mechanisms for the emerging link between obesity and increased intestinal permeability. *Nutrition Research.* 2012; 32(9), 637-47.

Zonulin Levels are Increased in Obese Subjects and Type 2 Diabetics

Int J Endocrinol. 2013;2013:674106. doi: 10.1155/2013/674106. Epub 2013 Jul 18.

Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects.

J Nutr. 2016 Sep;146(9):1694-700. doi: 10.3945/jn.116.235358. Epub 2016 Jul 27.

Gut Microbiota Richness and Composition and Dietary Intake of Overweight Pregnant Women Are Related to Serum Zonulin Concentration, a Marker for Intestinal Permeability.

PLoS One. 2012;7(5):e37160. doi: 10.1371/journal.pone.0037160. Epub 2012 May 18.

Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance.

Eur J Endocrinol. 2015 Jan;172(1):29-36. doi: 10.1530/EJE-14-0589. Epub 2014 Oct 21.

Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation.

GENOVA DIAGNOSTICS

Preventing and Treating Intestinal Permeability

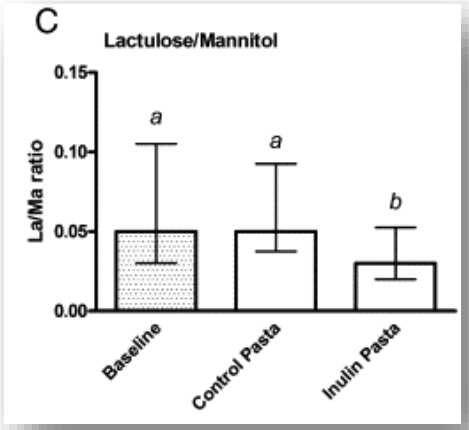
- Since intestinal permeability is rarely a “stand-alone” diagnostic criteria, there are few studies that investigate this as a primary outcome
- Many studies looking at in-vitro changes in barrier function (Ussing Chamber) and animal studies looking for influences of nutrients
- Few human clinical trials so far

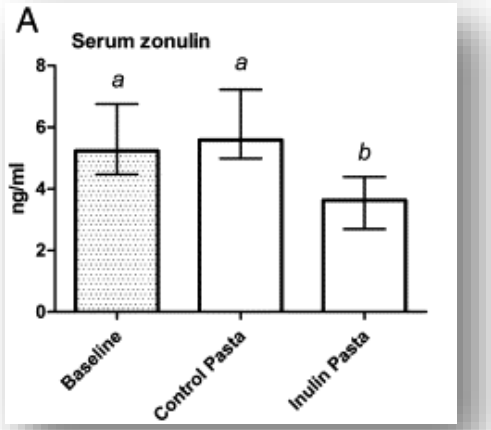
Dietary Interventions

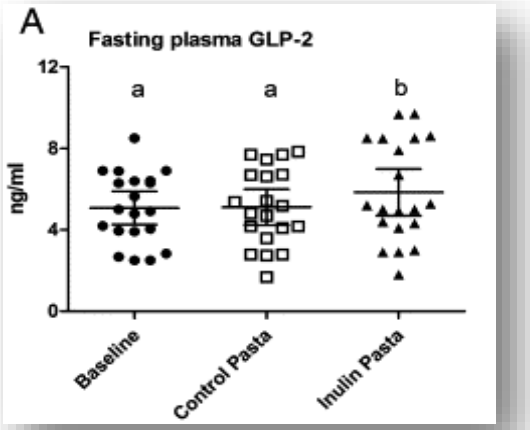
- It is assumed that common dietary patterns that induce poor microbiome balance, inflammation and chronic disease (excessive fat, refined carbohydrates, phytonutrient poor) are linked with increased intestinal permeability
- Few studies using dietary interventions have looked at changes in gut permeability

Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers

- 5 week crossover design with 8-week washout
- Healthy young (mean age 18.8 years old)
- 100 grams pasta/day
- Wheat pasta with or without 11% inulin (prebiotic from chicory)







GENOVA DIAGNOSTICS

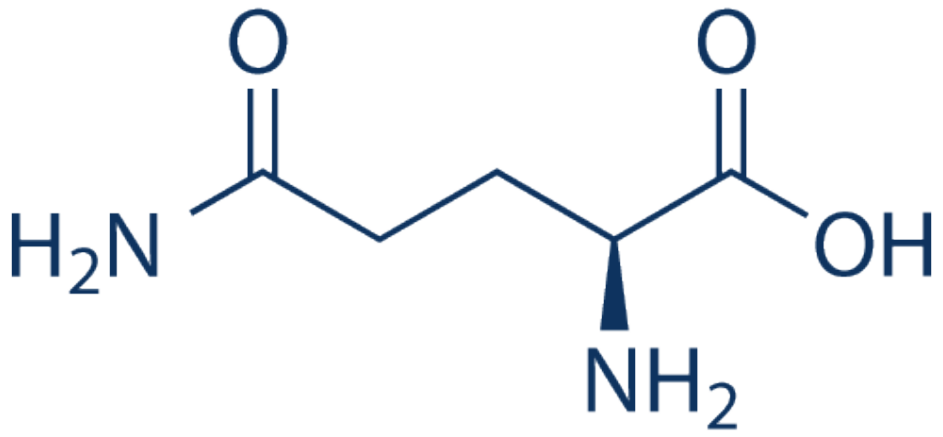
Russo F, Linsalata M, Clemente C, Chiloiro M, Orlando A, Marconi E, Chimienti G, & Riezzo G. Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers. *Nutrition Research*. 2012; 32(12), 940-6.

Micronutrients and Barrier Function

- Compromised barrier function is related to deficiencies in:
  - Vitamin A / beta carotene
  - Vitamin D
  - Zinc
  - Iron
- Intervention studies are limited to vitamin A + zinc supplementation in malnourished children (improved barrier function) and zinc in IBD and exercise induced permeability

- Chen P, Soares AM, Lima AA, et al. Association of vitamin A and zinc status with altered intestinal permeability: analyses of cohort data from northeastern Brazil. *Journal of Health Population and Nutrition*. 2003; 21(4), 309-15.
- Goto K, Chew F, Torún B, Peerson JM, Brown KH. Epidemiology of altered intestinal permeability to lactulose and mannitol in Guatemalan infants. *Journal of Pediatric Gastroenterology and Nutrition*. 1999; 28(3), 282-90.
- Roy SK, Behrens RH, Haider R, et al. Impact of zinc supplementation on intestinal permeability in Bangladeshi children with acute diarrhoea and persistent diarrhoea syndrome. *Journal of Pediatric Gastroenterology and Nutrition*. 1992; 15(3), 289-96.
- Santucci NR, Alkhouri RH, Baker RD, Baker SS. Vitamin and zinc status pretreatment and posttreatment in patients with inflammatory bowel disease. *Journal of Pediatric Gastroenterology and Nutrition*. 2014; 59(4), 455-7.
- Davison G, Marchbank T, March DS, et al. Zinc carnosine works with bovine colostrum in truncating heavy exercise-induced increase in gut permeability in healthy volunteers. *American Journal of Clinical Nutrition*. 201; 104(2), 526-36.

L-Glutamine and Barrier Function



- Long list of mechanisms
- Numerous animal models to show GI barrier benefits
- Most human data related to severe injury/burn victims
- Frequent use within integrative/functional medicine community (anecdotal success)
- Recent limited research in traditional gut barrier human clinical research

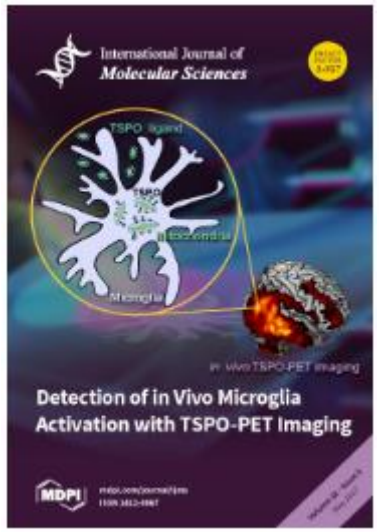
Glutamine

Some mechanisms linking glutamine with intestinal barrier functions:

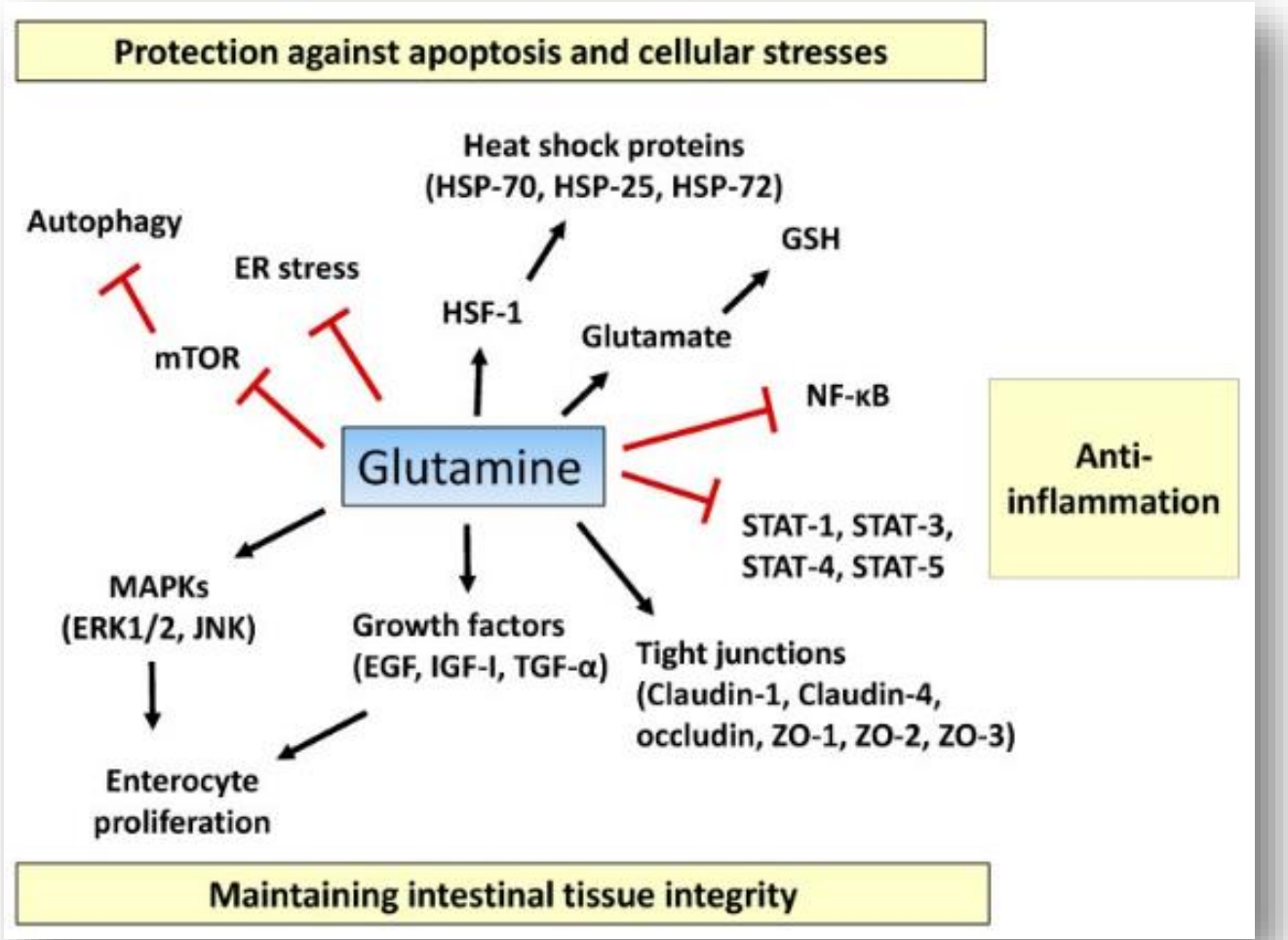
- Needed for the development of the gut epithelium during early life supplementation (used in neonates to improve gut barrier function)
- Critical substrate for metabolites within enterocytes including ATP, glutathione and DNA/RNA
- Important secondary signaling molecule within enterocytes, affecting critical metabolic and proliferative pathways in the cell
- GLN has been shown to modulate TJ proteins, phosphorylation and assembly, using both GLN deprivation and supplementation studies
- GLN contributes to favorable alterations in the gut microbiota
- GLN maintains intestinal structure and function during aging
- GLN promotes sIgA secretion via direct (immunomodulatory) and indirect (microbiota) signals
- GLN modulates the GI permeability effects of HPA axis stress (i.e., CRF)
- GLN modulates the gastrointestinal permeability effects of intensive exercise

GENOVA DIAGNOSTICS

TG Guilliams (2016). *Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*. Stevens Point, WI: Point Institute



The Roles of Glutamine in the Intestine and Its Implication in Intestinal Diseases

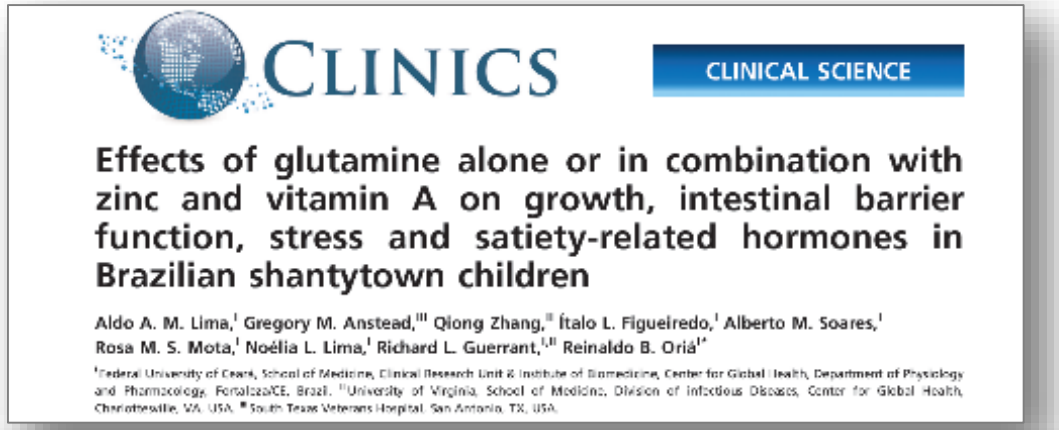


GENOVA DIAGNOSTICS

Kim MH & Kim H. The roles of glutamine in the intestine and its implication in intestinal diseases. *International Journal of Molecular Sciences.* 2017; 18(5), 1051-66.

GLN Studies in Undernourished Children or Severe Illness

Limited data on “functional” GI situation



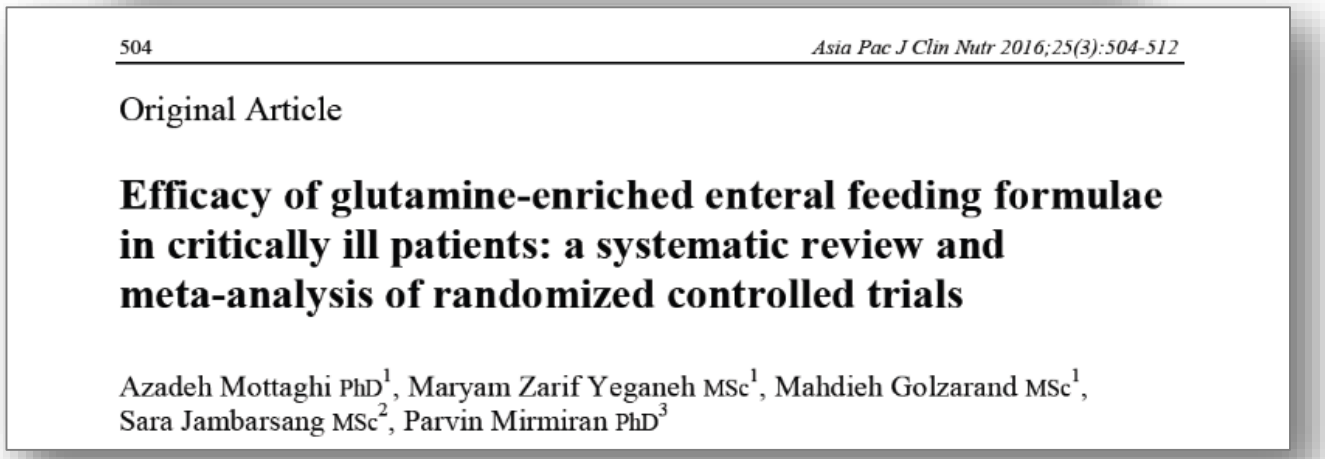
CLINICS

CLINICAL SCIENCE

Effects of glutamine alone or in combination with zinc and vitamin A on growth, intestinal barrier function, stress and satiety-related hormones in Brazilian shantytown children

Aldo A. M. Lima,[I] Gregory M. Anstead,[III] Qiong Zhang,[II] Ítalo L. Figueiredo,[I] Alberto M. Soares,[I] Rosa M. S. Mota,[I] Noélia L. Lima,[I] Richard L. Guerrant,[I,II] Reinaldo B. Oriá[I*]

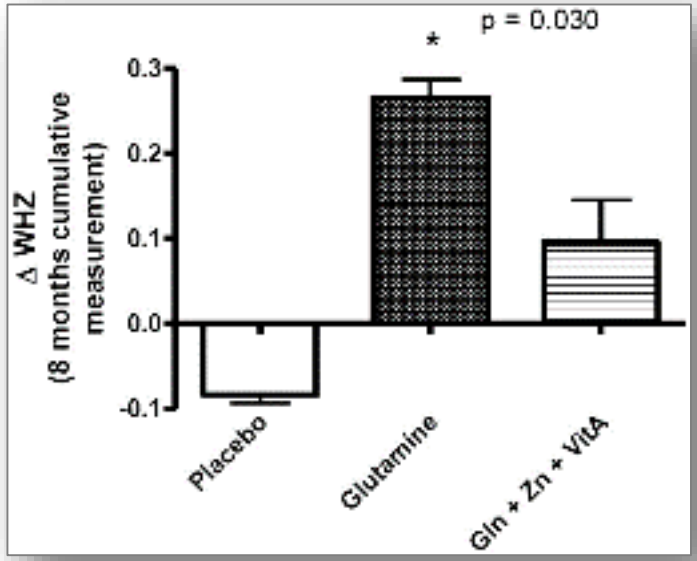
[I]Federal University of Ceará, School of Medicine, Clinical Research Unit & Institute of Biomedicine, Center for Global Health, Department of Physiology and Pharmacology, Fortaleza/CE, Brazil. [II]University of Virginia, School of Medicine, Division of Infectious Diseases, Center for Global Health, Charlottesville, VA, USA. [III]South Texas Veterans Hospital, San Antonio, TX, USA.



Original Article

Efficacy of glutamine-enriched enteral feeding formulae in critically ill patients: a systematic review and meta-analysis of randomized controlled trials

Azadeh Mottaghi PhD[1], Maryam Zarif Yeganeh MSc[1], Mahdieh Golzarand MSc[1], Sara Jambarsang MSc[2], Parvin Mirmiran PhD[3]



Lima AAM, Anstead GM, Zhang Q., et al. Effects of glutamine alone or in combination with zinc and vitamin A on growth, intestinal barrier function, stress and satiety-related hormones in Brazilian shantytown children. *Clinics*. 2014; 4(2), 225-233.

Mottaghi, A, Yeganeh MZ, golzarand M, Jambarsang S, & Mirmiran P. Efficacy of glutamine-enriched enteral feeding formulae in critically ill patients: a systematic review and meta-analysis of randomized controlled trials. *Asia Pacific Journal of Clinical Nutrition*. 2016; 25(3), 504-512

GENOVA DIAGNOSTICS

Glutamine and Whey Protein Improve Intestinal Permeability and Morphology in Patients with Crohn's Disease: A Randomized Controlled Trial

Jaya Benjamin · Govind Makharia · Vineet Ahuja · K. D. Anand Rajan · Mani Kalaivani · Siddhartha Datta Gupta · Yogendra Kumar Joshi

Received: 2 May 2011 / Accepted: 8 October 2011 / Published online: 26 October 2011

Abstract

Background Increased intestinal permeability (IP) has been implicated in the etiopathogenesis, disease activity and relapse of Crohn's disease (CD). Glutamine, the major fuel for the enterocytes, may improve IP.

Aim We evaluated the effect of oral glutamine on IP and intestinal morphology in patients with CD.

Methods In a randomized controlled trial, consecutive patients with CD in remission phase with an abnormal IP were randomized to a glutamine group (GG) or active control group (ACG) and were given oral glutamine or whey protein, respectively, as 0.5 g/kg ideal body weight/day for 2 months. IP was assessed by the lactulose mannitol excretion ratio (LMR) in urine, and morphometry was performed by computerized image analysis system.

Results Patients (age 34.5 ± 10.5 years; 20 males) were assigned to the GG ($n = 15$) or ACG (n = 15). Fourteen patients in each group completed the t[illegible] [median (range)] in GG and ACG at 2 mo[illegible] (0.006–0.090) and 0.033 (0.009–0.077), respectively, with $P = 0.6133$. IP normalized in 8 (57.1%) patients in each group ($P = 1.000$). The villous crypt ratio (VCR) [mean (SD)] in GG and ACG at 2 months was 2.68 (1.02) and 2.49 (0.67), respectively, ($P = 0.347$). At the end of 2 months LMR improved significantly in GG from 0.071 (0.041–0.254) to 0.029 (0.006–0.090) ($P = 0.0012$) and in ACG from 0.067 (0.040–0.136) to 0.033 (0.009–0.077) ($P = 0.0063$). VCR improved in the GG from 2.33 (0.77) to 2.68 (1.02) ($P = 0.001$), and in ACG from 2.26 (0.57) to 2.49 (0.67) ($P = 0.009$).

Conclusions Intestinal permeability and morphology improved significantly in both glutamine and ACG.

J. Benjamin · G. Makharia · V. Ahuja · Y. K. Joshi (✉)
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G. Makharia

40 grams of glutamine (80kg-176 lbs)

GENOVA DIAGNOSTICS

Glutamine Supplementation and Immune Function During Heavy Load Training

- 24 athletes given 10 gram/day GLN or placebo for 6 weeks
- GLN was able to attenuate immunosuppression triggered by intense heavy-load training compared to placebo
- Gut permeability was not assessed in these subjects

GENOVA DIAGNOSTICS

Song QH, Xu RM, Zhang QH, Shen GQ, MA M, Zhao XP, Guo YH & Wang Y. Glutamine supplementation and immune function during heavy load training. *International Journal of Clinical Pharmacology and Therapeutics*. 2015; 53(5), 372-376

Glutamine for Gut Support

- One of the most common recommendations for supporting gut barrier, though with limited published support (considered to be very safe for nearly all subjects)
- Dose recommendation starts at 4 to 8 grams/day, but may need much higher doses for desired outcome
- Often provided in powder rather than capsules to allow for higher dose therapies

Phytonutrients

- Flavonoids (of all kinds) have been shown to promote strong tight junction formation when tested in vitro (directly signaling enterocytes)
- Flavonoids have been shown to create a diverse and healthy gut population (indirect benefit on barrier)
- Many supplemental flavonoids are potent anti-inflammatory agents
- Diverse diet is best option, followed by range of flavonoids via supplementation (dose not as important as long-term use)

GENOVA DIAGNOSTICS

TG Guilliams (2016). *Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*. Stevens Point, WI: Point Institute

Berberine

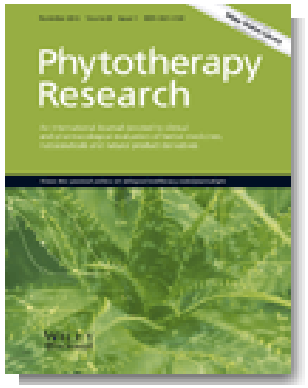
frontiers in Pharmacology

Berberine Attenuates Intestinal Mucosal Barrier Dysfunction in Type 2 Diabetic Rats

- Popular alkaloid for antimicrobial and metabolic-related outcomes (LDL-C, FBG, Met-syn, BP, etc.)
- Numerous in vitro and animal studies suggesting potent affects on improving TJ formation and function
- Berberine has affect on microbiome and type 2 diabetes, both known to affect or be affected by gut barrier issues

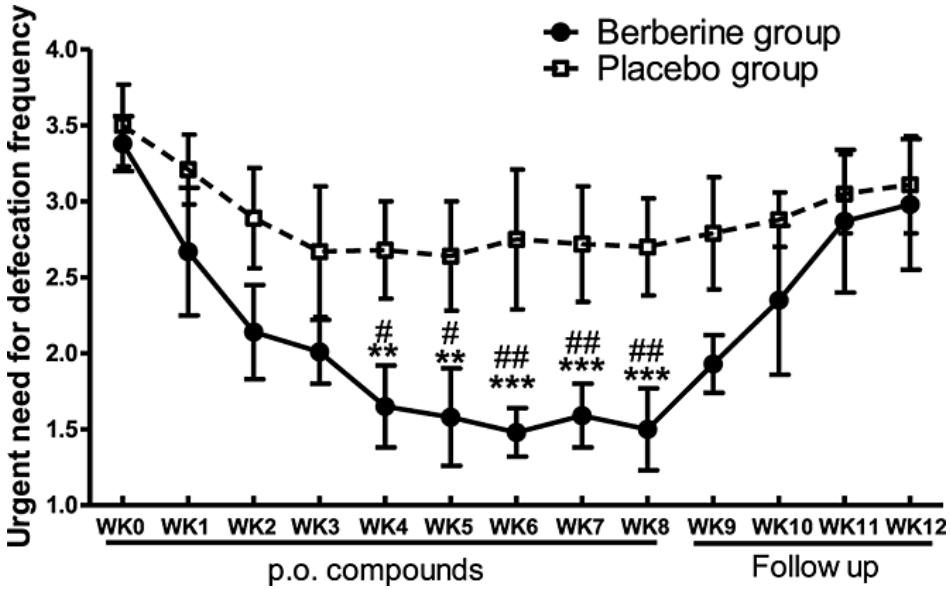
GENOVA DIAGNOSTICS

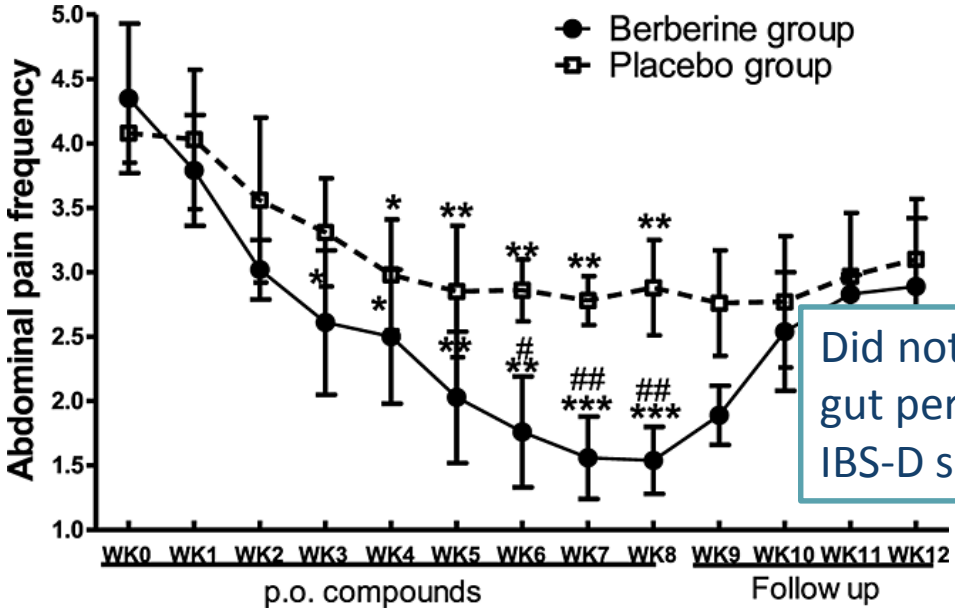
Gong J, Hu M, Huang Z, et al. Berberine attenuates intestinal mucosal barrier dysfunction in type 2 diabetic rats. *Frontiers in Pharmacology*. 2017; 3(8), 42-54



A Randomized Clinical Trial of Berberine Hydrochloride in Patients with Diarrhea-Predominant Irritable Bowel Syndrome

400 mg/day berberine





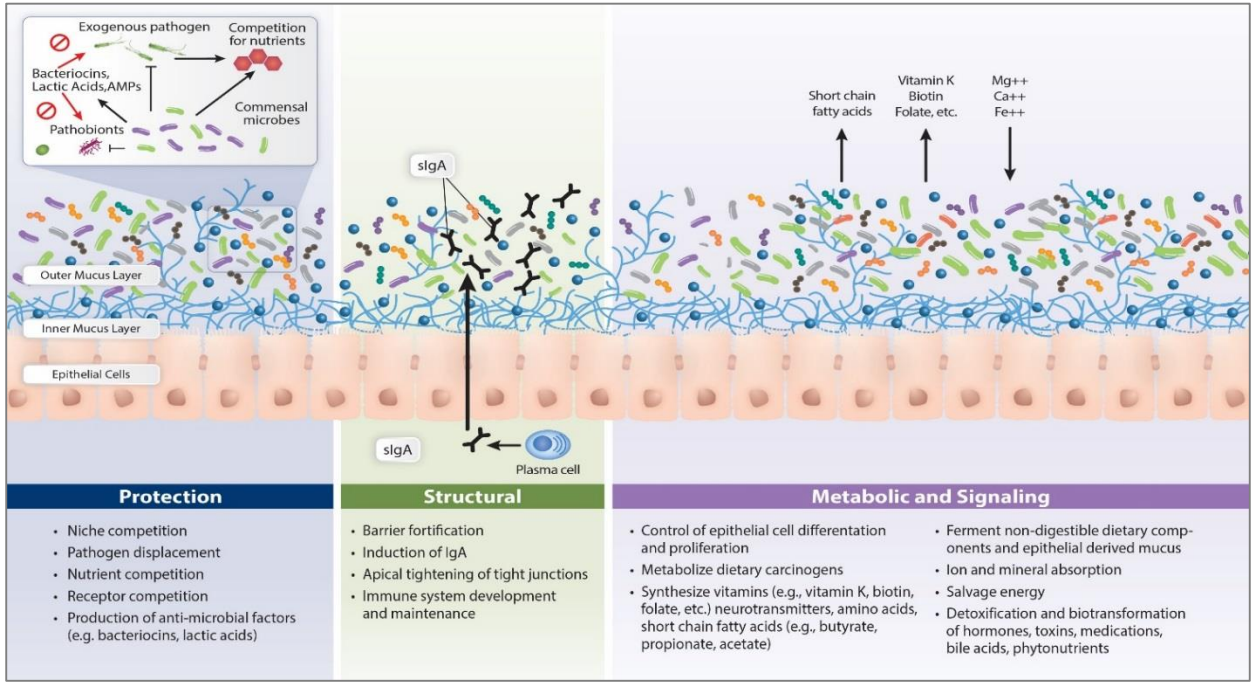
Did not measure gut permeability in IBS-D subjects

GENOVA DIAGNOSTICS

Chen C, Tao C, Liu Z, et al. A randomized clinical trial of berberine hydrochloride in patients with diarrhea-predominant irritable bowel syndrome. *Phototherapy Research*. 2015; 29(11), 1822-27

Probiotics

- A wide-range of probiotics are likely to help balance the microbiome, benefit immune function and improve gut barrier function…however
- Few clinical trials actually measure gut barrier function as an outcome in probiotic clinical trials



GENOVA DIAGNOSTICS

TG Guilliams (2016). *Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*. Stevens Point, WI: Point Institute

Many In-Vitro Studies

Physiol Rep. 2015 Mar;3(3). pii: e12327. doi: 10.14814/phy2.12327.

Strengthening of the intestinal epithelial tight junction by Bifidobacterium bifidum.

Hsieh CY[1], Osaka T[1], Moriyama E[1], Date Y[2], Kikuchi J[3], Tsuneda S[4].

Vet Immunol Immunopathol. 2016 Apr;172:55-63. doi: 10.1016/j.vetimm.2016.03.005. Epub 2016 Mar 5.

Protective effects of Lactobacillus plantarum on epithelial barrier disruption caused by enterotoxigenic Escherichia coli in intestinal porcine epithelial cells.

Wu Y[1], Zhu C[2], Chen Z[2], Chen Z[2], Zhang W[2], Ma X[3], Wang L[3], Yang X[3], Jiang Z[4].

Inflamm Bowel Dis. 2016 Dec;22(12):2811-2823.

VSL#3 Probiotic Stimulates T-cell Protein Tyrosine Phosphatase-mediated Recovery of IFN-γ-induced Intestinal Epithelial Barrier Defects.

Krishnan M[1], Penrose HM, Shah NN, Marchelletta RR, McCole DF.

J Pediatr Gastroenterol Nutr. 2017 Mar;64(3):404-412. doi: 10.1097/MPG.0000000000001310.

Secretions of Bifidobacterium infantis and Lactobacillus acidophilus Protect Intestinal Epithelial Barrier Function.

Guo S[1], Gillingham T, Guo Y, Meng D, Zhu W, Walker WA, Ganguli K.

J Crohns Colitis. 2017 Feb 22. doi: 10.1093/ecco-jcc/jjx030. [Epub ahead of print]

Saccharomyces boulardii CNCM I-745 restores intestinal barrier integrity by regulation of E-cadherin recycling.

Terciolo C[1], Dobric A[1], Ouaissi M[2], Siret C[1], Breuzard G[1], Silvy F[1], Marchiori B[3], Germain S[1], Bonier R[1], Hama A[3], Owens R[3], Lombardo D[1], Rigot V[1], André F[1].

GENOVA DIAGNOSTICS

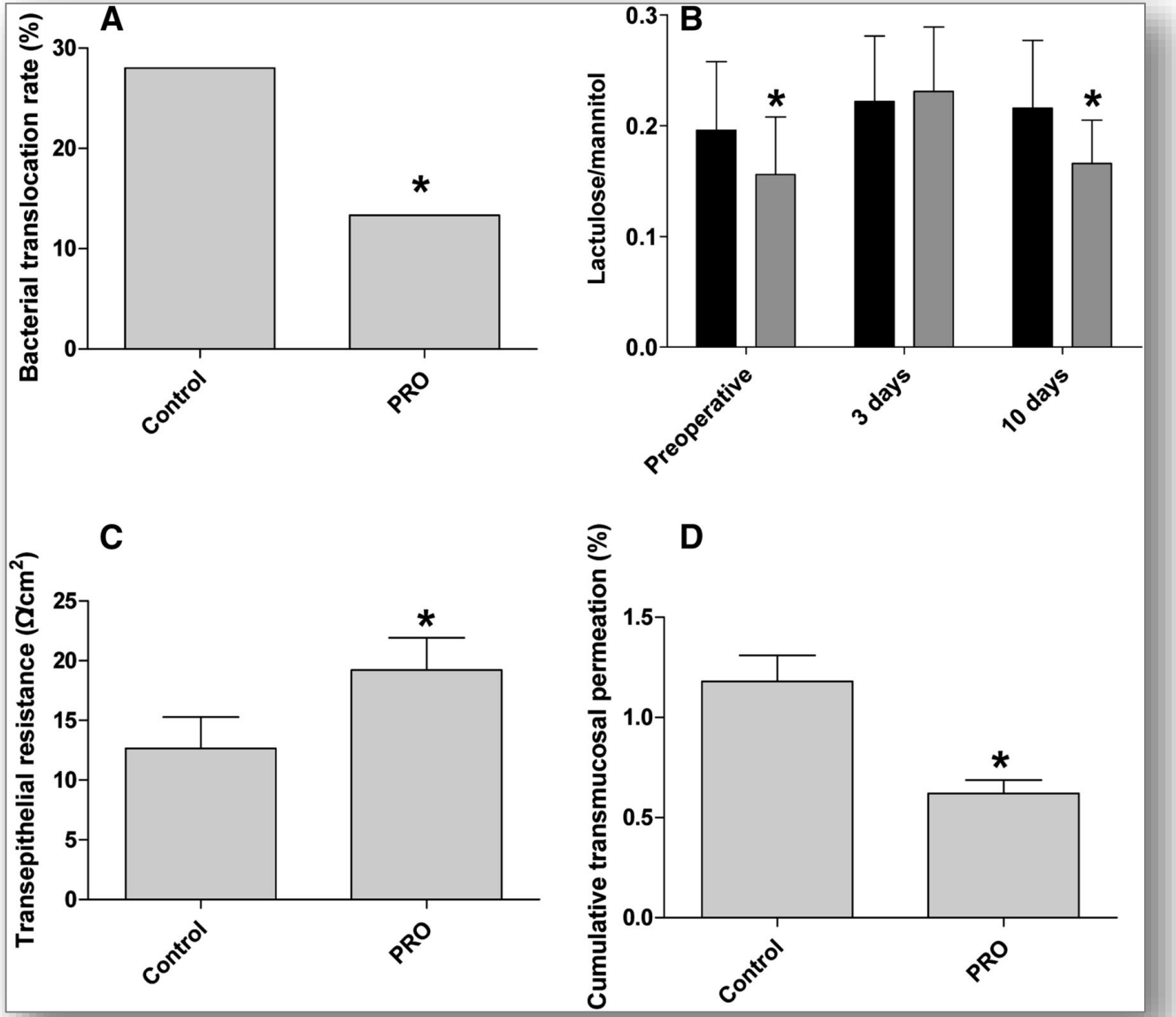
Effects of Probiotics on Intestinal Mucosa Barrier in Patients With Colorectal Cancer after Operation: Meta-Analysis of Randomized Controlled Trials

- Most studies published in Chinese (poor quality-low JADAD score)
- Most probiotics showed improvement in variety of TJ, inflammatory markers and L/M when measured
- Large heterogeneity of probiotic strains and doses

GENOVA DIAGNOSTICS

Dun L, Xiao-Ying J, Lan-Shu Z, et al. Effects of probiotics on intestinal mucosa barrier in patients with colorectal cancer after operation. *Medicine*. 2016; 95(15), 1-14

- 160 Subjects given probiotic or placebo for 6 days prior to surgery and 10 days after
- The probiotic was delivered in acid-resistant capsules containing 2 g of a mixture of *L. plantarum* (>10^{11} CFU/gram), *L. acidophilus* (>7x10^{10} CFU/gram) and *B. longum* (>5x10^{10} CFU/gram)
- The postoperative serum zonulin concentration in the control group (1.08 ± 0.28 ng/mg protein) was significantly higher than that in the probiotics group (0.39 ± 0.26 ng/mg protein; P = 0.001



GENOVA DIAGNOSTICS

Liu ZH, Huang MJ, Zhang XW, et al. The effects of perioperative probiotic treatment on serum zonulin concentration and subsequent postoperative infectious complications after colorectal cancer surgery: a double-center and double-blind randomized clinical trial. *American Journal of Clinical Nutrition*. 2013; 97(1), 117-26

Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial

- The probiotic supplement contained six probiotic strains: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Enterococcus faecium* W54, *Lactobacillus acidophilus* W22, *Lactobacillus brevis* W63, and *Lactococcus lactis* W58; equivalent to 10^{10} CFU/day- for 14 weeks
- Fecal zonulin decreased with supplementation from values slightly above normal into normal ranges (<30 ng/ml) and was significantly lower after 14 weeks with probiotics compared to placebo ($p = 0.019$)…. At baseline, both groups showed considerably higher TNF-α concentrations than normal. After 14 weeks TNF-α was tendentially lower in the supplemented group ($p = 0.054$). IL-6 increased significantly from pre to post exercise in both groups ($p = 0.001$), but supplementation had no effect
- **Conclusions** The probiotic treatment decreased zonulin in feces, a marker indicating enhanced gut permeability. Moreover, probiotic supplementation beneficially affected TNF-α and exercise induced protein oxidation. These results demonstrate promising benefits for probiotic use in trained men

GENOVA DIAGNOSTICS

Lamprechet M, Bogner S, Schippinger, et al. Probiotic supplementation affects markers of intestinal barrier, oxidation, and inflammation in trained men; a randomized, double-blinded, placebo-controlled trial. *Journal of the International Society of Sports Nutrition*. 2012; 9(1), 45-58

ORIGINAL ARTICLE

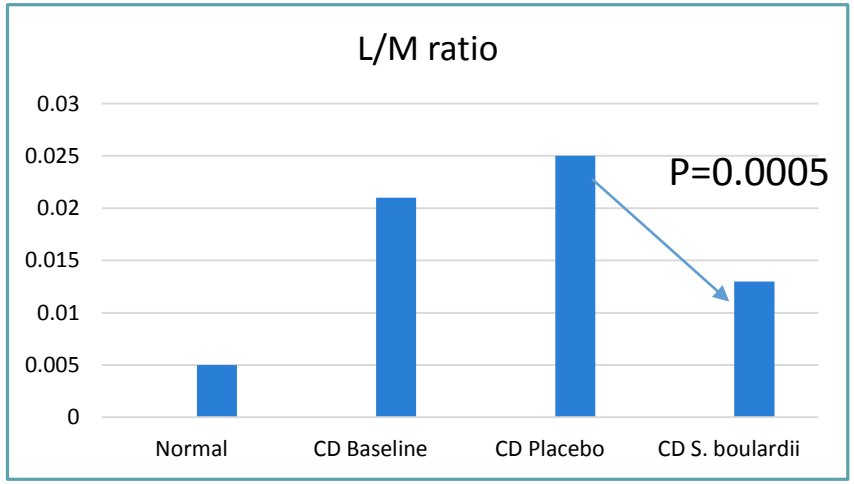
Influence of *Saccharomyces boulardii* on the intestinal permeability of patients with Crohn's disease in remission

Eduardo Garcia Vilela, PhD, Maria De Lourdes De Abreu Ferrari, Henrique Oswaldo Da Gama Torres, Ademar Guerra Pinto, Ana Carolina Carneiro Aguirre, Fabiana Paiva Martins, ...Show all

Pages 842-848 | Received 03 Nov 2007, Published online: 08 Jul 2009

Download citation http://dx.doi.org/10.1080/00365520801943354

- 34 Crohn's patients randomized for treatment with either placebo or *Saccharomyces boulardii*. Baseline medications (mesalamine, azathioprine, prednisone, metronidazole and/or thalidomide) were maintained
- 400 million CFU of *S. boulardii* every eight hours
- Intestinal permeability (lactulose/mannitol ratio) was evaluated immediately before the beginning of treatment and at the end of the first and third treatment month
- Fifteen healthy volunteers were also submitted for the intestinal permeability test



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Garcia VE, De Lourdes AF, Oswaldo GT, et al. Influence of Saccharomyces boulardii on the intestinal permeability of patients with Crohn's disease in remission. *Scandinavian Journal of Gastroenterology*. 2008; 43(7), 842-48

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Article

Oral Supplementation with Bovine Colostrum Decreases Intestinal Permeability and Stool Concentrations of Zonulin in Athletes

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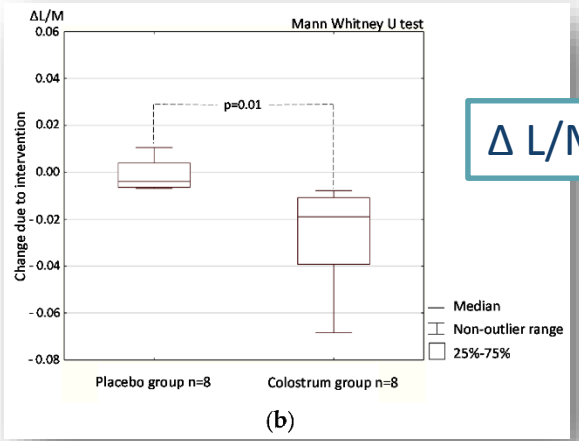
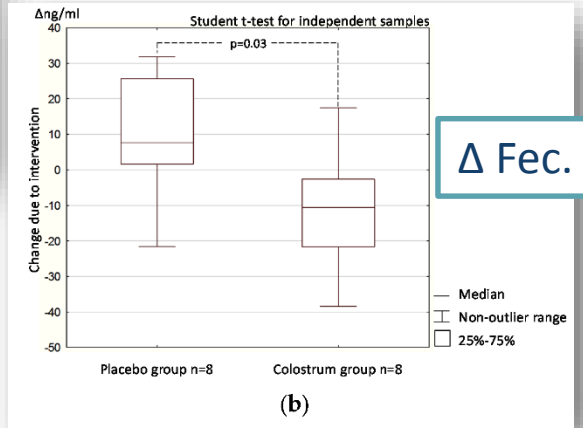
Abstract: Increased intestinal permeability has been implicated in various pathologies, has various causes, and can develop during vigorous athletic training. Colostrum bovinum is a natural supplement with a wide range of supposed positive health effects, including reduction of intestine permeability. We assessed influence of colostrum supplementation on intestinal permeability related parameters in a group of 16 athletes during peak training for competition. This double-blind placebo-controlled study compared supplementation for 20 days with 500 mg of colostrum bovinum or placebo (whey). Gut permeability status was assayed by differential absorption of lactulose and mannitol (L/M test) and stool zonulin concentration. Baseline L/M tests found that six of the participants (75%) in the colostrum group had increased intestinal permeability. After supplementation, the test values were within the normal range and were significantly lower than at baseline. The colostrum group Δ values produced by comparing the post-intervention and baseline results were also significantly lower than the placebo group Δ values. The differences in stool zonulin concentration were smaller than those in the L/M test, but were significant when the Δ values due to intervention were compared between the colostrum group and the placebo group. Colostrum bovinum supplementation was safe and effective in decreasing of intestinal permeability in this series of athletes at increased risk of its elevation.

- 16 Athletes given either placebo (whey) or 500 mg of bovine colostrum (both with 500 mg of desiccated banana) for 20 days
- Tested during peak training when it is known that gut permeability is compromised



Δ L/M Ratio



Δ Fec. Zon.

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Halasa M, Maciejewska D, Baskiewicz-Halasa M, et al. Oral supplementation with bovine colostrum decreases intestinal permeability and stool concentrations of zonulin in athletes. *Nutrients*. 2017; 9(4), 370-81

Therapeutic Summary

- Discover and avoid foods known to cause increased intestinal permeability
  - May include: gluten, dairy/lactose, capsicum/spicy foods, FODMAPs, etc.
  - Test for (and avoid) food allergens (IgE/mast cell stimulation)
- Cease NSAID use if possible
- Assess HPA axis stressors and treat accordingly - stress directly influences gut permeability
- Avoid strenuous physical activity/exercise or pay special attention to supporting gut and immune health before and after such activities - moderate exercise is helpful
- Avoid processed foods with artificial colors and flavors
- Eat abundant amounts of fresh fruits and vegetables to maximize the amount and diversity of phytonutrients

Therapeutic Summary Cont.

- Consider the following nutrients for supplementation:
  - Omega-3 fatty acids, ALA, EPA, DHA (through diet and supplementation)
  - Glutamine (4 to 8 grams daily)
  - Vitamin D (1,000 IU minimum daily; best to test and dose to desired serum levels)
  - Probiotics (mixed strain combination 20-40 billion CFU; consider high doses for long-standing intestinal barrier issues or when associated with IBD)
  - Prebiotics (precursor for important short-chain fatty acids - may be contraindicated if FODMAPs are to be avoided)
  - Zinc (25 mg daily with other minerals)
  - Iron (only when iron deficiency is confirmed)
  - Flavonoids (for quercetin and related compounds, dose not as important as consistent daily consumption from foods and supplementation)
  - Colostrum/Lactoferrin/IgG
  - Berberine (consider adding 1 g/day when subject is obese, insulin-resistant or has type 2 diabetes)

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TG Guilliams (2016). *Functional Strategies for the Management of Gastrointestinal Disorders: Principles and Protocols for Healthcare Professionals*. Stevens Point, WI: Point Institute

Michael Chapman, ND
Moderator

Thomas G. Guilliams, PhD
Presenter

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Thomas G. Guilliams, PhD
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  - A full day event with Dr. Todd LePine
    - *October 28th, 2017*
    - **Register @ attend.gxd.net**
  - Topics included:
    - Learn how gut bacteria impacts health and disease
    - Learn how impaired digestion and absorption can affect GI bacteria and nutrient status
    - Discover the importance of GI barrier function and the immune system reactions…*and more!*

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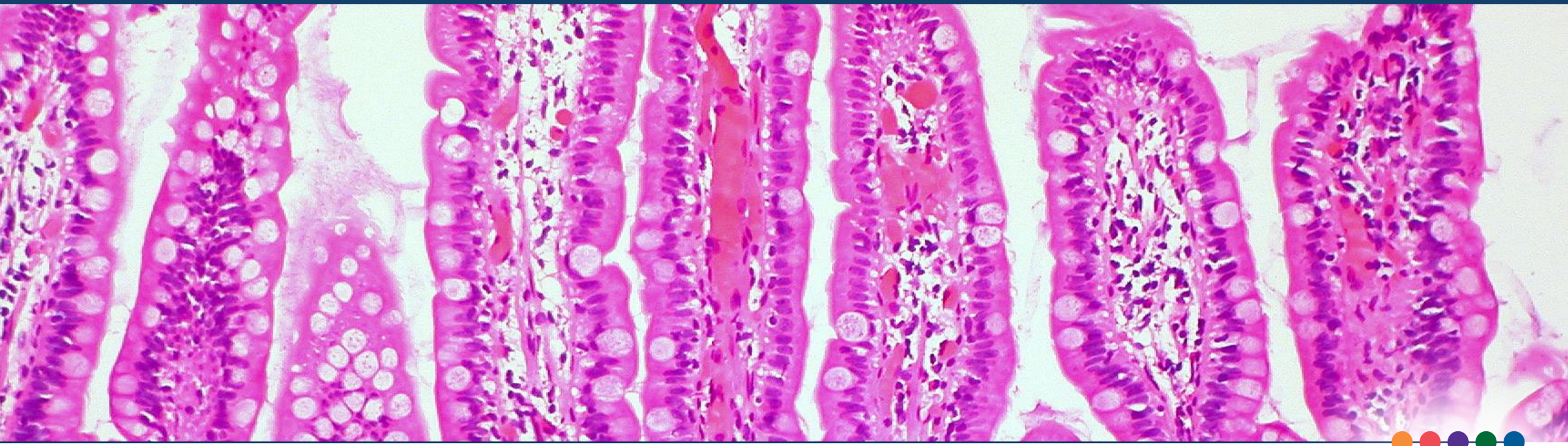

Supporting Gut Barrier Function

Thomas G. Guilliams, PhD

Point Institute- Stevens Point, WI (USA)

September 27th, 2017