The Human Microbiome

What We Know About It
and How We Can Manipulate It

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Disclosures

None

*Companies in Microbiome Space, no financial relationship

Historic Context

1796: Edward Jenner

1860: Louis Pasteur

1928: Sir Alexander Fleming discovers *Penicillium*.

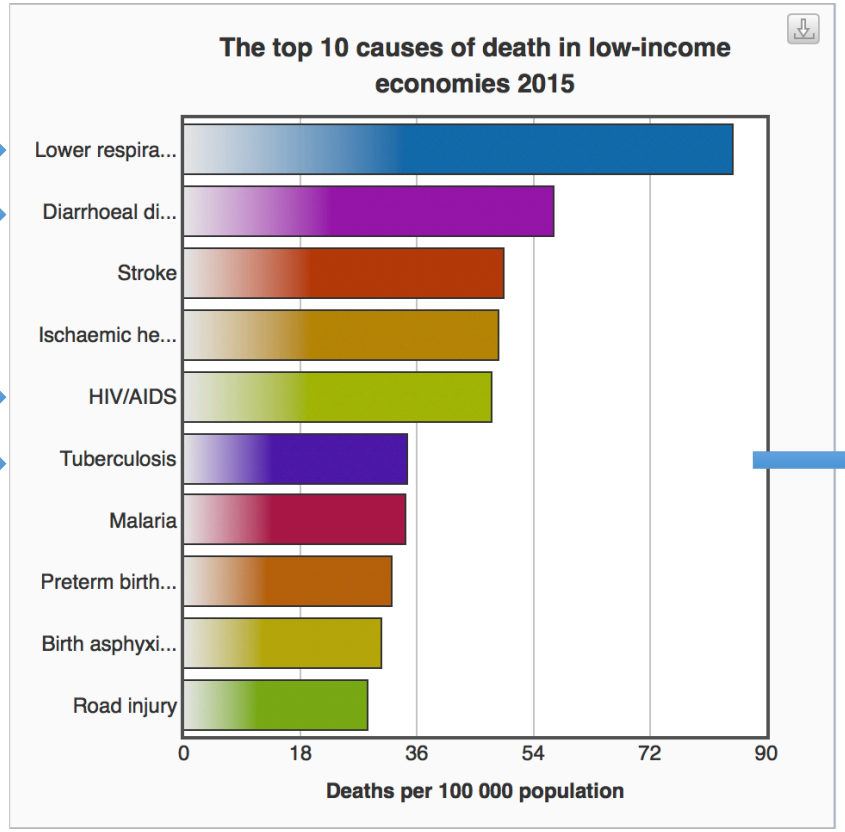
1942: Manufacturing process for Penicillin

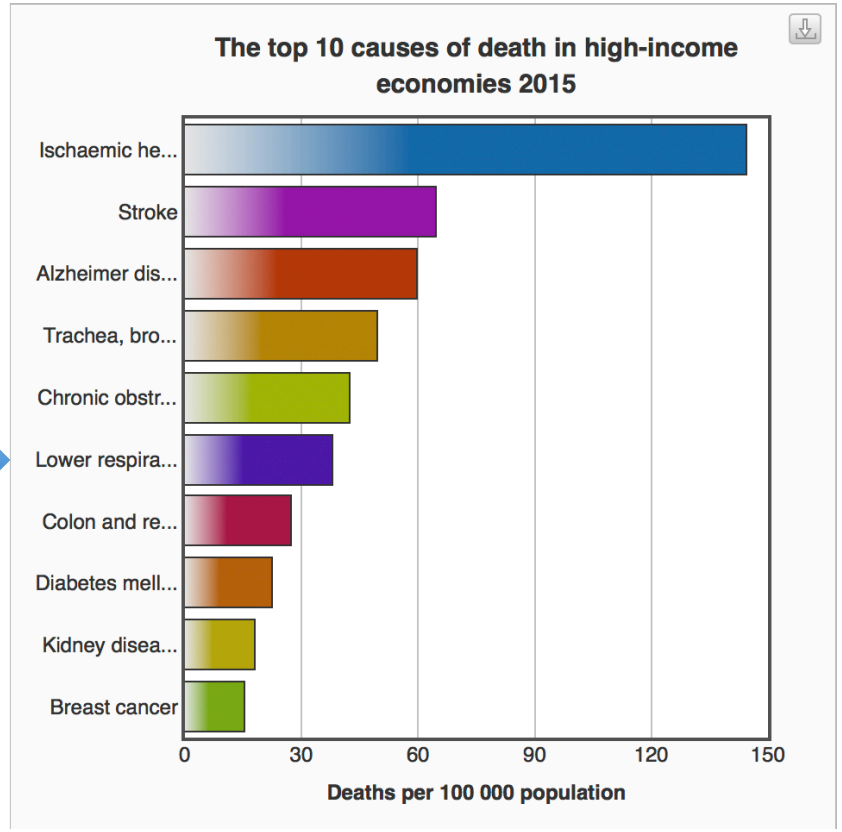
The Age of Antibiotics: Killing Bad Bugs is Good

3.1 million

2 million

1.5 million

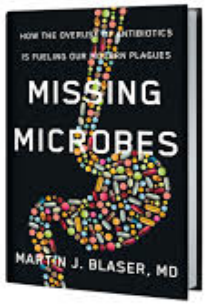


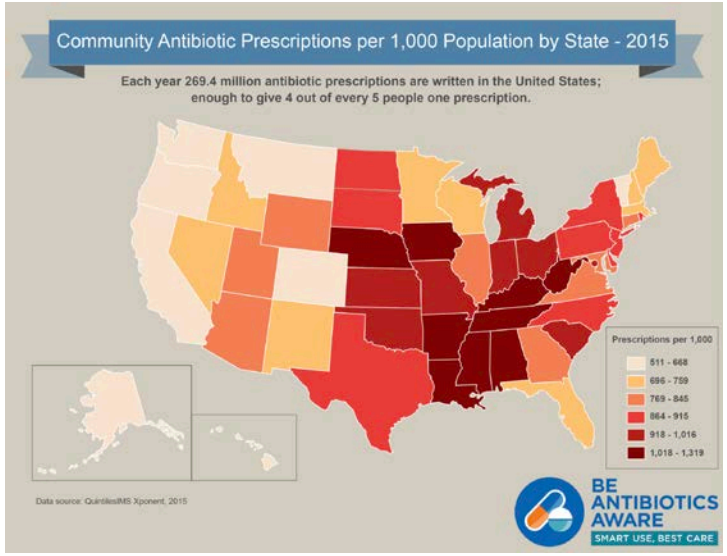


WHO, 2015

The Human Microbiome: an Innocent Bystander?

- Antibiotics are generally broad spectrum
- Americans receive, on average, ~18 rounds of antibiotics by age 20.
- Regional variation in antibiotic usage suggest cultural practices as opposed to medical necessity





CDC, 2015

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Unintended Consequences?

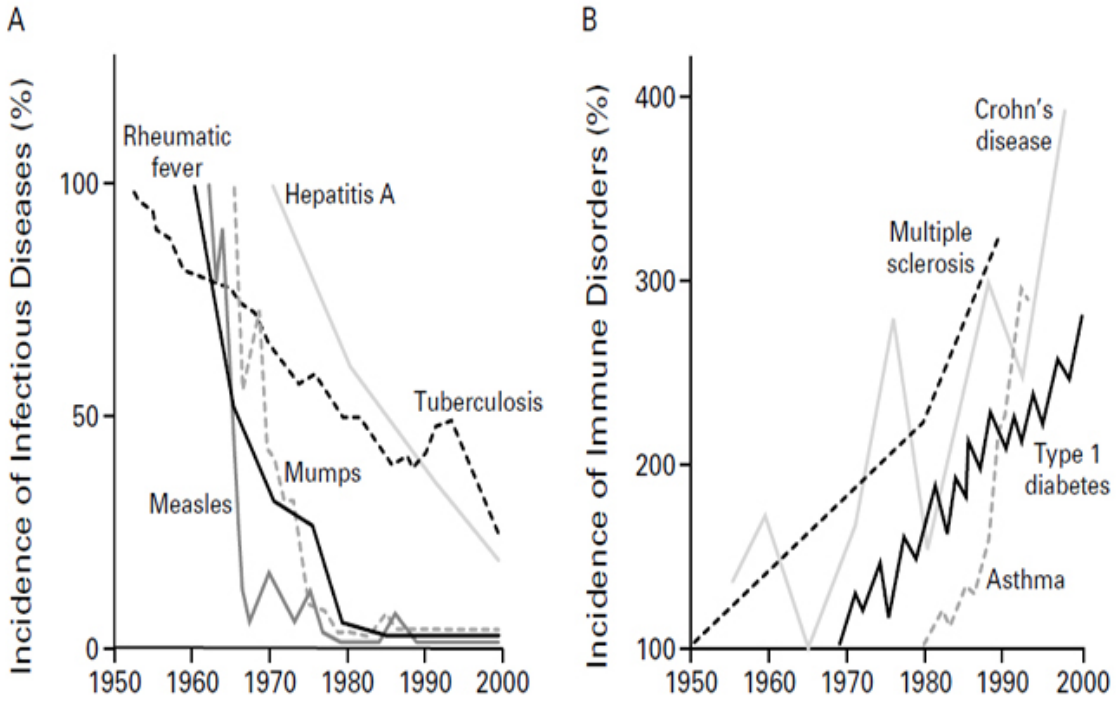


Figure 1. Inverse Relation between the Incidence of Prototypical Infectious Diseases (Panel A) and the Incidence of Immune Disorders (Panel B) from 1950 to 2000.

In Panel A, data concerning infectious diseases are derived from reports of the Centers for Disease Control and Prevention, except for the data on hepatitis A, which are derived from Joussemet et al.[12] In Panel B, data on immune disorders are derived from Swarbrick et al.,[10] Dubois et al.,[13] Tuomilehto et al.,[14] and Pugliatti et al.[15]

Bach. New England J. of Medicine 2002

Ancient Relationships

The Human Microbiome

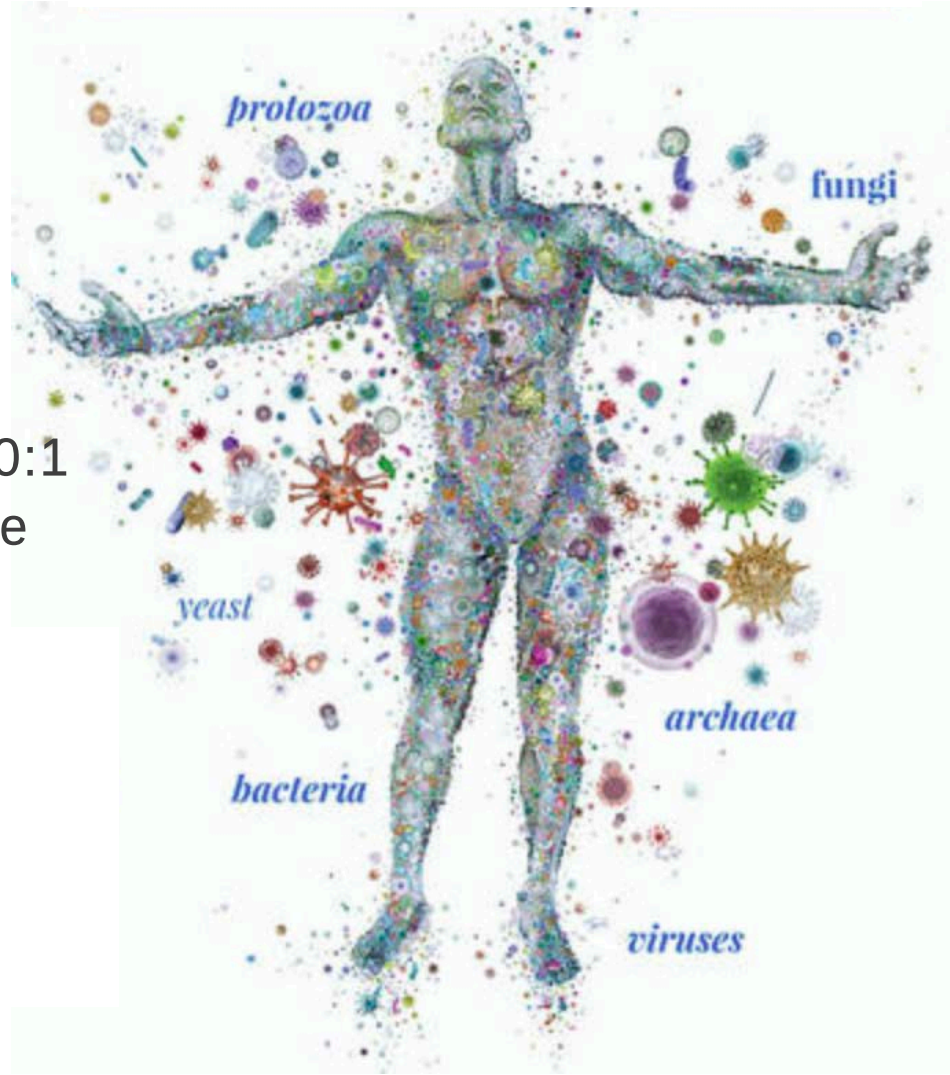
Human body is composed of 30 trillion cells

It harbors *2-10 x* as many microorganisms

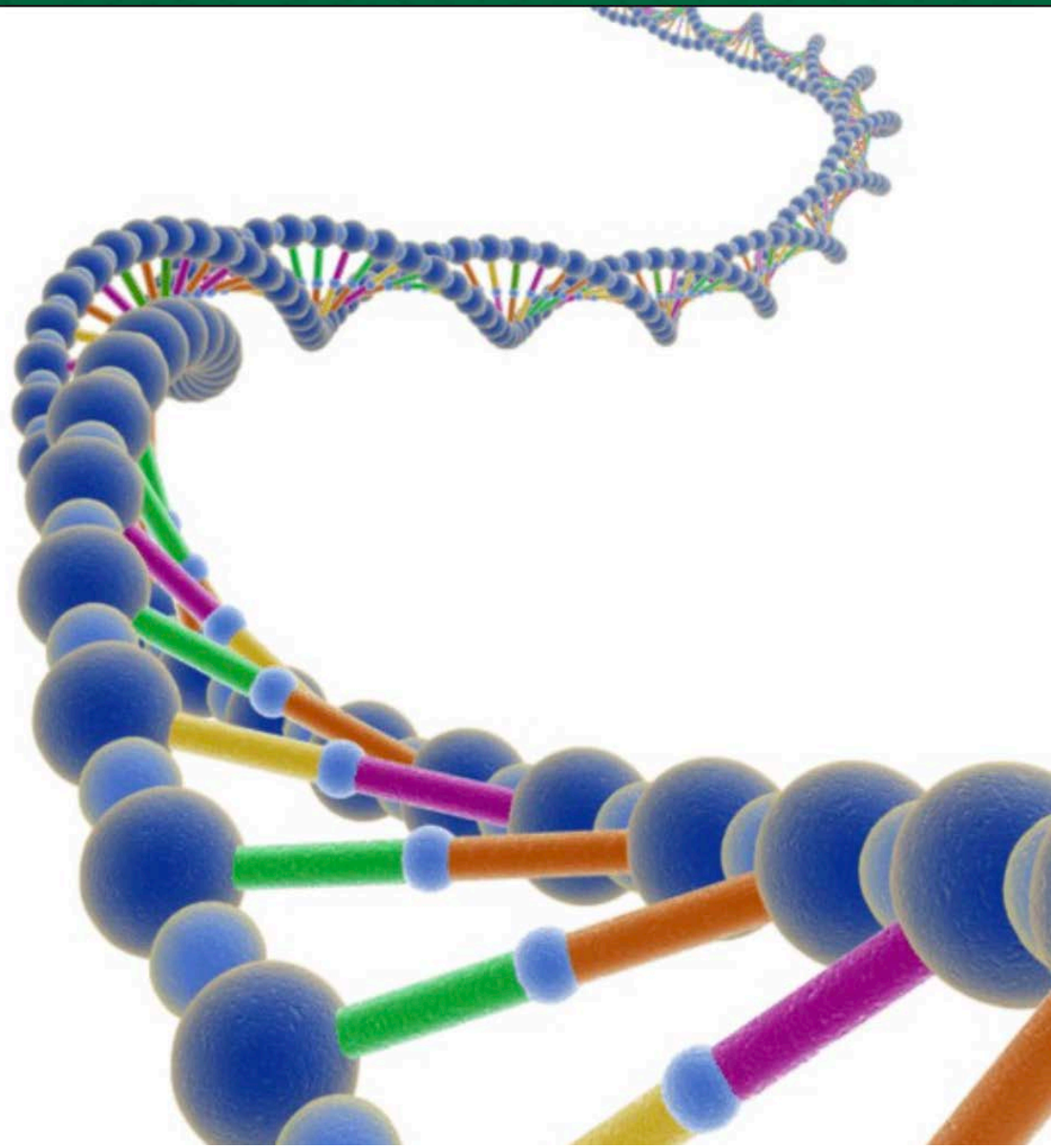
- 3 lb of bacteria
- Genetic material outnumbers that of human genome 150:1
- “Second Genome” - One that we can shape and cultivate

The Human as an Ecosystem

Microbiota – community of microorganisms
Metagenome – collection of genes contained by entire microbiota
Microbiome – microbiota + host

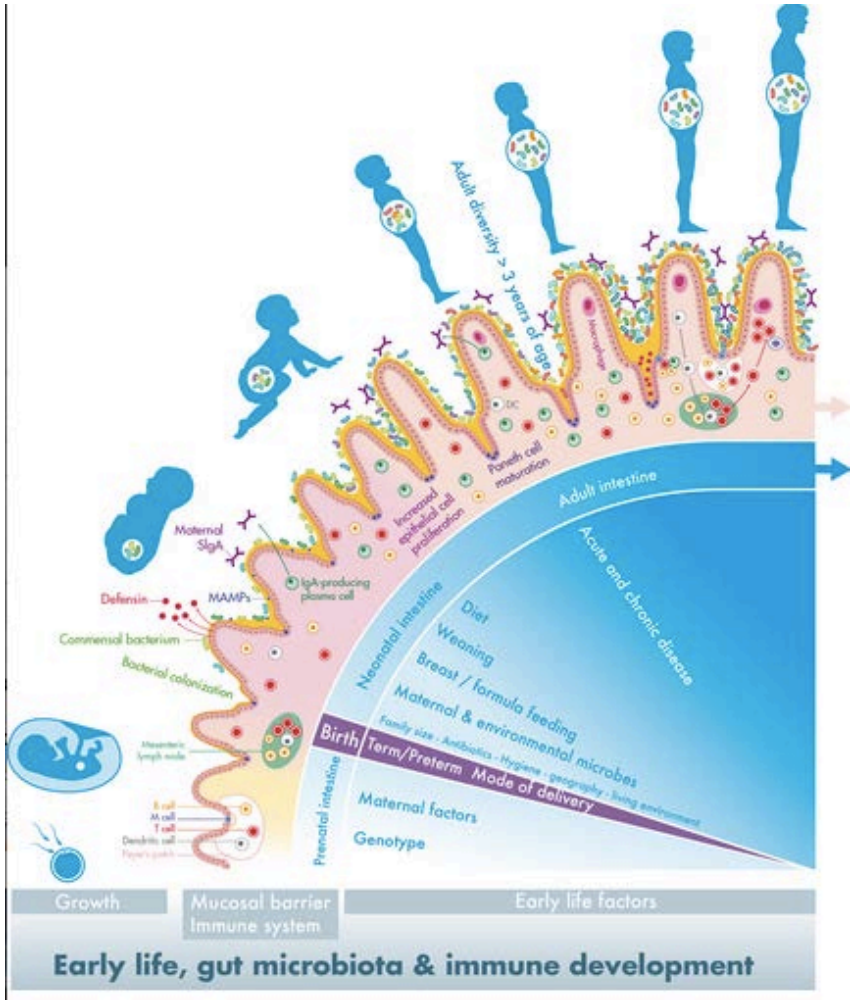


Why Now?.....
Modern Genomic Technology



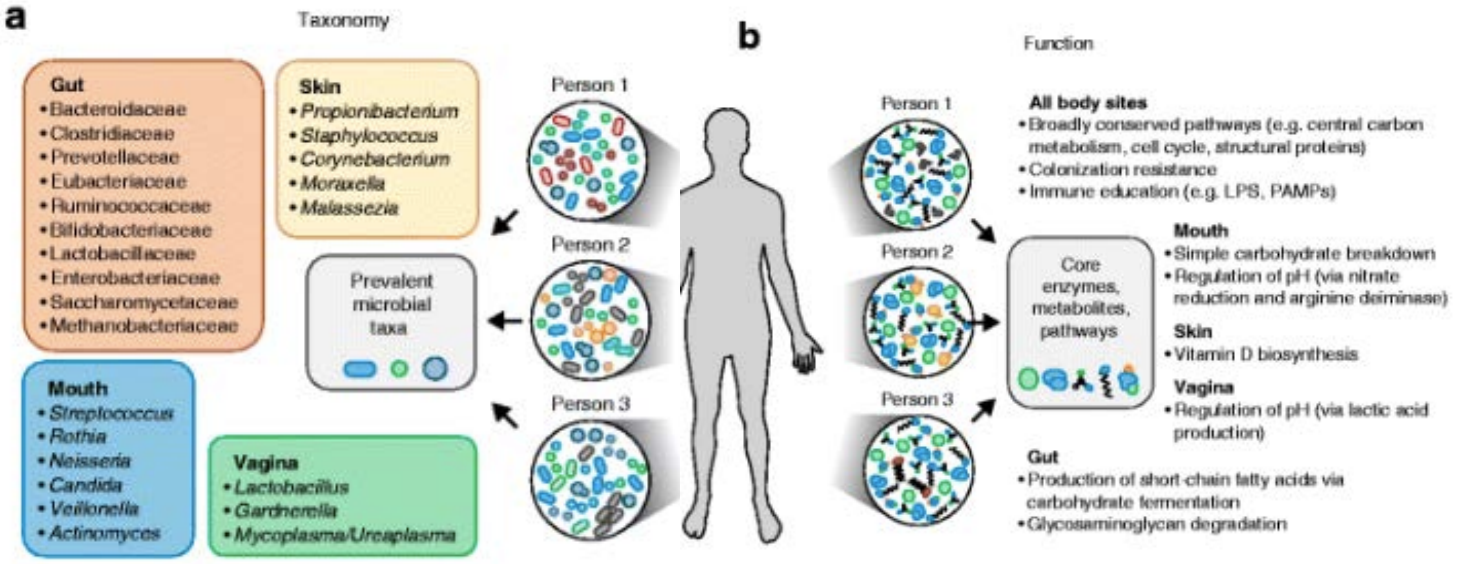
Normal Development of the Human Microbiome

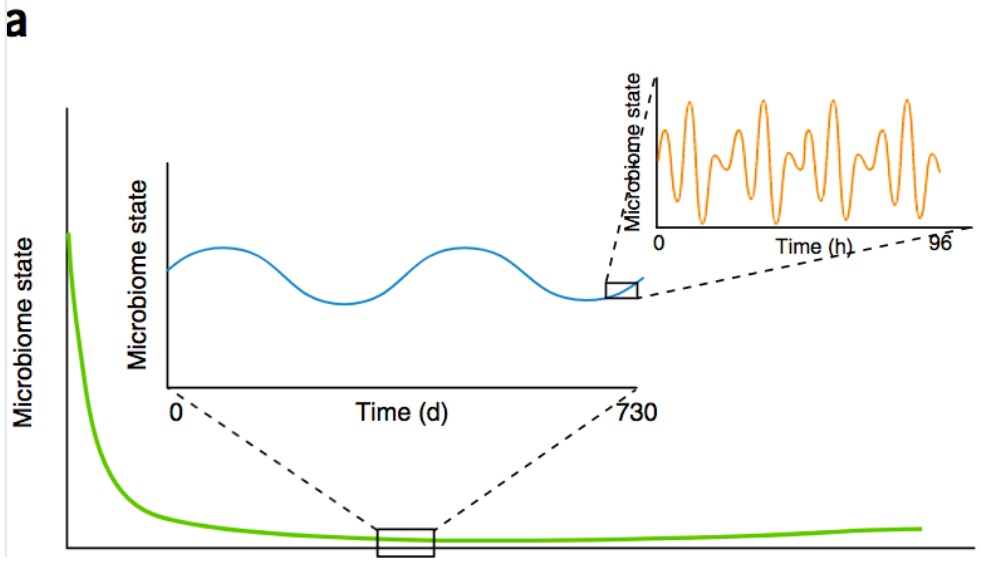
- Neonatal period is generally sterile*
- Birth and colonization
  - Mode of delivery
  - Breast Feeding
- Volatility and increasing diversity (0-2 yrs)
- Stability and resilience (2 yrs-adulthood)
- Decreasing diversity and return of volatility (elderly)
- Each individual is unique
  - *personalized medicine



Wopereis, 2014

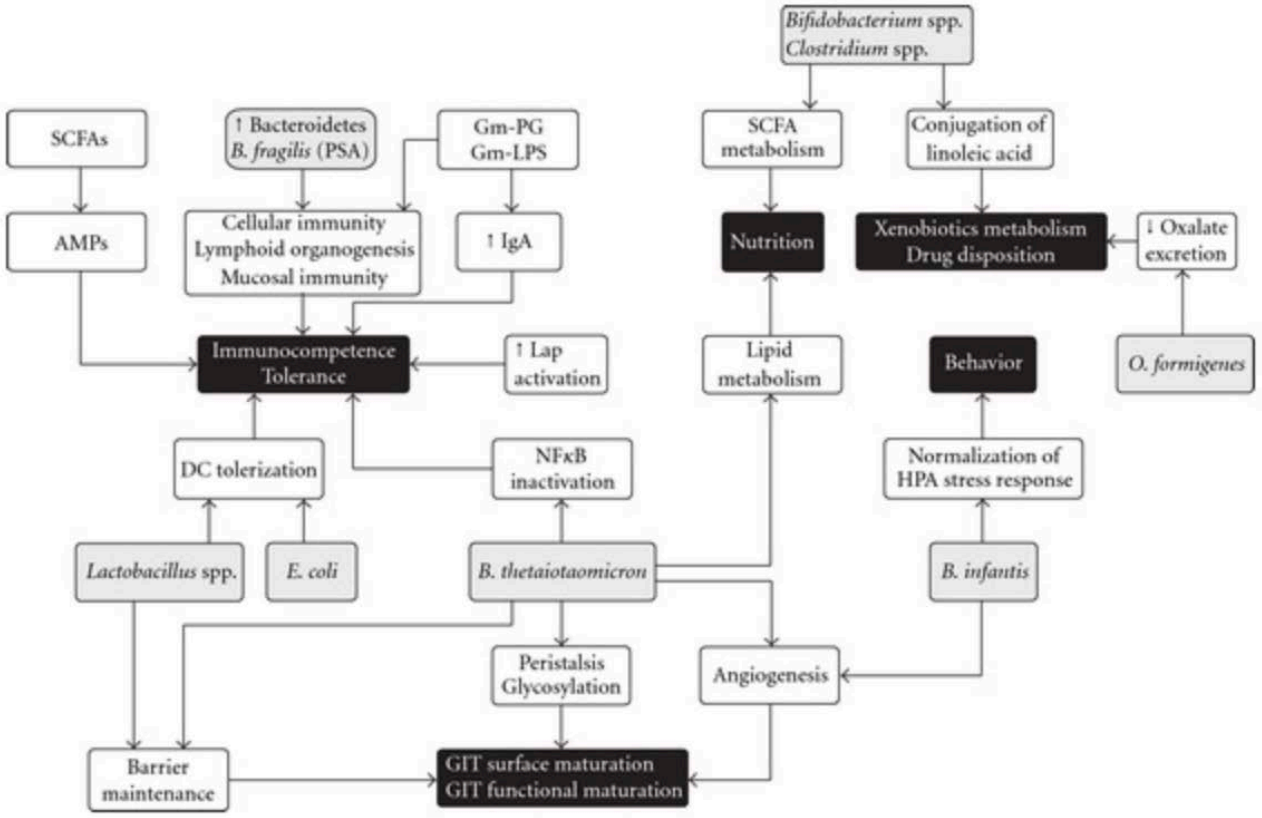
The Rhythms and Environmental Niches of the Human Microbiome





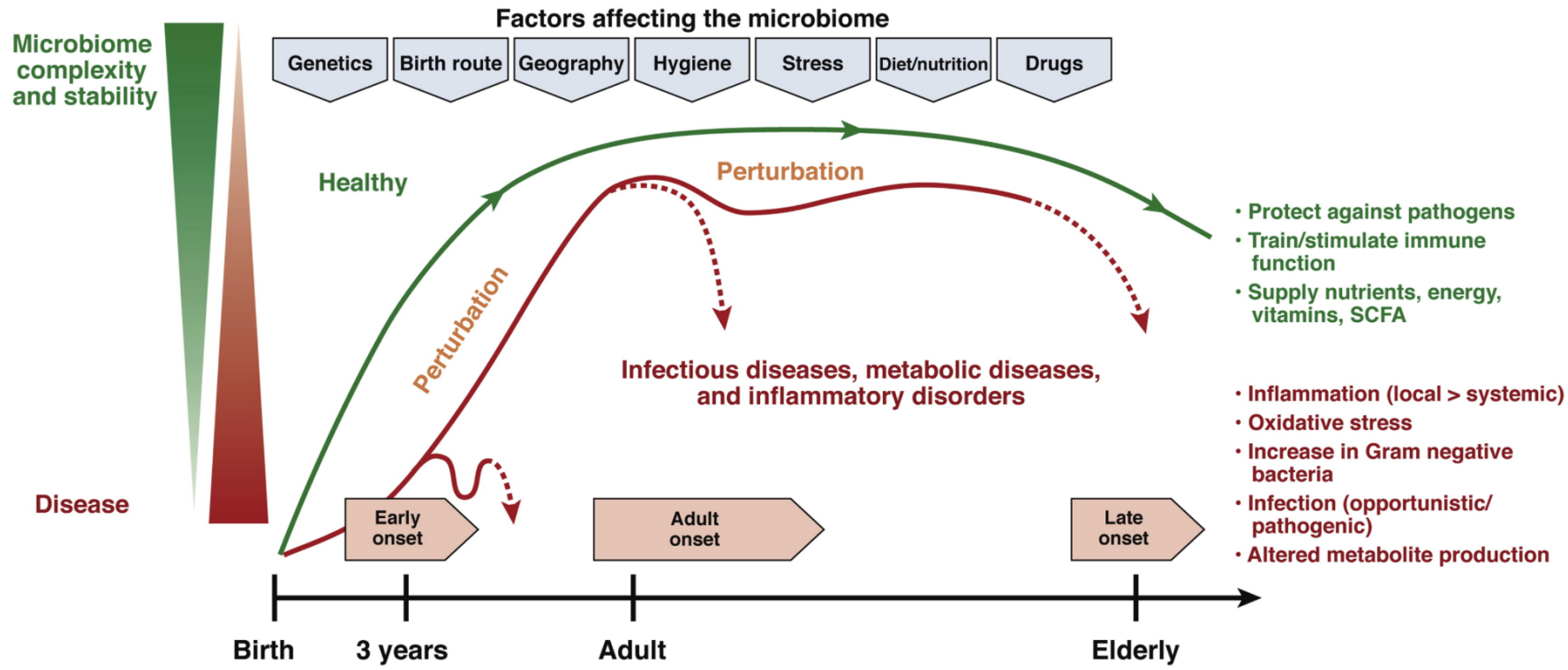
Gilbert. Nature Medicine 2018.

Host Physiology and the Microbiome



Phys Rev 2010 Sekirov et al.

Disruption of the Human Microbiome: Dysbiosis



Kostic, et al. Gastro. 2014; 146(5): 1489-1499

Dysbiosis: Cause or Effect?

Nat Rev Rheumatol. Author manuscript; available in PMC 2012 Feb 8.
Published in final edited form as:
Nat Rev Rheumatol. 2011 Aug 23; 7(10): 569–578.
Published online 2011 Aug 23. doi: 10.1038/nrrheum.2011.121
PMCID: PMC3275
NIHMSID: NIHMS352

The microbiome and rheumatoid arthritis

Jose U. Scher and Steven B. Abramson

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Oncotarget. 2017 Jan 31; 8(5): 8890–8899.
Published online 2016 Oct 28. doi: 10.18632/oncotarget.12985
PMCID: PMC5352451

Cross-talk between microbiota and immune fitness to steer and control response to anti PD-1/PDL-1 treatment

Andrea Botticelli,[#2] Ilaria Zizzari,[#1] Federica Mazzuca,[2] Paolo Antonio Ascierto,[4] Lorenza Putignani,[3] Luca Marchetti,[5] Chiara Napoletano,[1] Marianna Nuti,[#1] and Paolo Marchetti[#2]

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Subgingival microbiota dysbiosis in systemic lupus erythematosus: association with periodontal status

Jôice Dias Corrêa,[1] Débora Cerqueira Calderaro,[2] Gilda Aparecida Ferreira,[2] Santuza Maria Souza Mendonça,[1] Gabriel R. Fernandes,[3] E. Xiao,[4] Antônio Lúcio Teixeira,[2] Eugene J. Leys,[5] Dana T. Graves,[#4] and Tarcília Aparecida Silva[✉#1,6]

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Interactions between Gut Microbiota, Host Genetics and Diet Modulate the Predisposition to Obesity and Metabolic Syndrome

Siegfried Ussar,[#1,2] Nicholas W. Griffin,[#3,4] Olivier Bezy,[#1] Shiho Fujisaka,[1] Sara Vienberg,[1] Samir Softic,[1] Luxue Deng,[5] Lynn Bry,[5] Jeffrey I. Gordon,[3,4] and C. Ronald Kahn[1]

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Front Neurosci. 2017; 11: 490.
Published online 2017 Sep 15. doi: 10.3389/fnins.2017.00490
PMCID: PMC5605633

Cross Talk: The Microbiota and Neurodevelopmental Disorders

John R. Kelly,[1,2] Chiara Minuto,[1,2] John F. Cryan,[2,3] Gerard Clarke,[1,2] and Timothy G. Dinan[1,2,*]

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Gastroenterology. Author manuscript; available in PMC 2015 May 1.
Published in final edited form as:
Gastroenterology. 2014 May; 146(6): 1534–1546.e3.
Published online 2014 Jan 7. doi: 10.1053/j.gastro.2014.01.001
PMCID: PMC3995897
NIHMSID: NIHMS553868

Gastrointestinal Malignancy and the Microbiome

Maria T. Abreu[1] and Richard M. Peek, Jr.[2]

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Gut. 2017 Apr; 66(4): 633–643.
Published online 2016 Mar 18. doi: 10.1136/gutjnl-2015-309595
Original article
PMCID: PMC5529966

Tumour-associated and non-tumour-associated microbiota in colorectal cancer

Burkhardt Flemer,[1,2] Denise B Lynch,[1,2] Jillian M R Brown,[1,2] Ian B Jeffery,[1,2] Feargal J Ryan,[1,2] Marcus J Claesson,[1,2] Micheal O'Riordain,[3] Fergus Shanahan,[1,4] and Paul W O'Toole[1,2]

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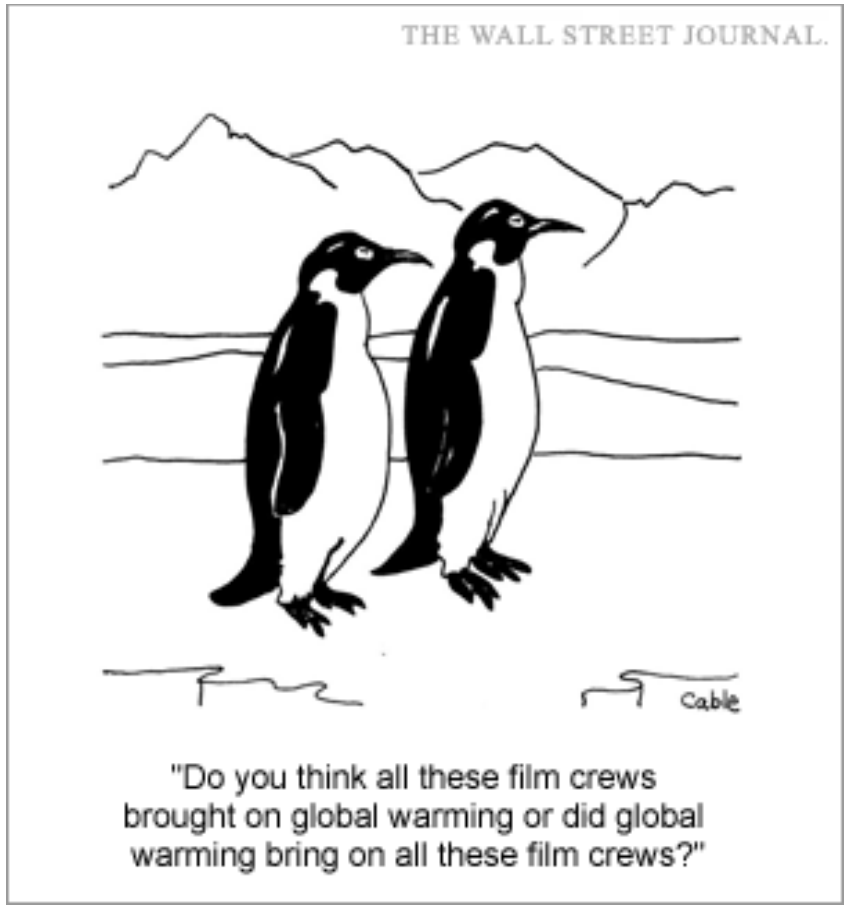
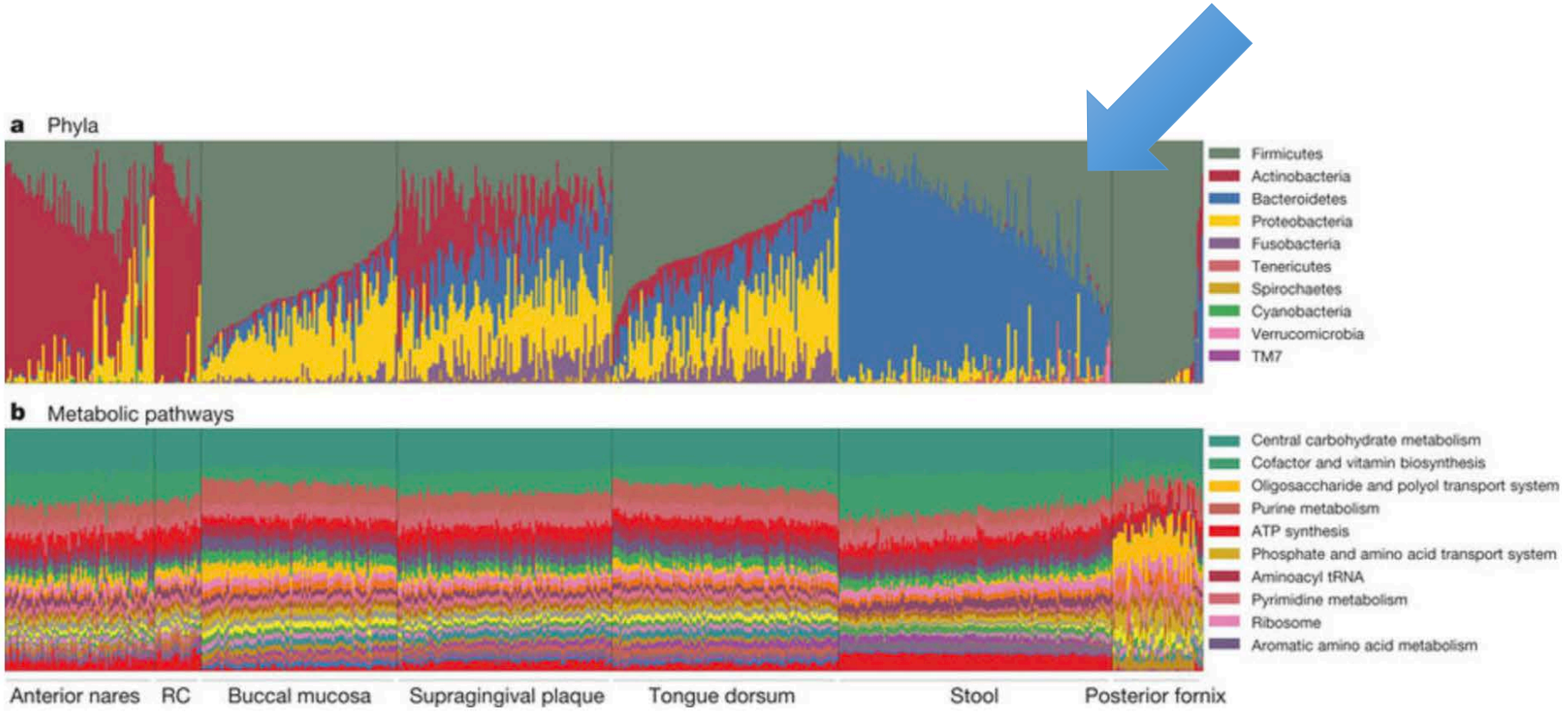
Correlation ≠ Causation

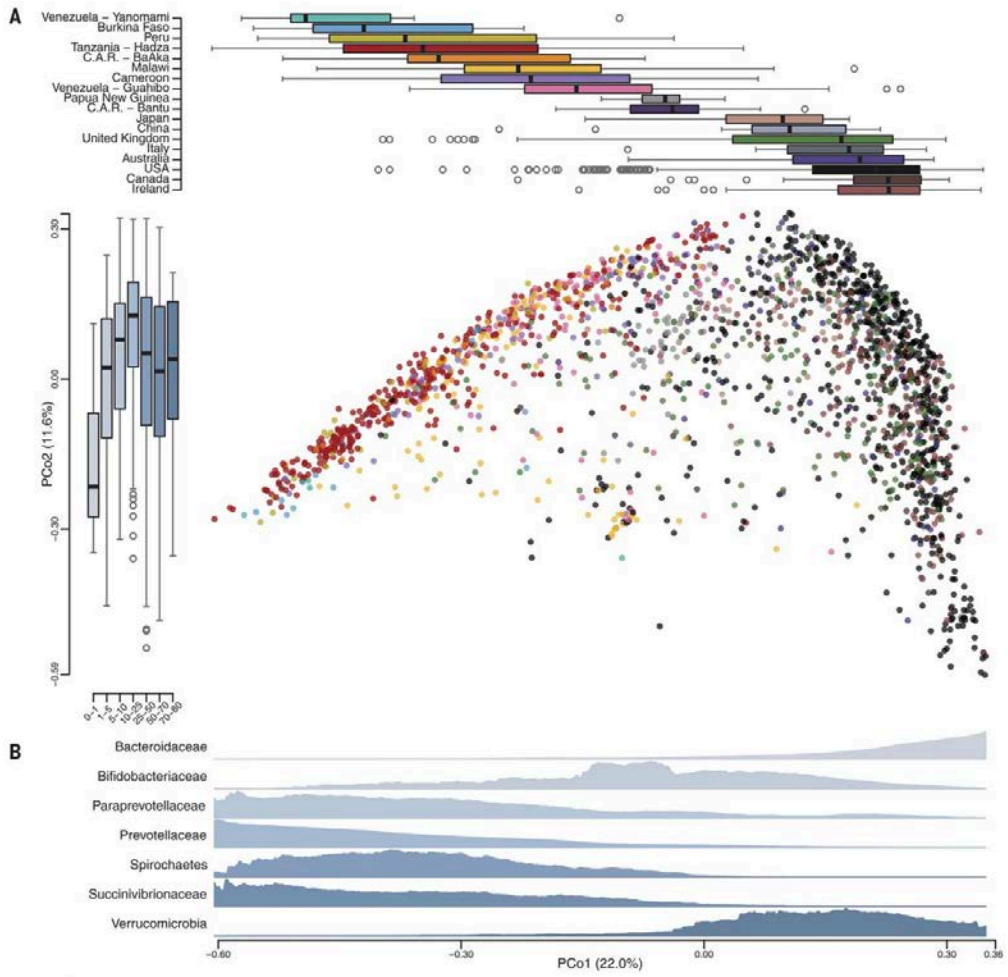


The Human Microbiome: What is Normal?



HMP. Nature 2012

Evolution of the Human Gut Microbiome



Smits, et al. Science. 2017

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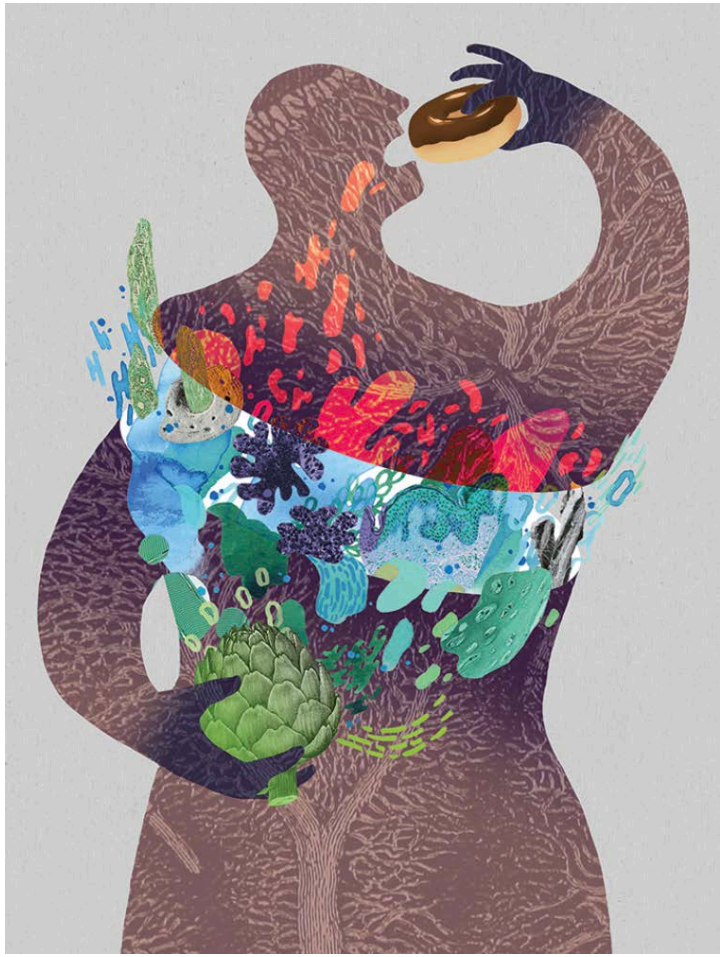
The Modern Gut Microbiome

- Urbanization, housing
- Sanitation
- Modern Medicine
  - Antibiotics

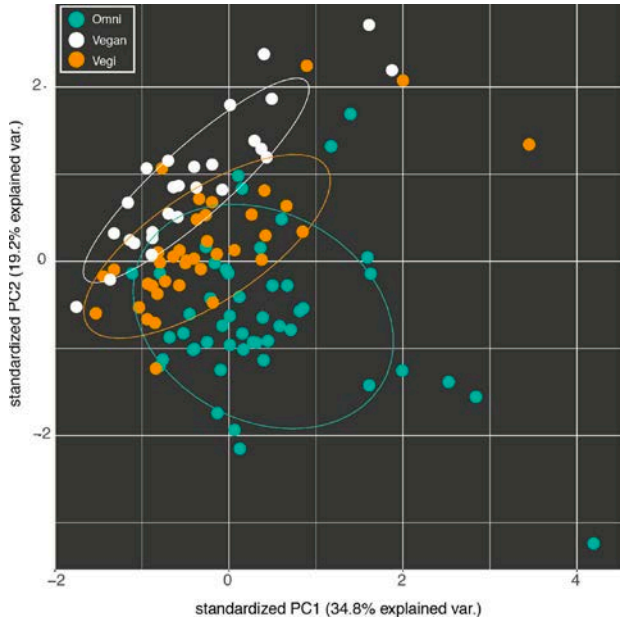
- **Diet**
  - Easy access to historically rare foods (sweet, salty)
  - Processed Foods
  - Dietary fiber: average American 15 g/ ADA 30 g/ Hadza 300 g

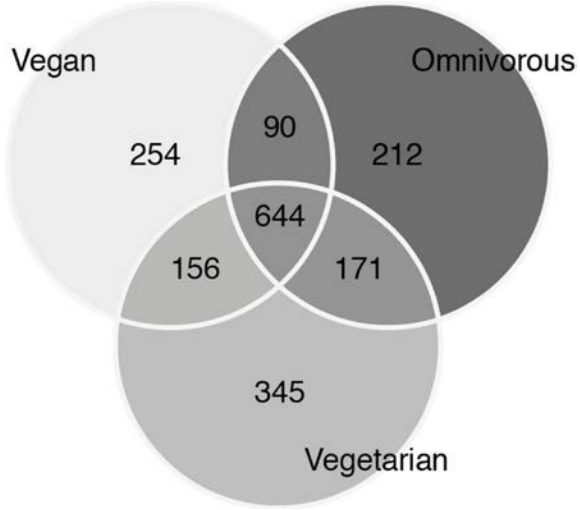
Diet: Major Influence Shaping the Gut Microbiome



Diet and the Gut Microbiome

- "Enterotypes"
  - Meat vs Plant-based diet
- Controlled feeding interventions
  - Shift within days of dietary change
- Immigration studies
- Japanese and seaweed





Losasso, et al. Front. Microbiol., 05 March 2018

Prebiotics vs Probiotics

Prebiotics: Food for your gut bacteria

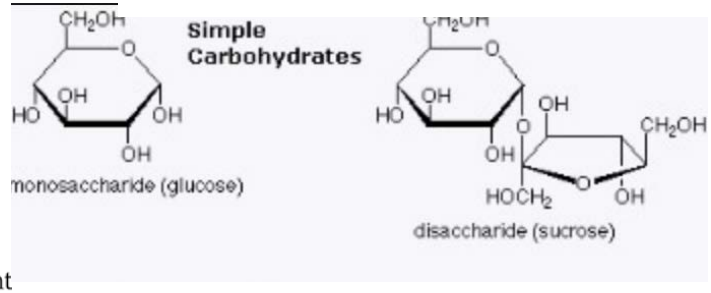
Microbiota-accessible carbohydrates (MACs)

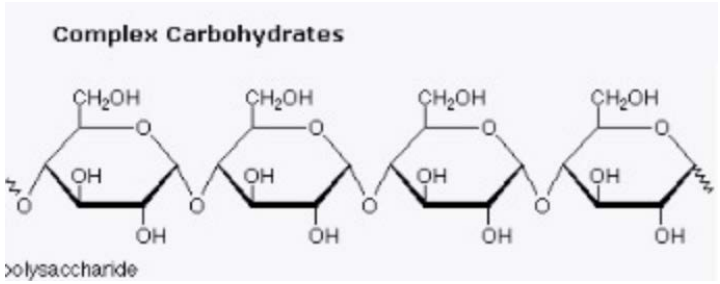
Dietary: Fermentable fiber

Host-derived: mucosal glycans

Probiotic: Live organisms consumed for a health benefit.

Dietary Fiber





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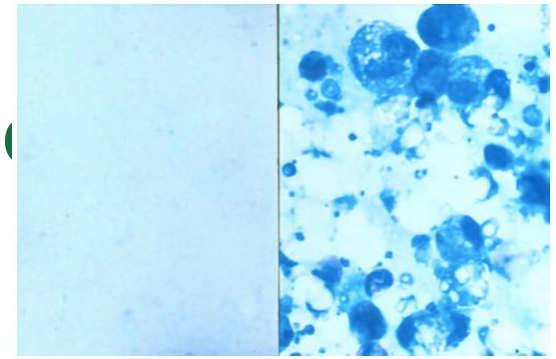
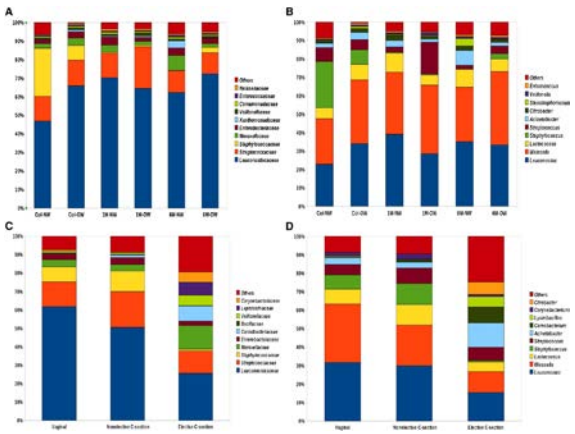
Breast Milk: The First Probiotic + Prebioti



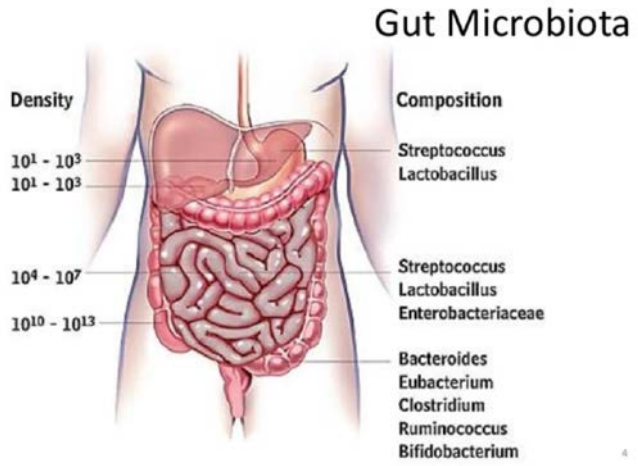
- Breast Milk
  - Cytokines, Immunoglobulins, Growth factors, Lysozymes, Lactoferrin, and...
- Microbiota
  - Bacteria, archaea, viruses, fungi, and protozoa
- 21%: Oligosaccharides (complex carbohydrates)
  - Selects for bacteria (i.e. *Bifidobacterium longum)* to begin cultivation of the baby's gut.



Raul Cabrera-Rubio et al. 2012

Probiotics: Challenges

- The bug
  - Aerobic manufacturing (vs anaerobic gut)
  - Storage and preservation (heat killed, temperature)
  - FDA regulation



- The host
  - “Drop in the bucket”
  - Colonization niches (pass on through vs. fill an unfilled niche and last)

Effects of the Modern Western Diet on the Gut Microbiome

Decreased complex fiber

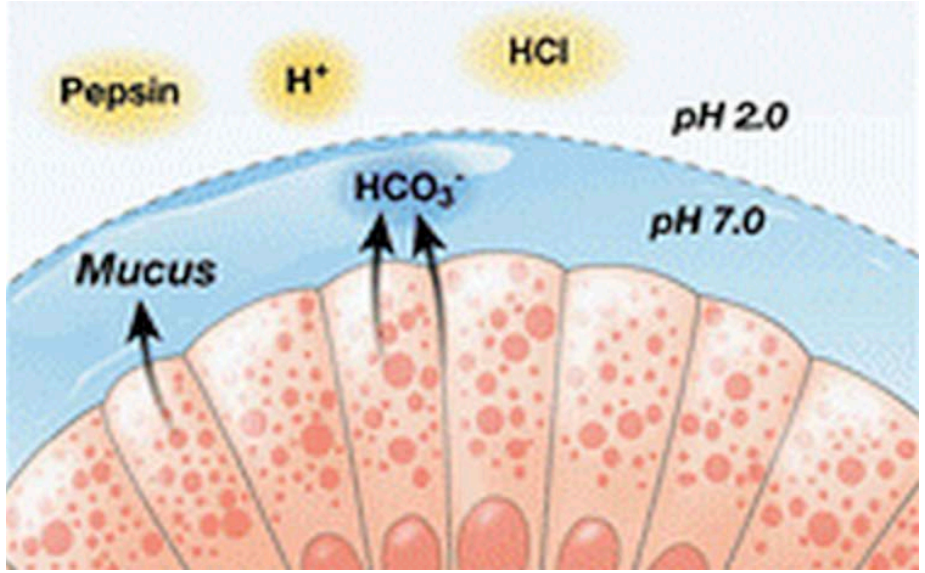
- "Hungry" bugs metabolize host glycans (mucus layer) instead
- Thinning of the protective mucus layer => Microbes closer to the epithelium => Immune activation

Artificial Sweeteners (sucralose and saccharin)

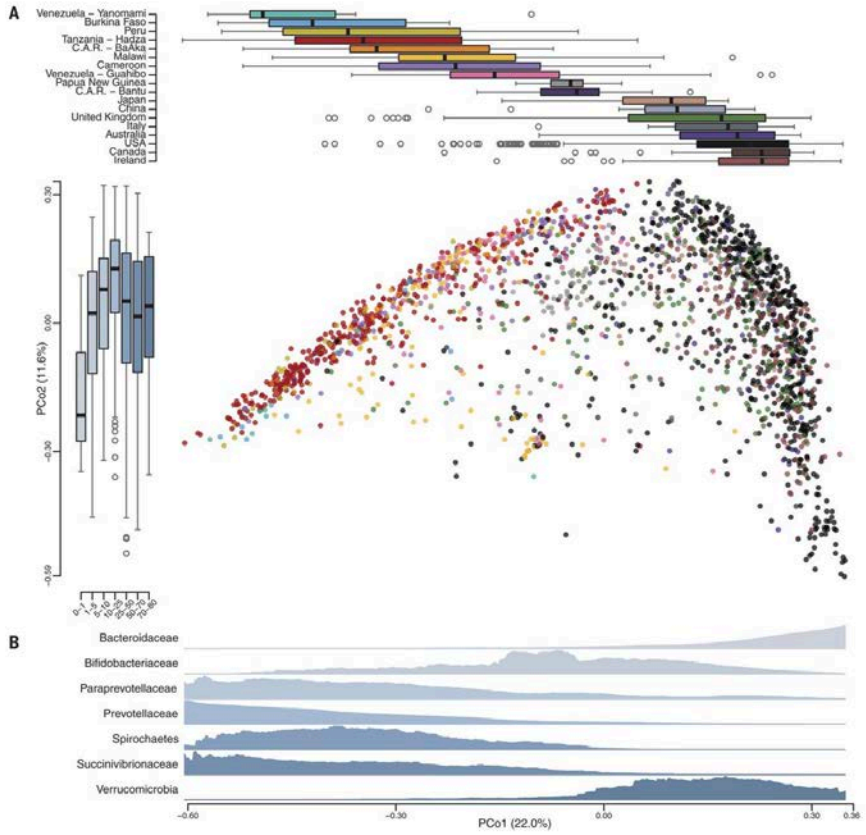
- Metabolized by microbes instead of host
- Results in microbial shifts
- Associated with metabolic changes in mice

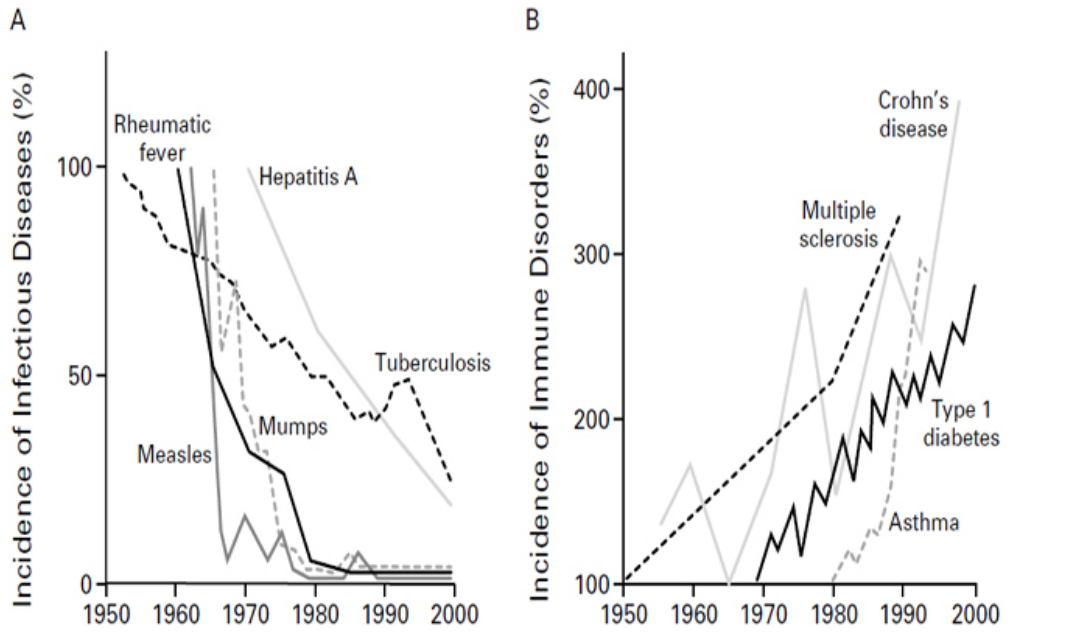
Emulsifiers

- Thin host mucus layer in mouse models



Is the Modern Gut Microbiome the (or Part of the) Link?





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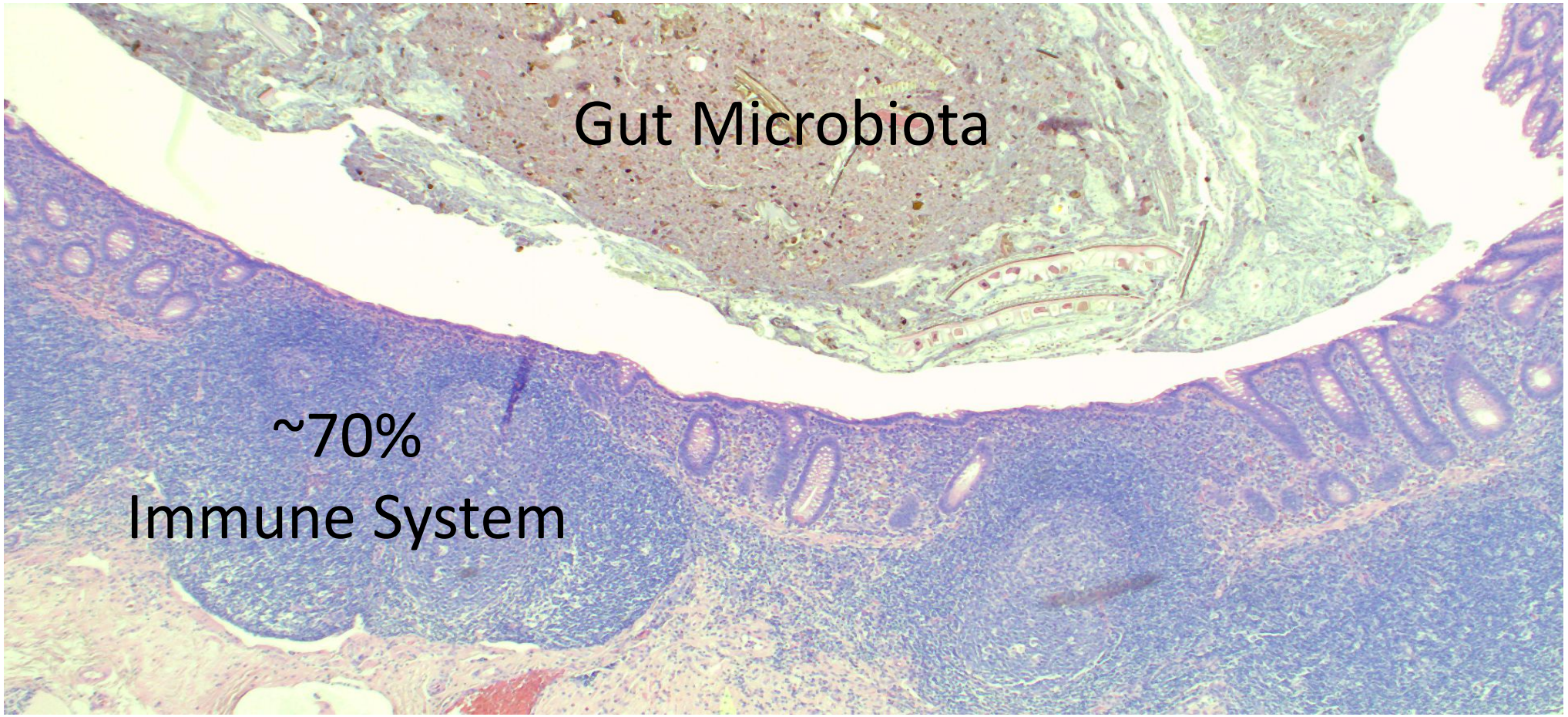
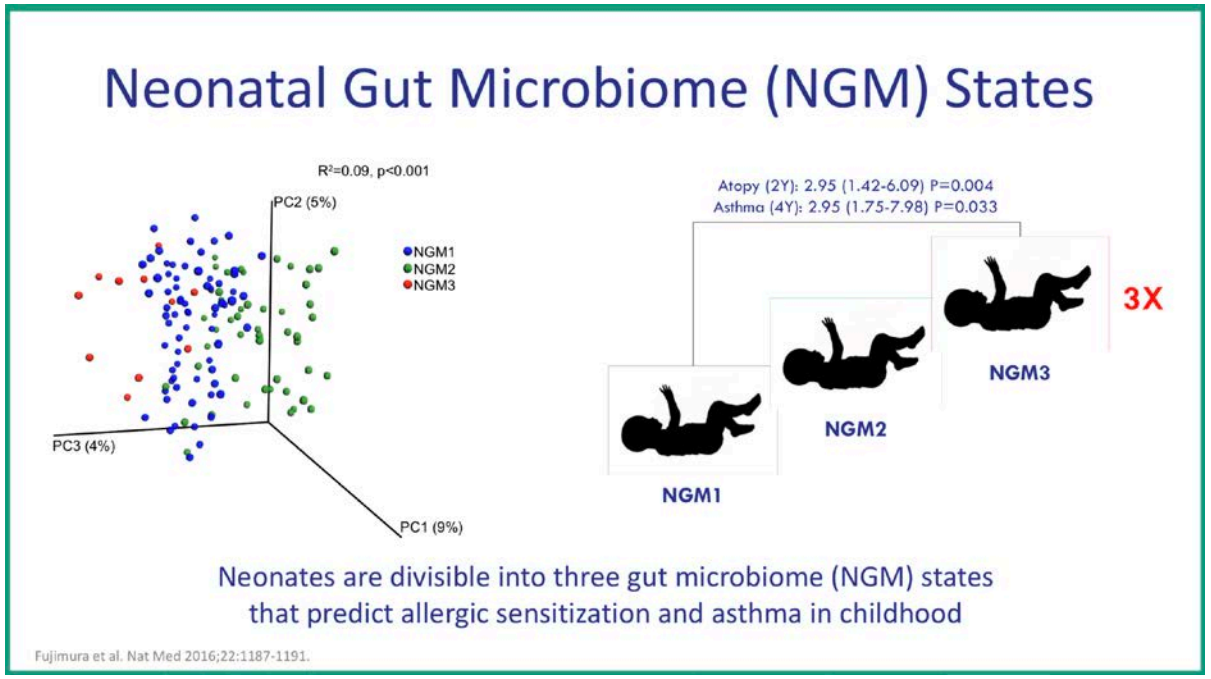
The Gut Microbiome and Immune Education

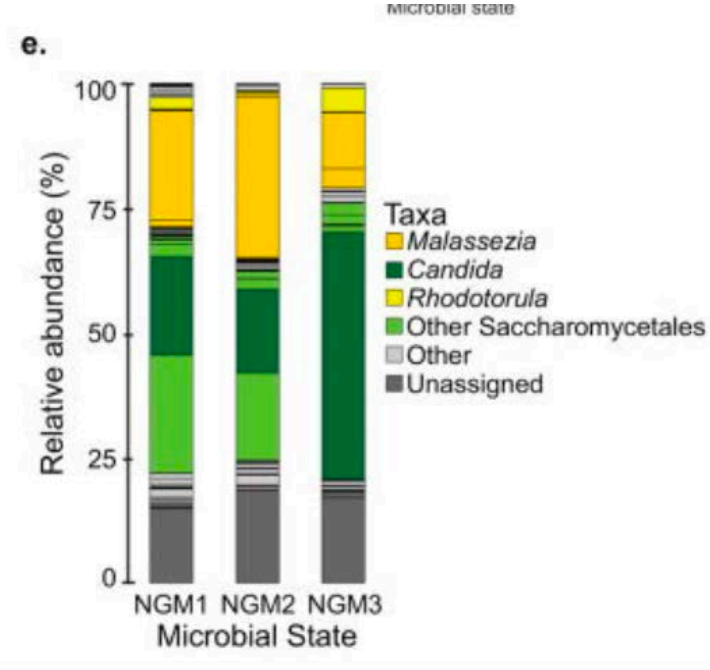


The Gut Microbiome and Immune Education

Prospective, birth cohort

Primary outcomes: Multi–sensitized atopy at 2 yo; Asthma at 4 yo





R. Valladares. Mol. TriCon. 2018

Fujimura 2016

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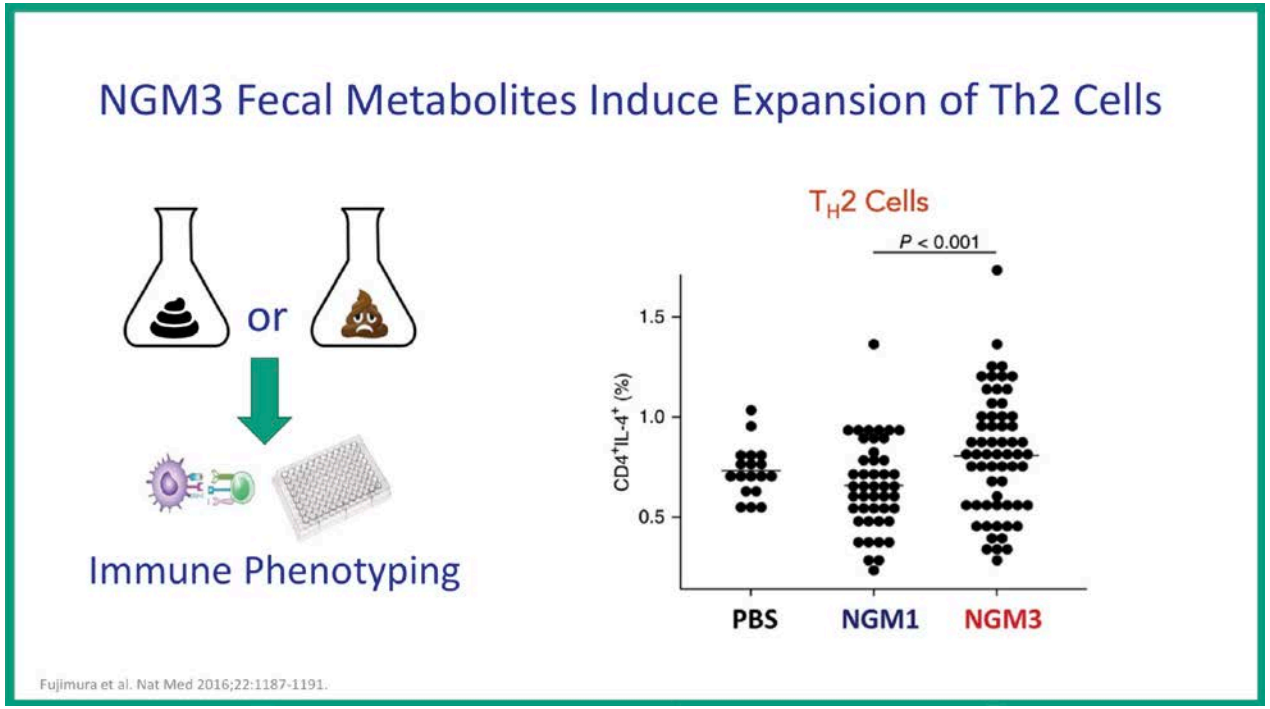
The Gut Microbiome and Immune Education

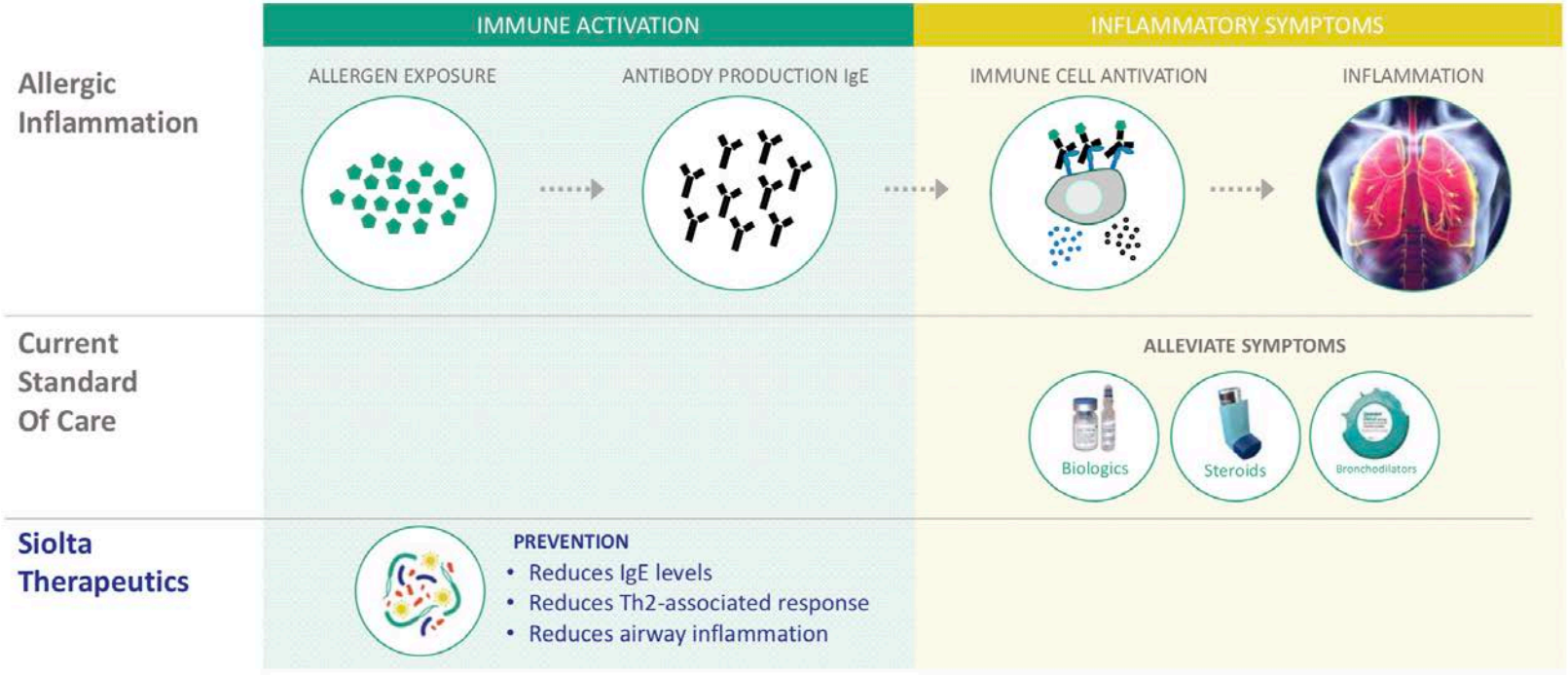
- Exposure of immune cells to sterile fecal water of “high risk” neonates =>
  - T cell activation (increased Il-4) and
  - Decreased immune regulatory cells.



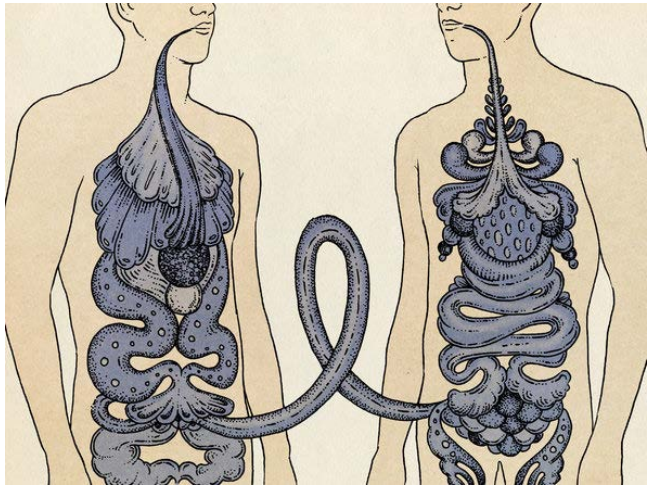
R. Valladares. Mol. TriCon. 2018

Using the Gut Microbiome to Prevent Disease: Asthma

Siolta Therapeutics



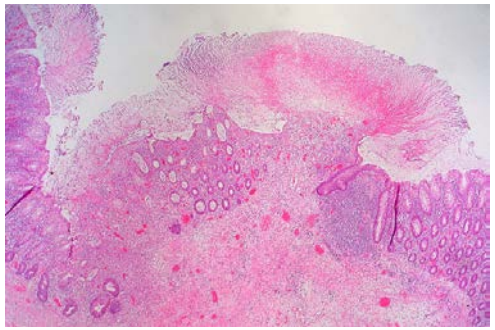
Using the Gut Microbiome to Treat Disease: Fecal Microbiota Transplant



Clostridium difficile Colitis

- *C. difficile* colitis is characterized by profuse, watery diarrhea, abd pain, fever
  - Incidence ~ 500,000
  - Mortality ~ 14,000
  - Morbidity includes drug toxicity 2° Abx use, toxic megacolon, total colectomy

- Deaths linked to C. diff increased fivefold between 1999 and 2007.
- Risks for developing colitis include antibiotic use, increasing age, long term care facility
- Recurrent C. difficile colitis (rCDI)
  - 1st reoccurrence: ~25% of patients,
  - Of those, 35-65% will suffer multiple episodes



Fecal Microbiota Transplant: *C. difficile* Colitis

The NEW ENGLAND JOURNAL of MEDICINE

- **Prospective, randomized, controlled trial**

1) **FMT**: Short-course of vancomycin (500 mg orally q6 x 4d) =>FMT

2) **Standard vancomycin**: 500 mg orally q6 x 14 days

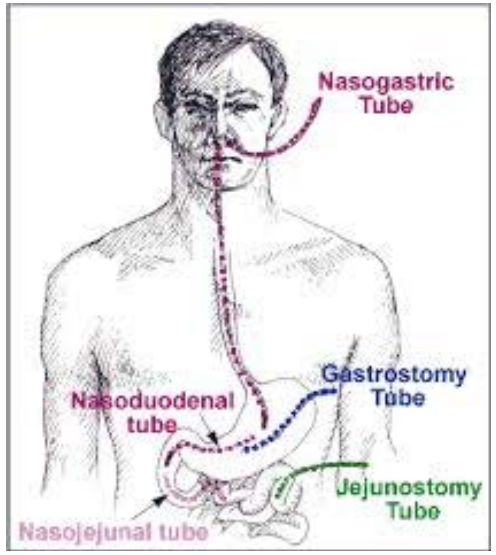
3) **Vancomycin with bowel lavage**: Bowel lavage performed on d 4

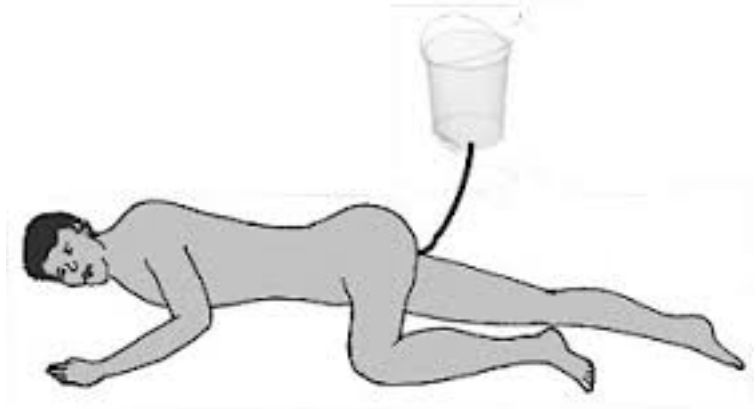
van Nood, et al N Engl J 201333

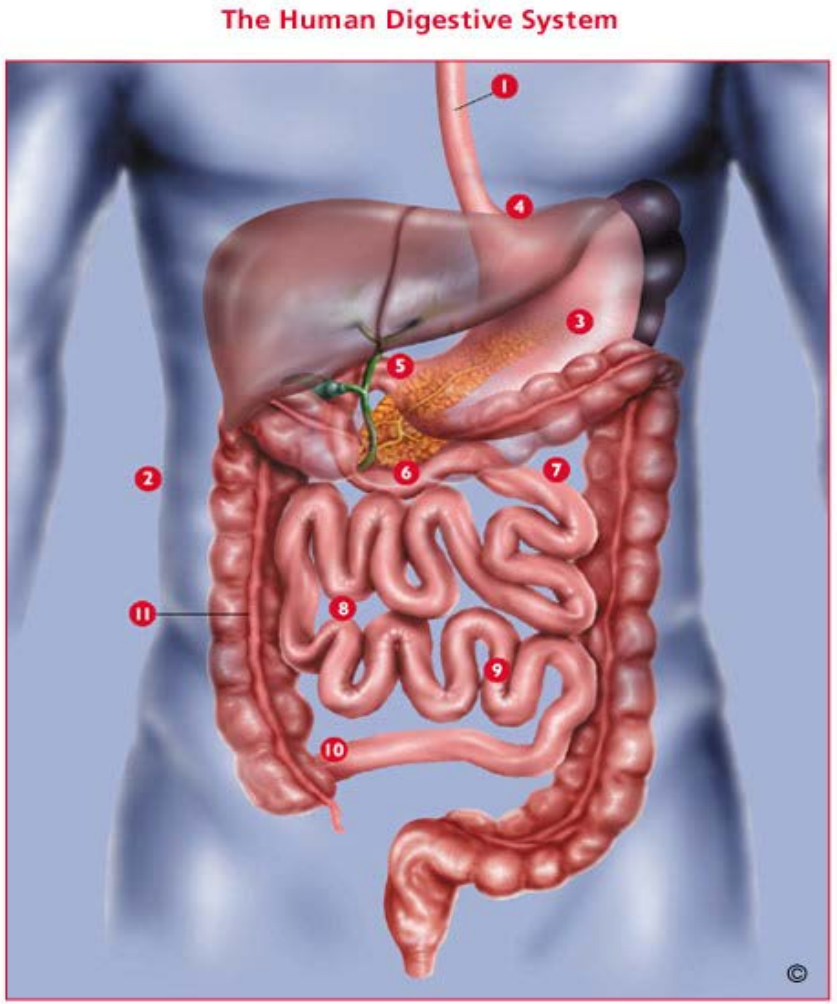
Fecal Microbiota Transplant: *C. difficile* colitis

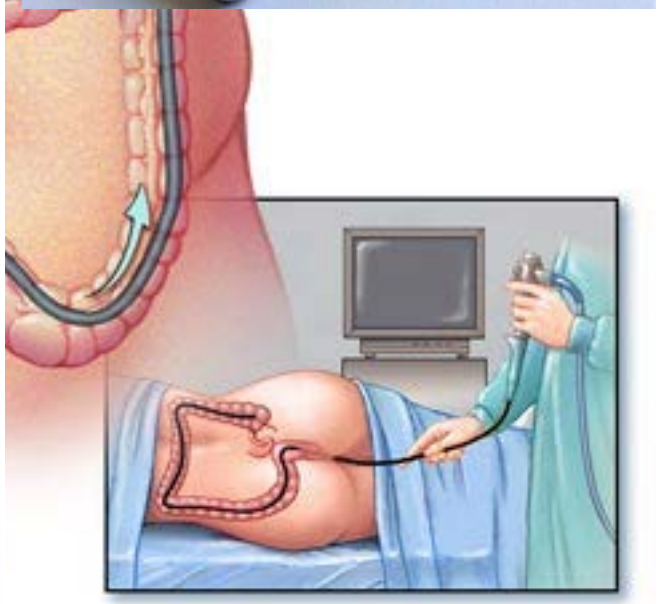
- The **study was stopped** after an interim analysis.
  - 13/16 (81%) resolved with 1 FMT, 2 of 3 remaining patients resolved after 2nd FMT.
- Recurrence rate 5 weeks following treatment:
  - 62% in vancomycin alone
  - 54% in vancomycin + bowel lavage
  - 1 patient (6%) in FMT
- Average cure rate: 93%
- No serious adverse events to date have been reported.

Fecal Microbiota Transplant: Delivery









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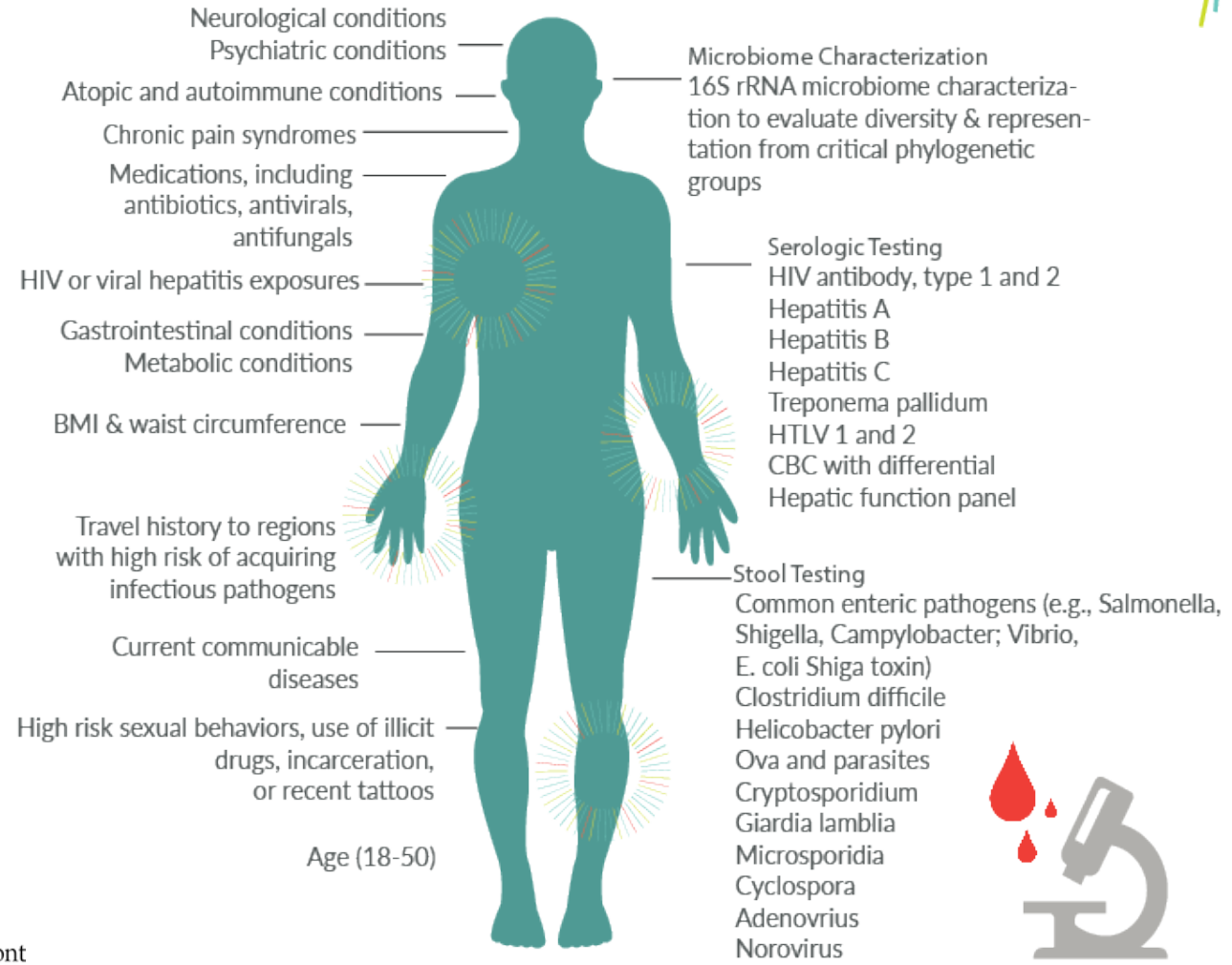
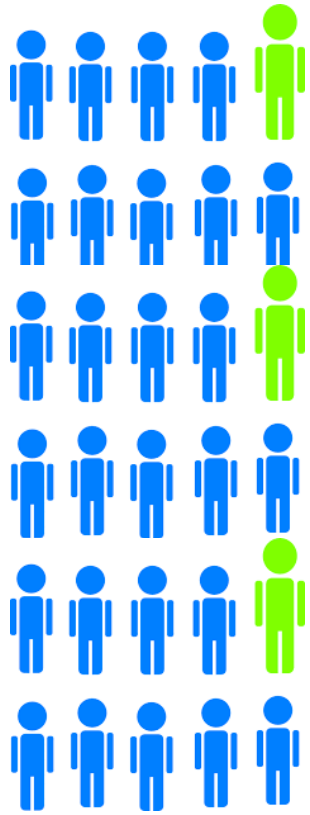
Fecal Microbiota Transplant: Donor

OPENBIOME





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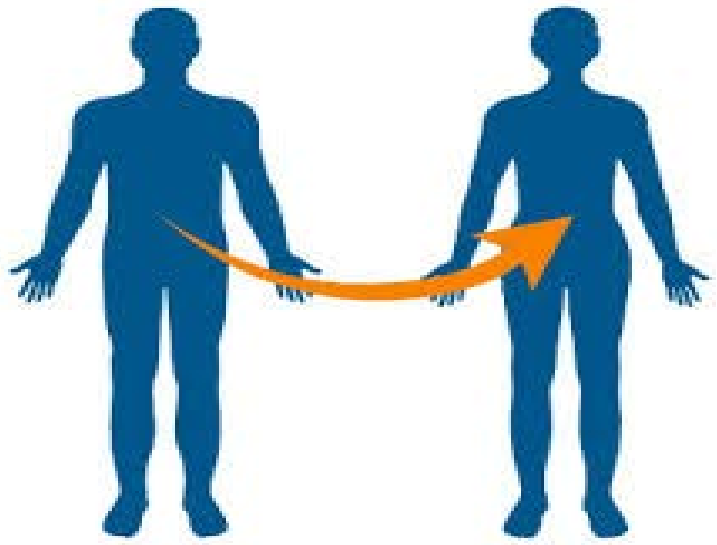
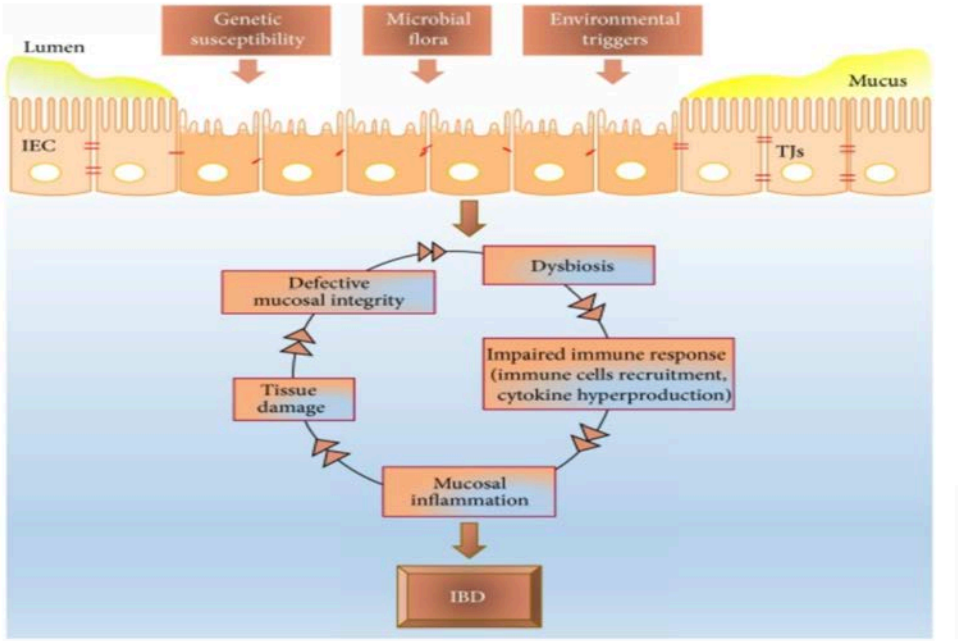
Fecal Microbiota Transplant in the Treatment of Ulcerative Colitis

- Clinical Trial #NCT02390726
- Principal Investigator: Peter L Moses, MD
- Multidisciplinary
- Study Design: Randomized Control Trial
- Intervention Model: Parallel Assignment
- Masking: Double Blind (Subject, Investigator)

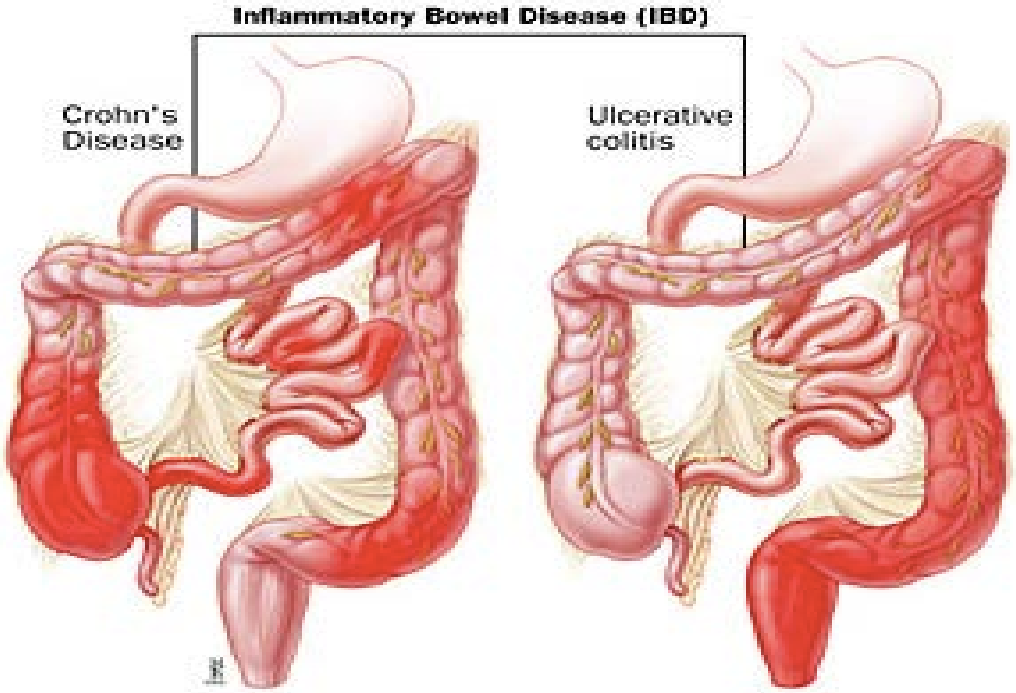


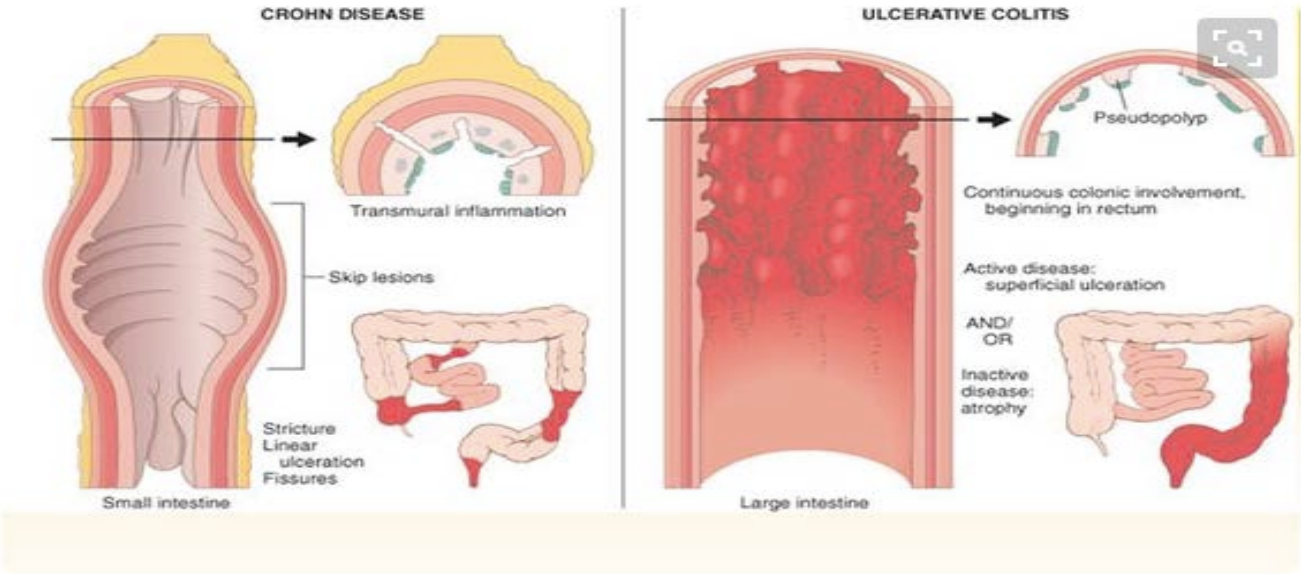
Using the Microbiome to Treat Disease: Fecal Microbiota Transplant in Inflammatory Bowel Disease (IBD)

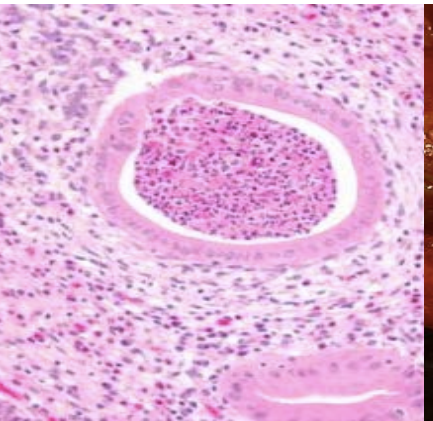
- Includes both Crohns Disease & Ulcerative Colitis
- US incidence ~ 1.6 million
- Peak age of onset ~ 2nd-3rd decades

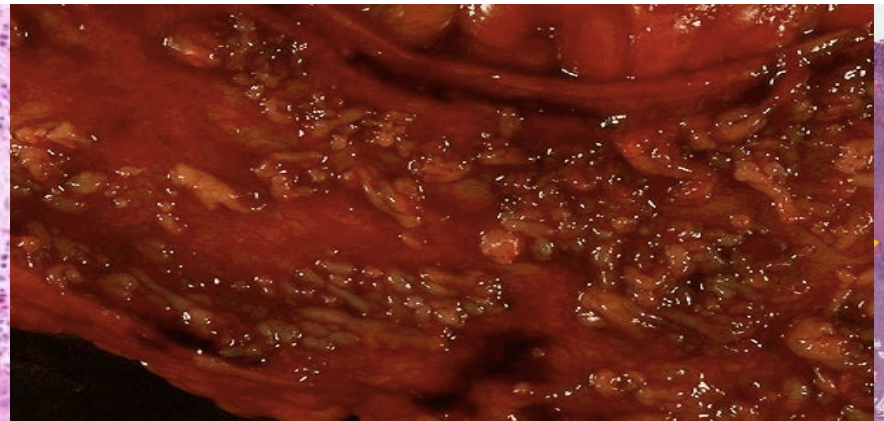


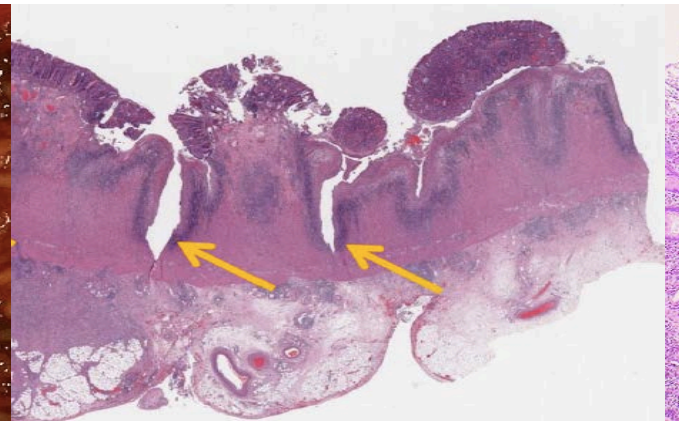
IBD: Crohn's vs Ulcerative Colitis

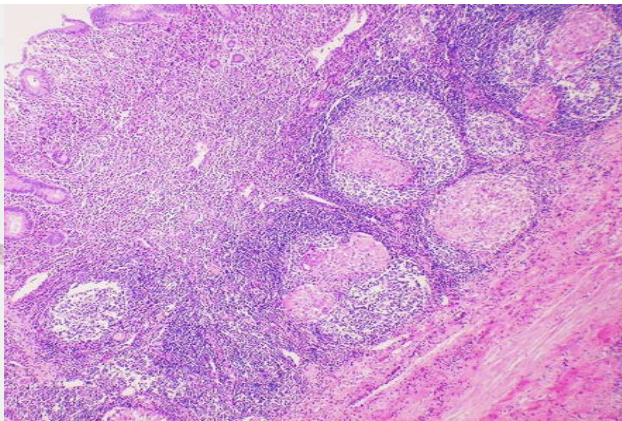




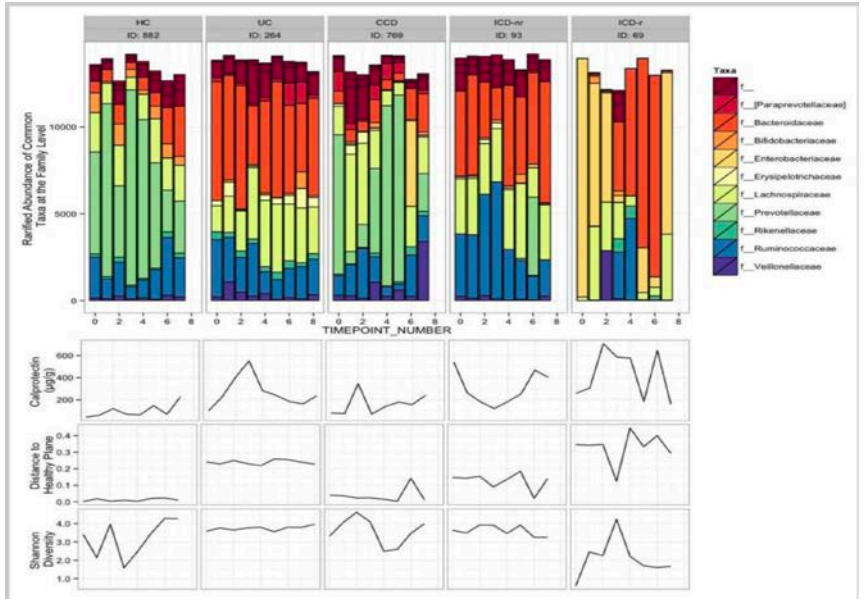




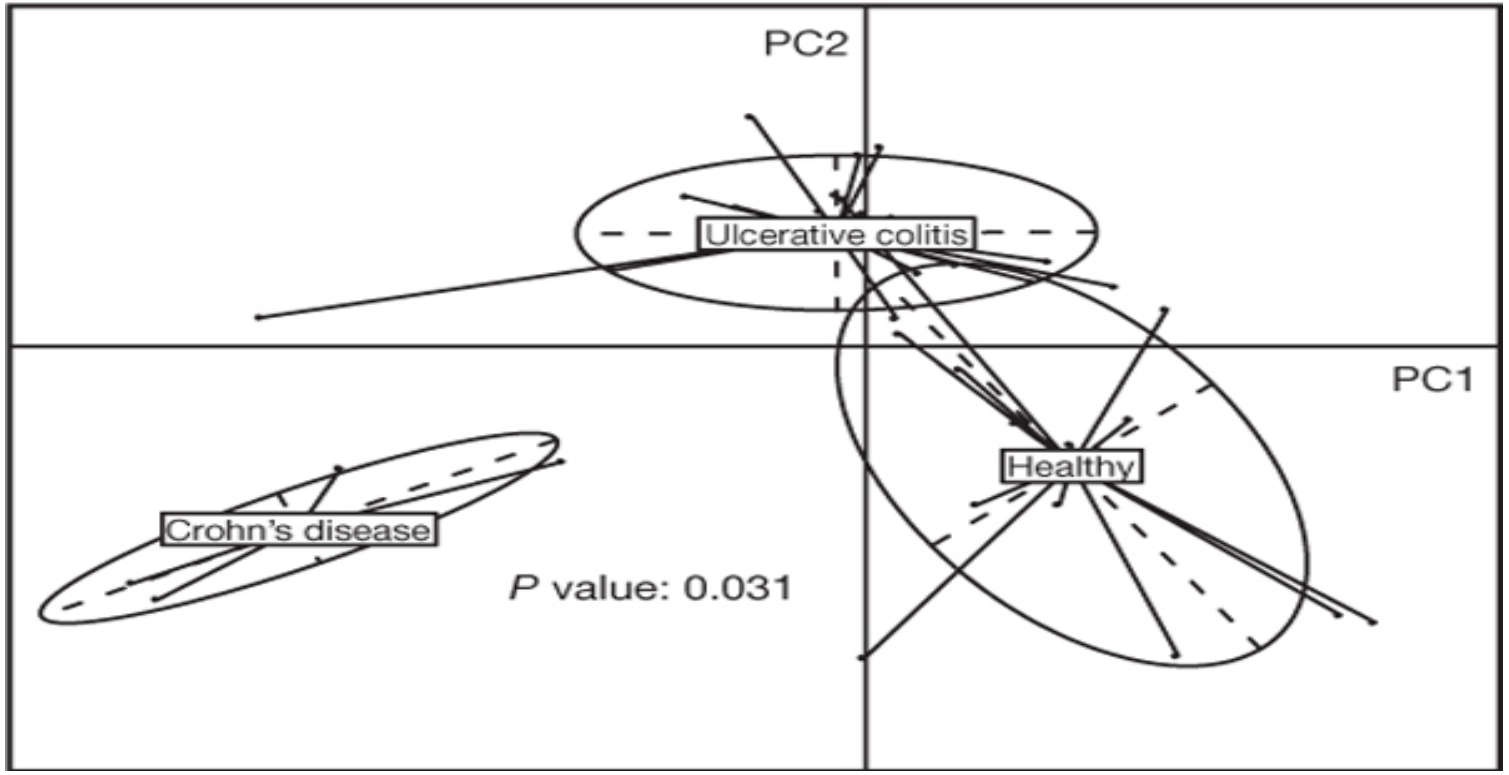




Gut Microbiomes of IBD patients vs. healthy individuals



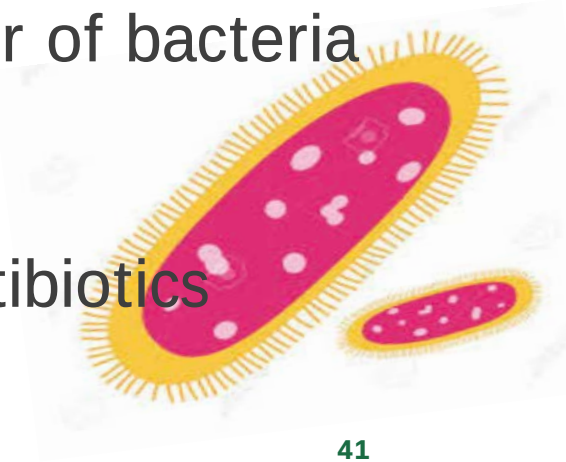
Halfvarson. 2017



JJ Qin *et al. Nature* **464**, 59-65 (2010) doi:10.1038/nature08821

IBD: Evidence for Microbial Pathogenesis

- IBD patients display aberrant T-cell activation, high levels of mucosal IgG, AB cytokine responses to intestinal bacteria
- Risk increased by agents suspected of disrupting mucosal barrier and normal microbiota composition.
    - Antibiotics, enteropathogenic exposures
- IBD pts have decreased mucus layer and increased number of bacteria directly adjacent to epithelial surface.
- Effective treatments include: Diversion of fecal stream, Antibiotics



Fecal Microbiota Transplant in the Treatment of Ulcerative Colitis

Antibiotic pretreatment (Both Arms)

- ciprofloxacin 250mg PO q12 and metronidazole 500mg PO q8 x7 days

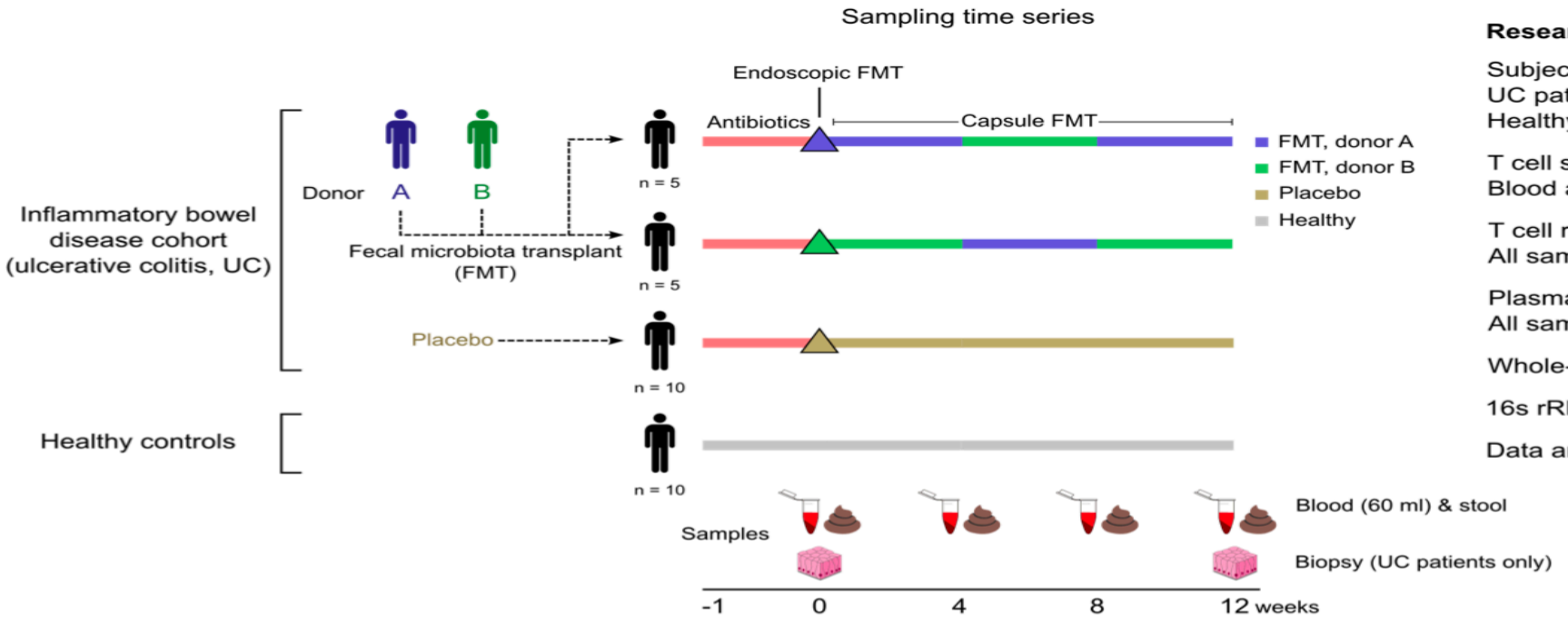
Treatment Arm:

- FMT Induction by colonoscopy plus microbial maintenance plus standard therapy

Control Arm:

- Sham FMT and Sham Microbial Maintenance plus standard therapy

Study Design



Research Locations

Subject recruitment and sampling:
UC patients @ UVMMC
Healthy controls @ MIT

T cell sorting:
Blood and biopsy samples sorted @ UVMMC

T cell receptor sequencing:
All samples @ Juno Therapeutics

Plasma metabolomics:
All samples @ the Broad Institute

Whole-shotgun metagenomics @ the Broad Institute

16s rRNA sequencing @ the Broad Institute

Data analysis @ MIT and UVMMC

Sample analyses

- Whole-shotgun metagenomics
- Plasma metabolomics
  T cell sorting (Th1, Th17, Treg)
  T cell receptor sequencing
- T cell sorting (Th1, Th17, Treg)
  T cell receptor sequencing

Patient Groups are Similar at Baseline

	Study Number	Age	Sex	Initials	Primary Donor	
7	1	44	M	KDZ	A	
	7	46	F	JHR	A	
	10	38	M	AMR	A	
	14	20	M	AJS	A	
	3	22	F	ECT	B	SCREEN FAIL
	8	35	F	JCJ	B	
	11	65	F	JFE	B	
8	2	27	F	BAE		SCREEN FAIL
	4	65	M	AZN		
	5	68	M	MCT		
	6	47	F	CLR		
	9	31	F	LEH		
	12	40	F	LLM		DROPPED OUT
	13	58	M	GLF		
	15	57	M	CSE		SCREEN FAIL

	Group		
Variable	Active	Placebo	P value
N	7	8	
Age	39 (15)	49 (15)	0.21
Sex	4 (57%)	4 (50%)	1.00
Race	6 (86%)	7 (88%)	1.00
BMI	25 (3)	29 (4)	0.04
CRP	2 (29%)	3 (38%)	1.00
Fecal calprotectin	513 (607)	306 (301)	0.47
Fecal lactoferrin	7 (100%)	6 (75%)	0.47
Endo UCEIS score	6.6 (2.0)	7.4 (2.6)	0.51
Endo Mayo score	1.4 (0.8)	1.8 (1.2)	0.55
Mayo symptom score	4.6 (1.8)	4.4 (1.1)	0.80
IBDQ bowel system	4.4 (0.7)	4.2 (0.8)	0.67
IBDQ emotional health	4.6 (1.0)	4.7 (1.0)	0.91
IBDQ systemic systems	4.5 (1.1)	4.2 (1.1)	0.70
IBDQ social function	5.1 (0.5)	4.9 (1.2)	0.60
IBDQ total score	147.3 (19.3)	144.1 (25.1)	0.79

ITT n = 15

Adverse Events:
No difference between groups

Adverse Event	Cases	Relatedness	Severity	Group Designation
Fever	2	Not Related, Possibly Related	1	Active, Active
Worsening Disease	2	Possibly Related	1	Active, Placebo
Abdominal pain	1	Not Related	1	Active (not treated)
Epitaxis	1	Not Related	1	Placebo
URI	1	Not Related	1	Placebo
Head Cold	1	Not Related	1	Active
Nausea	1	Probably related	1	Placebo
Post- Anethesia Myocolonic Jerks	1	Probably Related	3	Active (not treated)
Sore throat	1	Not Related	1	Placebo

6/7 vs 5/8 *p = 1.0 fischer's exact test*

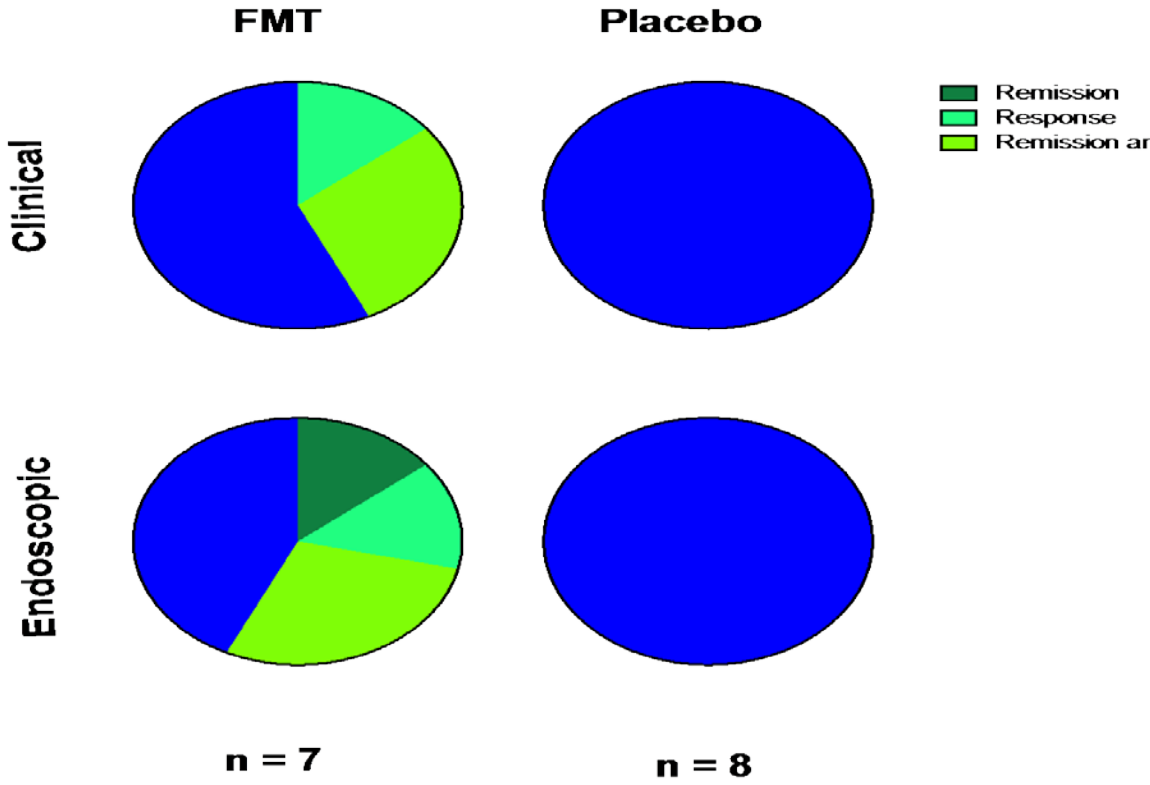
Primary Clinical Outcomes

Clinical Remission: 29% vs 0% *(p=0.20)*

Clinical Response: 43% vs. 0% *(p=0.08)*

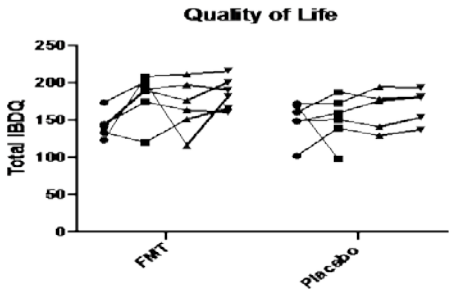
Endoscopic Remission: 43% vs 0% *(p=0.08)*

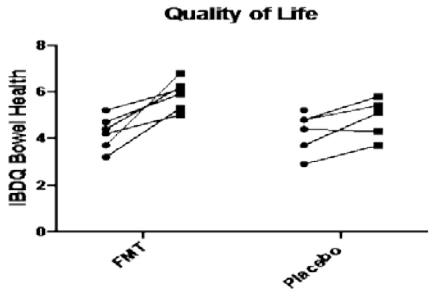
Endoscopic Response: 43% vs. 0% *(p=0.08)*

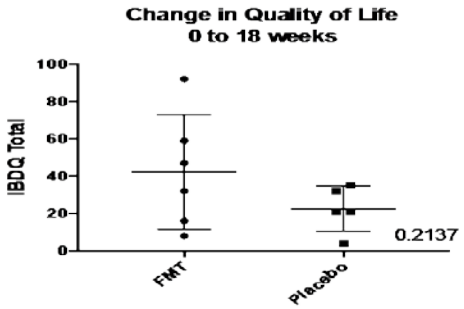


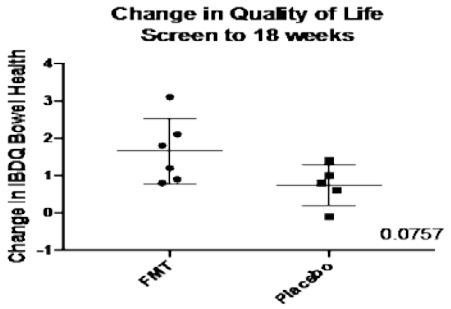
**Either Endoscopic Remission of Response: 57% vs 0% (p= 0.03)*

FMT patients reported enhanced bowel health







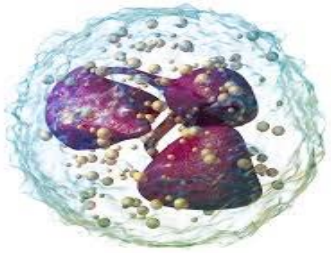


- **IBDQ** (Inflammatory Bowel Disease Questionnaire)
  - Validated
  - Disease-specific

FMT patients have a decrease in stool markers of inflammation (fecal calprotectin and lactoferrin)

Variable	Group		Screen or Procedure	4 week	12 week	18 week	P value*
CRP	Active	Adjusted %	27%	30%	79%	9%	0.99
	Placebo	Adjusted %	31%	37%	71%	7%	
Fecal calprotectin	Active	Adjusted mean (SE)	447 (39)		184 (43)		**0.03**
	Placebo	Adjusted mean (SE)	417 (34)		396 (41)		

			Visit			
Variable	Group		Screen	4 week	12 week	18 week
Fecal lactoferrin	Active	#(%) positive	7 (100%)	5 (83%)	4 (67%)	3 (50%)
	Placebo	#(%) positive	6 (75%)	5 (83%)	5 (100%)	5 (100%)
	P value		0.47	1.00	0.45	0.18

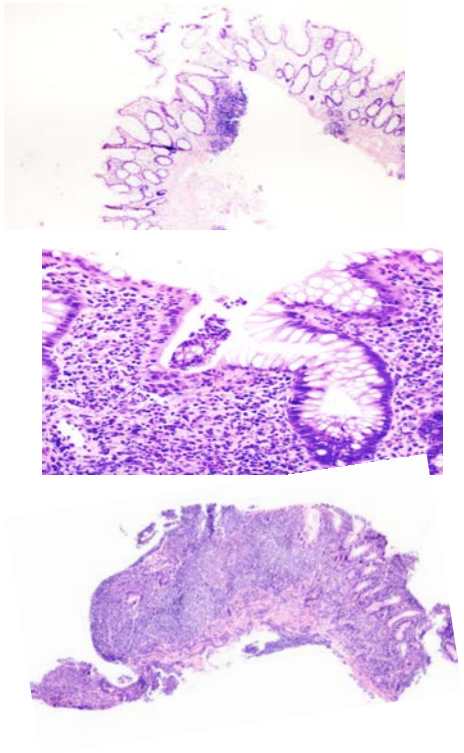


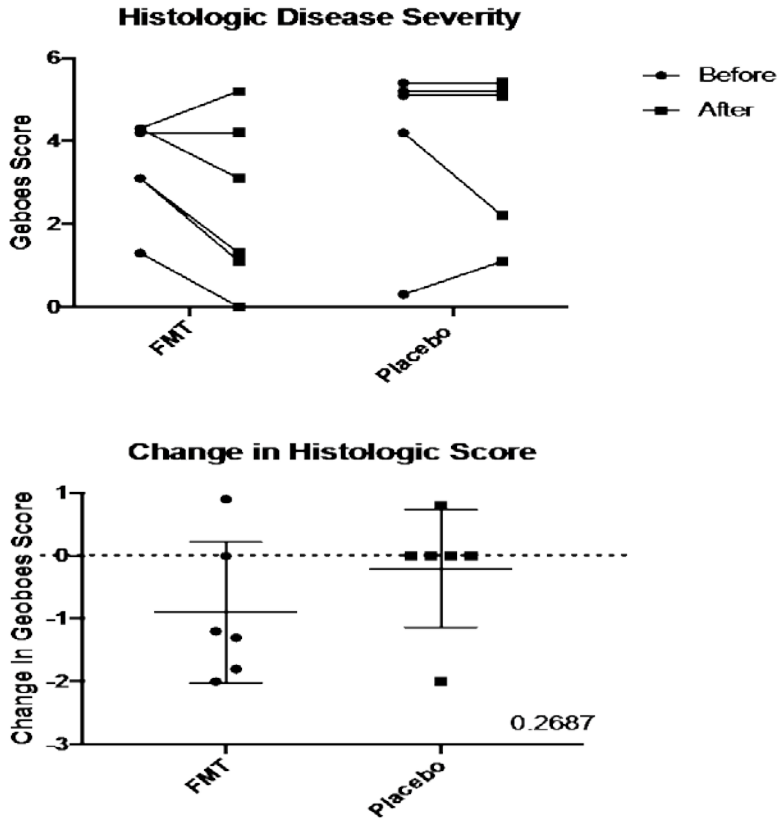
C-Reactive Protein:
Nonspecific, acute phase reactant
Method: Immunoturbidimetric Assay
Ref Range: <10mg/L

Fecal Calprotectin:
Heterodiner of S100A8 and S100A9. Member of the calcium-binding protein family. Primarily expressed by neutrophils
Method: ELISA
Ref Range:
< or =50.0 mcg/g (Normal)
50.1-120.0 mcg/g (Borderline)
or =120.1 mcg/g (Abnormal)

Fecal Lactoferrin:
Fe+ binding protein.
Antibacterial.
Secreted by neutrophils
Method: ELISA
Ref Range: negative

FMT patients trend toward decreasing histologic evidence of inflammation





Global Assessment:
The Super Responders and Non Responders

Global Response:

3/6 (50%) vs 2/6 (33%)

								Clinical		Endoscopic				Histologic		Inflammaotry		
	Study Number	Age	Sex	Extent of Disease	Duration of Disease (yrs)	BMI	Primary Donor	Total Mayo B	Change in Total Mayo	UCEIS B	Change in UCEIS	Mayo B	Change in Mayo	Geboes B	Change in Geoboes Score	Fecal Lactoferrin	Fecal Calprotectin	Escalation of Therapy
FMT	7	46	F	pan-colitis	5.5	20.9	A	1	-7	4	-2	1	0	0.1	-3	PNNN	285=>0	
	8	35	F	pan-colitis	7.5	27.8	B	1	-3	5	-1	2	0	0	-1.3	PPPP	336=>147	
	14	20	M	pan-colitis	3.8	25	A	4	-1	4	-1	1	0	1.1	-2	PPPP	385=>221	*Mesalamine 4.8 mg at wk 26 11/15/17*
	11	65	F	L-Sided	26.2	20.9	B	8	3	7	0	2	1	3.1	-1.2	PPPN	?=>375	Prednisone 40 mg at 13 wks
	1	44	M	pan-colitis	0.2	25.6	A	8	1	7	-3	2	0	5.2	0.9	PPPP	>1000=>>1000	Prednisone 10 mg at 14 weeks
	10	38	M	pan-colitis	10.2	25.2	A	6	-3	10	2	2	-1	4.2	0	PPNN	119=>72	Prednisone 40 mg at 6 wks
	3	22	F	pan-colitis	6.9	27.7	B	SCREEN FAIL										
Placebo	5	68	M	pan-colitis	4.4	28.8		4	-2	5	-1	2	0	1.1	0.8	PNPP	196=>64.8	
	13	58	M	L-Sided	27.8	26.9		6	1	8	0	2	0	2.2	-2	PPPP	129.6=>133.3	
	4	65	M	L-Sided	0.4	36.15		8	0	8	0	2	0	5.1	0	PPPP	286=>360	Adalimumab 40mg 13 wks
	6	47	F	pan-colitis	8.8	29.2		7	-1	10	0	3	0	5.4	0	PPPP	873=>846	Mercaptopurine 50 mg QD at wk 12
	9	31	F	pan-colitis	0.8	29.1		6	0	7	-1	2	0	5.2	0	PPPP	579=>442	Budesonide 9 mg QD at 13 wks
	12	40	F	pan-colitis	16.3	25		DROPPED OUT DUE TO WORSENING DISEASE ACTIVITY								PP	383=>	Prednisone 40 mg at 2 wks
	2	27	F	pan-colitis	5.2	23.8		SCREEN FAIL										
	15	57	M	pan-colitis	11.9	32.9		SCREEN FAIL										

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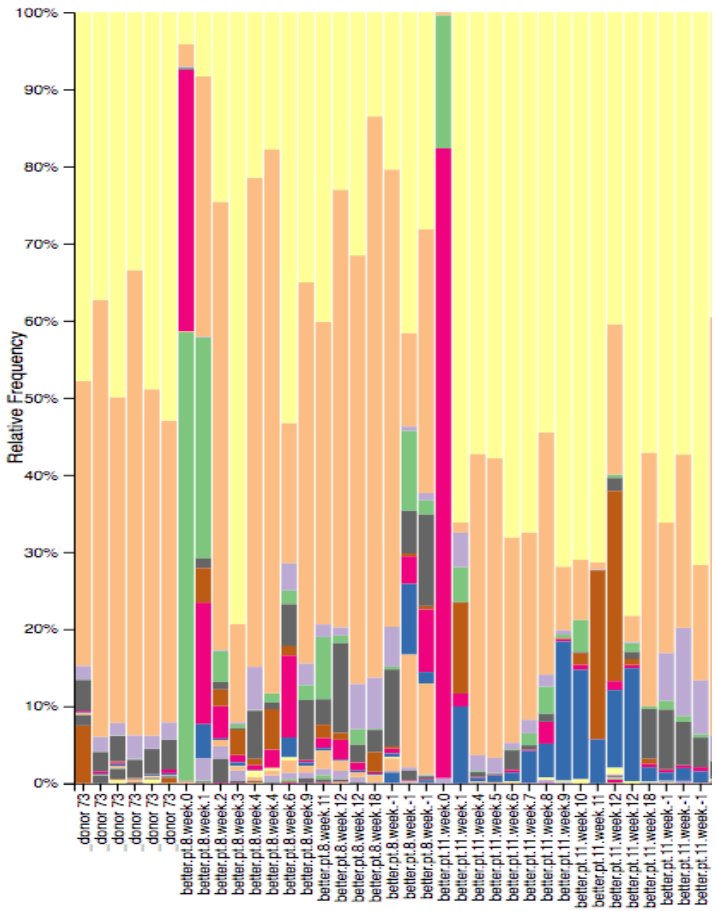
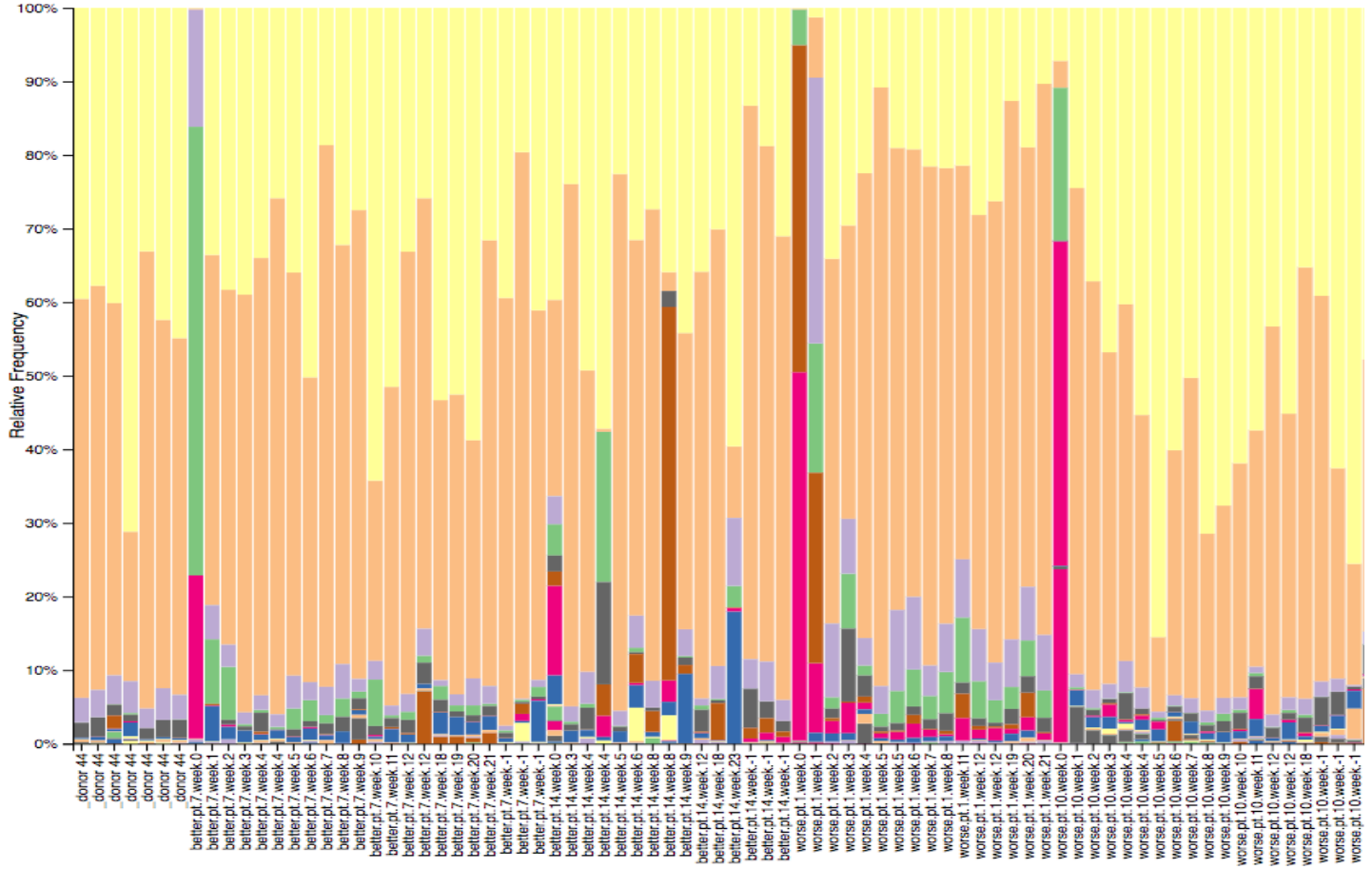
Changes in the Gut microbiome of FMT patients





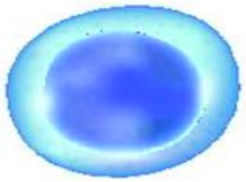
Immunologic Investigation of FMT: Mechanism of Action Patient Stratification

CD45

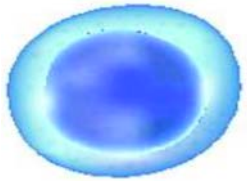
CD4

CD8

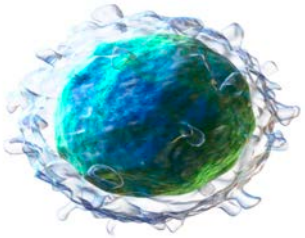
CD25
(T-reg)



MR1 (MAIT tetramer)



TCRγδ



CD14/CD13
(monos/ macs)

IFNγ
Th1
Inflammatory

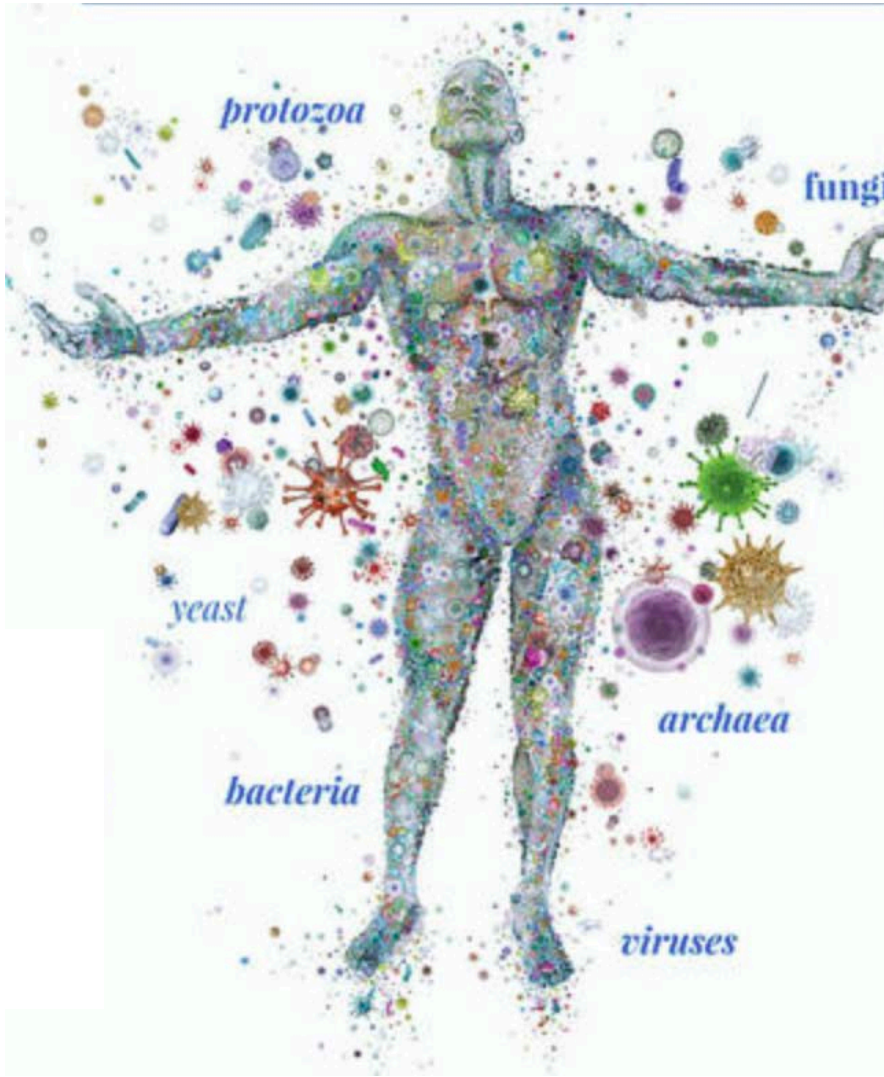
IL-17
Th17
Neutrophil recruitment

IL-10
T-reg
anti-inflammatory
reparative

While we work out the Science…

Eat and live like your ancestors (when appropriate)

Honor your ancient relationship with your microbes



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