2011 ISAPP Meeting: Summaries of Sessions

Group 1: Bioactives 2. George Fahey

As a follow-up to a discussion of "Bioactives" at ISAPP 2010, a second round of discussions on this same topic occurred at ISAPP 2011. **Bacteriocins** have application in important areas such as food quality, food safety, veterinary medicine, and human medicine. Bacteriocins can be thought of as "colonising peptides", "killing peptides", and "signalling peptides" that may affect the gut lumen, the large bowel microbiota, the intestinal epithelium, and (or) the intestinal immune cells. **Other peptides** are found in milk and certain of these have ACE inhibitory activity in probiotic fermented milk. In the area of **prebiotics**, it was shown that **short-chain and long-chain fructooligosaccharides (FOS)** have somewhat different potential in reducing intestinal inflammation in HLA-B27 transgenic rats (with the short-chain FOS resulting in a lower histology score and a lower concentration of IL 1-beta). The Bacteroides group, the Bifidobacterium spp. group, and the Clostridium cluster XI group were most impacted by FOS supplementation of these rats. In a human clinical trial, positive outcomes resulted from feeding FOS included the microbiota profile and the production and uptake of butyrate. **Isomaltooligosaccharides (IMO)** were shown to positively impact beneficial gut microbiota but were poorly metabolized by potential pathogens. Alpha-1,2 glycosidic branching was believed to be important in positively impacting the gut microbiota and the short-chain fatty acids produced as a result of IMO fermentability. Vaccination is the gold standard for evaluating the effect of interventions on **immune function.** A double-blind, placebo controlled randomized study is underway to determine the effects of **prebiotics, probiotics, and synbiotics** on the immune response to influenza vaccination and fecal microbiota concentrations in healthy adults. A second study involved use of **galactooligosaccharides,** five grams per day of which resulted in a 40% reduction in percentage of days with cold and flu for those college students with a healthy body mass index (BMI; 64% of participants had a BMI between 18.5-24.9). **Polyphenolics** and their metabolities have antioxidant, anti-microbial, anti-inflammatory, and potential **prebiotic** properties, and research is underway to determine those with the greatest potential. **Fibrous carbohydrates** in select fruits also are being evaluated for their ability to modify the colonic ecosystem vis-a-vis the microbiota composition and end-product formation. Finally, the **industry scientists** in Discussion Group 1 identified several issues important to this general area of science:

(a) Better define "bioactive" as it relates to **prebiotics** and **probiotics** (relate "bioactivity" to the mechanism of action of a **prebiotic** or **probiotic**).

(b) Better educate and communicate the science of **prebiotics** and **probiotic**s, especially to health care professionals.

(c) Work on overcoming the conflict in global regulations related to **prebiotics** and **probiotics** that is making claims more difficult.

(d) Include more "quality of life" assessments in future human studies of **prebiotics** and **probiotics** since consumers are seeking "feel the difference" outcomes.

(e) Identify the specific gut microbial populations associated with health outcomes.

(f) Work towards a complete understanding of the mechanism of butyrate action.

Group 2. Guidelines for Safety Evaluations Regarding the Addition of Live Microorganisms in Food. Jim Heimbach.

Group 2 developed a decision-tree model to provide guidance in meeting regulatory requirements for assuring that probiotics intended for addition to foods or dietary supplements are safe. The model includes recommendations regarding confirmation of the identity of the strain and genomic analysis. Based on the findings of the genomic analysis, history of human exposure to the strain, membership in a species accepted as possessing status of Qualified Presumption of Safety (QPS), presence of potentially transferable antibiotic resistance, and intended use (particularly with regard to target populations), different pathways to safety determination are specified requiring greater or lesser levels of additional research. Additionally, the guidance addresses issues regarding the selection of animal models and the design of human studies that provide evidence of safety as either primary or secondary endpoints. The group intends to further develop this guidance, including a greater level of specification than was possible during a one-day meeting, and incorporation of Bayesian mathematical principles to aid in formalizing and lending a higher degree of objectivity to defining the evidentiary burden on the basis of the level of presumption of safety available a priori. The goal is to publish this guidance in a peer-reviewed scientific journal.

Group 3. Culturing the unculturable, or as was decided during the discussion, '**Culturing the not-yet cultured'. Karen Scott.** The discussion started off with some updates on the current knowledge on the microbial composition within the human gastrointestinal tract (GIT), and the changes that occur through life, from infancy through adulthood to old age. Differences between breast-fed and formula-fed babies were recognised, and attributed at least in part to differences in the oligosaccharide content of the milk. The clear succession of colonisation by different bacterial genera can be established through work with gnotobiotic animals. The stability of the microbiota was also debated, with clear evidence that diet-induced changes do occur. Changes that can be associated with the development of disease were also debated, and the difficulties in deciding if the microbial changes are the cause or consequence of the disease.

Despite the fact that new molecular tools have been developed within the last decade that mean that we now know much more about the diversity present within the Human GIT, there have been no similar advances in our ability to culture the obligately anaerobic bacteria that are the most abundant and active residents in the large intestine. In fact it became clear that the most successful methods are still

those that were developed in the 1960's for culturing anaerobic bacteria from the rumen, namely the Hungate technique using a rumen-fluid based medium, with pure CO_2 in tubes, which can be supplemented by the use of anaerobic cabinets. This is at least partly because relatively few labs in the world have the appropriate facilities and know-how, and are actually involved in culturing new anaerobic gut bacteria. However, comparing the prevalence of different bacterial species (assessed using molecular techniques) with the identities of bacteria that have been cultured, there are actually cultured representatives of most of the abundant bacteria, and it is the more diverse, less numerous groups for which cultured isolates are lacking. In fact based on metagenomic and phylogenetic data the Human Microbiome Project have created a list of the '100-most wanted' bacterial isolates, which correspond to sequences frequently encountered in (meta)genomic libraries but for which there are no sequenced, cultured representatives. The merits of using novel new technologies to facilitate culturing these low abundance bacteria were discussed, including encapsulation prior to growth, as well as more traditional options including enrichment cultures. The latter could be helped by utilising metagenome data to identify key growth requirements for some of these hard-to-culture isolates. Other 'omic technologies could also help in identifying important bacterial activities, and metabolic pathways. It is also likely that some pairs of bacterial species live in such close symbiosis, that it will be extremely hard, and may even be impossible, to separate them. The difficulties of getting such bacteria identified as new species were debated. Culturing techniques frequently do not mimic conditions in the large intestine, where there may be little food and bacterial multiplication times are low, and bacteria live as part of microbial communities often in biofilms.

Further discussion points focussed on the bacterial interactions that determine overall bacterial activities in the GIT, and the relative pros and cons of focussing on single strain probiotics, or developing multiple strain ecobiotics (defined bacterial mixtures containing abundant commensal bacterial groups). The latter approach is a more controlled version of faecal transplants, which have had considerable success in treating patients with eg. recurrent *C. difficile* associated diarrhoea. However regulatory issues are currently a problem in this area, and there clearly have to be informed discussions between scientists, clinicians and regulators to reach a satisfactory conclusion.

The main outcome of the discussion was the optimistic message that the group did not believe that gut bacteria were actually unculturable, but rather that we had to try harder to define selective media, and methods to reach the low abundance bacteria, some of which could have important metabolic activities. There was enthusiasm amongst the scientists that it was worth it to try and culture these bacteria, and from industry that any potential novel probiotic bacteria that were isolated could be taken forward to the market place.

10 scientific experts and 5 industry representatives contributed to the lively, interactive discussion.

Group 4. Signaling processes interconnecting microbes and host immune cells

Our discussion section at the ISAPP 2011 annual meeting entitled Signaling Processes Interconnecting Microbes and Host Immune Cells brought together experts in from diverse backgrounds. Some of the

recognized outstanding questions in this area that we articulated were: A) What microbial genes, structures and metabolites are altering the immune system? B) Is it specific members of the microbiota or the emergent properties of the whole community that impact the immune system? C) What tools do we need to measure the impact of the gut microbiota on T cell development or other markers of adaptive immunity?

Our first group of speakers discussed microbial and dietary factors that can signal to the innate and adaptive immune system. Federico Rey, presented work in a simplified gnotobiotic model of the gut microbiota, containing *Eubacterium rectale*, a known butyrate producer and *Bacteroides thetaiotaomicron*, a microbe that is known for the diversity of plant and host polysaccharides that it can digest. In combination this pair resulted in significant increased expression of Mcp-1, and importer of butyrate as 500+ host genes (compared to a mere 5-11 when either microbe colonized by themselves). The theme of short chain fatty acid signaling to the host was carried further by Nathalie Delzenne presenting data in the iflammation based model of metabolic disease. In this model, prebiotics directly impact adipocyte size and adiposity through a GPR43 SCFA mediated signaling pathway. Together these results demonstrate that the output of the microbial community is a key influence on the host and with the prebiotic approach, suggest that the community behaves in a way that reflects the impact of selective growth of some organisms, in this case likely Bifidobacterium.

Susan Lynch then further described an experiment where the addition of a single organism to the microbiota (in this case *L. rhamnosis*), had a significant impact of the total microbial community, with 361 taxa changing significantly. She introduced the concept of “keystone” species to the discussion, where on or a few microbes impact the total composition and emergent properties of the community as a whole. To follow up on how Lactobaccilus may be influencing the microbial community, Maria Marco presented microbial genetic data that has identified a number of *L. plantarum* genes, particularly bacteriocins that could influence the immune system, perhaps directly or indirectly through changing the microbiota.

Wendy Garret presented a model of colitis that happens in the abcence of T and B cells when there is Tbet deficiency. Examining the microbiota in these mice identified a number of changes, including an erichment for Klebsiella and a loss of microbes including Bifidobacterium. Interestingly, when these mice are fed a diet with fermented milk containing B. lactis and other Bifidobacterium and Lactobacillus species there is a dramatic improvement in histological colitis scores in these mice. Notably, cecal pH is much lower in these mice, yet the SCFA that are increased were not the lactic acid that you would expect from the milk fermenting microbes, but acetate, propionate and butyrate. This reinforced the concept of keystone microbes, impacting the emergent properties of the micrbiota through changing both the composition as well as the output of the community as a whole.

As we moved the discussion towards the adaptive immune system, Keichiro Suzuki, presented work on the follicular dendritic cells (FDC) of Peyer’s patches of the gut, that are strong inducers of IgA class switching and production in the gut through their interaction with B cells. Using a gene-chip approach he discovered that the pathway that creates these specialized IgA inducing cells requires both an innate signal through TLR signaling, but also a dietary factor, vitamin A/ retinoic acid, there together these

signals drive the FDC to develop into gut FDC. Likewise he demonstrated that IgA is also crucial for controlling the microbiota, as he had previously demonstrated that mice without IgA, had dramatic shifts in the microbiota, specifically with the expansion of SFB (segmented filamentous bacteria).

Ivo Ivanov, demonstrate how the SFB, a single organism, could have a dramatic impact on the immune system, being single handedly responsible for the presence of Il-17 producing CD4 cells in the colon of SFB positive mice. This single organism can provide non-specific protection from pathogens and can promote autoimmunity both. A true double edged sword. His dramatic electron micrographs visualized for all of us the intimate relationship that exists between host and microbe and it is easy to see how this bacterium poking into the epithelia can really make things happen. He reported to recent results of sequening the genome, which reveals what appears to be genome reduction, which may be the consequence of moving from the competitive colonic environment into the small intestine or the parasitic relationship it may have evolved to become dependent on host metabolism as has been seen in other organisms like H. pylori.

While the specificity of Th17 cells induced by SFB has not been measured, Yingzi Cong provided a great story of cBir1 specific T cells that recognize a common flagellar antigen. An interesting antigen that contains both the T cell epitope, but is also a TLR-5 ligand, that can drive activation of the innate and adaptive immune response. These T cells (cBir1 specific can induce colitis only when the mice have the correct microbiota present in the GIT. Finally Chyi Hsieh described his new model of examining gut microbe reactive T cells. Using a DNA sequencing approach to identify T cell receptors from the colon (in T regulatory cells), he tranfered both specificity to gut microbes to hybridomas in vitro, but also demonstrated that these same Tcells when activated and put into mice can induce colitis.

The presentation of Ivanov, Cong, Suzuki and Hsieh, demonstrated that the gut microbiota can have an enormous impact on T cells in both homeostasis and disease. These models show new and innovative ways to address the non-specific signaling and antigen specific adaptive immunity to gut microbes. These presentations demonstrated the change in mucosal immunology over the last 7-8 years moving from anonymous microbes and undefined specificity in the T and B cells, to modern approaches that define both of these through advances in immunology and non-culture based analysis of the gut microbiome.

The discussion in this session focused in part on the exciting questions that now can be addressed, and those that may appear to be too big to tackle even with today technology. Defining where knowledge today stands is a difficult process given the cacophony of results that emerge from the diverse areas that affect mucosal immune signaling; nutrition, microbiology and immunology being 3 of these major fields. Two major questions emerged from the discussion: A) How do we define specific and non-specific immune systems responses to diet, probiotic and prebiotic studies, when we don't have the tools to measure specific and non-specific immune cell responses. B) How do we separate the impact of individual microbes that are acting directly or indirectly as "Keystone" species. With these as a guide, we agreed that the future is bright and full of opportunity to pursue these questions.

Group 5. Importance of 'beneficial' microbes in vaginal health. Gregor Reid

Urogenital diseases, especially infection and cancer, are major causes of death and morbidity in females. Yet, millions of women in the developing world have no access to basic urogynecological care, and diagnosis and treatment of widespread aberrant bacterial conditions (bacterial vaginosis (BV) and aerobic vaginitis (AV)) remain sub-optimal the world over. High throughput sequencing is revealing the diversity of bacteria in the vagina and how they fluctuate over time, and switch between healthy and aberrant conditions. Unfortunately, diagnostic methods are inefficient and too often outdated therapies are incorrectly administered. The net result is sub-optimal care and recurrent disease that adversely effects quality of life. This viewpoint outlines a scientific and translational roadmap designed to improve cervico-vaginal health and treatment of disease. This comprises (1) improving education of women and physicians on the vaginal microbiota; (2) having agencies target funding for research to improve diagnosis and test new therapies; and (3) making sure that new approaches are accessible in developing countries, empowering to women, as well as being acceptable and appropriate for different populations.

Group 6. Probiotics and Prebiotics in Neurogastroenterology. Francisco Guarner

Brain-gut axis allows bi-directional input and thus links emotional and cognitive centers of the brain with peripheral functioning of the bowel, and vice versa, signals arising from the gut can influence brain centers. Recent experimental work suggested that the enteric microbiota may have an impact on the brain-gut axis. Thus, the ability of gut microbiota to communicate with the brain and influence behavior is emerging as an exciting concept.

A group of experts convened by ISAPP discussed around the role of gut bacteria on brain functions and the implications for probiotic and prebiotic science. The experts presented data and discussed topics such as the role of microbia on epithelial cell function, motor bowel function, visceral sensitivity, perception, and behavior. The data suggest interaction of gut microbia not only with the enteric nervous system but also with the central nervous system, either via neural, neuro-endocrine or humoral links. Experimental work indicates that colonization by the gut microbiota impacts mammalian brain development and subsequent adult behavior. In mice, the presence or absence of conventional microbiota influences behavior, and is accompanied by neurochemical changes in the brain (Neufeld et al, 2011). Germ-free mice have increased locomotor activity and reduced anxiety, and this behavioral phenotype is associated with altered expression of critical genes in brain regions implicated in motor control and anxiety-like behavior. It has been shown that some behavioral characteristics of mice are linked to the strain they belong. Interestingly, when germ-free mice are reconstituted with a microbiota from mice belonging to another strain, they display similar behavioral characteristics as the donor mice strain (Bercik et al, 2011). Microbial transfer was also associated with changes in brain chemistry. Thus, experimental work clearly shows that the enteric microbiota can affect brain function.

These findings provide novel insights for a better understanding of the potential role of gut microbial communities on psychiatric disorders, most particularly in the field of psychiatric co-morbidities

associated with functional bowel disorders like the irritable bowel syndrome (IBS). Studies with probiotics and prebiotics have already shown promising results for alleviating IBS symptoms.

Experts in the meeting concluded that a better knowledge on the gut microbiota structure and function should provide windows of opportunity for interventions (probiotics and prebiotics) in order to produce beneficial effects on brain development, bowel function, abdominal well-being and behaviour, in the future. Translational studies are needed. It was emphasized the common defects of human intervention studies are the lack of definition of phenotypes in sample population in an area with wide heterogeneity, the limitation of current tools (mainly questionnaire-based, lack of biomarkers), and the deficient collection of metadata sets (diet, natural environment, stressors, etc.)

Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil. 2011 Mar;23(3):255-64.

Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, Verdu EF, Collins SM. The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology. 2011 Aug;141(2):599-609.

IAC/BOD meeting. Arthur Ouwehand

During the BOD/IAC meeting the following questions were discussed in five groups:

a) Are faecal bifidobacteria levels a marker for health?

b) Why are faecal bifidobacteria (not) a marker for health?

Three of the groups answered the first question with an unambiguous 'no'. One of the groups argued it might be a marker for 'likelihood of health,' while one group noted that different perspectives exist among different parties. The regulatory views differ among countries (FDA and EFSA 'no', Health Canada 'yes') and the scientific view differs among experts. This group indicated that yes, from a scientific view, bifidobacteria could be considered a biomarker for health, but causality remained to be established. As with all biomarkers, correlation is not 100%.

As for the second question; there was unanimity in the groups that there is not enough evidence on causality. To achieve this, long-term longitudinal studies would be needed.