

Probiotic Use in Preterm Infants and Children Differentiating Between Health and Disease



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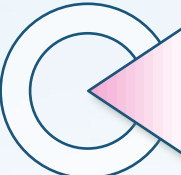
For complete financial interest disclosures, see the course CE statement.



Learning Objectives



Discuss immunity and its interaction with the microbiome in the developing child



Evaluate the impact of dysbiosis in infancy and childhood on long-term health outcomes



Review evidence for prebiotic and probiotic use in preterm and term infants

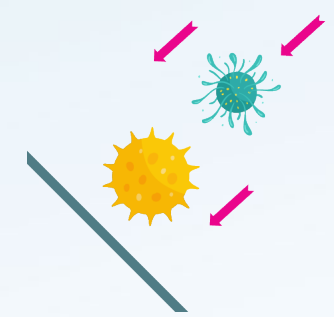




Immunity in the Developing Child and the Gut Microbiome



Immune System



➤ The immune system is a network of cells, tissues, and organs that work together primarily to defend the body against attacks by “foreign” invaders.

➤ Designed to carry out rapid, specific, and protective responses against harmful pathogens or their biologic products.

➤ The mechanisms of immunity function across a broad spectrum of clinical conditions spanning from resolution of infectious disease, recognition and rejection of tumors, tolerance or rejection of transplanted tissues or organs, autoimmunity, and allergy.



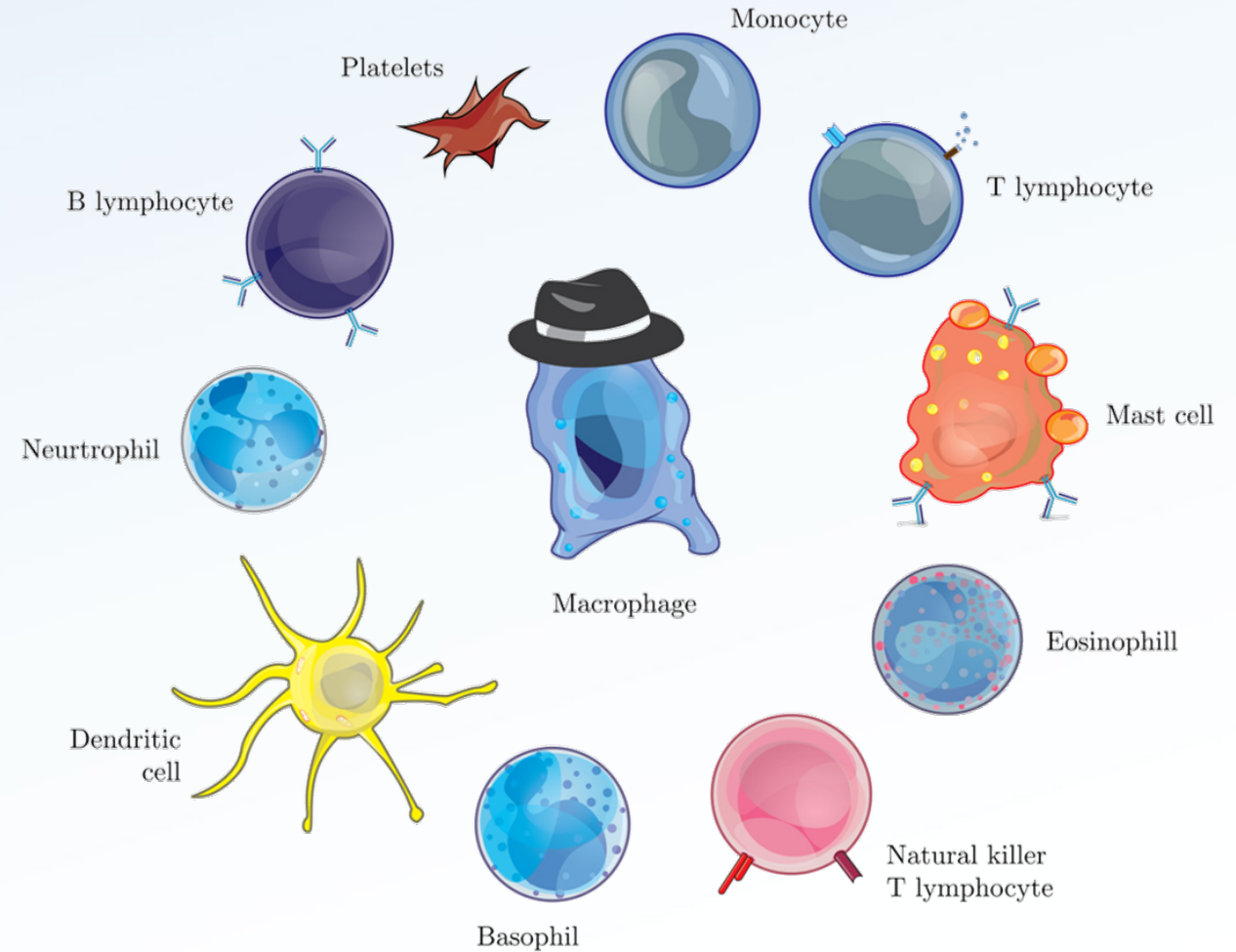
Functions of the Immune System

Function	Normal	Hyperfunction	Hypofunction
Defense	Antimicrobial activity	Allergy Autoinflammatory disorders	Immune deficiency
Homeostasis	Removal of damaged cells	Autoimmune disease	
Surveillance	Removal of mutant cells	None	Malignancies



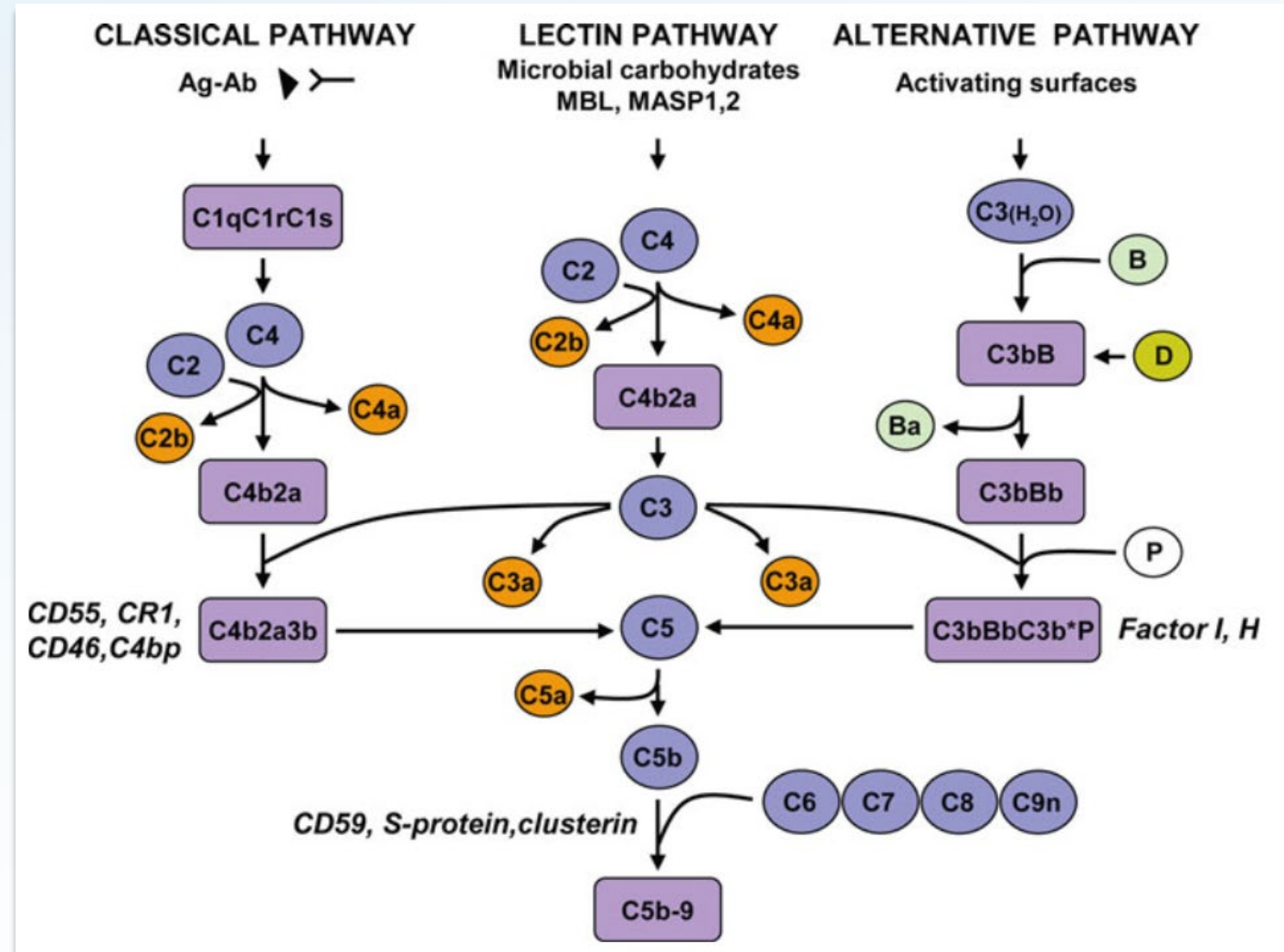
Innate Immune System – Immunity You Are Born With

- Phagocytic cells (macrophages, polymorphonuclear leukocytes, eosinophils, & dendritic cells)
- Mediator cells (mast cells & basophils)
- Natural killer (NK) cells
- Complement



Innate Immune System – Immunity You Are Born With

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Innate Immunity – Mechanism of Action

Two basic strategies of immune recognition:

Recognition of **microbial non-self**.

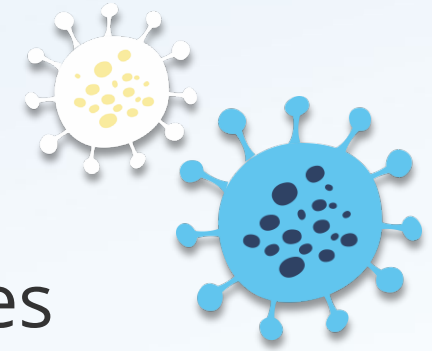
Recognition of **missing self**.



What Is the Adaptive Immune System?



Adaptive Immune System

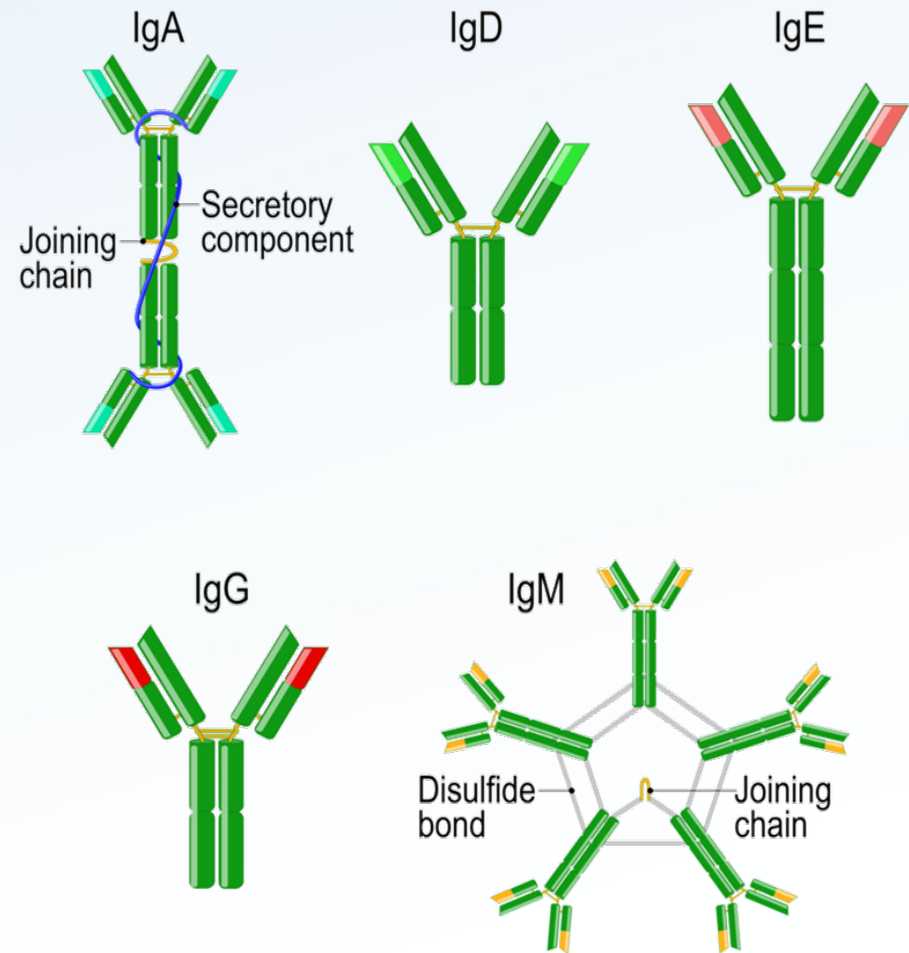


- Adaptive immune cells originating from lymphocytes differentiate to recognize specific antigens.
- As rearrangements within the genes in the immune cells occur during this developmental process:
 - Antigens present in the host (self-antigens) interact with the emerging cell population to eliminate those adaptive immune cells that would attack the host
 - Only those cells that will target any non-self-antigens are retained



Adaptive Immune System Components

- T cells (Th1, Th2, Th17 and Treg)
- B cells
- Th2 → B cells → IgE
- Th1 → B cells → IgG, IgM, IgA



Adaptive Immune System – Cytokines and Chemokines

- Cytokines are a group of protein and peptide that comprise the intercellular communication network of every cell system of the body, including the immune system.

They function as signaling molecules to regulate the growth, differentiation, activation, and inhibition of all cellular aspects of both the innate and adaptive immune responses.

- Chemokines are a specialized subset of cytokines that function to induce directed cell movement, ie, chemotaxis, in nearby responsive cells; they are chemotactic cytokines, hence the name “chemokines.”



Adaptive Immune System – Cytokines and Chemokines

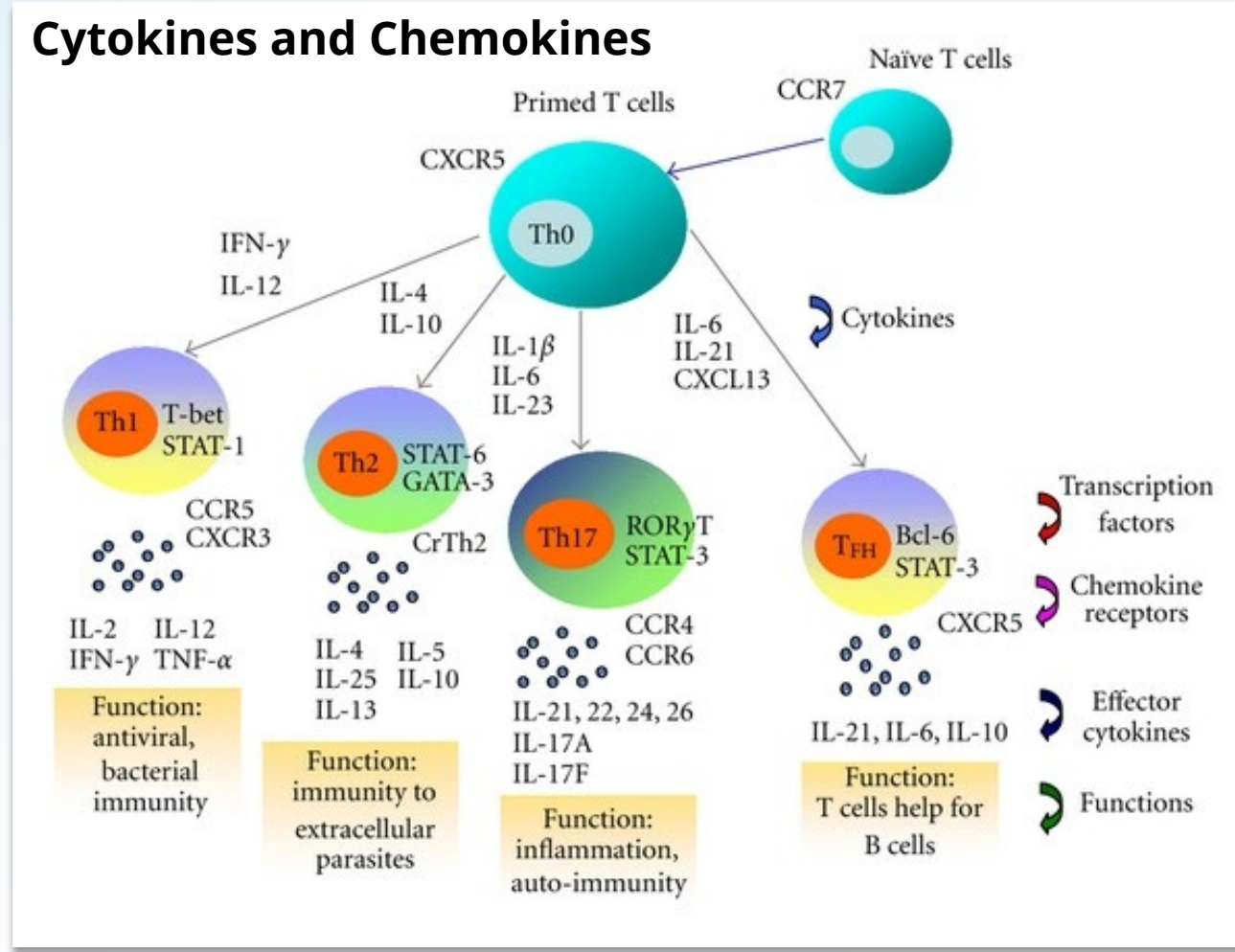


Image courtesy of Yu SL et al. *Clin Dev Immunol.* 2012;2012:715190. Used under the terms of a Creative Commons license [CC BY 3.0](https://creativecommons.org/licenses/by/3.0/).



Messaging From Innate to Adaptive Immune System

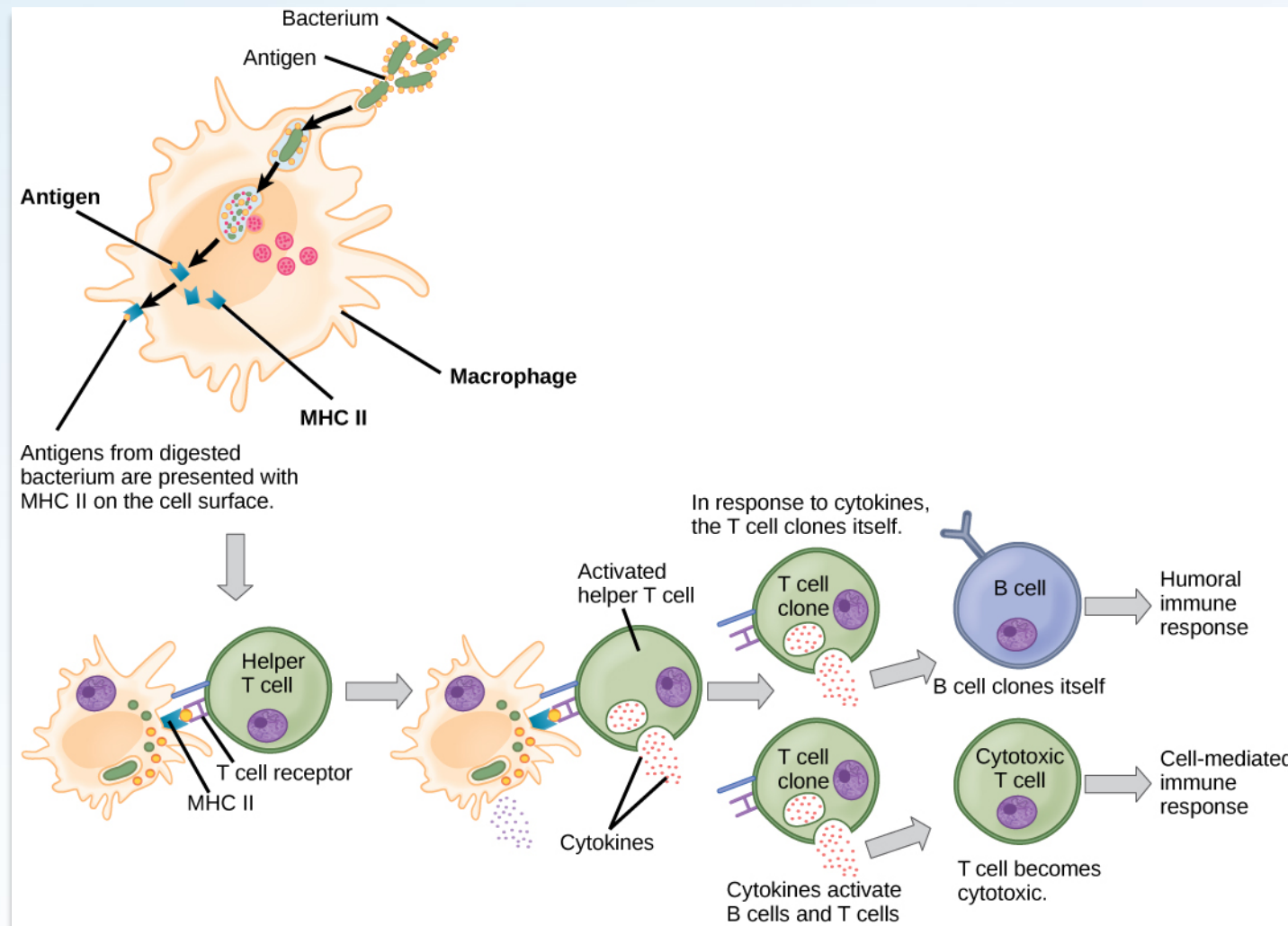
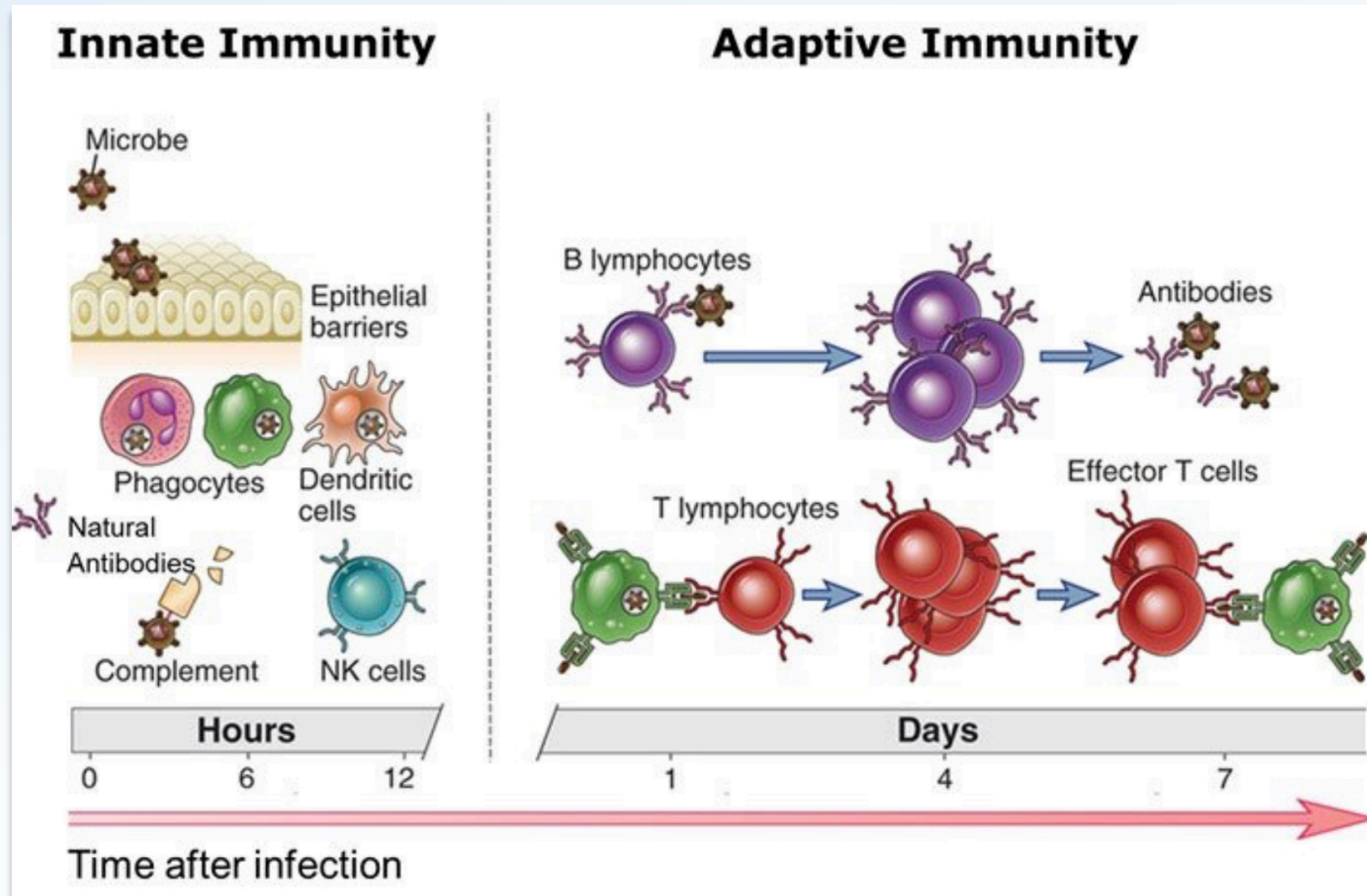


Image courtesy of Molnar C, Gair J. In: *Concepts of Biology - 1st Canadian Edition*. <https://opentextbc.ca/biology/chapter/12-3-adaptive-immunity/>. Used under the terms of a Creative Commons License [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/).



Innate and Adaptive Immunity



Adapted from Abbas AK, et al. *Cellular & Molecular Immunology (8th Edition)*. Elsevier, 2014.



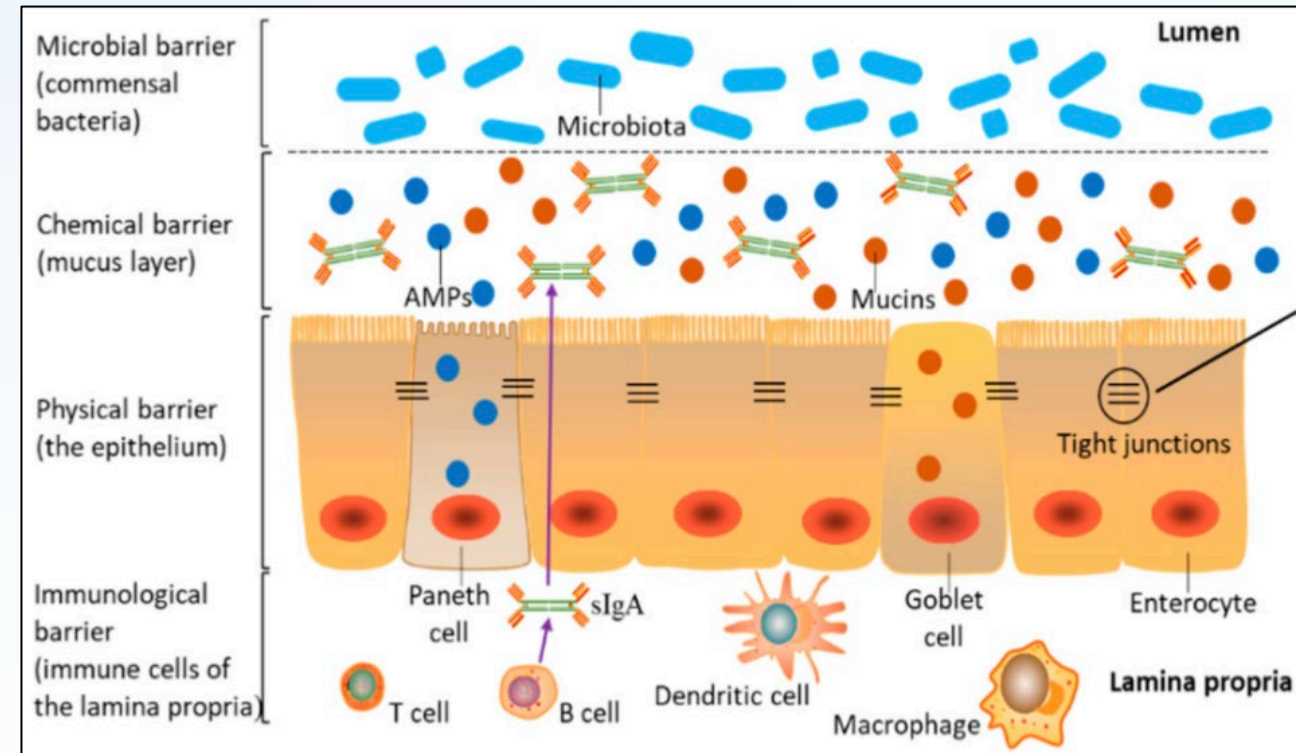


Microbiota, Microbiome and Normal Development vs Dysbiosis



Role of GI Barrier – Provides First Line of Defense

- Chemical barrier: Layer of mucus, barrier to pathogens, accommodates commensal bacteria
- Physical barrier: Column of epithelial cells with junctions between the cells, controls permeability
- Immunological barrier:
 - Contains gut-associated lymphoid tissue (GALT)
 - Builds tolerance to antigens and defends against pathogens
 - Produces secretory immunoglobulin A (sIgA): prevents pathogens from adhering to and penetrating the epithelium



Analysis of the fetal intestine suggests limited bacterial colonization with potential immunological functions

Andreu Prados • 27 April 2020

Whether bacterial colonization starts in utero is still a matter for scientific debate. The study of the fetal intestinal content collected from terminated pregnancies reveals some evidence for early bacterial colonization linked to distinct immune imprinting.



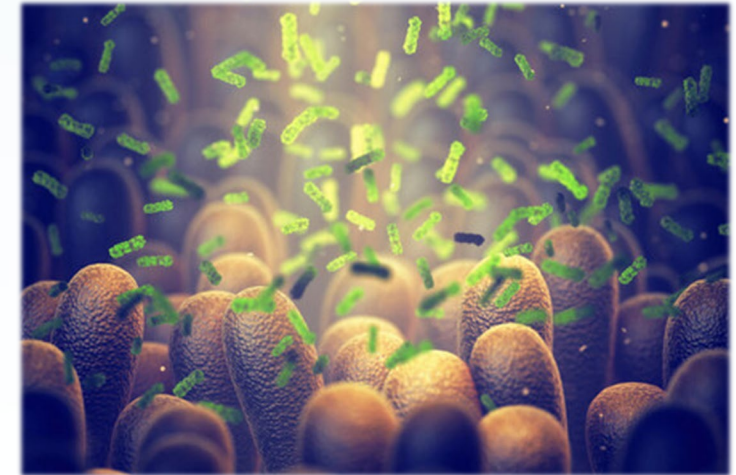
The Significance of the Gut to the Immune System

- Bacteria-like structures in pockets of human fetal meconium at mid-gestation; electron microscopy, 16S ribosomal ribonucleic acid sequencing
 - 3 bacterial profiles identified in the fetal intestinal content samples
 - Associated with distinct gene expression, distinct patterns of T-cell composition
- Fetal *M. luteus* isolates promoted immune regulation by
 - Inducing tolerogenic antigen presenting cells in the lamina propria
 - Reducing the pro-inflammatory interferon-gamma production by fetal memory T-cells



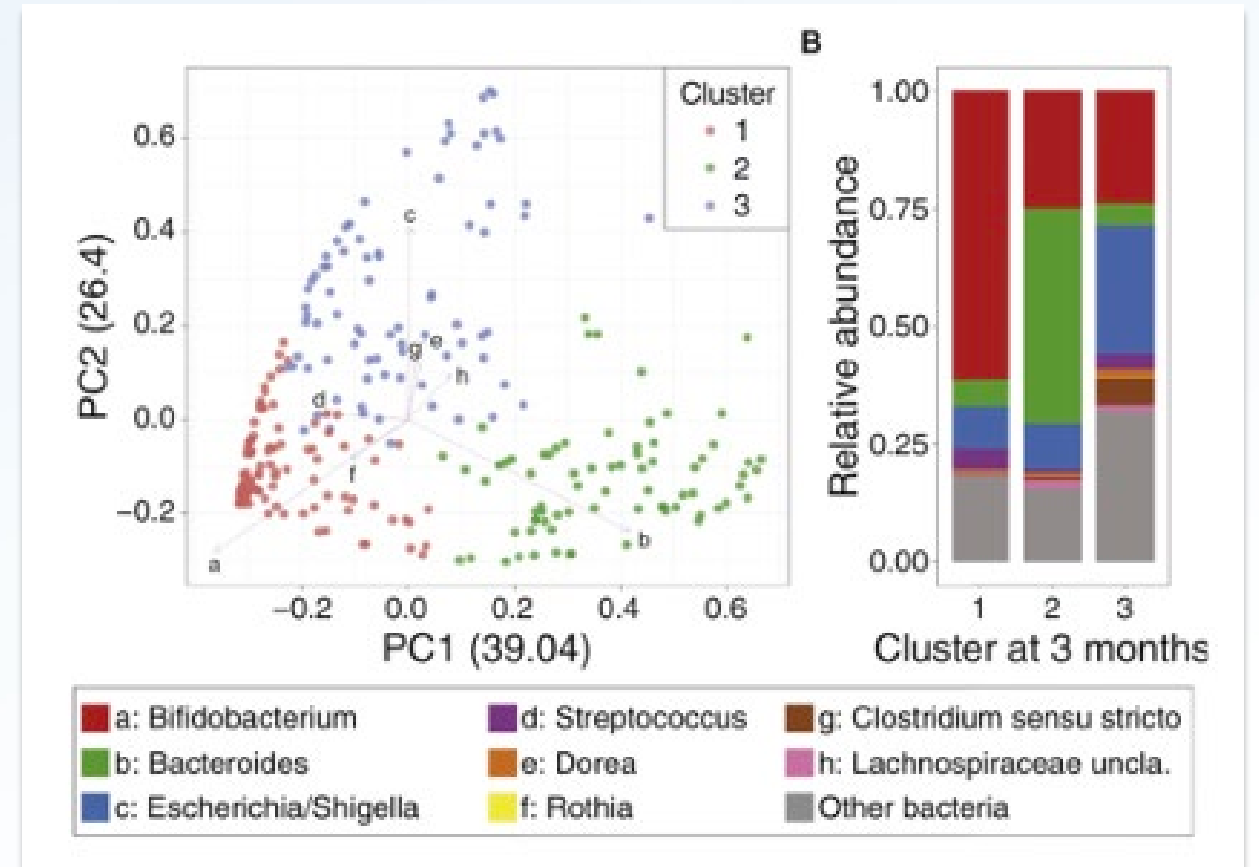
Diet a Key Influencer of Infant GI Microbiome

- Human milk directly contributes to the establishment of the microbiome
- Gut microbiota composition differs between breastfed and formula-fed infants
- Breastfed infants: Characterized by higher abundance of bifidobacteria and lactobacilli
- Formula-fed infants: Increased amounts of bacteroides, clostridia, and Enterobacteriaceae, including opportunistic pathogens such as *Clostridium difficile* and *Escherichia coli*



Diet Contributes to Evolution of the Infant Gut Microbiota

Authors from the EAT study investigated a nested cohort of infants undergoing randomized introduction of allergenic solids as part of a randomized controlled trial to prevent food allergy.



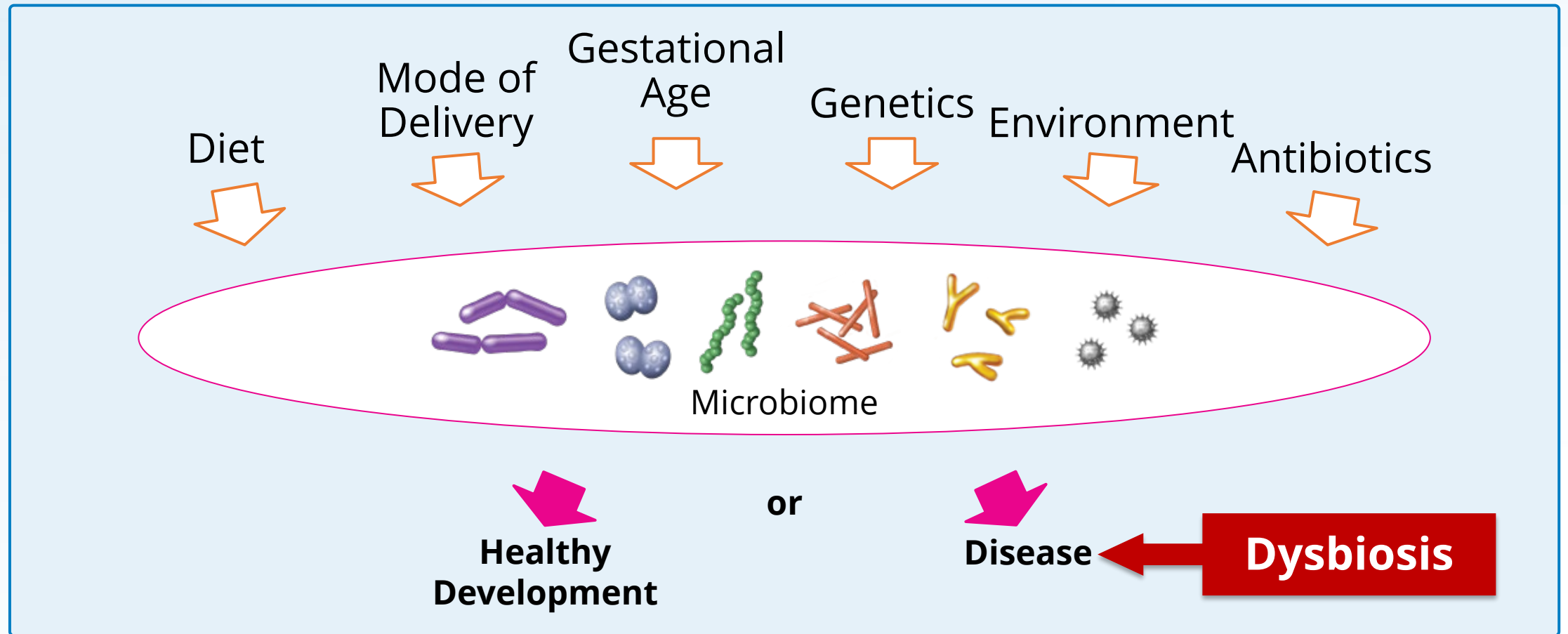
Diet Contributes to Evolution of the Infant Gut Microbiota

- In the EAT study, early peanut and egg introduction, if consumed in sufficient quantity, was shown to protect against the development of peanut and egg allergies between age 1 and 3 years
- It has been demonstrated that the early introduction of allergenic foods alongside ongoing breastfeeding between age 3 and 6 months led to an increase in overall gut microbiota, in particular promoting an influx of various microbes including *Prevotellaceae* and *Escherichia/Shigella*
- Interestingly, the presence of *Prevotella* has been shown to be associated with high-fiber diet, including in remote villages with less frequent chronic inflammatory disorders



Each Person Develops a Unique GI Microbiome

Influenced by:



Functions of the Intestinal Microbiota

Functions	Mechanisms/Effects
Digestive and metabolic functions	<ul style="list-style-type: none">• Vitamin production• Fermentation of nondigestible CHO → SCFA• Dietary carcinogens metabolism
Neuronal development	<ul style="list-style-type: none">• Modulation of brain gut axis during neuronal development• Motor control and anxiety behavior
Protective functions against pathogenic bacteria	<ul style="list-style-type: none">• Pathogen displacement• Nutrient competition• Production of antimicrobial factors• Activation of local immune response• Contribute to the intestinal barrier function
Immune development	<ul style="list-style-type: none">• IgA production• Control of local and general inflammation• Tightening of junctions• Induction of tolerance to foods

CHO, carbohydrates; SCFA, short-chain fatty acids; IgA, immunoglobulin A.



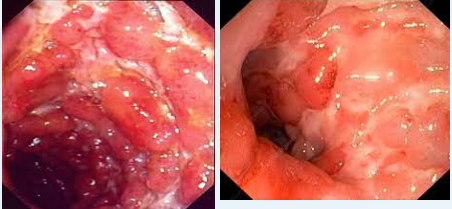
Dysbiosis

Dysbiosis is any perturbation of the normal microbiome content that could disrupt the symbiotic relationship between the host and associated microbes, a disruption that can result in diseases, such as inflammatory bowel disease and other gastrointestinal (GI) disorders, including gastritis, peptic ulcer disease, irritable bowel syndrome, and even gastric and colon cancer.

- Perturbation of normal microbiota
- Disrupts symbiosis
- Associated with short- and long-term health implications, including inflammatory and autoimmune diseases

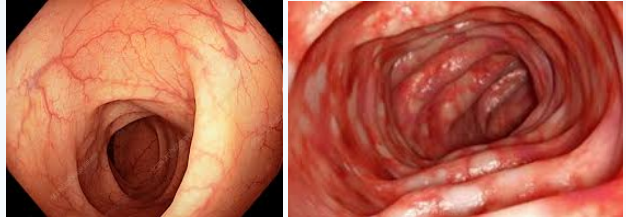


Where Are the Microbes Causing GI Disease With Dysbiosis?

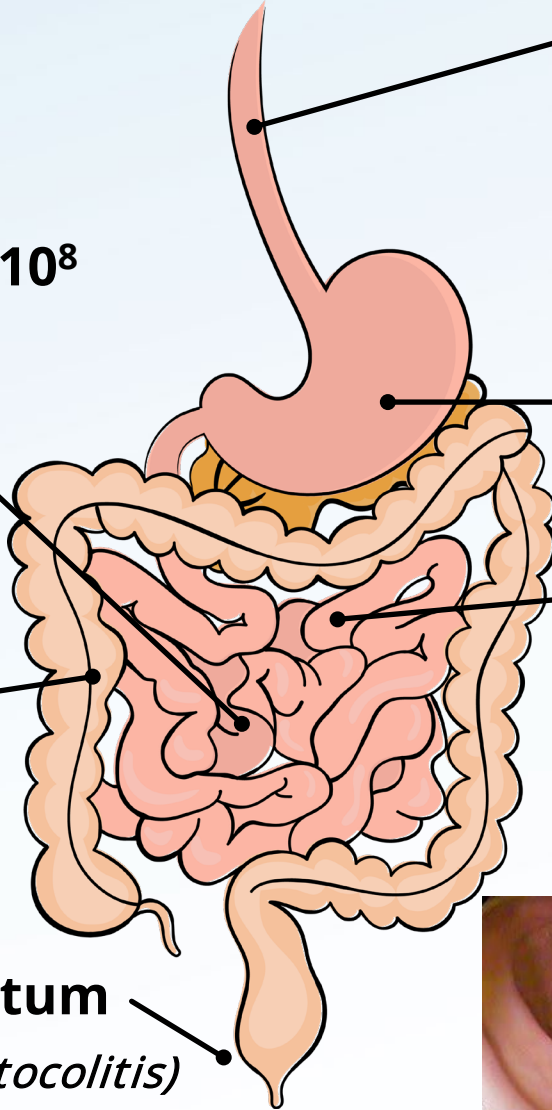


Distal Ileum 10^7-10^8

(NEC, IBD)



Colon 10^{11}



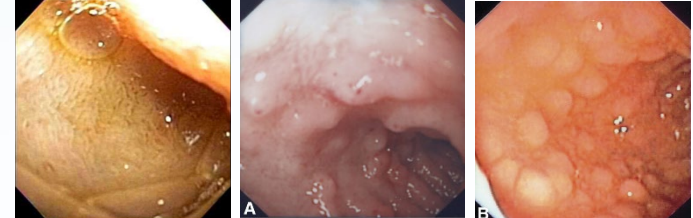
Esophagus $0-10^2$
(Eosinophilic Esophagitis)



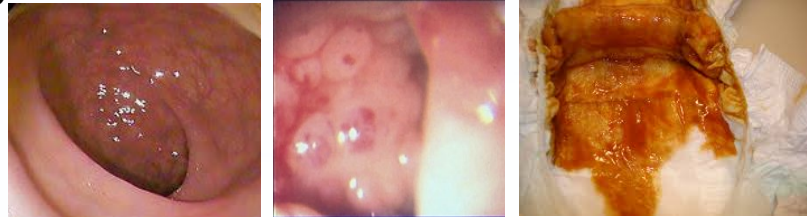
Stomach $0-10^2$
(H. pylori Infection)



Jejunum 10^2
(Celiac Disease)

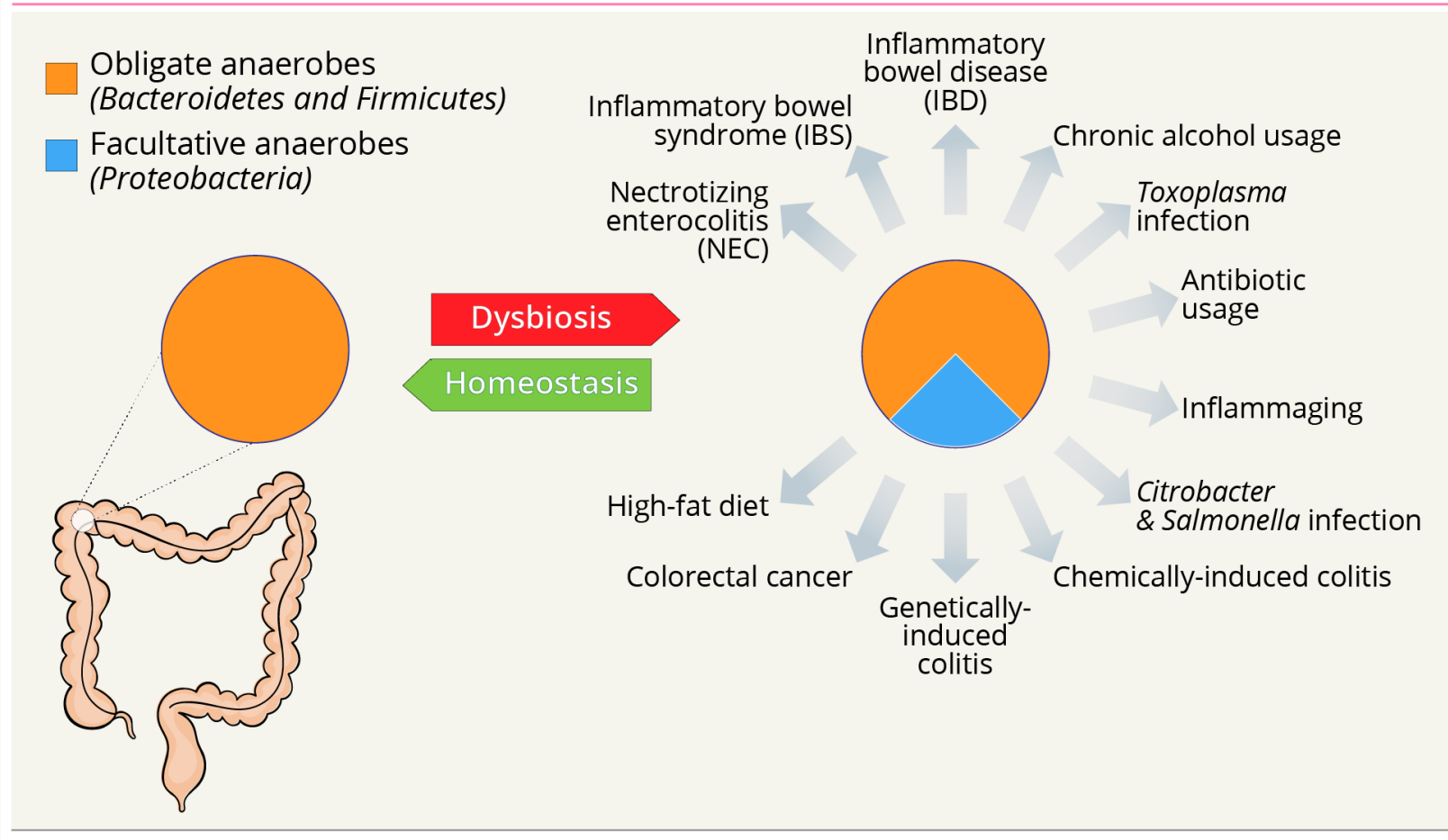


Rectum
(Allergic proctocolitis)



Where Are the Microbes Causing GI Disease With Dysbiosis?

Figure. **Dysbiosis: From Fiction to Function**



Adapted from Tiffany CR and Bäumer AJ. *Am J Physiol Gastrointest Liver Physiol.* 2019;317:G602-G608.



Vaginal delivery – a major source of bacteria for the infant



Why so many C-sections have medical groups concerned

Jen Christensen • 8 May 2014



Your Biggest C-Section Risk May Be Your Hospital

Consumer Reports finds that your risk of a cesarean section can be more than nine times higher depending on the hospital you choose

Tara Haelle • 16 May 2017



C-Section Rates by Gestational Age in the US

Births: Final Data for 2015

by Joyce A. Martin, M.P.H.; Brady E. Hamilton, Ph.D.; Michelle J.K. Osterman, M.H.S.; Anne K. Driscoll, Ph.D.; and T.J. Mathews, M.S., Division of Vital Statistics

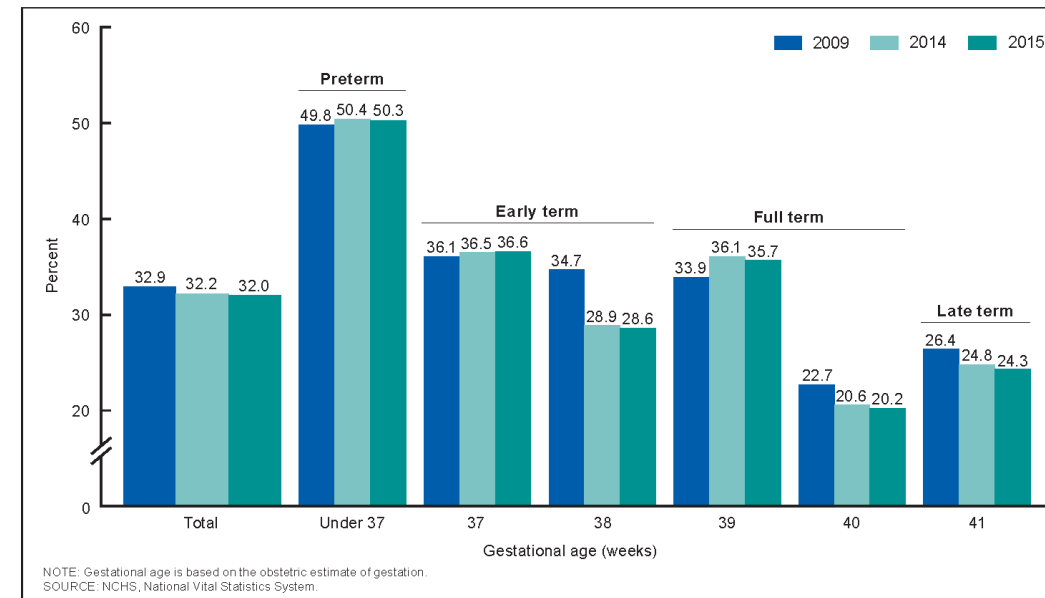
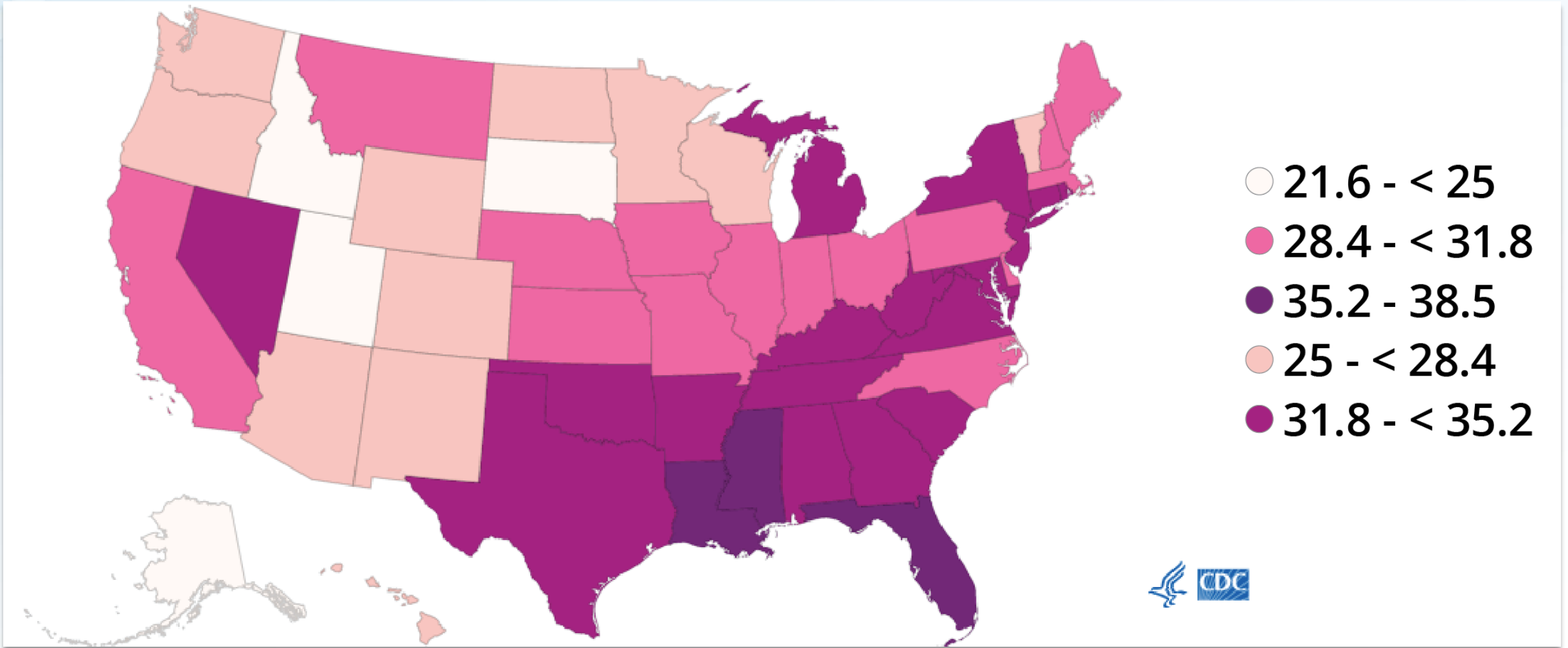


Figure 1. Cesarean delivery, by gestational age: 2009, 2014, and 2015



C-Section Rates in the US by State

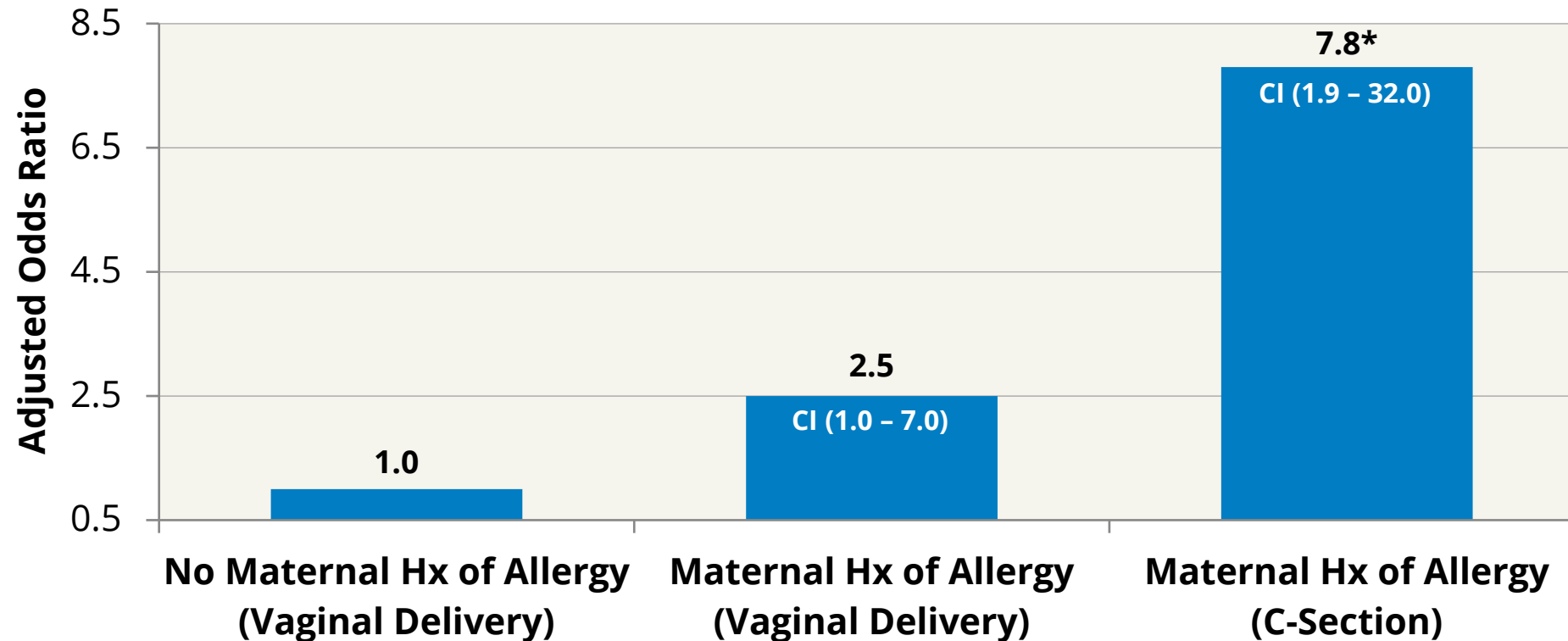


Centers for Disease Control and Prevention. Accessed April 26, 2021.
https://www.cdc.gov/nchs/pressroom/sosmap/cesarean_births/cesareans.htm.



Influence of Cesarean Delivery on Relative Risk of Childhood Food Allergy

Table. Food Allergy and Mode of Delivery



Food allergy to egg confirmed by testing at age 1-2 year. *p<0.01; adjusted for covariates

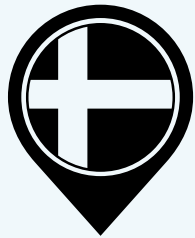


Influence of Cesarean Delivery on Relative Risk of Childhood Food Allergy

Medical Xpress | IMMUNOLOGY

C-section children run increased risk of developing food allergies – as opposed to very preterm children

Örebro Universitet • 10 September 2018



1.09 million newborns
2001-2012
Sweden

Percent
diagnosed with
food allergy

2.4%

Baseline risk

Vaginal/full-term
delivery
n = 901,262

2.9%

21% higher risk

Cesarean
delivery
n = 185,117

1.9%

26% lower risk

Very preterm
birth
n = 7741



Influence of Cesarean Delivery on Relative Risk of Childhood Food Allergy

Medical Xpress | IMMUNOLOGY

NewScientist | Health

Boost C-section babies by giving them vaginal bacteria


Jessica Hamzelou • 1 February 2016

nature medicine

Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer

Maria G. Dominguez-Bello, Kassandra M. De Jesus-Laboy, Nan Shen, Laura M. Cox, Amnon Amir, Antonio Gonzalez, Nicholas A. Bokulich, Se Jin Song, Marina Hoashi, Juana I Rivera-Vinas, Keimari Mendez, Rob Knight & Jose C Clemente • 1 February 2016



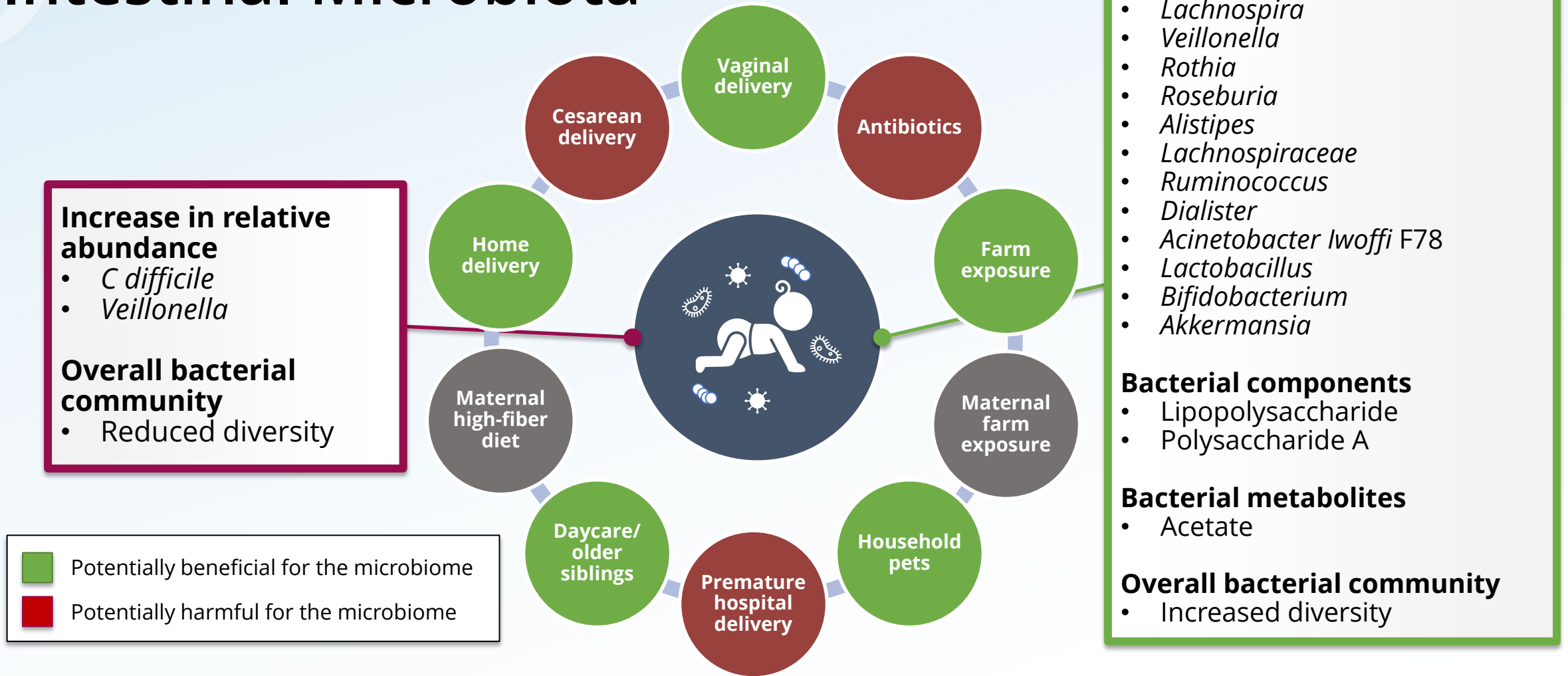


When the Newborn Microbiome is Changed...

Increase in Autoimmune, Allergic, and Inflammatory Diseases



Infant Exposures Help Define Their Intestinal Microbiota



Adapted from Sbihi H, et al. *Allergy*. 2019;74:2103–2115.





Prebiotics, Probiotics, and Neonatal Care



ISAPP Definitions for Terminology

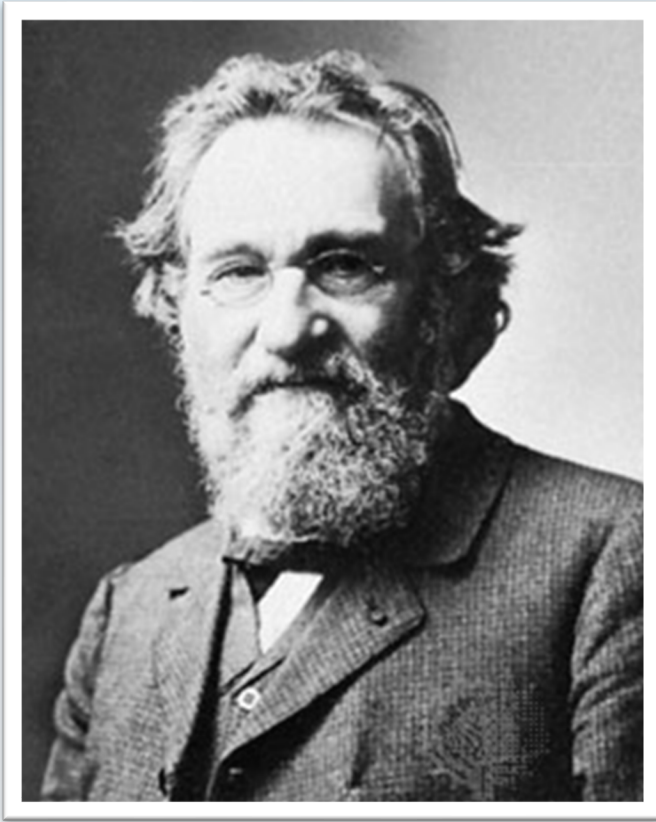
- **Probiotic:** "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [1]
- **Prebiotic:** "a substrate that is selectively utilized by host microorganisms conferring a health benefit" [2]
- **Synbiotic:** "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" [3]

ISAPP, International Scientific Association for Probiotics and Prebiotics.



1. Hill C, et al. *Nat Rev Gastroenterol Hepatol*. 2014;11(8):506-514.
2. Gibson GR, et al. *Nat Rev Gastroenterol Hepatol*. 2017;14(8):491-502.
3. Swanson KS, et al. *Nat Rev Gastroenterol Hepatol*. 2020;17(11):687-701.

Ingestion of Bacteria Proposed as Beneficial



Elie Metchnikoff
(1845-1916)

- Suggested that ingested bacteria could have positive influence on microflora in the intestinal tract
- Hypothesized that lactobacilli were important for human health and longevity
- Promoted yogurt and fermented foods as healthy



Ingestion of Bacteria Proposed as Beneficial



Lactobacillus sp^{1,2}

L acidophilus

L brevis

L delbrueckii

L fermentum

L gasseri

L johnsonii

L paracasei

L plantarum

L reuteri

L rhamnosus GG (LGG)

L salivarius



Bifidobacterium sp^{1,2}

B bifidum

B breve

B infantis

B longum

B adolescentis

B lactis (Bb12)



Other microbes²

Escherichia coli Nissle 1917

Saccharomyces boulardii

Saccharomyces cerevisiae

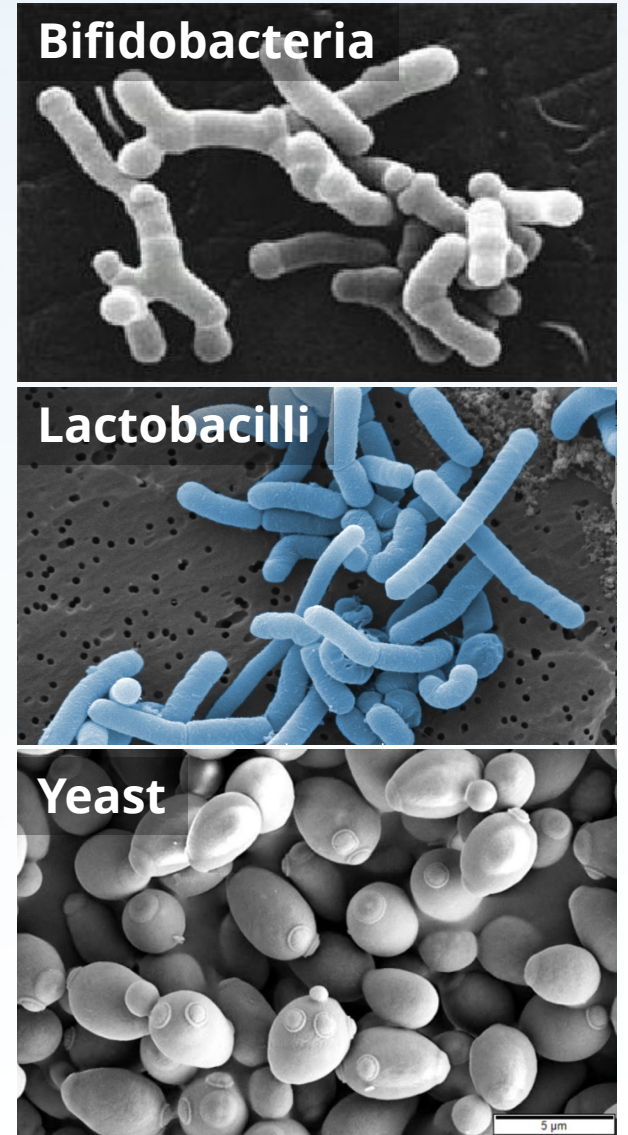
Enterococcus sp



Probiotics

- Nonpathogenic, live microorganisms in the food supply that, when **consumed or ingested** in adequate amounts, are capable of conferring a health benefit to the host
- Main genera used in commercial probiotics:
 - *Bifidobacteria*
 - *Lactobacilli*
 - Yeasts (*S boulardii*)—not yet cleared as safe by FDA Generally Recognized as Safe (GRAS) panel

Image of bifidobacteria from https://commons.wikimedia.org/wiki/File:Bifidobacterium_longum_en_microscopie_%C3%A9lectronique.jpg.
Image of lactobacilli from https://commons.wikimedia.org/wiki/File:Lactobacillus_paracasei.jpg. Image of yeast from https://commons.wikimedia.org/wiki/File:Saccharomyces_cerevisiae_SEM.jpg.
Used under terms of a Creative Commons License [CC BY-SA 3.0](https://creativecommons.org/licenses/by-sa/3.0/).



Prebiotics

- Food ingredients that benefit the host by stimulating the growth or activity of components of the gut microbiota
- Typically nondigestible oligosaccharides that are fermented by colonic bacteria
- Certain bacteria generate energy from these fermentation products



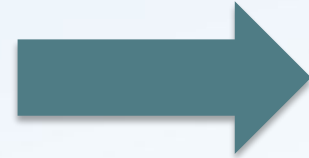
Breastmilk HMOs Can Help Balance the Microbiota and Support the Developing Immune System (Prebiotic Effect)

- Balance the microbiota
 - Enhance growth of bifidobacteria
 - Inhibits adhesion of pathogens
- Support the infant's developing immune system
 - Circulate in the bloodstream
 - HMOs influence lymphocyte maturation and promote a shift in T-cell response



Premature Infants: Set-up for an Altered Microbiota and its Potential Consequences

- C-section birth
- Less chances of being breast fed
- NICU microbes
- Antibiotics



Delayed establishment of microbiota
Aberrant composition of microbiota



Inadequate GALT development and maturation
Decreased gut barrier (mucin, permeability)
Poor humoral and cellular immune response



Contributors to and Consequences of Gut Dysbiosis

Contributors (all infants)

- Hospital environment
- Maternal microbiota
- Mode of delivery
- Feeding type
- Home environment
- Antibiotics
- Feeding tube biofilms (preterm)

Dysbiosis-associated diseases

Preterm infants:

- Necrotizing enterocolitis (NEC)
- Late-onset sepsis

All infants:

- Neurodevelopmental impairment
- Colic
- Atopic and autoimmune diseases
- Type 1 diabetes
- Metabolic disorders and obesity



Premature Infants: Set-up for an Altered Microbiota and its Potential Consequences

- Early recognition and aggressive treatment of necrotizing enterocolitis (NEC) has improved clinical outcomes
- NEC accounts for substantial long-term morbidity in survivors of neonatal intensive care
- NEC is particularly significant in preterm very low birth weight (VLBW) infants (BW <1500 g)

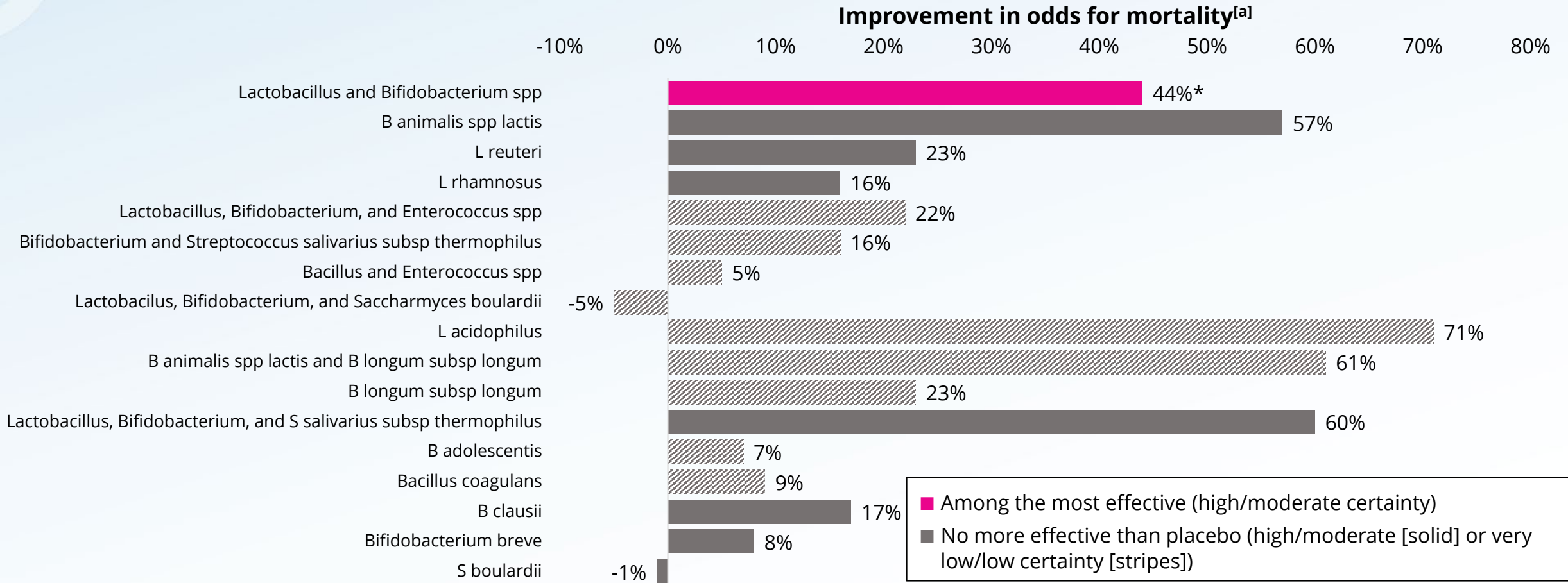


Probiotics for Prevention of Necrotizing Enterocolitis in Preterm Infants (Update)

- Meta-analysis of 24 trials
- Probiotic supplementation was shown to:
 - Reduce severe NEC (typical relative risk [RR], 0.43; 95% CI, 0.33-0.56)
 - Reduce mortality (typical RR, 0.65; 95% CI, 0.52-0.81)
- Probiotics had no effect on:
 - Nosocomial sepsis (typical RR, 0.91; 95% CI, 0.80-1.03)
- Probiotics containing either lactobacillus alone or in combination with bifidobacterium were effective
- Head-to-head studies are needed "to assess the most effective preparations, timing, and length of therapy"



Probiotic Effect on Mortality in Preterm Infants

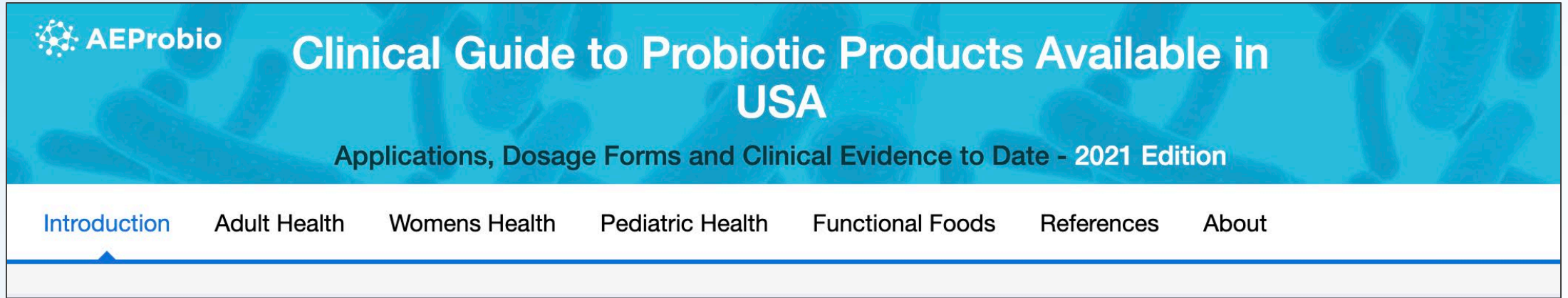


a. In a meta-analysis of 63 trials of preterm infants (n = 15,712) comparing probiotics with placebo

*Statistically significant



Clinical Guide to Probiotic Products Available in the US



For more information, visit <http://www.usprobioticguide.com/>



Summary

- Provided an overview of how the infant's immune system develops and the interaction with the gastrointestinal microflora
- Noted the importance of the gastrointestinal microbiome in health and disease
- Reviewed how prebiotics and probiotics can alter intestinal microflora and potentially change health outcomes



Clinical Practice Guidelines

AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders^[1]

Key guideline recommendations:

- For preterm (born before 37 weeks), low birthweight (<2500 g) infants, specific probiotics can prevent mortality and necrotizing enterocolitis, reduce the number of days required to reach full feeds, and decrease the duration of hospitalization.
- Certain probiotics should be considered for the prevention of *C. difficile* infection in adults and children who take antibiotics and for the management of pouchitis, a complication of ulcerative colitis that has been treated surgically.



Clinical Practice Guidelines

AGA Clinical Practice Guidelines on the Role of Probiotics in the Management of Gastrointestinal Disorders^[1]

Key guideline recommendations:

- Probiotics do not appear to be beneficial for children in North America who have acute gastroenteritis — they should not be given routinely to children who present to the emergency room due to diarrhea.
- There was insufficient evidence for AGA to make recommendations regarding the use of probiotics to treat *C. difficile* infection, Crohn's disease, ulcerative colitis or IBS. For these conditions, AGA suggests that patients consider stopping probiotics, as there are associated costs and not enough evidence to suggest lack of harm.



Key Concepts

Where are the microbiota?

- Found throughout the GI tract
- Organized into specific compartments on the mucosal surface and within the gut lumen

Who are they?

- The microbiota of early childhood is very unstable and highly susceptible to environmental influences
- In adults, *Firmicutes* and *Bacteroidetes* are the 2 predominant phyla
- Great deal of diversity at the species level that defines a unique microbial signature for each of us

What are they doing?

- Nutrition and metabolism
- Regulation of the immune system
- Maintenance of gut structure

How can they be manipulated?

- Prebiotics/diet
- Probiotics/fecal transplantation
- Synbiotics (combination of pre- and probiotics)

