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Gastrointestinal Dysbiosis and Inflammatory Conditions

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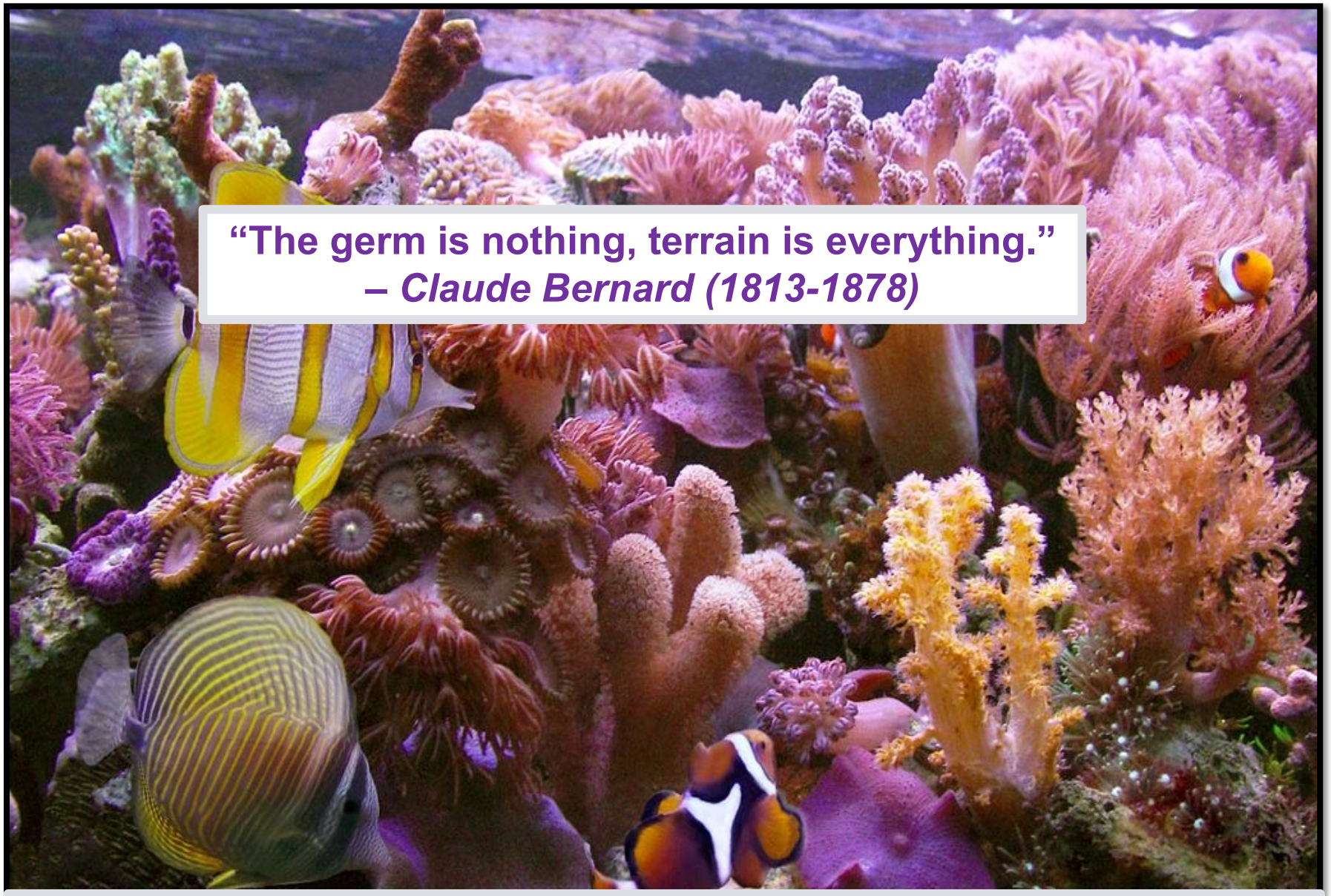
Disclosure

- David Quig is employed by Doctor's Data, Inc.

The central role of the gastrointestinal (GI) microbiota in local, and systemic low-level inflammation

- Recognize the complexity of the intestinal barrier **system,** and develop plans to support it
- Describe the mutual relationships between the microbiota and the mucosa with respect to local, and chronic low-level systemic inflammation
- Interpret laboratory results for stool biomarkers of GI inflammation, and identify a non-organic inflammatory dysbiosis



Network of mutual interactions among and between organisms and their barrier system

Intestinal Barrier

- Multilayered and highly integrated system
- Primary functions
  - H_2O/electrolyte balance, **prevent** entry of pathogens/toxins, food-derived antigens, and environmentally-derived toxicants
  - Regulate ***appropriate*** inflammatory/immune responses
- "Two way street"- mutual regulation
  - Commensal bacteria facilitate optimal status of barrier components.
  - Barrier components provide **surveillance**, **protection** and **selection** of commensal bacteria.

BMC Gastroenterol(2014)14:189 Eur J Pharmacol(2011)666:516-22
Sci (2012)336:1268-73 Nature(2012)489:231-41

Multifaceted Intestinal Barrier System

- Intestinal microbiota –high abundance key players

  communications command center

- "Biochemical" barrier

  Digestive secretions, sIgA, antimicrobial peptides/proteins (lysozyme, defensins), reactive oxygen species, cytokines

- Structural/physical barriers

  Mucus *gradient*

  Glycocalyx- membrane tethered mucins

  Epithelial lining, tight junction proteins

  Lamina propria

Clin Nutr(2015)34:1080-7 BMC Gastroenterol (2014)14:189-213
J Clin Endocrinol (2012)46:512-17 Neurogastroenterol Motil (2012)24:503-12

Modified from World J Physiol(2012)3:27-43

Gut lumen

Obesity, high fat diet
↓ mucus, ↑ permeability and bacterial penetration

Pathogens

Mucus "blanket"

Outer loose

Inner firm

peristalsis

sIgA

Antimicrobial peptides, ROS, cytokines

Glycocalyx

Primary Commensals

Clostridium spp.
Lactobacillus spp.
Bifidobacterium spp.
F. prausnitzii
****A. muciniphila***

Mucosa

Epithelial cells

Tight Junctions

Endosome

Lamina propria

Dendritic and T- cells, macrophages, lymphocytes

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sIgA, Barrier Function and the Microbiota

- sIgA protective functions- "housed" in mucus
  - *Immune exclusion* of microbes (including viruses) and toxins
  - *Binding to pathogens* - decreases ATP production→ loss of motility and biofilm production
  - *Antiparasitic augmentation* via activation of eosinophils
  - *Regulates the composition of the microbiota*
  - ***Anti-inflammatory*** *- neutralizes LPS in apical recycling endosomes*

- Sustained elevations common up to 4-6 weeks after a GI virus
- Want higher sIgA with pathogenic microbes
- Chronic Candidiasis is often associated with low **s**IgA despite normal serum IgA levels (sIgA specific protease activity)

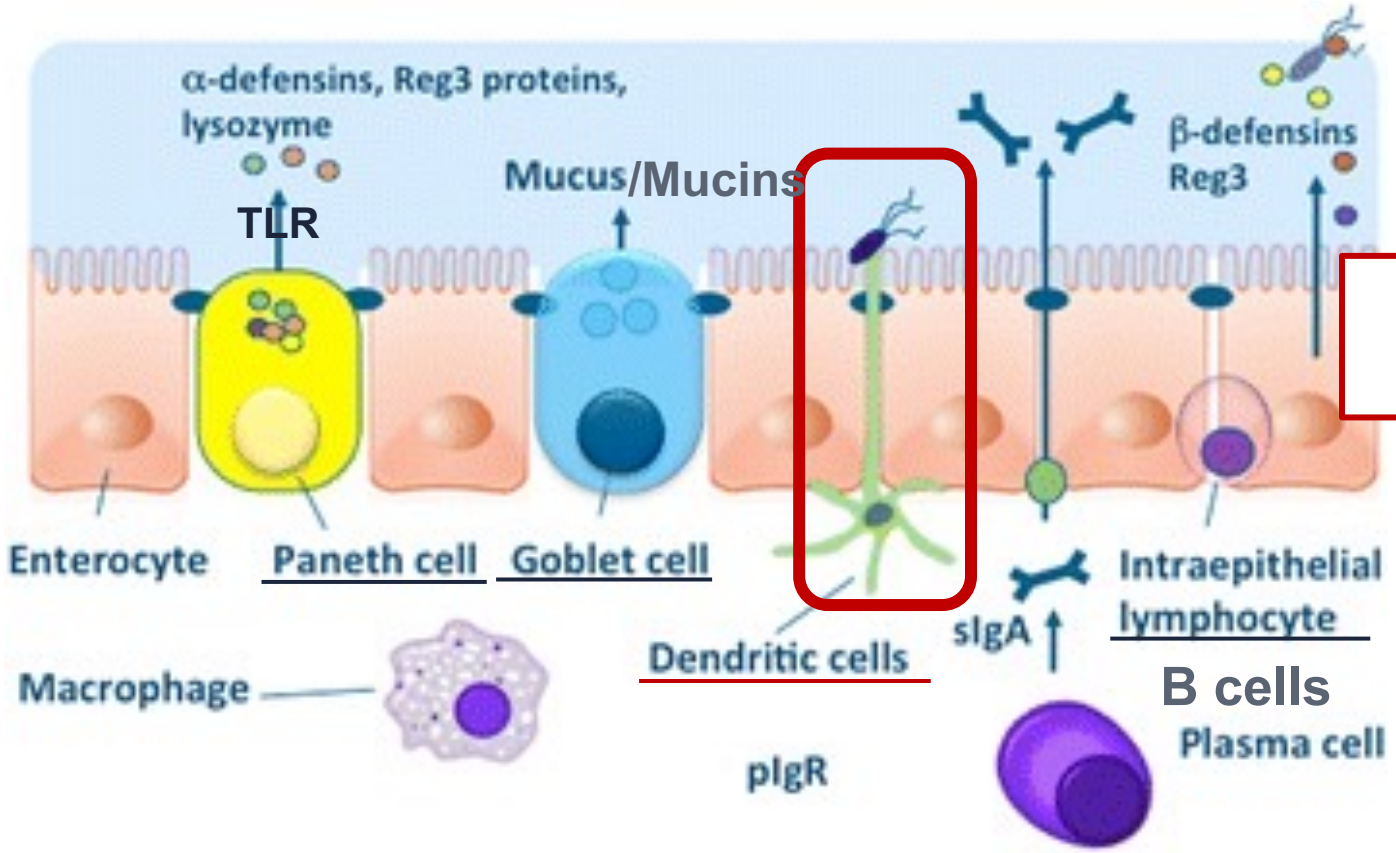
Front Immunol(2013)4:1-11 Cell Host(2007)2:28-39 JBC(2011)286:17239-47
Infect Immunol(2013)81:653-64 Gig Sanit(2012)3:27-29

Intervention for Low sIgA

- Ω-3 fatty acids, olive oil, A, D and Zn
- Probiotics
  - *Lactobacillus rhamnosus* GG* *and Bifidobacterium lactis* Bb-12 (↑ *number of sIgA secreting cells in formula-fed infants*)
  - *S. boulardii- ~75% ↑ sIgA* (germ-free mouse model)
- Prebiotics Soluble fiber
- Glutamine (dosing concerns)
- **Elevated cortisol and low DHEA *decrease* sIgA (chronic stress)**

Clin Nutr(2015)34:1080-7 Amino Acids(2015)47:2143-54 J Steroid Biochem Mol Biol(2015)148:179-83 Eur J Nutr(2013)52:1089-98 Am J Surg(2010)199:35-42

Mucosal Specialists- Sense, Report, Secrete



Surveillance/reporter function

- **Finely tuned network of immune mechanisms for microbial recognition; selection and eradication**

Microbial-host Crosstalk

- Fermentation of soluble fiber and endogenous mucins to short chain fatty acids by commensal bacteria
- Fuel for enterocytes, maintenance of barrier functions, and glycemic regulation
- Body "listens" to butyrate
  - G-protein-coupled receptors (immune and epithelial cells)
  - Butyrate mediates the release of anti-inflammatory cytokines, gut-derived peptides and antimicrobial peptides
- *Faecalibaterium prausnitzii,* Lachnospiraceae*,* and some Clostridium spp. are major butyrate producers
- Butyrate producers are more abundant with regular intake of chick peas, inulin-like fructans, and partially hydrolyzed guar gum (galactomannan).

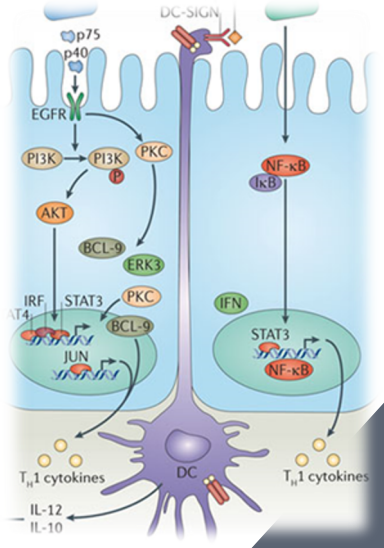
AJCN(2019)109:1683-95 Nutrients(2020)12:1257 Immunity(2014)40:128-39
Nature(2015)518:S3-11

Clostridia Phobia is Misguided

- Clostridium spp. are normally ≈ 5 % of total microbiota and provide continuous cross-talk with the mucosa.
- Commensal Clostridium spp. are key butyrate producers
- **Low** abundance of Clostridium *spp.* occurs with IBD and colorectal cancer
- **Colonization in *breastfed infants* during the 1st month of life**

  (*C.blautia, C. collinsella, and C. veillonella*)

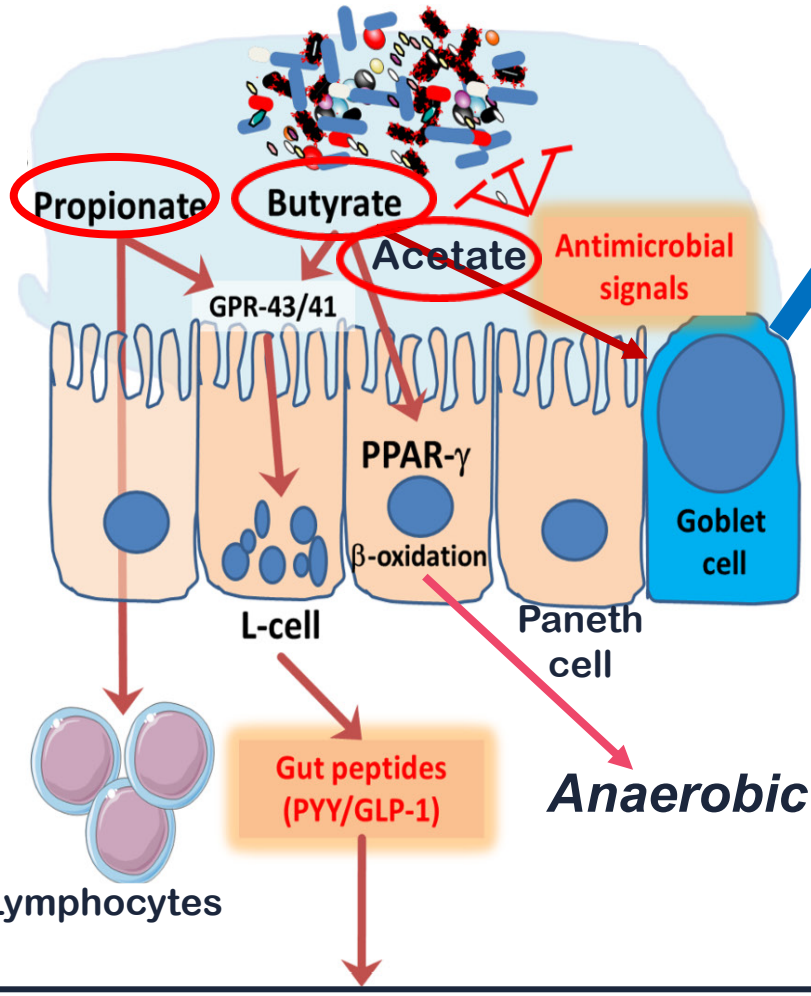
  Vertical transfer; Maternal gut→ lymph → breast milk → infant's gut



Gut Pathog(2013)5:23 AJCN(2013)97:1044-52 J Nat Cancer Inst(2013)105:1907-11
PLOS ONE(2015)doi:10.1371/journal.pone.0124599 Pediatric Res(2015)77:220-28

Microbiota-normoglycemia Connection

Abundant/diverse microbiota, sufficient dietary prebiotics



Mucus barrier (≈ 5 L/day)

Lamina propria

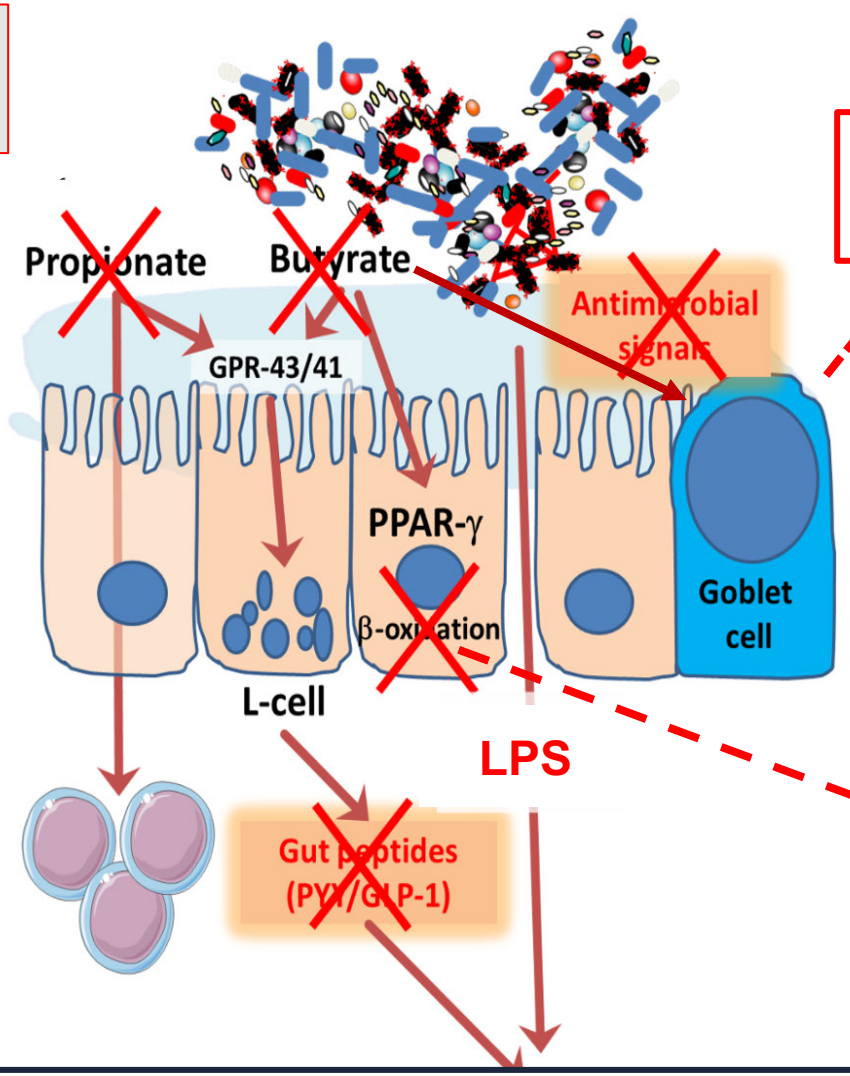
Anaerobic microenvironment

Glycemic control, satiety, antimicrobial, anti-inflammatory

Modified from Gut (2018)67:1716–25

Breakdown of Microbial-Host Crosstalk

↓ abundance/diversity
Inadequate prebiotics



↓ mucus thickness (sIgA)

↑ facultative anaerobes (Enterobacteriaceae)

↑ appetite, hyperglycemia, permeability, metabolic endotoxemia, adipocyte inflammation

The LPS Breach

- Lipopolysaccharides (LPS)- essential for all gram-negative bacteria (70% of GI bacteria)
- Metabolic syndrome- sterile LPS elevation to about 0.5-2.0 times > normal
  (10-50 times lower than during septicemia)
- Assimilation of LPS from the gut
  Normal- *transient* post-prandial ↑ with high fat meals *(chylomicrons)*
  Abnormal- breach of the endothelial barrier (*serum* Zonulin, L: M)
- The set up for metabolic endotoxemia:

  Inflammatory Dysbiosis and paracellular permeability

J Endocrinol Metab (2015)100:3427-35 Diabetes Care (2012)35:375–382 Metab(2017)69:43-50
J Nutr(2016)146:1694-700 Eur J Nutr (2017)57:2985-97

What Tells Us Directly About Intestinal Inflammation?

Inflammation

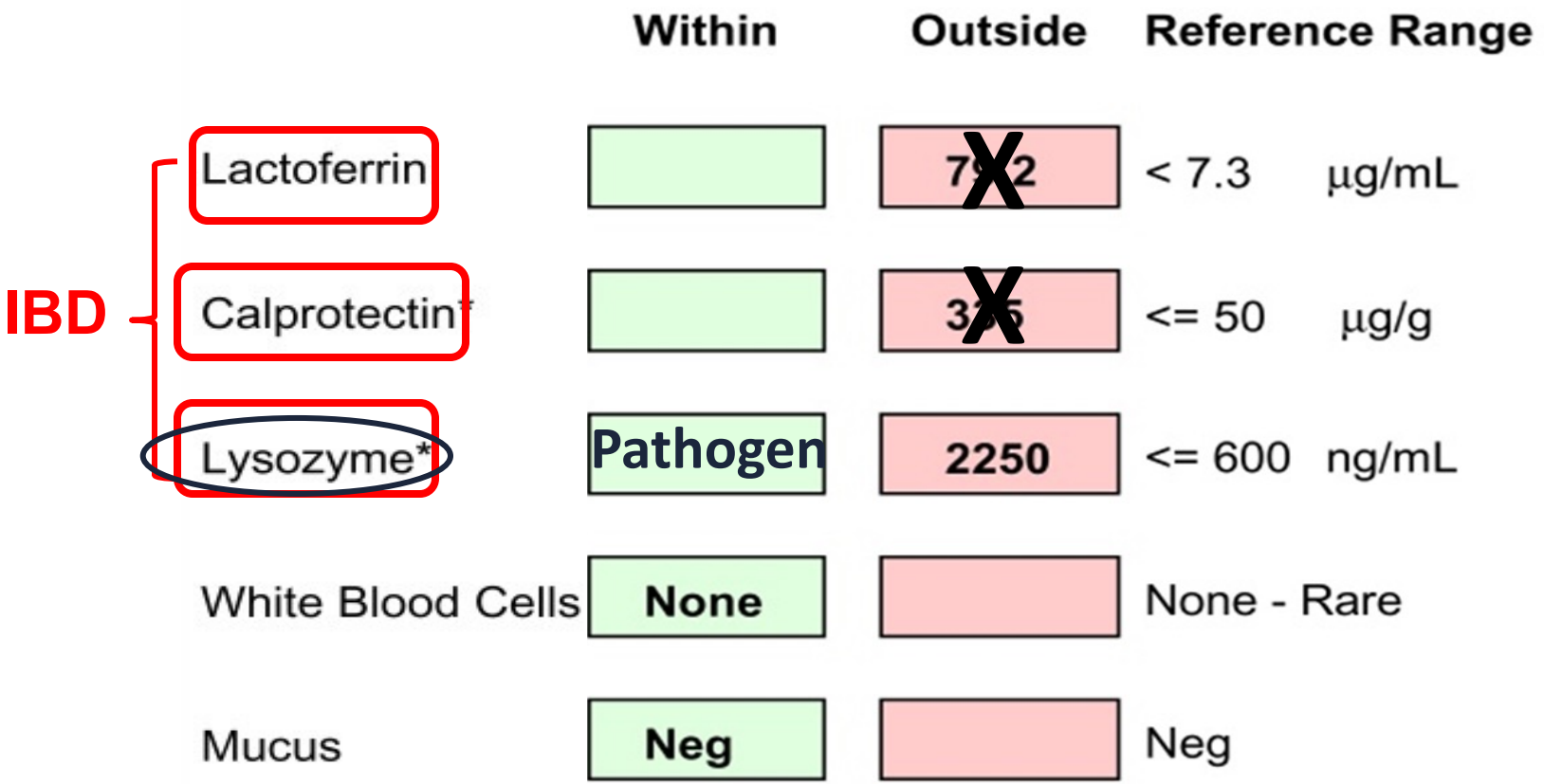
	Within	Outside	Reference Range
Lactoferrin		23.6	< 7.3 µg/mL
Calprotectin*		92	10 - 50 µg/g
Lysozyme*		1400	<= 600 ng/mL
White Blood Cells	None		None - Rare
Mucus	Neg		Neg

Specific Biomarkers of Intestinal Inflammation

- Provide *noninvasive* assessment of active inflammatory responses
- Neutrophil-derived proteins measured by immunoassays
- Specific proteins may be elevated in stool in association with:
  - Inflammatory bowel diseases (Lactoferrin and/or Calprotectin, and Lysozyme)
  - Intestinal infections (viral, bacterial or parasitic)
  - NSAID abuse
  - Colorectal cancer
  - *Possibly* Celiac disease (very poor clinical specificity)

Gastroenterol(2011)140:1817-26 Dis Rectum Colon(2007)50:1697-1706

Gastrointestinal Inflammation



World J Gastroenterol(2008)14:5584-89 Acta Gastroenterol Belg(2013)76:322-8
Clinica Chimica Acta(2012) doi: 10.1016/j.cca.2012.11.008

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What if Lactoferrin and / or Calprotectin are High?

- If marginal *and* pathogens detected-

  Consider retesting about 1 month after **eradication of pathogen(s)**
- If high Lactoferrin (LF) and/or Calprotectin (CP)-

  Repeat with the same laboratory (stand alone tests)
  Endoscopy recommended if 2 elevated results 4-6 weeks apart
- Utilize to monitor the efficacy of anti-inflammatory therapy

Clin Chim Acta(2012) doi: 10.1016/j.cca.201 Inflamm Bowel Dis(2008)14:40-6
Clin Chim Acta(2007)381:63-8 Am J Gastroenterol(2008)103:162-9

Fecal LF Levels Elevated During Remission

Group	# observations	µg/ml (X±SE)
Controls	55	**2.8** (0.4)
IBS	31	**1.3** (0.3)
Inactive UC	41	**67** (24)
Active UC	31	**815** (389)
Inactive CD	26	**239** (83)
Active CD	51	**672** (242)

Am J Gastroenterol(2003)98:1309-14

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Textbook Flaming IBD

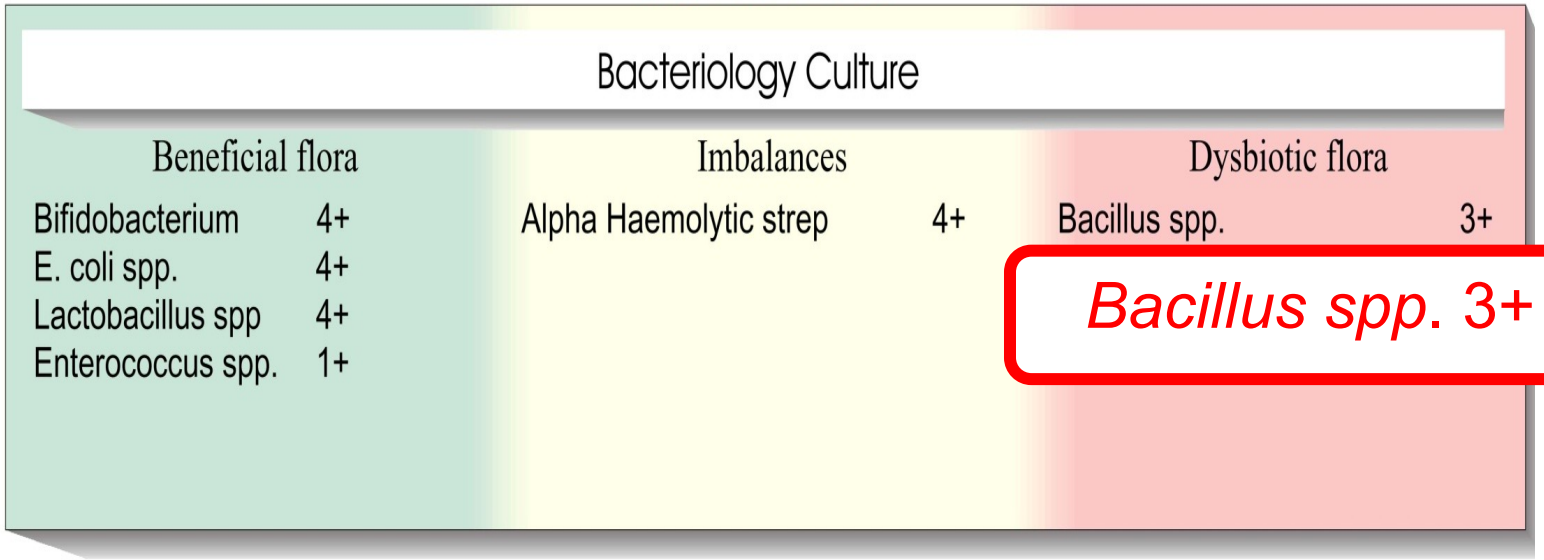
13 yof, inability to gain weight, multiple loose BMs/day, abdominal pain
Culture *only* indicated 4+ *Proteus mirabilis*

	Within	Outside	Reference Range
Lactoferrin		492	< 7.3 µg/mL
Calprotectin*		1506	<= 50 µg/g
Lysozyme*		11300	<= 600 ng/mL
White Blood Cells		Many	None - Rare
Mucus	Neg		Neg

Post-colonoscopy diagnosis of Ulcerative colitis leading to complete proctocolectomy

Dysbiosis or IBD? You make the Call

41 y.o.m., chronic diarrhea, wt. *loss for 1.5 years*



GI Inflammation

	Within	Outside	Reference Range
Lysozyme*		**1170**	<= 600 ng/mL
Lactoferrin		**596**	< 7.3 µg/mL
White Blood Cells		**Many**	None - Rare
Mucus	**Neg**		Neg

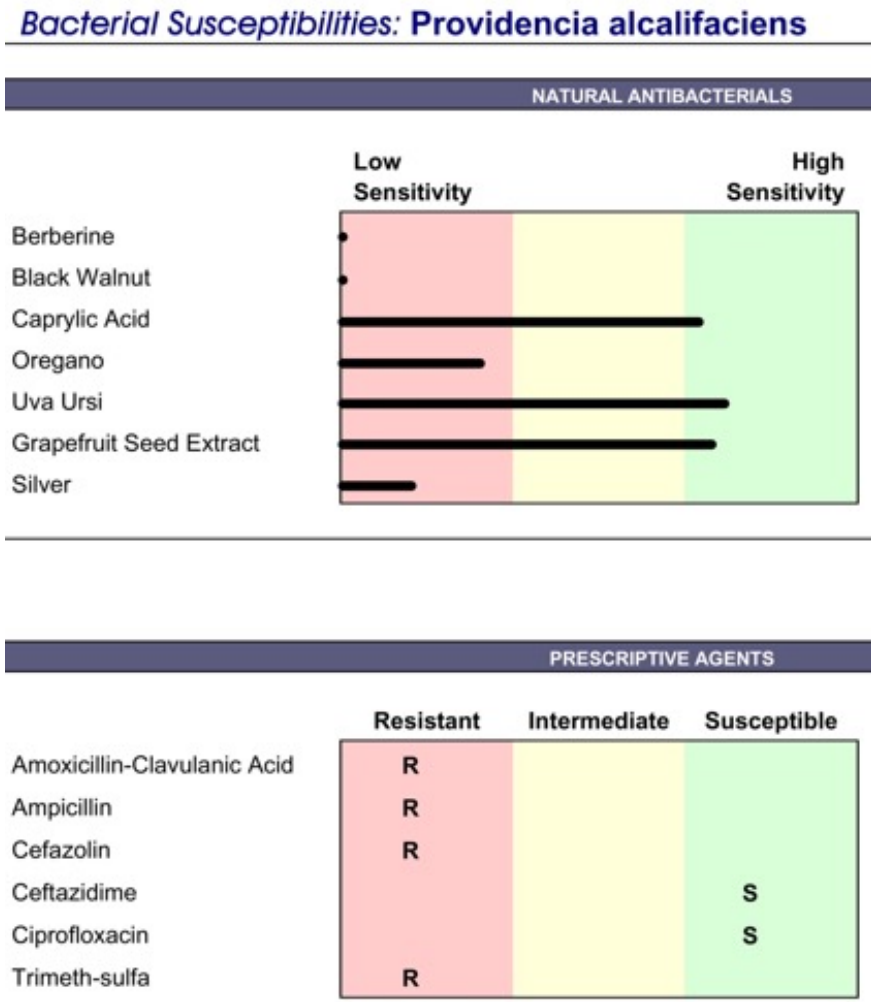
Classic Pathogen-induced Inflammation

	Within	Outside	Reference Range	
Lactoferrin		11.1	< 7.3	µg/mL
Calprotectin*		69	<= 50	µg/g
Lysozyme*		1170	<= 600	ng/mL

BACTERIOLOGY CULTURE		
Expected/Beneficial flora	**Commensal (Imbalanced) flora**	**Dysbiotic flora**
2+ Bacteroides fragilis group	2+ Citrobacter freundii complex	3+ Providencia alcalifaciens
NG Bifidobacterium spp.	1+ Enterobacter cloacae complex	
3+ Escherichia coli	2+ Gamma hemolytic strep	
2+ Lactobacillus spp.	1+ Klebsiella pneumoniae ssp pneumoniae	
2+ Enterococcus spp.	2+ Kluyvera ascorbata	

Viruses	**Within**	**Outside**	**Reference Interval**
Adenovirus F40/41	Negative		Negative
Astrovirus	Negative		Negative
Norovirus GI/GII		Positive	Negative
Rotavirus A	Negative		Negative
Sapovirus (I, II, IV and V)	Negative		Negative
Parasites			
Cryptosporidium		Positive	Negative
Cyclospora cayetanensis	Negative		Negative
Entamoeba histolytica	Negative		Negative
Giardia duodenalis (AKA *intestinalis & lamblia*)		Positive	Negative
Diarrheagenic *E. coli/Shigella*			
Enteroaggregative *E. coli* (EAEC)	Negative		Negative
Enteropathogenic *E. coli* (EPEC)	Negative		Negative
Enterotoxigenic *E. coli* (ETEC) *lt/st*		Positive	Negative
Shiga-like toxin-producing *E. coli* (STEC) *stx1/stx2*	Negative		Negative
E. coli O157	Negative		Negative
Shigella/Enteroinvasive *E. coli* (EIEC)		Positive	Negative
Pathogenic Bacteria			
Campylobacter (jejuni, coli and upsaliensis)		Positive	Negative
Clostridium difficile (Toxin A/B)	Negative		Negative
Plesiomonas shigelloides	Negative		Negative
Salmonella	Negative		Negative
Yersinia enterocolitica	Negative		Negative
Vibrio (parahaemolyticus, vulnificus and cholerae)	Negative		Negative
Vibrio cholerae	Negative		Negative

Eliminate the Pathogen(s)



- Requires live microorganisms (bacteria, yeast)
- Provides *guidance*
  - No biofilm
- Combo of natural agents- Dose/duration?
- Probiotics
- "Starve" specific microbes (*Klebsiella*, yeast)
- Consider concomitant anti-inflammatories
  - e.g. curcumin, berberine

- Standard dosing guidelines for Rx agents

Inflammatory Dysbiosis and Permeability

- Dysbiosis- compromised microbiota abundance and diversity
- *Inflammatory* dysbiosis, and increased permeability associated with IBDs, obesity, diabetes, excessive gestational weight gain, and high-fat/keto diets

High abundance of proinflammatory LPS-rich Proteobacteria, Enterobacteriaceae, Shigella, Escherichia

Lower abundance of *Faecalibacterium prausnitzii, Eubacterium rectale, Clostridium* spp., *Bifidobacterium* spp., Lachnospiraceae and *A.muciniphila* (Amuc_1100)

- Associated with low saccharolytic fermentation and fecal butyrate (SCFAs)

Compromised barrier integrity, permeability and metabolic disruption

J Obesity (2019) doi:10.1155/2019/4608315 Am J Physiol Gastrointest Liver Physiol (2017)312:G327-39
Int J Endocrinol (2013)2013;674106 PLoS One (2012)7:e37160 Mol Cell Biochem (2014):388:203–10

PCR-based Dysbiosis Test

- Dysbiosis Test- differentiation of normobiotic subjects and dysbiotic patients
- Dysbiosis index scores compared across healthy subjects (n=297), IBS patients (n=236) and IBD patients (n=135)
- Dysbiosis score > 2 indicates deviation from the reference normobiotic profile

Parameter	IBD	IBS	Healthy
Dysbiotic	74%	73%	16%
Normobiotic	26%	27%	84%

Cohort	% Dysbiotic
Healthy	16
IBS	73
IBS-D	76
IBS-C	73
IBS-M	67
IBS-U	72
IBS-A	70
IBD naive	70
CD	80
UC	64
IBD remission	80
CD	89
UC	75

IBD = inflammatory Bowel Disease (scoped/biopsies)
IBS = Irritable Bowel Syndrome (Rome III criteria)

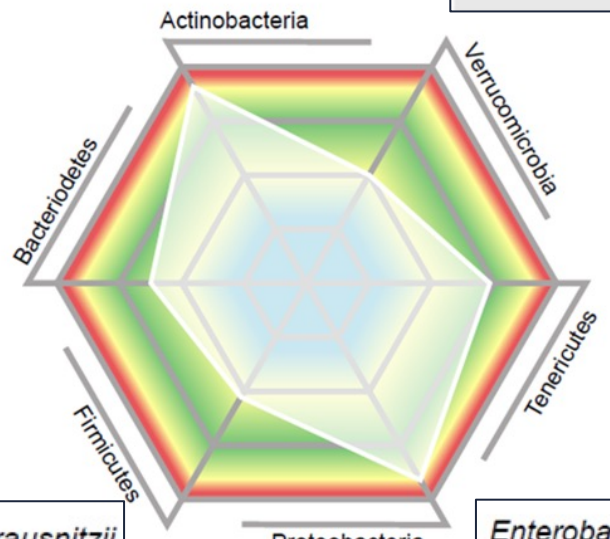
Aliment Pharmacol Ther (2015) doi: 10.1111/apt.13236

IBD, Pathogen-induced, or Inflammatory Dysbiosis?

Inflammation	Result	Unit	L / WRI / H	Reference Interval
Lactoferrin	124	µg/mL		< 7.3
Lysozyme*	814	ng/mL		≤ 500
Calprotectin	12	µg/g		≤ 50

Yeast	Result	NG / 1+ / 2+ / 3+ / 4+	Reference Interval
Candida albicans	2+		0+ – 1+
Candida lusitaniae	2+		0+ – 1+
Rhodotorula mucilaginosa	1+		0+ – 1+

Akkermansia muciniphila



Faecalibacterium prausnitzii

Lachnospiraceae

Enterobacteriaceae

Escherichia spp.

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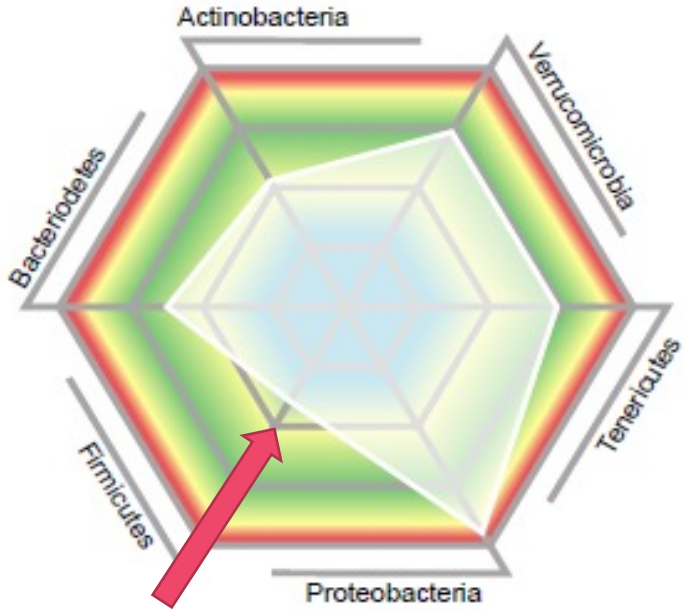
Inflammatory Dysbiosis- Cardiometabolic Syndrome

- 68 year old sedentary female with family history of CAD
- 5’5,” 200 lbs., excessive visceral fat (BMI= 32.3)
- Typical SAD diet
- Fatigue, hypertension, shortness of breath
- T2DM- on insulin

Dysbiosis Score

- GI inflammatory biomarkers- **WNL**
- Elevated hsCRP and leptin, and low adiponectin (high L:A ratio)



Enterobacteriaceae
Escherichia

Firmicutes, Very Low	↓
Proteobacteria, Very High	↑
Bacilli Class, Very Low	↓
Escherichia spp., Very High	↑
Clostridia Class, Very Low	↓
Eubacterium hallii, Very Low	↓
Faecalibacterium prausnitzii, Very Low	↓
Lachnospiraceae, Very Low	↓
Veillonella spp., Very Low	↓

Short Chain Fatty Acids‡	Result	Unit	L	WRI	H	Reference Interval
% Acetate	53	%				50 – 72
% Propionate	26	%				11 – 25
% Butyrate	17	%				11 – 32
% Valerate	4.1	%				0.8 – 5.0
Butyrate	0.59	mg/mL				0.8 – 4.0
Total SCFA's	3.4	mg/mL				5.0 – 16.0

Take-Aways

- “Feed” the commensal bacteria so they can communicate with the mucosa, and support the barrier system (“terrain”).
- Integrate ***all*** components of the patient’s comprehensive stool analysis when interpreting **specific biomarkers** of GI inflammation.
- Identify and ameliorate **Inflammatory Dysbiosis** for patients with the non-organic inflammation associated with cardiometabolic syndrome.