Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

COURSE TRANSCRIPT

Probiotic Use in Preterm Infants and Children

Differentiating Between Health and Disease

Overview

The gut microbiome plays a key role in the development of childhood immunity, and gut dysbiosis has broad-reaching implications for health. In this course, **Benjamin D. Gold, MD**, and **Jonathan Malka, MD**, provide learners with a comprehensive overview of the evolving immune system from infancy through childhood, with a focus on the complex interplay between the gut microbiome and immunity. In addition, participants will learn about the impact of gut dysbiosis on allergy and autoimmunity, the ongoing efforts to modify these effects, and the impact that prebiotics and probiotics can have on the developing immune system, with an emphasis on current best-practice guideline recommendations.

This activity is part of the curriculum, *Understanding Food Allergies in Infants and Children: The Symptoms, Diagnoses, and Management*.

Target Audience

This activity was developed for pediatric physicians, nurses, advanced practice clinicians, dietitians, allergists, and other healthcare providers who have an interest in newborns, infants and toddlers.

Learning Objectives

At the conclusion of this activity, participants should be better able to:

- 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.

Faculty

Benjamin D. Gold, MD, FACG, FAAP
Attending Physician
GI Care for Kids, LLC
Children's Center for Digestive Health Care, LLC
Atlanta, Georgia

Jonathan Malka, MD, FAAAAI
Director of Allergy and Immunology
Pediatric Allergy
Pediatric Associates
Miami, Florida

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Advisor/Speaker	Mead Johnson Nutrition

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Stanley A. Cohen, MD (Curriculum Chairperson)

Advisor:	Nutrition4Kids
Consultant:	AbbVie, AstraZeneca, Janssen, Mead Johnson Nutrition, Medtronic, QOL
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The estimated time to complete the activity is 1.25 hours.

This activity was released on June 11, 2021, and is eligible for credit through June 11, 2023.

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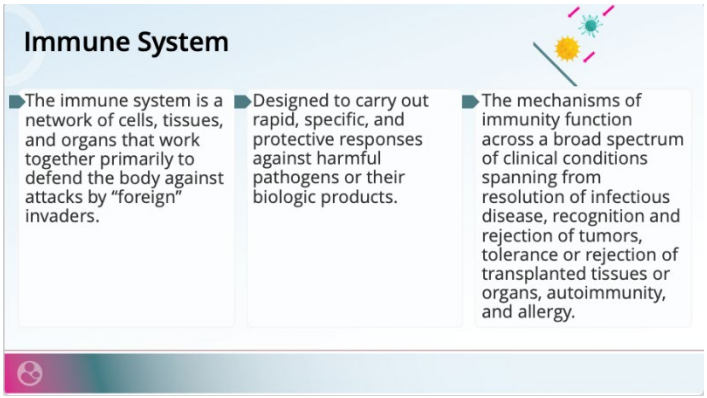
Editor's Note: This is a transcript of an online presentation broadcast on May 6, 2021. It has been edited and condensed for clarity.

Jonathan Malka, MD: Today's objectives: we'll have a great discussion starting with the immune system and how the immune system develops in children [and] interacts with the microbiome, which will lead then to an overview of the microbiome, which all link together. The gut becomes more and more a relevant organ for the immune system. Therefore, when you have a gut problem, or a gut organ, as a regulator, it may have either benefit or disease. The disease may be dysbiosis. Factors that affect the microbiome. Outcome of dysbiosis (diarrhea, malnutrition, allergies, and autoimmune diseases). Prebiotics and probiotics, and the use of prebiotics and probiotics in preterm infants and other common conditions.

Immunity in the Developing Child and the Gut Microbiome

To understand a little bit about the immune aspect of the gut, we need to start with understanding what the immune system is. The immune system is a network of cells, tissues, and organs. They all work together to primarily defend the body against attack by foreign invaders. [The immune system] is assigned to carry out specific, rapid, protective responses against harmful pathogens or their biological products. The mechanisms of immune function across the broad spectrum of clinical conditions can span from resolution of infectious diseases, recognition of rejection or tumors, tolerance or rejection of transplanted tissues, or autoimmunity and allergy. Like anything in our body, we have to have homeostasis. We have to have a good balance between good and bad in order for the system to work (Slide 1).



Slide 1 – Immune System

The immune system—when it's in good condition—a healthy immune system [protects] you against antimicrobial activities, such as bacteria or viruses, coronavirus for example.

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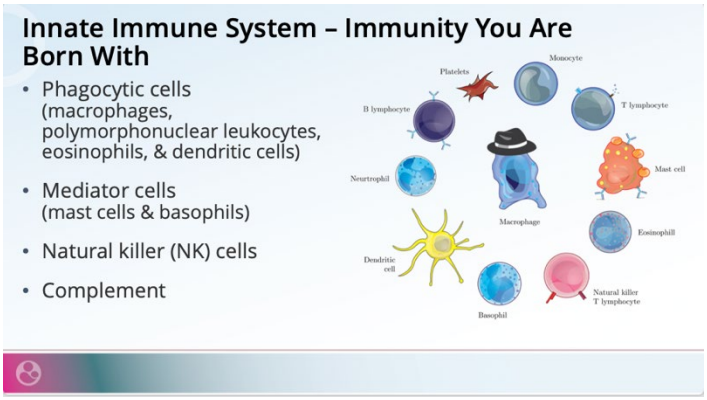
[The immune system] has to have homeostasis; it has to keep the good cells and remove the damaged cells, and it has to keep having a surveillance system in order to remove any mutant cells that may activate the immune system to go against its own cell.

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

Table 1 – Functions of the Immune System

When you have a hyperfunctioning immune system (Table 1), you then will have, for example, rather than have a defense, you'll have an allergic component. For example, a lot of patients come to the clinic and ask me, "Does my child have asthma? Does he have an immune deficiency?" The answer I always give them is that if it's an allergic type of disease, they have a hyperactive immune system. We have to work on taking care of the inflammation that your immune system is producing on your own body. If you lose homeostasis, you will have autoimmune diseases. Lupus, for example, is a common one, along with many of the gut disorders, which we will see later. And you may not have good surveillance, that may lead to more difficulties in recognizing your own cells. If you have decreased immune function, you then tend to have all the immune deficiencies, and potentially will be more prone to malignancies, because of the increased prevalence of mutant cells or cancer cells that are not being removed by [a functioning system].

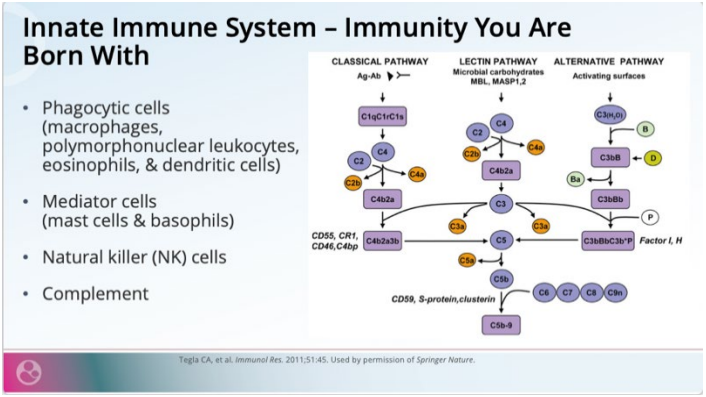
The immune system is quite complex. You have different parts of the immune system that will play different roles. The innate system is composed of many of the cells that we actually are encountering every day when we do our complete blood counts (CBCs) or testing, or are worried about our patients' health. Those include, for example, macrophages, which are the big eaters, right here in the middle (Slide 2). You have dendritic cells, [which fight bacteria, viruses, and fungi]; neutrophils, which help to fight bacteria [and fungi]; B lymphocytes [of the adaptive immune system]; platelets; monocytes; T lymphocytes [of the adaptive immune system]; mast cells, which become allergic cells, [along with eosinophils and basophils]; and the natural killer cells [which fight viruses and cancer cells].



Slide 2 – Innate Immune System – Immunity You Are Born With

All of [the immune cells] are working in conjunction with other parts of the innate system, such as the complement system (Slide 3). I'm sure you guys will recall from your medical school years, all this crazy sequence of C1, C4, C2, it's an alphabet of Cs that I cannot for the life of me recall, but all those have different pathways of the complement system: the classical pathways, the lectin pathway, and the alternative pathway. All of them are linked together in a way, with the cells mentioned previously, that will promote tolerance or

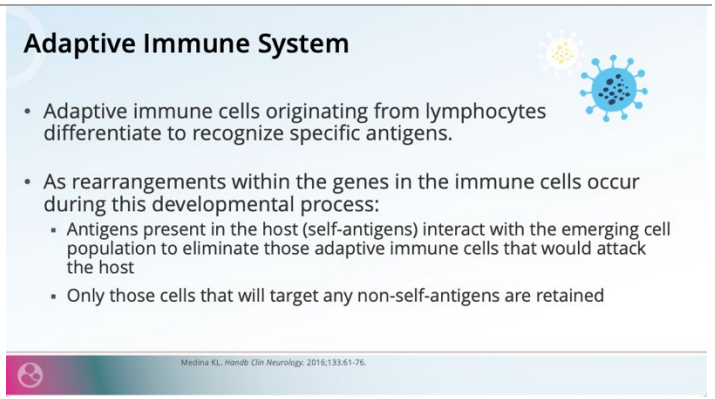
protect your system by having an innate immune system that basically recognizes microbial non-self. So it [distinguishes] microbes from your own good bacteria.



Slide 3 – Innate Immune System – Immunity You Are Born With

What Is the Adaptive Immune System?

Now, the innate system is somewhat robust. It's very quick to work, but it does not last long, so you have to have an adaptive immune system (Slide 4). As we get exposed to certain antigens, we will then promote the adaptive immune system. The adaptive immune system's cells originate from lymphocytes that differentiate to recognize specific antigens. For example, you get the common coronavirus, or you may get a viral disease, you produce memory cells. Again, that's the IgG. Those cells will then protect you from reinfection, hopefully for some time, but they also may be lost.

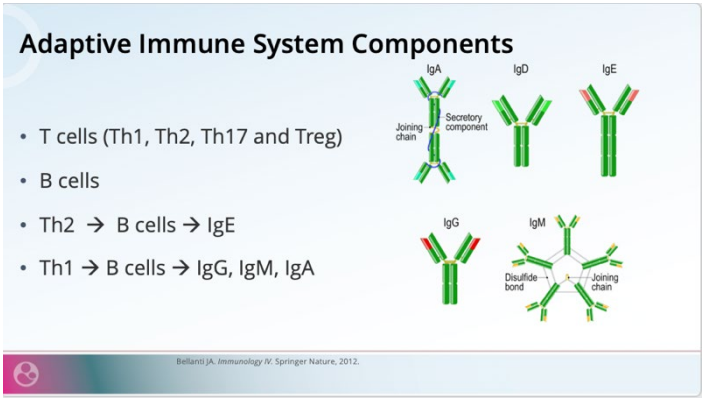


Slide 4 – Adaptive Immune System

The adaptive [immune response is developed by] a rearrangement, within the genes of the immune cells that, during the development process, will then activate and learn from the presentation of the host of the antigen. So it will attack the bad cells, but also recognize your own cells in order to not attack them. In a way, it differentiates between what you need to be exposed to—[things that are] good for your system, such as foods, for example. Foods become protein. The immune system has to learn to not react in order to not become allergic to the food. Allergic patients will not recognize the food as a good protein. It will develop an immune response that will lead to allergic response.

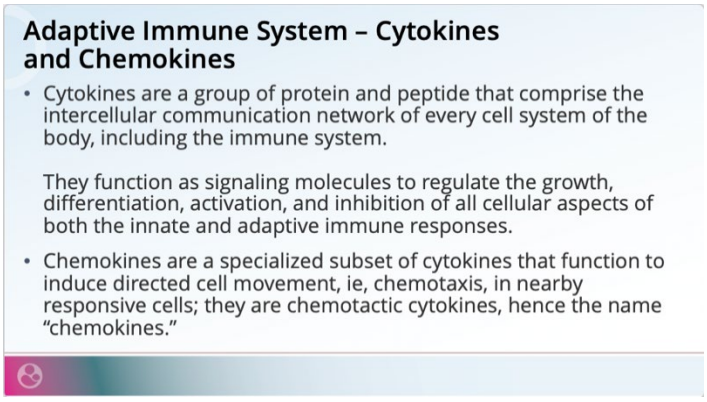
In order for the adaptive immune system to work—and we have to have significant work from this side of the immune system—you have many, many, immune globulins and you have many different pathways that we learn about more and more (Slide 5). You have the Th1, Th2, Th17, and T regulatory cells (Tregs). All of them becoming more and more important. Tregs are like the big police officers that truly drive the immune system one way or the other in health and disease. The Th17 is also a very big player. Th2 becomes more prominent in allergic

disorders. Th1 is more for viral-type disease [and also for bacterial infections]. You have the subclass of immunoglobulin still within their own IgG. You have 1, 2, 3, and 4, and each one of them will have different bacterial protection against different pathogens.



Slide 5 – Adaptive Immune System Components

In order for the adaptive system to communicate with itself and to the innate system—because they both have to work together to have a proper immune system work—you have to have mediators. Those are known as cytokines and chemokines (Slide 6). I see them, for example, as the hormones of our immune system. They basically give signals out, and those signals will lead to promotion of different cells within their own immune system.



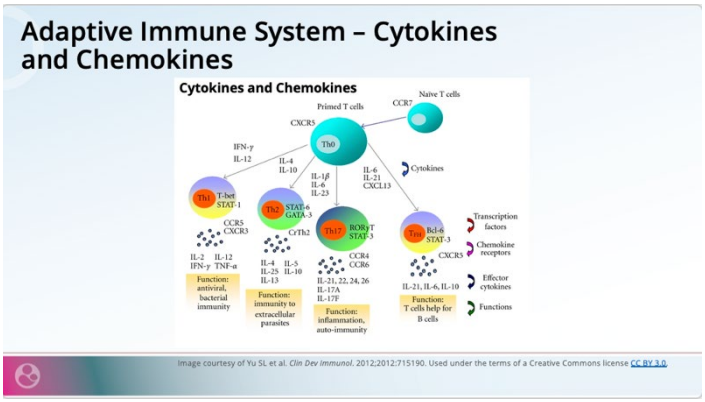
Slide 6 – Adaptive Immune System – Cytokine and Chemokines

Cytokines are a group of proteins and peptides that comprise the intercellular communication network of every cell system to the body, including the immune system. They function as signaling molecules to regulate growth, differentiation, activation, and inhibition of all cellular aspects of both the innate and adaptive immune responses.

The chemokines are a more specialized subset of cytokines that function to induce direct cell movement. For example chemotaxis attracts the cells to specific organs where they have to work, hence the name chemokines. They attract and they work with the cytokines. Now, these are very important, because these are now what we're using to target different new therapies for some of the allergic disorders and some of the autoimmune processes, as you will see in Dr. Gold's presentation.

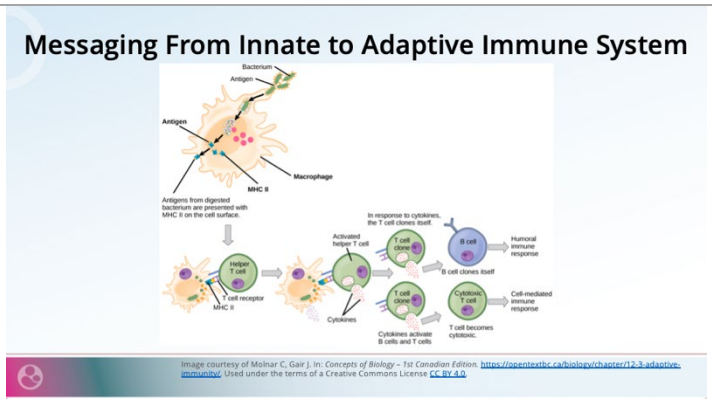
As you can see here, there are many, many, cytokines and chemokines (Slide 7). This is just an example of how complex the system is—how you have different IL-1, IL-4, IL-5. In the allergy world, the biggest ones, for example, are IL-5 and IL-13. IL-4, IL-5, and IL-13 become important. But it's so complex that you have different transcription factors. You have STAT-6 and GATA-3. You have different chemokine receptors, so you can work at the specific receptor. You can work on a transcription factor; you can work at the effector cytokines. So depending where an agent blocks, it may decrease the overactive immune system in a way, for example, in allergy disorders. But the problem, if you block too high [up the cytokine cascade], you may also block a good component of the immune system and lead to some subsequent infections or other problems

when you deal with these hyperimmune dysfunctions.



Slide 7 – Adaptive Immune System – Cytokines and Chemokines

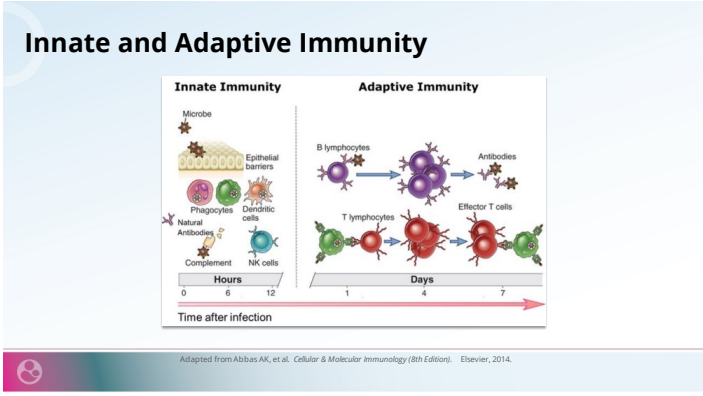
As you can see here (Slide 8), if you look at how bacteria or antigens come into your system, we produce our immune response. You have here, the bacteria come in contact with the antigen. It gets to the macrophage, which is the “Pac-Man” who brings it into the rest of the immune cells. They have specific receptors, the major histocompatibility complex (MHC) II, [which express the antigens]. The particles get expressed, and it goes to a naïve cell. The T helper cell then says, "Okay, this is part of the innate system." The helper gets in, says, "Okay, there's a protein here. I don't know you. Let me get the B cells to come in." The B cells come in. The helper T cells become activated. And then, you have different types of cytokines being released at each of these steps along the way.



Slide 8 – Adaptive Immune System

Then, you have different activation of either B cells or T cells. The B cells become more of a humoral response, which is more the bacterial [and viral] type of response. The cytotoxic T cells become more the cell-mediating response, more like interferon gamma production, which is more for tuberculosis, viral diseases, and other types of infections.

Here you can see the complexity between the innate and the adaptive immune systems (Slide 9). Actually, I mentioned earlier, the innate system, and I'll mention this in a minute, is part of our membranes. It's part of our lungs, our gut. It is what we have. It works really quickly. It's very robust, not very specific. So, it comes in quickly, leaves quickly, within 12 hours it's gone. Therefore, the adaptive immune system becomes the one that will produce the long-term response and produces the antibodies, which is what will produce the memory cells over time. Therefore, either a protein will be tolerated or the protein will not be tolerated, and then the immune system will attack it and become more of the immune response in order to protect you.

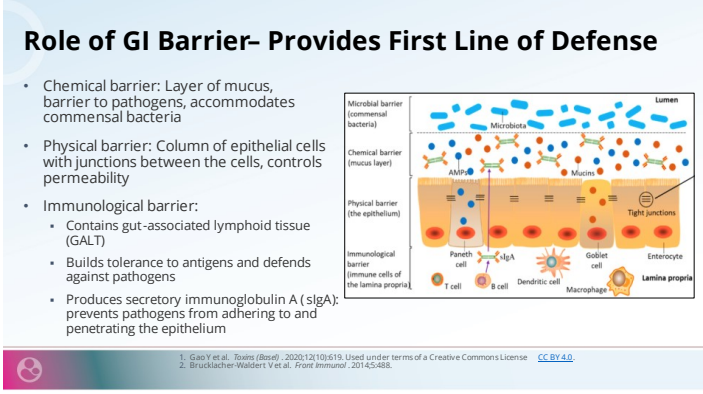


Slide 9 – Innate and Adaptive Immunity

Microbiota, Microbiome and Normal Development vs Dysbiosis

Now, as we have progressed, and the reason why Dr. Gold and I are here together, is because we have learned over time that the microbiome—the microbiota and the gut—are really major organs that play a role in the immune system. One of the biggest organs we have, other than the skin, is actually our gut. Now to the role of the gastrointestinal (GI) barrier. The GI barrier provides a first line of defense. It's quite a complex and interesting organ, because it really has to work on filtering the good and the bad. So, you have to get the nutrients in, but you also have to remove the bad pathogens or other bacterial viruses that may actually have harmful effect.

It is important because it's associated with a broad range of either disease or protection. For example, in disease you have celiac disease, you have inflammatory bowel diseases (IBDs), colon carcinomas. You may have type 1 diabetes. It has to do this function. The GI system is composed of several elements that aid in function and it includes a physical and immunological defect barrier (Slide 10). So, you can see the microbial barrier is the one with commensal bacteria on the top. It is the first one to come in contact with the pathogen. Then, you have the epithelial cells, which have an important tight junction between them that will control the permeability and will also be very, very, important as a way to protect us from the pathogens that we may be exposed to.

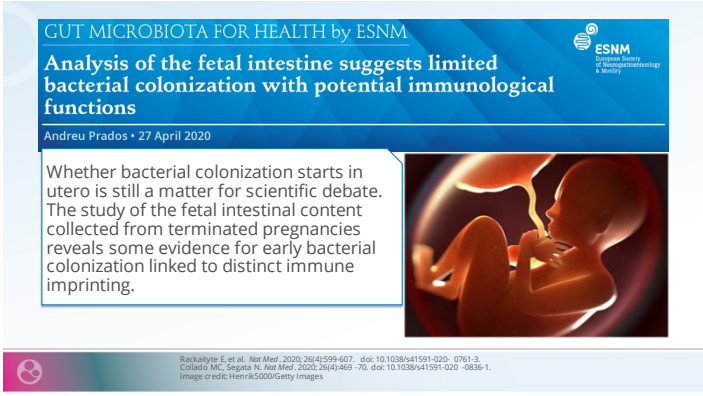


Slide 10 – Role of GI Barrier – Provides First Line of Defense

And then, within the physical barrier and the lamina propria, you'll have different types of immune components, such as gut-associated lymphoid tissue (GALT), which is divided into 2 categories. It either has an organized GALT, which is made up from follicles such as Peyer's patches or mesenteric lymph nodes, or you may have diffused GALT, which is single T and B cells, macrophages, eosinophils, basophils—different types of cells that are present in order to have the first step of immunity. Here, you can see the gut is truly our first barrier for protection against bacteria, but also it has to divide between protective effects and the beneficial effects of what it's being exposed to. It has a very complex function.

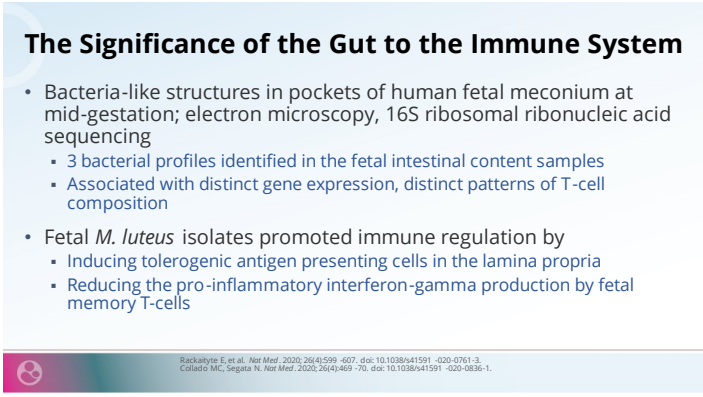
Now, the microbiome is an important part, as you can see in Slide 10. It is known that it is very important in many conditions, such as food allergies, such as IBD, and others. But we truly don't know when the microbiome starts (Slide

11). There is a debate about it in the literature. Does it start in utero? When does it start in utero? What bacteria do we get? What bacteria don't we have?



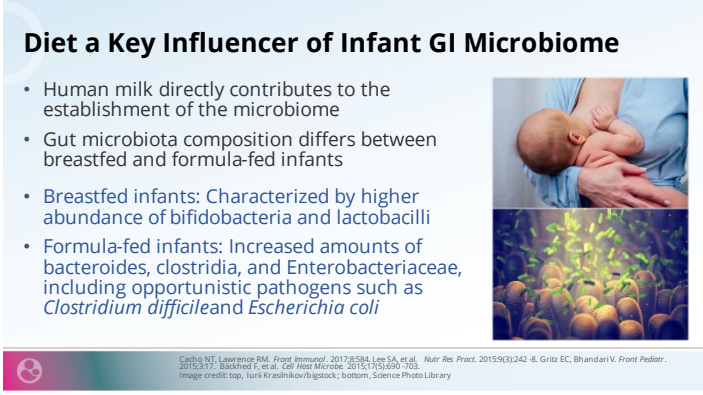
Slide 11

Studies have shown that, for example, looking at fetal meconium, mid-gestation, they found 3 different bacteria profiles identified in intestinal content samples (Slide 12).[1] They found that *M. luteus* isolates promote immune regulation, which is the one that actually seems to survive the longest in utero. It has an important function [in reducing] proinflammatory production of interferon gamma, which is T cell–mediated function, so it protects you in a way. It also induces [tolerogenic] antigen-presenting cells.[1]



Slide 12 – The Significance of the Gut to the Immune System

There are key factors that influence the microbiome (Slide 13). Human milk directly contributes to the establishment of the microbiome. The microbiome differs between breastfed formulas and kids who are formula fed. The breastfed infants usually have higher levels of Bifidobacteria and *Lactobacillus* vs formula-fed infants, who have increased amounts of bacteroides, clostridia, and Enterobacteriaceae.[2,3]

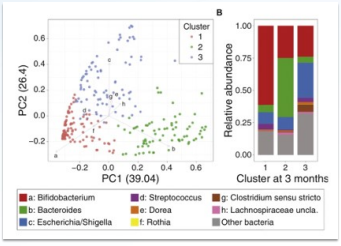


Slide 13 – Diet a Key Influencer of Infant GI Microbiome

Now, it is interesting, because we have learned, for example, in food allergies, before we get more into the probiotic component, that depending on when the food is [ingested and] exposed to the patient's microbiome, you may actually develop some tolerance to it or not. In Slide 14, I'm showing a study, the EAT study, which is Early Acquired Tolerance, that looked at different foods being given to children at a very early age to see if they can actually have tolerance earlier and not develop a food allergy. They looked at the microbiome of those patients.[4]

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.



Slide 14 – Diet Contributes to Evolution of the Infant

They found that in the EAT study, early peanut and egg introduction, if consumed in sufficient quantity, protected against the development of peanut and egg allergies between ages 1 and 3 years (Slide 15). It has been demonstrated that the early introduction of allergenic foods alongside ongoing breastfeeding between 3 and 6 months led to increased overall gut microbiome, particularly promoting influx of various microbes, including *Prevotellaceae*, and it increased *Escherichia* and *Shigella*. The prevalence of *Prevotellaceae* has been shown to be associated with higher-fiber diet, including remote villages with less frequent chronic inflammatory disorders.[5]

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 promotingan 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

Slide 15 – Diet Contributes to Evolution of the Infant

Again, it's not just when the microbiome is present, it's how we promote the microbiome. Can we have a better microbiome by introduction of certain foods early? That depends on the diet and exposure of the patient, depends on breastfeeding vs not breastfeeding. Each person has his own microbiome, which is influenced by many factors, like diet, mode of delivery, gestational age, genetics, environment, antibiotics.

It is quite important to understand that the microbiome has to be developed early and should be influenced early. Because, by 3 years of age, it has been shown that the child's microbiome will be part of the adult microbiome, and that cannot be changed. Therefore, the early exposure is quite important.

Now, moving forward with functions on the intestinal microbiome and other roles of the gut, I'll leave it to Dr. Gold.

Benjamin D. Gold, MD: Thank you, Dr. Malka. I appreciate the first part of the presentation and the opportunity now to take part. From the GI standpoint—or as a poop, vomit, burp, and fart doctor—I think the world revolves around the GI tract. What Dr. Malka brings in from the allergy perspective, and what he has provided for you in the first part of the presentation with respect to the overview of immunity, and then introducing the concept of the microbiome and the particular factors shown and highlighted on Slide 16, which influence the microbiome, result in either the arrow at bottom left, which is the healthy environment, or this concept, which I want you to take home tonight, which is the pathway on the bottom right: dysbiosis or disease.

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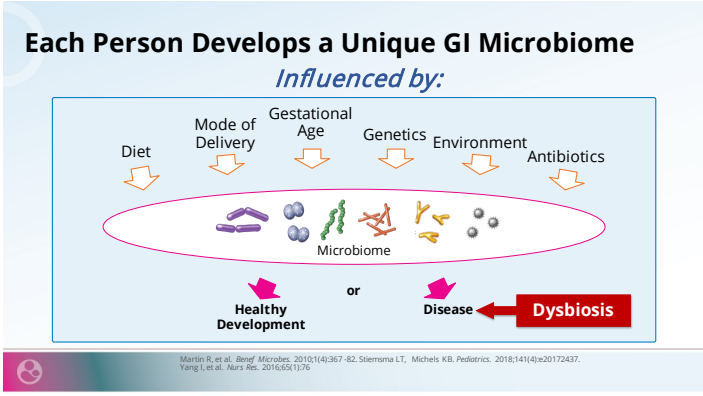


Slide 16 – Each Person Develops a Unique GI Microbiome

The microbiome, by definition, is made of 2 major components. First, the microbes that are there, which can be bacteria. They can be parasites or protozoa. They can be viruses, and they can be fungi. The combination of those microbes makes up the microbiota. And then, together with their byproducts, are what makes up the microbiome. They all live in this very tightly controlled community of multiple types of organisms in the different compartments of the body. The more diverse the microbiome is, influenced by the factors that Dr. Malka just briefly touched on, the better the result, or the more robust they are, in interacting with the intestinal epithelium and the host immune system to result in a good, healthy, robust immune system. The less diverse the microbiome, then the less healthy or more dysbiotic, if you will, the immune system is.

In terms of functions of the intestinal microbiome, there's a whole host of them. I think if there's anything that I feel has been one of the most exciting advances in science and medicine, it's really understanding that right there living within our body and on each of the different compartments of our body, there's a unique microbiome that, interacting with the host, actually [influences] outcomes.

As you heard from Dr. Malka's part of the presentation, it's early on, even prior to delivery, that the foundation species of microbes are getting established. They interact with the developing immune system to then result in either health or disease.

As shown in Table 2, the intestinal microbiota do things like metabolic functions, vitamin production, fermentation of nondigestible carbohydrates. They're involved in protection against pathogenic bacteria, which is what used [to be believed to be the] only role for the microbiome, but that's really just a part of what they do. They're involved in immune development, as you heard from Dr. Malka's elegant presentation, and in particular with the different components of immunity resulting in either an exaggeration or abundance, which then results in disease, or downregulating the immune system and a balance, and therefore health.

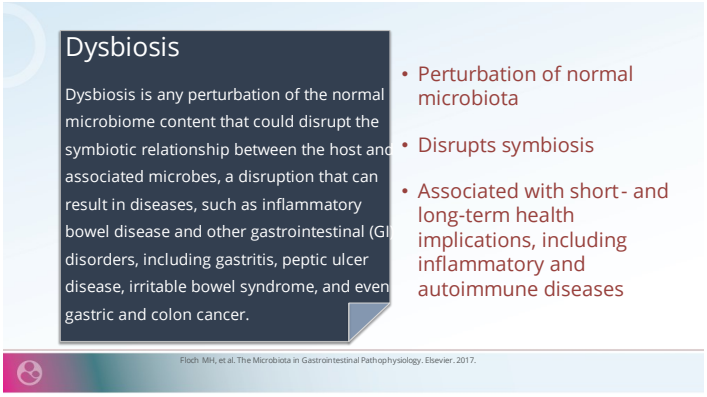
Functions	Mechanisms/Effects
Digestive and metabolic functions	• Vitamin production • Fermentation of nondigestible CHO → SCFA • Dietary carcinogens metabolism
Neuronal development	• Modulation of brain gut axis during neuronal development • Motor control and anxiety behavior
Protective functions against pathogenic bacteria	• Pathogen displacement • Nutrient competition • Production of antimicrobial factors • Activation of local immune response • Contribute to the intestinal barrier function
Immune development	• IgA production • Control of local and general inflammation • Tightening of junctions • Induction of tolerance to foods

Table 2 – Functions of the Intestinal Microbiota

What I think is very exciting is the whole relationship between the brain and the gut. And that is neuronal development, both by direct interaction between the microbes and the neuroenteric nervous system, and indirect by neurotransmitters either produced by the bacteria that live there or neurohormones, neurochemokines, or signals, which then result in a brain-gut influence. Ranging from things

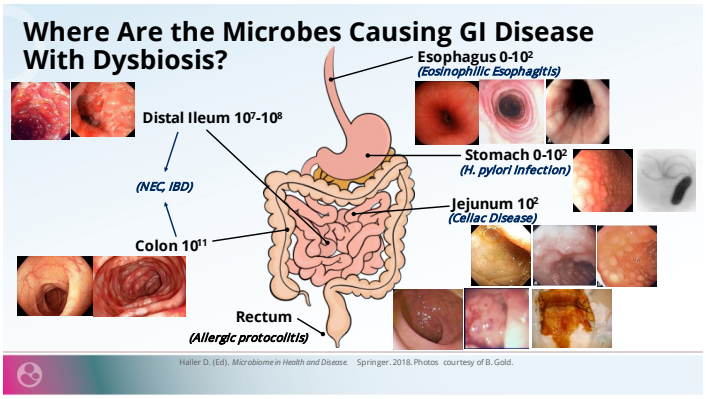
like functional GI disease and even emotional and psychiatric disease shaped by the gut microflora.

Now, this concept of dysbiosis (Slide 17), which I want to you take home, is important, because really the pathogenesis, or the basis, for most diseases, when you're talking about the role of the microbiome, is when the normal, rich, diverse, microbiome gets changed—perturbation of the normal microbiome and its content results in a disruption of the symbiotic relationship between the host, human, and the associated microbes. That disruption can result in a number of different disease outcomes, ranging from IBD (Crohn's disease and ulcerative colitis) to disturbances in the motor function of the GI tract, and irritable bowel, to even gastric and colon cancer. There's both short- and long-term implications resulting from the decrease in the diversity of the gut microbes and dysbiosis.



Slide 17 – Dysbiosis

That's shown particularly in Slide 18, where if you go from the top of the schematic, starting from the esophagus, then the stomach, the small bowel, and then the colon or the large bowel, you can see that in each of these areas highlighted by photomicrographs, taken at the time of endoscopy, of different disease states. First, there's the esophagus. The esophagus has its own unique microbiome called, believe it or not, the esophagiome. There've actually been elegant studies, which have shown that a disruption or change in that microflora in the esophagus, can actually be more associated with things like eosinophilic esophagitis (EoE), shown in those top 2 figures. There's a normal esophagus on the top left. And then, in the middle and the right are 2 figures of children, that were endoscoped in our center, with EoE.



Slide 18 – Where Are the Microbes Causing GI Disease With Dysbiosis?

Moving down into the stomach, you can see the electron micrograph of an *H. pylori* organism. And then, the lumpy bumpy, or cobblestoning, of the stomach, which is not normal. And that is *H. pylori*-associated gastritis. There are actually elegant data dating back to early studies in original neanderthal peoples that demonstrate that *H. pylori* may be as old as humans. And that at one point in time it actually was a part of the normal gastric microbiome. Over time, the bacteria have evolved, as have humans. That relationship has become dysfunctional, if you will, and resulted in disease.

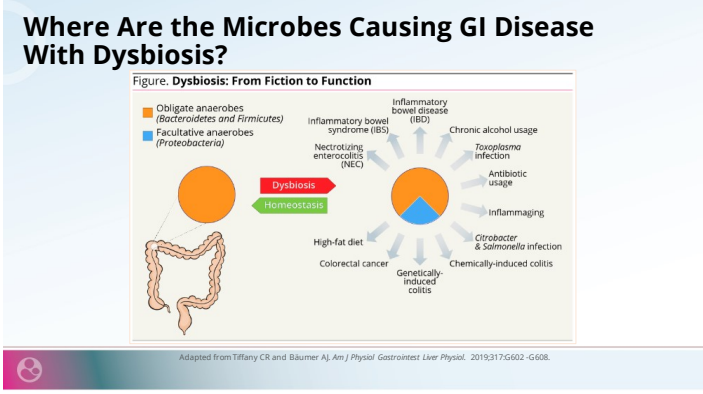
Moving further down the GI tract and the small bowel. Again, moving down on the figures on the right. You have a normal jejunum on the

left. And then the 2 middle ones showing a nodular type of jejunum and a duodenum. That results in celiac disease. In fact, there's been some elegant studies now looking at how dietary control, and more importantly control of the microbiome, in patients at risk for celiac, may actually be eventually used to prevent celiac disease in at-risk individuals. Alessio Fasano and his group at MassGeneral Hospital, and others, have been doing this elegant work.[6]

And then, if you move further down the GI tract to the distal ileum, or the colon, or even the rectum, things like Crohn's disease and ulcerative colitis have been shown to have distinct, unique, signatures of their microbiome compared with normal GI, and even in the baby that presents with bloody, mucousy stools. I know this is not good at dinner time. The bottom right 3 micrographs were taken from a 4-month-old that had cow's milk protein allergy—a dysbiotic microbiome can be associated with disease.

Now, where are these microbes that are causing the GI disease with dysbiosis? There've now been a number of studies, actually, literally an explosion, over the last 5 or so years that have started to characterize the normal microbiome of each of the different compartments within the GI tract. In fact, in other compartments of the body too: the skin, the middle ear, the eye.

As you'll see on this particular figure (Slide 19), it's a balance between obligate anaerobes, *Bacteroidetes* and *Firmicutes* being the predominant ones, and facultative anaerobes, *Proteobacteria* being in blue. It's a normal balance where there's predominantly obligate anaerobes that result in homeostasis and health. And it's an imbalance, or a decreased diversity and an enhancement of the facultative anaerobes, the *Proteobacteria*, that then results in a range of diseases, from IBD, necrotizing enterocolitis in the preterm baby, to colorectal cancer.



Slide 19 – Where Are the Microbes Causing GI Disease With Dysbiosis?

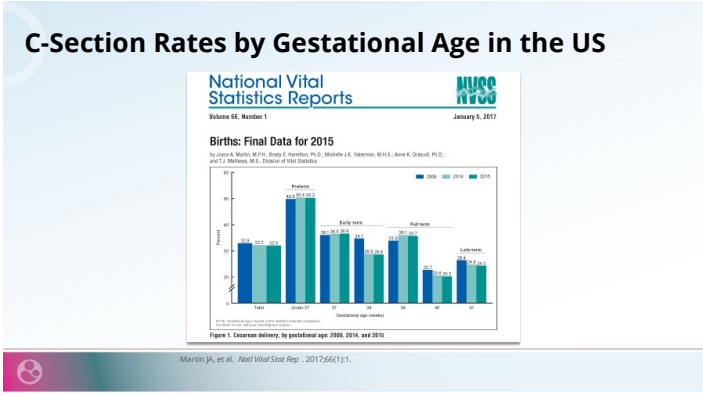
Now, as Dr. Malka pointed out in his presentation, there's been a lot of exciting research and controversy in terms of when the microflora actually begin [to develop], whether it's right in utero, just before birth, or early on in pregnancy. A number of studies are now beginning to point out that in fact these foundation species, if you will, may occur earlier on (Slide 20). The mode of delivery clearly has been shown, in a number of large epidemiological studies from multiple hospital institutions, to have a direct effect, when you control for every other factor influencing bad outcomes, as compared with vaginal delivery, which is a major source of bacteria for the infant.

Two particular highlights from the lay health media are shown in Slide 20. CNN from 2014, "Why so many C-sections have medical groups concerned." Or *Consumer Reports* about 3 years later, "Your Biggest C-Section Risk My Be Your

Hospital." What this particular report alluded to is that, depending upon the zip code that you fall in, you may be better able to get an elective C-section than a nonelective C-section and a vaginal delivery.

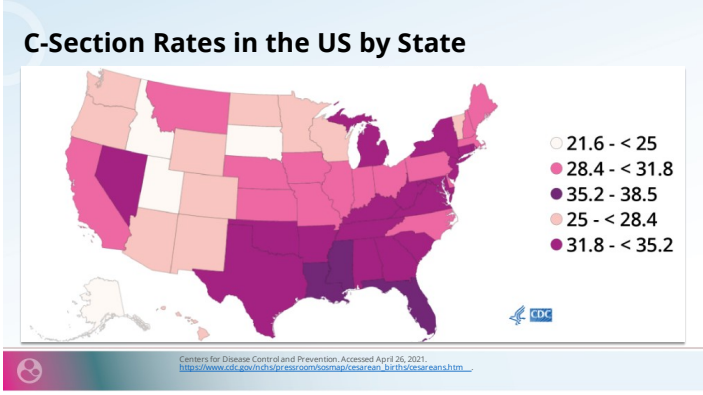
Slide 20 – Vaginal delivery – a major source of bacteria for the infant

Data speak louder than words, and that's important to see on Slide 21. This slide shows you C-section rates by gestational age. The dark blue, 2009 cohorts. The light blue, 2014. And the between-shaded blue, 2015. You can see, if you look at the figures on the far left that the Y-axis is percent, and the X-axis looks at different gestational age cohorts. The average C-section rate across this country is about 30%. That dramatically changes based on your particular zip code.[7]



Slide 21 – C-Section Rates by Gestational Age in the US

That's shown in the next slide, where you see C-section rates, where the white is ~21% to less than ~25%, and the dark violet is ~31% to ~35%.[8] The bottom line is that's not good when it comes to the microbiome.

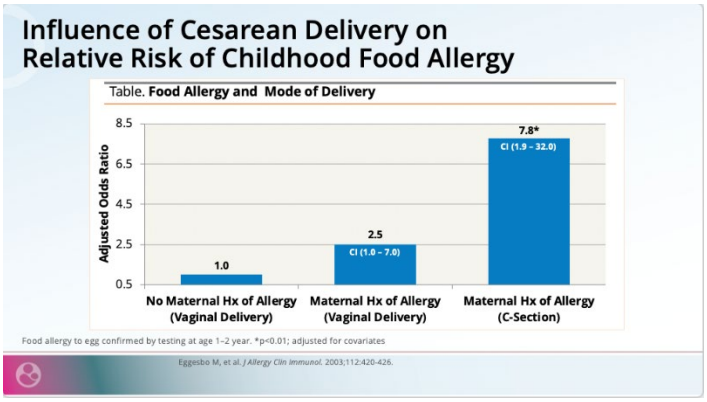


Slide 22 – C-Section Rates in the US by State

Why is this so? You can see in this particular seminal study (Slide 23), and I'm one of those, and Dr. Malka is the same, that we don't like to show data unless it's within the [last] 5 years. I think this study highlights an important point that has been duplicated multiple times. This is really one of the original seminal studies. For your orientation, this looks at the influence of C-section on childhood food allergy.[9] The Y axis is adjusted odds ratio. If you remember from your statistics class, or if you didn't take a statistics class, when you're looking at the risk of C-section and the outcome is allergy, if you've got an odds ratio of 1, that means there's no risk. If you have an odds ratio greater than 1, that means if you were born by C-section, you have a greater risk for food allergy. If you have an odds ration less than 1, that means if you were born by C-section, then you have a decreased risk, or actually it's protective against food allergy.

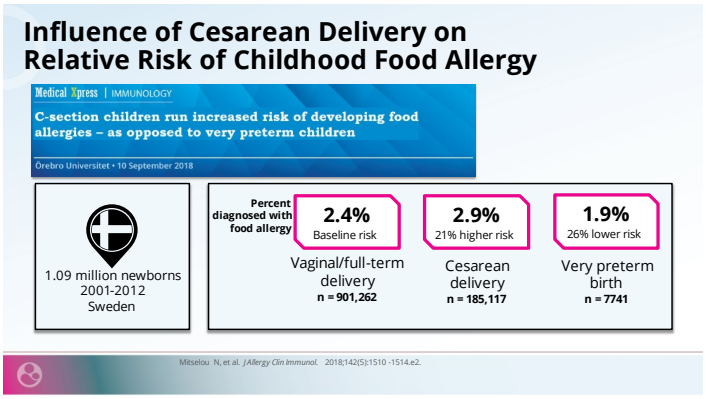
For slide 23, in your orientation, they adjusted the odds ratio. So, if mom didn't have a history

of allergy and baby is born by vaginal delivery, there's an odds ratio of 1. Now, if you just look at vaginal delivery, and a mom has a history of allergy, then that infant has a 2½ times higher likelihood of getting food allergies—this is actually demonstrated by challenge at age 1 to 2 years of age to egg—than those where the mother didn't have a history of allergy. If you look at the bar on the far right, and this is what's really the most important data from this slide, if mom has a history of allergy and baby's born by C-section, there's an almost 8-fold increased risk that that infant, by the time they hit 1 or 2 years of age, is going to have demonstrable reactions to egg challenge and allergies to that particular food protein. So this is really compelling and important data that now has been duplicated in multiple studies after this particular one was published that shows that C-section clearly can influence outcomes.[9]



Slide 23 – Influence of Cesarean Delivery on Relative Risk of Childhood Food Allergy

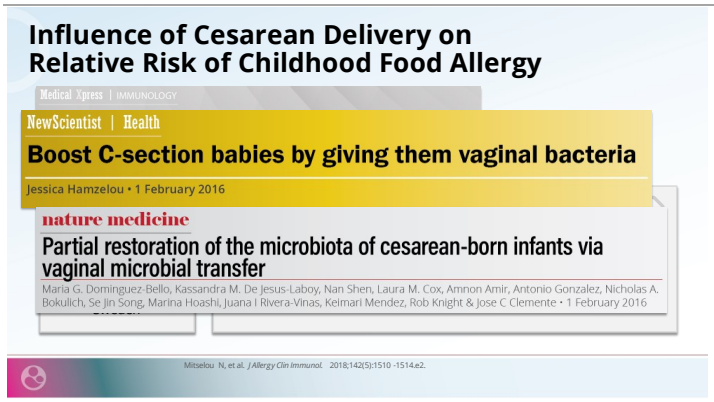
If you look at it in this particular study just published a couple of years ago, and this is a study in Sweden that looked at C-section compared to vaginal delivery,[10] it showed an increased risk of developing allergies as opposed to those that were born very preterm in terms of outcomes of specific food allergy: 2.4% was the baseline risk if they were not born by C-section, 2.9% was the baseline with C-section (that was the number), with a 21% higher risk. Preterm babies had a 26% lower risk.



Slide 24 – Influence of Cesarean Delivery on Relative Risk of Childhood Food Allergy

A number of studies, since that original landmark study, show that C-section babies have an increased risk of allergy and autoimmune diseases, and that we need to think about ways to supplement those bacteria. There are a number of clinical trials. If you go to clinicaltrials.gov, you can see studies ranging from giving flora or giving fecal flora in order to restore the restoration of the microbiota in C-section infants, in order to decrease eventual risk of allergy development. These 2 are shown here.[11]

Probiotic Use in Preterm Infants and Children



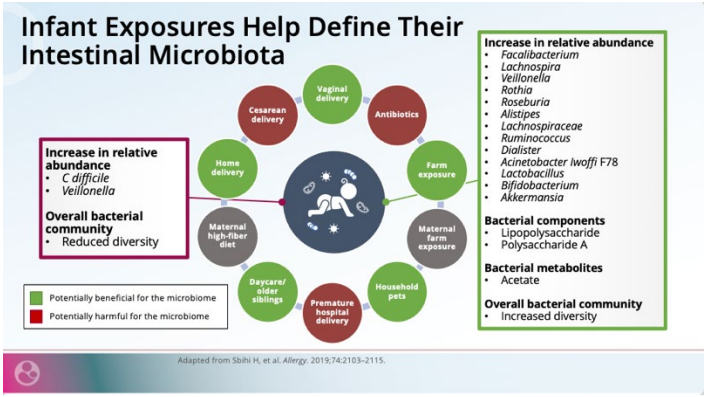
Slide 25 – Influence of Cesarean Delivery on Relative Risk of Childhood Food Allergy

If you look at specific association between delivery and microbial characteristic, it's important to recognize that the mode of delivery is a great distinguishing factor, whether or not you a have a *Bifidobacterium,* good bacteria-rich microbiota, or an *E. coli/Shigella*-rich microflora. If you introduce foods, you're going to increase the propensity for bad bacteria later on in life vs introducing foods early in life.

Prebiotics, Probiotics, and Neonatal Care

I hope I've taken you through this now so you get the first section talking about immunity, then the whole role of the microbiome and interaction with the gut and intestinal layer, and then the immune system, and then this concept of dysbiosis. When the newborn microbiome is changed, you have a dramatic increase in autoimmune, allergic, and inflammatory diseases.

Schematically, that's shown in this elegant study published in *Allergy* about a year and a half ago (Slide 26).[12] In this schematic, green is good and red is bad, at least in terms of conferring risk in a bad intestinal microflora. Home delivery, vaginal delivery, farm exposure, household pets, and daycare all confer a more rich, diverse, microbiome and enhance other metabolites and overall immune health. It might explain why the fact that if you have a child who's born into the house with a dog or a cat, those children are less likely to develop cat dander allergies by their 10th birthday.

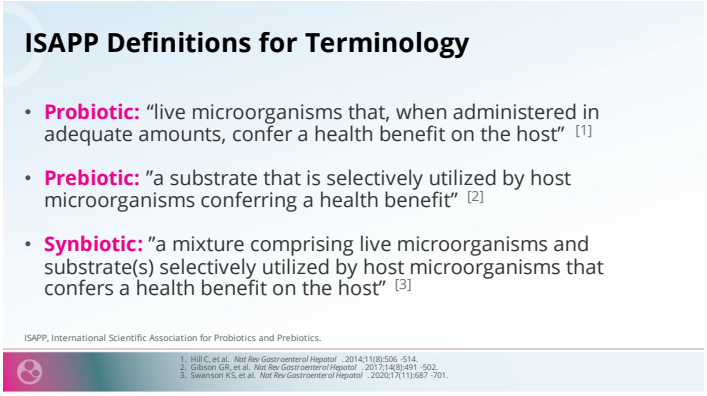


Slide 26 – Infant Exposures Help Define Their Intestinal Microbiota

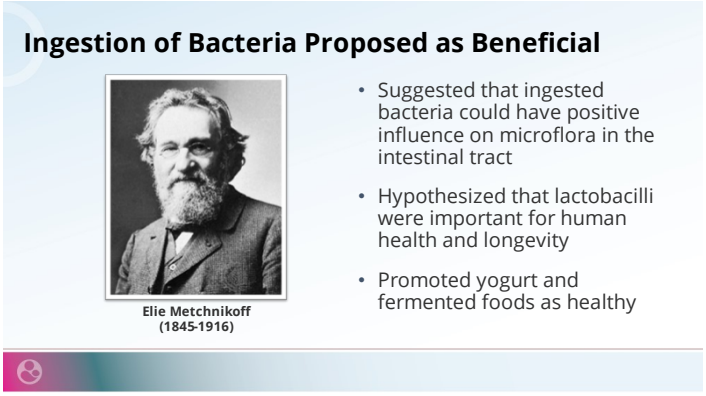
Another epidemiological study that had looked at place of residence showed that if you have a child reared on a farm or born into a farm life and a rural environment, they are less likely to develop allergy and autoimmune disease than those that were born in the city. So, C-section red, antibiotics red, premature hospital delivery—all can confer an increase in bad bacteria, *C. difficile*, and more importantly a reduced diversity in the gut microflora.

As a purpose of definition (Slide 30), the ISAPP defines a probiotic as live microorganisms that, when administered in adequate amounts, confer health benefit on the host.[13] A prebiotic is a substrate that is selectively utilized by host microorganisms that confers the health benefit.[14]

Probiotic Use in Preterm Infants and Children



Slide 27 – ISAPP Definitions for Terminology

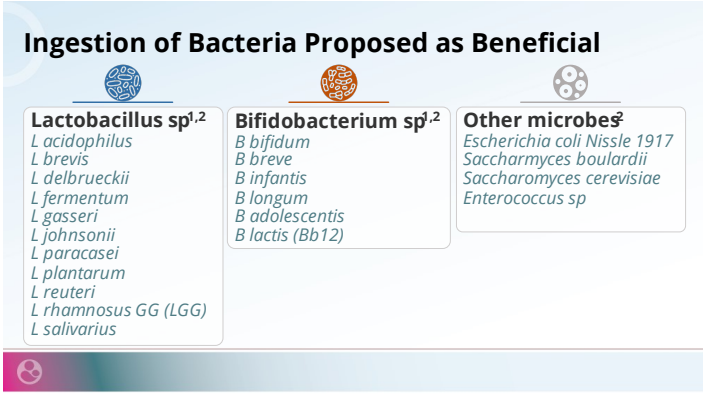


Slide 28 – Ingestion of Bacteria Proposed as Beneficial

So, prebiotic is like a food substrate that then enhances the growth of good microbiota. Probiotics are actual live microorganisms that, when administered in adequate amounts, confer health benefits. A symbiotic is actually a mixture of both the prebiotic and the probiotic,[15] and that when given to a certain individual, confers a health benefit.

I had the blessing of actually sitting on the Food & Drug Administration Generally Recognized as Safe (FDA GRAS) panel, some 25 years ago, where the genus *Lactobacillus* and the genus *Bifidobacterium* were designated as generally recognized as safe for human consumption down to birth/up to the elderly. This is not a new concept. Elie Metchnikoff, who lived between 1845 and passed over a century ago, proposed that the ingestion of bacteria was actually a good thing, and that in fact long before we had yogurt and fermented foods, he hypothesized that Lactobacilli, good bacteria, were important for human health and longevity. He promoted yogurt and fermented foods as healthy.

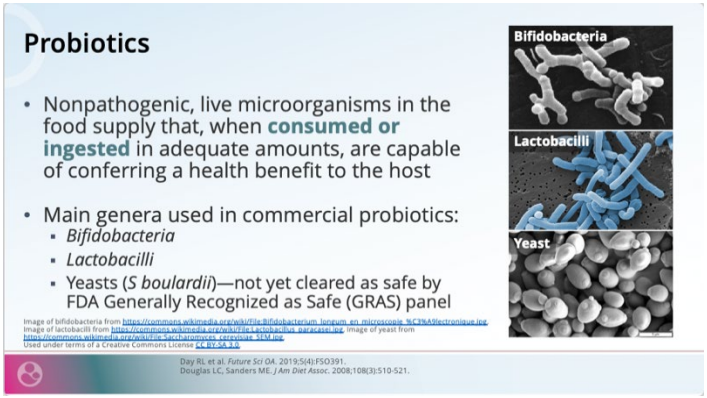
There's been a whole host of different strains of probiotics. I can tell you, when I got into this field some 28 years ago, it was still, at least in the US—much more so than in Canada and Europe where they're more predisposed to thinking about the gut microflora and dietary approaches—at least in the US, it was really not a common topic in terms of thinking about probiotics. Now, you can go into almost any place, from a quick trip to Whole Foods, and you'll see walls of probiotics. Now remember, this is an unregulated market, and the majority of these are single strains, and there have been no head-to-head trials. But you can see there's a whole host of *Lactobacillus* species, a whole host of *Bifidobacterium* species, and other microbes that have been used in different clinical trials (Slide 29).



Slide 29 – Ingestion of Bacteria Proposed as Beneficial

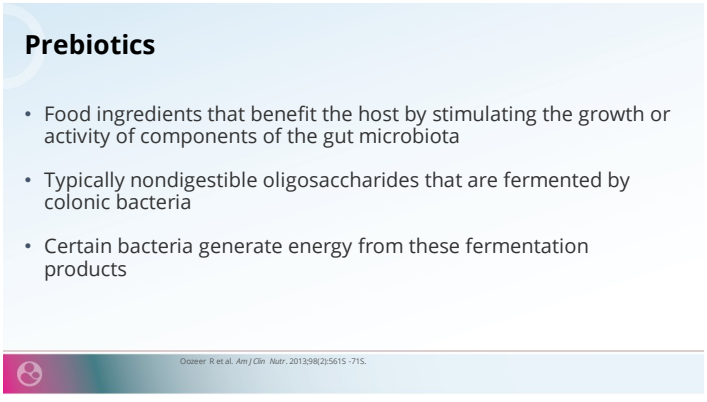
Probiotic Use in Preterm Infants and Children

Probiotics, which are nonpathogenic, live organisms, when consumed, are capable of conferring a health benefit. The most commonly used in commercial probiotics are *Bifidobacterium, Lactobacillus*, and Florastor, which you may be aware is a yeast, *Saccharomyces boulardii*, which at least at the GRAS panel didn't quite give GRAS status (Slide 30).



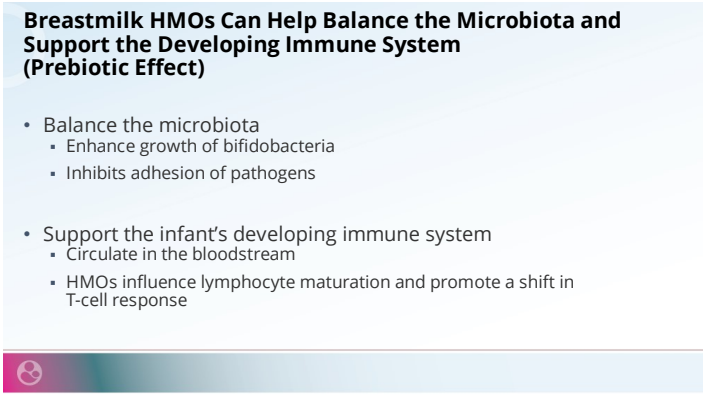
Slide 30 – Probiotics

Prebiotics are food ingredients that benefit the host by stimulating the growth of activity of components microbiota, typically nondigested. Although, the saccharides that ferment the colonic bacteria promote the growth of good bacteria and generate energy (Slide 31).



Slide 31 – Prebiotics

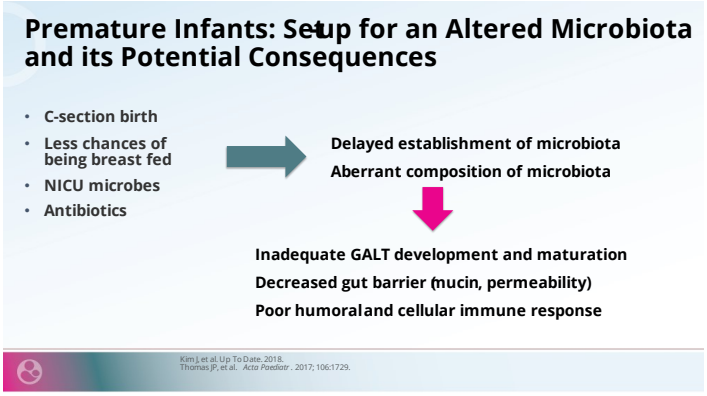
Breast milk, as alluded to by Dr. Malka in his part, have these prebiotics called human milk oligosaccharides (HMOs), which can help balance the microbiota by enhancing the growth of good bacteria, inhibiting adhesion of pathogens, and supporting the developing child's immune system (Slide 32).



Slide 32 – Breastmilk HMOs Can Help Balance the Microbiota and Support the Developing Immune System (Prebiotic Effect)

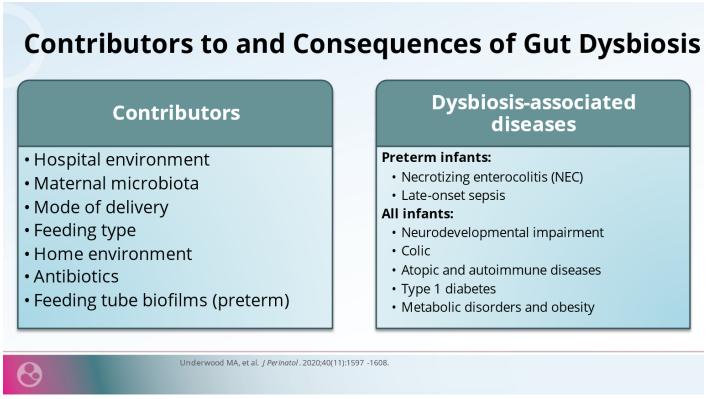
Why is this important when we think of infants, and in particular premature infants? If you think about it, C-section birth, on which I provided data for you in the earlier part of my presentation, results in a setup for a less diverse, less robust microbiome (Slide 33). Oftentimes, premature infants don't have the chance of being breastfed. They're exposed to microbes that, I think, walk around with cigarettes rolled up in their sleeves, and say, "Look, you can hit me with anything," and lots of antibiotics early on in life. Well, these factors then delay establishment of the microbiota. It becomes dysbiotic. You get an inadequate development of GALT, which Dr. Malka explained, and you develop poor humoral and cellular response.

Probiotic Use in Preterm Infants and Children



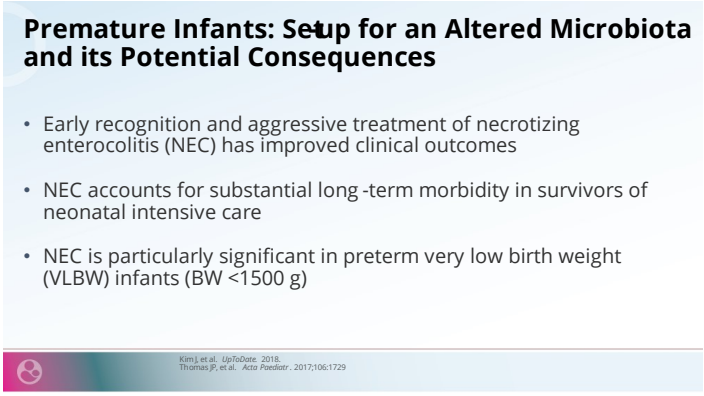
Slide 33 – Premature Infants: Set-up for an Altered Microbiota and its Potential Consequences

Contributors and consequences to gut dysbiosis (Slide 34). The contributors are on the left. And then, the specific diseases are associated on the right. You can see things from hospital environment, to feeding type, to feeding, to biofilms in the premature infants, and a number of specific diseases that have been contributed to by gut dysbiosis.



Slide 34 – Contributors to and Consequences of Gut Dysbiosis

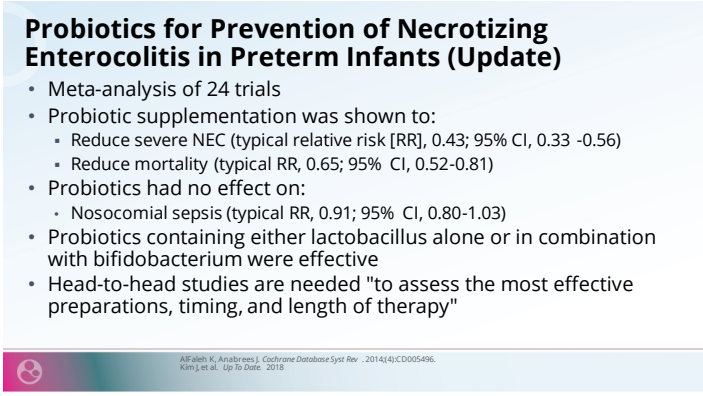
Now, with respect to premature infants, particularly those born before 35 and probably 30 weeks gestation, and under 1,500 g, early recognition and aggressive treatment of this condition called necrotizing enterocolitis (NEC) has improved clinical outcomes (Slide 35). These babies that had NEC resulting in major surgeries, oftentimes taking out a great portion of their bowel, have long-term morbidity, and often long-term ICU stays where they're on nutrition by vein rather than by their gut, and then with lot of other comorbid factors.



Slide 35 – Premature Infants: Set-up for an Altered Microbiota and its Potential Consequences

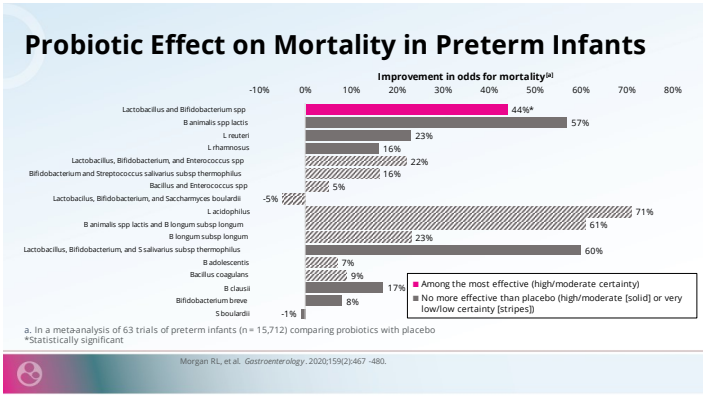
How can this be changed? There's been some wonderful sets of studies, actually a Cochrane analysis. For those of you who don't know, Cochrane is a think tank of investigators, scientists, biostatisticians, and researchers that look at a specific problem and the world's best available data and come up with recommendations. There's been a Cochrane analysis published in 2008,[16] another one published in [2014],[17] and one of the most recent just published[18] and reviewed in *UpToDate* by Jae Kim (Slide 36), who is the head of neonatology at Cincinnati Children's, shows that probiotic supplementation in preterm infants reduced severe NEC incidents, new cases of NEC, and all-cause mortality, and that probiotics had no effect on enhancing sepsis. So they didn't cause any adverse effects. Most of these were either using *Lactobacillus* alone or in combination with *Bifidobacterium*. There's a lot of active research in the neonatal networks looking at head-to-head trials and seeing how,

potentially, we can further reduce the incidence of NEC.



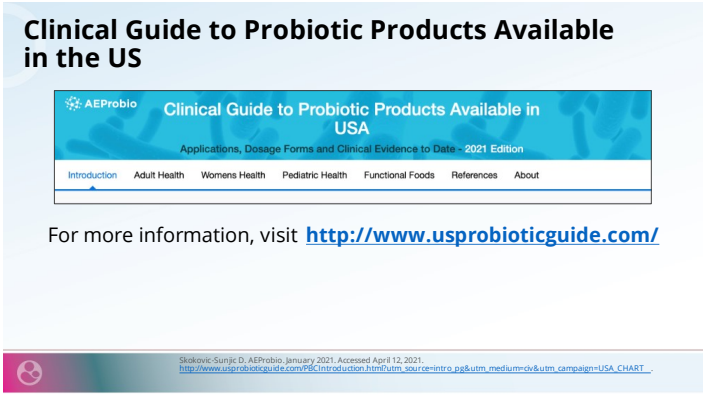
Slide 36 – Probiotics for Prevention of Necrotizing Enterocolitis in Preterm Infants (Update)

If you look at probiotic effects on mortality shown in Slide 37, so the X axis is improvement in the odds—the risk for mortality [goes down as the bars increase].[19] You can see that there is a whole range from the *Saccharomyces boulardii,* which did not show improvement either by itself or when combined with *Lactobacillus* and *Bifidobacterium*, but if you combine *Lactobacillus* with *Bifidobacterium*, you had a significant reduction in overall mortality in this large meta-analysis of over 15,000 babies in 63 different trials.



Slide 37 – Probiotic Effect on Mortality in Preterm Infants

And then, to summarize and then bring it to the point of Q&A, if you want some further reading, and actually something which I think is a wonderful resource if you're thinking about probiotics and what are specifically good for use and have evidence in particular disease states, there's this reference, the *Clinical Guide to Probiotic Products Available in USA* (Slide 38). It is a really important one for you to take home. The URL is www.usprobioticguide.com.



Slide 38 – Clinical Guide to Probiotic Products Available in the US

And so, finally, to summarize, what I hope in this presentation, both from the allergist perspective and the gastroenterologist perspective, really talking about the very same issue from 2 perspectives, we provided an overview of how the infant's immune system develops, and in particular the important interaction with the gastrointestinal microflora, even in utero. Actually, [there is additional evidence for probiotics that we did not describe], including some of the studies that have looked at administration even in the last trimester of pregnancy in women whose infant was at risk for development of food allergies based on family history, showing that they could actually decrease the risk of allergy in those infants later on in life. We've tried to describe the growing problem in epidemiology of allergy worldwide, just briefly touched on that. That's going to be reviewed in other

presentations that are shown in this series. We've noted the importance of the GI microbiome in both health and disease.

Finally, in this last section, we tried to give you an overview at a higher level of how prebiotics (food substrates that promote the growth of good bacteria) and probiotics (live organisms that have multiple functions), when they get incorporated into the normal microbiome, can enhance the diversity of the microbiome, alter intestinal microflora, and potentially change health outcomes.

Audience Question & Answer

Editor's Note: This is a transcript of audience questions together with presenter responses from the May 6, 2021, audio webcast.

What is the evidence for the use of probiotics at other developmental stages outside of preterm infancy?

Dr. Gold: Yes, where do I not begin! The short answer to your question is that there are a number of elegant studies, which is actually why the FDA was able to demonstrate a compelling level of scientific evidence that the genera *Lactobacillus* and *Bifidobacterium* were generally recognized as safe for human consumption. They didn't pass judgment on efficacy, but we reviewed a lot of efficacy studies. And so, there's been conditions ranging from actual cow's milk–associated colitis in newborn infants, either as supplemented in infant formula or probiotics given alone. There's been studies that have looked at probiotic administration in functional abdominal pain. There's been studies looking at probiotic administration at helping infant colic, reducing severity of crying episodes and frequency of crying episodes. There's been studies looking at probiotics and helping with constipation or regurgitation episodes. And then, finally, there's been studies that have looked at probiotics for decreasing the incidence of antibiotic-associated diarrhea, as well as probiotics for shortening duration and severity of diarrheal episodes and acute gastroenteritis.

What's amazing is there's been a whole host of studies. And now what needs to be done are studies that compare one probiotic to another to see if there's a better efficacy with one. That now, because we have the capacity to look at both microbial outcomes, to actually look at what happens to the gut microflora, as well as clinical outcomes. And then, finally, on that really good question, we need to do studies where we actually look longer term. All of the studies of the different disease states that I just reviewed rapidly for you, were really clinical trials of 2 to 4 weeks, or at most 8 weeks duration. What we really don't know is what happens with long-term administration.

I get asked that question all the time by parents of my patients, colleagues, and referring pediatricians. I think that where we need to start doing investigations is looking at really more long-term use.

Dr. Malka: Now, to add to Dr. Gold's excellent answer and presentation, it's quite fascinating. When we have so many probiotics, it's hard to compare head-to-head, because they're not all made equal. As we mentioned, if you go to the supermarket, you get 20 different types. One is a billion, and one is a trillion, and one is in the fridge, one's outside the fridge. Even if you know something about probiotics, you get confused. There is no black and white response to this. What I would say, at least in my field,

especially in the allergy world, there are some studies looking at starting at 3 to 6 months pregnancy and after.

The most important factor we have seen in the allergy world is that it has decreased the incidence of eczema or severity of eczema. That's quite important, because we now believe that eczema becomes the predisposing factor for food allergies, rather than what we thought before that the food allergy causes the eczema. If we can decrease the incidence of eczema, which has been shown specifically with *Lactobacillus rhamnosus*, then you can hopefully decrease the incidence of food allergies, so you have a healthier microbiome, a healthier gut, and less permeable, better tight junctions. As I mentioned earlier, you have less chance of having food allergies.

To that point, I think data show starting at 6 months of age, especially in siblings or mothers who have a high family history of atopic dermatitis, it becomes useful. Data show maybe 3 months after—again, long-term data we haven't had. I think the studies are needed in the future, because we need to give a more robust or specific recommendation. It will be hard, because [probiotics] are not made equal, and because, as we all know, the host response may be different. That will be, at least from the allergy perspective, where it has become more useful, and that is the incidence or severity of eczema.

At what age are fermented foods, such as beets or carrots, safe in children?

Dr. Malka: I'll start with allergy part. Fermented foods, in general, for the allergic components, we're not really worried about. The vegetables you can introduce whenever you can start feeding. I mean, as long as your motor development is correct, I think you're good to go. I don't have a specific recommendation for beets. I'm sure Dr. Gold's going to mention that. Beets, at least from the allergy perspective, have become one of the common causes of food impaction in patients with EoE. I think, down the road, we're more afraid of beets getting stuck as a diagnosis for EoE. But I don't have a specific recommendation from the allergy perspective, at least, of delaying the foods, other than when you have improper motor development. Ben, do you have a different approach from a GI perspective?

Dr. Gold: I think that you highlighted that well. I mean, beets are becoming one of those things that we see, at least in children (and what we didn't go through, but I know it's addressed in another presentation that's done in the series of webinars) for IgE vs non-IgE-based allergy. At least in the children that we see, with GI-specific allergies, EoE or food protein-induced enterocolitis (FPIES), these children can develop a beets allergy. We typically don't see it in infants and typically haven't seen a problem in terms of early introduction.

If we were to give probiotics to an infant who was born C-section and breast- or formula-fed to decrease autoimmune disease, how long should it be given?

Dr. Malka: I don't think I'm going to have the answer. I don't know if there is an answer for that. Dr. Gold may be more specific on if there's an answer. I would say, again, from the allergy perspective and what I've learned, I can maybe project to other conditions. It's not the specific microbiome component. It's when the microbiome component is present that makes

a difference. You can argue and say that already when the baby is born by C-section, you're already at a disadvantage, because you already had the C-section process, so the microbiome was not property formed. Even if you start at that point, you can argue and guesstimate that that may not be already a proper time.

In some studies, for example, we have seen that patients that outgrow food allergy to milk in the Food Allergy Research & Education's studies, they have seen that [the microbiome at certain ages can influence the chance of outgrowing milk allergy].[20] You have a disadvantage in the C-section, as Dr. Gold mentioned in his very excellent presentation, but I'm not sure you can actually decrease autoimmune disease if you're prone to it. But you may have a greater chance of not [developing autoimmune disease] if you supplement with [probiotics]. But I think it's the timing that is of the essence. I don't know if we have the answer to that question specifically.

Dr. Gold: Yeah, I would just add, and I think that's a great way to think about it, and it's an excellent question, Jessica, in the end sometimes moms fail to progress in terms of the delivery process, and due to circumference of the head and pelvic size, they need to be delivered by elective C-section. I wouldn't spend the rest of that child's life worrying about the development of autoimmune disease. There are lots of different things that they can do thereafter, both in terms of feeding, in terms of other types of exposures. I think, really, Dr. Malka's concluding thought was the best one and spot on and it is that we really still need to do much better designed longer studies to see whether there's a way to mitigate against potential development of autoimmune disease in children born by C-section, and other factors that we could potentially control.

There's even data from a multidisciplinary collaboration that's ongoing right out of the UK that has demonstrated that you can actually influence the microflora even beyond age 3 in certain patient populations. I still think we just need to wait, and larger population research with rigorous study designs needs to be done in order to give us answers to that question.

Dr. Malka: To add one more thing, and I want to add to Dr. Gold's point. It's very important. You don't want parents to be fearful of, "My God, I'm having a C-section, this kid's going to have allergies, autoimmune disorders. He's going to have IBD. He won't be able to eat egg or milk." Because the microbiome can be affected by many things. I mean, we didn't talk about it, but even dental procedures during pregnancy, antibiotic use during pregnancy, may actually alter the microbiome. It can be altered by many, many reasons. You can't lose sleep over C-sections. If you could avoid it, great. But if you can't, look, there are other things you can do in the process.

Do you use a specific probiotic that you recommend for preterm infants with *Lactobacillus* and *Bifidobacterium*? The second part of the question is about, what about kids with eosinophilic esophagitis.

Dr. Gold: In terms of preterm infants, I first need to give the disclaimer that, at least at present, although there's really compelling data, now 3 different Cochrane analyses, I think it's important—the reference was shown on the slide to read Jae Kim's *UpToDate* article just published about a year and a half ago,[21] because it's not really at a time where we can

definitively say, in babies, all preterm babies, we need to sprinkle probiotics on every single bit of formula that they need to have, and they all need to be on donor breast milk, which we may not be able to get to them.

I do think, in terms of what would we use, there really are very few probiotics that have truly good data. There's a group of investigators that came out of UC Davis that looked at a specific probiotic, a *Bifidobacterium*. Evivo is the name of it. It's trade name. Evolve Biosystems is the company it's proprietary to. There's really been some very elegant data looking in even term and preterm infants and enhancing the microflora, changing the relative pH of the microflora, and enhancing the diversity in the microflora.[22] There have been a number of studies that are ongoing actually right now looking at the use of LGG or *Lactobacillus rhamnosus* (Culturelle is what we know it as commercially) in preterm infants. And then there've been other studies looking at the use of the combination of *Bifidobacterium* Lactobacilli.

At present, there are relatively few that I'll recommend. And then, I have an individual relationship with each of the neonatologists if I'm particularly consulting in the NICU and they ask the question about which probiotic would I give. In particular, the ones that we recommend that have data, so Evivo; the Gerber Soothe Drops, which is *L. reuteri*; and Culturelle, which is LGG or *Lactobacillus rhamnosus*. There's been some studies that it used be called VSL#3, and now it's called Visbiome. That's actually one of the few that has multiple strains that have been looked at in preterm babies.

Beyond the preterm era, those are typically also the same ones that we recommend. Because there are data at least with these specific probiotics in the patient populations in which they're given.

Dr. Malka: I think LGG, like with Culturelle, has been the most studied, at least the most reproducible studies of all of them, again with no head-to-head comparison because it hasn't been done. I think LGG has been the one that has been shown the most effective, at least in the allergy perspective.

Dr. Gold: That's one of the reasons why we included that reference and URL (Slide 38). This is updated annually. If clinicians are interested in seeing what probiotics have been approved and/or, more importantly, what conditions they're being used for, there's really some interesting and exciting data that are even looking at some of the non-*Lactobacillus*, non-*Bifidobacterium* probiotics that have some very promising data, with Akkermansia being one of those.

Probiotics can be expensive, especially if we serve lower socioeconomic status patients. Is there any advice or ways to help this population?

Dr. Gold: Awesome. First, now I'm going to wear my advocacy hat and the work that I do was part of the North American Society For Pediatric GI, Hepatology & Nutrition (NASPGHAN). There is a current bill that is making it's way through that actually has, believe it or not, bipartisan support called the Medical Nutrition and Equity Act. In that particular bill, it talks about insurance companies paying for medical foods. In this particular circumstance, germane to this

presentation, probiotics, if written as a prescription, would count as a medical food. Right now, and we try, but it's very rare that you're going to get insurances to cover probiotics. They did a trial when the first phase of the Medical Nutrition and Equity Act was going through, and TRICARE tried this, and actually showed improvement in overall health and decrease in health care costs, when people were actually given appropriately prescribed nutrition interventions. And so, the question is spot on.

I think it's one of the important things that we have to look at in terms of disparities of healthcare and some of the access for populations. Because these are not cheap! I mean, just go to any store, and you can see in the whole wall of probiotics, look at how much they cost for any of these. It is not cheap. If this type of legislation goes through, then it's going to mandate that insurance companies cover these, as long as they've been written as a prescription from the physician.

Now mind you, I'm a nothing ventured, nothing gained, type of guy. Dr. Malka knows this. That's one of the reasons why we get along so well, because he's the same. I would not hesitate to try to write a prescription for the insurance company, because you never know. If you give a specific medical reason, you give specific ICD-10 codes for the disease for which you're writing it, you may get it approved. In fact, we have, in certain circumstances been able to at least get it underwritten to a certain extent. Long-winded answer just to answer your question, but a very good one that at least, right now, would be difficult to pay for.

Dr. Malka: I always say that, and I think Dr. Gold mentioned it, the hygiene hypothesis, we believe that when you have lower socioeconomic status, and growing up in Venezuela, we know these communities that basically grow up playing with dirt all day and exposed to an environment that in the Western society we don't have, they for sure have a different microbiome that we need to compare data on Western society vs non-Western society in the use of probiotics. Because they may not actually need as much or may need different probiotics. They will have a significantly different microbiome and significantly different immune system, immune regulatory cells. To me, it's a fascinating question. In addition to the question, what we need to ask is what type of microbiome do they have? Do they really need the same amount of probiotics that Western society needs?

Can introducing probiotics within days of a C-section of an allergic mom lessen the 8-fold increase and likelihood of allergies in the child?

Dr. Malka: I think it goes back to the same question we had before. I would say, look, would I go to my clinic tomorrow and promote probiotics to every single patient that walks in? No. I don't think I need to. I think there's a role for specific at-risk populations that will benefit from the use of probiotics. Like I mentioned earlier, there are some studies that have shown that introducing [probiotics] during pregnancy has become much more effective that actually doing afterwards, even in vaginal deliveries. This is what I basically said before, I think you can do it, as long as you do it in the right population at risk, you may promote a

tolerance. It won't hurt. I think it will work. Specifically, right after C-section, I don't have the answer.

I can tell you that there's studies ongoing right now. For example, we in the food allergy world, we have patients with food allergy to egg, and we're able to introduce baked goods. Because we can denature the protein. There are studies now looking at baked products with supplemented probiotics. You sprinkle probiotics in it, you bake it, and then you give it. We're trying different types of different forms at different times to actually make it work. I would answer saying, if it doesn't hurt, it can only help.

Dr. Gold: I mean, yes, not to belabor this, or delay on this topic, but I think that we really need to do more studies looking at that. I agree with Dr. Malka. I'm not ready to every time I see somebody eating, go and grab my probiotic and start sprinkling on their food so that they get an extra dose during the day—in particular, for somebody born by C-section, immediately start giving them probiotics to see that they can decrease the risk of allergy. Mind you, the risk of giving [probiotics] is not very great. So, you may not do any harm. In medicine, that's what we're supposed to do first and foremost is to do no harm. But I think that we really need to do a lot more studies. I mean, I think there's some really compelling ones, but a lot more studies looking at what we're really trying to influence and potentially alter when they give probiotics, and for how long.

Because, buried in that question is, okay, so you can start giving probiotics, but what are you going to do? Give it for the first month, 2 months, 4 months? Are they going to be breastfed or bottle fed? I mean, you can actually enhance their microbiome in children born by C-section by having them breastfeed, because they get skin microflora, they get probiotics from the breast milk. They get other wonderful factors from breast milk. That's what's really the best source of nutrition for infants anyway. So, I think we're not yet at a stage where we can say, across the board, everybody that's born by C-section needs a probiotic, but I do think that there are a number of things that you can potentially do right now, even without giving a probiotic, to enhance the microflora.

Probiotic Use in Preterm Infants and Children

Abbreviations

EAT	Enquiring About Tolerance	**IBD**	Inflammatory bowel disease
EoE	Eosinophilic esophagitis	**ICU**	Intensive care unit
FDA	Food & Drug Administration	**IL**	Interleukin
FPIES	Food protein-induced enterocolitis syndrome	**MHC**	Major histocompatibility complex
GALT	Gut-associated lymphoid tissue	**NASPGHAN**	North American Society For Pediatric Gastroenterology, Hepatology & Nutrition
GI	Gastrointestinal	**NICU**	Neonatal intensive care unit
GRAS	Generally recognized as safe	**STAT-6**	Signal transducer and activator of transcription 6
HMO	Human milk oligosaccharide		

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