Pediatric Nutrition
CONTINUING EDUCATION FOR CLINICIANS

The Intestinal Microbiome and the Developing Immune System

✦ Course Transcript ✦

Overview

The gut microbiome is recognized as playing a key role in infant health. **Alessio Fasano, MD,** world-renowned for his research on the gut microbiome, focuses on the ways in which the gut microbiome influences allergy and autoimmunity. The effects of infant nutrition on the composition of the microbiome are also discussed, including the impact of feeding mode and probiotic and prebiotic supplementation. Dr. Fasano highlights the most recent clinical evidence supporting the use of key probiotic strains, including *Lactobacillus rhamnosus* GG (LGG), *Bifidobacterium animalis* subsp. *Lactis* (BB-12), and others, to inform clinicians about the current and emerging roles of probiotics for allergy management.

Target Audience

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

Learning Objectives

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

- Describe the association between gut microbiota imbalance and autoimmune disease
- Identify strategies, including the use of probiotics, that can regulate the gut microbiota
- Optimize the feeding strategy for an infant with persistent food intolerance

Faculty

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Faculty

Alessio Fasano, MD

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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.

This activity was released on June 16, 2022 and is eligible for credit through June 16, 2024.

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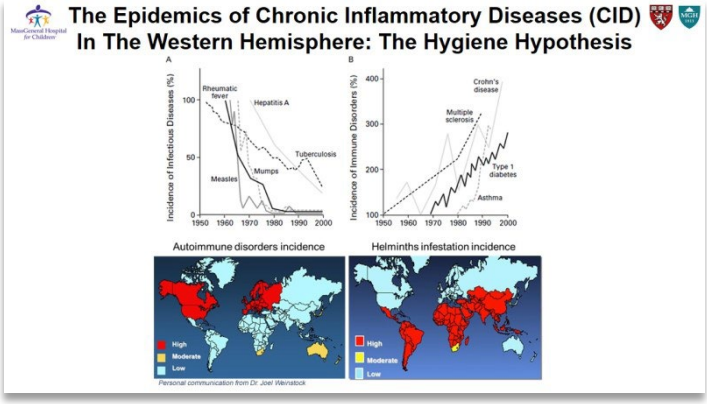
Editor's Note: This is a transcript of a live webcast presented online on May 20, 2022. It has been edited and condensed for clarity.

Intestinal Microbiome

Alessio Fasano, MD: The next 45 minutes or so, we are going to go over what is the current knowledge in terms of the role of the intestinal microbiome to shape, program, and dictate the function of the immune system. And I would like to start—this is a use and abuse of slides of what was formulated a few decades ago as the hygiene hypothesis (Slide 1)—because this has a lot to do with the discussion that we're going to do today. But just to put this in perspective, the human species for almost 99% of the time of its evolution during the past 2 million years had been getting sick and died by [a] single reason: infections. And indeed, the morbidity and mortality associated with infectious disease was the main reason why we got sick and died. Then finally, we were able to have a little bit more insight into the nature of this infection, the pathogenesis of this clinical situation and science—in particular pathogenesis—took the front line.



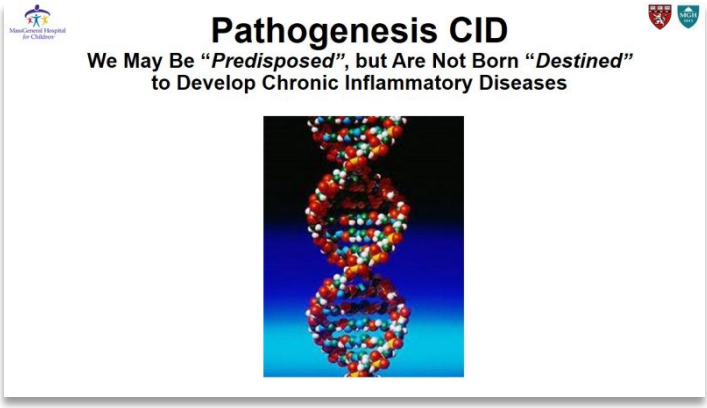
Slide 1

And of course, in understanding the mechanism related to infections, we were able also to deploy diagnostic and therapeutic options that were not available before that, including of course, the advent of antibiotics, the implementation of hygiene, vaccines, and so on and so forth. And in the past 4 decades, particularly in the Western hemispheres, we've seen a dramatic drop of the incidence and prevalence of these conditions. Diseases that were extremely impactful, like measles, hepatitis A, cholera, and so on and so forth, just plummeted. But during the same time, interestingly, at the same latitudes, we saw this equally dramatic increase in noninfectious chronic inflammatory diseases.

Bottom line, if you embrace it, the Western lifestyle looks like this: we don't die fast of infectious diseases, we die slowly of chronic inflammatory diseases—until mother nature threw another curve ball, like the COVID-19 pandemic, and a level starts again, even in the Western hemisphere. If you want to look at this epidemiological data with a negative point of view, the conclusion is that we are really destroying the environment. We are doing something too fast and changing environments for us, as human beings, to adapt genetically. And this was based on, of what at that time was the paradigm to develop any disease in humankind, 2 absolutely essential and necessary elements, [which] were genetic predisposition and exposure to an environmental trigger.

These epidemiologic data materialize in a very short period of time, these chronic inflammatory diseases, including autoimmunity, neurodegenerative diseases, cancer and so on and

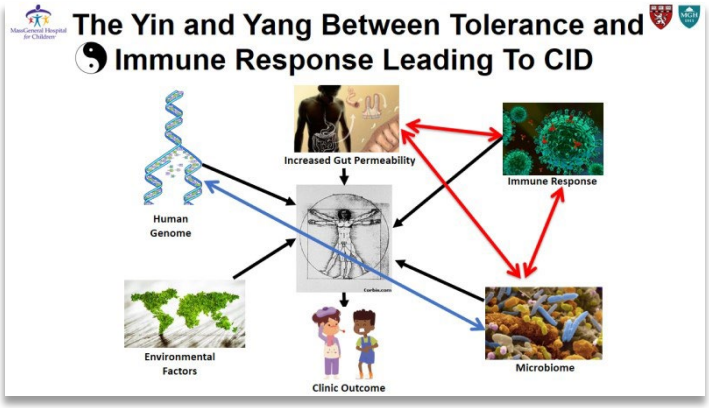
so forth, really materializing in such a period of time that we cannot blame genetic mutations to be responsible [for] this. So again, the acceleration of these conditions is teaching us another lesson, contrary to what we believed before. The fact that I'm born with genes that put me at risk for colon cancer or breast cancer or Alzheimer's disease is not a destiny that I will develop this condition. If I do, or do not, depends on how I play my genetic cards, opening a new paradigm of possibilities in terms of personalized medicine and, most importantly, primary prevention (Slide 2).



Slide 2

The other thing that we learned in the past few years is absolutely that genetics is important; that of course the environmental exposure to triggers is instrumental. They are in other words necessary, but not sufficient. There are at least another 3 elements at play that put us in that perfect storm to start that march from genetic predisposition to clinical outcome (Slide 3). A third element is the law of compartmentalization. In other words, the capability to keep at bay these enemies, these environmental triggers at the interface with the environment. At least here, the intestinal gut permeability, because the GI tract, it's the largest interface between us and environment. And it's the one that [has] been best studied. But, you know, the lung, the airways in general, the genitourinary tract, the skin, all interface with the environment that there can be a play in this break of protection. Of course, the fourth element, by definition, since we're talking about inflammatory diseases, is the immune system that becomes hyper-belligerent, generating chronic inflammation. And last, but definitely not least, the microbiome: this ecosystem that we coevolved with, for which we knew very little until the recent past, but has an instrumental importance in dictating how we indeed play our genetic cards.

Now, these 5 elements that I put artificially in silos here, are highly interconnected, particularly the last 3. As a matter of fact, now there is strong evidence suggesting that, and you will see some examples of this, that if I lose my barrier function, my microbiome can go off balance and vice versa. An imbalanced microbiome is one of the key elements to lose barrier function. If I lose barrier function, there is an increased antigen trafficking that in turn will make our immune system exposed to antigens, instigating inflammation. And vice versa, inflammation can increase gut permeability. In other words, you got the concept.

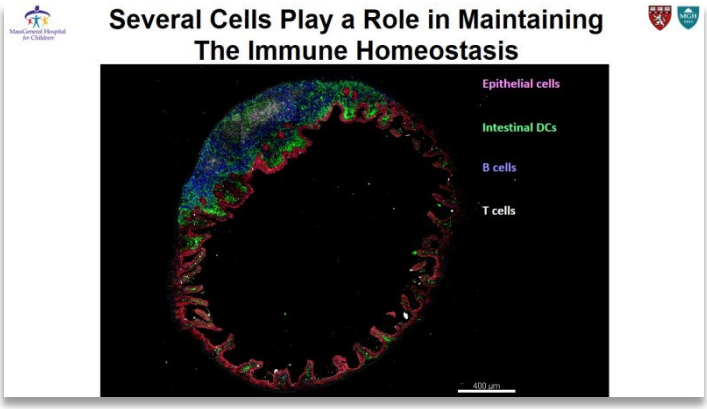


Slide 3

This triangulation is highly interconnected, but the most important element that now is really surfacing from all these studies, is the capability [of] the microbiome to influence if, when, why, and how our genes will be turned on and off, starting, indeed, this march from genetic predisposition to clinical outcome. This is what we call, technically, epigenetics: the pressure from the environment

through the microbiome to put in motion these genes so that eventually we will develop these diseases. Even mother nature, when we talk about the interface with the environment, suggests that this arrangement, these 5 elements, are extremely important. What you see here [in Slide 4] is the cross section of a gut (happens to be of a mouse, but a human being will be absolutely the same). The big black hole in the middle is the intestinal lumen that is separated; therefore, the external environment that separates us as a human being by this single layer of epithelial cells that you see here decorated in red. Very reductive, in a reductive vision, we always consider the cells important just to digest and absorb food stuff. Of course, this is an extremely important function, but they do much more than that. Indeed, they offer this barrier to prevent this uncontrolled trafficking of antigens, nutrients, or elements from microorganisms to come through and instigate [the] immune system.

But also, they have the capability to have sensors on their surface, what we call pattern recognition receptors that will sense the presence of possible enemies or friends, and based on the signal that they receive from the environment, they can prompt the immune system in the gut (that is the largest immune system in our body) to start a possible war. And like in any army, there are subgroups of immune cells. They do different functions. For example, the one that you see decorated in green there are intestinal dendritic cells. They are the first comers when the epithelial cells sense a possible signal of danger in the intestinal lumen. And eventually they coordinate the action to deploy an immune response that is then eventually transmitted in another subgroup of cells. What we call the B cells, or B lymphocytes.



Slide 4

They are extremely important because they produce immunoglobulin, particularly IgA, that can be secreted in the lumen, becoming secreted IgA and fighting this enemy outside of the body. But at the same time, they seem to have an extremely important function in decorating a subgroup of elements, the microbiota, so changing their function and therefore how our microbiota interact with us also depends on this secreted IgA. And last, but definitely not least, the last group of immune cells, the T cells, the heavy weaponry—these are the ones that make a lot of danger and a lot of damage. When activated, they deploy heavy weaponry that translates into severe inflammation. If they stay on the battlefield (ie, the intestine), they create inflammation there. And that's typically the case of diseases like inflammatory bowel disease, like Crohn's, ulcerative colitis or celiac disease, but most of the time they're programmed to leave the intestine and go anywhere in the body. And we have evidence that they can go in the skin and you develop urticaria; they can go in the joints and you develop rheumatoid arthritis; the brain and multiple sclerosis; the pancreas and type 1 diabetes, and so on and so forth.

All this to say that this orchestration of epithelial cells with their sensors, their viral functions, the immune cells and again, these microbiota that are created in the intestine—it can really dictate the balance between health and disease. A while ago,

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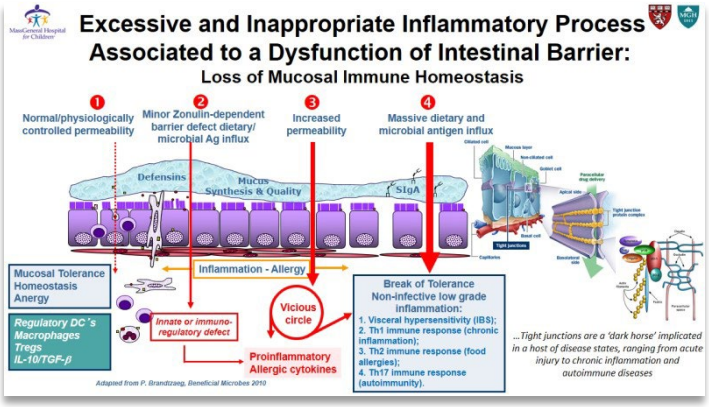
we put forward a working hypothesis,[1] as summarized in this cartoon (Slide 5) to answer the question: how can I switch from a genetic preposition to clinical outcome? Where are the mucosal events that will break tolerance and will start that march from genetic preposition to clinical outcome? If you go from left to right, this seems to be the steps that we go through.

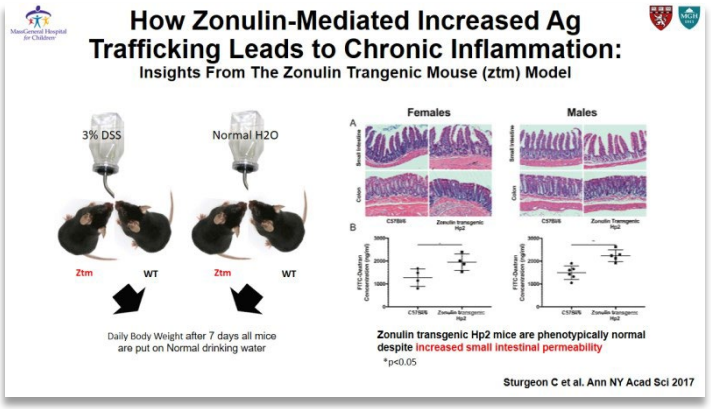


Slide 5

Physiology, step number 1, everything works. The barrier works fine. There is a tightly controlled antigen trafficking. We want some of this model to come through, but only under specifically tightly controlled mechanisms so that we can know our surroundings and have the immune system to develop what we call the mucosal tolerance or anergy. In other words: stable, do nothing with that. The problem arises when we move from step 1 to step 2. There is an increased permeability of the gut, mainly in-between cells, what we call the paracellular pathway. This was a pathway that was naïve in terms of knowledge to us for many, many decade'. It's only in the recent few decades that we understood that, rather than sealed, the space in between cells is regulated by a very complex structure that we call tight junctions, which we can conceptualize the sort of gates. Most of the time they're closed, so everything that needs to be negotiated has to go through the cell, but once in a while, they can be open for this antigen sampling and immediately closed. If they get stuck open, now you have the flux of antigens coming through, and that creates the syndicate of instigated immune system to fight, because now this flood of enemies, they come in, and when you fight, you have collateral damage. There is inflammation.

This inflammation is mediated by specific molecules, particularly pro-inflammatory cytokines that, per se, can increase your gut permeability. And that makes you move from step 2 to step 3. In other words, you have this vicious circle of antigen trafficking, inflammation, pro-inflammatory cytokines that, per se, increase gut permeability with more antigen to come through until we break tolerance. And we switch from genetic preposition to clinical outcome.

The kind of disease that we develop depends on our genetic makeup. If we are predisposed for functional diseases, we develop conditions like irritable bowel syndrome. If we're more skewed toward the Th1 immune response, we will develop a condition like cancer or chronic inflammation; Th2, food allergies; Th17, autoimmunity; and so on.



Slide 6

What was not known was how you move from step 1 to step 2. And this was our contribution almost 20 years ago, we developed, we discovered this molecule that's called zonulin (Slide 6). That turns out to be a molecule that remains the only physiological modulator of this gut permeability. And when it's produced in excessive amounts, it

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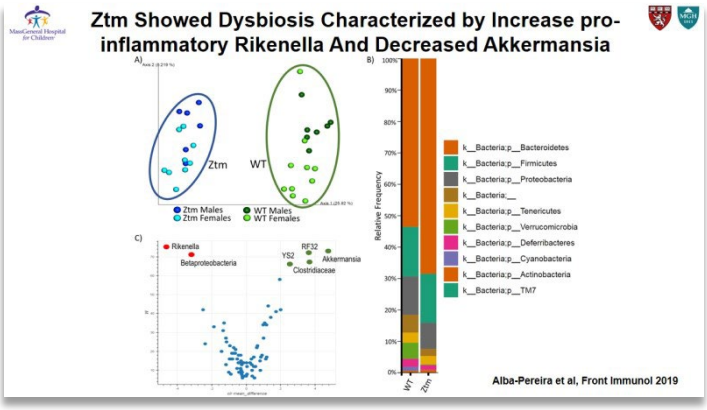
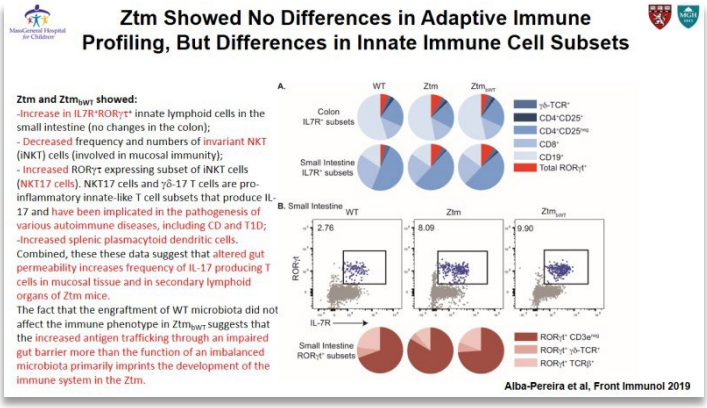
creates the syndicate, starting this uncontrolled antigen trafficking. Now, this triangulation that I was telling you, the mutual influence of gut permeability, the immune system, and the microbiota, is simplified in an animal model that produces a lot of this zonulin. This is what we call a transgenic mouse that has genes that turn on the zonulin production.[2] So the gut of these animals leaks, even if the structure, as you see from the picture, is normal. So there is no damage of the intestine, but ultra-structurally speaking, the spacing between cells is loosened up. And therefore there is an increased gut permeability.

This translates to a totally different set of microbiota in these animals (Slide 7).[3] You see on the right in green, the microbiota distribution in normal mice. And on the left, you see in blue, the microbiota composition in zonulin transgenic mice. What makes a difference that can segregate these 2 microbiota aside from each other, is that the microbiota of the zonulin transgenic mice is characterized by loss of protective elements of the microbiota, particularly *Akkermansia*, that we know that is beneficial for gut health and for many other things. But also for the enrichment of pro-inflammatory components like *Rikenella*. In other words, the microbiota of these animals seems to be prone to create inflammation, seems to be skewed toward inflammation. And when we take a look at the immune profiling, I don't want to go into details here, but it's suffice to say that also there we see differences.



Slide 7



Slide 8

In these animals, simply because they lost the barrier function, their immune system distribution and cell subtypes have been changing (Slide 8).[3] So this animal lost a subgroup of immune cells that put the brakes on inflammation, while they have enrichment in another subgroup of immune cells that are more pro-inflammatory. In other words, these animals are skewed to really more mount a severe immune response.

When instigated by external stimuli—there is, for example, a chemical stimuli that we use to create gut inflammation—the normal mice (the wild type mice), their gut inflammation, they become sick, but eventually they recover once the effect of this chemical is gone. In the transgenic mice, they suffer up to 70% mortality (Slide 9).[2] So, they got so sick that they would not recover. And if you give to this animal an inhibitor of zonulin, so you stop the

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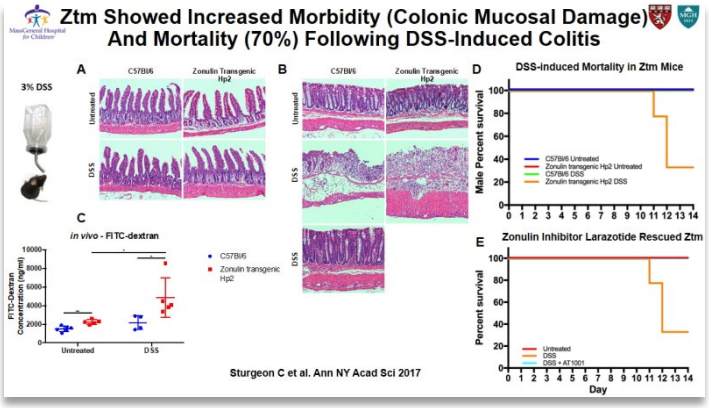
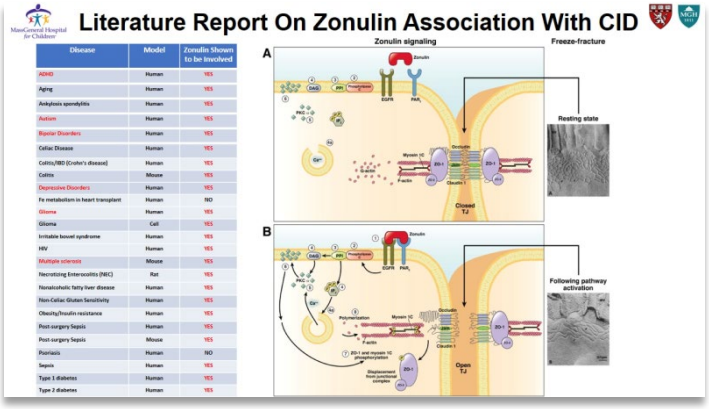
antigen trafficking, these animals are rescued. Meaning that it's the excessive, exaggerated antigen trafficking in these animals, that when exposed also to an environmental stimuli, they may be put over the edge and develop chronic inflammation.



Slide 9

Now in the literature, there have been many, many conditions that have been reportedly associated with exaggerated zonulin production (Slide 10)—this is not even an exhaustive list of conditions—that goes from aging, to liver inflammation, to neurodegenerative diseases, to cancer, to infections, and so on and so forth. This seems to be all totally unrelated with each other. What is the common denominator here? Chronic inflammation. But again, this brings me back now to the microbiome, because again, the strongest stimuli for zonulin release is indeed an imbalanced microbiome. So it's a 2-way situation.



Slide 10

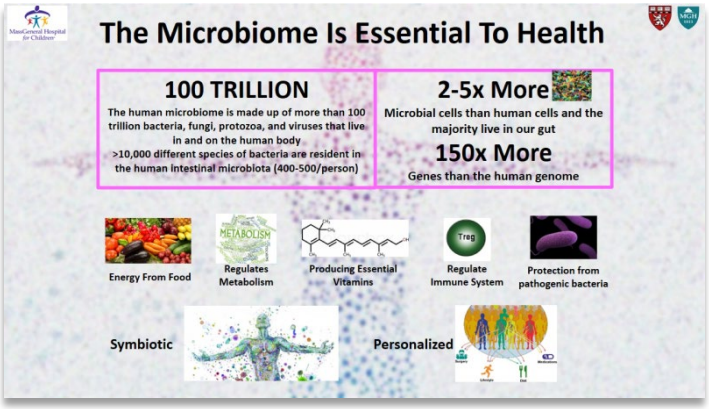
I started this chat by making the point that we were, as a human species, affected, mainly in terms of morbidity and mortality, by infection diseases. And therefore, that brought us to the state of mind that microbes are enemies. We need to fight against them at all cost.

Slide 11

We didn't realize that the enemies, the pathogens, the ones that make us sick and eventually cause mortality, are the minority of a much more complex ecosystem that now we call microbiota (Slide 11). They co-evolved with us from birth to death. They are extremely important for our health. I don't want to go into these details because I'm pretty sure you all know, but we have at least the same number of our own cells, and compared to the microbiome (microbes that live with us), they generate many more genetic materials than we do.

Why do we want to be in this friendly symbiotic relationship with them? Because there is mutual advantage to that (Slide 12). Again, we are the way that we are, with 2 eyes and a nose and the kind of hair distribution on our body, because we were programmed through this exchange with the microbes to be built like this. We provide them hospitality and food in exchange they do stuff for us. They scavenge extra energy from nutrients that we could not use, such as fibers. They help us to regulate our metabolic pathways. And this is 1 of the most important functions that they do for us. They can supply vitamins that we don't have access to

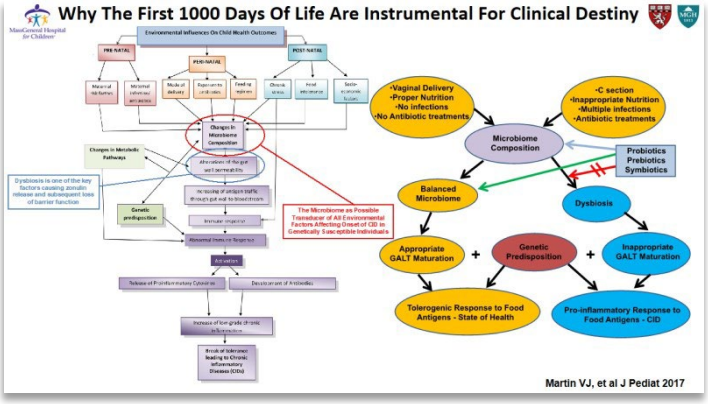
through the diet. They regulate the immune system, as I told you, and you will see more examples of that. Most importantly, they protect us against the bad guys, the pathogens—after all, penicillin is nothing more than a molecule produced by bacterium to kill another bacterium.



Slide 12

But 1 point that I want to make straight right now is that as we are genetically all different—even monozygotic twins, epigenetically, they are different. So is our microbiome. There is no such thing as the normal microbiome, a microbiome that is good for everybody. It has to be compatible with our genetic makeup. The symbiotic relationship between us and our microbiome, it's something that is extremely personalized. The outcome is the same. We all need to have the blood pressure within the same range or the glucose level in some certain range in our blood and so on. And this is achieved thanks to the interplay between the human genome and the microbiome. They regulate specific metabolic pathways.

Now, why, in the Western hemisphere, do we have this increase in chronic inflammatory diseases? The hygiene hypothesis has been revisited and put under scrutiny, because it didn't hold—some of the elements. I'm afraid that you will not be able to see this slide (Slide 13). The point that I want to try to make with this is the following: no matter what kind of environmental trigger you want to consider (eg, prenatal, mom's lifestyle, where she lives, rural vs urban, does she smoke, does she drink, prenatal birth or C-sections, infections, a visit to the intensive care unit or postnatal nutrition, stress, whatever you want to consider), these are all impinge on a bottleneck change of the microbiome. And if the microbiota change in composition, and therefore, in fact, in function, as I discussed with you before, that can lead to increased gut permeability or increased permeability of the interface with the environment.



Slide 13

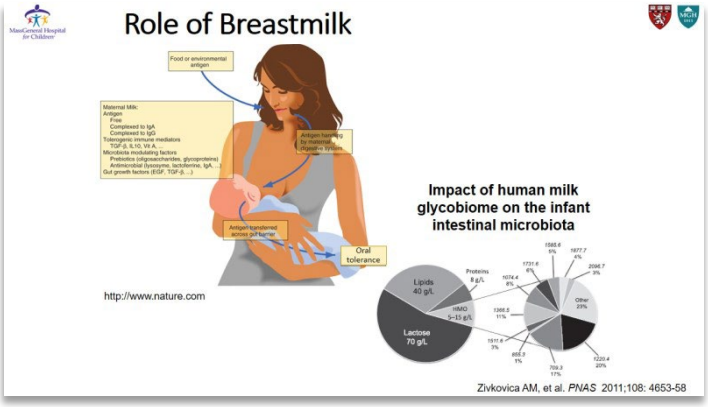
And this starts the march from genetic predisposition, with this chain of events, to the clinical outcome. Why do we see much more [inflammation] in the Western hemisphere vs developing countries? Because we, with the Western hemisphere lifestyle, really departed from what was the plan of evolution. We were planned to be born by vaginal delivery, to give our kids real food and not junk, no antibiotics (because there were no antibiotics), very few infections (because if you got infected, you die), 2 million years ago. And that, in other words, brought the right maturation and engrafting of a healthy microbiome. And it should not come as a surprise that the microbiome is instrumental to programming our immune system. Remember that 2 million years ago, our average survival age was 13 or 14 years. You either died by an infection or a dinosaur would eat you. There was no time to develop cardiovascular diseases or cancer.

And the microbiome was instrumental as the interface with the enemy that, eventually, the immune system has to face, to program the immune system to decide where to put the bar to fight—where to put the bar to develop inflammation. Inflammation is nothing more than creating a barrier-style environment for microbes to grow. It is too hot. There are chemicals like cytokines and chemokines that will kill you. There are cells like immune cells that will eat you. In other words, it's a very bad environment for microorganisms. Of course, you pay a price. The tissue that is inflamed will die, but the whole organism will survive. So, a balanced microbiome will make sure that the immune system is programmed to unleash inflammation only when we are severely in danger, under attack of microbes, and will turn off inflammation when the enemy has been defeated.

When does this programming happen? Very early. The famous first thousand days of life from conception to 2 years of age is when the microbiome is in charge of programming the immune system to decide where to put the bar to generate inflammation.

Now we depart for that plan. We are on the right-hand side [of Slide 13]. Much more frequently, we are born by C-section. Again, we feed junk to our kids. Use and abuse of antibiotics or multiple infection. This now has put the microbiome in imbalance (dysbiotic stage). This will make the immune system program inappropriately. The bar is set very low. We have inflammation even when it's not necessary. And, on a specific genetic background, this continuous instigation to generate inflammation, sooner or later it will lead to disease.

Even mother nature is telling us that taking care of the microbiome is top priority. The composition of the breast milk. For us, nutrition has been a puzzle for many, many decades. Why? Because the human manno-oligosaccharides, the most abundant sugars into the breast milk. We had no idea what they were doing there. They are not apt to provide nutrition to babies. Babies cannot make use of these manno-oligosaccharides. What are they there for then? They nourish the proper microbiome (Slide 14). They help to shape the compatible microbiome with the baby, so that there is that searching each other for a healthy symbiotic relationship.



Slide 14

When we start to engraft the microbiome, of course, we always believe this starts at birth (Slide 15). You know, when a baby passes the vaginal canal if she's lucky enough to be born by vaginal delivery. Well, it looks like this starts much earlier than that. Even during conception, there is a contribution from the male's counterpart to provide some input to the microbiome. There is a lot of controversy, and there is a discussion, far from being settled, that there is a placental microbiome. There is some beginning during pregnancy, particularly the last trimester for initial engraftment.

And if that's the case, anything that the mother is exposed to that affects that proper transmission from mothers to babies of the microbiome can create problems in the proper engraftment of a compatible microbiome. Of course, the major shift is at birth. If you are born by C-section, you will not receive the mother's microbiome that has been already preselected because it is genetically compatible with the host, and therefore with the baby. Rather, the microbiome comes from the skin.

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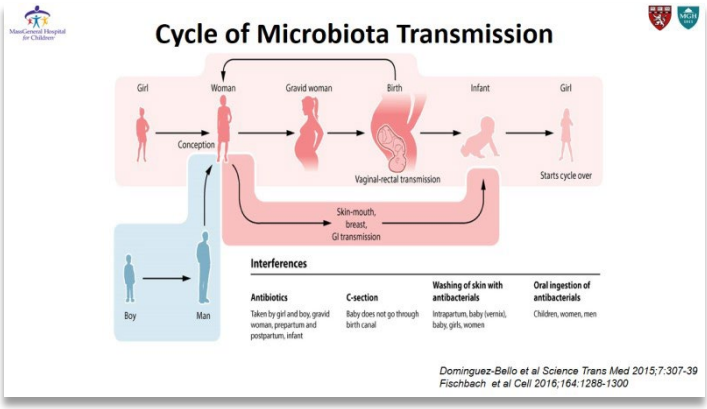
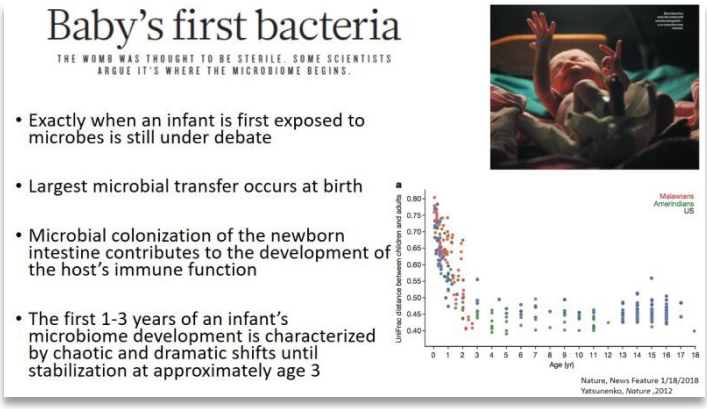
And of course, the rest of the first thousand days of life, anything that will derail from there, will be impactful in the optimal symbiotic relationship with the baby host.

If we analyze these changes since the birth of the microbiome, it seems like complete chaos (Slide 16). It seems completely random. At the beginning, there are some microbes that are engrafting in the GI tract of this baby. Then, they leave, and other microorganisms come in. And then, when baby food comes into the picture, there is another shift. And then finally everything quiets done around age 2, 3, when the microbiome matures as an adult microbiome. Is this chaos? Is this random? Absolutely not. This is dating. This is searching each other to try to find that ideal relationship that mutually benefits the host and the microbes, so that there is going to be this ideal symbiotic relationship.

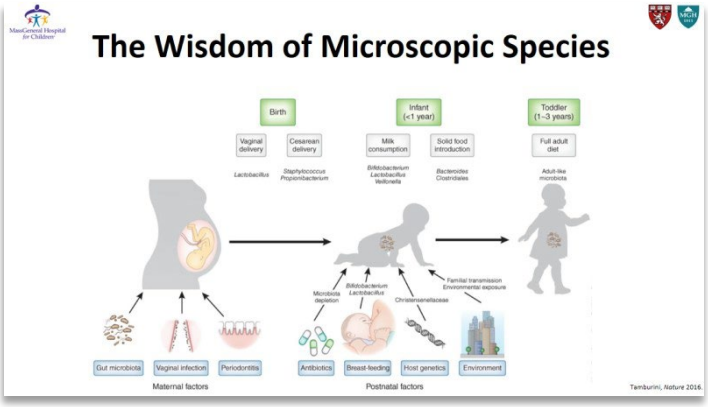


Slide 15



Slide 16

So, you visually realize that they're searching each other, if disturbed by external elements. That can be again during pregnancy or during/soon after the birth. And this can be exposure to antibiotics, can be exposure to pollutants, can be a lack of exposure to animals. In other words, elements that were not planned by evolution that can create a disturbance of these very finely tuned, extremely tightly regulated way that we search each other (Slide 17).

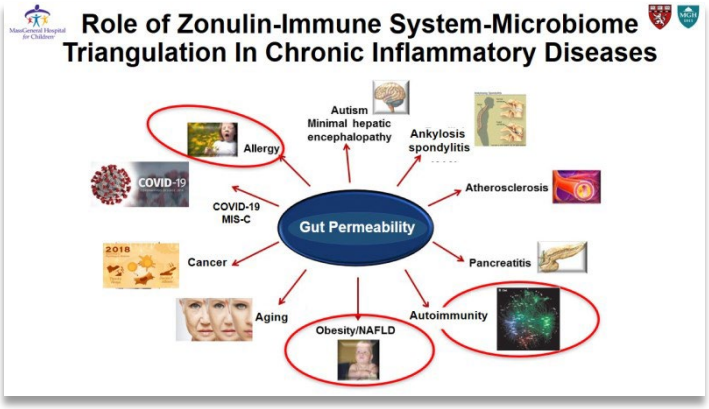


Slide 17

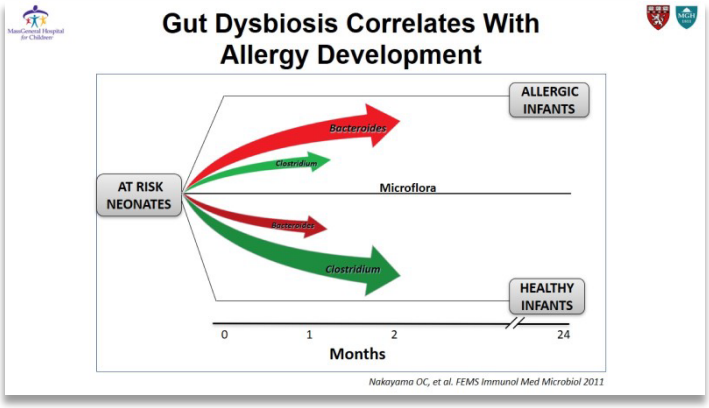
The other thing that needs to be said is that, even if all this affects the GI tracts of the microbiome, and the GI tract is the one that can be affected by these external elements, the consequence are not limited to the GI tract. Every tissue or organ in our body can be affected by reprogramming the genetic function of this organ system, by this inappropriate engraftment, by this nonideal interaction with the microbiome.

What are the outcomes of this triangulation (ie, increased gut permeability, chronic inflammation because of the immune system hyper-belligerence, and dysbiosis)? What is the outcome of all this? This is not even an exhaustive list of possibilities [in Slide 18], and I don't have the time to go through all this, but I want to give you as an example, a few of them like food allergies, obesity, autoimmunity. Just as you know, elements to give you an idea of what we're talking about.

The Intestinal Microbiome and the Developing Immune System



Slide 18

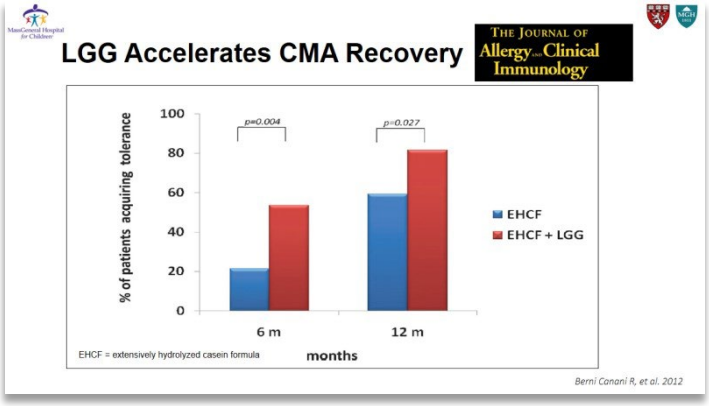


Slide 19

Food allergies, like any other chronic inflammatory disease, we have seen a surge of food allergies in the past decades. We do analysis of the microbiome, and we see, definitely, there are changes in the microbiome when kids have food allergies, or allergies in general, compared to the ones that do not have food allergies. So, there is change, definitely a difference in the microbiome composition (Slide 19).

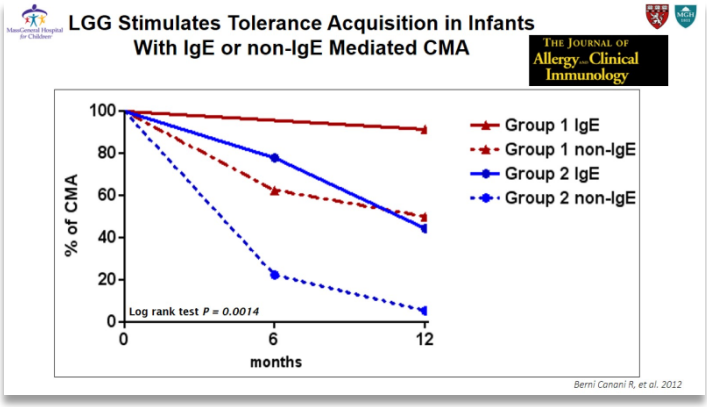
And again, we have seen some elements suggesting that eventually, this shift that distinguish cases of food allergies and controls can be manipulated by external intervention, particularly with intervention with *Lactobacillus casei* or even better LGG, as we will see in a moment. So, restoring the microbial health is something that can now be done by different means. One being by using specific elements of good bacteria, what we call probiotics like LGG, that have been proven to restore a more normal microbiome composition in infants that have cow's milk allergy.

This is a study that is not recent but definitely still of great value (Slide 20).[4] They compare extensively hydrolyzed formula with and without LGG. And you will see, at both 6 months and 12 months, that if you add the probiotics to this formula, the percentage of kids that reacquire tolerance to cow's milk proteins is much higher. So, it seems that reestablishing a balanced microbiome will accelerate the resolution of food allergies.

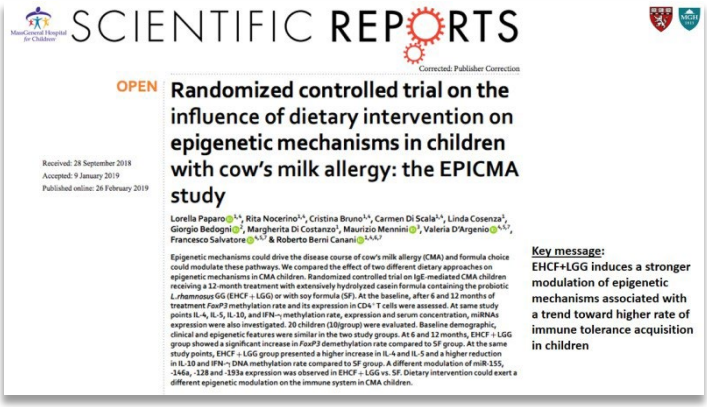


Slide 20

The Intestinal Microbiome and the Developing Immune System



Slide 21



Slide 22

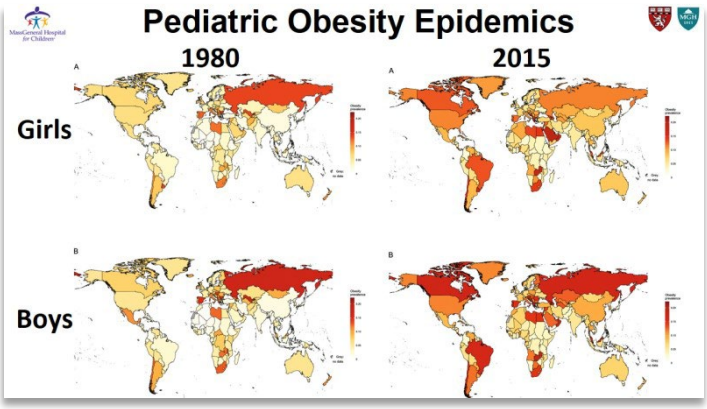
And again, in the same [study] (Slide 21), if they are IgE- or non-IgE–mediated, you see the same kind of effect, if you add the probiotics. This definitely accelerates, eventually, the regaining of tolerance. And again, this is information that I'm giving to you to make the point that there is a way to eventually rebalance the microbiome. In this case, for example, this was a randomized controlled trial with the dietary intervention on epigenetic mechanisms in kids with cow's milk allergy.

This study (Slide 22), for example, shows that if you use a hydrolyzed formula plus LGG, it gives a stronger modulation of epigenetic mechanism, associated with a trend toward higher rate of immune tolerance.[5] In other words, you can reprogram the immune system epigenetically to have a faster reacquisition of tolerance to cow's milk. So these are all proven concepts that were heavily studied preclinically, and now even in a clinical setting to show that, again, there is a way that we can influence what we've done wrong in creating dysbiosis, by intervening, in this case with probiotics.

Obesity

Obesity, of course, for us—I say us because I'm a pediatrician and also a nutritionist, but I know [in] the audience we have neonatologists, we have dieticians, we have pediatricians and so on and so forth, nurses—is a pathology that unfortunately we see increasing exponentially in the pediatric population.

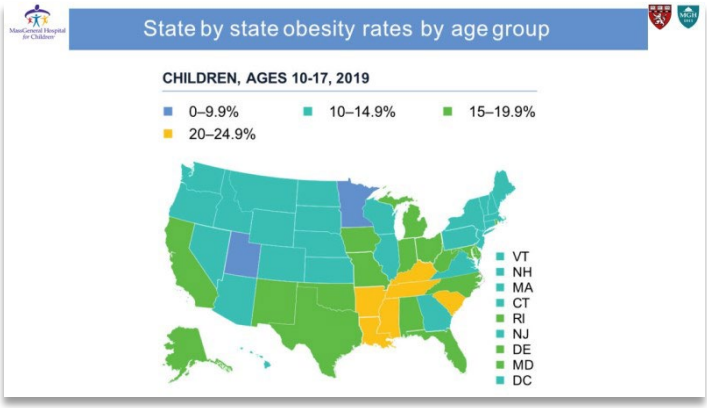
I don't have to twist your arms to be sympathetic with this data in which we see that, in only 25 years, this unbelievable explosion of obesity cases worldwide (Slide 23).[6] But just look at North America or the United States, we, of course are the darkest. The [darker the] site—the more impactful the percentage of obesity.



Slide 23

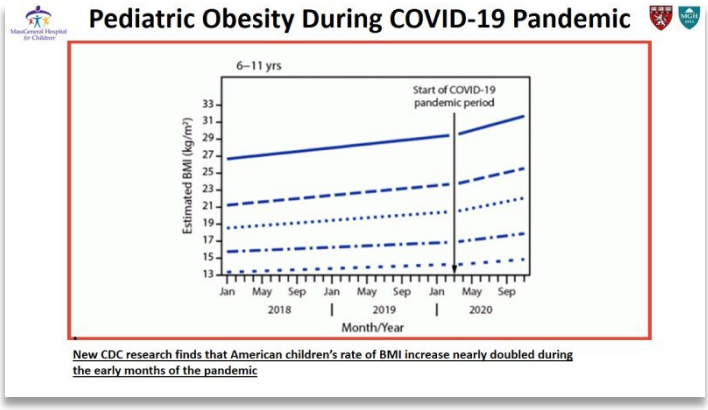
And we see that again, unfortunately we are paying a dear price, both boys and girls, in obesity, in the

United States (Slide 24). What is unfair, and I believe concerning, is who pays the highest price: the people that have low socioeconomic status because they cannot eat well, they cannot eat healthy. And you see, in this US map, the states in the South, where the average income is lower, are the ones that pay the dear[est] price.[7] So the paradox is that, while 2 or 3 centuries ago, obesity and its complications (like gout, for example) were the diseases of the wealthy, now obesity and its complications are the diseases of the poor.



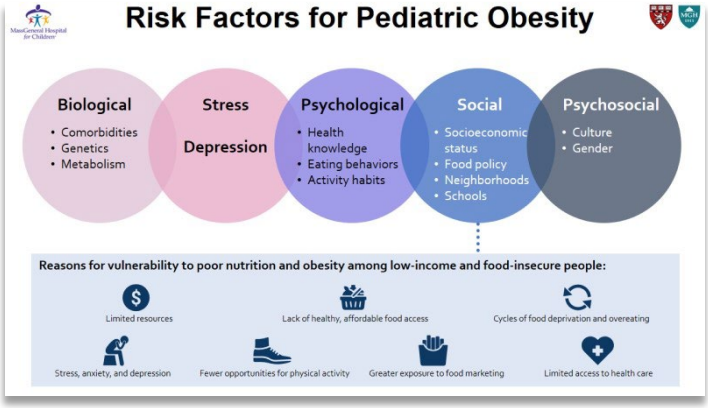
Slide 24

The other thing, that again, is extremely impactful and worrisome is that the rate of pediatric obesity further increased during the COVID-19 pandemic (Slide 25).[8] So of course, because the kids were locked in and stayed home, they were prevented from having physical activity. Of course, being home, you are exposed to ingestion of food, specifically junk food all day, all day long. And this has been the consequence. So this is another dear price that we pay as a consequence of pandemics.



Slide 25

But of course, I can't just make the point that obesity is simply a disease of poor nutrition. It's much more complicated than that: the biological component, of course, stress—and therefore state of mind—also has an impact (Slide 26). The psychological component I mentioned about the social, psychosocial component.



Slide 27

So there is a lot of stuff that eventually creates this perfect storm that leads to [obesity], but among the others, definitely there is the microbiome. We know from animal studies and now evidence in humans, that there is what we call an obesogenic microbiota (Slide 28). It can be transferred vertically from mom to babies. So a mother who is overweight will transmit to the offspring, an obesogenic microbiota, and therefore, will make the offspring obese. Of course, during evolution, 2 million years ago, when food was scarce, that was an advantage. Now, it's a

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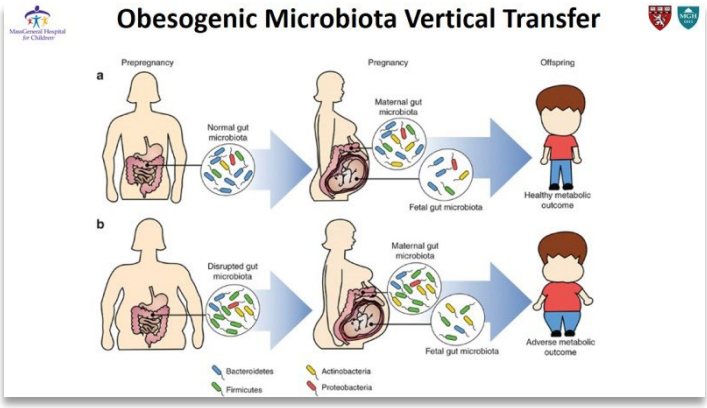
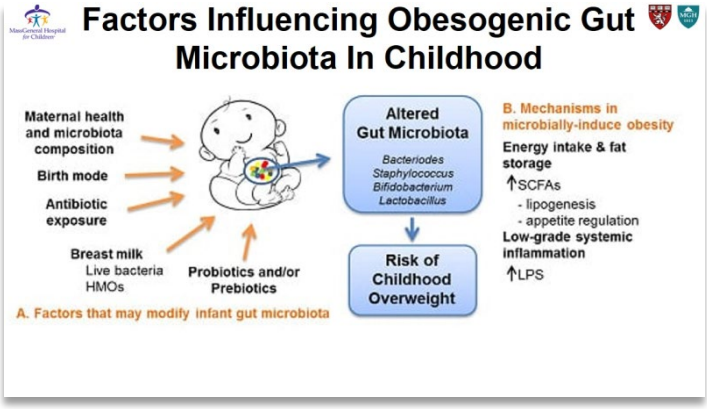
liability of course. And the factors that influence the obesogenic gut microbiota in childhood are multiple. One, that again, I was telling you about mom (Slide 29). The birth mode, antibiotic exposure, if fed breast milk or not, probiotics and prebiotics—they all alter [the microbiome] for better or for worse, depending on the microbiota, and therefore, can eventually increase or decrease the risk of childhood overweight.



Slide 28

Even here, based on what we understand (leaky gut, state of inflammation, the hyper-belligerent immune system, dysbiosis), this provides specific possible targets to eventually manipulate the system, including the manipulation of the microbiota through probiotics or other means.



Slide 29

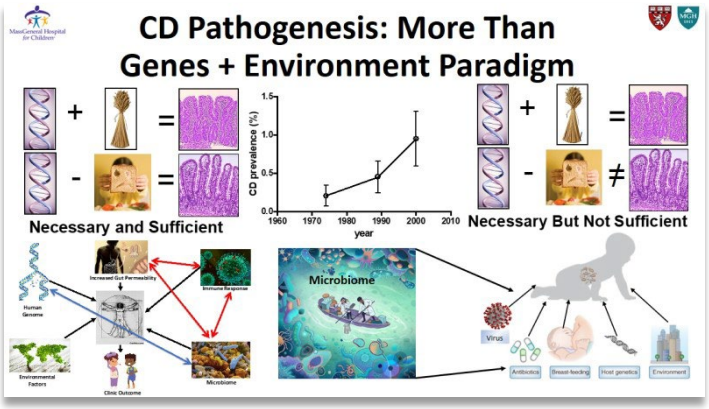
AUTOIMMUNITY

And finally, autoimmunity, I don't have time to go through all this, but one I want to give as an example is celiac disease. Celiac disease is an autoimmune disease that you know well, instigated and triggered by exposure to gluten. That is the environmental trigger.

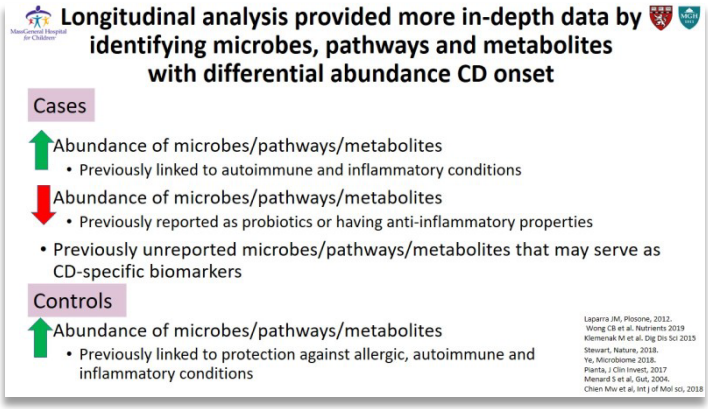
Slide 30

And we have this project now, for almost a decade, in which we have been following from birth more than 600 kids at risk for celiac disease (Slide 30).[9] And we've done all these multiomic analyses in these kids that I don't have the time to go through, but this was the classical example in which we understood the genome environment that we thought was necessarily sufficient to develop any disease, including celiac disease, was not the case. Because we've seen, like many other chronic inflammatory diseases, this increase of incidence of celiac disease over time (Slide 31), and the rate of doubling every 15 years. The genes would not change in such a short period of time. The grains will not change. So definitely, we understood that there were other elements, and the microbiome definitely was 1 of them.

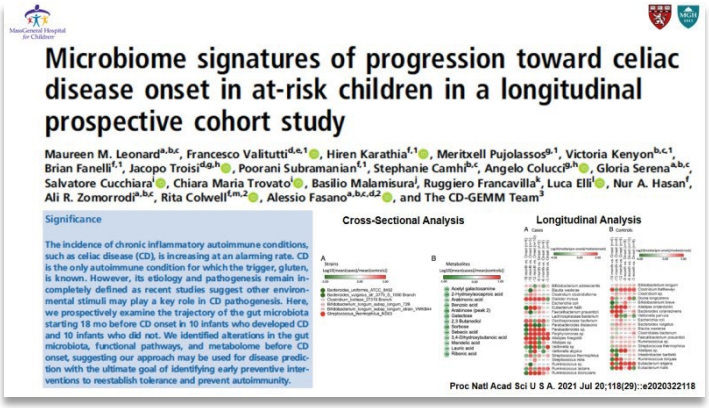
The Intestinal Microbiome and the Developing Immune System



Slide 31



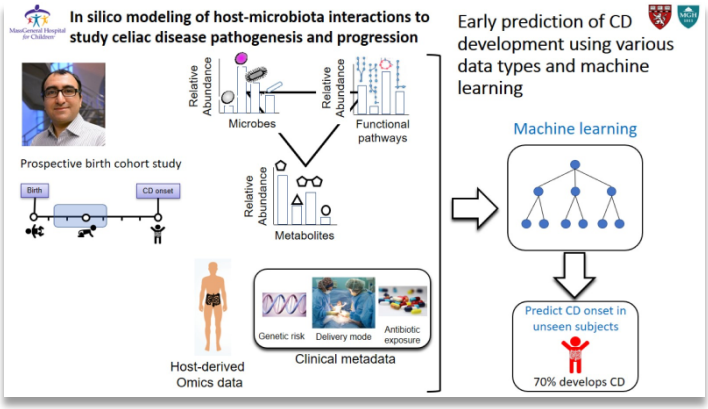
Slide 33



Slide 32

This is a study that we just published in *PNAS* that, again, as a proof concept, we were able to have an idea of a specific signature in the microbiome that will predict, on a specific genetic background and therefore risk, who will develop celiac disease and who will not (Slides 32 and 33).[10,11] Bottom line, what we discovered is that in cases of kids that eventually end up developing celiac disease, we saw an increased abundance of microbes, pathways, and metabolites previously linked to autoimmune inflammatory conditions. And when I say we saw it, we saw it months before the onset, as we saw, months before the onset, the loss of [the] protective component of the microbiome.

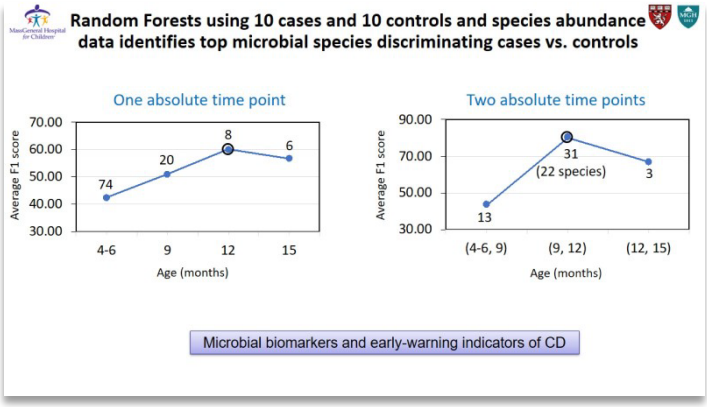
Through mathematical modeling and artificial intelligence, some of our colleagues, Ali R. Zomorrodi being the lead of this competition biology lab, were able to eventually predict, again through machine learning based on a specific genetic background, the host who will eventually go on to develop celiac disease (Slide 34).



Slide 34

[Slide 35] gives you an example. Through this mechanism (again, it's a proof of concept), he told us that some elements of the microbiome, depending the way that you do analyze this, but a handful of microbes will predict 9 to 12 months before the onset of disease who will develop disease with good accuracy—like 70%, 80%—who will develop, months or a year down the road, celiac disease. I don't know if you appreciate [the] magnitude of what I just said. This means that again, if these data are confirmed, we have a target for

intervention: manipulating the microbiome with probiotics, prebiotics, synbiotics, diet, or whatever, to eventually derail this trajectory towards celiac disease and bring these kids back to a more friendly interaction with the microbiome, so that epigenetically they don't put in motion that condition that will bring intolerance to gluten and therefore the onset of celiac disease.

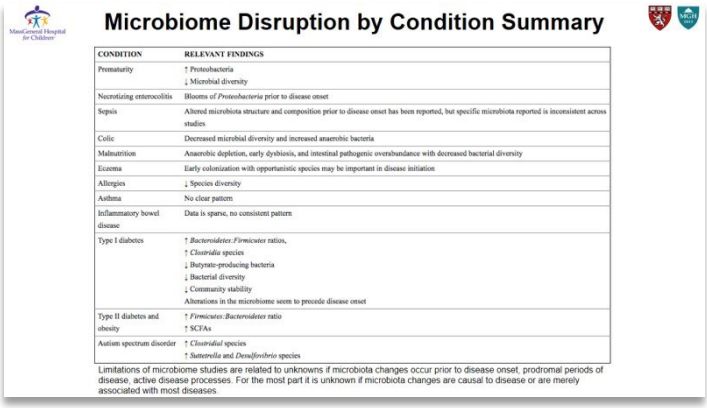


Slide 35

Manipulating The Microbiome

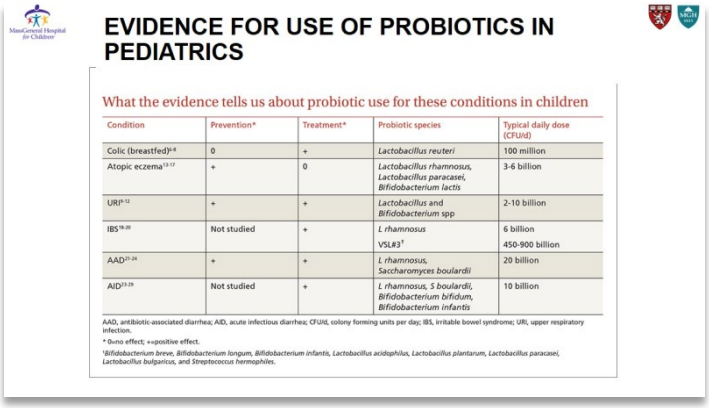
This brings me to the last part of this chat, how can we manipulate the microbiome to treat this?

Again, there are now—still descriptive, but moving toward mechanistic distribution and link between specific microbiome imbalance and conditions. And these are the most important [conditions] in pediatrics that you can read [in Slide 36].



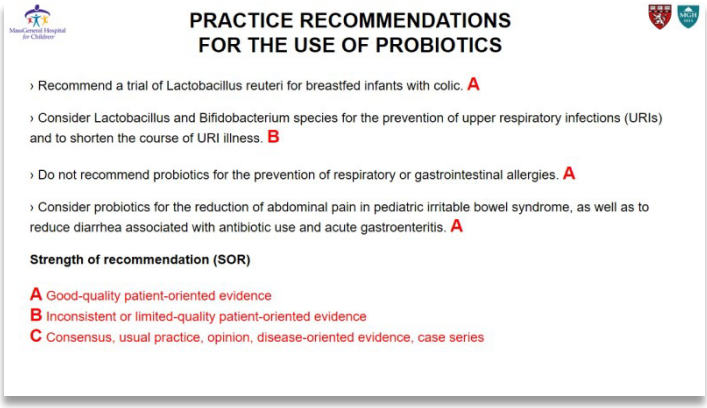
Slide 36

But we talk about colic, of course. We discuss cow's milk allergy. We talk about post-antibiotic era and so on and so forth. And there are several products already out there that have been proven to have efficacy in the specific use of probiotics in pediatric conditions (Slide 37).



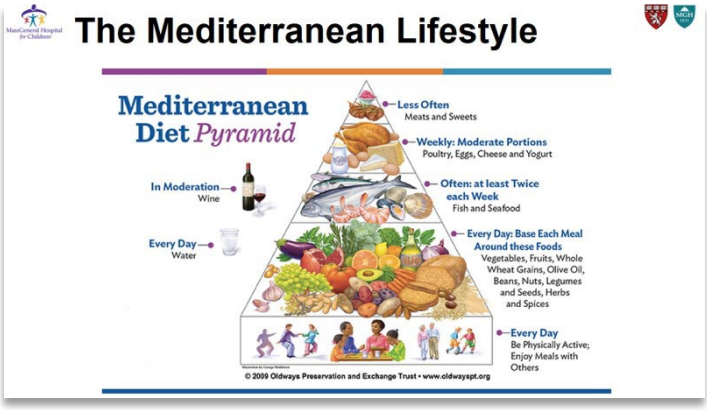
Slide 37

So there is evidence out there (Slide 38).[12] And the evidence—they're not all strong right now—there are recommendations for the use of probiotics classified by what [the] evidence says. "A" for good-quality patient-oriented evidence; "B," inconsistent; "C," consensus, usual practice, opinion and so on and so forth. And you see, in different conditions, for example, the use of *Lactobacillus* for breastfed infants with colic and again, *Lactobacillus* or *Bifidobacteria* for upper respiratory infection, and so on.



Slide 38

So you see that again, this is the beginning of a revolution. However, I want to finish, for my friends and dieticians and nutritionists, that all the elements that can influence the microbiome, particularly making the microbiome off-balance (antibiotics, the way you're born, exposure to pollution), these are all point elements. We eat 3 times a day. By far, nutrition is the most impactful way that the microbiome can be derailed from what was the plan of evolution. And among all the studied diets, probably the Mediterranean diet is the one that has been studied the most. It is a lifestyle rather than a diet.



Slide 39

Again, as you know, nutrition is important, but it's not the only thing that needed to change. And this most resembles the way that we evolved as a species (Slide 39). In other words, the gatherers, hunters—until 10,000 years ago, we ate a lot of fruits, vegetables, nuts, tubers. Why? Because you pick; they are there. And meat, of course, yes, but you have to catch the animals, so it's rare. And it's lean meat because these are animals that escape their predators. And the Mediterranean lifestyle has that kind of distribution in terms of quantity and quality of food.

Slide 40

At the basis of health is not even food. We need to lead a stressless life. In other words, if we have stress, no matter how you eat, it doesn't matter. So the Mediterranean lifestyle is no-stress; the level of adrenaline with these people is low because they have a low, slow pace. They grow together. They don't take life too seriously. They laugh at themself. They dance. They eat together. They grow all together. This is all stuff that gives stability. They sleep well, and it's on and so forth. And then of course, nutrition is important.

And I want to finish with 1 last element. The Mediterranean diet is a diet that a group of pediatricians in the region where I am right now, in Campania, decided to use for weaning for babies (Slide 40). Rather than introducing the typical solid food, they decided to start weaning babies at 4 to 6 months of life with the Mediterranean diet. Fruits, vegetables, nuts, olive oil, and so on and so forth. Why? Because the travesty here is that the rate of obesity in this region is one of the highest in Europe.

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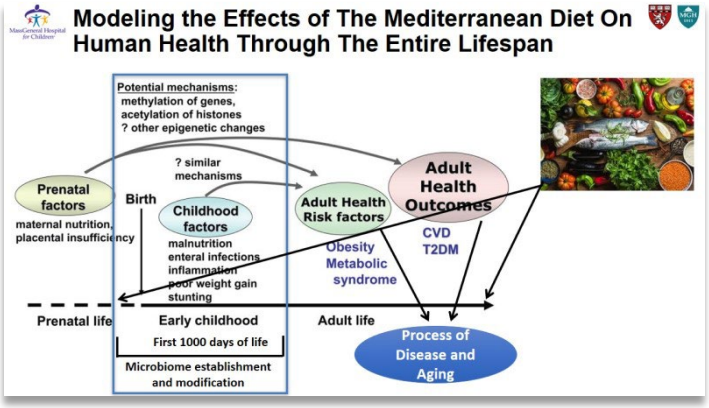

Slide 41

And this is the cradle of the Mediterranean diet. That is a counter sense. So, in only 2 years, these people, they saw a decrease in obesity in this region by 25%, simply changing the weaning to Mediterranean diets. You say, well, it seems to be unbelievable. The reason why is because they are used to the dirt taste; they're used to eating a lot of fruits and vegetables by the time they are toddlers, and then teenagers. They've been used to that kind of diet. They don't crave junk food because they only know that they think they know is to eat that way.

[Slide 41] is to say we all, because of our job, because we are in the pediatric domain, we hold the tremendous responsibility to really invert this tendency of this increased chronic inflammatory diseases, because we can intervene in this famous [1,000] days of life, pre-birth until 2 years of age. That change, that maturation of microbiome and ideal interaction with the host will really dictate the destiny of this individual, not just in the pediatric [age range], but throughout his or her entire lifespan. Because if we have a microbiome to program the immune system to fight only when necessary, we will eventually decrease the chance as an adult or elderly to develop conditions like cardiovascular diseases, metabolic disorder, obesity, and so on and so forth, that will materialize if we don't play our genetic cards well.

Some of the data that I presented to you is the fruit of a very talented group that I have the privilege to direct at MGH Harvard Medical School and our Mucosal Immunology Biology Research Center. You see the crew over there (Slide 42). And a series of sponsors, public and private, that allow us to do this kind of studies. So again, [I] want to thank them for their commitment and their support.

Slide 42

Question & Answer

Editor's Note: This is a transcript of audience questions together with presenter responses from the May 20, 2022, webcast.

What in a pregnant mother's diet and post-delivery can be focused on to optimize the infant's gut microbiome?

Alessio Fasano, MD: That's a good question. You know, again, so far we have descriptive information, but because again, the technology and the knowledge that we have right now is really focused on shifting from a descriptive to a mechanistic personalization of intervention, including diet. In lieu of this technology that we have right now at the research level that would not be cost effective at a clinical care level, the common sense is a balanced diet. And again, I'm a broken record here, but probably the Mediterranean diet seems to be the most logical of all. Favoring fruits, vegetable, olive oil, short-chain fatty acids, like the ones that you can

get from foods like salmon and so on and so forth, seems to be the most logical way to go.

Zero-mile, in-season is also common sense, because of course you know having food in season ensures that you have lots of produce, a lot of products that you will have access to. When we come to products that we consume routinely, organic seems to be a logical approach. Of course all this implies that this is not a cheap diet. So that, again, brings us back to the socioeconomic part of the story.

Would there be a risk of iron deficiency for infants weaning with a Mediterranean diet?

I don't think so, because again, the Mediterranean diet is rich in legumes like lentils, for example. That's a huge source of iron. And I don't want to leave this false sense that the Mediterranean diet bans meats by all means. We are omnivores, so we have to have meats. The problem that we're facing with this chronic inflammatory diseases is that we shift from an occasional meat ingestion to a daily meat ingestion. That was not part of the evolution.

So if you allow me a parallel, assuming that the microbiome is a sort of farm in which you have chicken, you have cows, you have horses, you have sheep, and so on and so forth. And you want a farm in which you want 1,000 chicken and 10 cows. They do different things. But if you shift, and you have now much more food for cows and not enough for the chickens, guess what's going to happen, you can lose chickens and therefore you're not going to make the number of eggs in your plan. And now you have an excess of cows and you don't know what to do with that. All that can be detrimental to your farm.

Do you recommend avoiding gluten at all?

I do not. I mean, of course the gluten is, it's detrimental for the people that will have consequences when they consume it. Until the recent pass, we thought that gluten was detrimental only for people with celiac disease. Now we know there is a spectrum of gluten-related disorders, celiac disease being the autoimmune response to gluten. There are people that suffer an allergic reaction to gluten, and so that we call an allergy, or there's this other form of gluten reaction that we call non-celiac gluten sensitivity. Those definitely need to be gluten-free because otherwise they will have clinical consequences. The rest of the people again, they can tolerate gluten. There is no rationale to eliminate that. But once again, it's the same story, with balance and moderation, because everybody that will eat an excessive amount of gluten will pay a price, even healthy stuff. Like, I don't know, turkey meat that is good for you, but if you eat too much, you fall asleep. So it's all a matter of balance and moderation.

Are you using probiotics at your center? And if so, what strain are you using? When are you starting it?

We do, actually. Again, it depends. What is the use of probiotics? Some of the use I showed you. So we have kids with cow's milk allergies, or they have to have an antibiotic treatment because it's clinically indicated. That's the time in which we use probiotics. You know, if there is a history of allergic disease, sometimes we use that as preventive intervention. In general, we favor multistrain probiotics because until we can personalize by having access to an economically feasible analysis of the microbiome to customize what kind of probiotic we give to that particular patient, we have to just give-it-a-try kind of business. So that's the reason why multiple strains can be a more logical choice for us. And, of course, make use of natural sources. You know, yogurt, for example, is a great source of balanced, healthy microbiota and probiotics that can be very beneficial for the microbiome.

Specifically for a child or infant with severe IgE-mediated cow's milk allergies, is there anything

else that you recommend or is it still the multistrain approach?

Again, I don't want be biased, because I know that this educational initiative is sponsored by a company that makes products in that domain. But I personally believe the evidence-based data is the one they have to guide us until we have the next wave of information and knowledge to be deployed on a clinical care. So I gave you the example of LGG in formulas because it is probably the most studied strain of probiotics related to cow's milk allergy. But there are other strains, like *Bifidobacterium* that eventually have a similar positive impact. There is also a multistrain formulation that seems to be equally effective.

It is a matter of what is the evidence that has been reported in the literature. What kind of products are in the market? If you have something that is like the one that I presented, a formulation which you have already a hydrolyzed formula that you got to give kids with cow's milk allergy, plus the probiotics, you take 2 birds with 1 stone, so that you're not going to have to do an extra intervention. So it's a matter of convenience and availability, out there, supported by clinical data.

Do you recommend any interventions for women who have C-sections to increase bacterial exposure for their newborns?

There was a practice of taking a vaginal swab soon after birth and just imprinting in baby's mouth as a way to mimic a vaginal delivery. And this has been discouraged by obstetricians, because of the risk of transmitting some infections with specific strains that may be present on the vaginal canal, that may not be impactful in passing or would be more impactful in terms of the amount, if you do the swab. So I will say that right now, if the C-section is necessary for medical necessity, and that's extremely welcome, the best way is really to eventually keep an eye on this child, particularly in terms of diet with breast milk—that will be the top priority. Or using formula that they fortify with probiotics, but the swab approach that was implemented and studied in the past seems to be discouraged now by obstetricians.

Abbreviations

GI	gastrointestinal	**MGH**	Massachusetts General Hospital
IgA	immunoglobulin A	**PNAS**	*Proceedings of the National Academy of Sciences*

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