**Background/Inspiration:**

In 2012 Chung et al. (Kasper Lab) asked if small intestinal immune maturation depended on a host-specific microbiota.

To answer this question they did a series of experiments where they transferred human, mouse, or rat feces into germ-free mice and then measured levels of various immune cells (e.g. CD4 and CD8 T-cells). They found that the gnotobiotic mice treated with human stool over all had less immune maturation compared to mice treated with mouse stool. *This suggests that there are specific signals that members of a specific species’ microbiome provide to their co-evolved host.*

**Major Caveat:**

* The bacterial OTUs/species differed greatly between the human and mouse stool used to colonize the germ-free mice.
  + Is this just a case of different bacteria do different things in a given environment?

In addition, Mark Koenigsknecht also has data that suggests that some epithelial genes are differentially regulated in germfree mice colonized with a human vs. a mouse strain of *Clostridium clostridioforme.*

**Hypothesis**: Bacterial members of the gut microbiota that are from one host species are better suited to interact with that host than another host species.

**Aim:** Determine if HIO epithelial gene expression is different if it is colonized with the same species of bacteria but isolated from a human vs. mouse.

Experimental design:

1. Isolate/obtain recently isolated closely related bacterial strains from mice and humans.
   1. Colonic or Illeal?
   2. Isolates will represent ‘major’ phyla in colon with 3-4 members from each phyla
      1. Bacteroidetes
         1. Bacteroides acidofaciens?
         2. Parabacteroides?
         3. Barnesiella-like clone ?
      2. Firmicutes
         1. *Clostridium clostridioforme*
      3. Proteobacteria
         1. *E. coli (human, mouse, non-host associated?)*
      4. Verrucomicrobia
         1. *Akkermansia muciniphila*
2. Grow bacteria to same growth phase
   1. Determined by: OD?, mid-log, stationary?
3. Inject HIOs – for each strain there will be the following conditions:
   * 1. Media alone
     2. Filtered culture supernatant
     3. Un-filtered culture supernatant
   1. Incubate HIOs for 12 hrs. post injection
      1. The lukovac et al. paper incubated for 3hrs.
   2. Harvest HIOs into Allprotect (Qiagen: 76405) .

**Potential Measures:**

1. HIO gene expression by microarray or RNAseq
   1. With specific interest in
      1. anti-microbial peptide production
      2. mucin
      3. cellular metabolism?
2. Bacterial gene expression
3. Epithelial barrier function
   1. Resistance to toxin mediated damage?
4. Mucin structure
5. IF for tight junction proteins
6. Determine bacterial genomes

