Gut microbiome, HIV-exposure, and vaccine responses in South African infants

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What we know:

- **Gut Microbiome** plays a key role in the normal development of the immune system development (Mackie et al., 1999; Clemente et al., 2012).

- **Feeding affects and gut microbiome** (Ardeshir et al., 2014).

- **Maternal Microbiome influences neonatal microbiome** (Joann Romano-Keeler and Jörn-Hendrik Weitkamp., 2014).
• HIV-infected adults have altered gut and vaginal microbiome (Vujkovic-Cvijin et al., 2013; Dillon, 2014; Mutlu, 2014).

• HIV-exposed infants receive cotrimoxazole (CTM) prophylaxis against Pneumocystis jirovecii pneumonia (PCP) at 6 weeks.

• HIV-exposed uninfected (HEU) infants have altered vaccine responses compared to HIV-unexposed (HU) infants (Epalza, 2010; Koyanagi 2011; Kidzeru et al., 2014).
Hypothesis:

HIV-exposed uninfected (HEU) infants have altered immune responses due to dysbiosis of the gut microbiome

Aims

• To characterize the infant gut microbiome of HIV exposed uninfected (HEU) infants versus HIV unexposed (HU) infants.

• To relate vaccine-specific T cell responses with the gut microbial profiles (A pilot study).
Hypothesis: **HEU have impaired immune responses due to dysbiosis of the gut microbiome**

**Aims**

- To characterize the infant gut microbiome of HIV exposed uninfected infants versus HIV unexposed infants.
- To relate vaccine-specific T cell responses with the gut microbial profiles.
Larger African cohort study (INFANT Study)

Babies
- All born vaginally
- HIV Negative; DNA PCR at birth

500 HIV-exposed uninfected (HEU) exclusively breastfed infants

100 HIV-exposed uninfected (HEU) formula fed infants

150 HIV-unexposed (HU) exclusively breastfed infants
Methodology:

Fecal DNA extraction (Power Soil DNA Isolation Kit - MO BIO)

PCR amplified V6 hypervariable region of 16s rRNA

Sequenced; Illumina Hiseq platform

Data analysis: QIIME 1.8.0
- Pick OTUs at 97% ID
- Assign taxonomy (GreenGenes)
- Compute diversity

Statistical Analysis: R
Preliminary analysis:

HIV exposed uninfected (HEU) breastfed infants (n = 65)

Samples analyzed (52)

HIV exposed uninfected (HEU) formula fed infants (n = 31)

Samples analyzed (25)

HIV unexposed (HU) breastfed infants (n = 17)

Samples analyzed (16)

Time points

• D4-7
• WK 4
• WK 7
• WK 15
• WK 36

• 113 samples were sequenced (Illumina Hiseq)
• 18 samples had < 100 000 reads (cutoff) and were excluded from downstream analyses, leaving 95 samples.

• Across the 95 samples, 5712 OTUs were identified.
• Mean reads/sample : 562605.558
Shannon alpha diversity measures for HEU and HU (All time points)
Beta diversity: MDS analysis showing clustering for HU (All time points)

HIV exposure
- HIV unexposed (HU)
- HIV exposed (HEU)

Feeding
- breast
- formula
Relative abundance of gut microbiome between HEU and HU (All time points)

- Difference in the most abundant taxa observed at multiple levels.
- Here shown at family level between the two groups.
Unsupervised hierarchical clustering showing differentially abundant OTUs (all time points)

- Controlled for feeding
- OTUs filtered prior to differential abundance testing.
- OTUs included when present in 25% of samples (metagenomeSeq)

- Log2 Transformed OTUs
- FDR ≤0.05
Linear Discriminant analysis at Week 1

Shows potential biomarkers for HIV unexposed infants at week 1
Aims

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A Pilot study: Gut microbiome and vaccine (BCG) responses in vaginally delivered, HIV unexposed infants at 6 weeks of age
Gut microbiome versus BCG vaccine specific T cell responses (HIV unexposed (HU) at 6 weeks)

Parameters measured:
- T Cell proliferation (Ki67)
- Intracellular cytokine production (IFNg, IL-2, IL-13, IL-17)

Findings:
- Multiple organisms significantly correlated with increased BCG-specific CD4+ and CD8+ T cell proliferation and cytokine expression
OTUs differentially abundant (FDR ≤0.05) between HU infants with high or low CD4 IL-2 expression at 6 weeks.

- OTU_48
- OTU_40
- OTU_86
- Bacteroides
- B. fragilis
- CD4+ IL-2+
- Lactobacillus
- Clostridium
- Prevotella
- Bacteroides
- B. fragilis

No clustering by feeding.

FDR ≤0.05

Feeding
- BF
- MF

CD4+ IL-2+
- high
- low
B. Fragilis diff. abundance by “high” vs “low” CD4+ IL-2+ expression at week 6

B. fragilis has been shown to induce Foxp3+ Tregs subsets - could dampen immune responses. (Round and Mazmanian et al., 2010).
Conclusion

• There are differences in diversity and relative abundance of gut microbiota between HEU and HU

• An altered gut microbiome is associated with impaired immune responses to early childhood vaccines in HU infants

• We will further investigate whether the gut microbial profiles are associated with the altered vaccine responses observed in HEU
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