Module 1, Lecture 5 – Microbiome, part 3

Goals:

1. Define the major conclusions of the HMP so far - microbial community, part 3
2. Explain the interactions between the immune system and microbiota
3. Explain weighted and unweighted UniFrac

Learning outcomes:

1. Be able to describe the basic structure of a host microbial (bacterial) community
2. Develop hypotheses about the differences that may change or manipulate microbial host-associated community structure/function
3. Be able to explain how the microbiota influence immune function and how the immune system recognizes microbes at epithelial surfaces.
4. Be able to explain why weighted vs unweighted UniFrac matters

**A. Microbial Community Analysis – The HMP**

HMP (Nature, 2012)

* A total 242 screened and phenotyped adults (129 males, 113 females)
* Categorized as ‘healthy’, by consortium-defined clinical sampling criteria
* Women were sampled at 18 body habitats, men at 15, at five major body areas
* Major body areas : oral cavity, oropharynx, skin, stool, vagina
* 10 Gb per sample = millions of reads/sample 🡪 E. coli at less than 1% is completely represented and other rarer are partially represented.

**Remember one of the central questions to understanding the community is how those of individuals are related to each other?**

**Which depicts the real microbiome in humans or other species like our gulls?**

**And how much do the differences impact their physiology? Susceptibility to disease?**

**Metabolic pathways might be more important!**

At functional level, there may be significant similarities 🡪 has parallels to macroecosystems.

* **Diversity and abundance of each body habitat’s signature microbes varies widely, even among healthy subjects – we are all unique**
* **Primary clustering is by body area, with oral, gastrointestinal, skin & urogenital habitats separate; but nares bridges oral & skin habitats**
* **Carriage of metabolic pathways appeared stable among individuals, despite variation in community structure/ taxon composition**
* **Ethnic/racial background proved to be one of the strongest associations of both pathways and microbes with clinical metadata.**

But**, culturally, things seem to be quite different.** Studies of very different cultures parallel the differences seen between carnivorous and herbivorous animals.

***The new question now seems to be, do enterotypes exist?***

Data is unclear – some studies show yes, others no

***And how much does resilience play in structuring communities?***

Dietary changes produce differences, but small compared to variation between individuals 🡪 can longer term changes induce state shifts or regime change to a new stable state?

Resilience seems to vary a lot between individuals according to antibiotic studies.

Transplantation studies are suggestive, but studies to understand the factors governing stability and the ability to establish a healthy microbiota by succession will be key to engineering therapies using microbial ecology.

**B. Immune interactions with the microbiota**

*What does the gut community provide for the host?*

**We mentioned barrier function, but did not go so far as immune priming**

Intestine reaches 1012 bacteria per gram of luminal content in distal gut.

200m2 in the gut

**A. Are the microbiota involved?**

1) In rodent models of GI inflammation, inflammation (immune response) is not present in the absence of microbes in the gut nor does gut immunity develop properly.

2) Animal model or human polymorphisms associated with disease frequently involve mucosal immune response

a. in pathways of innate receptors (NLR or TLR), or

b. in signaling molecules (eg – IL-23) that remove inhibition or take away a conditioning response (i.e. – constant signaling regulated by blunted responses).

3) “Colitogenic” microbes are found in human and animal disease, but there is little evidence that these microbes can cause disease in an otherwise healthy host (in other words, there must be some sort of immune breakdown or insult first).

**B. How do the microbiota regulate immune response?**

1) Induction of Innate mechanisms

2) Induction of T cell regulatory balance

**Specific microbes may be responsible for these effects (Treg induction)**

1. Nonenterotoxigenic Bacteroides fragilis –

a. Monocolonized mice are protected via induction of IL-10 producing Treg induced by polysaccharide A (PSA) produced by B. fragilis.

b. PSA producing but not non-PSA producing B. fragilis suppress effector T cell function.

c. Unlike other TLR ligands, PSA acts directly on Treg cells and ultimately induces lower IL-17 production via close contact in the epithelial crypts.

🡪 Role of B. fragilis might be only local though in letting it colonize crypts

2. Clostridium species

a. Germ free mice provided an SPF flora produce Treg cells in colon dependent on gm+ spore forming bacteria (which include Clostridia and not B. fragilis).

b. Colonization with different cocktails of bacteria showed that SFB and Bacteroides and Lactobacillus weren’t capable but certain Clostridial groups were, independently of TLR, NLR, and Dectin signaling (KO mice) of inducing Treg cells providing protection.

 c. However, this may only be in the colon

3. SFB

a. SFB seem to prep the gut in the opposite way. Mice who don’t colonize SFB are deficient in IgA and do not develop as much Th17 pro-inflammatory cells. For instance, SFB-less mice don’t control *Citrobacter rodentium* infection.

b. SFB attach tightly to epithelium in Ileum and at Peyer’s patches and may allow intimate stimulation of pro-inflammatory responses without overdoing it. Perhaps not coincidentally, many pathogens do the same thing.

**Take home message 🡪 clearly complex, but specific interplay is important for initiation and progression of disease, over and above normal immune homeostasis of the mucosal surfaces.**

**D. UniFrac Analysis – continued**

Major axes:

A. Alpha diversity – how many taxa/lineages in one sample

Beta diversity – how taxa/lineages are shared

*Remember the human microbiome sites – Turns out that* ***Alpha and beta diversity*** vary but are similar across body locations.

Eg – vaginal community is lower diversity than GI tract, but variation between samples is similar to other sites.

B. Qualitative – presence/absence (aka - community membership)

Quantitative – relative abundance (aka - community structure – might include spatial and temporal too)

So method, like unweighted UNIFrac is a qualitative, phylogenetic, beta diversity metric.

Human gut microbiota seems to be relatively few deep-branching taxa with enormous diversity at the tips.