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MICROBES AND MAMMALIAN BIOLOGY – A SUMMARY OF OLD HERBORN UNIVERSITY SEMINAR 35

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INTRODUCTION – MAMMAL AS HOST AND BENEFICIARY

Our annual tradition of the Old Herborn University Seminar (OHUS) series highlighted our microbial world yet again in 2023. In this latest seminar, our emphasis was on the mammalian host, in contrast to plants as hosts in OHUS 33. In OHUS 35, our aim was to explore the bidirectional interplay of mammals and microbes, while considering mammalian species as both hosts for diverse microbes and as beneficiaries of microbes in their roles as messengers and catalysts. In the broader context of mammalian physiology and systems biology, it is striking how profoundly mammal-associated microbes impact early life development, local and systemic immunity, and the communication stream between the gut and the central nervous system.

Evidence supports exposure to microbes and/or microbial metabolites during foetal development and immediately after birth. During the neonatal period, infancy and early life, the microbial ecosystem fluctuates and expands gradually in terms of microbial composition and function. The intestinal microbiome fluctuates dramatically during infancy and the first 3 years of human life, and it continues to evolve and change during childhood. We know that the human microbiome reaches a relatively steady-state or equilibrium by early adulthood, but these microbial communities continue to change or shift during adulthood depending on the influences of diet, medications, hygiene and a diverse array of xenobiotic compounds in the environment (built or natural). Microbes are intimately associated with different body sites throughout the mammalian lifespan, and ongoing shifts in microbial composition and function may contribute to the nature of the aging process.

In addition to changes over time during an animal's lifespan, we must consider fluctuations in microbial composition and function at different body sites (in space) and whether microbes send signals extending beyond local targets to remote locations. Shifts in microbial ecosystems at specific body sites such as the oral cavity may impact the placenta via the bloodstream. Another example prominently displayed at OHUS 35 was the nature of communication between the intestinal microbiome and the brain via the peripheral / central nervous system. This microbiome-gut-brain axis highlights the importance of microbe-generated signals affecting remote targets in mammalian systems via bidirectional communication.

GROWTH, DEVELOPMENT AND INFECTIONS

Kjersti Aagaard and colleagues described the importance of maternal-foetal communication during early mammalian development. The "Developmental Origins of Health and Disease" hypothesis (*Barker*, 1986) links maternal exposures during pregnancy to adverse outcomes later in life for offspring. The mammalian microbiome

serves as a mediator and messenger conveying signals from maternal exposures such as diet with lasting effects on foetal and early postnatal life development. In nonhuman primates, a high fat maternal diet during pregnancy has lasting effects on microbial composition and function after birth (*Ma* et al., 2014; *Bolte* et al., 2022). The effects of the high fat diet on the gut microbiome seem to be dependent on the diet quality, and not the number of calories. Another example in humans of the potentially profound impact of dietary compounds is the example of the sugar alcohol, xylitol, and its application to prevention of preterm birth in humans (*Aagaard* et al., 2022). Xylitol in chewing gum may be used preferentially as a carbohydrate source by pathogens in the oral microbiome, thereby suppressing proliferation of pathogenic streptococci and oral inflammation (e.g., periodontitis). Suppression of oral inflammation may reduce the production of inflammatory cytokines and lower the risk of preterm birth perhaps by suppression of systemic inflammation. Martin Schwarzer cited studies by Gehrig and colleagues (*Gehrig* et al., 2019) showing that changes in therapeutic diets could result in increased abundances of growth-promoting bacterial taxa in juvenile mice, piglets and children. These findings highlight the potential importance of diet as a strategy to shift the biology of the microbiome in a more favourable direction for the mammalian host.

Microbial communities at different body sites may have cumulative effects on biological processes and different remote effects dependent on resident microbes and microbial mediators. Shifts in the oral microbiome may yield remote effects on the placenta and the foetus. We've come to appreciate the presence of low biomass microbial communities in body sites such as the placenta and the vagina, and in different body fluids such as amniotic fluid during pregnancy. We know that pathogenic microbes within the oral microbiome can spread via the bloodstream to infect remote sites such the placenta or the heart, so we cannot discount the possibility of bacterial colonization in one body site affecting another. Within amniotic fluid, the combination of human milk oligosaccharides and bacterial composition might affect the likelihood of preterm birth or timing of labour and delivery. Priming of the immune system and foetal immune development may be impacted by the various signals emanating from the microbiome. Martin Schwarzer and colleagues identified a single beneficial microbe, a *Lactiplantibacillus plantarum* strain (LpWJL), that can single-handedly rescue a growth-deficient phenotype in mice on a low protein diet. The problem gets flipped. Instead of studying how diet modifies the composition and function of the microbiome, (*Schwarzer* et al., 2016) studied how supplementation with a single gut microbe (LpWJL) rescues the stunted phenotype in mice as a result of chronic undernutrition. The punchline is that a cell wall component of this bacterial strain stimulates the pattern recognition receptor (NOD2) in the intestinal epithelium and promotes growth hormone (GH) signalling via insulin-like growth factor 1 (IGF-1) in the liver. Changes in microbial composition in the intestine result in modulation of signalling pathways in liver and muscle (remote effects) resulting in improved growth in early life.

Luigina Romani and colleagues elaborated on microbe:microbe interactions across bacterial and fungal kingdom boundaries. In addition to considerations of commensal bacteria and bacterial pathogens at different body sites, bacterial and fungal species (emphasis on yeast) interact with each other. *Candida albicans* can be considered a commensal of the gut microbiome, and *C. albicans* can modulate signalling pathways in dendritic cells and promote immune tolerance (*Romani* et al., 2002; *Romani*, 2011). *C. albicans* can also modulate bacterial composition in the gut microbiome, so we are left to conclude that bacteria and fungi are dynamically fluctuating

over time in terms of relative abundances. One example of a signal is the compound p-cresol that is produced by *C. difficile* and suppresses hyphal growth by *C. albicans* (*van Leeuwen* et al., 2016), demonstrating cross-kingdom signalling within the intestinal microbiome. Tripartite interactions between bacteria, fungi and mammals provide fascinating examples of cross-communication and influences on development of the immune system. The bacterial commensal organism, *L. reuteri*, can utilize the amino acid tryptophan (Trp) in the intestine and produces indole-3-aldehyde (*Morgan* et al., 2023). Indole-3-aldehyde is a ligand for the aryl hydrocarbon receptor (AhR) which is a key receptor of microbial signals within the intestinal epithelium. AhR signaling results in release of IL-22 by innate lymphoid cells, resulting in suppression of *C. albicans* proliferation via release of antimicrobial peptides. The key message is that bacterial metabolism of dietary components (amino acids) can promote immune function and resistance to fungal pathogens.

MICROBIOME-GUT-BRAIN AXIS

Microbes can have a profound impact on remote body sites in mammalian systems. A prime example is the microbiome – gut – brain axis and emerging insights with respect to communication between intestinal microbes and the mammalian central nervous system. Rochellys Diaz Heijtz described contributions of gut microbes and intestinal microbiology to neurodevelopment and autism spectrum disorder of childhood. As already mentioned in this summary, gut microbes may contribute to fundamental processes in prenatal and postnatal mammalian development. Postnatal microbial colonization promotes establishment of gut homeostasis, angiogenesis and immune system development (*Hooper* et al., 2012). The gut microbiota influences a wide array of neurodevelopmental processes such as the maturation of microglia and synaptogenesis (*Diaz Heijtz* et al., 2011). Interestingly, the metabolic capacity of gut microbes to produce metabolites such as vitamins B9 (folate) and B12 in childhood may support their contributions to neurodevelopment (*Hollister* et al., 2015). Similar to the work presented by Martin Schwarzer whereby cell wall components from *L. plantarum* bind to pattern recognition receptors such as NOD2, Rochellys Diaz Heijtz presented signalling pathways that included bacterial peptidoglycan (PGN) fragments interacting with NOD-like receptors and PGN recognition proteins. PGN can prime systemic innate immunity by interacting with Nod1 (*Clarke* et al., 2010). In a fascinating twist to the story, PGN recognition proteins were expressed in the developing mammalian brain. PGN recognition protein-2 (Pglyrp2) appears to modulate expression of autism risk genes in mouse models and may affect formation of brain circuits. Both Pglyrp2 and NOD2 appear to modulate mammalian brain function and behaviour, and such findings add evidence to the importance of microbial components in neurodevelopment and biology of the mammalian brain.

Intestinal microbes may produce neurotransmitters such as y-aminobutyric acid (GABA) and histamine, and gut microbes may stimulate mammalian enteroendocrine cells to produce serotonin. Rochellys Diaz Heijtz discussed the potential importance of microbial GABA in neurodevelopment with low levels of faecal GABA in young children associated with elevated risk for developing autism spectrum disorder. Gut microbes such as *Bifidobacterium* spp. may produce GABA and other gut microbes such as Clostridia may consume GABA so the relative balance of GABA producers and consumers may affect the relative risk of autism spectrum disorder. In addition to

GABA, histamine may be produced by intestinal microbes and contribute to abdominal (visceral) pain in irritable bowel syndrome (IBS) due to signalling via the histamine 4 receptor (H4R). Premysl Bercik described intriguing studies whereby mice colonized with IBS microbiota displayed greater visceral pain responses due to colonic distension (*De Palma* et al., 2022). One microbial strain, *Klebsiella aerogenes* MQ, was identified as an avid producer of microbial histamine in the gut. Clearly, production and release of neurotransmitters and amino acid metabolites such as GABA and histamine could have important consequences for neurodevelopment and pain signalling.

Autism spectrum disorder and Parkinson's disease represent well-known medical disorders with prominent neurologic/psychiatric and gastrointestinal features. Michael Zasloff described fundamental studies documenting the importance of gastrointestinal symptoms such as constipation and intestinal alpha-synuclein (aS) inclusions in Parkinson's disease. The gut-brain connection was emphasized by the Braak hypothesis of aS accumulation in the enteric nervous system as a possible initiator of Parkinson's disease pathogenesis (*Braak* et al., 2003). The cationic sterol, squalamine, was identified as a compound that could displace aS from neuronal membranes and serve as a drug candidate to ameliorate constipation and improve neurologic symptoms. In collaboration with John Bienenstock's team, Michael Zasloff and his team showed that squalamine could enhance intestinal motility and stimulate signalling from the enteric nervous system (ENS) to the brain in mammalian (mouse) models (*West* et al., 2019). These applications have been extended to human clinical trials. An important concept that was highlighted as part of our tribute to John Bienenstock was the notion of the "SSRI"code whereby distinct stimuli such as squalamine, probiotic lactobacilli, or a SSRI therapeutic all transmitted SSRI-like signals via the vagus nerve (*West* et al., 2021).

CELIAC DISEASE : DIET, MICROBIOME AND IMMUNITY

Elena Verdu shared insights regarding diet-microbiome-immune system interactions in the context of celiac disease. Celiac disease is instructive as a human disease model whereby specific dietary components contribute directly to human gastrointestinal pathology. Specific proteins from cereal grains known as gluten trigger intestinal inflammation in susceptible individuals (*Shan* et al., 2022). Deamidation of gluten peptides increases their affinity for HLA surface molecules – HLA-DQ2 and HLA-DQ8 – and highlights the importance of autoimmune responses associated with specific MHC class II antigens (specifically, HLA-DQ). The microbiome participates in gluten metabolism, and duodenal microbes are known to generate gluten peptides with enhanced or reduced immunogenicity. Elena Verdu raised examples of microbes such as *Pseudomonas aeruginosa* that produce proteases cleaving gluten into peptides with greater immunogenicity. Countering this phenomenon, other microbes such as *Lactobacillus rhamnosus* or *Lactobacillus fermentum* from healthy controls digested gluten to peptides with reduced immunogenicity. These findings highlight the importance of a relative balance in microbial composition as a predisposing or protective factor for autoimmunity. Gut microbes can profoundly affect digestion of dietary components such as plant proteins, thereby influencing protein metabolism and local immunity.

Beyond luminal proteins and peptides, amino acids such as tryptophan may be metabolized to specific signalling com-

pounds including indole derivatives. Luigina Romani highlighted indole derivatives from tryptophan metabolism as key elements of antifungal defence systems in mammals, while Elena Verdu mentioned indoles as key signals maintaining intestinal homeostasis and suppressing inflammation (*Verdu* et al., 2015). Indoles augment antifungal defence and modulate inflammation by signalling through a common pathway via the AhR. Tryptophan metabolism yields distinct compounds such as serotonin, kynurenine and indole derivatives, and the gut microbiome plays a pivotal role in these bioconversions from a single amino acid. Clearly dietary protein may yield peptides that stimulate the immune system, possibly resulting in immunopathology, or amino acids that can be converted to a variety of bioactive microbial metabolites. These experimental insights provide opportunities for new therapeutic strategies such as engineered bacterial enzymes, new probiotics that may suppress specific bacterial proteases, indole-generating microbes or host enzyme inhibitors.

CONCLUSIONS

OHUS 35 highlighted the importance of the mammalian microbiome in mammalian systems biology. The findings and insights shared at **OHUS 35** strongly support the holobiont concept that includes both microbial and mammalian cells as cellular components of one integrated mammalian system. Microbes and their corresponding enzymes and metabolites clearly contribute to early life development (both prenatal and postnatal) and proper growth and maturation. The microbiome-gut-brain axis has become a central theme in neurobiology and gastroenterology, while emphasizing the ways in which gut microbes can affect neurodevelopment and brain function remotely. Microbes may produce neurotransmitters or stimulate mammalian cells to produce neurotransmitters, and bidirectional communication via nerves such as the vagus highlight how the microbiome interacts directly with the central nervous system and vice-versa. The coordination of signalling via the microbiome as a bridge between diet and host is evident when considering the impact of diet during pregnancy or during pathologic states such as celiac disease. Microbes may modulate immunity by altering metabolism of dietary components, and microbes may also regulate visceral pain signalling by converting amino acids to signalling metabolites. Specific host receptors such as H4R and AhR may provide mechanistic pathways to explain microbial communication with mammalian cells. In short, we have witnessed many examples showing how the mammalian microbiome can profoundly impact mammalian biology. Our long-time colleague in Herborn and pioneering scientist, John Bienenstock (1936-2022), https://www.old-herborn-university.de/in-memoriam-john-bienenstock-1936-2022/, would not be surprised today to see so many insights extending from his seminal work in mucosal immunology and the gut-brain axis. As we close 2023 and OHUS 35, we say good-bye in appreciation once again to Professor John Bienenstock and to Old Herborn University Seminar 35.

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