Keystone microbiome meeting 2012: a mountain top experience

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The joint Keystone Symposia on 'Innate Immunity and the Microbiome' took place in March 2012 in Keystone, Colorado. Gabriel Nunez (U. Michigan, USA) and Akiko Iwasaki (Yale U., USA) organized sessions focused on innate immune sensing of microbe and damage signals, whilst Andrew Gewirtz (Georgia State U., USA), Fergus Shanahan (National U. Ireland, Ireland) and Ruth Ley (Cornell U., USA) organized the microbiome-focused session. Joint and concurrent talks, and poster sessions between the groups, made for a sensational meeting with active exchange between participants. This meeting point is focused on the microbiome meeting talks and joint sessions.

Ruslan Medzhitov (Yale U., USA) and Jeffrey I. Gordon (Washington U. School of Medicine, USA), leaders in the fields of innate immunity and the microbiome, respectively, delivered the keynote addresses. Medzhitov's scientific minimalism suffused his talk on a three-pillar framework of 'avoidance, resistance and tolerance' for conceptualizing host–pathogen interaction, reviewed in [1]. Medzhitov provided data drawing on historical examples, such as Typhoid Mary and streptococcal pneumonia mortality during the influenza pandemic of 1918, as well as unpublished data from his own group on influenza co-infection.

Gordon's presentation was notable for its breadth and depth, and highlighted the role of the microbiome in global public health challenges—spanning a continuum from western-pattern, diet-related obesity to child malnutrition in resource-poor countries. The talk ranged in scope from predictive modelling of the microbiota's response to diet in mouse models, to Gordon's efforts to involve social scientists and biologists in questions addressing the 'anthropology of microbes', reciprocal interactions between the microbial world, and human biology and culture [2]. Both keynotes were an energizing kick-off to the meeting.

Two major themes that structure much of microbiome research permeated the meeting: symbiosis and configurations of the microbiome in health and disease. Symbiosis-related talks fell into two categories: mechanisms for the establishment and maintenance of symbiosis between mice

and their gut microbiota, and consequences of microbial symbionts for mammalian metabolism and physiology.

Host–microbiota symbiosis

"The epithelium is 'where the rubber hits the road' in host–microbiota interaction", noted Lora V. Hooper (U. Texas Southwestern Medical Center, USA). Hooper and Andrew Macpherson (U. Bern, Switzerland) have written about the importance of good 'fences' for neighbourly relations between hosts and their microbiota. Several participants presented their insights into how the glycocalyx and epithelial subtypes contribute to epithelial barrier function. Gunnar Hansson (U. Gothenburg, Sweden) shared published and unpublished data on intestinal mucus. Hansson showed how the use of simple reagents such as charcoal in live video microscopy have made the clear mucus layers visible, providing insight into the assembly of the colonic mucus layers. He discussed the microbial contribution to mucus thickness by exploring colonic mucus from feral, laboratory strain and germ-free mice, and that host genetics might influence the O-glycan repertoire of mucin 2—the intestinal mucus building block. Hansson suggested that O-glycan differences affect gut taxa distribution between mice and humans, and between individuals, as O-glycan variation can influence bacterial adhesion to the outer mucus. Hansson has examined the mucus layers of numerous mouse models of colitis and enteritis and found defective mucus, although the details of how these defects arise in interleukin-10 and Toll-like

receptor (TLR) 5-deficient mice remain to be elucidated. Studies from the Hooper lab have demonstrated a key role for epithelial intrinsic MyD88 and MyD88-dependent RegIII γ production in limiting gut bacterial incursions into the mucus layer. The Hooper lab has made seminal contributions to understanding how the bactericidal C-type lectin RegIII γ functions as a key host symbiotic factor. Hooper shared unpublished RegIII γ cryo-electron microscopy images that shed light on its unique biological activity.

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A common thread in talks was the role of autophagy genes in epithelial cells and their contribution to host–gut microbial symbiosis. Genome-wide association studies and follow-up research have established the importance of the autophagy gene *ATG16L1* and the Thr300Ala mutation as a risk-associated allele in inflammatory bowel disease. Autophagy was a focus of presentations by Dana Philpott (U. Toronto, Canada), Herbert W. Virgin IV and Thaddeus Stappenbeck (both of Washington U. School of Medicine, USA). The three talks highlighted the complexity of the cross-regulation of autophagy and nucleotide oligomerization domain signaling, autophagy–microbiota–virome interactions, and the importance of autophagy in the symbiosis-promoting function of distinct epithelial cell subsets, respectively.

Beyond its function as a barrier, limiting contact with gut microbes, the epithelium functions as a crucial intermediary in conveying microbial signals to lamina propria immune cells. Maria Rescigno (European Institute of Oncology, Italy) contrasted how the human intestinal epithelium produces thymic stromal lymphopoietin (TSLP) to condition tolerogenic lamina propria dendritic cells, but in mice, dendritic-cell-derived TSLP influences the balance of gut T_H17 and T-regulatory (T_{Reg}) cells directly [3]. Published research from the laboratory of Dan Littman (New York U. School of Medicine, USA), has demonstrated the importance of segmented filamentous bacteria (SFB) in the development of mouse intestinal T_H17 cells. Electron micrographs from Littman's group and others suggest that SFB perturb but do not penetrate epithelial cells. Efforts are directed at understanding the consequences of this physical interaction for the epithelium, and uncovering the role of SFB-induced, epithelial-derived serum amyloid A as a regulator of lamina propria myeloid cell function.

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The epithelium is not the only barrier separating the gut microbiota from the host. Andrew Macpherson discussed his concept of firewalls—mesenteric lymph nodes—and the importance of compartmentalized immune responses for host–microbiota symbiosis. Macpherson presented work on how the mucosal innate and adaptive immune systems work in a continuum with the innate immune system, both quietly clearing microbial interlopers without triggering adaptive responses, and setting the threshold for engaging adaptive responses. Thoughtfully engaged in innovating and optimizing the mouse models used in the field—for example, reversible germ-free colonization by using an *Escherichia coli* auxotroph—Macpherson also discussed data emphasizing his view on the importance of using simplified microbial communities, such as the ‘altered Schaedler flora’, for understanding the contribution of gut microbiota to immune system development and function.

Microbiota contributions to the host

In the interest of not being overly host-centric, it is important to recap several of the presentations on the role of microbes in host physiology and symbiosis. Fredrik Bäcked (U. Gothenburg, Sweden) presented several stories from his group, many of which were in press during his talk: the role of the microbiota in promoting intestinal angiogenesis [4], the microbiota's role in regulating bone mass density [5], and a comprehensive expression profiling of gastrointestinal and fat tissues from conventional and germ-free wild-type and MyD88-deficient mice (<http://microbiota.wall.gu.se>). By using a spectrum of dietary and antibiotic interventions in mice, Martin Blaser's (New York U. School of Medicine, USA) lab is investigating how changes in human ecology over the past century are affecting health. Blaser presented unpublished data on adiposity redistributions and altered bone mineral density as a result of these perturbations. David Artis (U. Pennsylvania, USA) discussed a story published after the meeting that, like Blaser's, is illuminating how antibiotic usage might be increasing the prevalence of allergic diseases. Artis's lab has found that oral antibiotics increase serum IgE and blood basophil counts, and enhance allergic inflammation. His group has determined that microbial signals and B-cell intrinsic MyD88 influence basophil haematopoiesis and susceptibility to T_H2 cytokine-mediated inflammation and allergy [6].

Microbial symbionts and SAMPs

Fergus Shanahan stated that polysaccharide A (PSA) is a model *par excellence* of a symbiosis-associated molecular pattern (SAMP). Sarkis Mazmanian (California Institute of Technology, USA) and his lab have proven this to be the case, as their studies have provided key mechanistic insights into how PSA influences the immune response. Mazmanian reviewed his group's work on how PSA influences the development of enhanced T_{Reg} cells in a TLR2-dependent fashion, and that *Bacteroides fragilis* can bind to intestinal mucin and penetrate the outer mucus layer. Following up on these studies, Mazmanian shared unpublished data suggesting that in *B. fragilis* monoclonized mice, colon tissue association is PSA-dependent, whilst in the absence of PSA, neutralization of interleukin-17 promotes *B. fragilis* colonization of mucosal tissues. This suggests the importance of T_{Reg}

cells in regulating *B. fragilis* tissue association, and that *B. fragilis* promotes immune tolerance to its own antigens. Mazmanian shared an unpublished story on *B. fragilis* outer membrane vesicles (OMVs). PSA among other molecules are packaged into OMVs, and OMVs confer distinctive immunomodulatory effects on dendritic cells in a PSA- and TLR2-dependent fashion.

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Data from several labs are identifying the microbial taxa and elucidating the features they require to be successful symbionts in the mammalian gut. Jens Walter (U. Nebraska-Lincoln, USA) discussed *Lactobacillus reuteri*, a successful vertebrate symbiont, and shared studies from his lab describing the genomic evolution of rodent and human *L. reuteri* strains. By using comparative genomics, his group is unravelling the mechanisms underlying host specificity for the rodent and human gut. The theme of host specificity was discussed by Kenya Honda (U. Tokyo, Japan). Honda shared several unpublished results from his laboratory about SFB and *Clostridia* species. His group has observed that SFB have specificity based on the donor genus from which they derive, and this restricts the immune responses they elicit across hosts, whereas *Clostridia* spp. can elicit similar responses from across many distinct hosts. These talks made clear that there are ample opportunities for SAMP discovery and study.

Microbiome configurations in A–Z

How health and disease are shaped by the microbiota and whether the microbiota are a cause or an effect of a given disease are popular topics. A high point of the conference was a workshop session entitled ‘The Microbiota and Your Research: A How-to Guide to Designing and Executing Studies of the Microbiota in Your Favourite Niche’. The jam-packed interactive workshop was targeted towards investigators familiar with the microbiota literature but with limited hands-on experience conducting studies in the field. Ruth Ley, members of the Ley laboratory and Rob Knight (U. Colorado, USA) presented the steps involved in executing a

successful microbiota study: study design, sample procurement and preparation, DNA library construction and sequencing, and metagenomic data analysis.

In an invited talk, Knight demonstrated and explained the applications of several of the pipelines his lab has developed, including QIIME and Site Painter. Knight and his group deserve praise for their commitment to developing easily accessible, freely available, user friendly and responsive platforms. Metagenomic studies, especially 16S rRNA-based surveys, are now widely available and affordable. Knight, Ley and members of her lab spoke briefly on whole-genome shotgun sequencing and meta-transcriptomic studies, as these approaches are becoming increasingly accessible to researchers. Jeremy Nicholson (Imperial College, London, UK) is a proponent and leading exponent of metabonomics and its use to parse the complexity of gene–diet–microbe interactions in health and disease. Nicholson discussed metabonomic nuclear magnetic resonance and ultraperformance liquid chromatography mass-spectrometry based methods to identify biomarkers and targetable pathways in obesity, hypertension and liver transplantation. Nicholson also discussed pharmaco-metabonomics, which focuses on how the genetics and microbiome of the host function in drug metabolism and response. Although many of these metabonomic approaches might be out of the research scope of the vast majority of investigators, they should be on everyone's radar.

A bacterial-bias pervades microbiota in health and disease research. Forest Rohwer (San Diego State U., USA) reminded participants that whilst studying the virome might seem like “moving from the cone of light into the darkness”, we must be mindful of

viruses harboured by our resident intestinal microbiota—bacteriophages—and of the several billion open reading frames of our blood viromes. Frederic Bushman (U. Pennsylvania, USA) discussed published data from his group on the bacteriophage changes observed during dietary interventions, as well as a paper in press during the meeting, now published, on the human gut virome and the targeted hypervariation of these viruses [7].

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Microbiome-wide association studies (MWAS) are under way for many diseases: inflammatory bowel disease, irritable bowel syndrome, *Clostridium difficile* colitis, necrotizing enterocolitis, nonalcoholic fatty liver disease, HIV infection, metabolic syndrome, type 2 diabetes, chronic fatigue syndrome, asthma and rheumatoid arthritis. Fergus Shanahan's talk provided a comprehensive overview of several published and ongoing MWAS, and discussed Eldermet, a faecal microbiota survey of the elderly population of Ireland, of which Shanahan and Colin Hill (U. Cork, Ireland) are principal investigators. Philip Sherman (Hospital for Sick Children, U. Toronto, Canada) spoke about the gut microbiome of paediatric populations, and discussed the evidence and international perspective on probiotic interventions for paediatric gastrointestinal diseases. Ruth Ley presented a fascinating unpublished study

from her group on the microbiome associated with pregnancy. James Versalovic (Baylor College of Medicine, USA) presented data from ongoing MWAS in paediatric and adult populations, and drew attention to observations about the expansion of gamma-proteobacteria in several gastrointestinal disease states.

The microbiome field has exploded over the past few years and enthusiasm for the topics covered at the meeting was palpable. Participants took away a respect for the microbial and viral parts of themselves and their system models, and importantly sensed an openness and willingness of microbiome investigators to share ideas, tools and pipelines and engage researchers from across many disciplines.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Medzhitov R, Schneider DS, Soares MP (2012) *Science* **335**: 936–941
2. Benezra A, Destefano J, Gordon JI (2012) *Proc Natl Acad Sci USA* **109**: 6378–6381
3. Spadoni I et al (2012) *Mucosal Immunol* **5**: 184–193
4. Reinhardt C et al (2012) *Nature* **483**: 627–631
5. Sjögren K et al (2012) *J Bone Miner Res* [Epub ahead of print] doi:10.1002/jbmr.1588
6. Hill DA et al (2012) *Nat Med* **18**: 538–546
7. Minot S et al (2012) *Proc Natl Acad Sci USA* **109**: 3962–3966

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