

Discussion

Leader: Yuan Kun Lee, PhD, National University of Singapore

Dr Lee: I am sure you agree that we have had four exciting presentations to bring us up to date on studies of probiotics, prebiotics, and fatty acids as dietary components on the subjects we have been talking about. I would like to start the discussion by making a provocative statement for our experts here to comment on and see where we end.

Can one go so far as to say that what we have observed is nothing other than the presence of specific strains of commensal microorganism in our gastrointestinal tract and their relative ratio to each other? The role of prebiotics and oligosaccharide in breast milk and infant formulas and of fatty acids in the diet is to modify and modulate the intestinal microbial profile, which eventually imparts health benefits on us. Can we say, it ultimately points to the microbial profile in the intestinal tract? Without good knowledge of the microecosystems in our intestine, perhaps it is not productive to talk about prebiotics, nutritional components, and other subjects we are discussing here.

Can we have input from some of our expert participants as to your view on our present knowledge of the intestinal microbial profile—the interactions between microbes and us, leading to the facts about probiotics and prebiotics? This subject has been touched on briefly by Dr Kuitunen. Dr Kuitunen, are we at a stage at which we can interpret the effects of probiotics and prebiotics? Perhaps you can explain the contradictory observation in probiotic studies and some prebiotic studies. Is it because we did not take into consideration the microbial profile of these individuals?

Dr Kuitunen: In the microflora, there are several hundred types of bacteria. I doubt we can make permanent large changes in the microflora by giving one germ or a couple of germs. Maybe prebiotics affect a larger amount of good bacteria in the gut, but we do not know enough about

the balance of the microflora that should be targeted. This issue is not settled, and how do we study all of these 500 foreign types? Which should we give, what is deficient, and what do we have too much of?

Dr Brandtzaeg: As Dr Kuitunen showed, we do not have a consensus about the mechanisms of prebiotic and probiotic effects. Multiple mechanisms probably produce these effects, and that confuses the issue. We use several readouts, but we do not know which readout will be the proper one for the effect we are looking for.

Dr Lee: Can we go so far as to say that we cannot draw any conclusions at all, because we do not know enough?

Dr Brandtzaeg: I say that we can be optimistic, because there are some positive results. However, it is too early to conclude.

Dr Rueda: I think we need more studies to determine how the intestinal microbiota are able to metabolize specific substrates, what metabolites are produced by the intestinal microbiota, and at the end, what signal pathways are derived that affect different functional outcomes. This is not easy to demonstrate, but I think that these types of studies might give us some idea of what is happening regarding the effects of prebiotics and probiotics in the gut, at least related to one specific substrate and to some of the microbial species affected by that substrate.

Dr Brandtzaeg: You look at signal pathways before you know anything about the receptors employed. That is a little premature. It would be nice to know more about the receptors. Is there an in vitro study that can be used to dissect the Toll-like receptors or whatever would then create the signal pathways?

Dr Rueda: There are in vitro studies [van der Werf MJ, Venema K: *J Agric Food Chem* 2001;49:378-383]. They are not in vivo, but when you select the microorganisms that are

affected by a specific substrate and you know which pathways are affected, you might have an idea. Of course, you cannot interpret the rest of the interaction with the rest of the microflora, but I think that might help.

Dr McCoy: I agree that we need to know a lot more information about the real composition of the microflora, and that is difficult. Maybe the increased accessibility to high-throughput sequencing platforms will provide answers. We do not even know the whole microflora of mice. That research has just started. With more information, we might be able to look at the consortia of bacteria in the gut, how they interact with each other, and which are luminal and which interact on the mucosal surfaces. I think we are just beginning to understand this consortia.

Dr Lee: It does not mean that what we have done so far is a waste. We have taken the first steps to understand the whole picture of the interactions. Where do we go from here?

Dr Kuitunen: I referred to the early study by Agnes Wold and coworkers that showed that Pakistani children had a lot of change in *E coli* [Adlerberth I et al: *Acta Paediatr Scand* 1991;80:602-610]. That was a nice theory, but in a study in several European countries in which fecal bacterial profiles were measured and related to allergy, the results were disappointing. There were no clear indications of what was missing or what should be present. So we have a long way to go.

Dr Lee: Why do you think they could not draw conclusions from this study? What can we learn from that?

Dr Kuitunen: How many bacteria should you study to be able to show differences? That study offered no clear direction to go, and it was done in many countries with different environments.

Dr Bienenstock: Part of the problem is that there is a lot of in-the-box thinking—a lack of attention to all the variables that could be affected. I do not want to keep plugging the same

business, but to avoid attention to the effects of systems such as the nervous system and the endocrine system on the immune system is just closing your eyes.

Another problem is that fecal samples only tell you what is in the colon; they do not tell you anything about what is in the small intestine. If the small intestine is where most of the action is in terms of effects on the immune system, then we need a lot more information about what is in the small intestine. I would start to sample in animals because of the difficulties in sampling from humans—it is highly interventional. Nevertheless, the conclusions that have been drawn about the human microbiome need to be applied to the small intestine as well.

Dr Lee: As you say, it is difficult to work on humans. At times, however, what happens in animals does not really reflect what happens in human. Do you think there is a good animal model to allow us to perform this kind of study?

Dr Bienenstock: No, I am just referring to normal animals, not to animal models.

Dr Brandtzaeg: I am on the advisory board for a large international multisite microbiome project, the MetaHIT consortium, and I try to question the members, but they only want to sample feces. That is the only thing this big project is focusing on. It is impossible to change their attitude and take any other approach, despite the fact that fecal bacteria are not representative of the entire intestinal tract. Can you sample the small intestine using a noninvasive method?

Dr Bienenstock: Noninvasively? It is not possible.

Dr Lee: Would the Abbott faculty like to share anything from the point of view of your industry? I think this is important, because you are the ones who will apply this information to a product. I am sure that at times the research is confusing. One day, someone will say that a strain has an effect, and at another time, you see reports that conclude there is no effect. How are you going to tell your customer that, in fact, the product you are promoting has an effect?

Dr Miller (Abbott Faculty): I think this gets to the heart of what I heard at this conference. You have it down to some beautiful pathways, but you know the complexity behind them. We talk about the number of species, but then we talk about the fact that bacteria live in colonies of biofilms that have no relationship to cultured bacteria. The bacteria are in biofilms that are potentially like organs that the inside can pull to the outside and have no relationship in the way they interact. This is one of the reasons why you cannot get a decontaminated gut; you never will do that.

The neuroendocrine system and the way motility and all that encompasses is fundamentally unknown, but we know that probiotics can influence these systems. Some of the research results on motility are impressive, and they may then link back to the neuroendocrine axis. We also saw some beautiful clinical studies that we can take away from this conference, but it is still hit and miss.

I am going to propose something, and I hope it will be beneficial. The question is whether to do clinical trials in, for instance, Milan instead of other parts of Europe because of the environment, or in Pakistan instead of Western Europe. I am just outlining how to get to this “in-between” of clinical research and the reduction of science such that we have at least more predictability or a hypothesis we can act toward. When a company like Abbott puts out a product, we would like to know that the underlying research has reproducibility so that the information is relevant whether the product is in this city, that country, or this culture. I think we have learned some fundamental elements at this conference.

Dr Lee: Can our experts tell us where we are? How do we reach the point at which we are confident that the data apply broadly to multiple countries and populations?

Dr Moro: I think we are just at the beginning of the story, because we have discovered the importance of intestinal microflora in the last few years. No one was aware of it before. Now we know we can modify the composition of intestinal flora with some interventions such as prebiotics and probiotics. We do not know what type of probiotic is best, and we do not know the dosages, how long to give it, and the side effects. We do not know which type of prebiotic is best, and we do not know the dosage, but we do know that we can influence the composition of the intestinal flora, and we know that that influence can change the clinical history of the infant.

Dr Lee: This is the first step, and it points to a direction. We know we are on to something.

Q: We keep saying we do not know, we do not know, we do not know. However, probiotic products have been with us for centuries. At this point, what are the success stories of probiotic products—of the foods people have been consuming for centuries? What do we really know works? Do we look at Bulgarian peasants eating yogurt or something like that? Do we know that it worked for them, and what it did?

Dr Lee: We know that probiotics work because we see their positive effects. The question is, should we look beyond that because we need to know more to have better control and a better grip on an important subject?

Dr Kuitunen: We know there is quite good evidence that probiotics work in infectious diarrhea. There are also some promising results in the fields of allergy and inflammatory bowel disease. I think the most important thing as we enter this area of research is that no matter whether the work is in vivo, in humans, in animals, or in vitro, that we have some kind of proof of concept. We are at the beginning, but we have chosen a path that, I hope, will be fruitful.

Dr Montalto (Abbott Faculty): There are two sides to every coin, and we have seen pros and cons of research results. We have seen an attempt to try to understand different methodologies and

different mechanisms. They tend to be, in some cases, isolated to certain factors, disregarding other possible components. So it is hard to know the specific mechanism. There are a lot of possibilities. I think we are trying to have some assurance that we are going to improve patient outcomes with what we do, and, first, do no harm. I think there are many chapters yet to be written in this whole story. We have a lot of research yet to do, but good research will lead to more research that will answer more questions. I think we need more long-term funding and repetitive trials, because one trial is not definitive. As Dr Kuitunen's presentation showed, sometimes you get the effect, and sometimes you do not. I think we will have to have a preponderance of evidence that what we want to do with foods will be the right thing for small children, older people, and everyone in between.

Dr Kuitunen: I also think that we should be very cautious, as scientists, about giving advice too early because this advice may not prove to be right. As Dr Bienenstock pointed out, we should refrain from black-and-white thinking. Probiotics are not all good or all bad; they have both sides. It is the same with prebiotics and the interventions we heard about today.

Dr Lee: The problem is that the consumer wants an answer.

Dr Rueda: About Dr Miller's previous comment, we are focused mainly on the interaction between the microflora and the immune system. I think that neuroimmunomodulation is going to change a lot of different concepts, and studying that interaction will get us closer to understanding some of these mechanisms.

Dr Brandtzaeg: There has been little discussion about helminths and microbial cell-wall products. Would that be a more potent approach than studying *Streptococci* and *Lactobacilli*? We see striking results in inflammatory bowel disease with the pig pinworm and actually better

results than with traditional medicine. Are there other approaches that might be better than *Lactobacilli* and fecal bacteria?

Dr Lee: There are reports on parasites in the prevention of atopic diseases. They show interesting results, but the question is, can we get a safe parasite? We have *Lactobacillus* and *Bifidobacteria*, which are harmless, if not beneficial, so they are safe for us to use. Do we have a safe parasite to use? If we can have that, perhaps it is worthwhile for us go in that direction. Can we consult the clinicians here about whether this is possible?

Dr Bienenstock: Why do we need a live parasite? I think that is Dr Brandtzaeg's point. We heard Dr Versalovic talking about immunomodulins and products that Mazmanian and Kasper have been describing with exopolysaccharides. There is no reason to think that this pinworm may produce the sort of molecules that Dr Versalovic described with *L reuteri*. That is thinking out of the box. That approach is crucial to our study. Ultimately for academic researchers, however, the question is, who is going to fund this? This area of gut microbiota and mucosal immunology is just starting to become mainstream. We also are waiting for the technology that is coming. That technology is amazing in terms of the quantity of information we will have and the ability to analyze, not only the human intestinal microbiome, but also the mouse microbiome. At the present rate of progress, the next couple of years will bring us enormous possibilities to study these things.