The Interplay between the Gut Microbiota and the Immune System

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The Microbiota

- $10^{14}$ microorganisms live in association with humans
  - Contain 100-fold more genes than the human genome
  - Concept of a “super-organism”: a combination of human genes and the genes of our microbial partners, the microbiome

- Distinct taxa are found across human environment
  - Main phyla include:
    - Bacteroidetes
    - Firmicutes
    - Actinobacteria
    - Proteobacteria
  - At lower taxonomic levels great variation is seen among individuals

The Gastrointestinal Microbiota

- Diverse community
  - Main phyla: Bacteroidetes, Firmicutes
  - Variation among individuals and over time

- “Core” microbiome
  - What taxa are shared in healthy subjects?
  - Enterotypes: distinct community types present in the human population, each defined by a dominant genus
    - *Bacteroides*, *Ruminococcus*, *Prevotella*

Dethlefsen et al., *Nature*, 2007

Arumugam et al., *Nature*, 2011
The Gastrointestinal Microbiota: Nutrition and Metabolism

- Host and microbiota have co-evolved

- Microbiome provides essential functions to host
  - Evidence from gnotobiotic mouse models
    (Backhed et al., 2004; Turnbaugh et al., 2009)

- Diet/nutrition can shape the microbiota
  - Microbiome reflection of dietary habits?
    (Wu et al., 2011; De Filippo et al., 2011)
The Gastrointestinal Microbiota and the Immune System

- The immune system shapes the microbiota
  - Mucus layer
  - Antibacterial proteins
  - IgA

- The microbiota impacts immune system development
  - Mucosal immune system:
    - Formation of organized lymphoid tissue
    - Regulation of innate lymphoid cells
  - Systemic immune system:
    - CD4, T<sub>reg</sub>, T<sub>h</sub>17 cells

Hooper et. al., *Science*, 2012
Shigellosis: An Endemic Disease

- Caused by *Shigella*, a mucosally invasive bacterium
  - Bloody diarrhea, fever, stomach cramps
  - Transmitted fecal-oral route

- Restricted to humans and non-human primates
  - Cynomolgus monkeys can serve as an animal model in vaccine development

- Evidence for effective live-attenuated vaccines administered orally (Levine et al., *Nat Rev Micro*, 2007)
  - No approved vaccine, but current vaccine trials are underway
  - Variability in vaccine response observed in different global populations
    - Unknown cause (diet, environment, genetic)
    - Role of gastrointestinal microbiota has not been investigated
• Oral live-attenuated *Shigella* vaccine trials with cynomolgus monkeys
  – Collect stool samples pre- and post-immunization and after WT challenge
  – Characterize the microbiota post-immunization and post-infection

• How does exposure to an enteric pathogen affect the intestinal microbiota?

**HYPOTHESES:**

Wild-type and live-attenuated strains of *Shigella* will alter the gastrointestinal microbiota

Composition of the intestinal microbiota may affect the outcome following immunization, challenge
16S rRNA Studies: Molecular census of the microbiota

• Most of the microbiota cannot be cultured

• High-throughput, multiplex, parallel sequencing and analysis technology has enabled characterization of microbiota

• 16S rRNA Surveys: Use 16S rRNA hypervariable region for bacterial identification
  – Universal gene in prokaryotes
  – Variable regions can be used for assigning taxa and phylogenetic relationships
  – Conserved regions can be used to design universal primers to amplify specific regions

Gary Andersen, Todd DeSantis, Lawrence Berkeley Lab
Relative abundance (%)

85-90% prevalence:
- Treponema
- Sarcina
- Unclassified Erysipelotrichaceae
- otu 1998 (Catabacteriaceae)
- Adlercreutzia

90-95% prevalence:
- Streptococcus
- Bulleidia
- Coprococcus
- Blautia

95-100% prevalence:
- Lactobacillus
- Prevotella
- Clostridium (clade 1)
- otu 2159 (Ruminococcaceae)
- otu 2087 (Lachnospiraceae)
- Oscillospira
- Ruminococcus (clade 1)

Study 1 - Indochina/Indonesia/Philippines or Admixed
Study 2 - Mauritius
Study 3 - Philippines
Study 4 - Indochina
Enterotypes in human GI microbiota

- Identification of enterotypes within humans
  - Limited number of community types within human population, dominated by *Prevotella*, *Bacteroides*, or members of the phylum, Firmicutes
  - Stable over time

- Determined by multidimensional cluster analysis from Jensen-Shannon divergence (measures similarity between probability distributions of genera)
Enterotypes within the cynomolgus monkey microbiota

Cluster analysis reveals 4 distinct community profiles
Each is characterized by a dominant genera
Genetic diversity in cynomolgus macaques

• Previous studies indicate allelic differences within cynomolgus macaques of different geographic origin (Krebs et al., 2005; Mee et al., 2009; Florese et al. 2009; Mitchell et al. 2012)
  – Indochinese/Indonesian: high level of diversity within MHC regions I and II
  – Mauritian: geographically isolated, restricted genetic diversity in MHC region

• MHC haplotype has been shown to be important in disease susceptibility in cynomolgus macaques (Wiseman et al. 2007; Florese et al., 2009)

• Analysis of non-MHC and MHC genotypes in our cynomolgus macaque population
  – Analyzed 24 short tandem repeats (STRs) to determine geographic origin
  – Analyzed seven microsatellite regions spanning the MHC
  – Determine whether genetic diversity correlated with differences in the microbiota
Genotype analysis using 24 non-MHC STRs from peripheral blood lymphocytes
Macaques from Mauritius cluster together
Comparison of STR data to macaques of known geographic origin confirmed macaque origin
MHC allele repertoire in macaques from different geographic origin

Genotype analysis of seven microsatellites spanning MHC region (both class I, class II)

→ Broad range of alleles for regions tested
→ As with non-MHC alleles, macaques from Mauritius cluster together
→ Macaques from Mauritius exhibit unique profile compared to macaques from Indonesia/Indochina/Philippines
Impact of live-attenuated and wild type *S. dysenteriae* 1 strains on microbiota composition

- Does vaccination and/or WT challenge alter microbiota composition?
  - Changes in diversity
  - Changes in community types
  - Clinical and immunological outcome following these events
  - Are observed changes the same for all populations
Measures of diversity:
Does vaccination, challenge affect microbiota?

**Study 1:**

- Significant change in Shannon diversity index following immunization in study 1 macaques and compared to study 4 macaques (untreated).
- No change in diversity estimates in study 2 macaques!
- Differences observed in macaques from study 1 and 2.
→ Both vaccination and challenge induce anti-LPS IgA, IgG antibody production (observed in both studies 1 and 2)

→ Difference in community type, clinical response between study 1 and study 2 macaques:
  -- Study 1:
    -- changes in community type, diversity measures following both vaccination and challenge
    -- Exhibit clinical symptoms
  -- Study 2:
    -- persistence of initial community type (high diversity)
    -- do not exhibit clinical symptoms

→ Different community types in monkeys of different geographic origin

Community types over time:
Does vaccination and/or WT challenge affect microbiota?
Increase in normally rare genera is associated with clinical symptoms following challenge. Increased abundance of *Pedobacter*, *Yersinia*, *Flavobacterium*, and *Comamonas* reads following challenge. Only in monkeys that exhibit clinical disease symptoms -- study 1 and 3 macaques (non-Mauritian).
Immune response following immunization and WT challenge

• Both immunization and challenge induce an immune response
  – Observed in both study 1 and 2 macaques
  – However, only study 1 macaques exhibited clinical shigellosis
    • Immune response as measured here not a determinant of protection—so what is?
      – Potential role for microbiota

• Are there correlations between strength, type of response and microbiota?
  – Utilized the statistical model, LSA (Local Similarity Alignment)
    • Time-dependent correlations
    • Used for intra-study comparisons
Correlation between immune responses and the microbiota community over time

Study 1:
→ Dense network of several genera related to anti-LPS IgA, IgG and stool score
   -- complex relationships between genera
   -- stool score correlated with many rare genera
Correlation between immune responses and the microbiota community over time

Study 2:

→ Less dense network
  -- reflection of high community stability for these macaques
  -- many observed correlations among core genera

→ Shared correlations among studies:
  -- *Weissella*
  -- *otu2998*
  -- *Clostridium* (clade 1)

→ Differences in vaccine regimen, however, make it difficult to determine how these may be related
We observe 4 enterotypes within the cynomolgus macaque gastrointestinal microbiota
- 2 “healthy”, 2 “transient”
- control macaques (study 4) are stable over time

Different enterotypes are present in macaques of different geographic origin:
- study 1:
  - Indochinese/Indonesian/Philippine origin
  - post-vaccination: change in community type, diversity
  - post-challenge: change in community type, increase in normally rare organisms
  - exhibited clinical symptoms
- study 2:
  - Mauritius origin
  - no changes post-vaccination, post-challenge
  - did not exhibit clinical symptoms
- STR and microsatellite genotypic analysis suggests a unique genotypic profile in Mauritian macaques

Vaccination and challenge induced immune response in both studies

Role of genotype in shaping microbiota composition
High-diversity community type: protective against *Shigella*
Need for characterization of the microbiome in future vaccine studies
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