2010 ISAPP Meeting: Summaries of Sessions

Group 1. Where Pathogenesis and Commensalism Meet. Chair: Todd Klaenhammer, Co-chair: Colin Hill.

Whereas pathogens that cause infectious disease harbor uniquely distinct properties from commensals and probiotics, pathogens and commensals do meet in the gut and face a number of shared environmental challenges. These include survival through gastric juice and bile, competition with the existing microbiota, attachment/retention in the intestinal mucosa, interactions with the immune system, and impacts to health – positively for commensals and negatively for infective pathogens. As a result, the two groups share very similar strategies for survival and competition in this niche, such as pili, fimbriae, bile salt tolerance mechanisms, IgA proteases, IgA binding proteins, and oxidative stress genes. Because many of these factors were first discovered in pathogens, they are often referred to as virulence factors on the basis that inactivation usually impacts the virulence of the pathogen. However, it is not surprising that many innocuous commensal bacteria also share these features. With the application of more genome sequencing and high throughput technologies, it is likely that many genes encoding structures or strategies previously associated with 'virulence' will be identified in commensal bacteria. It is important that a clear distinction is retained between true 'virulence factors' (pathogen specific factors such as toxins which damage the host, or internalins which facilitate entry into host cells) and those shared survival and colonization strategies employed by all gut-associated bacteria. This discussion group proposed that a more accurate description of these shared structures or strategies would be "survival, tolerance, or competition" factors.

Group 2. Gut Microbiota and Disease. Chair: Francisco Guarner, Co-chair: James Versalovic.

Presentations and discussion in group 2 aimed at describing characteristics of a "normal" gut microbiota in terms of structure and functions, i.e. microbial composition and activities that are considered to be commonly present in human subjects. A second aim of the group was to review clinical conditions associated with dysbiosis, i.e. associated with abnormal characteristics of the gut microbiota. This double approach was considered as the practical way of gathering the relevant information in order to eventually define a "healthy" gut microbiota. The group included a number of scientists actively involved in projects from the International Human Microbiome Consortium (IHMC, www.human-microbiome.org). These projects are currently going on in the US, Europe and China. The MetaHIt study suggests that up to 3-4 million microbial genes and about 20,000 functions encoded by these genes are present in the human gut microbiota (Qin et al. 2010). The NIH Human Microbiome Projects have investigated in depth samples from healthy individuals. These studies have clearly detected age related differences between healthy children and adults. Data from different studies on IBS, IBD, type II diabetes and obesity are providing information about consistent changes in gut microbiota composition. This information can be applied to rational remodeling or "tailoring" of human-associated microbial communities and their associated functions (Preidis and Versalovic. 2009). The group concluded that it is still too early to define the structure of a "healthy" gut microbiota. However, markers associated with

disease (microbial signatures) are expected to be available soon. These markers may eventually be useful as diagnostic tools. Prospective studies will be needed to provide information about cause-effect relationships.

- Qin J, Li R, Raes J, et al. 2010. A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464(7285):59-65.
- Preidis GA, Versalovic J. 2009. Targeting the human microbiome with antibiotics, probiotics, and prebiotics: gastroenterology enters the metagenomics era. Gastroenterology 136(6):2015-31.

Group 3: Bioactives, the Grand Canyon of our Field. Chairs: Nathalie Delzenne and Glenn Gibson

Each group participant was invited to choose a topic related to bioactivity of probiotics and prebiotics.

Galactoglucomannan oligosaccharide [GGMO]. This is an oligosaccharide that has demonstrated functional properties in animal foods. This new candidate prebiotic is isolated from waste materials. The unique galactoglucomannan oligosaccharides may play a role in digestive health and immune function. Animal and in vitro models have been used for testing temulose. The GGMO is a promising prebiotic as based on current evidence. Human trials are to be considered for the future.

Bacteriocin from Lactobacillus salivarius UCC118: The bacteriocin-producing probiotic *Lactobacillus salivarius* UCC118 alters the composition of the gut microbiota in diet-induced obese mice. Research has focused on (i) understanding the links between diet, gut health, inflammation and metabolic function and (ii) the identification of food components that impact the development of obesity and associated metabolic abnormalities and the underlying mechanisms by which these effects occur. UCC118 reduced Actinobacteria but increased Bacteroidetes in a model with diet induced obesity.

Gnotobiotic mouse models. This presentation addressed the role of the gut microbiota in host physiology and metabolism using gnotobiotic mouse models. Research is especially focused on the mechanisms by which the gut microbiota contributes to the pathogenesis of obesity, insulin resistance, diabetes and atherosclerosis. Mice kept under germ-free conditions have reduced adiposity compared with colonized mice. Lipidomics demonstrated that the gut microbiota had global effects on the host's lipid metabolism, characterized by increased hepatic and adipose triglyceride levels.

Butyrate. Butyrate is an important short chain fatty acid metabolite produced during anaerobic fermentation by gut bacteria, which helps maintain a healthy gut. Butyrate is the preferred energy source for gut epithelial cells, and induces apoptosis of cancer cells. Butyrate production also helps to maintain a slightly acidic colonic pH, thereby assisting in pathogen exclusion. Some of the more abundant gut bacteria produce butyrate, including *Faecalibacterium prausnitzii* and *Roseburia* spp. Other bacteria synthesise butyrate from other bacterial metabolites including lactate. Production of butyrate is substrate-dependent, and growth on starch and fructo-oligosaccharides (prebiotics) results in butyrate production.

Bile acid signatures. This research explored the influence of the gut microbiota on the bile acid signatures of host tissue compartments and the potential for the gut microbiota to modulate the signalling capacity of these trans-genomic metabolites. This work utilised a targeted High pressure liquid

chromatography-mass spectroscopy approach to characterise the bile acid signatures in the liver, kidney, heart and plasma of conventional, germ-free, and antibiotic-treated rats. In addition, work explored the impact of binge-drinking on cognition in humans. The aim of this study was to screen a wide range of urinary metabolites using a 1H nuclear magnetic resonance (NMR) spectroscopy-based metabonomic approach to establish the relationship between metabolic effects of alcohol consumption and cognitive impairment. The initial phase of this study has demonstrated an association between urinary markers and specific forms of cognitive impairment (spatial working memory). These markers are also linked to gut microbial metabolism.

Gut flora and metabolic syndrome. The field of interest is the role of the gut microbiota in the development of metabolic disorders, such as obesity, type 2 diabetes and low grade inflammation. Research covers the fundamental mechanistic aspects of the host-gut microbes interactions, as well as the impact of nutritional modulation of the gut microbiota by using pre- and probiotics. Special attention is given to the role of the endocannabinoid system and its impact on the control of gut barrier function and adipogenesis. Future strategies on the role of probiotics and prebiotics in metabolic syndrome were discussed.

Immunomodulation. The aim is to isolate and identify the effector molecule produced by *L. reuteri* that is capable of inhibiting TNF production from activated human myeloid cells. This has been done using a combination of mass spectroscopy and NMR techniques. The second objective is to understand the mechanism of probiotic-mediated TNF suppression.

Phenylpropanoid compounds. Current research focuses on phenylpropanoid-derived compounds in the diet that are released and transformed by the colonic microbiota to form anti-inflammatory metabolites. Particular compounds of interest are ferulic acid and its derivatives. These compounds undergo de-esterification hydrogenation, demethylation and dehydroxylation by the gut bacteria. Species responsible for these molecular transformations are being described with a view to development as potential probiotics.

Gut flora and xenobiotics. Research focuses on the metabolic interactions between the gut microbiota and its host metabolism, with a particular interest on the liver and the brain. The complex relationships between drugs, drug metabolism and the gut flora was described and the effect of dietary modulation. In this context, antibiotics as a means to modulate the host metabolism through alteration of the microflora and xenobiotic metabolism may lead to new research on pro and prebiotics.

Fatty acids and the gut. The focus was on the interactions between fatty acids and commensals in the gastrointestinal tract. This interaction between administered microbes and fatty acids could result in a highly effective nutritional approach to the therapy of a variety of inflammatory and neurodegenerative conditions. For the specific case of conjugated linoleic acid, its anti-proliferation effect was described. Species of bifidobacteria may produce conjugated linoleic acid at varying levels.

Immunoglobulins. Urinary metabolic and mucosal immunoglobulin responses of the pig to nutritional intervention around the weaning period indicated that *B. lactis* has a differential effect on both of these parameter sets depending on the initial weaning diet, even after a dietary washout period.

Antiadhesive activities of prebiotics. Prebiotics are generally thought of as fermentation substrates, manipulating the microbiota composition and activity. Oligosaccharides can, however, act as antiadhesive agents, preventing pathogens from binding to host cell receptors. These oligosaccharides are being developed as an approach to therapy, typically involving complex multivalent derivatives. There is, however, accumulating evidence that galacto-oligosaccharides also have the ability to prevent pathogens from binding to cells, although the evidence for an effect *in vivo* is currently lacking. This type of activity could, however, be a feature in design of future prebiotic oligosaccharides.

The group concluded with general discussion to ascertain whether bioactives were capable of inducing similar health benefits to probiotics and prebiotics and/or explaining mechanisms of effect. The question was raised as to whether bioactive compounds could be a new market with nutrition/medical applications.

Group 4: Probiotics and Prebiotics in Perinatal Nutrition. Chair: Michael Cabana; Co-chair: David Mills

This group discussed the increasing use of probiotics and prebiotics by infants and young children. One effect of these dietary agents is changes in the infant microbiota. Attempts to define the 'normal' or 'typical' infant microbiota, metagenome and metabolome were discussed. There are many confounding factors to these efforts, such as mode of delivery, antibiotic exposure, breastmilk exposure and gestational age. In addition, there are no accepted standards for stool collection. Stool itself may be an imperfect representation of the microbiota; however, given the limitations of current technology, it would not be ethical to use more invasive methods in well infants. Definition of the infant microbiota may allow us to find correlations with infant disease states, as well as develop biomarkers for clinical trials. Experiences were shared among group participants on attempts to manipulate the gut microbiota by probiotics and prebiotics. In addition, human breast milk as a delivery agent for prebiotics and microbes was discussed. The potential of adding human milk oligosaccharide mimics to infant formula was also discussed. In addition, the potential long-term impact of infant formula products supplemented by probiotics and prebiotics on long-term infant health was considered.

Group 5. Health Benefit Claims for Probiotic and Prebiotic Products. Chair: Mary Ellen Sanders, Co-Chair: Seppo Salminen.

This group discussion opened with brief descriptions of differences among regulatory frameworks for probiotics in the United States, Canada, Europe, China and India. All these geographical regions had in common the principle of consumer protection, although the means to this end varied. There was agreement that health claims should be substantiated by generally accepted scientific evidence, taking into account the totality of the available scientific data, and weighing this evidence to determine the strength of the support.

Of note, although both the US and Europe allow disease risk reduction claims, in the US foods have not been allowed to be used to reduce the risk of acute diseases, such as colds or flu, but in Europe this is in theory possible, if evidence is provided. India is in the process of developing specific probiotic guidelines and it is still possible to provide scientific input into this process.

Discussion about the wording of health claims emphasized that this is a very difficult endeavor. It often entails translating complicated scientific findings into claims that can be understood accurately by average consumers. Companies must first define what the most important message is, and then use wording that is simple, not vague, confusing or misleading and that accurately reflects the strength of the scientific evidence.

Another issue that was of concern regarding developing substantiation for claims is this issue of biomarkers. In Europe, this has a special importance, as the regulation specifically indicates that a change in a risk factor must be established in addition to convincing evidence directly on the endpoint, if needed. However, in the field of probiotics and prebiotics, few valid biomarkers are available for the types of endpoints these substances target. Furthermore, considering that regulators are focused on the role of foods in health, there is a great need for valid approaches for evaluating health instead of disease. Biomarkers would also be very useful for identifying subpopulations of responders and non-responders, to increase the focus of human studies.

Important themes that emerged during this discussion:

- Regarding the difficulty in measuring health, the focus of studies could be in measurement of homeostasis. From a statistical point of view, if a study were able to minimize the variation around the mean for a specific measure (even in the absence of changing the mean), it could be a reflection of improved health, assuming a biological rationale exists that tighter control of the parameter is physiologically advantageous. In other words, lessening the fluctuation around an individual's biomarker could be interpreted as contributing to improving health. This novel idea emphasizes the importance of homeostasis as a focus of studies on health, and provides a rationale based in solid statistical theory as a way to measure this.
- Another issue that emerged was the frustration about regulatory "boxes." Although scientists would agree that there is a continuum between health and disease, in regulatory terms these are distinct states. Likewise, there are numerous examples of foods having pharma-like properties, such as reducing the risk of acute infections. However, some regulatory authorities see such actions as only valid for "drugs." The consequences of such constraints can be significant for scientists and the studies they design, for consumers and how they might benefit from certain products.

Group 6. Probiotics and Prebiotics to Combat Enteric Diarrheal Diseases and HIV in the Developing World. Chair: Gregor Reid, Co-Chair: Dan Merenstein.

This discussion group comprised experts from the developing world, others who had worked on projects there, and participants committed to helping with this major morbidity and mortality problem. All were positive about the evidence to date and potential for probiotics and prebiotics to help lower the burden of disease and suffering in a cost effective manner that could engage and empower local people in

developing countries such as Tanzania, Kenya, Rwanda, South Africa, Ghana, India, Bangladesh, Pakistan, China, Brazil and Peru. This group aimed to build upon ISAPP's commitment to consider issues in developing countries and attempt to bring probiotic and prebiotic concepts and products to these regions. Furthermore, the group was interested in a follow-on discussion from the Gates Foundation meeting in London, UK (January 2010), which concluded that no funding was yet warranted for use of probiotics for diarrhea in developing countries.

The group addressed the following issues:

1. Determine the scientific rationale for using probiotics and/or prebiotics to combat diarrheal diseases and/or HIV/AIDS in the developing world.
2. Write practical recommendations for prebiotics and probiotics on issues such as choice of: probiotic strains, prebiotic, dosages, appropriate target diseases, test populations. Other issues to address include how to deliver the products in challenging settings, how to offset the cost of the treatment and use of unique, effective models.

With an eye to the issues stated above, there were three objectives for the session:

1. Try to set up collaborations leading to projects in the developing world.
2. Write a roadmap for how to bring probiotics to a developing country, even if only for research and not for profit.
3. Create project ideas that can identify plausible mechanisms whereby "biotics" combat diarrheal diseases and HIV.

Studies were proposed that would address the impact of pro/prebiotics on HIV/AIDS or on diarrheal diseases. Specific issues of best measurement endpoints, study population, confounders and other issues were considered.

Recommendations were made on how to facilitate progress in this area.

1. Products calling themselves 'probiotic' are slowly becoming available in developing countries, but the majority have never been clinically tested and shown to confer a health benefit. This is a major failing of companies and government agencies. Either products should be appropriately tested or the word 'probiotic' removed from the labels.

2. The group discussed studies showing that probiotics and prebiotics could provide viable tools for management of diarrheal diseases and HIV/AIDS.

3. Funding for research and young scientists is a major problem in the developing world. The suggestion was made that ISAPP could solicit funds from its Industry Advisory Committee members to set up a program to provide funding (~$40,000-50,000) for:

(i) Two studies per annum for developing world scientists, possibly in collaboration with ISAPP-selected scientists from developed countries, targeting this research area.

(ii) Fund an African junior faculty member, to enable him/her to spend 1 year working in an established program to learn methodologies, grant writing, collaboration skills, and how to

conduct independent research. This person would be selected by his/her department, with the stipulation that the department would provide ongoing research support.