Through the Microscope: A Closer Look at Human Milk

Summer Kelly, RN, MS, IBCLC

Mothers' Milk Bank of the Western Great Lakes

Disclosures

- I have no financial interest or other relationship with any manufacturer/s of any commercial products.

Objectives

1. List the three major macromolecules of human milk.

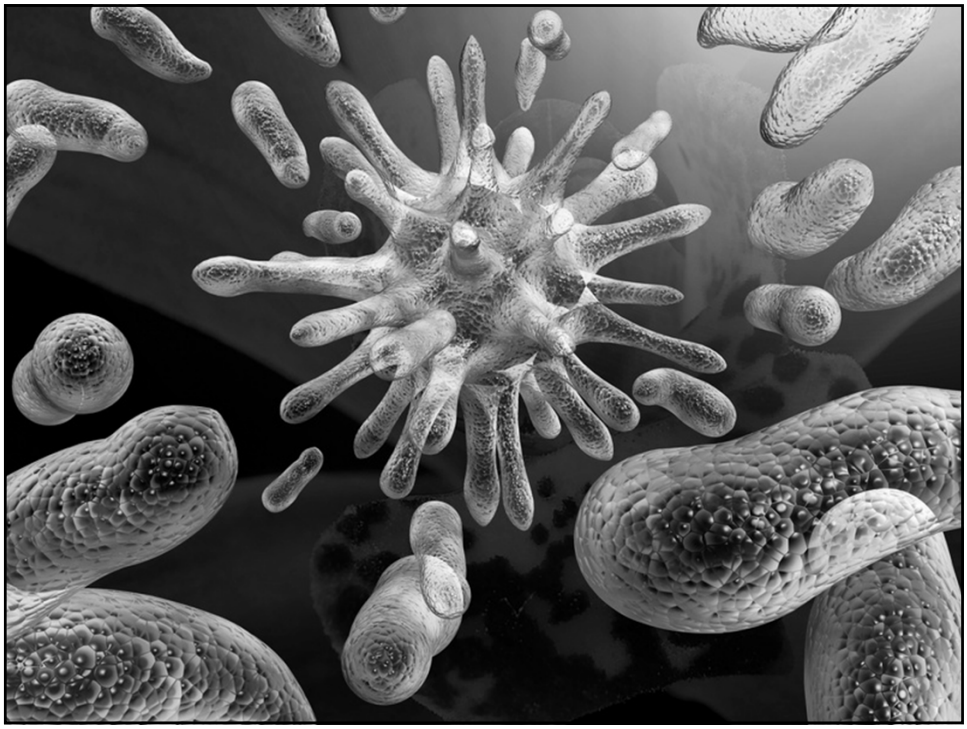
2. Identify at least two human milk immune factors that are not found in formula.

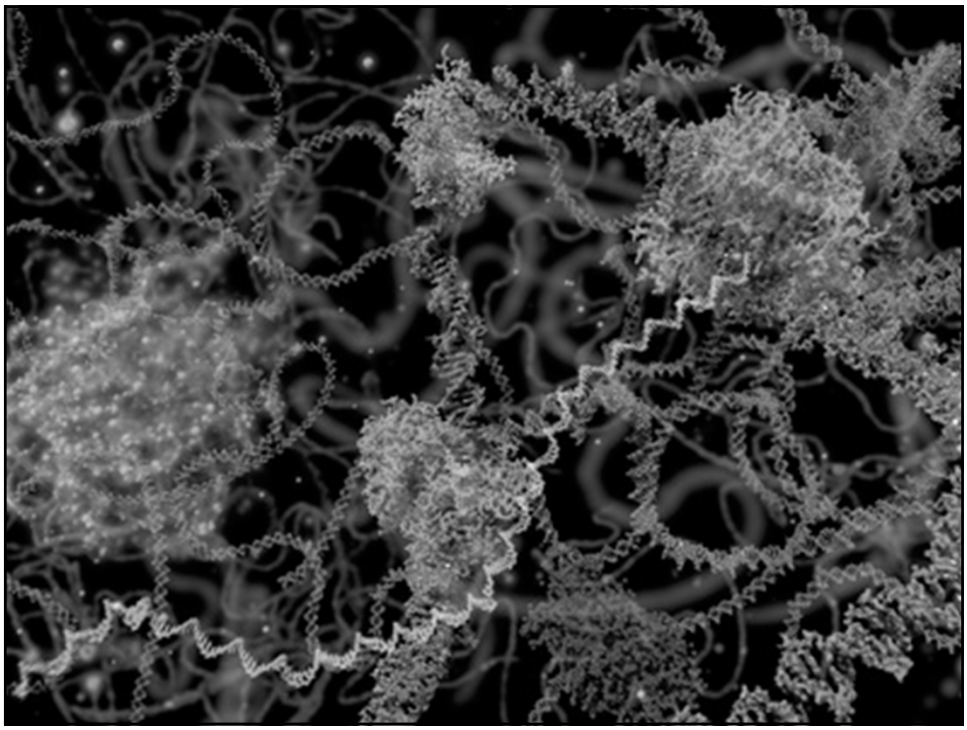
3. Define passive immunity.

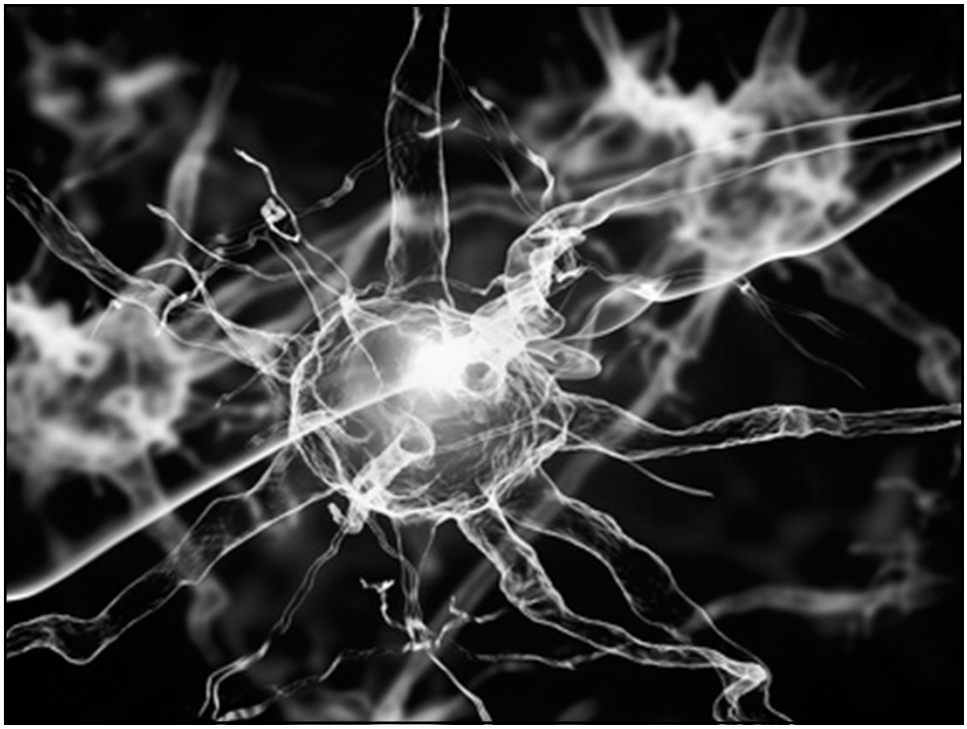
Think about human milk from an evolutionary and immunological perspective.

Topics

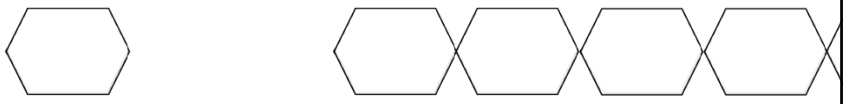
- Macromolecules of milk
- Evolution of milk
- Human milk immunology
  - Passive Immunity
  - Bacterial neutralization
- Human milk and the microbiome
- Clinical lactation implications







Monomers and Macromolecules



Glucose

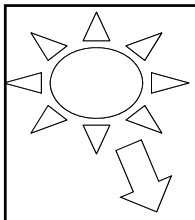
Starch

Monomer (*mono*=one)

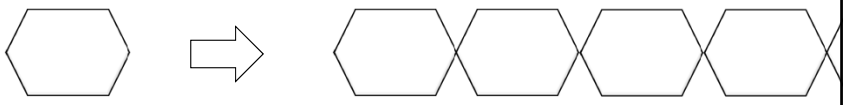
- Single unit, building block

Macromolecule (*macro* = large)

- Macronutrient, polymer



Photosynthesizers



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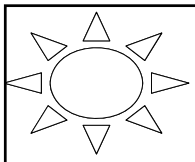
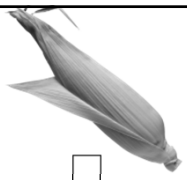
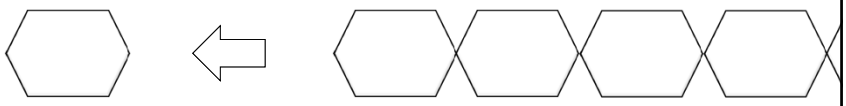


Animals





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Macromolecules = Macronutrients

Monomer	Macromolecule
Fatty Acid	Lipid
Monosaccharide	Carbohydrate
Amino Acid	Protein
Nucleic Acid	DNA and RNA

Human Milk Macromolecules

Monomer	Macromolecule
Fatty Acid	Lipid Milk Fat Globule
Monosaccharide	Carbohydrate Lactose, Human Milk Oligosaccharides
Amino Acid	Protein Lysozyme, Lactoferrin, α-Lactalbumin, Antibodies (sIgA)
Nucleic Acid	DNA and RNA miRNA

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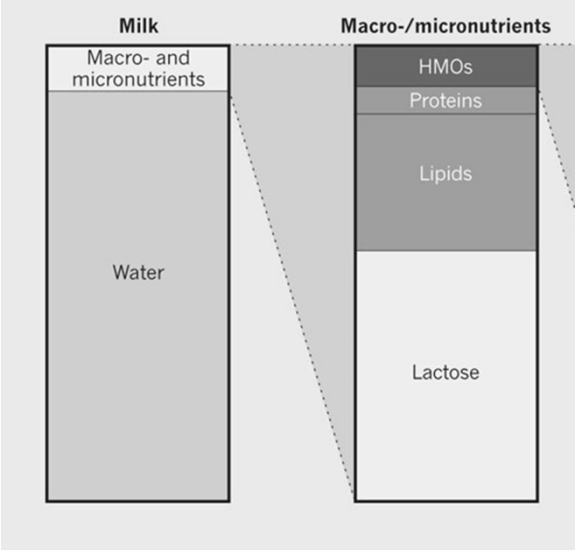
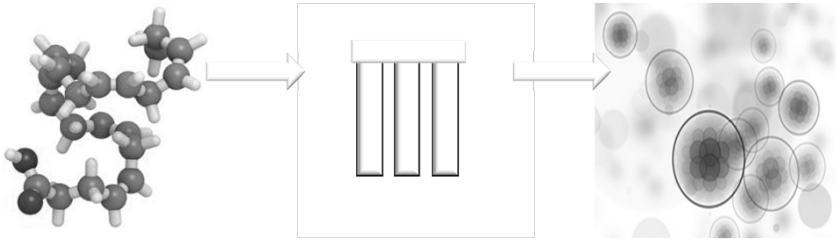
Human Milk Composition



Petherick (2010)

Lipids



Fatty Acid
DHA (Omega 3)

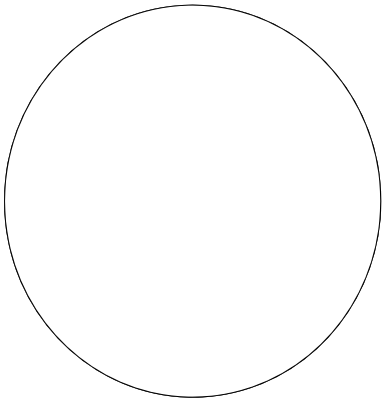
Triglyceride

Human Milk
Fat Globule

Human Milk Fat Globule

Important source of energy (50%)

- Fatty acids
- Fat soluble vitamins
- Cholesterol



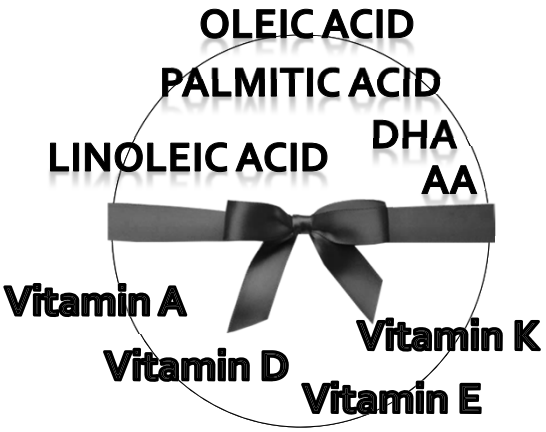
Lonnerdal (2014)

Human Milk Fat Globule

FATTY ACIDS

Fat soluble vitamins

Cholesterol



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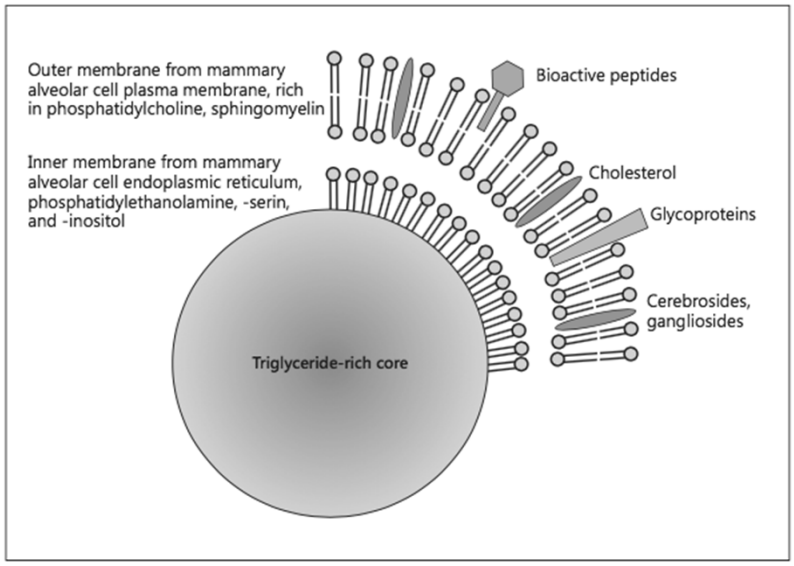
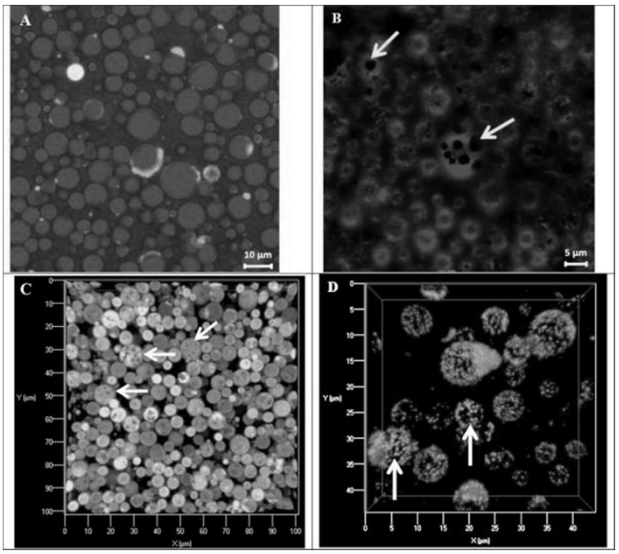


Koletzko, B. (2016)
Used with permission: S Karger AG, Basel

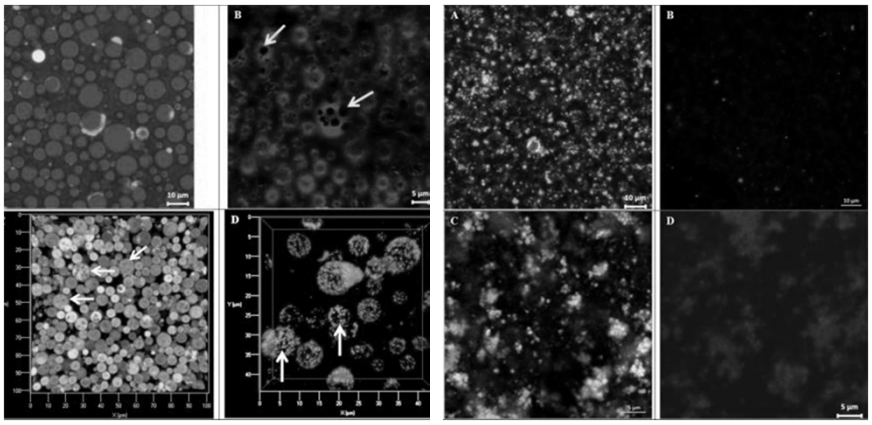
Human Milk Fat Globule: Confocal Laser Scanning Microscopy



Gallier et al. (2015)

Human Milk Vs. Formula

Human Milk **Formula**



Gallier et al. (2015)

Carbohydrates

Glucose Galactose Lactose

Lactose Fucose Human Milk Oligosaccharide

Lactose intolerance?

- Galactosemia: Rare autosomal disorder
  - Picked up by newborn screening or diagnosed clinically
- Lactose intolerance: Gene coding for lactase enzyme switches off around 4-5 years of age
  - Normal genotype turns off lactase (wild type)
  - Mutant genotype keeps lactase switched on throughout lifetime
- Cow milk protein allergy: allergic/sensitivity reaction
  - Symptoms: blood in stool, rash, colic symptoms, fussiness

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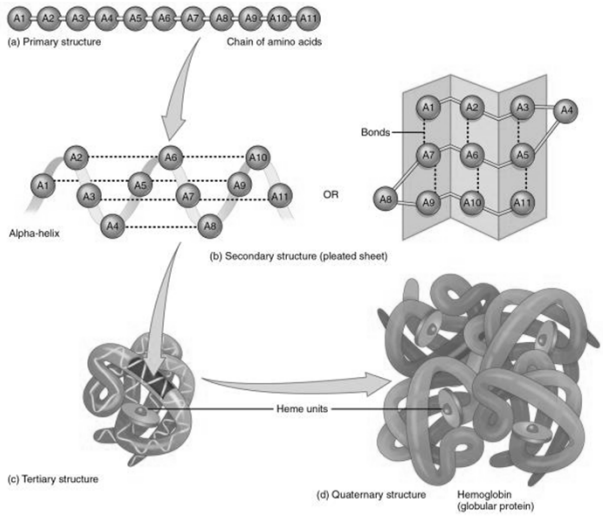
Proteins



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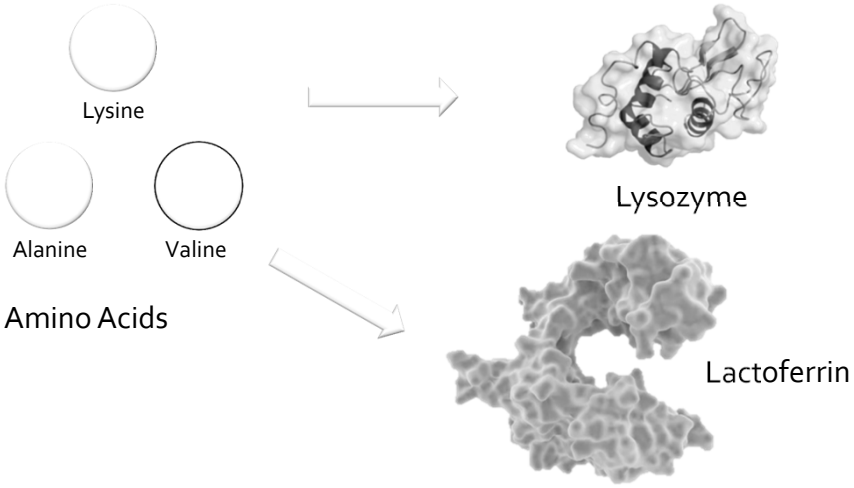
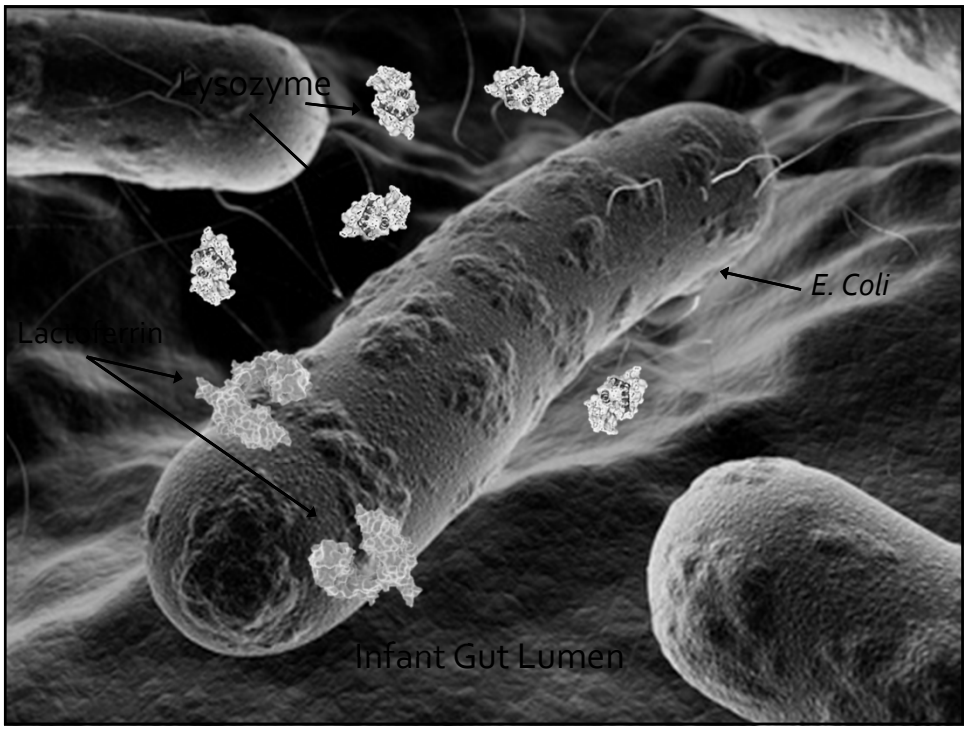
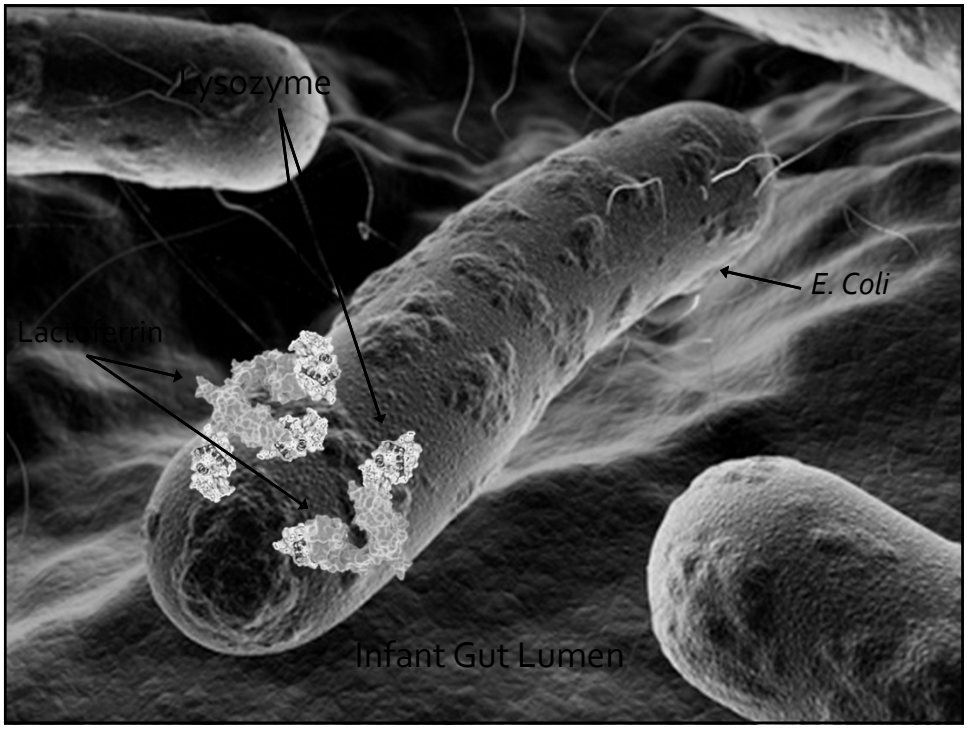


Lysozyme Image: By Yikrazuul [CC BY-SA 3.0 (https://creativecommons.org/licenses/by-sa/3.0)], from Wikimedia Commons





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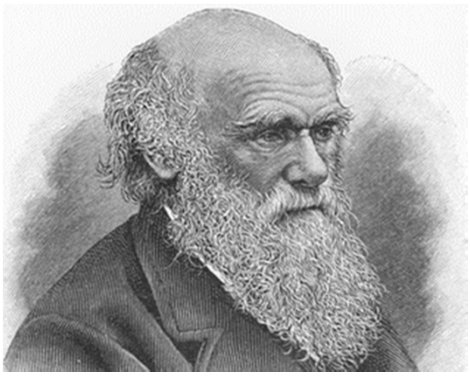
Evolution of Lactation

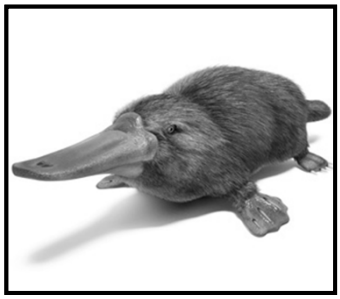


Charles Darwin

Lactation evolved because cutaneous gland secretions benefited eggs contained in brood pouches

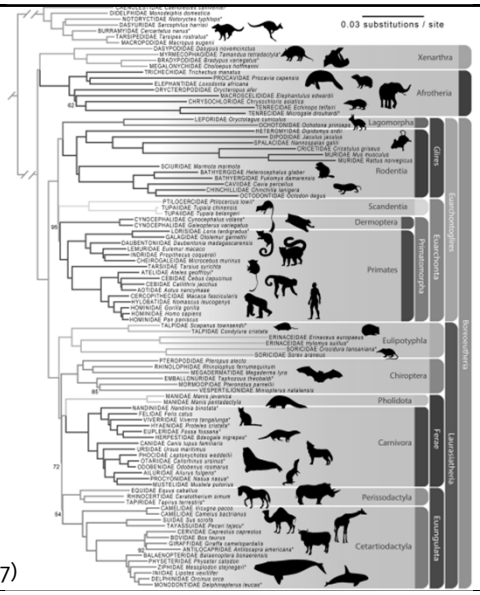
Mammalian Evolution

Monotremes



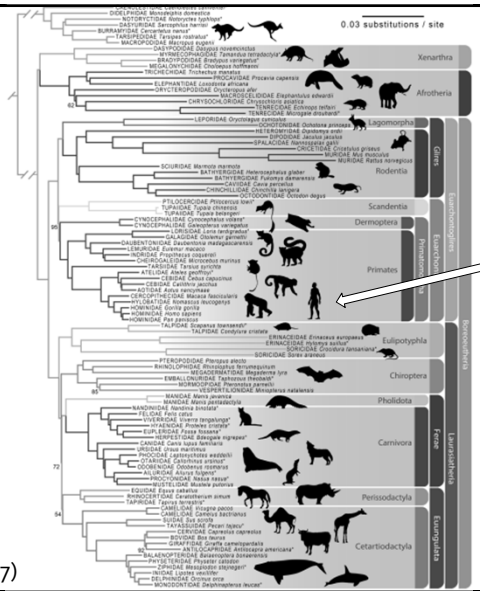
Marsupials

Placentals



Esselstyn et al. (2017)

From: Investigating Difficult Nodes in the Placental Mammal Tree with Expanded Taxon Sampling and Thousands of Ultraconserved Elements; Genome Biol Evol. 2017;9(9):2308-2321. doi:10.1093/gbe/evx168



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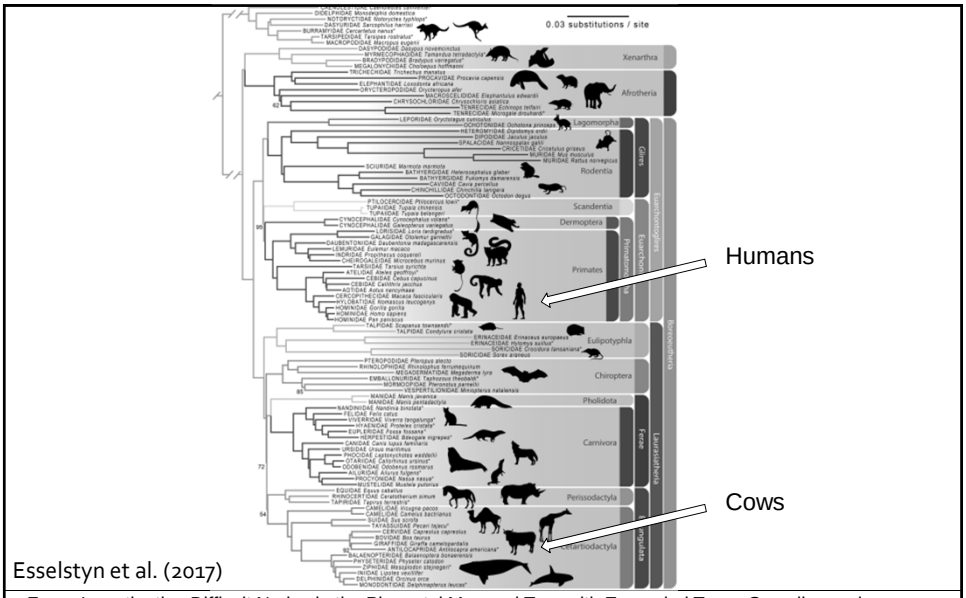

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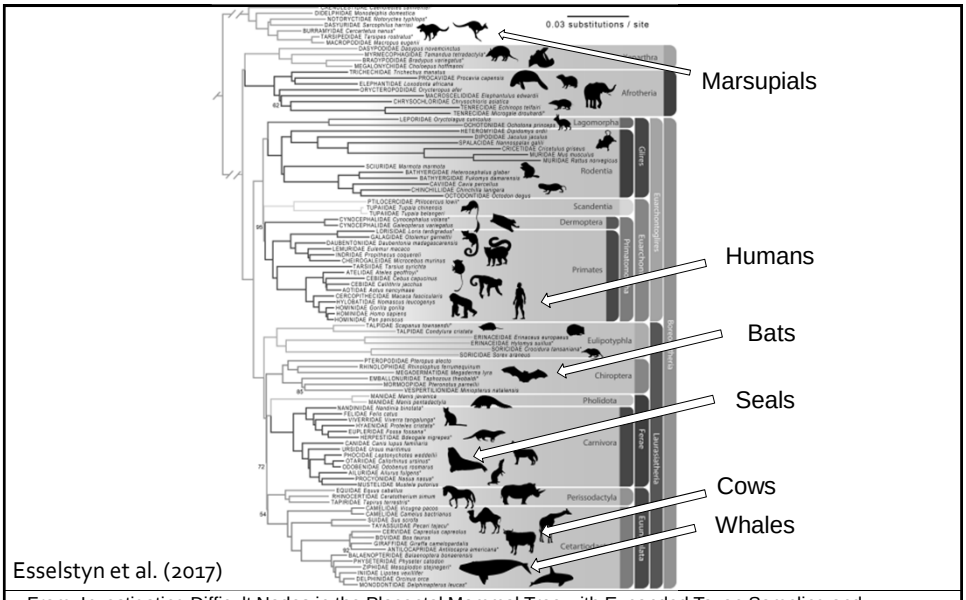

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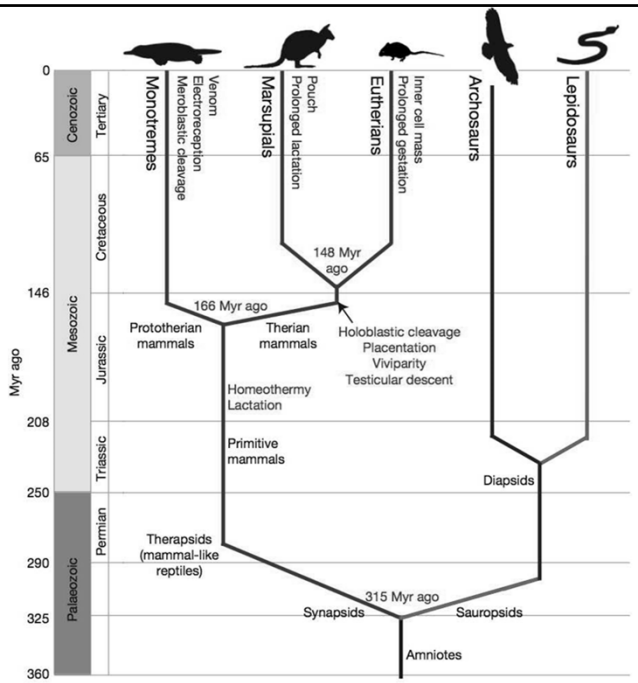

Warren et al. (2008)

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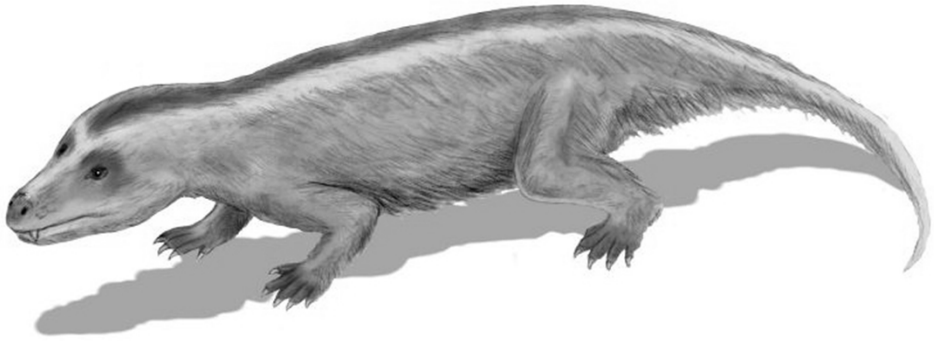
Therapsid

Keratocephalus moloch lived about 260 million years ago

Cynodont



Tritylodon longaevus lived about 200 million years ago.
By Nobu Tamura (http://spinops.blogspot.com) (Own work)

Egg Protection & Immunity First Theories

- Eggs had soft parchment-like shells.
- Secretions from skin or hair follicles could prevent against desiccation [1] and microbial attack. [2, 3]

1. Oftedal (2012) 2. Hayssen & Blackburn (1985) 3. Vorbach, Capecchi, Penninger (2006)

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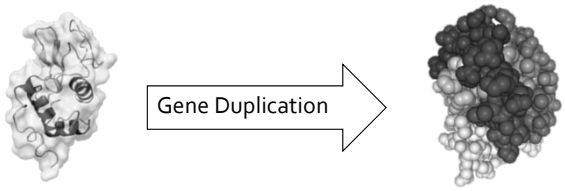


Lysozyme Alpha-Lactalbumin

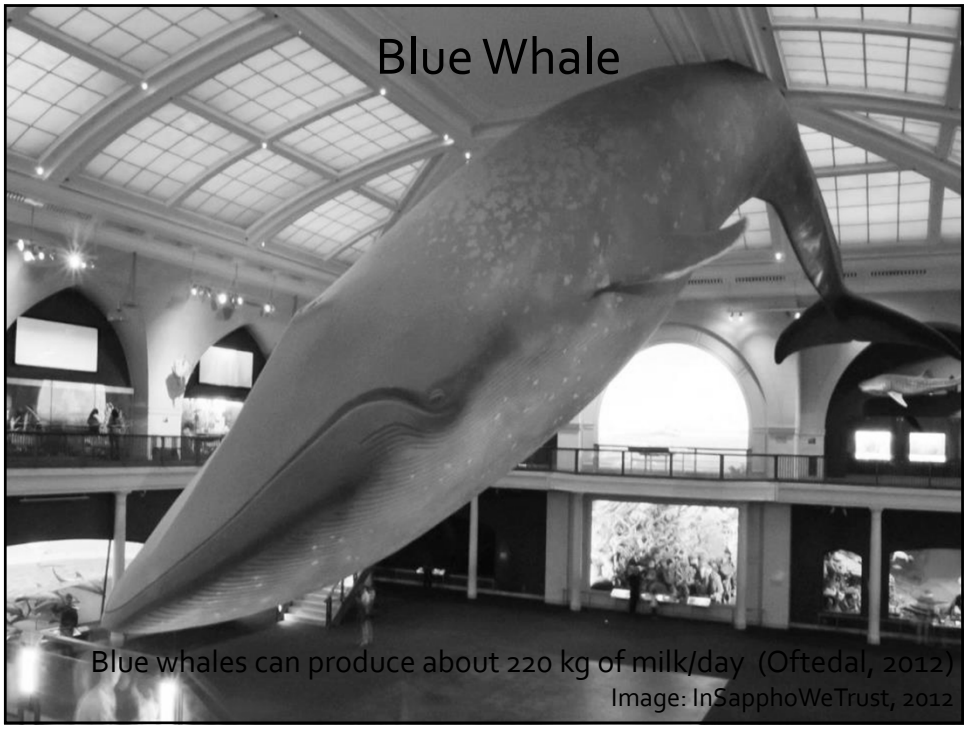
1. Oftedal (2012) 2. Hayssen & Blackburn (1985) 3. Vorbach, Capecchi, Penninger (2006)

Bats

- Bat pups may reach about 70% of maternal weight before they are weaned.
- Strong bones and muscles are required to deal with forces of flight (torque, pressure, and wind resistance).

Oftedal, 2012

Pipistrellus Bat



Phocid Seals

- Mothers stay with pups to nurse for short period of time (4-42 days) and then wean
- Mothers fast during lactation

Lefevre et al., 2010

Newborn Harp Seal

Pagophilus (Harp Seal)

Otariid Seals

- Mothers can forage for long periods (up to 23 days) in between nursing sessions
- Lactation lasts 4-12 months

Lefevre et al., 2010

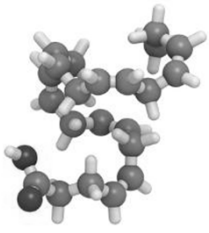
Fur Seal

Cows

- Calves stand and nurse soon after birth
- Milk is high in protein and IgG antibody
- Beef cows nurse for 6-8 months

Primates

- DHA and AA comprise 33% of the lipids in the human brain's grey matter[2]
- Human brain growth spurt
  - 3rd trimester – 2 years old



Milligan & Bazinet, 2008

Marsupials Nurse in Pouches

Kangaroo

Wallaby

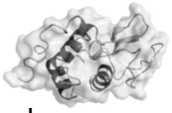
Quokka

Immunology

Innate Immune System

Generalists

- Cytokines
- Lysozyme
- Macrophage, neutrophil, natural killer cell

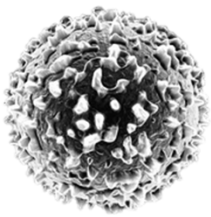


Lysozyme

Adaptive Immune System

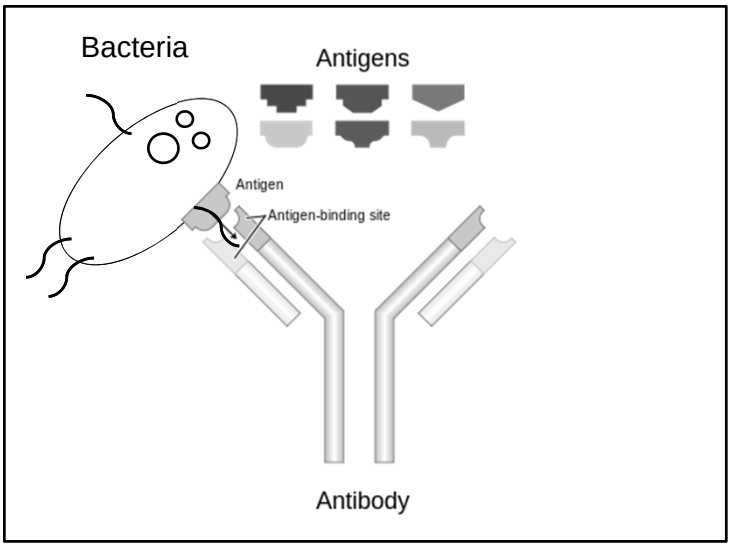
Specialists

- B cells & Antibodies
- T cells

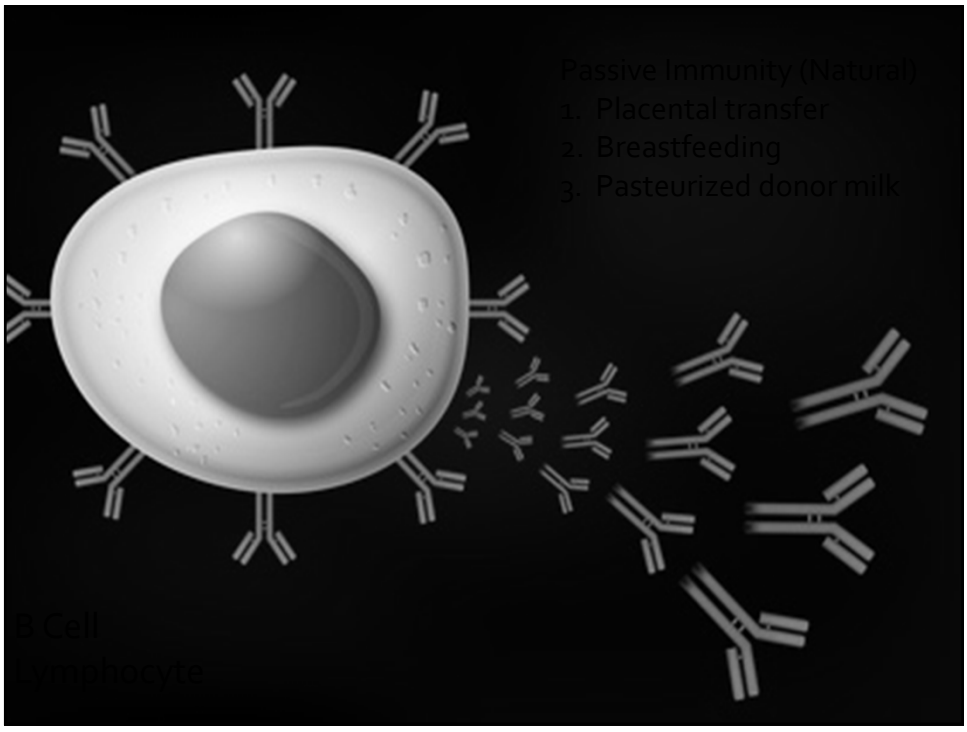


B Cell

Immunoglobulin = Antibody

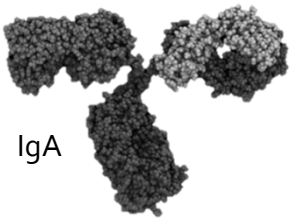


Altered image. Original image: Fvasconcellos, 2007: http://commons.wikimedia.org/wiki/File:Antibody.svg



Secretory Immunoglobulin A

- Major antibody in the human stomach
- Major antibody in human milk
- Secretory component protects IgA from digestion



IgA

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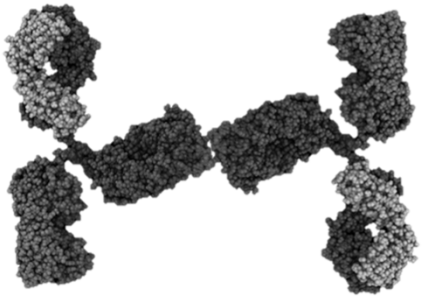

Dimeric IgA

Secretory Immunoglobulin A

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- Major antibody in human milk
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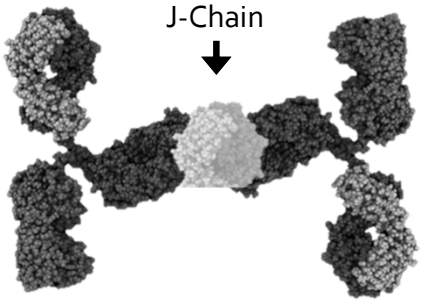

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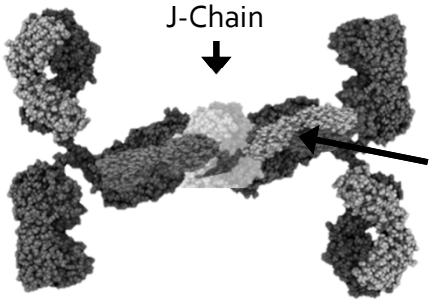
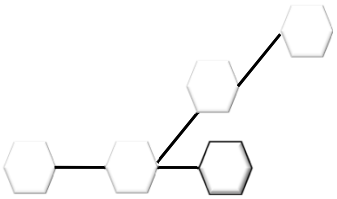
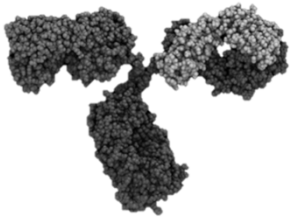


Bacterial Neutralization

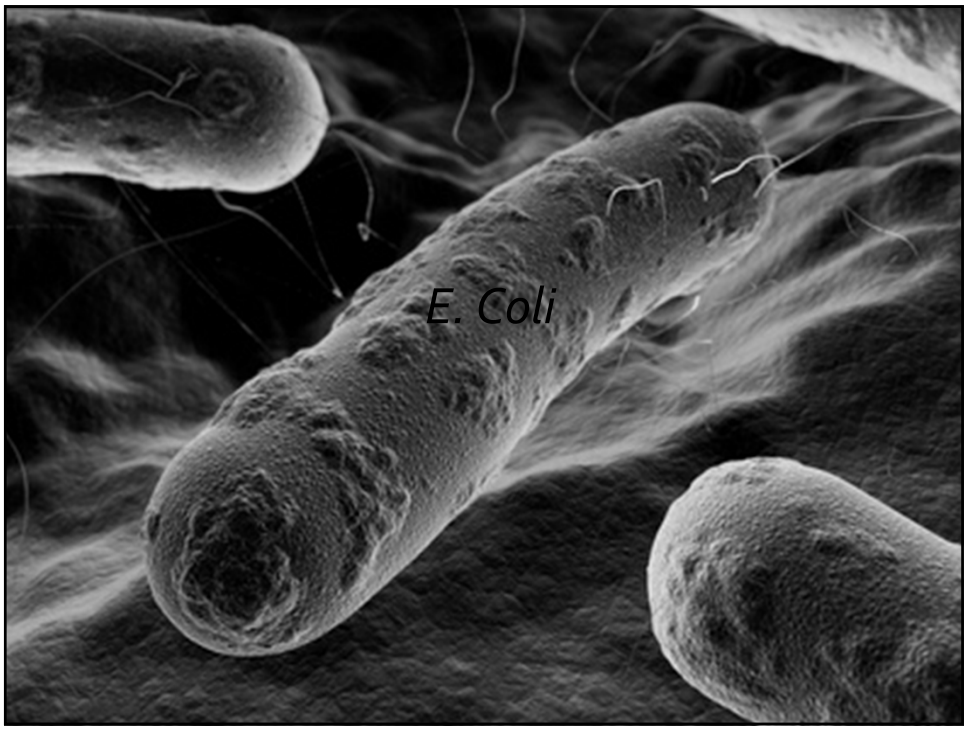
- HMOs and antibodies (sIgA) neutralize bacteria
  - Adhere to bacterium's binding site
  - Prevent attachment to the infant's gut

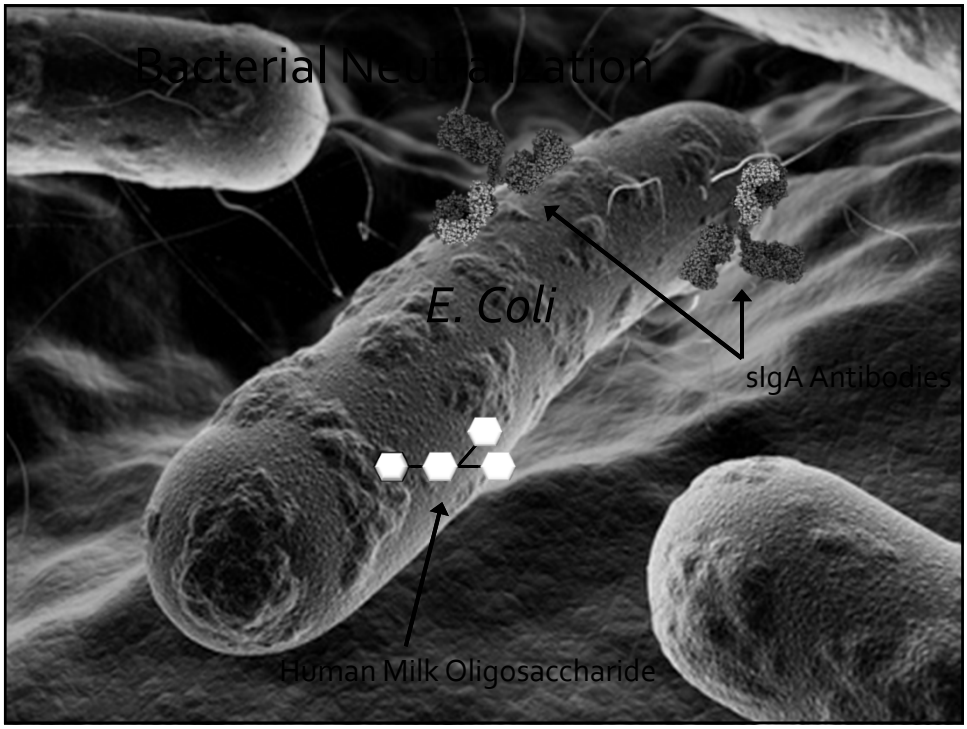


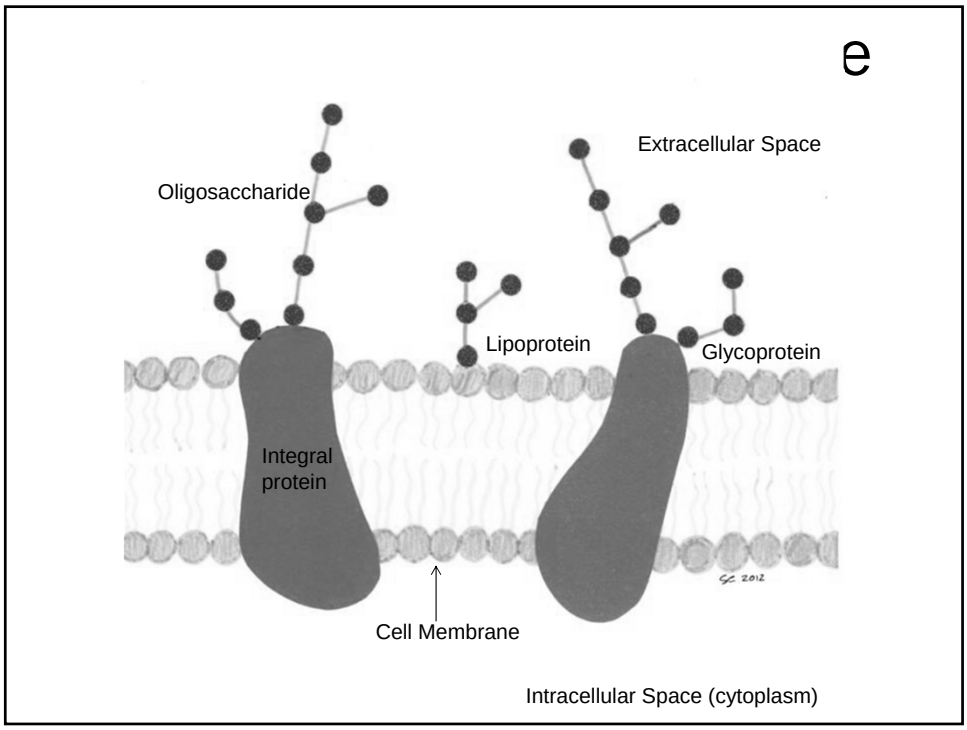
HMO

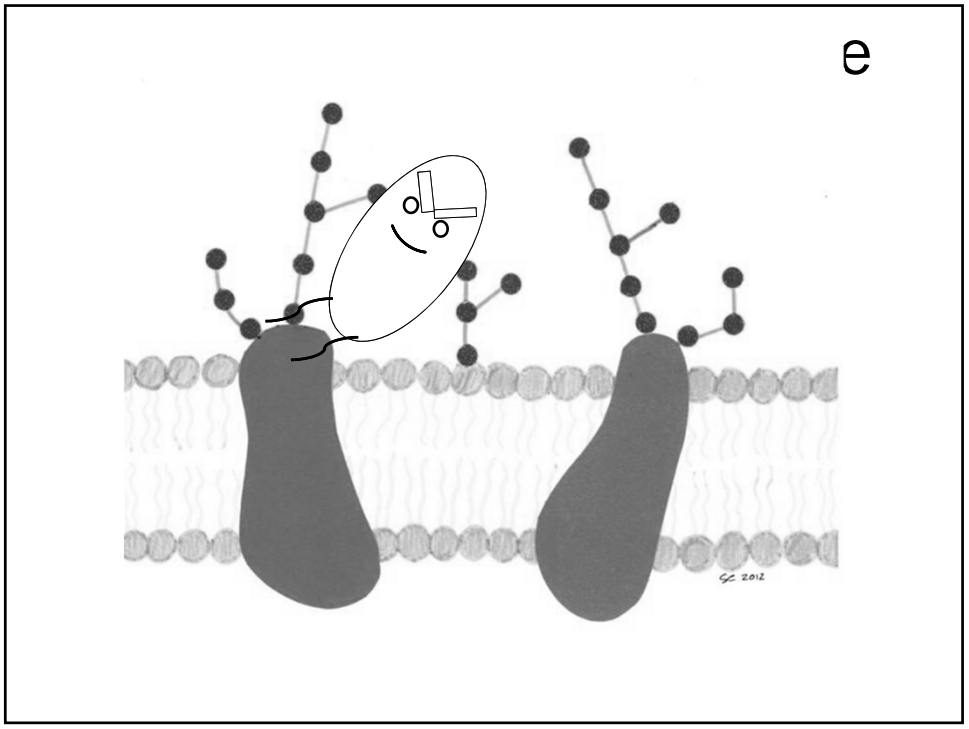


sIgA




Bacterial Neutralization
E. Coli
sIgA Antibodies
Human Milk Oligosaccharide


e
Extracellular Space
Oligosaccharide
Lipoprotein
Glycoprotein
Integral protein
Cell Membrane
Intracellular Space (cytoplasm)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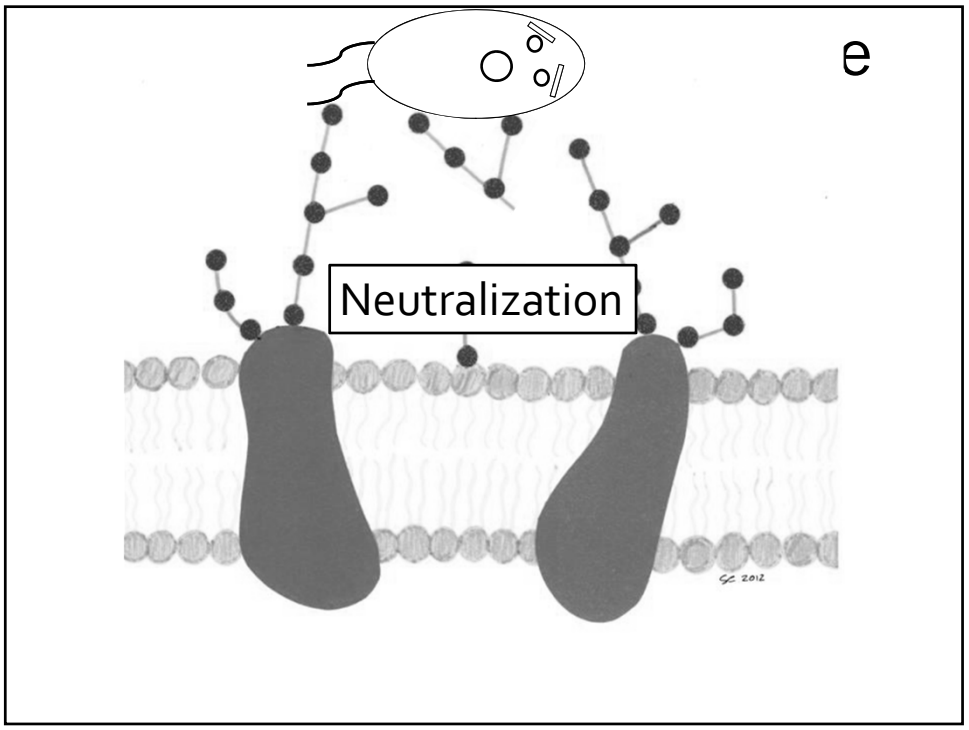
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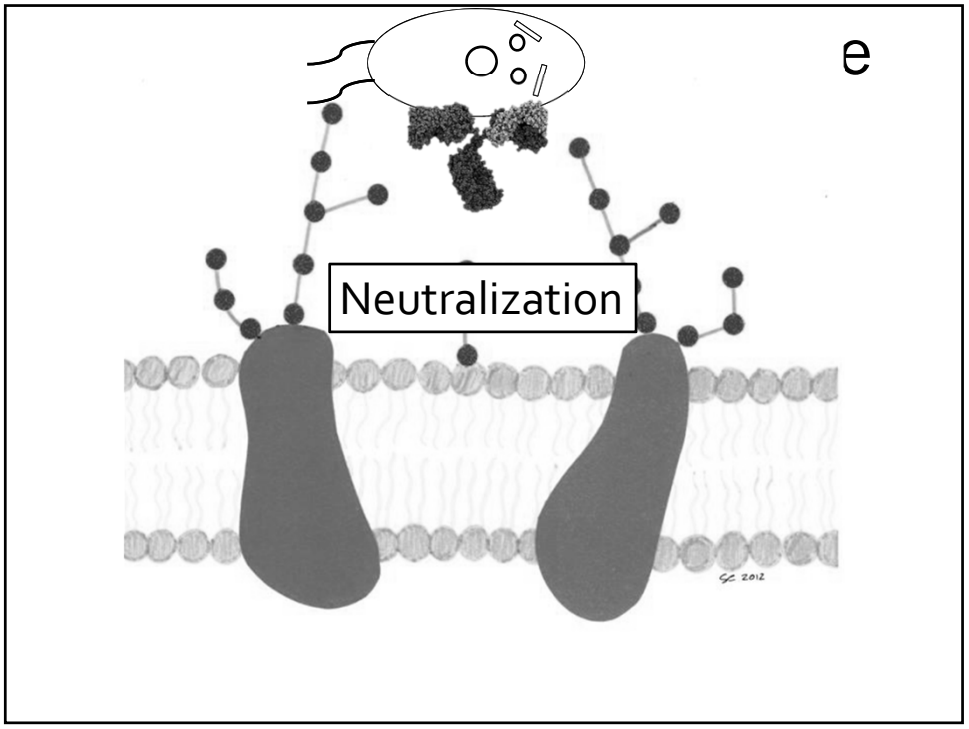
Neutralization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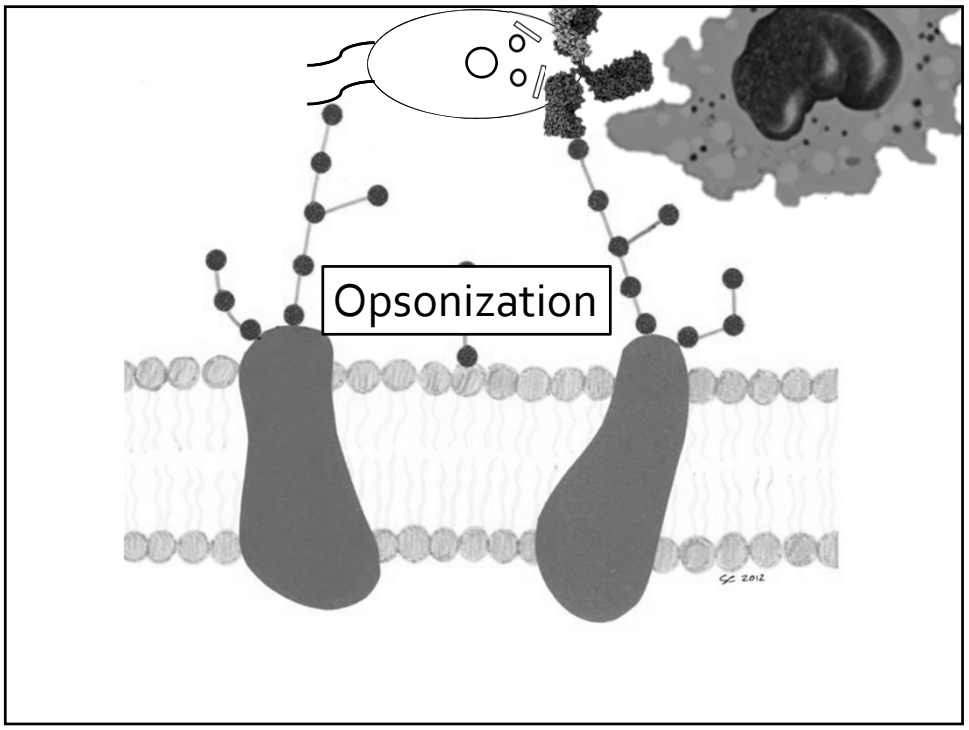
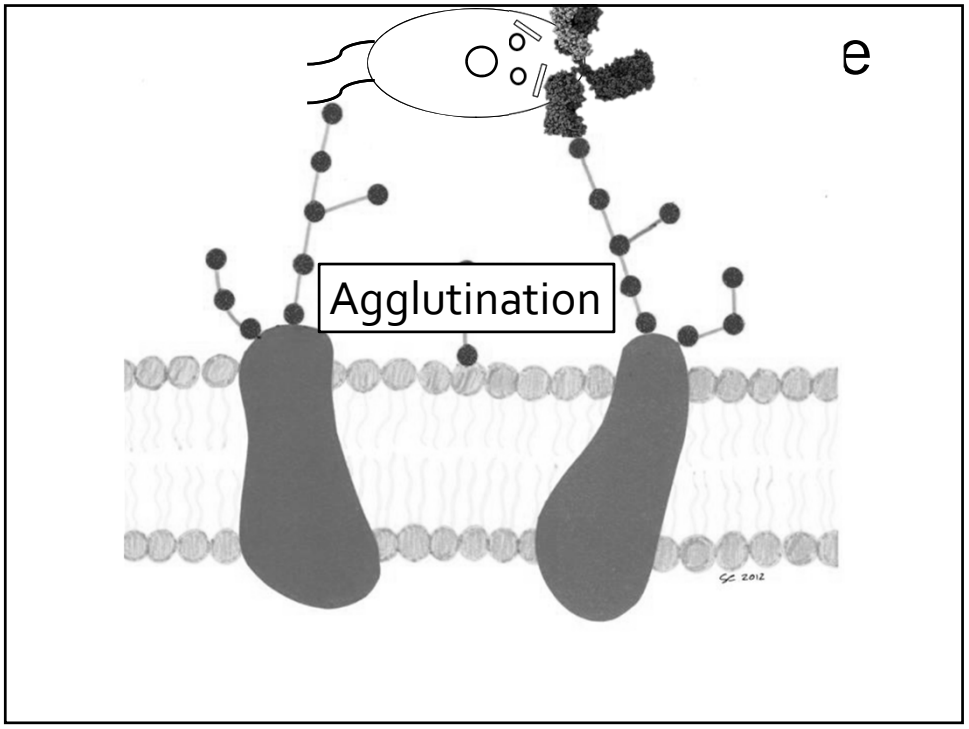
Neutralization


Opsonization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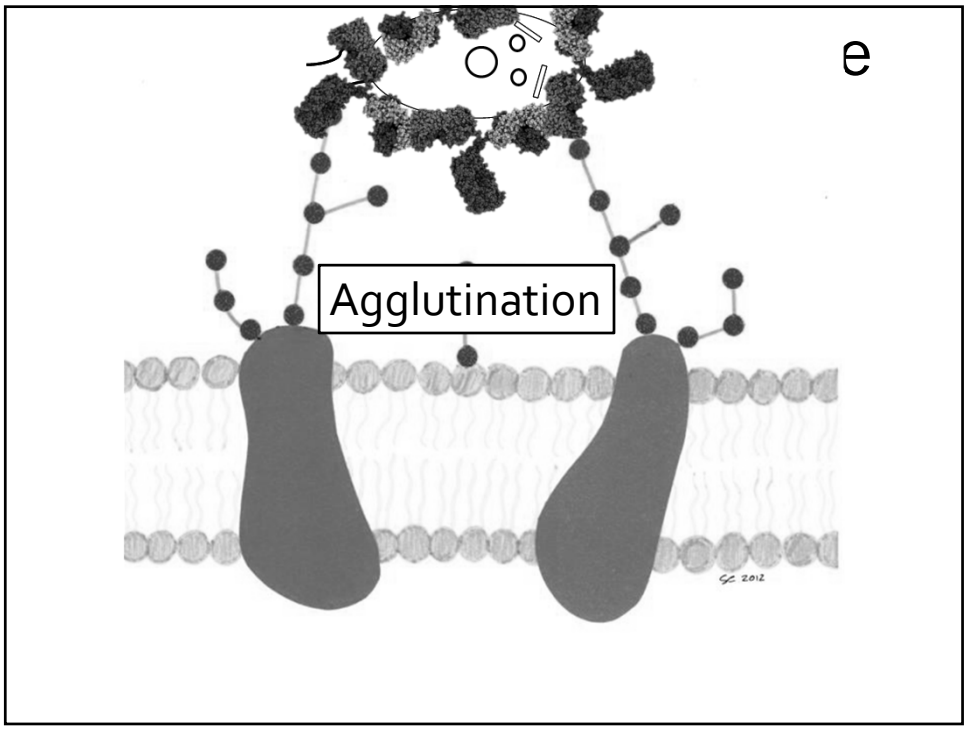
Agglutination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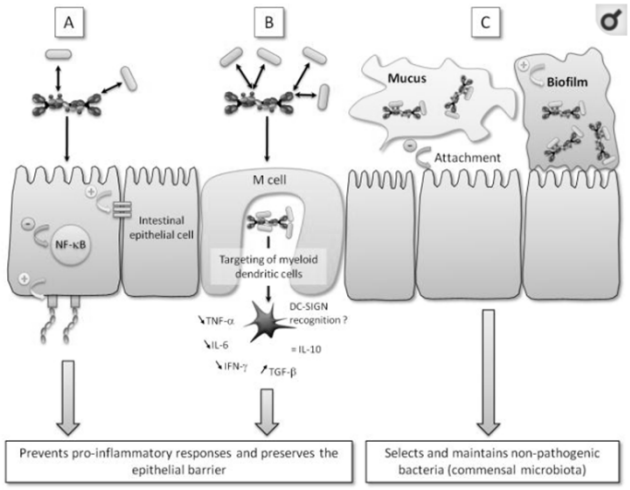
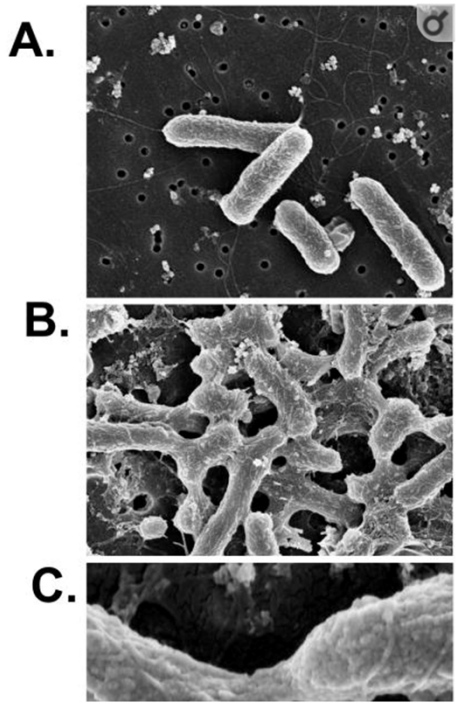
Agglutination



Mantis et al. (2011)



Mantis et al. (2011)

Microbiome: Bacterial DNA

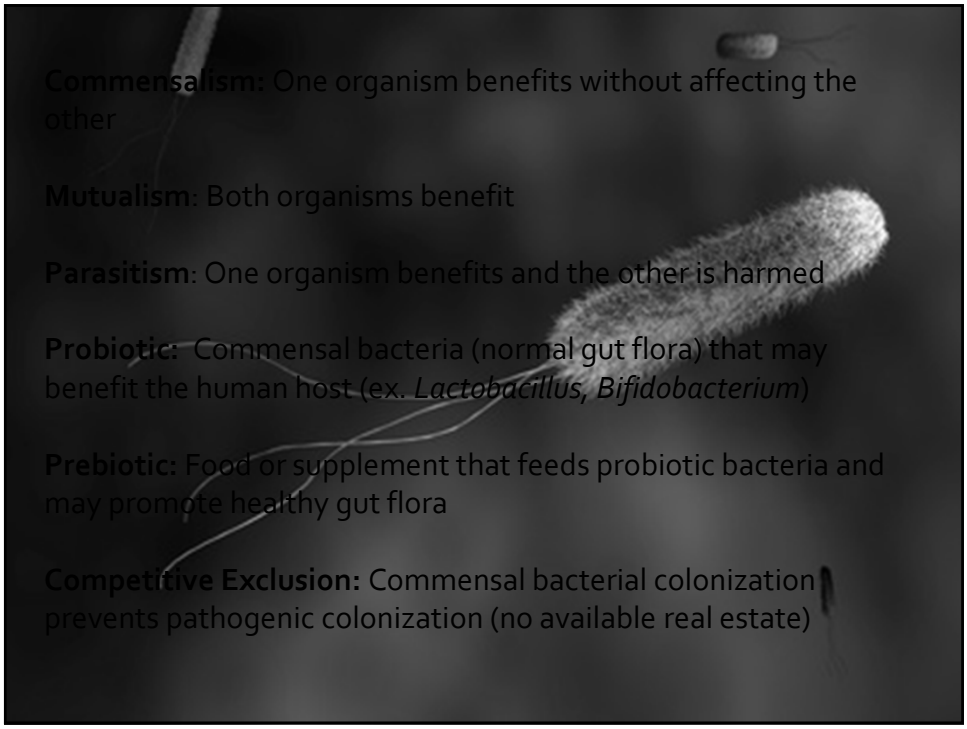
Microbiota: Bacteria

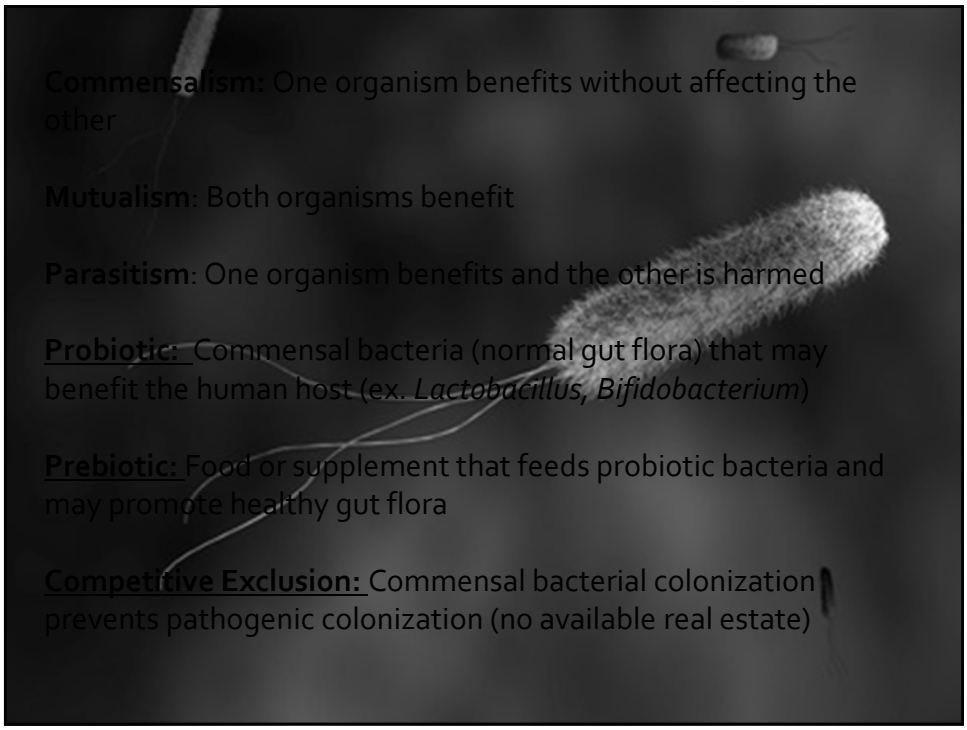
Human Microbiome Project (HMP)

- National Institute of Health (NIH) project launched in 2008
- Identify the human microbiome and health implications
- $150,000,000 budget
- Study mouth, gut, vagina, skin, and nasal cavity
- http://hmpdacc.org

NIH HUMAN MICROBIOME PROJECT

Turnbaugh et al., 2007


Commensalism: One organism benefits without affecting the other
Mutualism: Both organisms benefit
Parasitism: One organism benefits and the other is harmed
Probiotic: Commensal bacteria (normal gut flora) that may benefit the human host (ex. *Lactobacillus*, *Bifidobacterium*)
Prebiotic: Food or supplement that feeds probiotic bacteria and may promote healthy gut flora
Competitive Exclusion: Commensal bacterial colonization prevents pathogenic colonization (no available real estate)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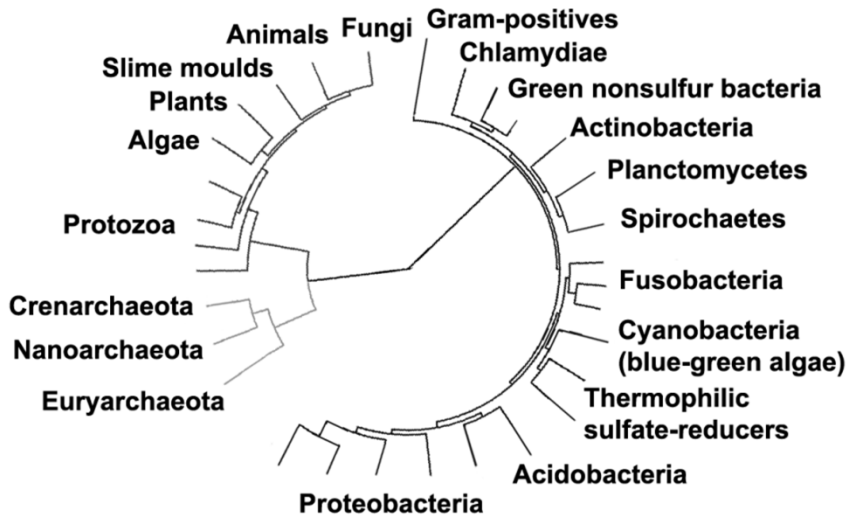
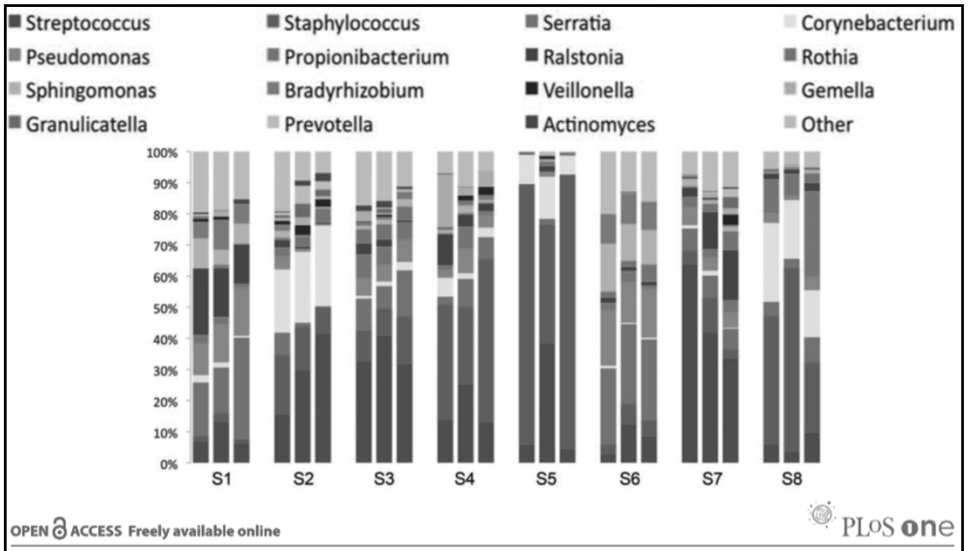


By TimVickers at English Wikipedia - Transferred from en.wikipedia to Commons., Public Domain, https://commons.wikimedia.org/w/index.php?curid=4423407

Breastfeeding Ecosystem

- Human milk contains millions of microbes
- Human milk oligosaccharides feed beneficial gut bacteria
- The breastfed infant's microbiome is significantly different than the formula fed infant's



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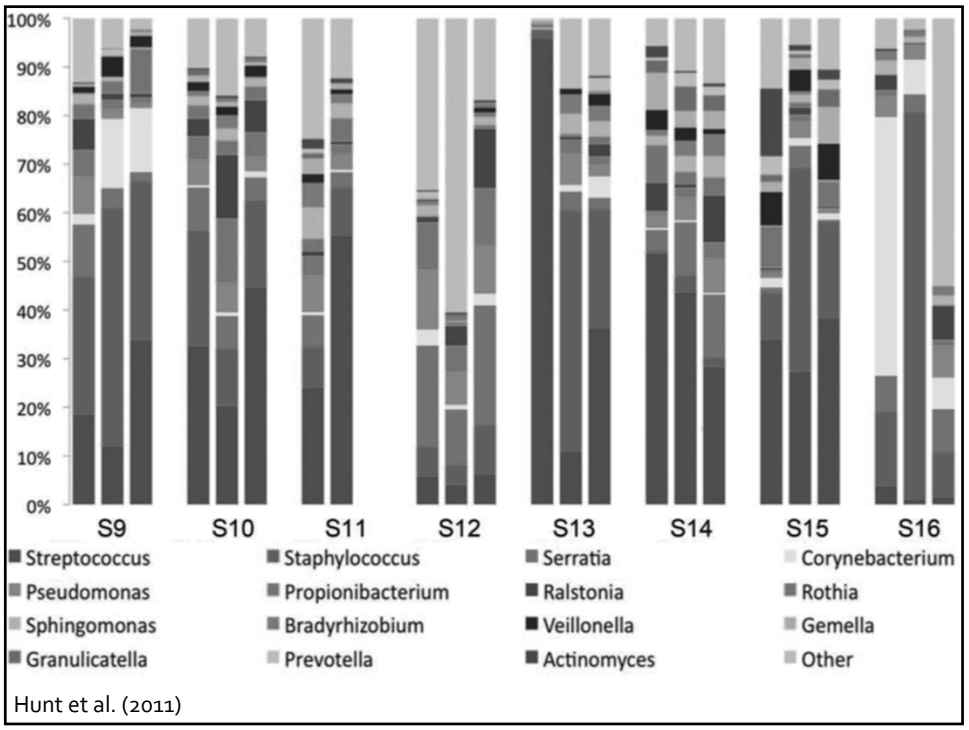
PLoS one

Characterization of the Diversity and Temporal Stability of Bacterial Communities in Human Milk

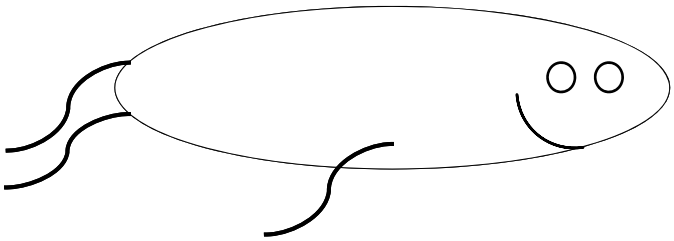
Katherine M. Hunt[1,6], James A. Foster[2,6], Larry J. Forney[2,6], Ursel M. E. Schütte[2], Daniel L. Beck[2,6], Zaid Abdo[3,6], Lawrence K. Fox[4], Janet E. Williams[1], Michelle K. McGuire[5], Mark A. McGuire[1,6]*



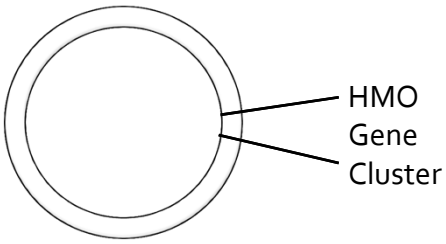
Hunt et al. (2011)

Bifidobacterium longum

- *Bifidobacteria* is a beneficial probiotic for babies.
- Colonization prevents pathogenic colonization via competitive exclusion
- Genomic sequencing in 2008 (Sela et al.)



Bifidobacterium Genomics

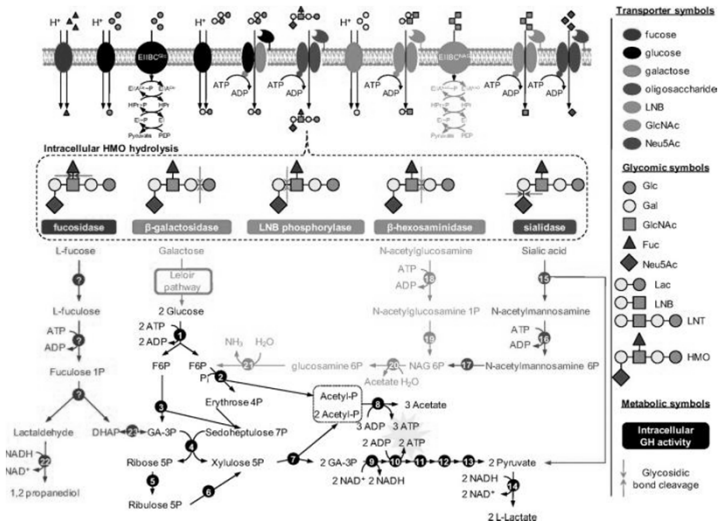


Bifido genome

- A 43,000 base pair cluster (30 genes) codes for several proteins that facilitate utilization of Human Milk Oligosaccharides (HMOs).

Sela et al. (2008)

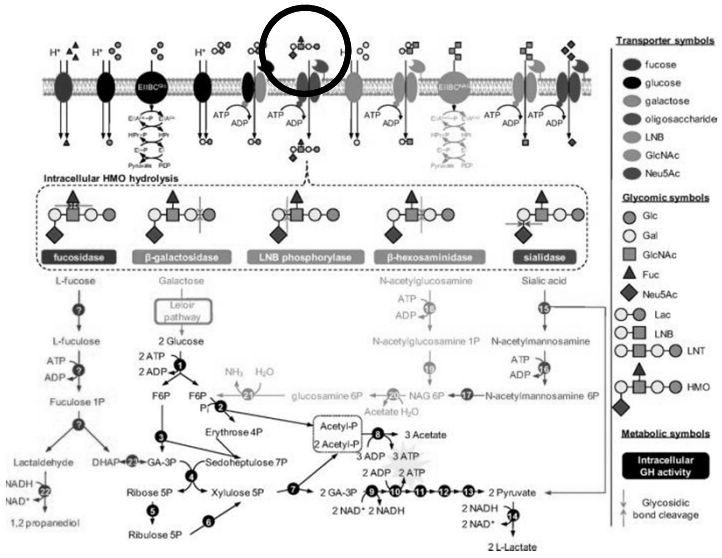
B. longum subsp. infantis metabolism of human milk oligosaccharides and derivatives.



D. A. Sela et al. PNAS 2008;105:48:18964-18969

PNAS

B. longum subsp. infantis metabolism of human milk oligosaccharides and derivatives.



D. A. Sela et al. PNAS 2008;105:48:18964-18969

PNAS

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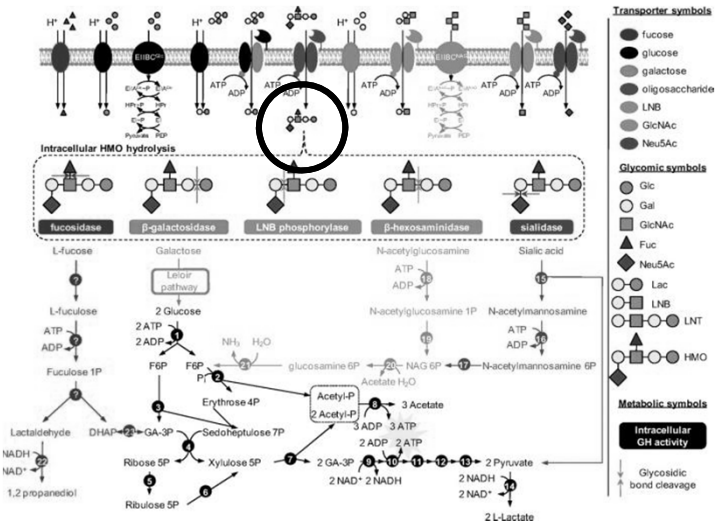
B. longum subsp. infantis metabolism of human milk oligosaccharides and derivatives.



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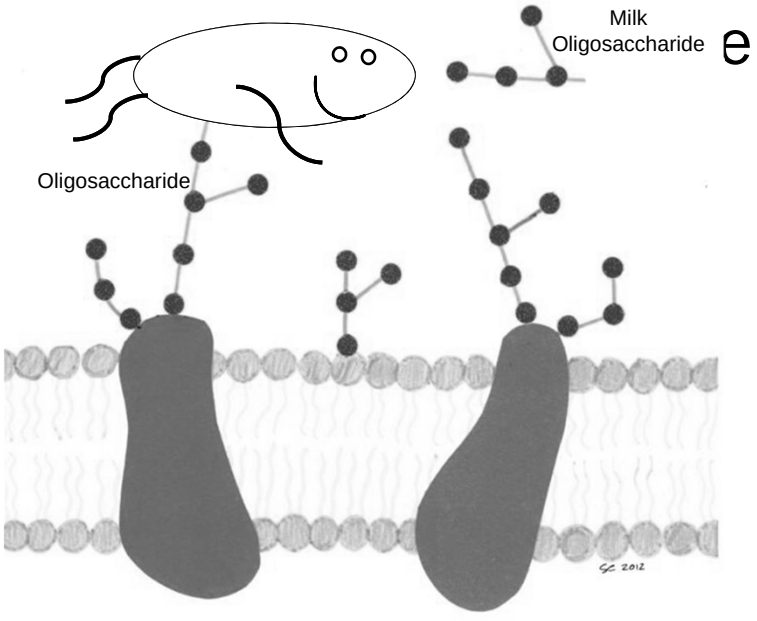
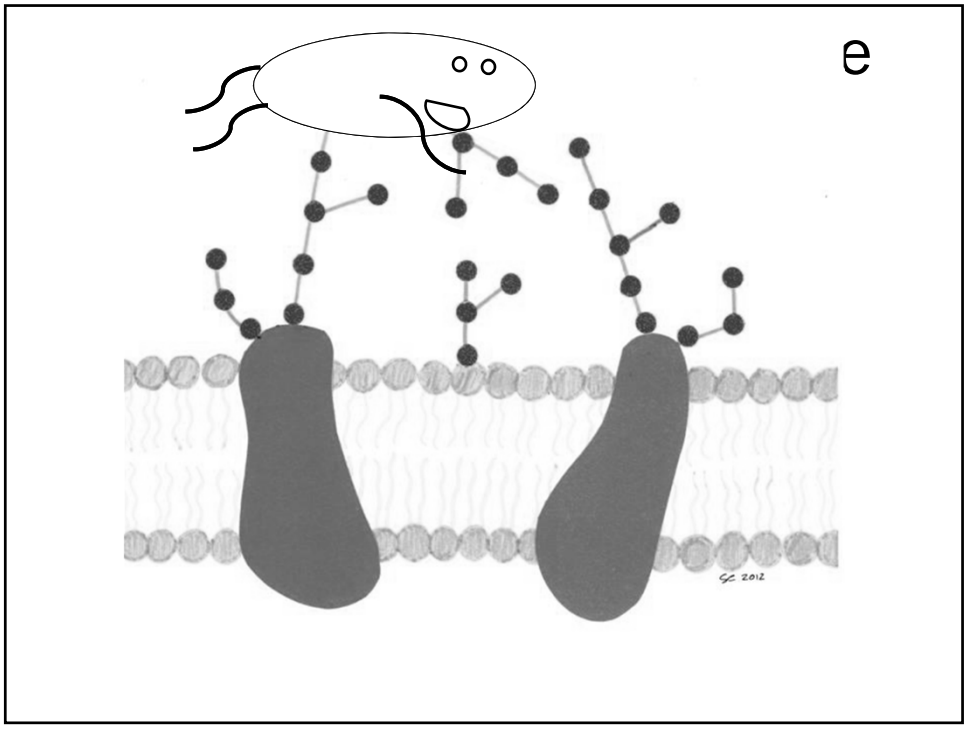
PNAS

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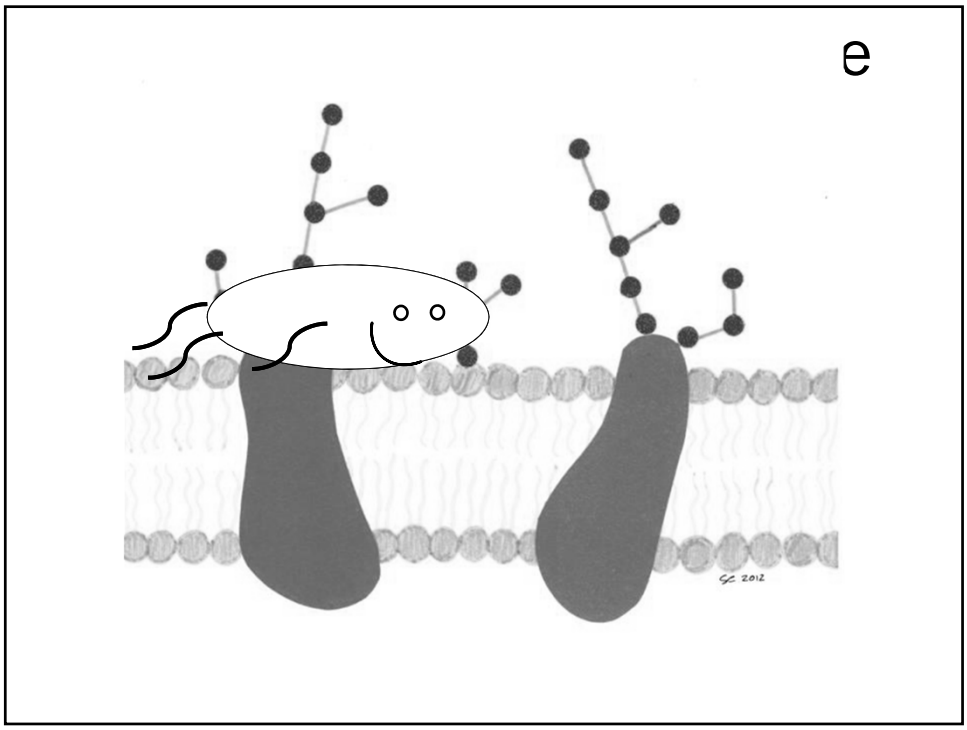
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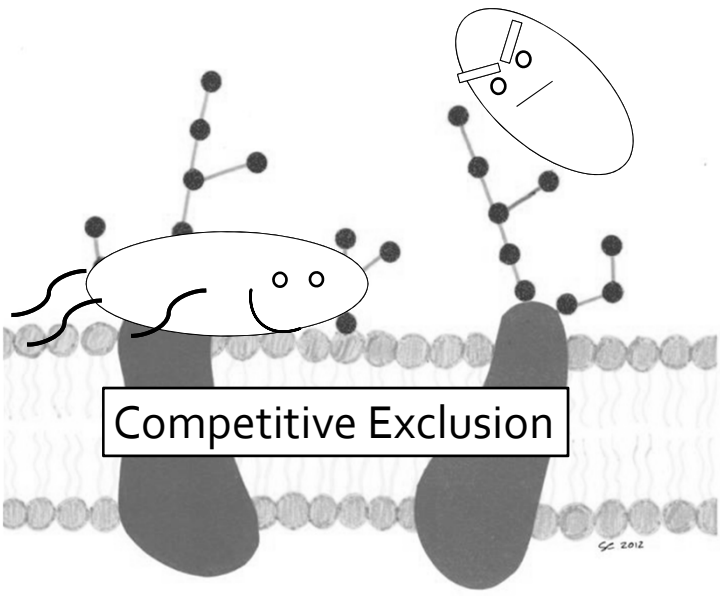
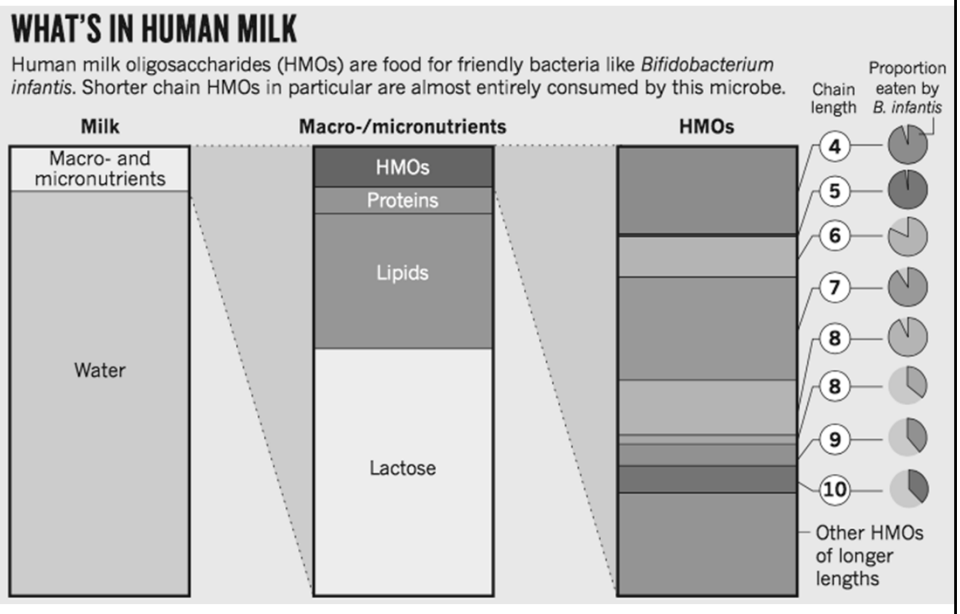


HMOs are the main prebiotic in human milk! HMOs are retained after pasteurization!

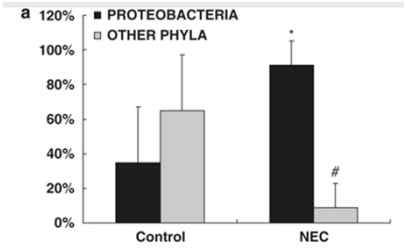


Petherick, 2010

Genomic NEC research

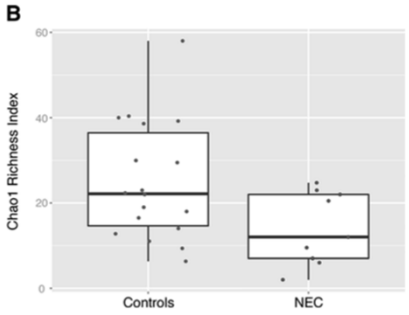
- Gut flora gene sequencing
- Bacterial signatures associated with NEC:
  - *Clostridium* or Proteobacteria bloom precedes onset
  - NEC patients have ↓ microbial diversity/ richness

Proteobacteria
Gram Negative
Escherichia
Klebsiella
Pseudomonas

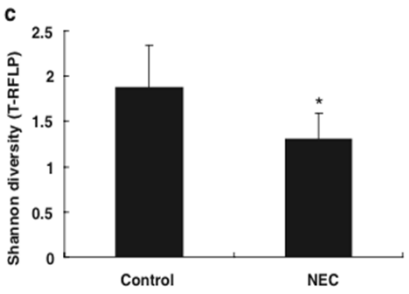


Wang et al. (2009)

Decreased microbial richness and diversity



Morrow et al., 2013



Wang et al., 2009

Infant Feeding Choices

	1. Mom's Milk	2. Donor Milk	3. Formula
Human Milk Oligosaccharides	100%	100%	0%
sIgA antibody	100%	70%	0%
Lysozyme	100%	75%	0%
Lactoferrin	100%	40%	0%
Bile salt stimulated lipase	100%	0%	0%
White blood cells	100%	0%	0%

Data from Tully et al., 2001

Milk Banking in the Prairie and Dairy States

Summer Kelly, RN, MS, IBCLC

Mothers' Milk Bank of the Western Great Lakes

"Breast milk cells are live when consumed by the infant during breastfeeding, suggesting that they confer modulatory and developmental benefits far beyond what is currently known… Further research will benefit from multidisciplinary collaborations that may view breast milk from the perspective of a complex biosystem rather than a food source."

Hassiotou & Hartmann (2014)

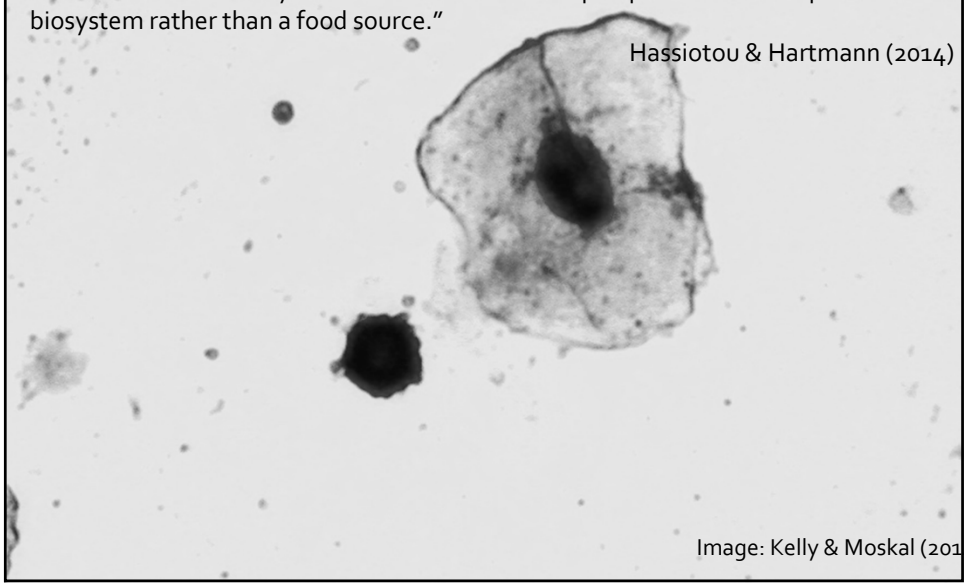


Image: Kelly & Moskal (201

Acknowledgements

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Thank you for sharing your knowledge and passion!

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- Dr. Booms
- Dr. Olfelt
- Dr. Schirmer
- Dr. Geddes
- Dr. Frankel

- Professors Herzog & Lendway (Wright College)

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