The Probiotic LGG® and Its Benefits to the Immune System

✦ Course Transcript ✦

Overview

The first 1,000 days are a critical period for maternal, fetal, infant, and toddler nutrition. A good microbiome allows for a healthy immune system. Renowned professor of pediatric gastroenterology and nutrition, and endowed chair in pediatric research, **Fayez K. Ghishan, MD,** discusses the important role gastrointestinal microbiota plays in the development of immune function. The properties of probiotics are strain-dependent and are not all formulated the same. Dr. Ghishan discusses the mechanisms and other factors that influence probiotics' clinical benefit to gut health and the immune system of newborns. He explains the unique properties of the probiotic strain *Lacticaseibacillus rhamnosus* GG and its impact on tolerance to cow's milk protein. Dr. Ghishan also reviews the most recent and pertinent studies of LGG®, while discussing the role of LGG® in the future of allergy management.

Target Audience

This activity was developed for obstetricians, pediatricians, neonatologists, nurses, advanced practice clinicians, dietitians, and other healthcare providers with an interest in infants.

Learning Objectives

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

- Describe the role GI microbiota plays in the development of immune function
- Discuss the importance of strain-specific identification among probiotics, focusing on *Lacticaseibacillus rhamnosus* GG
- Assess long-term outcomes and benefits associated with *Lacticaseibacillus rhamnosus* GG

Faculty

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Activity #171052

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Faculty

Fayez K. Ghishan, MD
Speakers Bureau: Mead Johnson Nutrition

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Victoria Anderson (Medical Writer)
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This activity is supported by an independent educational grant from **Mead Johnson Nutrition**.

This activity is an online enduring material. It has been edited to meet requirements for online learning. Successful completion is achieved by reading and/or viewing the materials, reflecting on its implications in your practice, and completing the assessment component.

The estimated time to complete the activity is 1.0 hour.

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This activity was released on June 17, 2022 and is eligible for credit through June 17, 2024.

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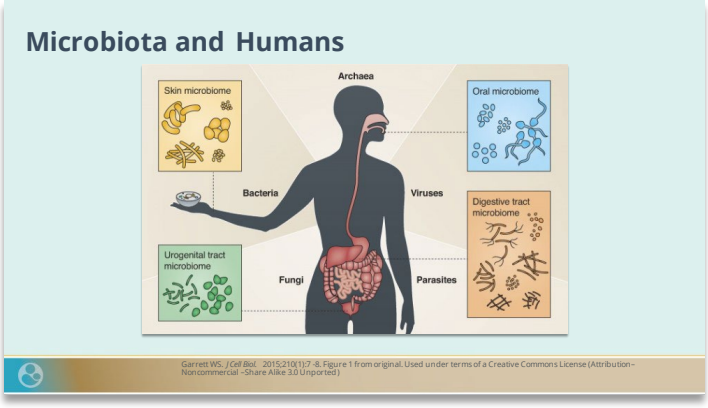
Editor's Note: This is a transcript of a live webcast presented online on May 31, 2022. It has been edited and condensed for clarity.

MICROBIOME

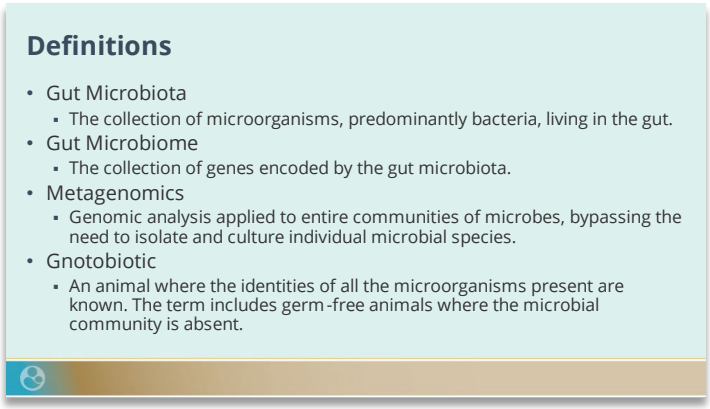
Fayez K. Ghishan, MD: I'm going to start with giving you a big picture of the microbiota of the gut. The first thing I want to share with you is that the microbiota and humans are interrelated. We have our skin microbiome. We have urogenital microbiome. We have the digestive disease tract microbiome, which is the largest of all, parts of the body, which contain microbes as well as parasite, fungi, viruses, and archaea. Of course, we also have oral microbiome.[1]

The collective of these is called the microbiota, which is the collection of microorganisms, predominantly bacteria living in our gut. Microbiome is the collection of the genes included by the gut microbiota.



Slide 1—Microbiota and Humans

Sometimes scientists will use microbiome and microbiota interchangeably, but nonetheless, metagenomics, that genomic analysis, applies to the entire community of microbes, bypassing the need to isolate and culture, individual microbial species. Gnotobiotic—I may mention this sometimes in my discussion—it's in animals where no microorganisms are present in their GI tract. They are delivered by C-section [Cesarean delivery], and therefore, we call them germ-free animals, but we use them extensively in our research. How is that going to help us understand the human? Because let's say, you have taken an FMT, fecal microbial transfer, from a patient with Crohn's disease and put it in these animals. You may develop colitis by using this tool. That's very helpful for us to understand how this gnotobiotic mouse works for us.



Slide 2—Definitions

The important thing is to remember we are super organism as humans. Why? Because we have entire microbes living in our gut. So, what is the relationship, mutualistic relationship between our gut microbiome and us as the host?

Let's first talk about us and what we provide for the microbe. We provide a nutrient-rich environment,

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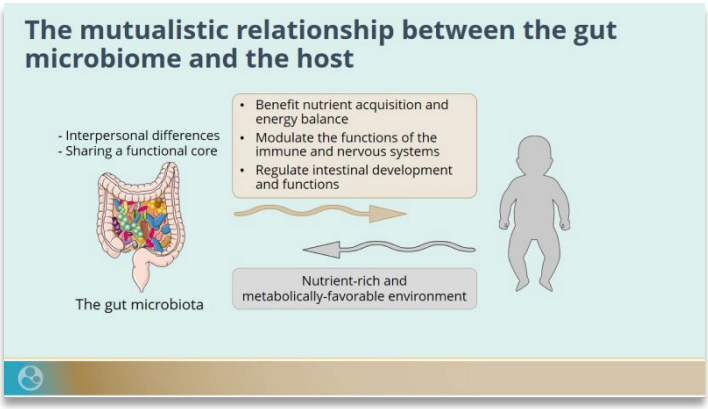
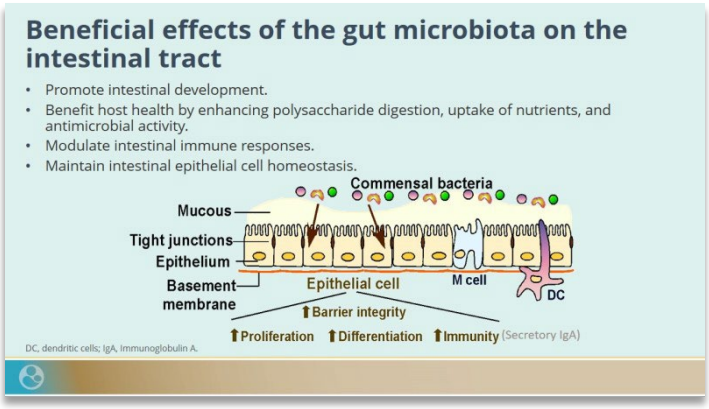
metabolically favorable environment for the bacteria to continue to grow. But on the other hand, what does the bacteria do for us? Well, they benefit us in nutrient acquisition and energy balance. They produce short-chain fatty acids: butyrate, propionate, acetate. Those are the end products of fermentation of the prebiotics, which is substrate for the probiotic to work. We use the short-chain fatty acid for energy in our colon. Our colon utilizes short-chain fatty acid as a tool for fuel for our colon. Second, the bacteria modulate the function of our immune system, as well as our nervous system. There is a connection between the brain and between the gut. I'll show you that in the next slide. It will regulate intestinal development and function, as well.



Slide 3—The mutualistic relationship between the gut microbiome and the host

It's clear we have to have a mutualistic relationship between the microbe in our gut and between the host. Remember, 20% of the vitamins you have in your body are made by the bacteria and gut. And this is the reason why we always give vitamin K in injectable form for every newborn baby. Why? Because the gut in the newborn does not have enough microbes to sensitize vitamin K. Therefore, if we don't give them [the newborn] vitamin K, they get hemorrhagic disease. This is a very important relationship between us and between the microbe in our gut.

This slide (slide 4) shows you what I have been saying in regard to this mutualistic relationship. Commensal bacteria in our gut produces metabolites, and those metabolites affect the tight junctions, improvement of the tight junction membranes, increase proliferation, differentiation, and increasing secretory IgA by increasing our immune system. It's clear **the gut microbe plays a major role in the development of our intestinal tract.**



Slide 4—Beneficial effects of the gut microbiota on the intestinal tract

I mentioned that we have a second brain in our gut, and that's absolutely true. When I tell you I have a gut feeling, that's true because this is the enteric nervous system that we have in our GI tract, which has more neurons than your entire spinal cord. There is a connection between the brain and between the gut microbiome. Let's say you have a patient who has depression, for example, while that altered their gut microbiome, and vice versa. Let's say, you have a change in your gut microbiome. Let's say, you got long COVID. That long COVID, through metabolites in the bacteria in your gut, will give you a foggy brain. There is a clear relationship. But let me also mention that there is a gut-skin interaction. There is a gut-lung interaction, so clearly our microbe in our gut has an influence on some of our other organs in our body.

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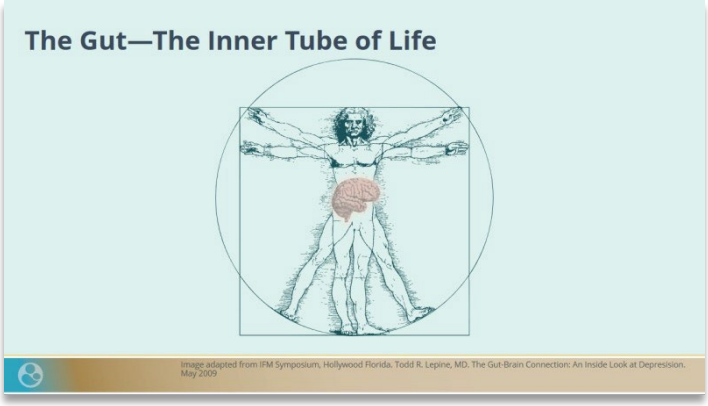
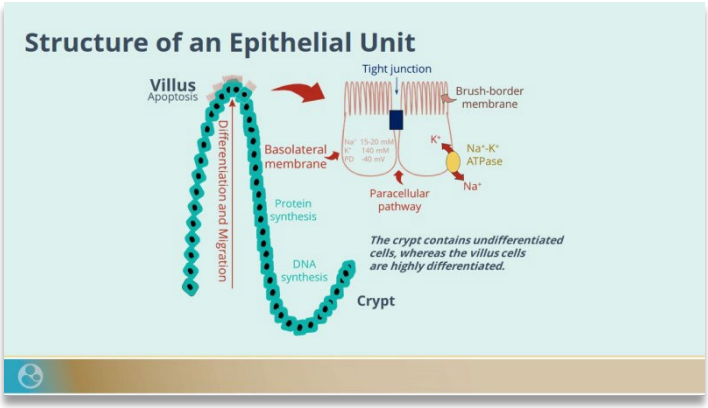


Slide 5—The Gut—The Inner Tube of Life

Epithelial Cells

This is a depiction of our epithelial cells in our gut (slide 6). Remember your GI tract and your skin are the 2 most highly productive organs in your body. Every 5 to 7 days, you change your epithelial cells in your GI tract. The tip of the villus is called cells, which are involved in absorptive function. The cells in the bottom of this, that's the crypt. That's where we have stem cells, which replaces the cell—at the tip of the villus—every 5 to 7 days. It's clear our GI tract is a highly productive organ involved in the transport function of nutrients across the GI tract.

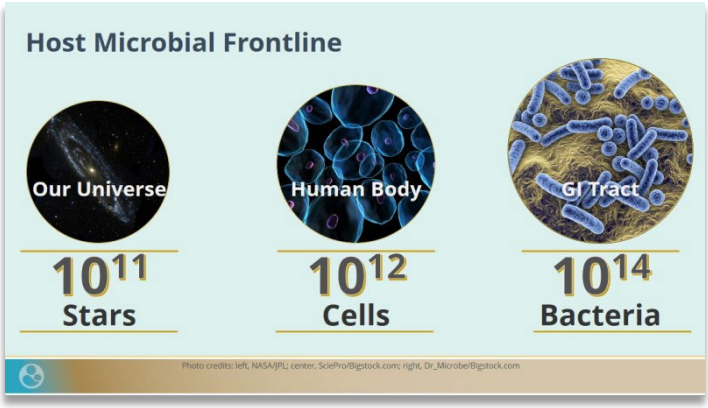


Slide 6—Structure of an Epithelial Unit

This is a cut section (slide 7) at the tip of 1 of the villi, which I have made. And then, looking at the tip of this villi, there is 1 cell here, and the 1 cell shows you, at the top is the brush-border membrane. Between the cells, there is paracellular pathway, and at the bottom, I'm showing to you that this is a capillary, with red blood cells ready to transport nutrients across the GI tract.

Slide 7—Cut Section of Villi

The question is, how many microbes do you have in your gut? Well, clearly 10^{14} we have bacteria in our gut. That's 100 trillion bacteria, literally, you have in your gut. However, the human body has 10^{12} of cells. And our universe has only 10^{11} of stars in our universe. So clearly, we have more bacteria in our gut than our cells in our body and stars in our universe.



Slide 8—Host Microbial Frontline

Literally, you have a 100 trillion organism, and 500 to almost 1,000 species have so far been identified, with 70 divisions. Ninety percent of the cells in our body are microbial cells. We have 100-fold more genes in our gut than the human DNA.[2] Our flora is clearly an integral part of our genetic landscape and

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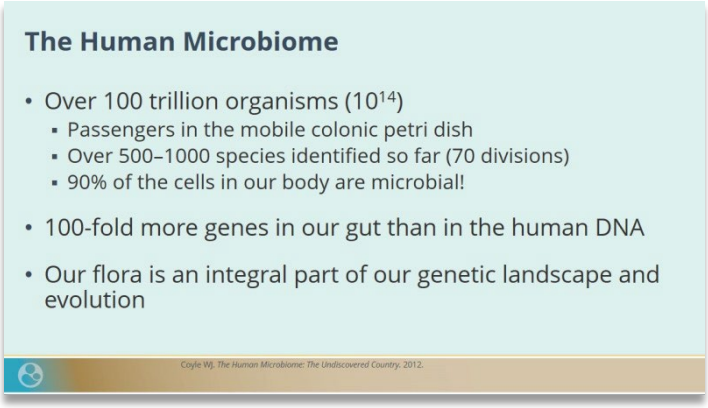
evolution. You have 3.3 million bacterial genes, while you have 23,000 human genes.[3]

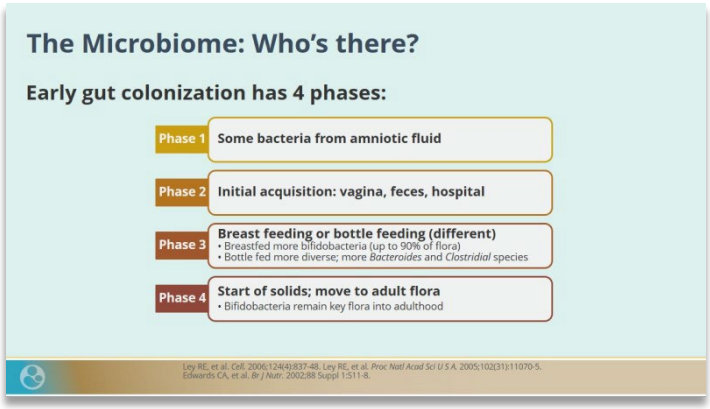


Slide 9—The Human Microbiome

So literally, our body has 10% of our DNA as a human, and 90% of our DNA is bacterial DNA. That tells you we have to take care of the bacteria in our gut, because 90% of our DNA is bacterial in origin.

Slide 10—The Human DNA

Where do we get this bacteria as humans? There are 4 phases of the acquisition of the bacteria (slide 11). Phase 1, there is some bacteria from… it used to be thought that the amniotic fluid is totally sterile. The problem is only 20% of the bacteria can be cultured, so when you do sequencing, you will see that there are some bacteria in amniotic fluid. The babies swallow some of the bacteria, but not enough. Phase 2, depends on vaginal delivery vs C-section. The initial acquisition if you have someone with a vaginal delivery, then the vaginal microbiome and the gut microbiome of the mom participate in the acquisition of bacteria. However, if you are born by C-section, then clearly you acquire the hostile microbiome or the skin microbiome from the mom.



Slide 11—The Microbiome: Who's there?

Phase 3, breastfeeding or bottle feeding. Breastfeeding, of course, is superior. The breastfed babies have Bifidobacteria, which account for more than 90% of the flora in these babies. Bottle-fed babies, they have more diverse *Bacteroides* and *Clostridium* species.[4,5,6] By the age of 2 years, you will move from infant flora to adult flora. Before the bacterium remain key flora into the adulthood; however, the food we eat changes your gut microbiome, which we will discuss a later.

Mode of Delivery

What are the differences in different microbiome based on mode of delivery? The left side (slide 12) is the vaginal delivery. In anticipation of the babies delivered by the mom, there is a change in her gut microbiome. There is a change in her vaginal microbiome. There is a change in her skin microbiome around the alveoli of the breast in anticipation of the baby to be breastfed by the mom. The vaginal microbiome is different than a skin microbiome, which is acquired when you're born by C-section.[7] See the number of different bacteria is different. I want to emphasize, vaginal microbiome, breastfed babies will acquire Bifidobacterium compared to C-section babies who

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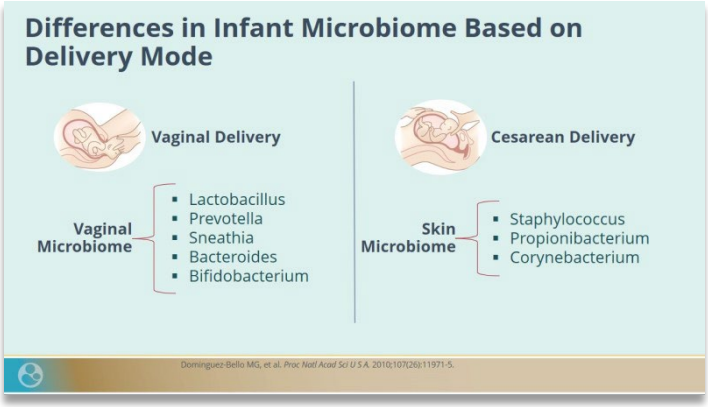
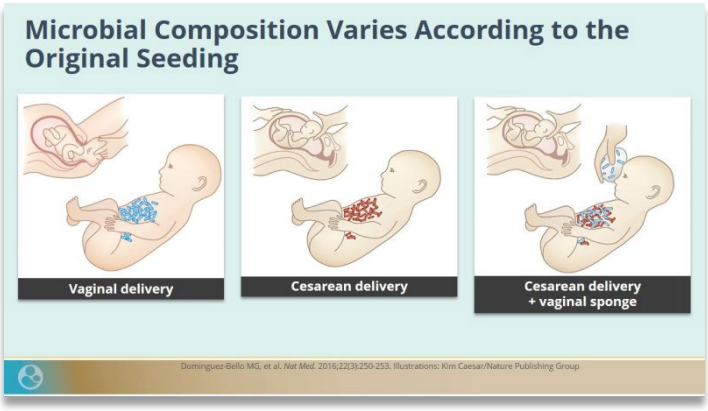
acquire the skin microbiome as well as hostile microbiome.[8]



Slide 12—Differences in Infant Microbiome Based on Delivery Mode

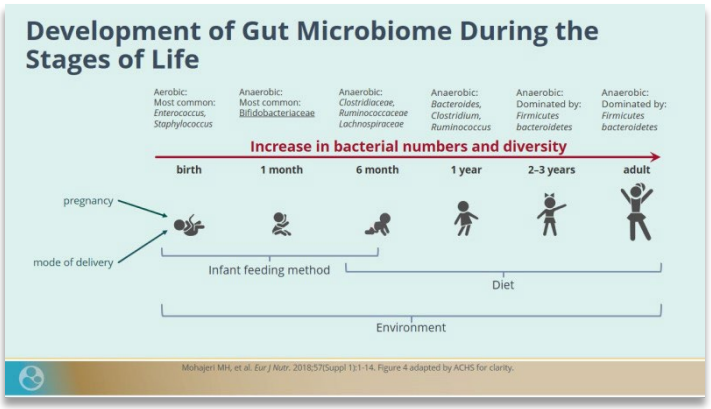
There's some evidence to suggest in vaginal delivery, to do seeding of the bacteria by doing vaginal swabs and swabbing the skin of the baby to potentially improve the microbiome of these C-section babies. There is a trial ongoing at this stage in the United States to try to address this concept.



Slide 13—Microbial Composition Varies According to the Original Seeding

We are now talking about 2 things: pregnancy and mode of delivery plays a major role in your gut microbiome. **Mom's nutritional status is very important, as well, delivering diverse microbiome to the baby.** We believe the healthier the mom's diet is during a pregnancy, such as omega-3 fatty acid, for example, that will be anti-inflammatory diet coming to the baby. Why? Because the mom's gut microbiome changes, and that will influence the baby's gut microbiome.

Mode of delivery, of course, we talked about vaginal delivery, which is much better for the babies. On top of the slide (slide 14), it shows you the start with aerobic, which is most common, and then moves to Bifidobacterium at 1 month of age, with the infant feeding method.[9] And then how the diet plays a major role.



Slide 14—Development of Gut Microbiome During the Stages of Life

Remember, the more diversified your microbiome, the better it is for you. We believe **the first 1,000 days**—from conception until 2 to 3 years of age—**are the most important, when we need to feed the babies a good, healthy diet.** There is evidence to suggest, which I'll show you a bit later, that will decrease autoimmunity when you are an adult.

Again, we're going to emphasize the first 1,000 days, from inception until the baby is about 1,000 days old, this is a very important time for nutrition. On the right side of the slide (slide 15), you see the growth of the central nervous system. This is the number of neurons, synaptic neurons, which are massive by days at 2 years. It's important for your brain, as well. Your brain grows 287% during the first 1,000 days.[10]

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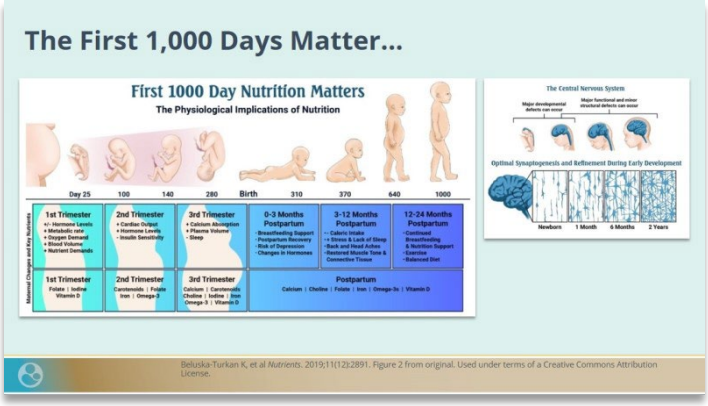
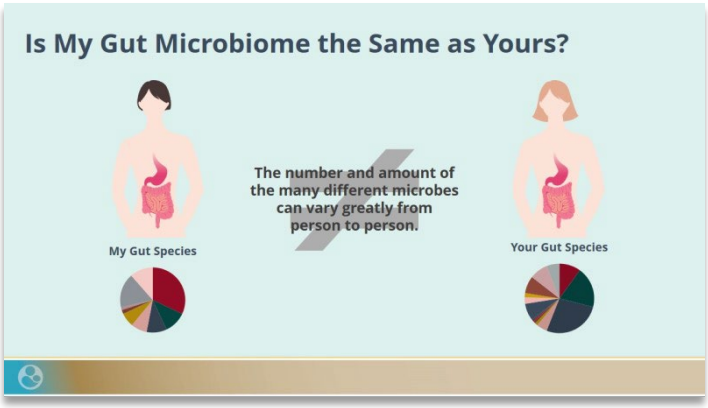


Slide 15—The First 1,000 Days Matter...

By the way, after 1,000 days, it grows only by 18%. It's clear the first 1,000 days are very, very important in regard to maternal nutrition, for general delivery, breastfeeding, healthier diet for our kids in the first 2 years of life, that will allow them to have a more diverse microbiome resulting in decreased, we believe—based on animal studies—a decrease in autoimmunity in later life.

Unique Microbiome

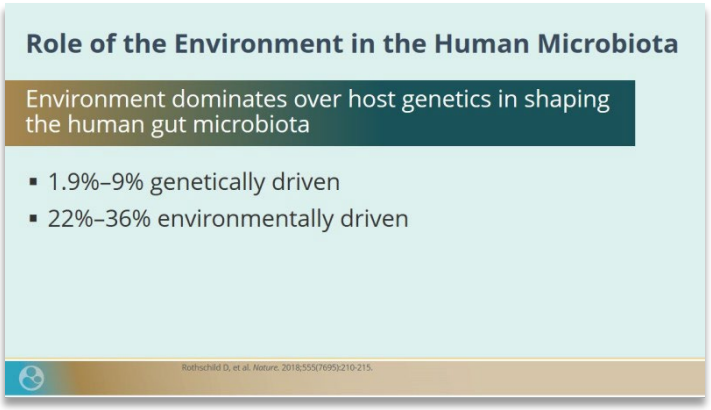
Is your gut microbiome like mine? No. The gut microbiome is like a fingerprint. Each one of us is different. Our microbiome is totally different between one another. The number and amount of the many different microbes can vary greatly from person to person, depending on 2 things: diet and lifestyle. These 2 important things dominate over genetics, which you'll see in this next slide.



Slide 16—Is My Gut Microbiome the Same as Yours?

First 1,000 Days

Someone has studied in a paper in *Nature*,[11] what is the contribution of genetics in shaping your gut microbiome vs environmentally driven? Clearly, it is environmentally driven. That is, **diet and lifestyle dominate over genetics in shaping your future.** Ladies and gentlemen, biology is not your destiny. Your destiny is what you do to yourself, what you do by exercise, what you do to have microbiome, what you do by eating a healthier diet, for example. Same thing for babies, early in life, the first 1,000 days. I cannot emphasize enough that this is very important compared to genetics.



Slide 17—Role of the Environment in the Human Microbiota

This is a picture from my lab (slide 18) to let you know that the number of bacteria we can culture is only 20%. The number we can sequence using this new technology, which is DNA sequencing, culture independent, is called next-generation sequencing. On the left side is something called MiSeq. That will give us a big picture vision. The NextSeq 550, that will give me metagenomics, ie, the species level. I can tell you the species level, what's happening in your gut. That's what we do in our lab. We recently acquired NovaSeq 6000, which is more important than the NextSeq 550. We can do a whole genome sequencing in less than 19 hours.

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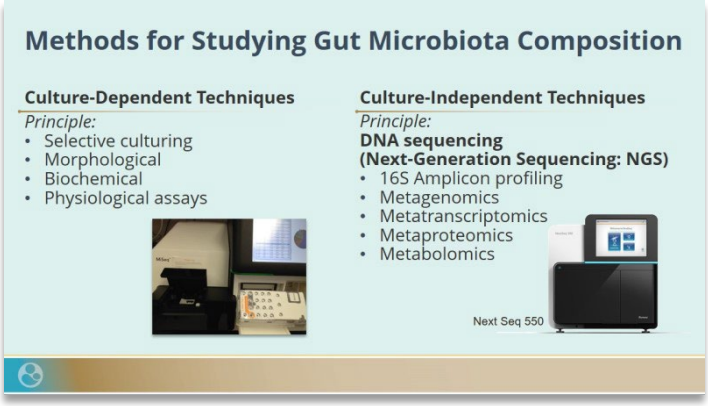
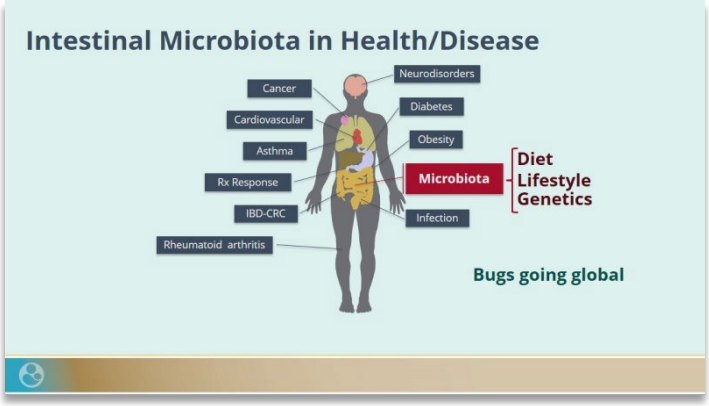
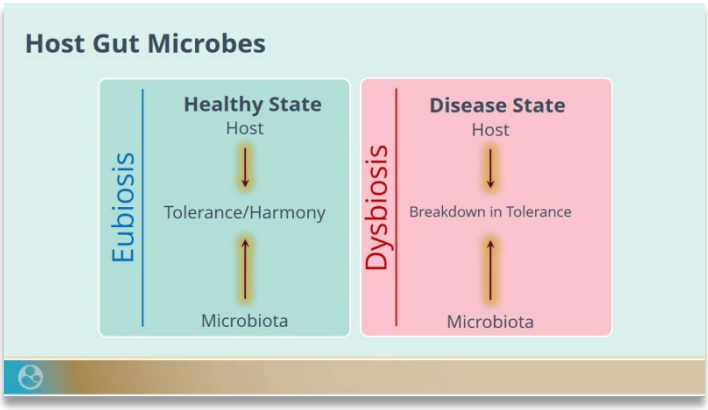


Slide 18—Methods for Studying Gut Microbiota Composition



Slide 20—Intestinal Microbiota in Health/Disease

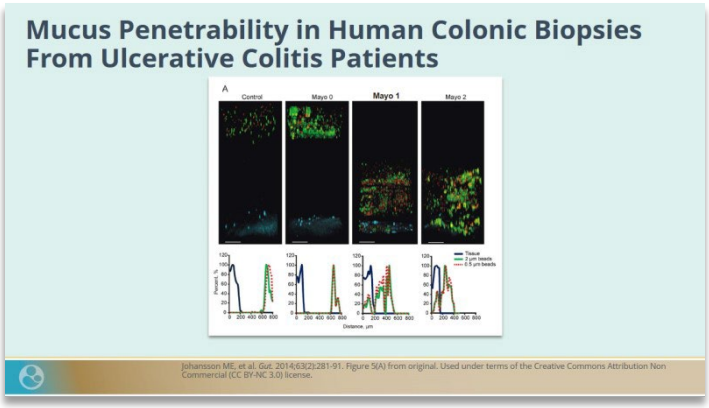
In summary, what I showed you so far: you need to be in eubiosis condition. Our babies need to be in eubiosis condition. We, as a host, we must have tolerance and harmony with gut microbiome. Disease state: you as a host, you have a breakdown in tolerance with your microbiome, that's called dysbiosis. We need to be in eubiosis condition rather than dysbiotic condition. Let me explain this to you.



Slide 19—Host Gut Microbes

Those are the diseases that are associated with a change in your microbiome, but again, diet and lifestyle dominate over genetics in shaping your gut.

This is research published by my lab in collaboration with our colleagues in Sweden,[12] (slide 21) on the left side, it's called control. The blue color on the bottom of the slide, it shows the lining of your gut. The mucus is green, and the red is bacteria. See the space, beautiful. That's what it should be. [However, on the] next image, beside it, that's a patient with ulcerative colitis. We treated them, and now this patient is in remission. Beautiful. Now, see Mayo 1, Mayo 2, look at this. The bacteria and the mucus layer is overlying the space in your gut. That's a dysbiotic state. That's autoimmune disease, like ulcerative colitis in patients, showing you there is overlap between your bacteria and your gut, the mucus and your gut, and your lining. The diet you eat, for example, can give you this type of picture.



Slide 21—Mucus Penetrability in Human Colonic Biopsies From Ulcerative Colitis Patients

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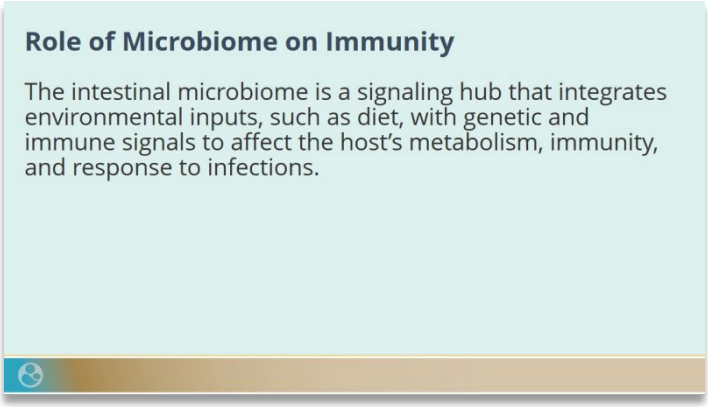
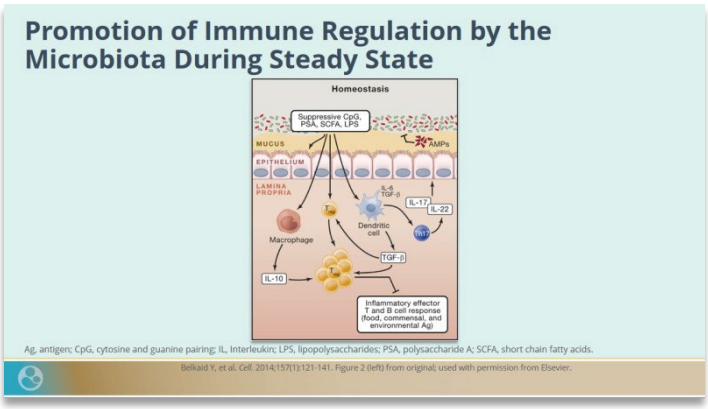
What is the role of the microbiome in our immunity? Clearly, the intestinal microbiome is a signaling hub that integrates environmental input, such as diet with genetic and immune signals to affect the host metabolism, immunity, and response to infection.



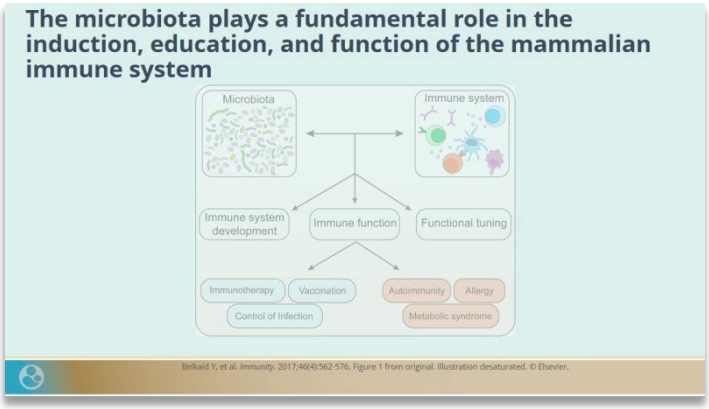
Slide 22—Role of Microbiome on Immunity

Our immune system relies heavily on the microbe in our gut. This slide (slide 23) shows you what I mentioned earlier, that the bacteria produces metabolites, and these metabolites alter your gut microbiome, alter your immune system.[13] Those metabolites can work on macrophages to produce IL-10, which is anti-inflammatory because they increase Tregs. T regulatory cells are anti-inflammatory as well, that are regulated by TGF-β [transforming growth factor β]. Again, **the important point here is our immune system depends on signals from our gut microbiome.**



Slide 23—Promotion of Immune Regulation by the Microbiota During Steady State

However, having said this, the immune system also affects your gut microbiome. If you have autoinflammation, autoimmune disease, that affects your gut microbiome, as well. There is an interaction between the microbe in your gut and the immune system.[14]



Slide 24—The microbiota plays a fundamental role in the induction, education, and function of the mammalian immune system

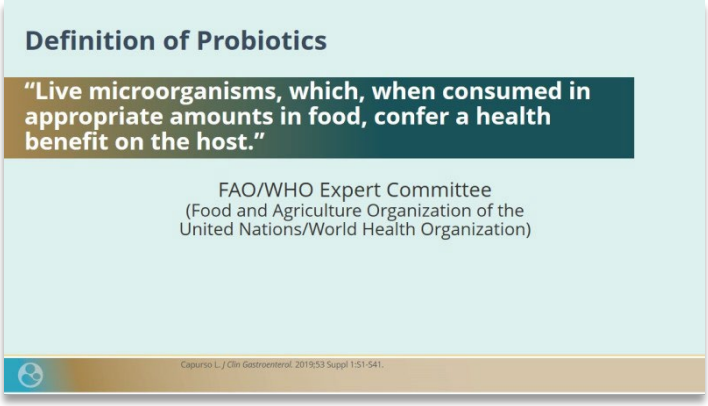
PROBIOTICS

Now, we will talk about probiotics. I explained to you first, the microbiome of the gut, how the microbiome alters your GI tract, immune system. Now, we will talk about how we're going to use probiotics. Pro, for life. The important point here that you need to remember is that we are talking about strain-specific probiotics. That is very important. *Lactobacillus rhamnosus GG* is one of the strains we will be talking about.

Defining Probiotics

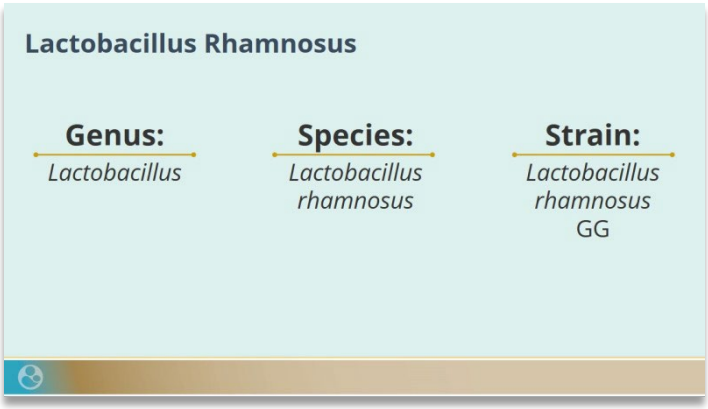
What is the definition of probiotics? Live microorganism, which when consumed in an appropriate amount, like 10^{10}, for example, of bacteria present in 1 capsule or 1 tablet, confer a health benefit to the host. That's the WHO [World Health Organization] definition of probiotics.[15] We need to make sure we are taking a good probiotic, which can deliver a good amount of microorganism to the host and confer a health benefit.

The Probiotic LGG® and Its Benefits to the Immune System



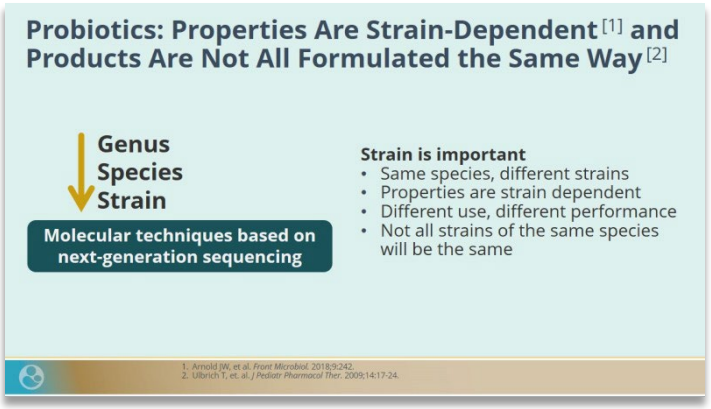
Slide 25—Definition of Probiotics

I'm explaining the strain-specific vs genus-specific. The genus is *Lactobacillus*. The species is *Lactobacillus rhamnosus*. The strain is *Lactobacillus rhamnosus* GG. So, what we are talking about, strain specific. Let's say you buy a probiotic for yourself. Are they talking about strain-specific or are they thinking about species or the genus or the phylum, for example, or something else? What you need to make sure of, whatever you do, is to look at the strain specific and see if there are studies done on that strain. That would be the important point.



Slide 26—Lactobacillus Rhamnosus

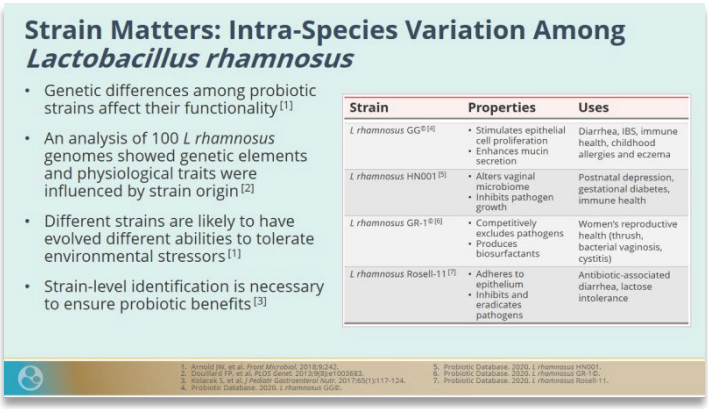
Strain is important, why? Because for some species, there are different strains, like *Lactobacillus*. *Lactobacillus* could be hundreds of *Lactobacillus* [strains], but we're talking about *Lactobacillus rhamnosus* GG, which is the strain. The properties are strain dependent, and different use provides different performance. Not all strains of the same species will be the same.[16,17] It is very important that molecular technique is based on next-generation sequencing, which can identify the strain, like I mentioned to you with NextSeq 550, which we use in our lab.



Slide 27—Probiotics: Properties Are Strain-Dependent and Products Are Not All Formulated the Same Way

We are talking about different strains. *Lactobacillus rhamnosus* GG is the one we are talking about, which stimulates epithelial cell proliferation, enhances mucin secretion. We use [this strain] in diarrheal disorders, IBS, for example, immune health, child allergies, and eczema. That's a different strain compared to *Lactobacillus rhamnosus* HN001 or *Lactobacillus rhamnosus* GR-1 or *Lactobacillus rhamnosus* Rosell-11, [15,18,19] because each one of them is different. The important point here: there are more than 100 *Lactobacillus rhamnosus* genome-shared genetic elements. What we are talking about, strain specific.

The Probiotic LGG® and Its Benefits to the Immune System



Slide 28—Strain Matters: Intra-Species Variation Among Lactobacillus rhamnosus

Probiotics vs. Prebiotics

Probiotics are like you and me. Number 1: it likes to live in a nice environment. Number 2, it needs food for it to eat.[20] Prebiotics is the substrate for the probiotics to grow. What is important for you and me, as adults, are the prebiotics. For example, if you eat fruits, eat it with the skin, not just the juice, because you need the fiber. The fiber is probiotics for adults.

For a baby, what is the probiotic? If breastfed, prebiotics are human milk oligosaccharides. That's it. That's the best prebiotic for the baby. However, if you have a formula-fed baby, what are you going to do? Infant formula manufactures now add in the following: GOS, which is galactose oligosaccharide, that's not digested, remember. All these prebiotics cannot be digested in the GI tract. It gives you substrate for the probiotics to work.

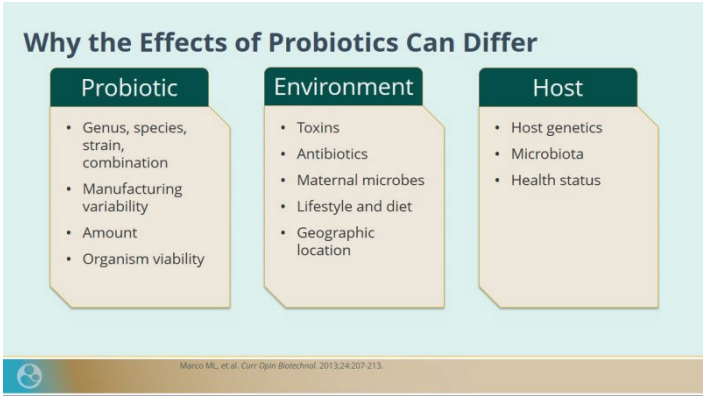
	Prebiotics	Probiotics
Definition	Nondigestible food ingredients that selectively stimulate the growth and/or activity of a limited number of bacteria in the colon that are thought beneficial to host health	Live microorganisms, which, when administered in adequate amounts, confer a health benefit for the host
Result of Use	Prebiotics enhance the gut microbiota already present	Probiotics add a specific colony to the gut microbiota
Purpose in Infant Feeding	Functional substitution for breast oligosaccharides	Specific probiotics are useful for specific conditions, including CMA [2] and diarrhea
Commercially Available Examples	• Galacto-oligosaccharide (GOS) • Fructo-oligosaccharide (FOS) • Human milk oligosaccharides (HMOs) • Inulin • Polydextrose (PDS)	• *Lactobacillus rhamnosus* GG© (ATCC 53103) • *Bifidobacterium animalis* subsp. *lactis* EVC001 • *Saccharomyces cerevisiae boulardii* • *Lactobacillus reuteri* (DSM 17398)

Table 1—Feeding the Microbiota: Prebiotics vs Probiotics

Fructose oligosaccharide, the human milk oligosaccharide, it's 2'-FL, Fucosyllactose, that's a synthetic milk oligosaccharide, synthetic (similar to human milk oligosaccharide), or something called inulin or polydextrose. Those are used by the infant formula manufacturers that allow the bacteria in your gut to grow because that's how the bacteria lives on prebiotics. Without prebiotics, the bacteria are not going to grow.

I want to emphasize 1 more point. Probiotics, like you and me, like to live in a nice environment. For example, take someone with IBS, irritable bowel syndrome, with SIBO, small intestinal bacterial overgrowth, and you take probiotics, it is not going to work. You need to clean the GI tract out, prevent the bacteria from growing again, and then give them probiotics. Then it will work.

What is the effect of probiotics and how is it be different from each other? Well, again, genus vs species vs strain, manufacture variability, organism viability, amount. 10^{10}; the amount is important. If the environment is good, maternal microbiome for lifestyle and diet again is important. Host genetics, microbiota, health status[21] [are all important]. As I mentioned to you, if someone has an autoimmune disease, active IBD, inflammatory bowel disease, well, there's a harsh environment, it's probably not going to work. Induce remission in this patient and then give them probiotics, then it will work.

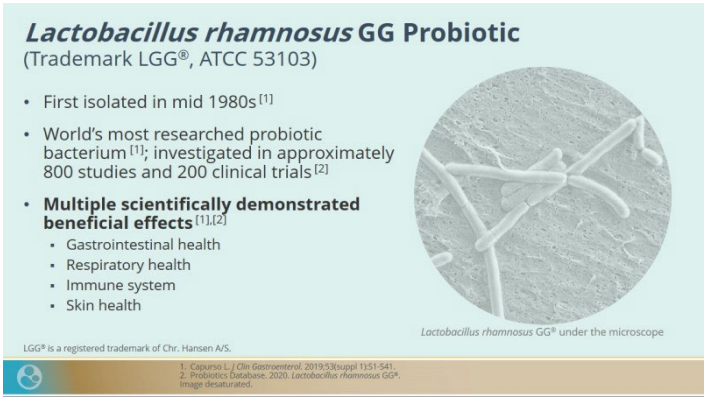


Slide 29—Why the Effects of Probiotics Can Differ

Benefits of LGG

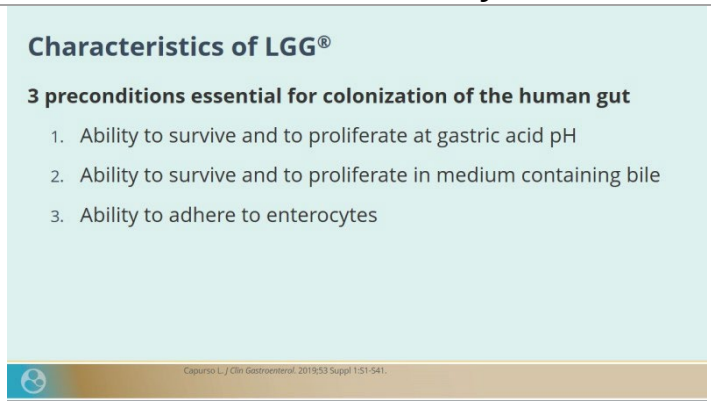
The Probiotic LGG® and Its Benefits to the Immune System

We are discussing *Lactobacillus rhamnosus* GG probiotics. It was first isolated in the mid 1980s and is the world's most researched probiotics bacterium, investigated in approximately 800 studies with more than 200 clinical trials. It has been shown scientifically to have benefits for your gastrointestinal health, for respiratory health, for the immune system, for skin health, as well like atopic dermatitis. It is very important.[15]



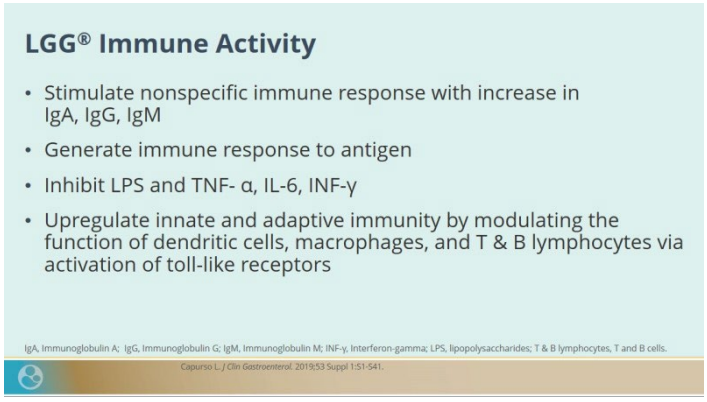
Slide 30—Lactobacillus rhamnosus GG Probiotic

What are the characteristics of LGG? Well, 3 preconditions are essential for colonization of your gut. One, the ability to survive and proliferate at the gastric acid pH. You don't want to take some bacteria and have it killed by the acid in your stomach. No. That's not going to work. The ability to survive and proliferate in a medium containing bile, because bile acids are detergent. Bile acids are essential for you to digest fat and disperses fat, and therefore emulsifies it, and then you have lipase and colipase enzyme make fatty acid from the lipids you eat. Third, you have to have the ability to adhere to enterocytes.[15]



Slide 31—Characteristics of LGG®

Does LGG have a role in our immune system? Yes, it does. It stimulates nonspecific immune response with increase in IgA, IgG, IgM. It generates an immune response to antigen, inhibits LPS, lipopolysaccharides, which are proinflammatory, TNF-alpha, IL-6, interferon gamma. It upregulates innate and adaptive immunity by modulating the function of dendritic cells, macrophages, and T and B lymphocytes via activation of toll-like receptors.[15] It's clear LGG has a role in our immune system.

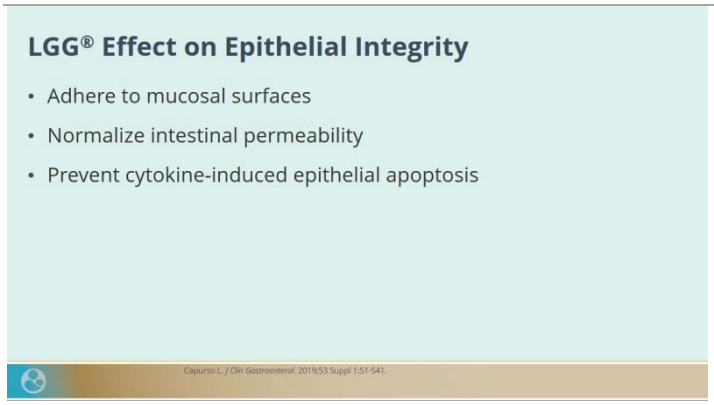


Slide 32—LGG® Immune Activity

Epithelial Integrity

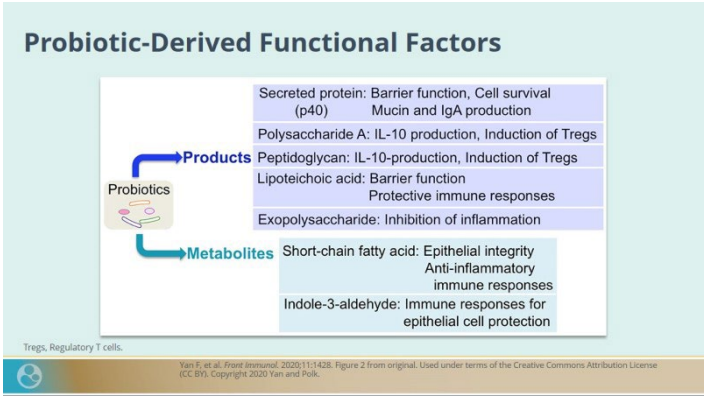
What about epithelial integrity? Well, it's clear that it adheres to mucosal surfaces, normalizes intestinal permeability—I'll show you a slide on that—and it prevents cytokine-induced epithelial apoptosis.[15] Apoptosis is associated with cell death.

The Probiotic LGG® and Its Benefits to the Immune System



Slide 33—LGG® Effect on Epithelial Integrity

How do probiotics work? Well, it's clear there is a product and metabolite of these probiotics. How do we know all these things? This is what we do. You take probiotics LGG, grow it in a culture media. Then, take the supernatant and run it over gel, and you will see that there is a protein called p40 kilodalton. That's one of the major secreted proteins by LGG that increases barrier function, cell survival, mucin production, and IgA production.[22] Now, also, there is a polysaccharide A, which is also involved in IL-10 production. IL-10 is anti-inflammatory, induces T regulatory cells, which are also anti-inflammatory. Peptidoglycan, IL-10 production as well, induction of Tregs. Lipoteichoic acid, that's barrier function protective. Exopolysaccharides, inhibits inflammation.



Slide 34—Probiotic-Derived Functional Factors

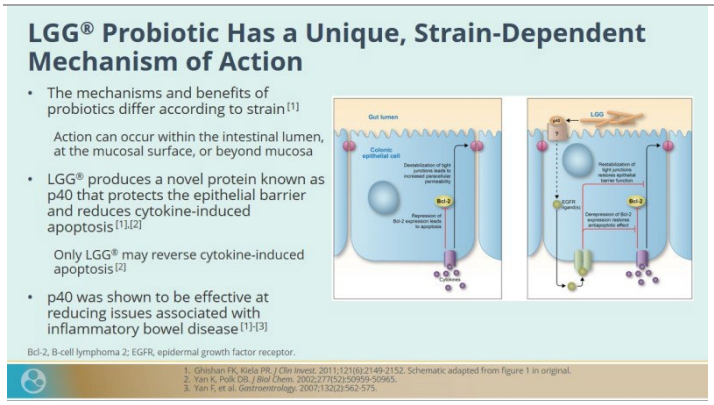
There are a number of products from LGG. Those are very well studied, but the most well-studied is the p40 protein. The probiotic LGG produces metabolites and short-chain fatty acid. Butyrate is the major short-chain fatty acid produced by probiotics that enhances epithelial integrity. It is anti-inflammatory because it increases Tregs, for example, and our immune system by increasing T regulatory cells. Also, short-chain fatty acid is important as a respiratory fuel for our colon. This is the reason why we always emphasize, even in babies, to feed them fruits and vegetables in their diet when they are several months old. Using this fiber-containing fruits and vegetables is very relevant for allowing the bacteria to make sure there is short-chain fatty acid.

Indole-3-aldehyde, for example, is also a metabolite. How do we work on this metabolite? There is something called metabolome. I can take the stool sample, for example, and take the supernatant and send it to the lab, which identifies metabolites from the stool. You can do blood metabolites, as well. Metabolome, it's called.

It's clear that our ability now is not only to do strain-specific level of probiotics and figure out their function, but it's also important that they produce metabolite, which we also can measure.

This is my work. I published this in JCI [*The Journal of Clinical Investigation*], and that's an editorial written when I reviewed a paper, which looks at p40 protein.[23] I postulated that this work by my collaborators, Fang Yan at Vanderbilt University, which shows that cytokines inhibit Bcl-2 gene, and that destabilizes our epithelial layer, which can result in leaky gut.[24] See the space here, leaky gut. Now through LGG, on the right side of the slide, LGG through p40 protein, which I just mentioned to you, works on the epidermal growth-factor receptor, which binds the ligand for the receptor. And that works on the Bcl-2, which inhibits it, destabilization of the tight junction. And you see here, epithelial integrity is restored with LGG.[23,24] This is how I think LGG works through this p40 protein.

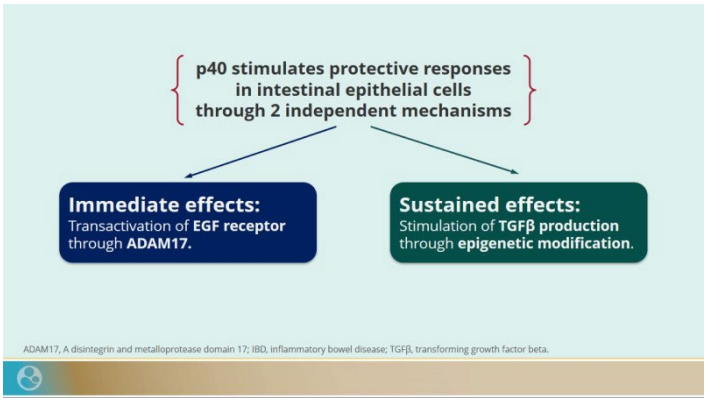
The Probiotic LGG® and Its Benefits to the Immune System



Slide 35—LGG® Probiotic Has a Unique, Strain-Dependent Mechanism of Action

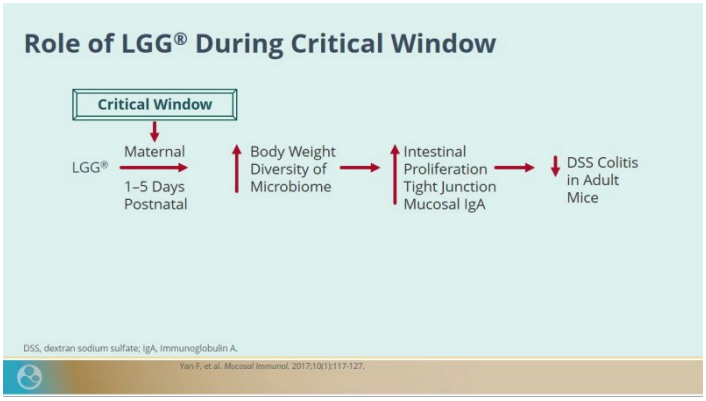
Effects of p40

p40 has 2 areas of importance I want to mention. One, the immediate effect of transactivation of epidermal growth-factor receptor through a transforming growth factor called ADAM17, and you'll get immediate effect on the epithelial layer.



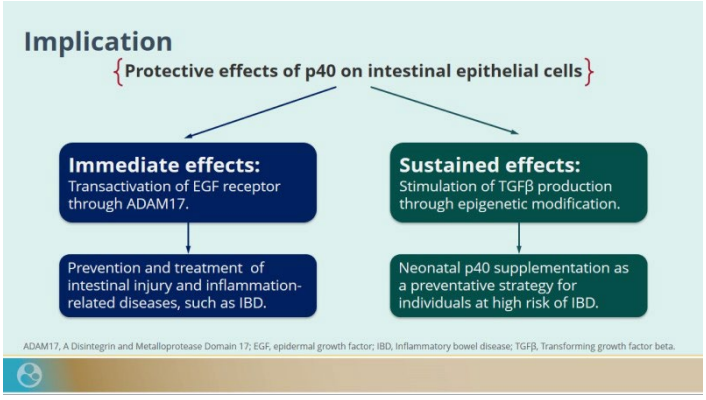
Slide 36—p40 stimulates protective responses

However, a sustained effect, stimulation of transforming growth factor β through epigenetic modification; you will have a sustained effect in the future. But the important point is what I'm going to show you next. There is a critical window for LGG to work during early life. In this study (slide 37), for example, again, published in *Mucosal Immunology* in 2016,[24] showed 1 to 5 days postnatally and maternal, LGG intake in that critical window increased body weight, diversity of microbiome, intestinal proliferation, tight junction, because of IGA increase. If you challenge these adult mice with DSS, dextran sodium sulfate, they're inflammation decreases. However, if you take LGG and give it later, 30 days postnatally, you don't have that effect. So, adjusting [with the knowledge] that there is a critical window, at least in animal models. However, in humans, we believe the first 1,000 days is the critical window where LGG has a long-term effect on decreasing colitis in adults.



Slide 37—Role of LGG® During Critical Window

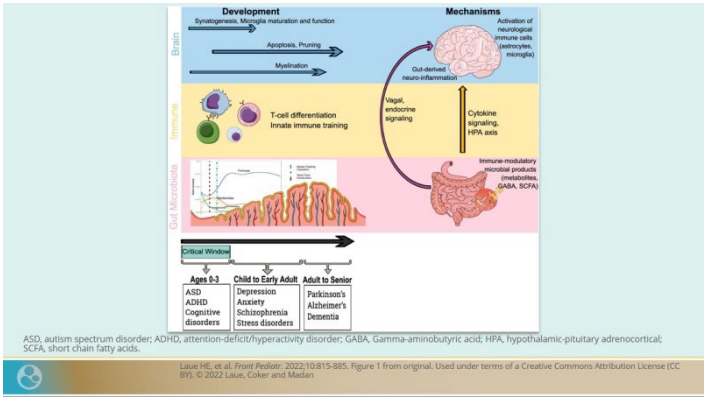
Here are the implications: there is a protective effect of p40 on intestinal epithelial cells. One is immediate that transactivation of EGF receptor through ADAM17 transcription factor, prevention and treatment of intestinal injury and inflammation-related disease. However, sustained effect, and during that critical window, stimulation of TGF-β transforming growth factor production and neonatal p40 supplementation as a preventative strategy for individuals with high risk of IBD.



Slide 38—Implication

The Probiotic LGG® and Its Benefits to the Immune System

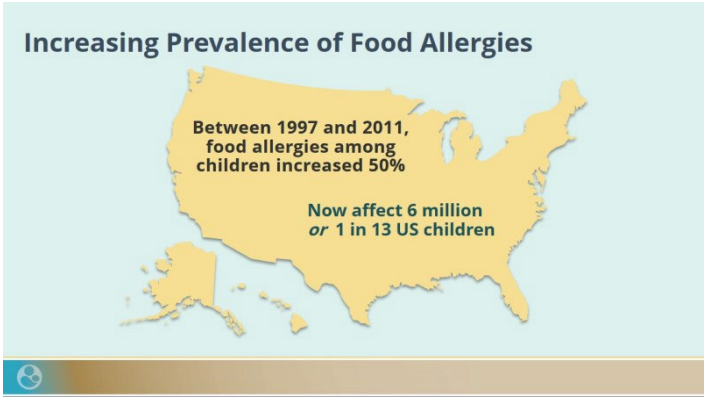
So, what about the brain? I told you about the brain-gut interaction. There is no question that this is very relevant (see slide 40). The critical window is, as I mentioned, the first 1,000 days. There is a decrease in activation of neurological inflammation, for example, during early life, if you use probiotics during early life.[25]



Slide 39—Microbiota, immune and brain development drive mechanistic pathways by which the gut microbiota influences the brain

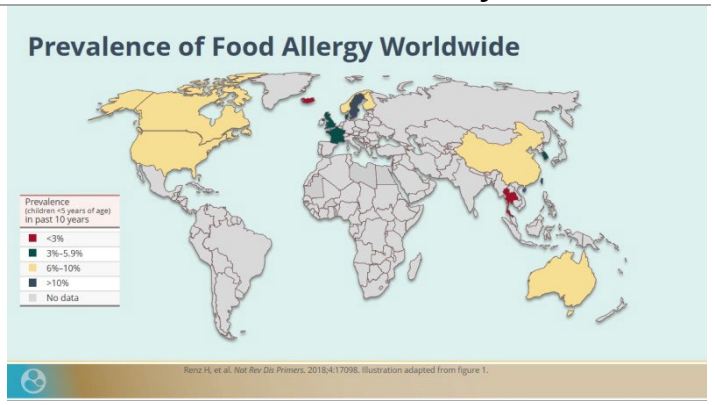
Understanding Allergy

Now we need to understand allergies, and that will be the final section of this talk. Allergies are increasing in the United States. One in 13 kids in the United States has allergies.



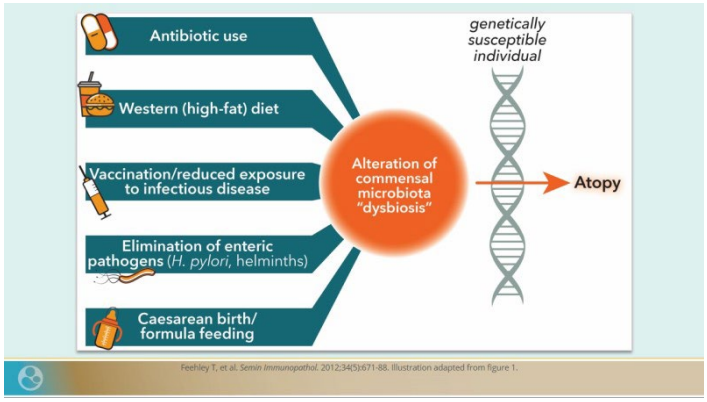
Slide 40—Increasing Prevalence of Food Allergies

What about the rest of the world? Similarly, there's increase in the Western world. In fact, in Sweden, more than 10% of the kids have allergies.



Slide 41—Prevalence of Food Allergy Worldwide

Why are allergies increasing? There are 2 reasons: one, genetic susceptibility. Second, is the change in your gut microbiome, dysbiotic state.[26] If you have a dysbiotic state from the use of antibiotic Western diet, for example, C-section and so forth, clearly that will result in an increase in atopic disease.[27]



Slide 42—Why is the prevalence of food allergy increasing?

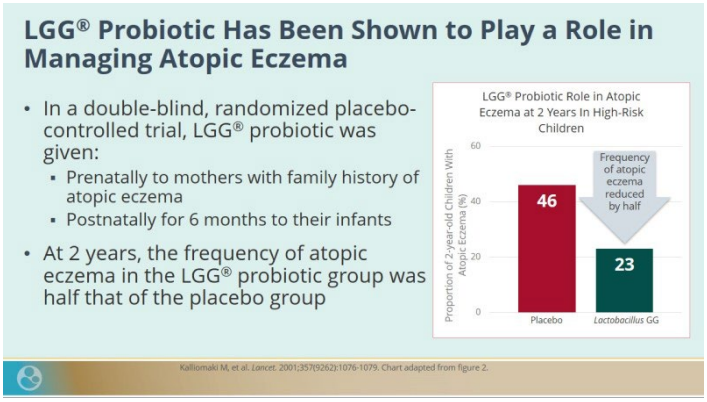
These are some of the studies (table 2) on probiotic and atopic disease management. Many studies have shown positive effects. In fact, more than 20 studies with LGG probiotic show positive results in regard to atopic diseases, such as atopic dermatitis.[15,28,29,30]

The Probiotic LGG® and Its Benefits to the Immune System

Author	Year	Study type	Probiotic type	Results	Result type
Ou	2012	R, PC, DB	LGG®	Issues of maternal allergic scores improved significantly	Positive
Nermes	2010	R, PC, DB	LGG®	Significant decrease in proportions of IgA- and IgM-secreting cells	Positive
Huurre	2008	R, PC, DB	LGG®	Protective effect against sensitization in infants with a high hereditary risk	Positive
Kalliomaki	2007	R, PC, DB	LGG®	Significant decrease in cumulative risk for developing eczema during the first 7 years of life	Positive
Viljanen	2005	R, PC, DB	LGG®	Decreased SCORAD	Positive
Kirjavainen	2003	R, PC, DB	LGG®	Decreased SCORAD	Positive
Majamaa	1997	R, PC, DB	LGG®	Decreased SCORAD	Positive

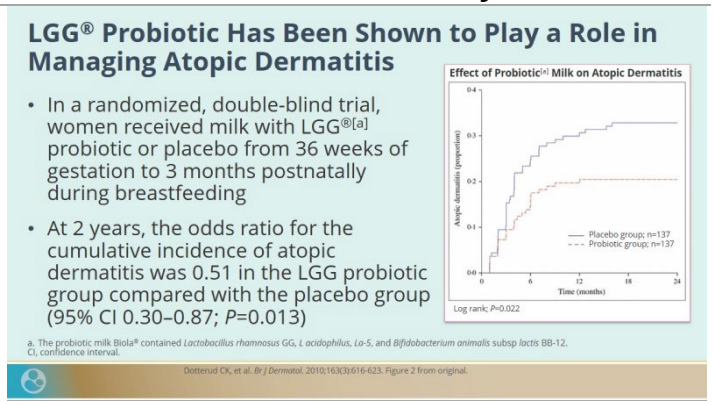
Table 2—Effects of Probiotics in Atopic Disease Management

This is, for example, 1 of the studies (slide 43), the use of LGG probiotics [in a child] at 2 years of age, shows there's almost a 50% decrease of atopic eczema using LGG in a double-blind controlled study.[31]



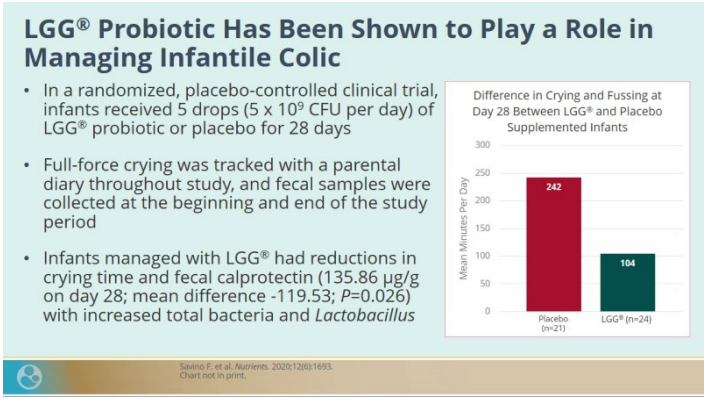
Slide 43—LGG® Probiotic Has Been Shown to Play a Role in Managing Atopic Eczema

What about putting probiotics over milk, like fermented milk? Clearly, in this study (slide 44), for example, 3 months postnatally, they are given LGG, and you could see almost a 50% decrease in atopic dermatitis using LGG probiotics added to milk, that is fermented milk.[32] Fermented milk has a role as well in managing allergies because of the role of LGG.



Slide 44—LGG® Probiotic Has Been Shown to Play a Role in Managing Atopic Dermatitis

I'm also showing you (slide 45) that LGG probiotic has a role in managing infantile colic. Giving 5 drops or 5 to the ninth part, for example, for babies with colic in the first 3 months of life has resulted in a decrease, for example, in crying and fussing episodes.[33]

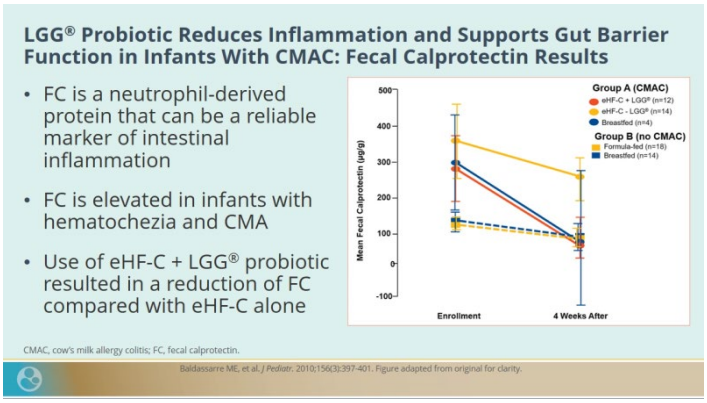


Slide 45—LGG® Probiotic Has Been Shown to Play a Role in Managing Infantile Colic

What about colitis, cow's milk colitis? In this study (slide 46), for example, they looked at calprotectin.[34] What's calprotectin? Calprotectin is 50% of the cytoplasm of the neutrophil. Calprotectin is a noninvasive measure of inflammation in your gut. In the red color (group A), extensively hydrolyzed formula, in this case, extensively hydrolyzed formula plus LGG resulted in a marked decrease in calprotectin compared to extensive hydrolyzed formula alone without LGG, which is in the yellow color.

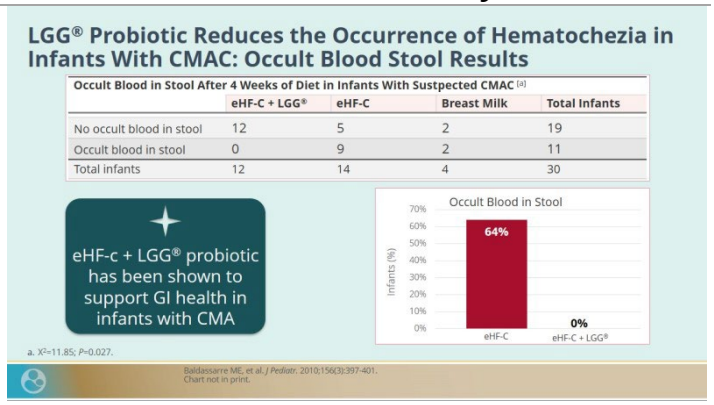
The Probiotic LGG® and Its Benefits to the Immune System

I want to mention, there are 4 breastfed babies in the study group A, which is the cow's milk allergy. In fact, some of them improved, but some did not improve, and the reason they did not improve is because the mom's milk may contain other antigens rather than cow's milk, for example, wheat for example, eggs, soy for example. That's the reason why you see differences here.



Slide 46—LGG® Probiotic Reduces Inflammation and Supports Gut Barrier Function in Infants With CMAC: Fecal Calprotectin Results

What about blood in the stool? Well, you could see here (slide 47) extensively hydrolyzed formula plus LGG, out of 12 patients, zero occult blood in the stools. However, extensively hydrolyzed formula without LGG, 9 of the 14 continue to have occult blood. Breast milk, 2 of them continue to have blood, because, as I mentioned, breastfed babies may have other than cow's milk allergy causing the inflammation in proctocolitis, as I mentioned, gluten, for example, or eggs, or something else.

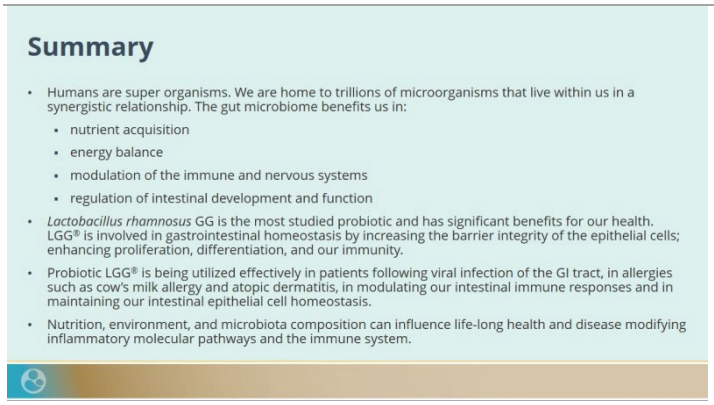


Slide 47—LGG® Probiotic Reduces the Occurrence of Hematochezia in Infants With CMAC: Occult Blood Stool Results

In summary, humans are a super organism. We are home to trillions of microorganisms that live within us in a synergistic relationship. The gut microbiome benefits us significantly by nutrient acquisition, such as short-chain fatty acid, energy balance, moderation of our immune and nervous system, regulation of intestinal development and function. And *Lactobacillus* GG, which is the most studied probiotic, has significant benefit to our health, is involved in gastrointestinal hemostasis by increasing barrier function as well as enhancing proliferation, differentiation, and IgA level. Probiotic LGG has been utilized in infants following viral infection of the GI tract; in allergies, such as cow's milk allergy and atopic dermatitis; and modulating our intestinal immune responses; and in maintaining our intestinal epithelial cell homeostasis.

Finally, most importantly, nutrition and environment dominate over genetics in shaping your gut microbiome. Enhancing microbiota for a diverse microbiome in the first 1,000 days can influence lifelong health and disease-modifying inflammatory molecular pathways and the immune system.

The Probiotic LGG® and Its Benefits to the Immune System



Slide 48—Summary

Question & Answer

Editor's Note: This is a transcript of audience questions together with presenter responses from the May 31, 2022, webcast.

Can you expand on the long-term outcomes and benefits associated with LGG?

Fayez K. Ghishan, MD: That's an excellent question. As I mentioned to you, the maternal diet is very important. Second, the first 1,000 days are very important in shaping your future. So, in the maternal diet, the more the mom takes omega-3 fatty acids, omega-3 fatty acids anti-inflammatory, it changes her gut microbiome, which is transmitted to the baby. During those important 1,000 days, breastfeeding would be the best. If the mom is not breastfeeding, my suggestion is they need to use probiotics. Probiotics in the first couple of years of life result in immediate effect on the epithelial integrity through epidermal growth-factor receptor.

However, late effect in that critical period through epigenetic mechanism involving transforming growth factor β, we believe, based on animal studies, that critical period can result in a decrease in inflammatory state in the future, such as IBD. We've proven this through animal work. However, in humans, it makes logical sense to shape the gut microbiome early in life in the first couple of years, and that is from inception until 1,000 days, that means the kid is about 2½ years old. During that critical period, there's a clear mechanism to enhance the gut microbiome, make it diversified, and it will, I believe, decrease autoimmunity, such as IBD, for example.

To touch on the data you presented in those last few slides, Dr. Ghishan, why are the studies so small? Is there a reason why the N is so small?

Fayez K. Ghishan, MD: Initially, we try to figure out in a smaller number of N whether we have some tendency for effect or not, and then we take it for a longer period of time, as well as for a larger end. So, that's the reason why some of those studies shown, at least in the humans using calprotectin in the stools, those numbers are small, definitely because it's clear that sometimes recruiting patients to these studies may be a bit difficult. But in animal studies we start using smaller numbers to figure out if there is an effect, and then move to the next level.

In addition to probiotics, what additional nutrients will help to diversify the microbiome in an infant?

Fayez K. Ghishan, MD: One of my colleagues has been studying this. We published great work together. He's been working on biotin, for example. So, he knocked out the gene which allows you to absorb biotin. And the change in the gut microbiome, the colitis, for example. I worked on sodium transport, and we knock out sodium transport, for example, and they have a decrease in sodium absorption... In my judgment, there are so many nutrients we have not studied yet. So many nutrients are very relevant. I think electrolytes is very important, and vitamins are very important. For example, trace minerals, zinc deficiency. Zinc is essential metalloenzyme required for DNA synthesis. Zinc deficiency can result in diarrhea because there is a change in the epithelial layers, because there is a decrease in enterocolitis in those kids. So, zinc is very essential, as well. We believe that adequate nutrition to involve electrolytes,

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minerals, trace minerals, vitamins are all relevant for a healthy gut microbiome.

How do you determine the appropriate amount of LGG to give?

Fayez K. Ghishan, MD: 10^{10}. Let's say, if you have a baby, you want to give him 10^5 in the morning, 10^5 in the evening. That will be adequate. But 10^{10}, that's really the magic number: 10^{10}. Anything less than that, it may not work. Because at the end of the day, you need the probiotics sustainability. Let's say, if you take probiotics for 2 weeks and then you stop, that's it. It's gone. You need resistant. Whatever you do in the microbiome space is this: maintain a persistent diet. Let's say, you eat a Mediterranean diet, stay on it. Don't use it for a couple of weeks and then go back and eat fast food. It's not going to work. Same thing with probiotics. Don't use it for a week or two and then you stop. No. Be persistent. But 10^{10} is the most studies done on those numbers.

Are there any precautions regarding the use of LGG or probiotics in general in children?

Fayez K. Ghishan, MD: Preemies are a problem. I'll tell you why. There are so many studies in preemies... Remember, if you look at the gut microbiome in preemies in the NICU units (and I consult in our NICU all the time), the microbiome of preemies is dominated by something called Proteobacteria. Proteobacteria is a pathogenic bacterium. So, probiotics are not going to work in that harsh environment. That's the reason why one of my colleagues who wrote in an editorial said, "In NICU babies, to prevent NEC, there are so many studies, but little progress." So, in preemies, especially in younger preemies, I will worry about using probiotics because of these risks of bacteremia or risk of septicemia in some of these extremely preterm babies, but healthy babies, no. There's no contraindication for using it. However, make sure in kids that they don't have an autoimmune disease, if they do have an autoimmune disease, first induce remission and then after the remission induction, then you use probiotics. Then the probiotic will survive.

Can you say more about the impact on the development of allergies when LGG is added to extensively hydrolyzed formula?

Fayez K. Ghishan, MD: Very good question. Most of the studies on atopic dermatitis, atopic eczema, for example, have 2 faults. One, if you have a family history of allergies, maternal LGG during trimester of pregnancy will help the baby because that is transmitted to the baby through the vagina or through the gut microbiome, or through the skin microbiome of the mouth. In those situations, using probiotic can reduce... shown in a number of studies, reduces the atopic dermatitis by almost 50%. The question is, if you have family history, use mom's microbiome as a tool, give probiotics to the mom, and then the baby probiotics. If they breastfeed the baby, then they have *Bifidobacterium*. Then the risk to the baby is very, very small of getting any allergies. That's my view. My view is pay attention to the maternal diet, maternal probiotics, as well as giving probiotics to these babies and decreasing the... Based on number of studies, there is a decrease in the incidence of atopic eczema by 50%.

Now, if they don't improve by the probiotics, the other 50%, there are newer therapies. As you know, monoclonal antibodies against IL-4 receptor, for example, which is TH2 helper, IL-4, IL-5, IL-13, there are newer medications, for example, that people are using.

Are there human studies in children that indicate LGG improves food allergies other than cow's milk allergy?

Fayez K. Ghishan, MD: Yes, definitely. The important thing is that allergies nowadays, the trick for allergies is tolerance. We need to allow patients to have tolerance. Let's say, if you have a family

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history of cow's milk allergy, then what you should do is give these babies, even if they are breastfed babies, you want to make them have tolerance for cow's milk. So, give them extensively hydrolyzed formula, for example, plus LGG, a small amount, 5 mL in the first month of life, then wait 3 days, then 10 mL, then 15 mL. If they tolerate it, then that will allow them to have tolerance. LGG plus extensively hydrolyzed formula will allow patients to have tolerance for cow's milk allergy, but you have to use it early. Strong family history, start early.

Abbreviations

ADAM17	A Disintegrin and Metalloproteinase 17	**IgA**	Immunoglobulin A
Bcl-2	B-cell lymphoma 2	**IgG**	Immunoglobulin G
DSS	dextran sodium sulfate	**IgM**	Immunoglobulin M
EGF	epidermal growth factor	**LPS**	lipopolysaccharides
FMT	fecal microbial transfer	**SIBO**	small intestinal bacterial overgrowth
GOS	galactose oligosaccharide	**TGF-β**	transforming growth factor β
IBD	inflammatory bowel disease	**Tregs**	T regulatory cells
IBS	irritable bowel syndrome		

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