# *BREASTMILK AND THE DEVELOPMENT OF THE NEONATE GI MICROBIOME*

UO-CHC 441H/431H: Microbes + Social Equity

Lecture 4

Dr. Sue Ishaq Pellegrini

# Learning objectives

- Learn about breastmilk microbial community
- Entrainment of neonate gut community

- **Discussion:** should we rethink pre- and postnatal care?

# MILK

# Colostrum

- Produced in first few days of milk
- Has more in it that regular milk
  - *protein*
  - *antibodies*
    - In humans, IgA which is localized to the gut
  - *fat*
  - *growth hormones and factors (other substances, ex. proteins)*
    - Stimulate growth and tissue repair
  - *anti-microbial compounds*
  - *bacteria and pro-bacterial compounds*

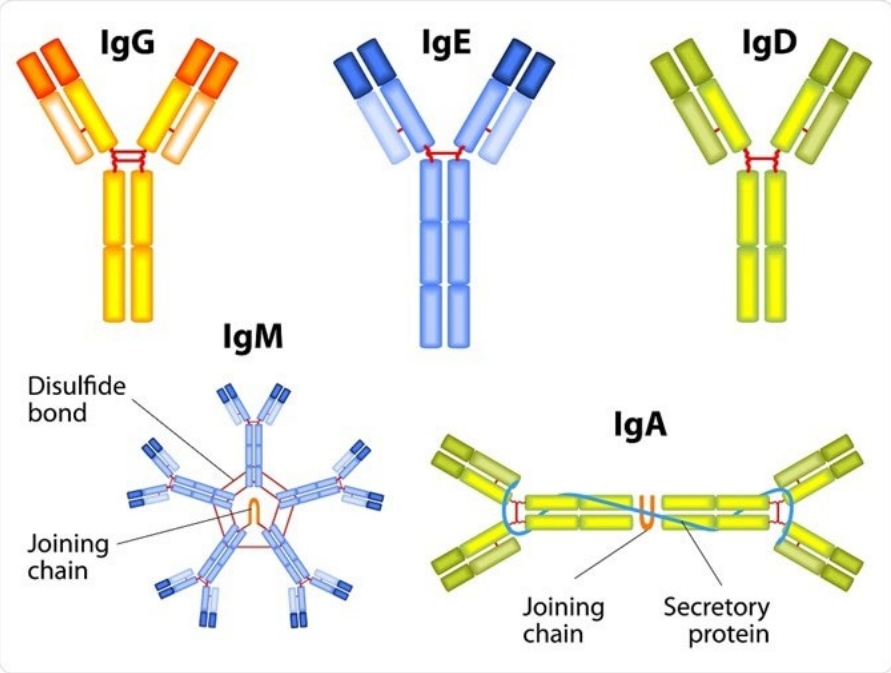
Milk Colostrum

| Bioactive Milk Component | Function |
|---|---|
| macrophages | Immune cells that engulf foreign material |
| Stem cells | repair |
| Immunoglobins A, G, M | Pathogen-binding inhibitors |
| Cytokines | Immune system factors which stimulate responses to pathogens (ex. inflammations) as well as inhibit response |
| Growth factors, chemokines, hormones | Stimulate cell growth and regulate cell metabolism |
| Oligosaccharides | Pro- and antimicrobial sugars |
| Mucin (major component of mucus) | Antimicrobial, infection prevention by physically protecting GI tract cells |

- Reviewed in Ballard_2013_human milk composition

# Immunoglobins

- Antibodies, special protein designed to bind to a target and allow it to be located by immune cells, flushed out of body
- Diff types have different targets and locations of action in the body




Antibody Types: IgM, IgA, IgD, IgG, IgE and Camelid Antibodies
News-Medical.Net

# Milk is species-specific nutrition

| Proximate | Water % | Protein % | Fat % | Ash % | Lactose % |
|---|---|---|---|---|---|
| Camel | 86-88 | 3.0-3.9 | 2.9-5.4 | 0.6-0.9 | 3.3 |
| Cow | 85-87 | 3.2-3.8 | 3.7-4.4 | 0.7-0.8 | 4.8-4.9 |
| Buffalo | 82-84 | 3.3-3.6 | 7.0-11.5 | 0.8-0.9 | 4.5-5.0 |
| Sheep | 79-82 | 5.6-6.7 | 6.9-8.6 | 0.9-0.1 | 4.3-4.8 |
| Goat | 87-88 | 2.9-3.7 | 4.0-4.5 | 0.8-0.9 | 3.6-4.2 |
| Human | 88-89 | 1.1-1.3 | 3.3-4.7 | 0.2-0.3 | 6.8-7.0 |

**Source:** (Al haj Omar *et al* 2010).

- Ash is the total of other nutrients which are unknown/not extracted (burned in a calorimeter)

# Milk nutritional components are somewhat adaptive to needs of offspring

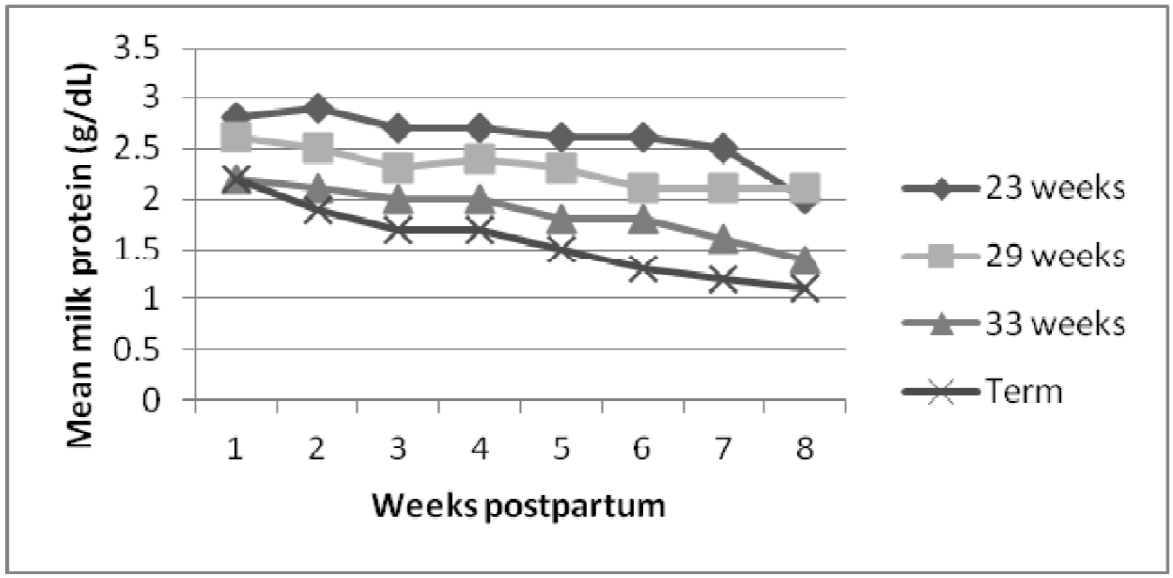



**Figure 1.**
Milk protein concentrations, comparing milk from mothers who delivered preterm and term, by gestational age at delivery and weeks postpartum (adapted from Bauer & Gerss, 2011).

# Milk prebiotics: human milk oligosaccharides (HMOs)

- Oligosaccharides are really large sugars
  - *often used as cell receptors (like TV antennae) because they are sticky*
- HMOs are a specific class that
  - *selectively feed beneficial microbes (probiotic)*
  - *Bind to pathogens which would otherwise bind to oligosaccharides on infant intestinal cells*
    - Including microbes that cause diarrhea
    - HIV (somewhat)
  - *NOT DIGESTED BY THE INFANT*
- Maternally- produced HMOs are universal
  - *Why breastmilk can be given to any infant*
  - *But genetic differences in mother creates different mix of HMOs*

# VERTICAL TRANSMISSION VIA MILK

i.e. milk probiotics

# Vertical transmission in animals

- **Vertical transmission – transmission (of something) from female to offspring during gestation or birth**
  - *Microorganisms (maybe infectious)*
  - *Genes (and phenotypic traits)*
  - *Mitochondrial genes (and phenotypic traits)*

Microbes transferred via

- Placental transfer
- Vaginal and fecal contact during birth
- Skin contact
- Milk

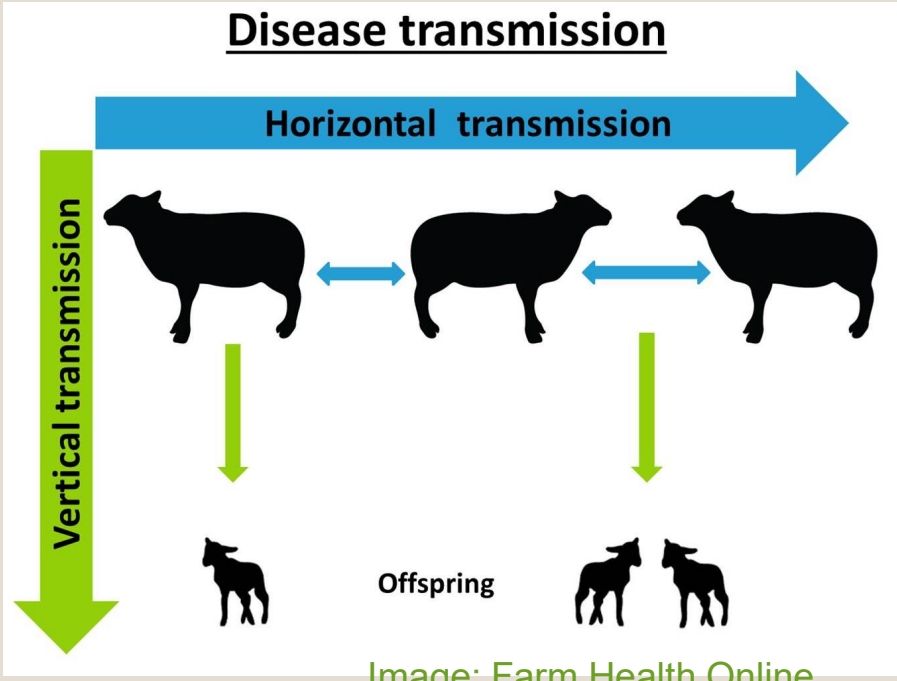
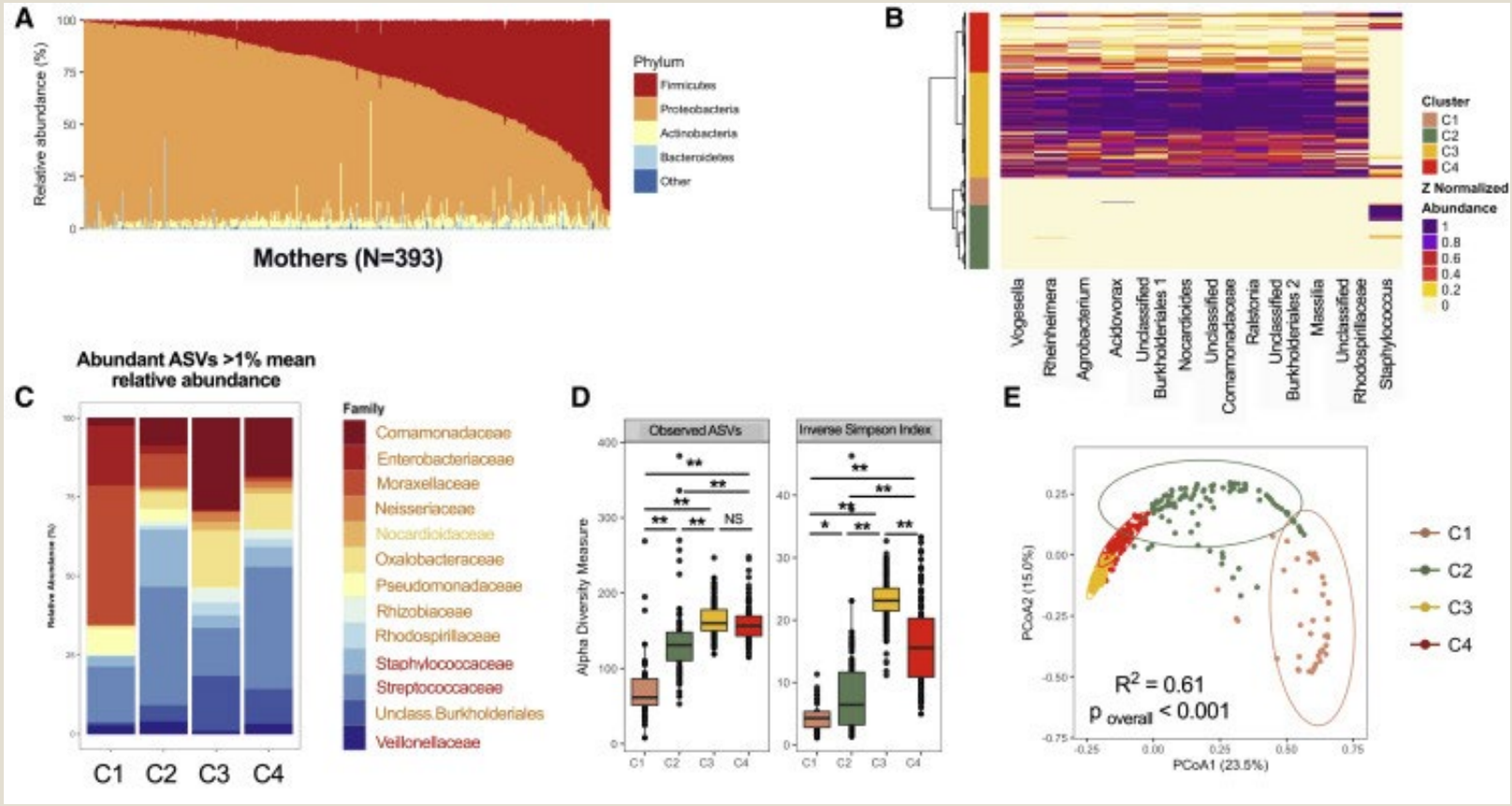



Image: Farm Health Online

# Bacterial community in breastmilk varies wildly by mother:

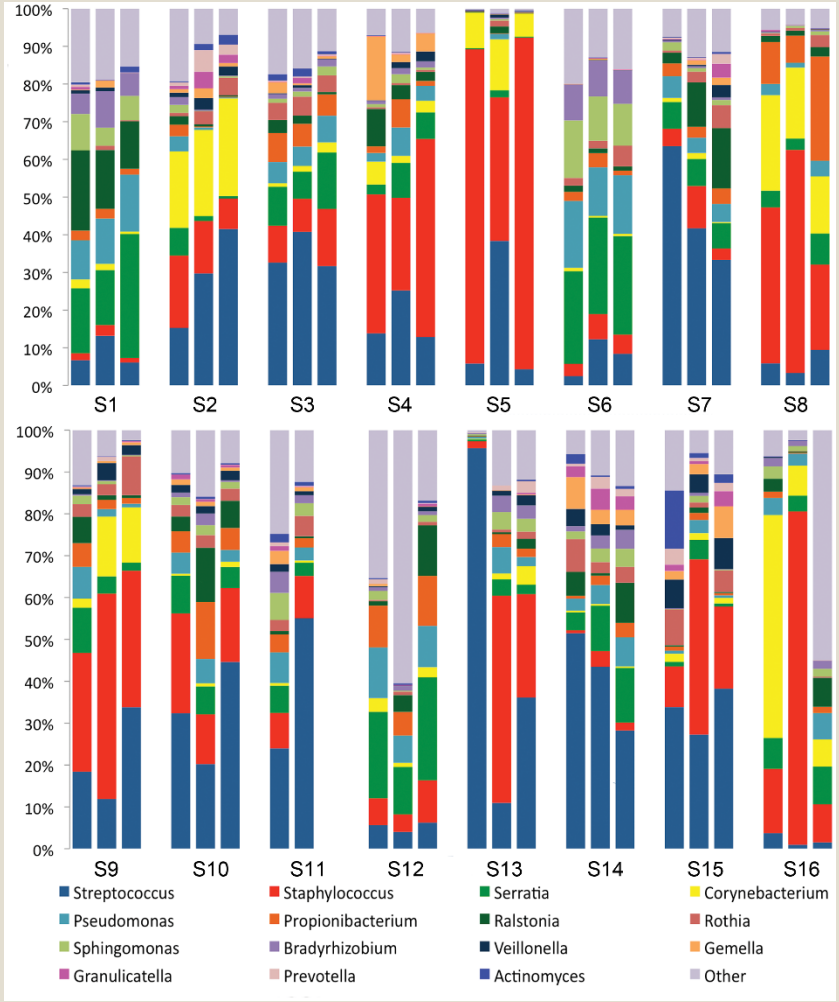
## study found 4 “types” defined by bacterial diversity




Moossavi et al. 2019

# Breast milk contains lots of bacteria

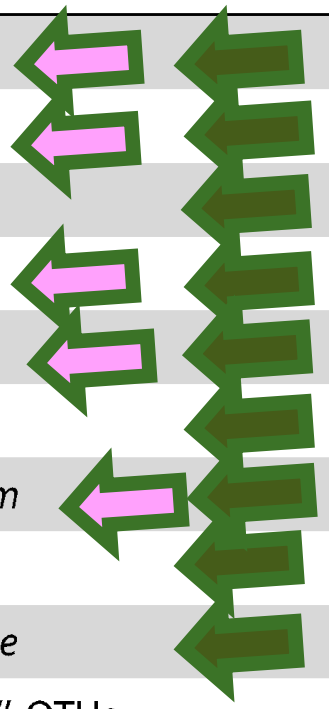
- Maternal factors determining breastmilk bacteria
  - BMI
  - parity (number of births)
  - mode of delivery
  - breastfeeding practices
  - milk components
- Within individual mothers, community stable over lactation




Hunt et al. 2011

# Bacteria in milk

| Core OTU Genera | Relative abundance of OTU in total community (%) |
|---|---|
| *Staphylococcus* | 15.8 |
| *Streptococcus* | 8.2 |
| *Serratia* | 7.6 |
| *Pseudomonas* | 4.5 |
| *Corynebacterium* | 3.8 |
| *Ralstonia* | 3.7 |
| *Propionibacterium* | 3.6 |
| *Sphingomonas* | 2.4 |
| *Bradyrhizobiaceae* | 1.9 |
| Sum of all "core" OTUs | 51.5 |



- Some bacteria in milk commonly found on skin (pink arrows)
  - *Expected would get sloughed off during milk production and feeding*
- Some bacteria in milk commonly found in environment, would likely be on skin (green arrows)

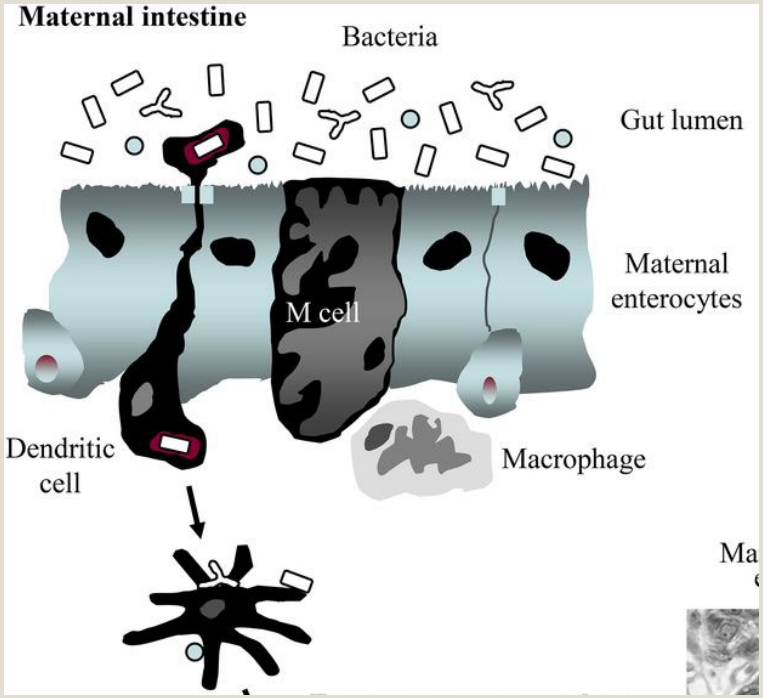
doi:10.1371/journal.pone.0021313.t001

Hunt et al. 2011

# Origin of bacteria in milk

- Maternal skin
  - *Mother's community*
  - *Transfers from infant mouth*
- Microbes in the mammary gland
- Maternal gut?

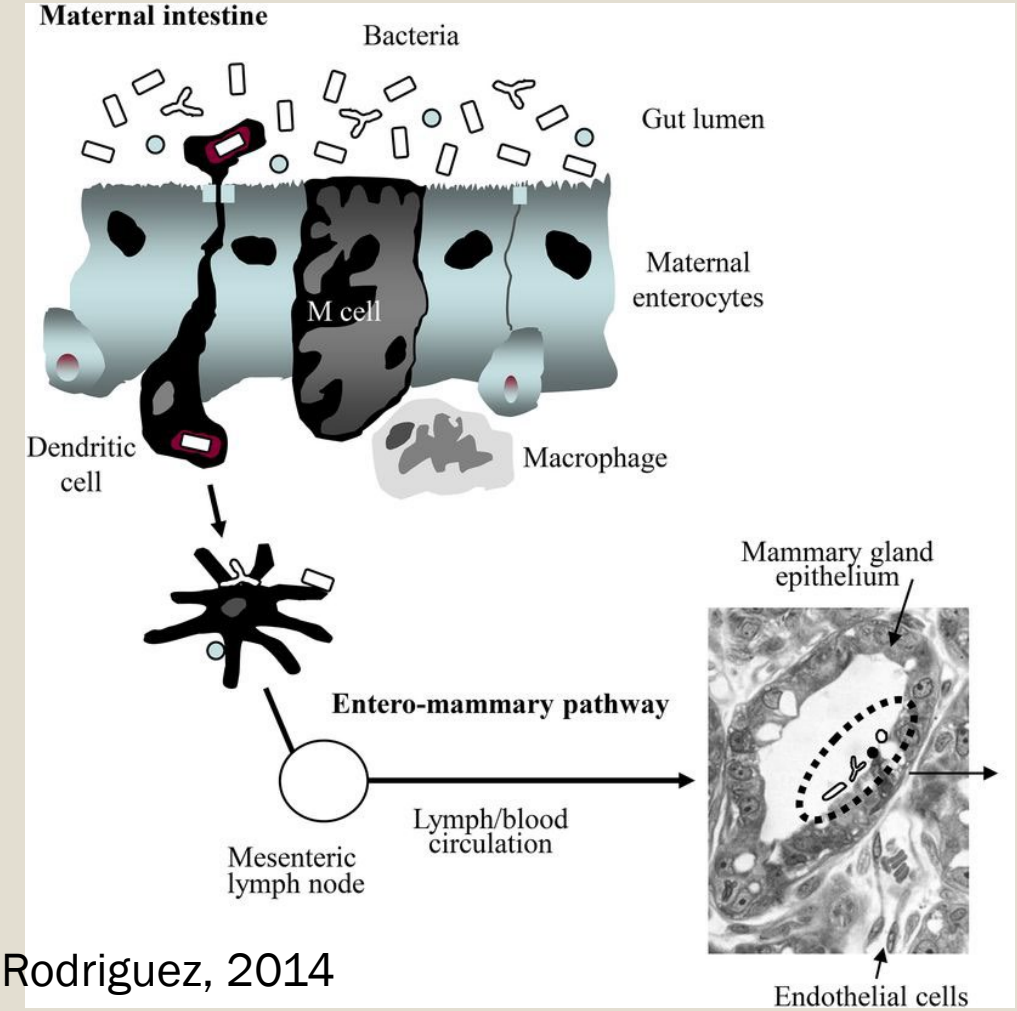
# Possible mechanism for transferring maternal gut bacteria to the mammary




1. Bacteria in gut attach to maternal dendritic cells
    1. A.k.a. antigen presenting cells, dendritic cells digest foreign cells and present pieces (antigens) to T cells to make antibodies against
2. Instead of chopping up gut bacteria, dendritic cells take them across intestinal wall into lymph network

Rodriguez, 2014

# Possible mechanism for transferring maternal gut bacteria to the mammary

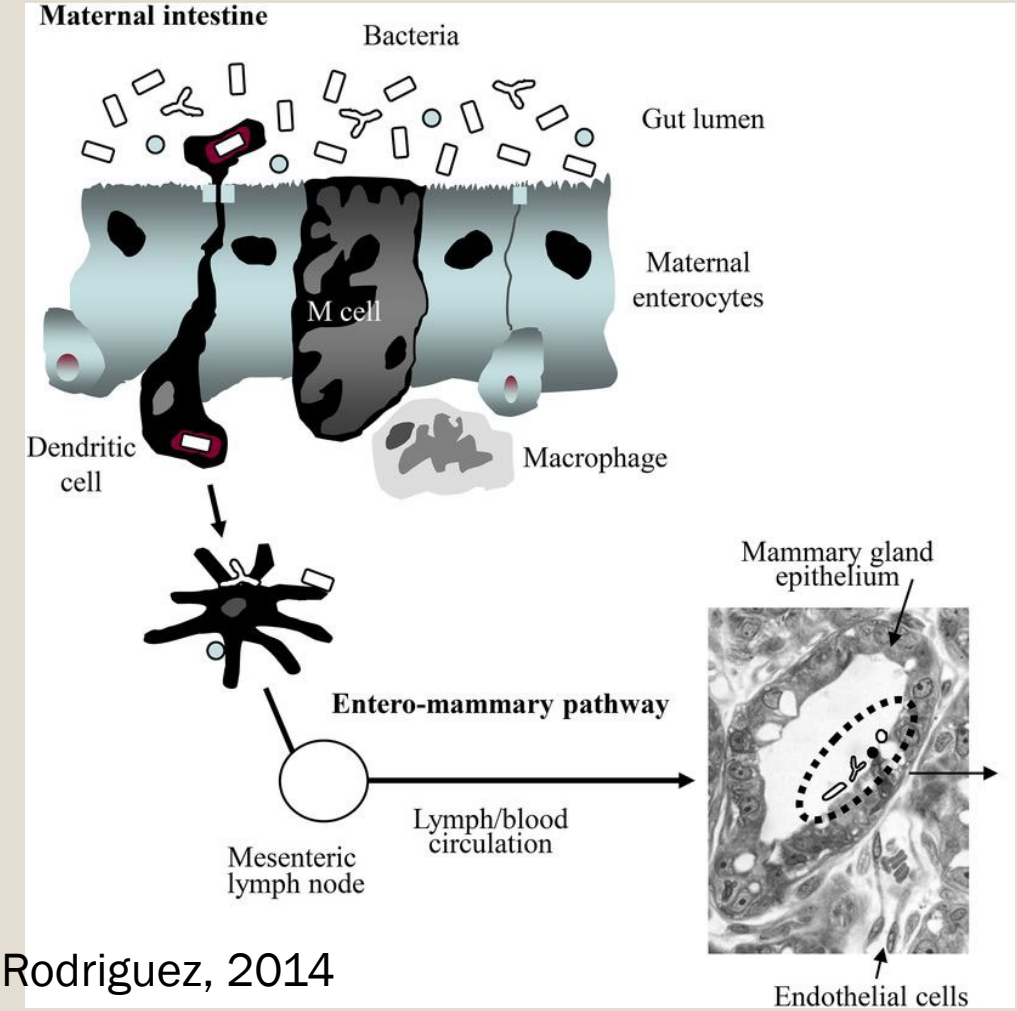



Rodriguez, 2014

Lymph circulation is for immune cell transport

- Connects lymph nodes
- Not through blood vessels but outlets to blood vessels
- Can take large molecules (like whole fats or protein) from gut and put them into blood circulation
  - One reason for heart disease, large fats from the gut enter blood stream right before go through the heart

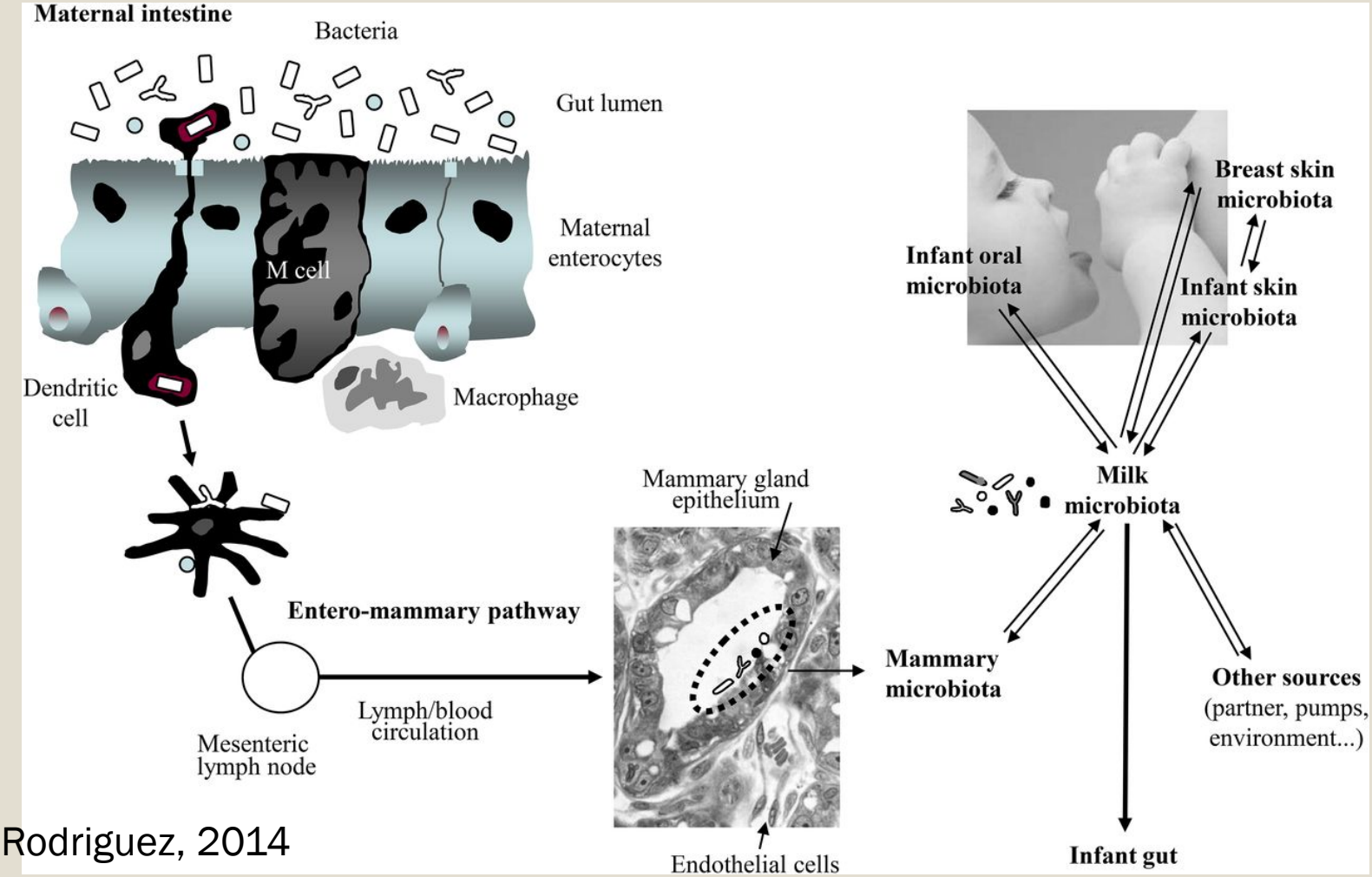
# Possible mechanism for transferring maternal gut bacteria to the mammary




Rodriguez, 2014

3. Lymph circulation may be used to transfer bacteria from gut to the mammary gland

# Possible mechanism for transferring maternal gut bacteria to the mammary

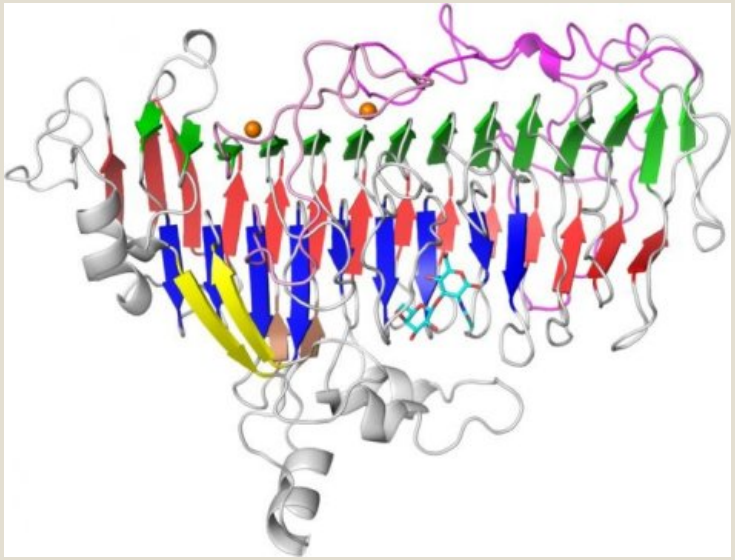



Rodriguez, 2014

# VERTICAL TRANSMISSION BENEFITS

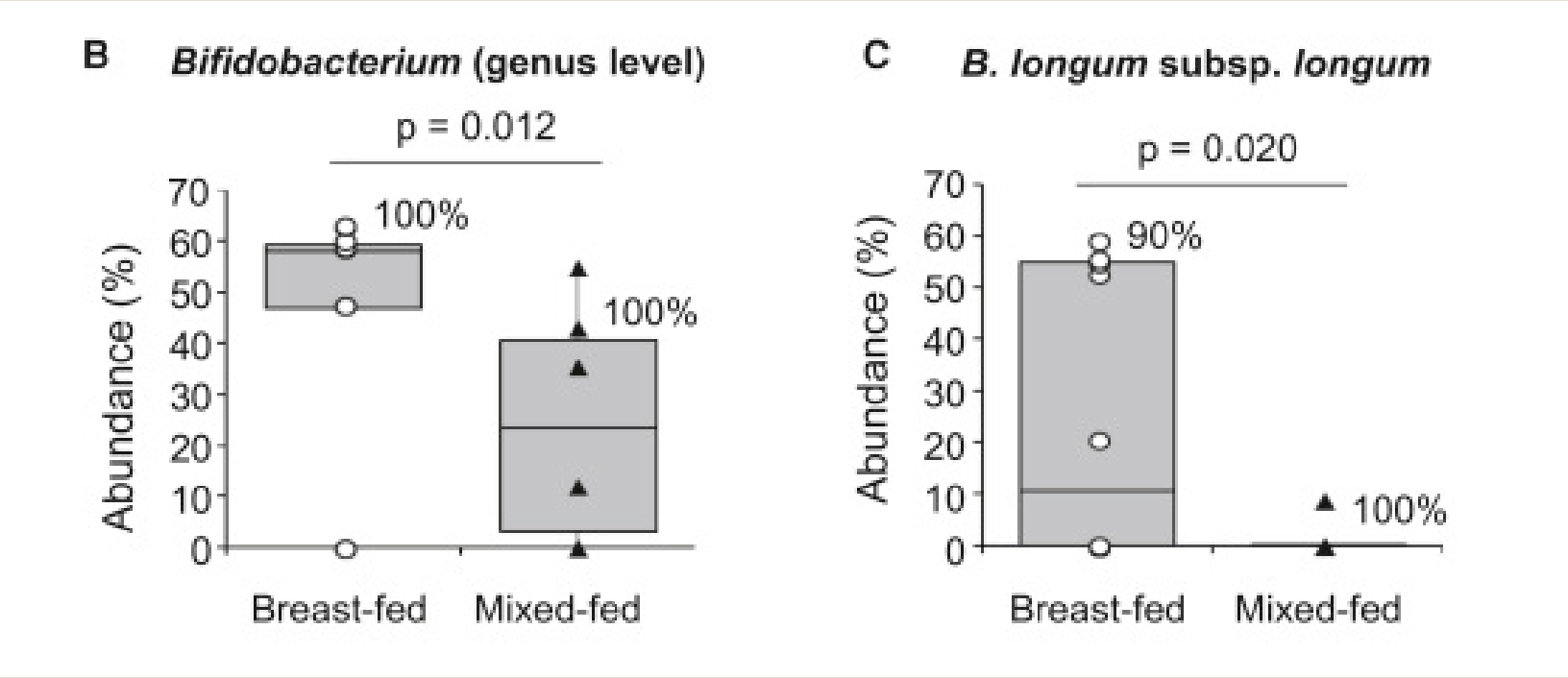
# Provide bacteria which aid in digestion of milk

- *Bifidobacterium longum*
  - *Commonly found in human infant feces*
  - ***Produces an enzyme to break down sugar found ONLY in HUMAN breast milk***
    - lacto-*N*-biose I structural unit (Gal-β1,3-GlcNAc; LNB or type-1 chain)
  - *Yamada et al. 2017*



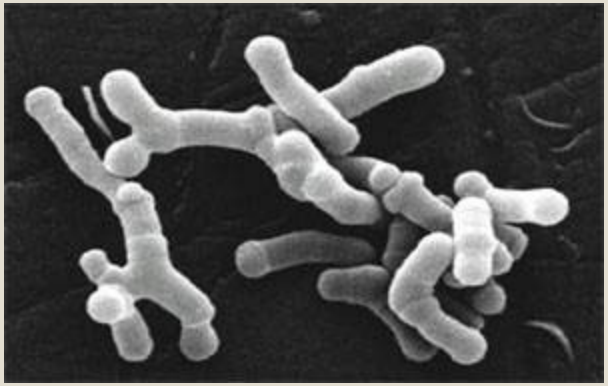
Molecular structure of lacto-N-biosidase LnbX, an enzyme from *B. longum. Image:* Yamada et al. 2017

# *Bifidobacterium longum* only in gut of breastfed infants, not from formula (mixed-fed)



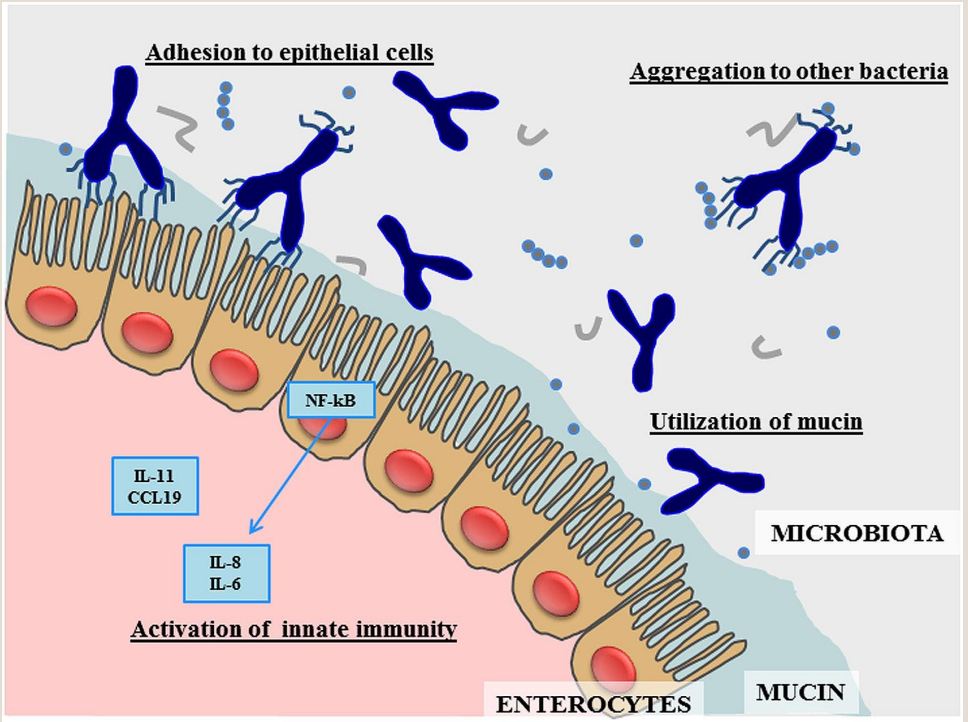

Yamada et al. 2017

# *Bifidobacterium longum*, a long-term symbiont



Bifidobacterium longum - microbewiki

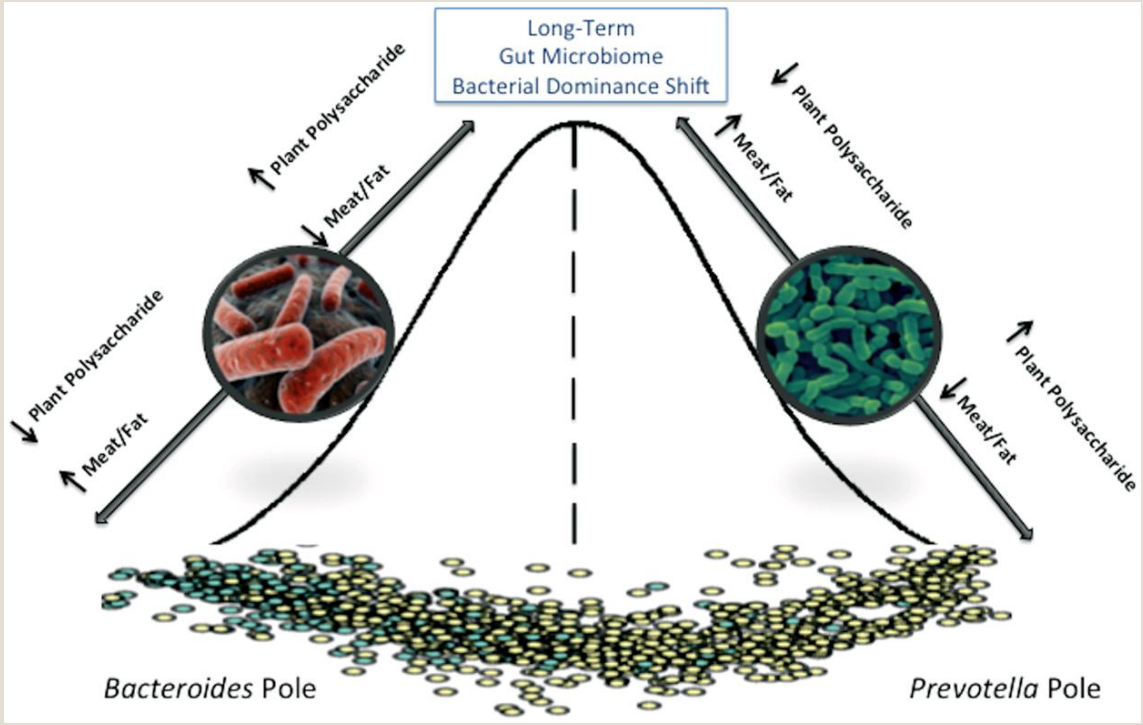
- Found in GI of infants, disappears in most adults
  - *Really well adapted to living in our gut*
- Ferments sugar into lactic acid
  - *Reduces the pH of GI tract*
  - *Makes it competitive against other microbes*
- Has a lot of genes related to building cell structures to allow it to attach to surfaces (like your gut)
  - *Helps it stay in colon*
  - *If attached to your gut cells, creates physical barrier for other cells that want to attach*
- Attaches to other bacteria and can help them be washed out of intestines
- Activates your innate immune system




Turroni et al. 2014

# Provide bacteria which aid in digestion long-term

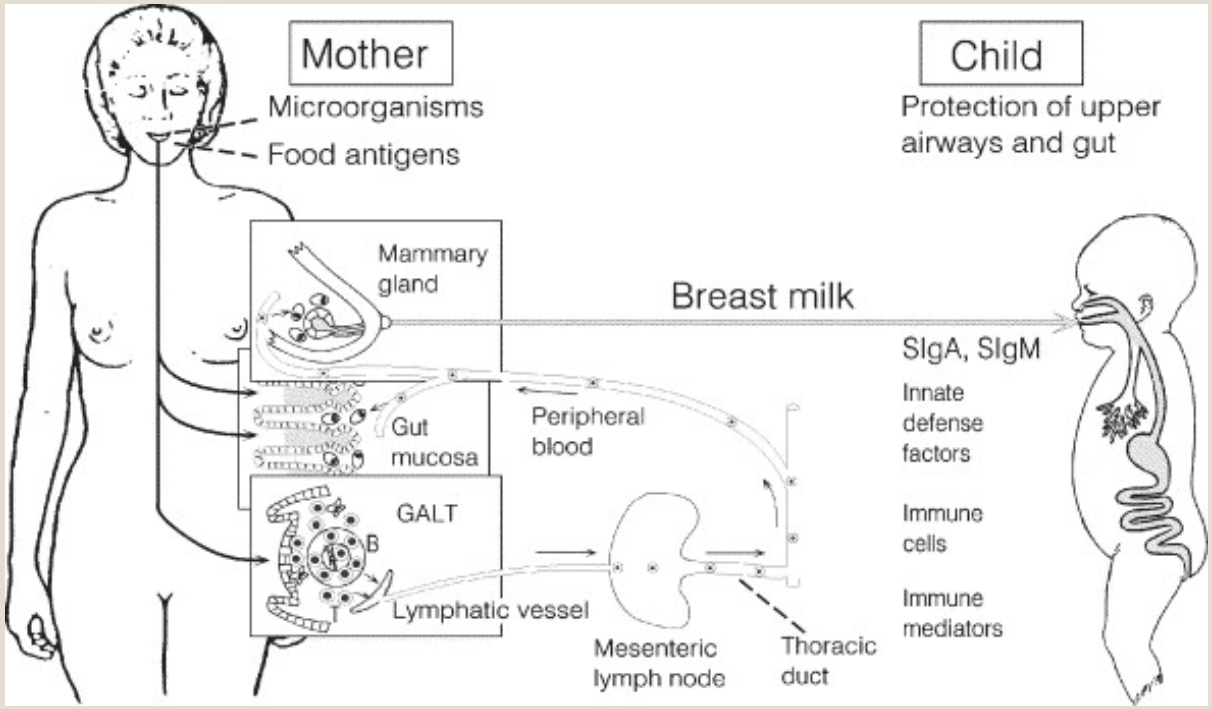
- *Ex. Prevotella* or *Bacteroidetes*
  - *Different species and strains break down a variety of carbohydrates (esp. fiber) and proteins*




Voreades et al. 2014

# Breastmilk contains immune supporting factors

- Ig = Immunoglobin antibody
- IgA = present in mucus, binds to things that infiltrate mucus barrier and call over immune cells to destroy the invader
  - Woof and Kerr 2004
- IgG = present in lymph, first responder to antigens




Brandtzaeg 2003

# Breastmilk contains immunosuppressive and anti-inflammatory agents

- Ex. Interleukin-10 and TGF-beta
  - *Immune factor which turns immune system down*
  - *Reduces inflammation*

- **Given at same time, helps turn down infant immune system and allow breastmilk microbes to colonize gut**

- Brandtzaeg 2003

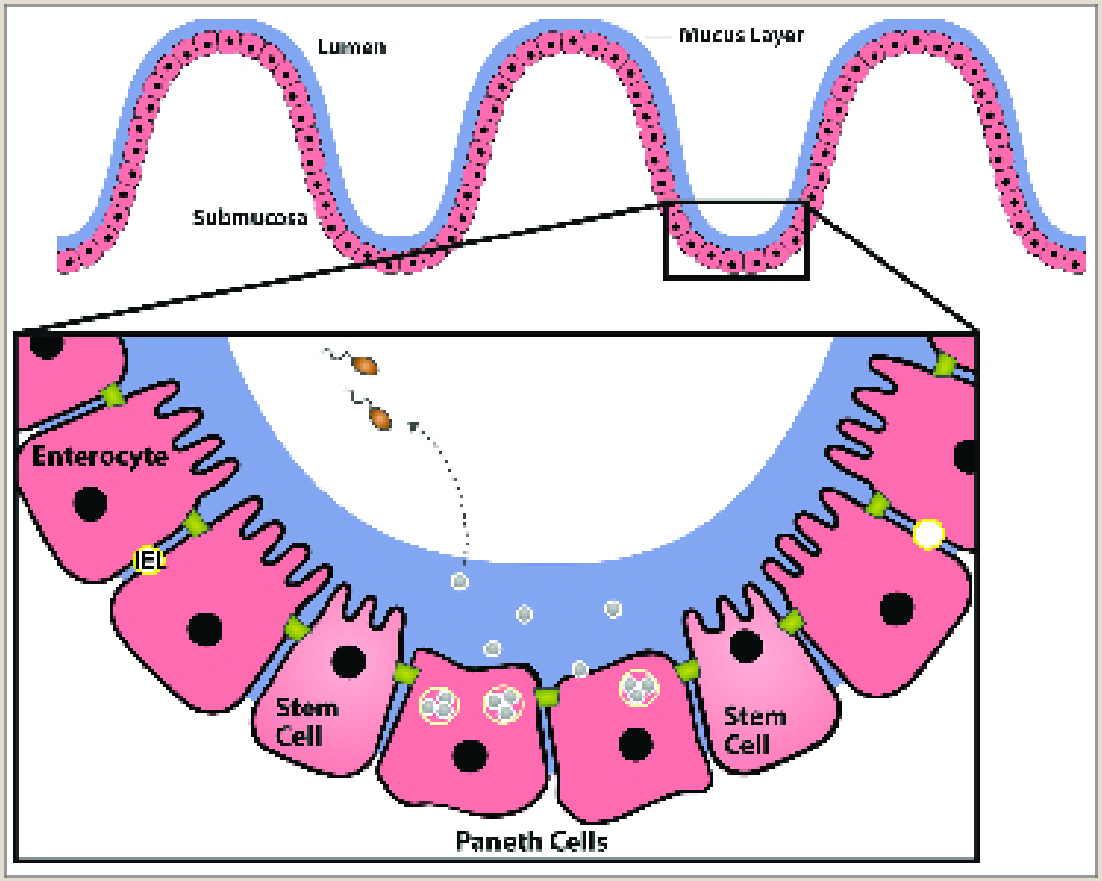
# The immune system must learn tolerance of microbes

- New immune systems only have maternal antibodies to work with
  - *No record of infectious or toxic things to watch out for*
- Learn friend from foe
  - *Don't want to kill off the helpful ones*
- Learn how to mount an immune response without going overboard
  - *Fever can disrupt your own enzymes, proteins, DNA*
  - *Inflammation can cause physical damage to your cells*

# THE HOST IMMUNE SYSTEM INTERACTS WITH (GUT) BACTERIA

# Why is the gut so important for host-microbe interactions?

- In the intestines, a single layer of host epithelial cells separate lumen (contents of intestines) from host tissues
- Layer includes
  - *Epithelial cells (called enterocytes in gut)*
    - Goblet cells
  - *Paneth cells*
  - *Stem cells*
  - *Intestinal epithelial lymphocytes (IEL)*




Halpern and Denning, 2015

# Host-microbe interactions in gut

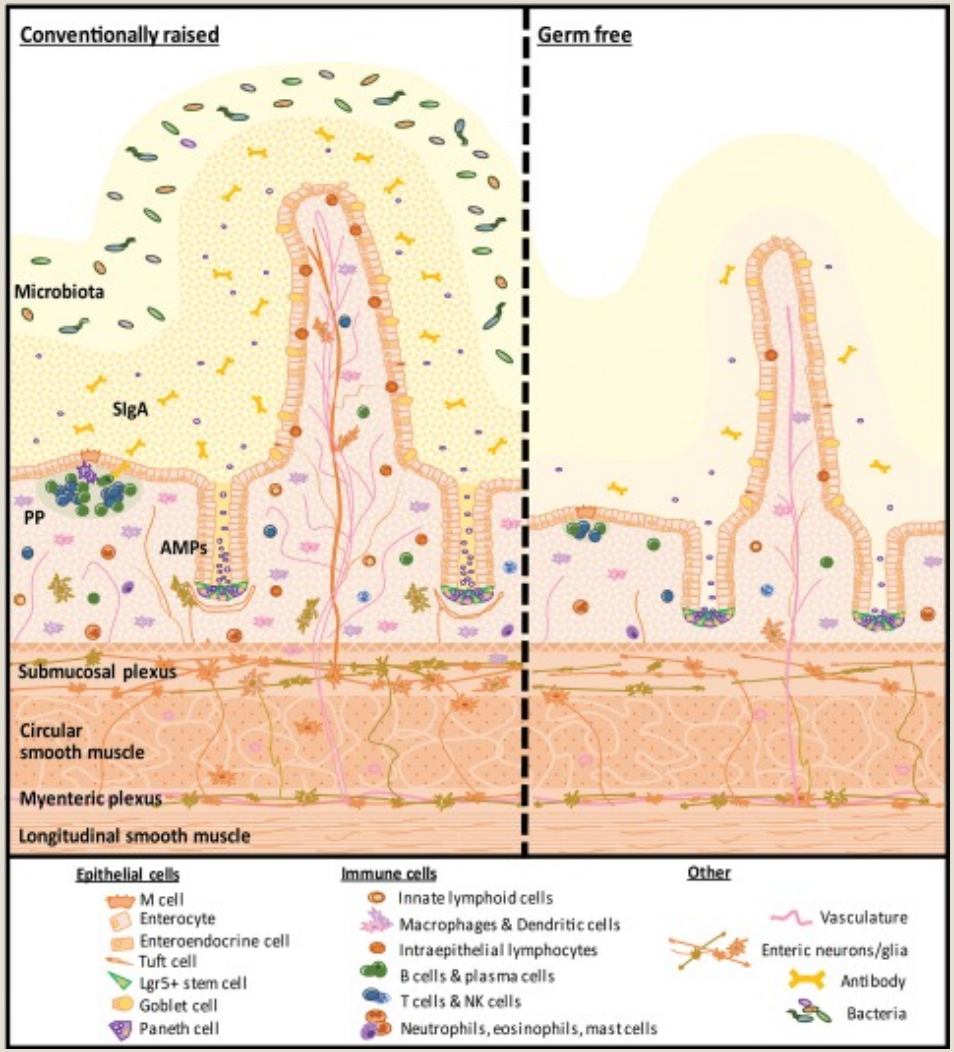
- Microbial colonization in the gut promotes…
  1. *Innate immune system such as more mucus*
  2. *Acquired immune system such as more immune cell monitoring of gut*
  3. *Host epithelial cell renewal to keep GI tract lining fresh*
  4. *Host epithelial cell barrier integrity to prevent microbe infiltration into body*
  5. *Host mucosal vascularization: more blood vessels which improves nutrient absorption*
  6. *Host mucosal innervation: more nerves to improve intestinal movement*

Parker et al. 2018

# 1. Microbial colonization in the gut promotes mucus production

Host goblet cells lining the intestine produce mucus

- Physical barrier between microbes and host cells
- Contains antimicrobial molecules
- Routinely sloughed by intestinal movement so physically removes microbes
- Mucus is a source of nutrition for some



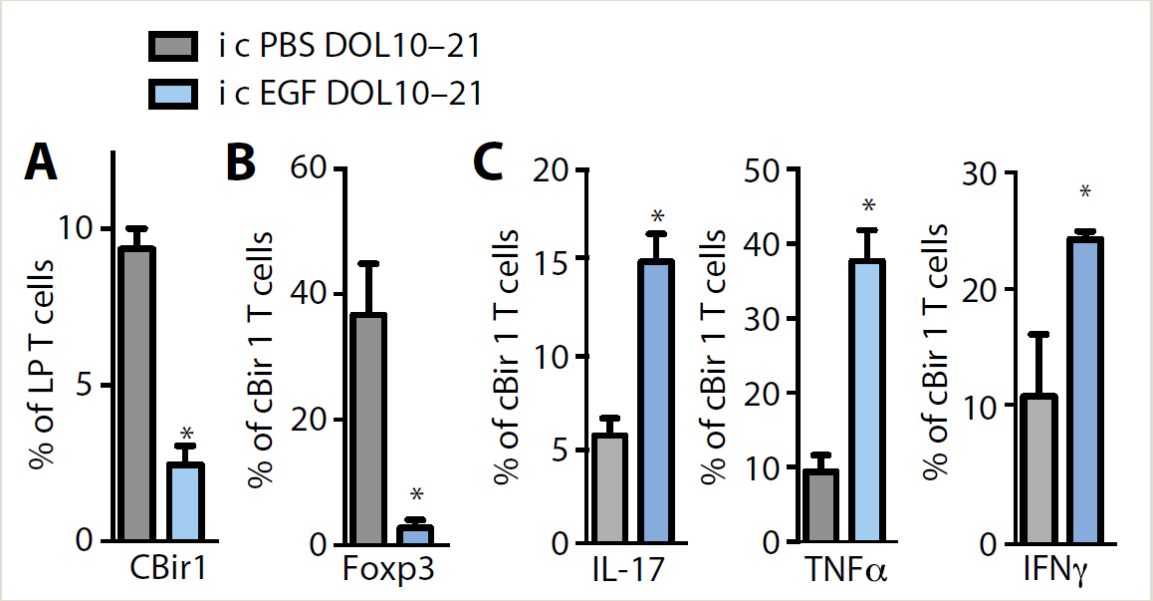

Parker et al. 2018

# 2. Microbial colonization in the gut promotes (active) immune system monitoring when exposure in first few weeks of life

- If bacteria (and antigens) **weren't encountered by mouse pups before weaning …**
  - *Prevented the development of a type of immune T cell (pTregs)*
  - *pTregs stops the immune system from overreacting to self or foreign cells*
  - *Without pTregs, mice developed colitis in response gut commensals later in life*

Grey: Normal mice

Blue: mice that don't have pTregs and now have overreacting immune system




Knoop et al. 2017

# 3. Microbial colonization in the gut promotes host epithelial cell renewal

- Ileum section of small intestine of **germ-free mice**
  - *Underdeveloped villi*
  - *Fewer host epithelial cells*
  - *Slower host epithelial cell growth*

- Ileum section of small intestine of **conventional mice**

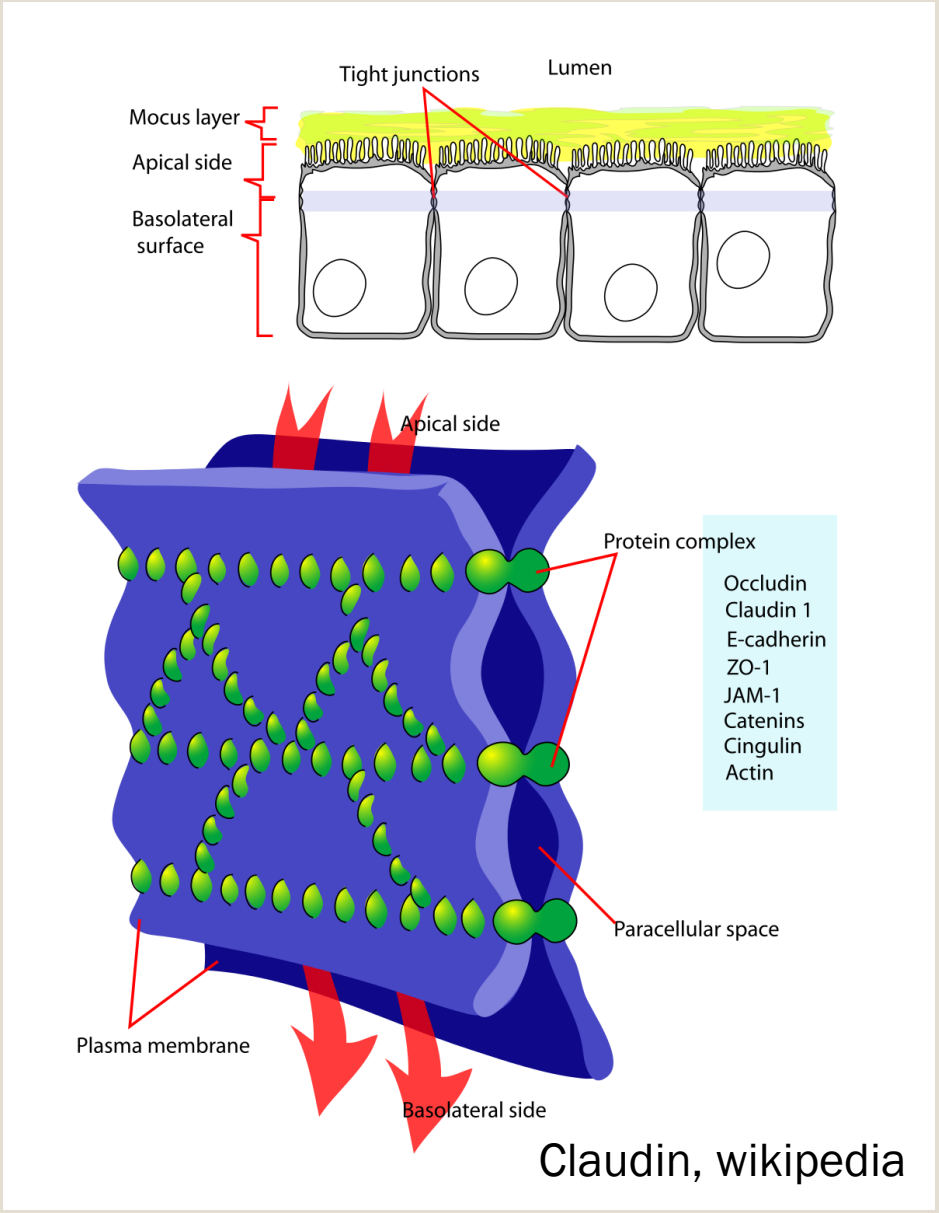
- Abrams et al. 1963


FIG. 1. Ileum of germ-free mouse. The lamina propria is poorly developed, with relatively few cells in its interstices. The crypts are shallow. Compare with Figure 2. Hematoxylin and eosin. ×111

FIG. 2. Ileum of conventional mouse. The lamina propria is well developed and contains numerous lymphocytes, reticuloendothelial cells, and plasma cells. The crypts are deeper than those of the germ-free mouse, and division figures are more numerous. Hematoxylin and eosin. ×111

# 4. Microbial colonization in the gut promotes host epithelial cell barrier integrity to prevent microbe infiltration into body

- Occludin is a protein that knits cells together
- Prevents bacteria from sneaking between intestinal cells

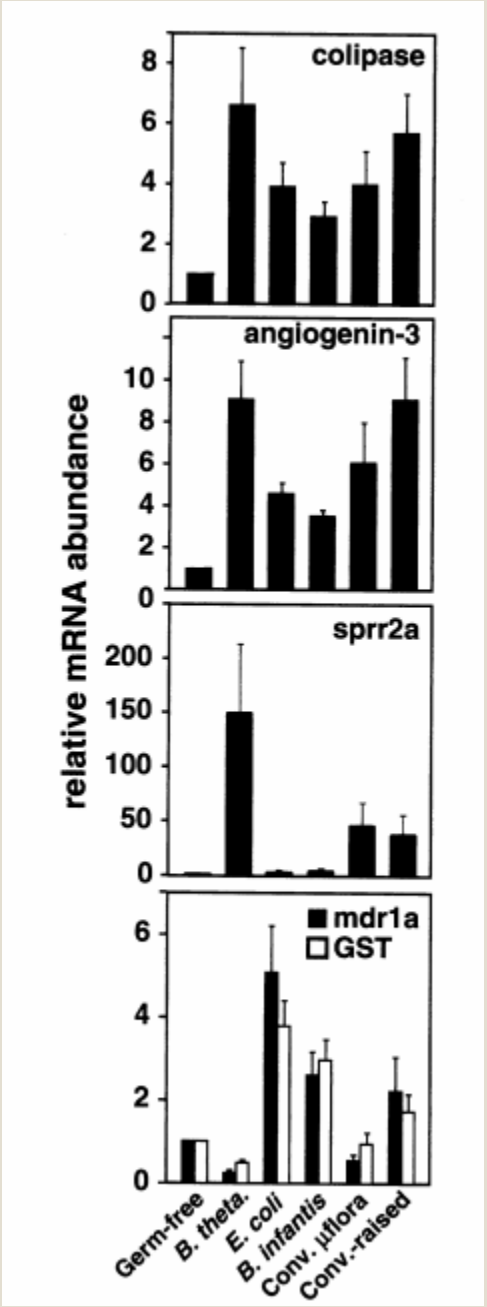



Claudin, wikipedia

# 4. Microbial colonization in the gut promotes host epithelial cell barrier integrity to prevent microbe infiltration into body

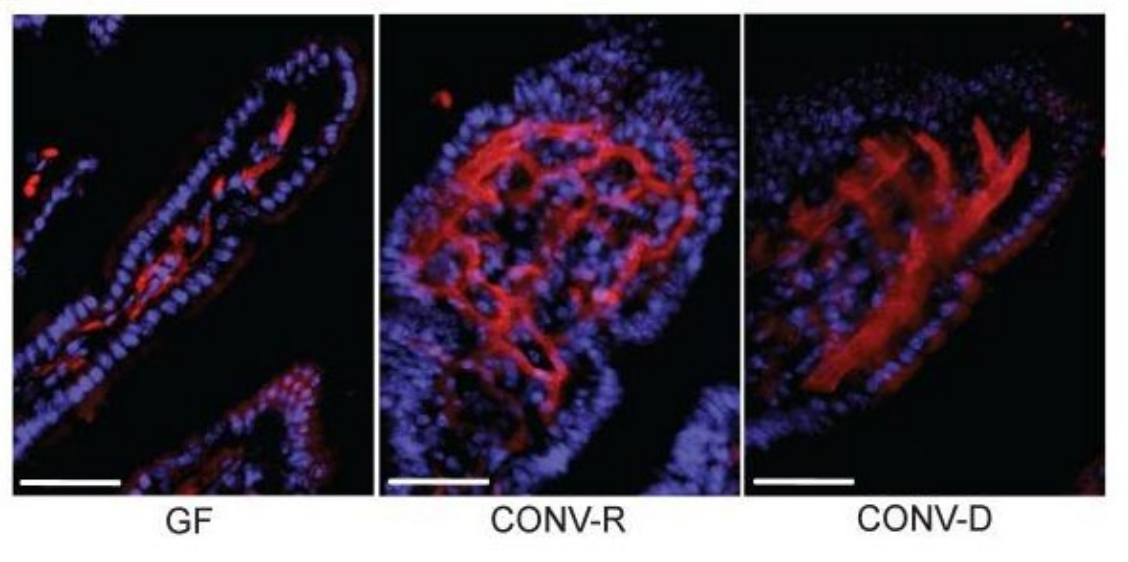
- The presence of microbes in the gut stimulates host cells to produce proteins involved in cell junction

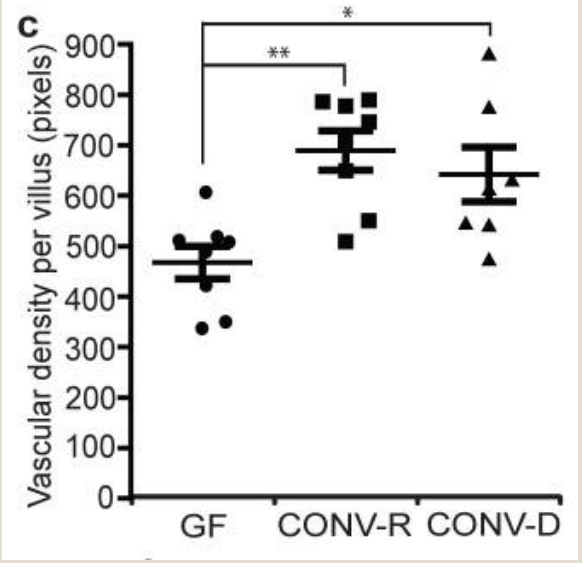
- Hooper et al. 2001



# 5. Microbial colonization in the gut promotes host mucosal vascularization (blood vessels)

- Conventional mice (CONV-R) and Conventionalized (CONV-D) mice had more blood vessels in intestinal villi than Germ-Free (GF) mice
  - *Larger villi*
  - *Improves nutrient absorption*



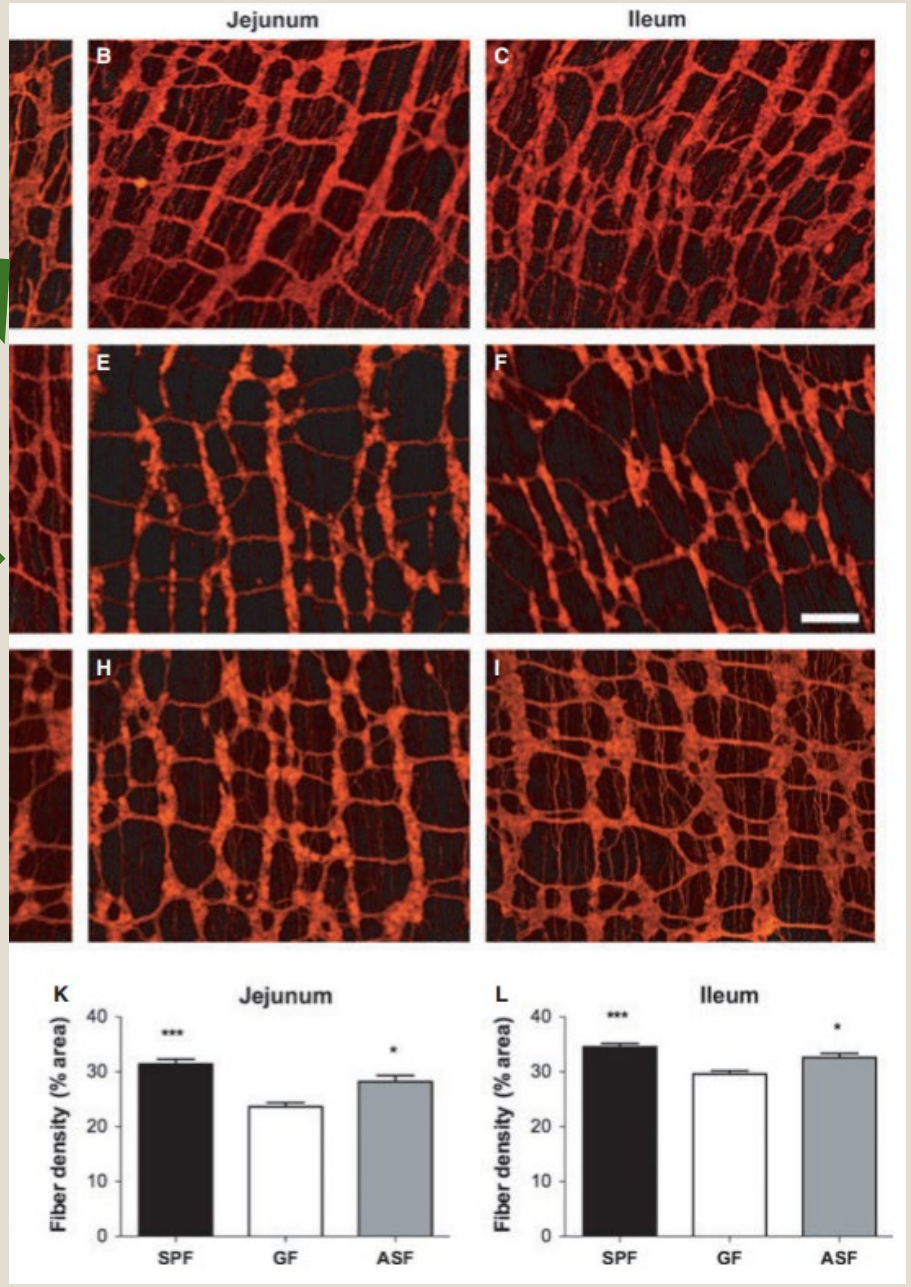

Reinhardt et al. 2012

# 6. Microbial colonization in the gut promotes host mucosal innervation (more nerves)

SPF: Specific Pathogen-Free mice

GF: Germ-Free mice

ASF: Altered Schaedler Flora mice (added community)

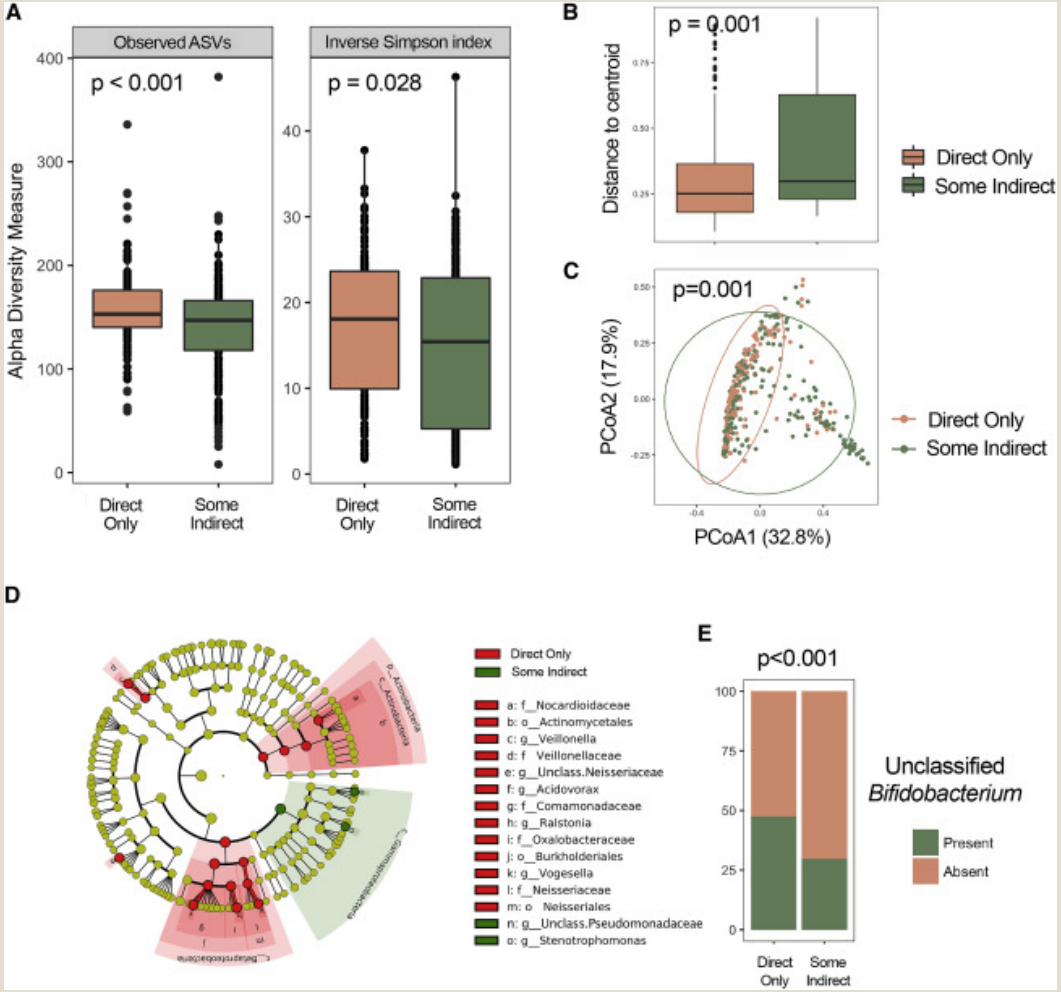



- More nerves improves intestinal movement and health
- Collins et al. 2014

# VERTICAL TRANSMISSION DRAWBACKS

# Fresh is best: effect of pumping

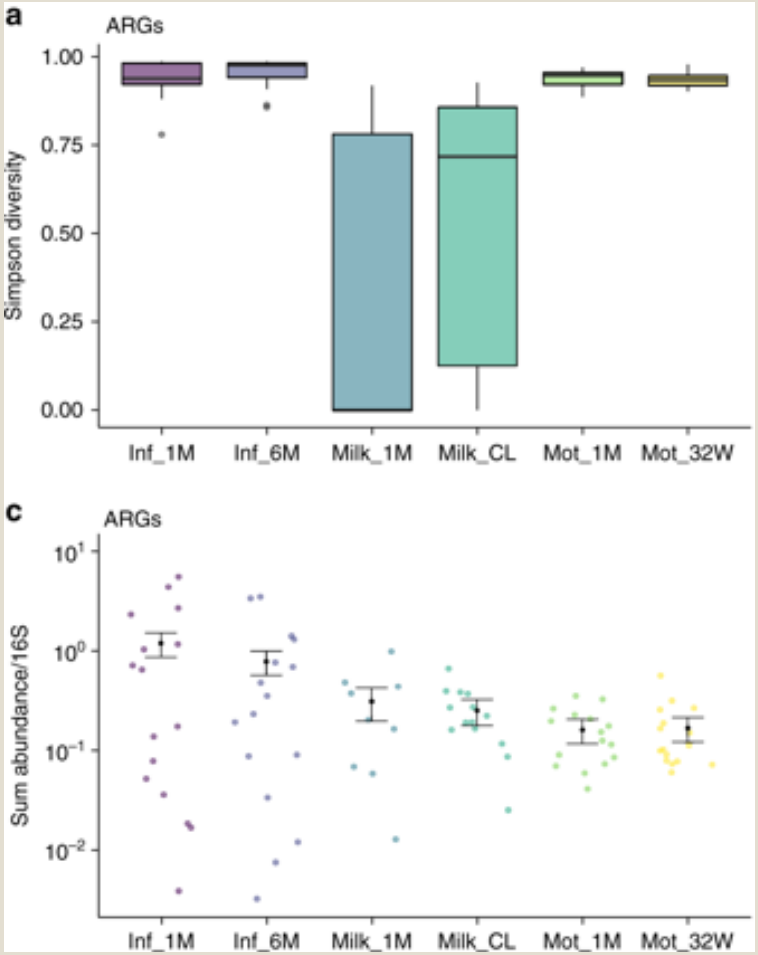
- Breastfeeding (direct) provided different community in milk than pumping (indirect)
  - *More bacterial diversity in milk (A and D)*
  - *More consistency in what that bacterial diversity was (B and C)*
  - *More bifidobacterial (E)*




Moossavi et al. 2019

# Antimicrobial resistance may accrue in infant gut over generations

- AMR genes and mobile element genes found in newborns that hadn't gotten antibiotics (Pärnänen et al. 2018)
  - *Mobile element (MGE) genes are easily traded between genomes, and provide survival benefits, like expansion packs*
  - *MGE Cropped from this graph*



# Antimicrobial resistance may accrue in infant gut over generations

- *E. coli* and other Gammaproteobacteria (subphylum) positively correlated with antimicrobial resistance load
  - *Not surprising, Proteobacteria known to have plastic genomes*
  - *Bifidobacterium negatively with antimicrobial resistance load*

| Genus | Class | Association with AMR in infant gut | P value |
|---|---|---|---|
| *Bifidobacterium* | Actinobacteria | -0.0231046 | 0.072 |
| *Lactobacillus rhamnosus* | Bacilli | -0.4554656 | 0.064 |
| *Escherichia coli* | Gammaproteobacteria | 0.0797925 | 0.0002 |

# Prolonged breastfeeding may reduce prevalence of antimicrobial resistance genes

- Breastfeeding for >6 months
  - *Reduced abundance of Gammaproteobacteria (like E. coli)*
  - *Reduced antimicrobial resistance and MGE-associated genes*
  - *Increased abundance of Bifidobacterium*

- Parnanen et al. 2018

# Breastfeeding is really good for the mother and offspring

Ip et al. 2007, Metanalysis of 9000 papers, breastfeeding was associated with:

## Infants

- a reduction in the risk of acute otitis media,
- non-specific gastroenteritis, necrotizing enterocolitis
- severe lower respiratory tract infections,
- atopic dermatitis, asthma (young children),
- obesity, type 1 and 2 diabetes,
- childhood leukemia,
- sudden infant death syndrome (SIDS),

## Mothers

- reduced risk of type 2 diabetes,
- breast, and ovarian cancer.
- Early cessation of breastfeeding or not breastfeeding was associated with an increased risk of maternal postpartum depression.

# DISCUSSION AND HOMEWORK

# Barriers to breastfeeding

- Health care practices
  - *Feeding formula, separated mother from infant, poor information, gift baskets with formula*
- Information
  - "…only 36 percent of participants thought that breastfeeding would protect the baby against diarrhea.[61]" (Surgeon General)
- Cultural/personal norms
  - *Opinions of the father drove prevalence for breastfeeding (Surgeon General)*
- Public breastfeeding shamers
  - *"…* 2001 found that only 43 percent of U.S. adults believed that women should have the right to breastfeed in public places.[98] " (Surgeon General)
- **Maternity leave or ability to bring infants to work**
- Access to quiet rooms/facilities

# Social implications

- Better breastfeeding infrastructure and policy
  - *More maternity leave*
  - *Public acceptance*
  - *Public places to feed*
    - The additional “away from home” time is important to infant mental and physical development!!

# Discussion

**Pregnancy and early life can have LIFELONG consequences for the health of the mother and offspring.**

**Do we need to rethink how we medically handle pregnancy to promote between early-life exposure to microbes?**

**Is this a public health/social equity issue??**

- *Better prenatal and early natal care?*
- *More time off and stress-reducing interventions?*
- *Maternal or neonatal probiotics? Repositories for vaginal microbiota the same way we have colostrum?*
- *Breastmilk vs. formula*
- *Recommendations for longer breastfeeding periods (1 year, 2 years)*
- *Postnatal contact in prisons?*

# Homework

- **Reading** (pick 2):
  - *Ballard_2013_human milk composition*
  - *Toscano_2017_breastmilk microbes and neonatal immune development*
  - *Parnanen_2018_breastmilk and AMR*
  - *Moossavi_2019_breastmilk_pumps*

- **Assignment:** Write a minimum 1-page, single-spaced essay on the importance of maternal microbes on offspring health and why this is a public health/social equity issue.
  - *Due 7/1.*
  - ***Include > 3 citations, and line numbers***
  - *You may choose any relevant, credible scientific article, review, or reference. You may incorporate your paragraph summary but the writing must be more sophisticated.*

# Citations


- Abrams et al. 1963 http://www.dtic.mil/dtic/tr/fulltext/u2/403101.pdf
- Ballard 2013 10.1016/j.pcl.2012.10.002
- Brandtzaeg, P. 2003 https://www.sciencedirect.com/science/article/pii/S0264410X03003384?via%3Dihub
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