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ARE STUDIES OF THE GUT MICROBIOME CLINICALLY RELEVANT?

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THE PURPOSE BEHIND THE LECTURE

I was asked by the organizers of this Old Herborn University Seminar to cast a critical eye on the field of research around which this seminar has been organized, namely the study of the intestinal microbiome. My task was to explore the "hard data" upon which assumptions are being made that extend to human health, both with respect to our understanding of the aetiology of certain diseases, their treatment, and the long term maintenance of human health. My motivation in undertaking this task was to explore for myself why so little of the information that has been gathered surrounding the human intestinal microbiome has, as yet, influenced the clinical practice of most allopathic physicians. Rather, information of this type generally is implemented by health care providers who practice "alternative" or "complementary" medicine, and is heavily promoted by companies that sell "health-benefiting" foods such as "yoghurt with active cultures."

SOME EXAMPLES WHERE COMMENSAL MICROBES PROVIDE A "BENEFIT" TO THE HOST

Few of us now doubt that, whether we like it or not, our bodies are the residence of great numbers of microbes, staggering in both numbers and species diversity. What I find remarkable is that our bodies have evolved with the expectation that certain microorganisms will naturally in-inhabit particular niches, whether it is the intestinal tract, the skin surface of our arm, or the corneal epithelium. Without these microbes, certain functions that characterize "health" fail to operate. We call the microbes that normally colonize our body "*commensals*."

Commensal intestinal microbes and epithelial homeostasis

For example, in the mouse large intestine, Gram-negative bacteria are required for the effective repair of the organ following an inflammatory insult (provoked by an oral dose of dextran sulphate) (*Rakoff-Nahoum* et al., 2004). Animals from which Toll-like receptors 2 or 4 have been genetically deleted are more likely than the wild type to experience a destructive DSS induced colitis. A similar phenotype can be demonstrated following DSS administration to animals depleted of colonic bacteria by broad-spectrum antibiotic treatment. The precise mechanism by which luminal bacteria enhance intestinal epithelial repair is not known, although dead bacteria (or LPS) can provide the necessary stimulus (*Rakoff-Nahoum* et al. 2004).

Antimicrobial peptides and harmonious co-existence with intestinal microbes

The mammalian intestine is invested with antimicrobial tools to permit us to live in harmony with our intestinal microflora (*Zasloff*, 2002). In the mouse and in man, antimicrobial peptides produced by the Paneth cells play a major role in protecting the delicate single cell layer that constitutes the intestinal epithelium from lumenal microbes (*Salzman* et al., 2007; *Vaishnava* et al., 2008). Paneth cells normally secrete high concentrations of defensin HD5 and lysozyme into the crypt-well and onto the epithelial surface, generating a protective antimicrobial microfilm. Microbes that access the lumenal surface of the enterocyte are killed rapidly as they attempt to attach to the epithelium. In certain human diseases, like Crohn's disease, this harmony is disturbed, in part as a result of impaired production/secretion of Paneth cell antimicrobial peptides. As a consequence microbes do attach to the epithelium, damage the enterocyte layer, invade into the lamina propria, and provoke a chronic secondary inflammatory response that causes much of the morbidity and pathology associated with Crohn's disease (*Salzman* et al., 2007). Our intestine has been designed to permit us to co-exist with microbes, and these microbes appear to be necessary for normal intestinal epithelial homeostasis.

Systemic bacterial commensals and viral resistance

Certain strains of *Drosophila* were found to be sensitive to infection by RNA viruses, while closely related parental strains were found to be resistant. The resistance trait was passed exclusively by the mother. Review of the origin of the sensitive strains revealed that the sensitive strain had been created from a parental line that had been treated with tetracycline (*Teixeira* et al., 2008). Careful microscopic study of the eggs of a resistant female revealed the presence of numerous cytoplasmic bacteria, not seen in the eggs of females sensitive to viral infection. The microbe was identified as a member of the genus *Wolbachia*. Examination of the tissues of both male and female resistant flies demonstrated the wide spread presence of this "commensal" throughout the animals' body. Sterilization of the viral resistant fly through antibiotic treatment converts it to a sensitive animal. The mechanism by which the presence of systemic *Wolbachia* confers resistance to viral infection is not known, but it does so without apparently diminishing the fertility, lifespan, or "health" of the uninfected fly. This example is presented to illustrate the unequivocal qualitative and quantitative "health benefit" that can be attributed to a "commensal" in certain animals (*Teixeira* et al., 2008).

In addition, I presented examples of bacteria serving a protective function to their host by elaboration of antimicrobial substances that controlled the growth of unwanted fungi. In one example, the embryo of a shrimp (*Palaemon* sp.) has been shown to be covered by a species of *Alteromonas* that secretes an antifungal substance, istatin. Istatin, in turn, prevents the fungus *Lagenidium callinectes* from infecting the embryo, for which it has a particularly great tropism (*Gil-Turnes* et al., 1989). A second example is from the story of the fungus-farming ants. Certain species of "farming ants" inoculate the leaves they harvest with a fungus, which serves as their principal food source. To protect this "farmed" fungus from parasitic microbes that can attack it (especially fungi of the *Escovopis* species), the ants inoculate their crop with a species of bacteria (*Pseudono-*

cardia) which produces a suite of antibiotics that specifically inhibit the growth of the "parasitic" microbial species (*Poulsen* et al., 2007).

GREAT EXPECTATIONS HAVE BEEN SET FOR THE VALUE OF THE KNOWLEDGE OF THE MICROBIOME FOR THE PRACTICE OF MEDICINE

What data exist that would help convince the critical and conservative clinician that human health requires a particular "optimal intestinal microbiome" and that distortion of this optimal collection of microflora can create disease?

In particular, I chose to focus my discussion on the claims made that certain species of intestinal bacteria ("probiotics") are beneficial to health. Highly regarded reviews have suggested that robust data exist to support the claims that:

1. we can effectively manipulate our intestinal flora to increase the proportion of probiotic bacteria, and
2. that such manipulation affords health benefits (*Preidis* and *Versalovic*, 2009).

A widely cited research study is said to have demonstrated that women (with at risk of bearing children with atopic disease) who consumed the probiotic *Lactobacillus rhamnosus* GG during pregnancy had a lower incidence of children with atopic eczema than women who did not consume a probiotic supplement, during the few weeks before delivery and 6 months postnatal (*Kalliomaki* et al., 2003). Careful reading of the original research report, however, would lead one to question the strong conclusion represented in the review, and perhaps, might make one question why the report itself was ever published in the Lancet in the first place. In the study referred to 159 pregnant women were randomly assigned to receive either the probiotic supplement or a placebo beginning 6 weeks before delivery, and continuing postnatal for 6 months. The highlight of the study was that while 24/56 children of mothers on placebo appeared to have atopic eczema at age 4 years, only 15/54 children of mothers receiving probiotics had eczema at that age. On the account of this single finding the paper was titled "Probiotics and the prevention of atopic disease". However, further examination of the data forces one to question the significance of the observation surrounding the apparent reduction in the risk of developing eczema. 10/54 in the treated group developed seasonal rhinitis, compared to 5/56 in the placebo group; skin prick reactivity to the common allergens did not significantly differ between the two groups. From my perspective, these data were unconvincing. Indeed I might worry that I might increase the risk of seasonal rhinitis through probiotic supplementation of mother and infant.

A second widely publicized study examined the impact on upper respiratory infections and antibiotic usage of feeding allergic infants a combination of prebiotics and probiotics (*Kukkonen* et al., 2007). The study is said " to have suggested that effective combinations of probiotics and prebiotics result in sustainable changes in microbial composition and benefits to the human host." (*Preidis* and *Versalovic*, 2009). But a careful review of the original report suggests that the claims exceed the realties reported. In this study, which was randomized and placebo-controlled, pregnant women at high risk for bearing children with allergy were

fed a mixture of 4 probiotics for 1 month prior to delivery, or a placebo. The infants of the treated mothers were then fed the probiotics along with a daily dose of 0.8 g of galacto-oligoscaccaride, a carbohydrate believed to help support the intestinal growth/carriage of the probiotic supplement, while the other cohort received placebo. The infants were followed up for 2 years. The "major" positive effect of treatment was noted between 6 an 24 months. The incidence of respiratory infections was reduced in the probiotic group compared with the control group (93% vs. 97%, p=0.023). The average number of infections was reported to have reduced in the treated vs. control cohorts (3.7 vs. 4.2 infections, p=0.009). And yet, examine the other data presented: similar prevalence of middle ear infections (72% vs. 76%); no effect on incidence of diarrhoeal disease or gastroenteritis; no effect on any infection being followed during the first 6 months while the infants were receiving the supplement; no impact on the incidence of infantile colic. Could one honestly conclude that probiotic therapy affords any benefit to infants at high risk of developing allergic disease?

ALTERING THE MICROBIOME OF A MAMMAL CAN INFLUENCE THE CONCENTRATIONS OF GUT DERIVED METABOLITES FOUND IN BLOOD AND FAECES, BUT IS THIS OF CLINICAL SIGNIFICANCE?

Powerful tools now exist that permit the quantitative and qualitative analysis of organic compounds in the vascular compartment. Several groups have demonstrated unequivocally that many metabolites found in the bloodstream and tissues of mice derive from metabolic conversions carried out by intestinal microbes (*Martin* et al., 2008). If mice are inoculated orally with probiotic bacterial species, distinct differences in metabolites present in blood, liver, and faeces can be correlated with the presence of this probiotic intestinal flora. Does it follow, as these investigators state, that "significant associations between host metabolic phenotypes a nutritionally modified gut microbiota strongly supports the idea that changes across a whole range of metabolic pathways are the product of extended genome perturbations that can be oriented using probiotic supplementation and which play a role in host metabolic health…."

In our studies in the transplanted human small intestine we have discovered that the presence of an ileostomy creates an aerobic environment that favours a microbiome that is enriched in organisms that can tolerate oxygen. In contrast, upon closure of the ostomy, and re-anstomosis of the ileum with the colon, a microbiome that consists of predominantly anaerobic species comes to populate the bowel (*Hartman* et al., 2009). In both settings, the bowel functions normally despite the different population of organism inhabiting the organ. We conclude that the bowel can accommodate different "alternative microbiomic states". Is one state more beneficial than another?

In the final segment of my presentation I reviewed the clinical data gathered to date by Danone, who produce the highly successful product, Activia. Activia is a probiotic-enriched yoghurt to which numerous health benefits have been ascribed. The promotional material used to market this product claims that benefits have been supported by clinical trials. We critically examined one of these published "positive" studies (*Guyonnet* et al., 2007). In a randomized placebo controlled double

blind study, 274 adults with constipation type irritable bowel syndrome were fed once daily for 6 weeks either the Danone product (which contains live organisms of a strain of *Bifidobacterium animalis*) or a heat inactivated control product. The subjects were assessed at week 3 and at week 6, as well as at the start. No clinically significant difference in any parameter measured was presented in the report. Of particular interest was a graph that presented the number of bowel movement/week, measured weekly throughout the course of the study. The graphs of the treated and control groups are indistinguishable. The data suggest that probiotic supplementation conducted in a well-controlled trial provide no significant clinical benefit to those suffering with IBS.

I ended my presentation by asking the participants to consider the following questions as the Seminar exploring the intestinal microbiome of man unfolded:

- With respect to humans: Are there good and bad microbiomes? (Exclude drug-bug interactions).
- If so, how are these two states manifest?
- Should stable differences in a microbiome be considered "Alternative" rather than Good or Bad?
- Prove that by changing an individual's gut microbiome we can impact the health of that individual.

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