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INTESTINAL MICROBIOMICS: NOVEL INDICATORS OF HEALTH AND DISEASE

SUMMARY OF THE SEMINAR DISCUSSION

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For a long time, we may have thought that we had a good view on our intestinal microbiota. The bacterial community, which inhabits the human gastrointestinal tract, is characterized by its high density and diversity. The colon contents support an estimated 1000 different species, with numbers as high as 10^{10} or 10^{11} bacteria/gram of content. These bacteria are in a continuous interaction with each other, and with the host, comprising a highly complex ecosystem. The ecosystem as such contributes to host health as an aid in the digestion of foods, by production of vitamins, supporting the maturation of the immune system, aiding in the digestion of foods, and forming a barrier for colonization of pathogenic bacteria.

Our understanding on the microbial ecosystem has led to practical applications. Food products are on the market that claim to alter the microbial composition in the gut and thereby promoting human health. Prebiotics and probiotics are the best examples for this. Prebiotics are defined as selectively fermented ingredients that allow specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health. The prebiotic concept was formed in the mid-nineties, when we had a rather premature view on the microbiota and its composition. At that time, bacteria were divided in harmful, neutral and beneficial species; the latter being promoted by prebiotic ingredients. Whereas prebiotics are substrates for beneficial bacteria, the probiotic concept is based on the consumption of beneficial bacteria themselves. Probiotic bacteria have an added value to the functionality of the normal microbiota. Although some pre- and probiotic products may be a commercial success, most health claims are under debate and so far, the medical world has not embraced the use of pre- and probiotics by implementation in standard protocols.

Despite years of research, and in contrast to the above, we seem to know relatively little on functionalities or species that really matter to health. In his opening presentation of the Old Herborn University Seminar, *Michael Zasloff* has stressed the importance of host-microbe interactions for some intestinal (mainly invertebrate) ecosystems, but also the apparent lack of hard evidence for a similar effect in humans. In many cases, only weak associations between microbiota composition and disease parameters could be made without a clear view on causality. To what extent individual species of the intestinal microbiota make a difference, or can be used as markers for a healthy microbiota is largely unknown.

The discussion session, of which the summary is presented here, started with an overview of the old concept of harmful and beneficial bacteria, and a description of the prebiotic concept. In addition, the following questions were raised:

- Do we have evidence that the microbiota composition reflects our health status?
- If the microbiota composition really matters, how can we turn this into practical tools for health care specialists?

Participants were challenged to provide examples on host- microbe interactions that are relevant for human health.
It is only recently that new techniques in molecular biology allow the detection of microbial species that are either difficult or as yet impossible to culture. Techniques such as fluorescent *in situ* hybridisation and denaturing gradient gel electrophoresis revealed first insight in intestinal microbiota composition and dynamics during health and disease. Novel techniques like microarray technology using chips spotted with probes that recognize different species of the human intestinal tract, and sequencing technology such as barcoded P454 pyrosequencing will provide further insight in the dynamics of microbiota composition.

During the discussion, it became clear that there are many factors that determine intestinal microbiota composition, some of which are of importance even directly after birth. Several of these factors were addressed during the various presentations of the seminar. Each author was asked to highlight the take home message of its presentation. These key messages, in the order of the seminar program were:

Lars Engstrand: Methodologies have become available today that allow us to assess the microbiota composition in very large cohort studies. Barcoded P454 pyrosequencing is a most promising technique that has been used to determine shifts in faecal microbiota, and is currently used in several other projects.

Joel Doré: Comparison of the microbiota of a group of volunteers reveals a number of species that are always present. This is referred to as the core microbiome. To what extend the core microbiome is conserved is subject of debate. Prevalence rather than absolute conservation is also a key-subject.

Michiel Kleerebezem: The small intestinal tract is the organ where most of the interactions between microbiota and host take place. We should therefore not forget this organ in our studies. In addition to the presence of species and their DNA, also the physiological state of these microbes is of importance.

Merete Eggesbø: Microbial colonization directly after birth likely has an impact on the health status of the host. In light with the hygiene hypothesis, Caesarean delivery is indirect a model for a distorted microbiota and has its impact on problems in later life.

Harro Timmerman: A Commensal Rat Ileum Bacterium (CRIB; spore former related to *Clostridium*) is found in a model for pancreatitis. Colonization levels were inversely associated with damage to the pancreas, which makes this a potential biomarker for a healthy microbiota.

Harry Flint: Core species are found in human subjects, the top ones actually described by *Moore* et al. (1996) after cultivation of these species. It suggests a functional redundancy, but this is probably not the case since ecological ability to maintain a high population level should come with a specific advantage. Cluster IV ruminococci are stimulated by starch in some individuals but not in all, as is often seen for dietary constraints. In non-responders, starch is detected in faeces.

Michael Blaut: Correlations between microbiota and health status can be seen but we need to be cautious in the point we make. There is a need to provide geneticists with better perspectives on key genes when relating microbiota functions and health, and we need reductionist models for this. A whole range of very good methods is available but need to be combined. This can fill the gaps between phylogeny and function in a few cases.

Hans Snel: There are organisms that have developed an intimate relation with host, as illustrated in mice with segmented filamentous bacteria. Adherence of these bacteria promotes priming of immune system as seen by T-cell proliferation and s-IgA production. This activation in turn reduced the number of adhering segmented filamentous bacteria.

John Penders: The host genotype is of importance when making associations between microbiota and health of the host. Early detection of (non-tox) *Clostridium difficile* is associated with all later outcomes of allergy, but this does not necessarily be a direct interaction between *C. difficile* and the host. An answer may come from prospective birth cohort studies, with possible later treatment as case-control sets.

Taken these key messages together, it becomes clear that the technology is getting in place to determine microbiota composition in great depth and at various parts of the gastrointestinal tract. These techniques have given us a first impression of important species (e.g. the concept of a human core microbiome, or presence of commensal rat ileal bacteria and segmented filamentous bacteria in rodents). We also get a better view on dynamics, and on factors like diet, antibiotic therapy and specific diseases that determine the final composition. Nevertheless, we still have difficulties in defining a healthy microbiota.

As a next step in the discussion, the concept of eubiosis (balanced microbiota) and dysbiosis (disturbed microbiota) was discussed. Eubiosis is based on both composition and dynamics of the microbiota. Regarding composition, we agreed that each adult faecal microbiota contains

- a few predominant conserved phyla (in varying relative proportions between subjects);
- over 1000 predominant species;
- a majority of species without cultured representatives;
- a majority of subject-specific species that are not necessarily being found in other subjects.

The core microbiome concept still needs further refinement. Regarding dynamics, this is highly dependent on the level of resolution of the analysis. However, the following characteristics are present:

- stability / resistance to change;
- resilience following stress (e.g. antibiotic therapy). To what extent the resilience is complete is still an open question.

There are so far no objective and generally accepted measures to describe dysbiosis. The time of sampling and other methodological aspects are important factors to consider. For Crohn's disease we seem to be closer to a general understanding of dysbiosis than for ulcerative colitis or allergies. Caesarean delivery is an interesting context promoting some dysbiosis.

Biomarkers for eubiosis are hardly present, and those available do require strengthening of evidence. Also biomarkers for microbiome functions rather than species are hardly present. As an example, one of the functions of the microbiota is to provide coloniza-

tion resistance, for which no adequate markers exist as yet. Some functions derive from single microbial species. There is still much to expect in this area, but we need more isolates for functions that have no representatives.

Early work on microbiota composition made a division between harmful and beneficial bacteria. This division is completely abandoned. Even pathogenic bacteria such as *Salmonella* spec. can be found in the intestines of apparently healthy persons. At present, there is no way to relate microbiota composition to a healthy state in absolute terms. There are, however, expectations to relate microbiota to disease risk. One example is the study of *Joel Doré* who presented data on the presence of *Faecalibacterium prausnitzii* that is associated with the absence of a relapse in Crohn's disease. At the moment, this is one of a few (if not the only) examples of microbiota elements that can serve as indicators for health or disease. This illustrates that it is possible to relate microbiota elements (present or missing) to disease risk. We do not know yet whether those elements are causally related to health or disease. No complete demonstration for that is yet available. Some bacterial "metabolites" are signalling to human cells and induce specific responses, and some "unknown signal metabolites" are protective against certain diseases in models.

What is needed?

To validate hypotheses on host-microbe interactions, the following studies and materials are necessary to draw proper conclusions:

- Prospective studies are needed since these allow a clear discrimination between microbial factors that precede a disease state, and factors that are the consequence of a disease state. Of critical importance for such studies are standardized sample collection and timing of collection. Some studies require collection of faecal material from children (e.g. for studies related to development of allergies) whereas other may preferably sample from adults (e.g. for studies related to colon cancer risk).
- There is a need for more studies on small intestinal microbiota since most host-microbe interactions take place in this organ. As this is the organ for immune sampling, bacterial interactions related to immunodevelopment likely are found here.
- Effect of bowel cleansing should be assessed as a way to study the impact of reduced bacterial numbers.
- There is a need for more studies on functionalities, e.g. butyrate production, rather than a focus on bacterial species. Most functionalities are not bound to one bacterial species, and therefore a change in microbiota composition at the species level does not necessarily result in changes in functionalities of the microbiota.
- We should biobank faecal and intestinal samples to have those available to test future hypotheses. So far, studies in which intestinal or faecal material is collected have not done so. The impact of storage conditions is largely unknown, and has to be considered since these samples need to be available for both microbial and biochemical analyses.

We know that some initiatives underway. In Europe together with China the MetaHIT project has been initiated, the United States has launched the Human Microbiome project, and Canada has the Canadian Microbiome Initiative. Also other national initiatives take place. We should learn from the out-

come of these projects to what extent the human microbiota contains elements that can be predictive for present and future health or disease.