A Systems Approach to the Modern Microbiome World:
Investigating Host-Microbiota Symbiosis
Webinar
24 September, 2014

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Slide 1

Sean Sanders: Hello everyone, and welcome to the *Science*/AAAS webinar, "A Systems Approach to the Modern Microbiome World: Investigating Host-Microbiota Symbiosis." My name is Sean Sanders, editor for Custom Publishing at *Science* and I have the pleasure of moderating today's webinar.

Microorganisms living in or on the body of a healthy human outnumber human cells by approximately 10 to 1. A large number of the 100 trillion microbes that compose the majority of life on Earth have coevolved a close relationship with the mammalian immune system. Therefore, by solely limiting research to either the host or bacteria, only half of the information required in microbiome studies is being seen. A delicate symbiotic harmony exists between the host and resident microbiota. The disruption of which can lead to an imbalance in homeostasis of immune cells leading to a variety of health disorders. It is becoming increasingly clear that to understand the complete story, the interaction between host and bacterial systems needs to be considered through a more holistic, systematic approach. This webinar will discuss just such an approach, examining the problem from the perspective of both the host and the microbiome.

It is my great pleasure to introduce our speakers for today's event to you, they are Dr. Oleg Paliy from Wright State University in Dayton, Ohio, and Dr. Yasmine Belkaid from the National Institutes of Health in Bethesda, Maryland. I'm very pleased that you could be with us today.

Before we get started I'd like to share some information for our online viewers, at the top right of your screen you'll find photos of today's speakers and if you view bio link which you can click on to read more details about their background and research. Underneath this line view is the resources tab where

you can find additional information about technologies related to today's discussion and the link to download the PDF of the slides.

Each of our guests will give a short presentation followed by a Q&A session, during which they will address the questions submitted by our live online viewers. So if you're joining us live start thinking about some questions now and submit them at any time by typing them into the box on the bottom of viewing consule and clicking the submit button. Please remember to keep your questions short and concise as this will give in the best chance of being put to the panel. You can also log in to your Facebook, Twitter, or LinkedIn accounts during the webinar to post updates or send tweets about the event just click the relevant widgets at the top right of your screen. For tweets, you can add the hash tag #sciencewebinar. Finally thank you to Affymetrix for sponsoring today's webinar.

I'd like to introduce our first speaker, Dr. Oleg Paliy…

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Dr. Paliy is an Associate Professor in the Boonshoft School of Medicine at Wright State University in Ohio, where he studies the roles of human intestinal microbiota in host health and disease. Dr. Paliy's research utilizes novel molecular techniques including polygenetic microarrays and next generation sequencing... Sorry, *phylo*genetic microarrays and next generation sequencing, which are combined with biocomputational modeling of microbial interactions and metabolic capacities.

A very warm welcome to you Dr. Paliy.

Dr. Oleg Paliy: Thank you Sean for introduction and thank you everyone for tuning in and listening to this webinar. So as you can tell from the title of my talk, my presentation will basically encompass talking about human gut microbiota. However, the same kinds of research studies are also done for all other types of microbes inhibiting our bodies, for example in the skin, in the mouth in our lungs, and so forth. So just to start with a very short introduction for the gut microbiota...

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This is a very complex community. We believe up to a thousand different species of microbes exists in every human GI tract. Every part of the GI tract would house some kinds of microbes. Majority of them would be bacteria, there are also some archaea, fungi, and protosomes also living there. The numbers of microbes increase from stomach to small intestine to colon and the types of microbes also are different in different sections of the gut: aerobic

bacteria are dominate more in the stomach and small intestine, whereas colon regions basically house almost exclusively obligate and facultative anaerobes because there was almost oxygen available to give microbes into that regional of the gut.

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So what we know about the function that these guts microbes play in our human life. On this slide I'm showing you, uhm, two sides of a coin: positive interactions of our cells are shown on the left of the slide, whereas the negative influences and associations are shown on the right. Among those which are positive: microbes participate in the fermentation/degradation of complex polysaccharides, which human cannot do, they modulate gut motility, they ensure proper homeostasis and development of immune function of the gut, they participate in the prevention of allergy development, and they also protect humans against pathogen invasion. However, when a normal gut community is perturbed in a human, in a person, what happens is that there are also some negative effect that this community can actually exert on human gut and generally human host. This includes, for the introduction, if microbes penetrate through intestinal barrier of the gut this can lead to sepsis and potentially person's death. There are also studies which are associations of perturbed gut microbiota to several different GI tract diseases such as colon cancer, obesity or malnutrition, inflammatory bowel disease, irritable bowel syndrome, and also there are obviously the different kinds of intestinal pathogens that can cause intestinal infections.

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Just to give you a little bit of introduction into this microbiota community which is in the gut, I'll give you two examples. The first example shown now on the left, you can see a compilation of a typical human gut microbiota as separated into different microbial phyla. Majority of microtomes belong to only two phyla, firmicutes and bacteroidetes, and there are some limited number of microbes from about maybe 10 other phyla, but they do not contribute more than maybe overall 10 to 15 percent of overall abundance in the microbial community in the gut. So a lot of studies looked at differences in gut microbial communities of among the different types of people. I'll give you just one example of number of very nice studies from Jeffrey Gordon's group looking at differences in gut microbes between lean individuals and who have high weight and are classified as obese. Dr. Gordon's group was able to show is there was a huge difference in the relative abundances of firmicutes and non-bacteriodetes phyla in the gut of obese people as you can see here the ratio is higher. And they also showed that this also leads to increased ability of gut microbes to derive energy from the same types of food, thus providing the source for maybe more energy every

single day. But what's also very interesting is that they took germ-free mice, these are mice that don't have any microbes in their gut and then transplanted the firmicute microbiota from either lean individuals or from obese individuals. And as you can see on this image, this led to a mice which had been given obese microbiota gaining high weight, while both types of mice had been maintained on the same diet. And so obviously there was something inherited in the gut microbiota in obese people which can be transferred through fetal microbiota transplantation into mice.

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Another way to work at gut microbiota is to look at the types of metabolic interactions that they might have with human host. Again I will just give you just two examples of site-potential interactions. One is a very well-known fact that a lot of microbes in the gut are able to produce short-chain fatty acids, which for microbes are end products of anaerobic fermentation. Generally speaking short chain fatty acids have very positive effects on the epithelial tissues in the gut and overall on the human physiology. Short chain fatty acids prevent mucosal inflammation—you can see here an arrow pointing down. When you decrease the amount of short chain fatty acids, this leads to an increase in inflammation. And short chain fatty acids also provide energy for the host, so when you have less of them, you provide less energy. Butyrate specifically is known to repress expression of proinflammatory transcription factor NFκb. And it also participates in enterocyte differentiation. Propionate is another example, is used in gluconeogenesis and it also can promote generation and development of specific types of T cells called Treg or T regulatory cells, which again prevent increased inflammation in the gut.

A second example, which basically shows how gut microbiota can influence a system's response of humans, is production of a particular compound called trimethylamine. This is a small chemical as you can see here, a small metabolite, which is produced by gut microbes during degradation of two compounds either L-carnithine or phosphatidylcholine. These compounds are prevalent either in meat or in very high fat diets. And what happen is that TMA can be actively taken up by enterocytes and passed into the blood. And then in the liver it is converted into a compound called trimethylamine N-oxide and TMAO has shown to actually facilitate development of atherosclerosis and cardiovascular disease. Thus, you can see here how the production of a particular metabolites by gut microbiota can have an impact on other different kinds of systems in human host.

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So how can we study gut microbiota-host interactions. So I'm going to speak mostly about the study of the microbiota side of things. And this slide shows you a number of tools that can be used to interrogate microbiota structure, which is shown at the top of the slide, with such techniques as phylogenetic microarrays, high throughput sequencing, quantitative PCR, and fluorescent in situ hybridization. For example, in our laboratory we developed a microbiota array based on the Affymetrix GeneChip platform, and this array contains thousands and tens of thousands of probes, which all target 16S rRNA genes of different phyla. This allows us to detect up to a thousand different species of gut microbes in a single sample by just utilizing this one microarray. We can also look at the metabolites and say, well we can determine what kinds of metabolites we can see in the same sample, for example, in a stool? And here's again several different techniques: nuclear magnetic resonance, which we use in our research, but you can also use mass spectrometry, gas or liquid chromatography, two measure different types of metabolites.

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So just to show an example of how all these techniques can be used in research, I'm going to tell you one story about gut environment in children with Irritable Bowel Syndrome. You can see that this has already been published in a number of reports and we have another manuscript in submission as well.

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So Irritable Bowel Syndrome is functional bowel syndrome, which can have a lot of different symptoms, but generally it is associated with abdominal pain, bloating, and changes in bowel movement or bowel habit. There's no visible damage or high-level inflammation observed, and therefore this is called an irritable bowel syndrome. Different subtypes are recognized by either the bowel movement habits, diarrhea-predominant (which is what our study has looked at), but they can also be constipation-predominant type and mixed type of IBS. This is a very prevalent disorder. Some estimates say that about 20 percent of the general population can be diagnosed with IBS, and it often manifests first during adolescence which is why we are very curious to study adolescent children who were diagnosed with this disease. And so, from many potential hypotheses about what causes IBS, one which would be relevant to our study is that of SIBO—small intestinal bacterial overgrowth. And this is hypothesis where we think that in some patients, microbiota numbers can increase disproportionately in the small intestine primarily in the ilium and, because there are many normal microorganisms there in a normal healthy gut, this can lead to some problems in that particular individual.

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So the sample that we had in this particular study had two groups of equal numbers, healthy adolescence humans with about 13 years of age, average—

we called them “kHLT” for healthy kids. And children with IBS, with a predominant type of IBS, which we designated as kIBS. Again, very similar average age and the same number of children were used. We profiled the gut environment by analyzing stool samples and profiled the microbiota with phylogenetic microarray and next generation sequencing techniques. And we also measured metabolites in those samples with proton Nuclear Magnetic Resonance.

Sean Sanders: Dr. Paliy?

Dr. Oleg Paliy: Yes.

Sean Sanders: Sorry to interrupt for a second. Some people are having some trouble hearing you. I wonder if you can just try move your microphone a little bit away from your mouth and let's see if we can improve this.

Dr. Oleg Paliy: Does this sound better?

Sean Sanders: Yup. I think that's all right. Thank you. Fantastic.

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Dr. Oleg Paliy: So the first slides will show our analysis of microbiota abundance. This is data from the microbiota array and we can interrogate about 115 genera on that microarray. Generally speaking, the human microbiota is dominated by about a dozen different genera and the majority of highly abundant genera had similar abundances between groups. However, when you look at, when you go deeper and look at not highly abundant genera but other genera is can play very important role in functions of different diseases and the different microbial communities. They identify about a dozen genera which were differentially abundant between healthy children and children diagnosed with diarrhea-predominant IBS. In the table I show you just five examples. You can see that the top four they are more abundant in kIBS, whereas *verrumicrobium* was more abundant kHLT samples. What's interesting about is the top four genera is that they all aerotolerant or facultative anaerobes, which means that they can actually tolerate a presence of oxygen and we also know that all of them are known to exist and live in the small intestine in humans. So this can potentially tell us about some potential link to the small intestine overgrowth hypothesis because this actually does confirm potentially, that that might be one of the reasons why IBS develop in people.

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If you look at… if you take all of this information, all the data of different genera and say, well, can we now separate… if you don't know what the sample is, can we now use statistical tools and microbiota profiling and tell if the sample belongs to IBS kids or to a healthy kid. Basically we're trying to say if we can separate samples, fecal samples, based on the microbiota abundance which we measured. And indeed what we show here are ordination plots that use statistical approaches to find differences among different samples. You can see that we used three different tools, a principal component analysis shown on the left. Principal coordinate analysis shown in the center and a supervised technique called orthogonal projections to latent structures discriminant analysis, shown in the right. In all three cases we could achieve a partial, but very statistically significantly separation, of healthy and IBS-D samples, which basically tells us that microbiota in this imminent fecal samples of healthy and IBS children in differential analysis, that we can separate these samples based just on the microbiota profile.

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What we are also interested in is to say: can we identify any associations among different microbial genera. So we used our data sets and we looked at the correlation matches in abundances among different genera and what you see currently on the slide is that network of abundance associations for healthy children. We found a total of 44 different associations which are presented by a line linking two different nodes. Each node is a particular genus, the color represents the phylum to which the genus belongs, the size of the node is proportionate to an average abundance of the genus in the stool sample. If the line is red it represents a positive association among abundances for a particular genera; if the line is green this represent a negative relationship between two particular genera. What you're seeing here is that generally there are a few hub here. *Ruminococcus*, which is the largest node on the image, has a lot of negative interactions with other members. *Prevotella*, which is a blue node in the lower left corner of the image, also has a lot of interactions and also *haemophilus* also had interactions linking different other genera in the healthy gut.

When we look at the IBS gut we saw quite a different picture. First of all, the number of associations was much lower: only 20 instead of 44. And you can see that the structure of associations is also quite different. We lose this hub structure, right? So the highest number of links is 3 versus, I believe eight, in a healthy gut. And we don't have any hubs anymore which would connect to many other genera. This can represent potentially a loss of microbe-microbe

interactions that we can see in IBS gut and this can also lead to some misbalance in maybe metabolites.

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So this gets me to the next part of my talk where I also wanted to see if there was any difference in metabolite level between healthy and IBS stool samples. What you see here are NMR proton spectra which were acquired and particular peaks on these proton mass spectra can be assigned to particular metabolites; so basically spiking and testing individual pure, pure metabolites so we know which peak corresponds to which metabolite. We can quantify these metabolites and so we obtain measurements of about 19-20 different metabolites in each stool sample.

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Generally speaking, what we saw in the gut of these people was the following. So what we wanted to see was, well, can we also separate sample bases on proton NMR. So for proton NMR, we can actually… the whole spectrum can we divide in about 300 individual bins, so we digitized all this information and this numerical array of values can now be used in those ordination plots which I showed you before for microbiota analysis. So you can see here on the left is the results of the principal component analysis based on this proton NMR data. And again you can see that even though the separation is not, full—it is on the partial—but it is statistically significant. We can use a supervised technique to basically… in a supervised technique you actually tell the program which samples are IBS and which samples are healthy—healthy children who donated a stool sample. And the program tries to find specific metabolites and specific links to separate the bins, and you can see that the separation is significantly better. Now because we also ran PCA on microbial abundances, one question that is obvious to ask is: is the separation of samples based on the metabolic profiling and based on microbiotic profiling similar or different.

To answer that question you can use the particular statistical tools called Procrustes analysis, which basically says we will take two separations and will rotate and scale them to find, to see, whether the sample are distributed equally or similarly in these two PCA analyses. What you can see here is a result of that analysis. It's probably going to be a little bit difficult to see on a small screen on a small number of pixels but the idea is that there are lines on that plot which connect the same sample either on a PCA analysis or base on metabolites which are colored-in dots, and based on the microbial abundance analysis which will be greyed diamonds. The smaller is the line connecting the dots the more congruent the separation of that sample in both analysis. So the whole take-home message out is that there is a statistically significant

congruence in the separation. And what this tells us is that basically two methods gives more or less very similar answer: that they separate these samples in a very similar way either based on metabolites or based on microbial abundance measurements.

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When we look at particular peaks and quantify specific metabolites, we can measure 19 of those metabolites and they found statistically significant differences. Specifically for IBS gut, they found it contained high levels of glucose and lactate and formate—these are all intermediates in the anaerobic fermentation process. IBS samples also contained high numbers of amino acids and also elevated total bile acids.

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Finally, what we could also say is, well, because we measure both microbes and metabolites in the same set of samples, can we now find a relationship among metabolites and microbes similar to the network analysis I showed you about five slides before. Similarly, we used non-parametric correlation followed by bi-clustering to find potential links.

What you see here on the left is an analysis result for healthy gut samples. Every column is a particular genus, every row is a particular metabolite. And each cell represents this non-parametric correlation value, which is a Spearman Rank Correlation. The red represents a positive relation between the particular metabolic and particular genus. Blue represents a negative relationship. So, the relationships which are statistically significant are highlighted with a yellow boundary box. So here we identify quite a few of these relationships in the healthy gut.

When we did the same analysis for an IBS gut, the picture was quite different. You can see that the colors are significantly more muted and we found absolutely not statically significant relationships among any metabolite to any of the genuses. This is again can represent a potential loss of microbial interactions in the gut which now perturbs the environment and also perturbs what kinds of metabolites are now available in the lumen and in the mucosal tissues of people with IBS.

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When we looked at the specifically statistically significant associations between microbes and metabolites is the healthy gut. And this is picture represents specific links, right. So you can see in the center are the metabolites, the color of the background of the metabolite represents the level of the metabolite.

Different genera are shown on the left and right from the metabolite. Again the node size represents and average abundance and the color corresponds to a particular phylum. Again the lines represent correlations, so the positive relationships would be in red and negative relationships would be in blue.

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I can just highlight just a few of these relationships. Some of them have already been experimental shown in the literature. For example, in *Ruminococcus* is known to release glucose during polysaccharide degradation, such as starch. And we found that *Ruminococcus* is positively associated with glucose, and this obviously makes sense but it can increase the amount of glucose when there are a lot of *Ruminococci* members in the gut. Similarly *Acidaminobacter*, *Coprococcu*, and *Prevotella* members produce acetate, valerate, and fumarate, respectively, and again we see positive associations between these metabolites and these genera. As an example of negative association, *Coprococcus* members are known to utilize glucose for growth and therefore this explain why we see a negative correlation between glucose and *Coprococcus*: because obviously when there is a lot of *Coprococcus* they will probably use a lot of glucose and so forth. Other interactions novel putative interactions and so we can go forward and test some of them experimentally to see what the association is, what the levels are and quantify these relationships.

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Finally… so the conclusions of our study are the following. Interrogation of fecal samples reveal differences both at the microbial and metabolite levels between healthy children and children with diarrhea-predominant IBS. The IBS disease state was associated with increased numbers of aerotolerant and facultative anaerobic members, many of which we know can reside and do reside in small intestine, as this provides a potential link to the small intestine bacterial overgrowth hypothesis for the IBS development.

The intestinal environment of IBS-D was characterized by increased proteolysis (because we found more amino acids in IBS lumen and fecal samples), incomplete anaerobic fermentation, and elevated levels of bile acids, which can also be another potential reason for IBS development. Finally, and very important for us, we measured both luminal and fecal metabolites in the same set of samples, and this allowed us to link microbiota composition with microbiota activity and disease-to-health status. Therefore, this can potentially also provides link between microbes, metabolites they produce or use, and host physiology, because we also know how some of these metabolites can influence health of human host.

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Finally in the last slide I would like just to acknowledge the students and researchers who did the work. I had the pleasure to work with a number of collaborators at Wright State University and outside, received funding from NIH, Wright State University, and Procter and Gamble. And I want to finish my presentation with a shout of microbial pride—microbes are the first to evolve and will probably be the last to survive. Thank you for listening.

Sean Sanders: Fantastic, thank you so much Dr. Paliy, very much appreciated. I do understand that some people are still having some audio issues and I do apologize for this, we're all working on it. Dr. Paliy I might ask you to move your mic a little bit service from your mouth and even possibly use your handset for the Q&A session a little bit later but we'll test that when we get to it. I'm going to move right on to our second speaker for today…

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And that is Dr. Jasmine Belkaid. In 2002, Dr. Belkaid joined the Children's Hospital Research Foundation in Cincinnati, Ohio, as an assistant professor, and in 2005 she became a tenure-track investigator in the laboratory of Parasitic Diseases at the National Institute of Allergy and Infectious Diseases (NIAID), part of the National Institutes of Health in Bethesda, Maryland.

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Since 2008, she has also worked as an adjunct professor at the University of Pennsylvania. Dr. Belkaid is currently the chief of the mucosal immunology section at NIAID, where her research focuses on the factors controlling immune responses to pathogens. Her work has defined fundamental mechanisms that regulate host immune responses to pathogens at mucosal and skin sites and revealed key roles for commensal microbiota and dietary factors in the maintenance of tissue homeostasis. A warm welcome to you Dr. Belkaid.

[0:30:07]

Dr. Yasmine Belkaid: Thank you very much for having me. So I'm gonna change a little bit topic and I'm going to discuss, ah, some of crosstalk between the microbiota and the tissue immunity. More particularly, discuss some concept of the crosstalk between the microbiota and pathogens.

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So I'm gonna move to my next slide and the first one I would like to highlight before we discuss the microbiota is we need to remember then tissue has evolved to develop immunity that is highly tailored to the site that is actually injured or infected. Which means that structural cells, antigen-presenting cells, tissue-specific factors, and the microbiota, will synergize to really allow the induction of a response in a way that allows the maintenance of tissue integrity

and function. Of course, dysregulation of any of these elements in the microbiota, for example, can lead pathological state. So one thing that we need to remember is that the microbes that reside in the tissue are one of the main factors that control this tissue specification.

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So what we also need to remember is the fact that, as was highlighted by the talk of Oleg, most tissue are colonized by highly specific microbiota and in each of these environments, the microbes are utilizing the mode interaction of the underlying tissue. In the GI tract, some work that was done by many authors including our groups, highlighted the importance of the good microbiota in the control of the immune system. It allows development of the tissue, its function, and the fine tuning of the cells in this environment. And because of this role, the gut microbiota has been shown to have not only a local role on the immune system, and acting as a local adjuvant to provide immunity, but can also in certain instance control systemic immunity at a distal site. For example, the gut microbiota can have influence on the capacity of the lung to control ciral infection. Another point that I would like to highlight before moving to the crosstalk between the pathogen and the microbiota…

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Is the fact that most microbes really exist in a continuum. We tried very often to distinguish the microbiota and approve them to pathogen but in reality most microbiomes can exist in different kinds of states. For example, *Mycobacterium tuberculosis*, clearly in pathogen in some individuals, really is a commensal in a large fraction of humanity. And so is the case for *Candida albicans*, for example, or *Staph aureus,* that can live in the skin of an individual without causing disease, but in the commensal state can trigger inflammatory responses. So we need to remember that most microbes in most microbiota in fact contain a large number of microbiota but in contextual a event can actually lead to pathogenesis. So in the context of this question we decided to explore for the last two years to crosstalk between the microbiota and pathogens.

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And one thing that we also would like to highlight is although we have, fo course, over the last two years really highlighted the importance of antibiotics, lifestyle, nutrition, and hygiene in the change that may have impacted upon microbiota and as a potential cause for the dysbiosis of certain developing countries. Infection and change in infection may be an important trigger in the change of the microbiota from healthy states to the one that maybe today the one amplifying inflammatory disorders.

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So as I mentioned before, the crosstalk between the microbiota and the pathogen is quite complex in one first thing that happened is most microbes that invade the host, invade the host via tissue that are constantly colonized by microbiota. So it was, for example, shown that the pathogen in the inflammatory process that is caused by the pathogen can lead to a dysbiosis of the microbiota and emergence of inflammatory microbes. For example, gammaproteobacteria can become enriched in the complex of acute inflammatory responses and acute infection in the gut. The microbiota can also, because of that, contribute to pathogenesis of infection. There are many incidences in which the microbiota become really part of the problem, and amplify the pathogenesis of the pathogen response. The microbiota can in a healthy manner promote immunity to pathogen and really act as adjuvant to immune responses, but in some inflammatory states pathogen can actually trigger aberrant response to the microbiota (and I'm going to discuss that later) and now induce abducted immune and inflammatory response to this microbe something that can have long-time consequences for the host. And finally as it was shown in the context of viruses and some new methods, microbiota can also promote transmission of pathogen by creating a milieu that favor the implementation of invasion of the microbe. And finally as it was preliminarily proposed in the context of probiotics, microbiota can also induce regulatory responses to [0:34:40][inaudible] infection. And I'm going discuss with you some other mechanisms of regulation.

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So one snapshot of a story that I would like to discuss with you is actually how infection can disruption the relationship with the microbiota. And this work was done by the very talented postdoc fellow, Tim Hand, in our laboratory, who explored the possibility that the host can no longer be able to distinguish between a pathogen and commensal in the context of inflammatory responses. So for that he utilized cells that were actually specific for commensal antigen and look at fate in the context of an acute inflammatory response to a pathogen, in this case *T. gondii* and was able to show that these cells are no longer be able to become regulatory or Th17, but when actually exposed to an environment that is actually highly Th1-mediated, are now able to adopt features of Th1 inflammatory cells, but these cell are in fact reactive to the commensals. Importantly, these cells were actually able to persist for the long-term and become truly memory cells able to be recalled upon secondary challenges. So this experimental highlighted the possibility than in some inflammatory states, the immune response may no longer be able to distinguish between pathogens and commensals, and established aberrant reactivity against the plethora of antigen that present in the microbiota.

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Another small story I wanted to discuss with you is how in the context of acute inflammation the microbiota can impose regulatory features to inflammatory cells and that was done by John Grainger in the laboratory, a postdoctoral fellow, who looked that the *T.gondii* infection and found then an inflammatory monocyte that accumulate at the site of inflammation in the gut are now able to produce a large amount of PGE_2. And what he was able to show is the fact that this PGE_2 is mediated and respond to the encounter with the microbiota in the gut and could suppress the capacity of neutrophil to produce inflammatory mediator that in turn trigger pathological responses. So the microbiota in the context of this acute inflammatory response were able to impose a regulatory program on the inflammatory cells and therefore suppress even the pathology. So clearly what we need to remember from this work, but also many others done by people in the field, is a fact that pathogen and microbiota history is already entwined. And that the microbiota control not only the induction of the responses, the pathogens of the infection, the memory responses, but also the resolution of the infection.

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So as I mentioned before, all different tissues are colonized by site-specific microbiota and although we know quite a lot in terms of the effect of the gut microbiota it is actually less clear the level in other tissues. And two years ago we embarked on the exploration of the role of the microbiota in the control of skin immunity that was done by a talented researcher still in the laboratory, Shruti Naik…

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And she was really intrigued by some of the beautiful stories that were emerging from the group of Julie Segre at the NIH that highlighted the complexity of human skin microbiota. In the skin these microbes reside in a certain niche they are on the surface of the skin, but also in the hair follicle and sebaceous glands, therefore clearly embedded in the skin tissue and not just at the surface of the tissue.

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She was able to show utilizing germ free mice, that is mice devoid of microbe—of live microbe—and she look at the T cell that resides in the tissue in the absence of microbe. There is a reduction of the capacity of the cell to produce inflammatory cytokines, in this case IL-17. Importantly, she was able to show that is effect of the skin microbiota occurred independently of the gut microbiota, so the effect of the skin microbes were actually tissue specific and was not influenced by other niches in particularly by the gut microbiota.

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So she did a very simple experiment to look at the function of these microbes using a pathogen, *Leishmania major*, in which she looked at the capacity of the microbiota to influence positively and negatively this response. And she was able to show that if she will infect the animal than is germ-free, the first observation effect that the inflammatory response that is observed in response to the infection is clearly reduced…

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…highlighting the fact that the inflammation is not due to the pathogen itself, but really to a reactivity to the microbiota in this site.

Of course having no inflammation can be a problem, because in the absence of inflammation you don't develop adaptive immunity, and indeed the mice that is germ-free is no longer able to develop adaptive immunity against *Leishmania* and has a poor response against his parasite and is not able to control parasitic load.

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She then performed an add-back experiment in which she utilized *staphylococcus epidermidis*. She colonized the germ-free mice with this microbe and was able to show then in this context she could recapitulate the capacity of the mice to develop adaptive immunity against the microbe and also to control the infection. This actually highlighted the fact that the skin microbiota, as well as the gut microbiota, was also able to locally control all the immunity against pathogen.

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Mechanistically, and I'm just going to give a summary of the findings that Shruti had, she was able show that the microbiota was able to manipulate two intriguing molecules. One is IL-1 receptor antagonist that is in fact an anakinra in terms of drug, but also to promote IL-1α production in the skin. And she was able to show then the skin microbiota was not changing the polarization of the cells, but was actually able to act locally on the cell that had entered the environment by promoting the capacity of the cell to produce inflammatory cytokines at the site of entrance in the tissue. So it's a very topical, local control in which the skin microbiota act exactly as the site where they are present, then licensing the cells to produce inflammatory cytokines, something that of course can have an important effect in the control of tissue immunity that could in certain instances promote inflammatory states.

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And I'm going to summarize some more recent work that we have done. This effect is actually not only seen with *Staph ep.* that seems to be able to create a largely area of lymphocytes in the skin, but also with the other microbes. Importantly we're able to show in relatively small survey of microbe that there is a high specificity in the capacity of each microbe to engage different lymphocytes in the tissue, highlighting the fact that there may be high specialization of interaction between unique microbes and the immune system—something that could be potentially leveraged in the context of therapeutic approach or to try to understand the potential association between defined microbes and inflammatory states.

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So to conclude, the skin as well as the GI tract and many tissues seems to be controlled independently by its resident microbiota. And the way by which the skin actually works in this tissue to tune the effect and activation tissue-resident cells. These commensals are able to promote immunity to pathogen and potentially to vaccine, something that may be interesting to explore, and to drive response locally via a mechanism that depends on IL-1 that is distinct and independent from the gut flora. Importantly this is not a saturated environment. If you add new microbes at the surface of the skin it can differentially alter locally immunity and inflammatory states of these individuals, of the mice.

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So I would like to finish by discussing how, in this tissue, the microbe can actually potentially control inflammatory states and disease. We could of course state in one of the primary element or determinant of the distributed reaction in the microbiota will be genetic predisposition. Having any kind of defect in barrier responses or regulatory mechanism is likely to impair the capacity of the host to just send these microbes as healthy partners and induce hyper reactivity against these translocating microbes. It could also be during the context of change in nutrition, the skin is extremely poor in nutrient and therefore the density of microbes is very low. In the context of metabolic syndrome an increase of nutrients in the skin, this also could lead to an increase of the density of microbe and maybe also the quality of microbe that together can promote inflammatory states. Co-infection can potentially allow the entrance of microbe in the tissue and actually in this case they could promote inflammatory responses. And finally, we could actually propose also that an increase in defined bacteria as it was shown in the context of [0:42:27][inaudible], may potentially be a causative agent of inflammatory states. However, I would like highlight the fact that in most cases the capacity of the host to develop disease in the context of microbiota is likely depending on many of these factors and not only one. And it's going be rarely the case that one single microbe is going

to be causative of the disease, but it's mostly a combination between a genetic disposition, states of the host, the age, co-infection, or increase of bacteria.

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So I would just like to conclude by thanking the people who have done the work. The work on the skin was essentially done by Shruti Naik in the laboratory, and Nicolas Bouladoux. She had some nice help by all the other members of laboratory. They had a great collaboration by Julie Segre and Sean Conlan as well as Heidi Kong and Giorgio Trinchieri. And thank you for all your attention.

Sean Sanders: Great! Thank you so much to Belkaid. And thank you to both of our speakers for their very enlightening presentations. We're going move right on to questions submitted by our online viewers. Just a quick reminder to our live audience that you can still submit your questions, just click the "Ask a Question" button below the slide window. So I'm just going to pull up my first question, which is actually going to go to both of you, but Dr. Paliy maybe I'll give this to you first. And this viewer asks, or they say that a fair amount of microbiome work to date has been to characterize microbial populations. Do you foresee a future trend that might include more host expression and genotyping studies?

Dr. Oleg Paliy: Right. So this actually a very nice question. I agree, I agree with the statement of the viewer that indeed the last decade probably saw a large number of studies that looked particularly to answer the question: who is there and how much of different kinds of microbes, and are there any differences between a different kinds of diseases and health. I think now we are well-positioned with a basic amount of knowledge we have about it to move into more of interact studies and try to basically combine looking at the microbiotic composition, at the micro biotic functionality (you know, what kinds of genes they express), what functions do they perform. Then look at the metabolites, right, so what do they produce? Maybe it's a metabolite, it might be some kind of a marker or inflammatory response elements, and then look also at the expression. For example, we can look at the gene expression in the epithelial tissues in the gut and can look at, first of all, what happens in different diseases. And we can also with the dynamics types where we can say, well, if there are some changes to the gut environment, you know, in my case, what kind of changes do we see at the host gene expression level?

[0:45:11]

Sean Sanders: Excellent. Dr. Belkaid, anything to add?

Dr. Yasmine Belkaid: Yes, uhm, I think what I may add also is, in fact, it's a really exciting time. I think for the first time we're gonna have probably the development of very integrated research in which you gonna have community of geneticists, bacteriologists, immunologists, biochemists, and hopefully oncologists that will work together to try to really understand the association and the mechanistic association between defined microbes or community of microbes, and the immune system and inflammatory states. So I really think that we are moving in this direction. It's actually moving slowly because it's an extremely complex question, but this is gonna be probably the next decade of work. It's gonna be really the mechanistic understanding of crosstalk between this microbe and immune system and physiological state in general.

Sean Sanders: Good. Dr. Paliy I'm going come with you to a couple of questions specific to your presentation. Ah, the first is referring to the slide that you showed on the nodes of interaction. How was the interaction between the microbes measured?

Dr. Oleg Paliy: Okay. So they are talking about the network analysis, right, so when we looked at what are the potential associations among microbes. So all of this is a statistical approach based on abundances and so, in a way you can think of this as a concurrence of microbes. So in this particular case, right, when we talk just about the microbial interactions we are saying that because we only look at the abundances of two different genera across all samples. If genus A is high in one sample and genus B is also high in that sample that's potentially that they might be connected and if you look at all the samples and they always go up and down in their abundance, you know, in concert, you can say, well there seems to be a relationship among abundances of these members. Now, whether this always can be because they interact physically among them, we don't know that. This can be due to metabolic reasons—maybe they are both very good at the use of a particular metabolite, but this allows us to also potentially reveal some associations. And so one example that I can think of is member A of the gut can degrade a very complex polysaccharide, such as *Ruminococcus*, and release glucose into the environment. Member B, some other member of the gut can actually use that intermediate to grow itself. You can imagine that you might have a metabolic cross-seeding system where, when there's a lot of member A this will lead to an increase in the ability of that polysaccharide to be degraded to an intermediate, which then will allow member B to again increase their numbers, because now they have a lot more of the nutrients that they can utilize.

Sean Sanders: So can you correlate the 16S abundance with metabolite abundance?

Dr. Oleg Paliy: Right, and so that was the second part of the talk, when we looked at already at the proton NMR analysis, and so the second part where we looked at the correlations was done between metabolites and abundances of different genera and members of the genera. And in this case it's a very similar approach: we look at the amount of particular metabolites in among all samples and the abundance of a particular genus among all samples. If we find a correlation whether it's negative or positive, this can again tell us presumably about some associations between metabolites and that, you know, that microbe. It can be that microbe can utilize that metabolite, it can be that the microbe maybe is able to release the metabolite as an end-product of its metabolism. You know, there might be also cases where metabolites reduces the grow of particular microbes such as changing of pH, being somewhat toxic to a microbe, and so forth. And all of these studies are very high throughput. They give us, you know, a lot of hypotheses. So you cannot really do mechanistic analysis by looking at everything at the same time. But they allow you to generate some hypothesis and generate some potential links and then you can do more mechanistic studies where you can actually do, you know, in vitro experiments and ex vivo experiments and you can look at these potential interactions between metabolites and microbes, between different kinds of microbes of course. And your studies actually are being done… some of them are being done in my laboratory, and there are a lot of other researchers in the field who are also interested in these kinds of analyses.

Sean Sanders: Excellent. Dr. Belkaid, I'm going to come to you with the next question: How might broad spectrum antibiotic treatment effect the microbial composition in your subject?

Dr. Yasmine Belkaid: You mean in the skin?

Sean Sanders: Yes.

[0:50:00]

Dr. Yasmine Belkaid: So that's actually interesting. We tried and it was a bit surprising that when we utilize very, very broad antibiotics, some kind of combination antibiotic treatment this, had actually no impact on the skin microbiota. And that's an interesting observation because that suggests that all the shift of microbiota that we see usually when we have this treatment orally are really not affecting the skin microbiota that remain quite stable at least under all the treatments we have tested. Again, this could be different in the context of inflammation in

which the barrier may be actually more leaky and there is more accessibility of this antibiotic, but under steady states there is actually no crosstalk and the microbiota of the skin remain stable.

Dr. Oleg Paliy: If I may add to this, to the answer. So this is very interesting, because there are a number of studies of the gut microbiota that show quite a lot of differences in the gut environment and microbial communities when broad spectrum antibiotics are taken orally. And so obviously there is a difference because antibiotics start acting in the gut and so there is direct physical contact probably between antibiotics and the gut microbiota. But, you know, different research and different studies show that the effect might last up to six months after you take, you know, maybe antibiotics for a week or just a few days.

Sean Sanders: Good. Excellent. So the next question, I guess may take a little long to answer, but this is obviously a common issue with many experiments. So I'm going to give to you Dr. Paliy: this viewer asks, can you conclusively say that the results that you're seeing show cause or effect for IBS?

Dr. Oleg Paliy: Right. So in such work as this it's unlikely that we are able to make such a strong conclusion. And the reason for this is that most of the studies that we do and are possible to do on human subjects are what we call associative studies. You obviously cannot do experiments on humans, right. So, we are limited in only looking at already developed disease. So you cannot tell how the disease is being developed because usually at that point, you know, a person is not aware of any potential problems that can actually happen, you know, in their gut. So what we can do is that through the associative analysis, we can say, well, when you look at the gut of a person with IBS, we can definitely see that there are different microbes in that gut compared to a healthy person and we can see that they produce different kinds of metabolites. Now, what we can do to answer these questions is a different kind of analysis, which we actually do but I didn't have time to talk about here, and that's in vitro or animal model studies. So for example, we can use something which we call an in vitro gut simulator, and this is an in vitro system of linked fermentation vessels where we basically can culture a very complex fecal microbiota derived from humans. But because this is not inside a human host, we can actually make any kinds of changes that we would like to test in that system. We can add for example antibiotics, we can add toxins, we can remove maybe selectively some members, and we can also test how different types of communities can… what kind of metabolites they produce. So this allows us to see maybe how the IBS community, which we can culture in the system and which use and produce different kinds of these metabolites.. can we find maybe a way to correct that IBS community into more

of a healthy state by utilizing maybe such interesting, I guess, medicines as probiotics, which are beneficial, basically microbes that can be added to yogurt and, you know, many companies now make yogurt which is active microbes and so forth. So the tool that I described and methods that I described cannot really tell what is a cause and what is an effect. However, there are other ways to prove it and what I haven't talked about much is also animal studies, where you can again do more complex experiments. You can perturb the system and study responses of animals and microbes towards this kind of perturbation.

Sean Sanders: Dr. Belkaid, a question for you. This viewer asks about the use of germ-free mice and wonders how accurate the conclusions are that you can draw since this mice clearly have an underdeveloped immune system? Could you talk a little bit about that?

Dr. Yasmine Belkaid: Of course. So germ-free mice actually a poor principle, I mean, it's absolutely clear that them being born and raised in the complete absence of microbes is not a physiological state. And by no means could they be conclusive or the definitive experiment. However, they to date the only way to just transfer a defined and controlled microbial community and to perturb it, for example, as it was described before. So they have limitations like all experimental systems, but I think they may be to date the only tool we have to go from observation to causation.

[0:55:07]

Sean Sanders: Great. The other question I have for you Dr. Belkaid is: have you looked at whether there is any connection between the skin microbiota and the gut microbiota?

Dr. Yasmine Belkaid: So as I mention before in the normal condition in the absence of inflammation this sites appear to not talk to each other, but I really think one thing that could be fascinating to explore is how this is actually disrupted in the context of inflammation and how this compartmentalization of responses may be lost in the context of inflammatory state. One very intriguing genealogical observation is the fact that a large number of children that developed atopic dermatitis, for example, will develop asthma. So how much actually the crosstalk exist between the different niches that are colonized by different microbes and how much this early interaction with microbiota in the skin can predispose inflammatory states may be interesting. By at the moment under steady state conditions that we have looked at and local infection, the systems appear to be independent of each other.

Sean Sanders: Dr. Paliy, what role do you think viruses might play in regulating and modulating the microbiota?

Dr. Oleg Paliy: So this is a fascinating question and something which different researchers have started to look at. So the viriome or basically general community of viruses in the gut and bacteriophages is very complex and might be more diverse than actually a microbial community of bacterial and fungal species. It is quite obvious that they are going to play a significant role in modulating the abundances maybe of different organisms, the composition of the community. I'm not sure we know enough about it at this point to make conclusive statements. We can say, okay, we know that they can influence microbiota. How they influence it, again, due to the large complexity of microbial communities and viral communities this will require, you know, a lot of investigations and again, this is something which is very exciting for the next decade or maybe even more to start to learn [0:57:09][inaudible] mechanistic interactions and how things influence each other.

Sean Sanders: So, we're almost no time for this webinar so I'm going to squeeze one more question in for both of you. There seems to be an evolution in the studies from basic comparison of child or adult microbiomes to linking microbiome populations to specific medical conditions and both of you talked about that to some extent. So what do you each see as the next evolutionary step in your microbiome studies. Dr. Paliy, let's start with you.

Dr. Oleg Paliy: So I think one of the interests for us and to some other researches in this field is trying to see how can we move all of this information we're getting through into the clinic, right. Can we help patients which are diagnosed with different kinds of diseases and how can we do it. And that can offer two potential applications. One is more the diagnostic way. So what we've done for example, and I haven't shown this, is you can actually use these data which you derive about metabolites and microbiomes in a stool sample, and you can actually create a diagnostic statistical model, which can help a clinician in, you know, in a hospital figure out whether a particular disease is more likely to be classified as IBS, IBD, or something else. And so that might be a very important tool to add to the diagnostic tools that clinicians have now in the hospital setting. The second part is, we use what we call personalized medicine and rational design to try to find a way to treat people *specifically*, not just assign a single antibiotic to everyone who comes to hospital with a particular GI tract problem, but maybe develop more personalized mixtures of beneficial microbes, probiotics, that can be given

to each individual person based on the analysis of their gut microbes. And then you can say: I know where the problem is. There is one particular microbiome that is lacking or there is some misbalance. So we can design sort of personalized mixture of good microbes and then give these good microbes in terms of pill or power, or some other way, to the patient and hope that, you know, maybe we can actually change the gut community to resemble more a healthy state.

Sean Sanders: Fantastic. So, Dr. Belkaid, last word goes to you.

Dr. Yasmine Belkaid: So what I would actually like to add for that is the fact than this is actually the case that dysbiosis is going to be maybe in some cases causative of inflammation but in many cases it's going to be very, very contextual. And I think trying to understand mechanistically how gene dysregulation in the form of polymorphisms can actually influence the ways we're going to sense distant communities of microbes remain to be explored. And I think there is a huge amount of mechanistic work that remains to be addressed before we can really make definitive conclusion about causative association. So I think it is time for us to just pair with microbiologists and biochemists and all people that can actually help us addressing these kinds of questions.

Sean Sanders: Excellent. Well, unfortunately we are out of time for this webinar and we are going to have to end there. So in behalf of myself and our viewing audience I wanted to thank our speakers for being with us today, Dr. Oleg Paliy from Wright State University and Dr. Yasmine Belkaid from the National Institutes of Health. Please go to the URL now at the bottom of your slide viewer to learn more about resources related to today's discussion and look out for more webinars from *Science* available at webinar.sciencemag.org. This webinar will be made available to view again as on the month presentation within about 48 hours from now. We're interested to know what you thought of the webinar. Send us an email at the address now up in your slide viewer: webinar@aaas.org. Again, thank you so much to our fantastic speakers and to Affymetrix for their kind sponsorship of today's educational seminar. Goodbye.

[1:01:11] **End of Audio**