Impact of the gut microbiome on immune responses to oral cholera vaccination and IgA coating of gut microbes

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Introduction
Cholera is a severe diarrheal disease caused by Vibrio cholerae. Explosive epidemics are increasingly large in size and occurring with greater frequency. Endemic cholera is less visible but causes most of the 3 million cases each year.

The World Health Organization recommends oral cholera vaccines as a tool for achievement of their goal to reduce the global burden of cholera by 90% by 20301.

The most commonly used cholera vaccine is a killed whole cell vaccine (Shanchol, ShanthaBiotechnic, and EuviChol, EuBiologics), which contains V. cholerae 0139 and 01 strains, and both Inaba and Ogawa serotypes. A randomized trial of a two-dose regimen in an endemic area reduced cholera in adults by 60% over five years, with less protection in children.

Responses to oral cholera vaccines vary for unknown reasons, especially between adults and children, and persons living in developing versus developed settings.

Limited data suggests that the gut microbiome may influence immune responses to oral vaccines, and the gut microbiome is known to influence susceptibility to V. cholerae infection2.

Hypothesis: Gut microbes at the time of vaccination influence immune responses to vaccination. By identifying IgA+ coated bacteria at the time of vaccination, we could identify commensals interacting with the mucosal immune response at the time of vaccination.

Methods

Stool and serum for measurement of the gut microbiota and vibriocidal titers were collected from 83 adults in Dhaka, Bangladesh on the day of cholera vaccination, and on follow up days as shown in Figure 1.

Microbial DNA was extracted using a modified PowerSoil DNA extraction kit (Qiagen). A mix of degenerate primers for the V4 region of 16S rRNA were used to amplify bacterial DNA for identification3. Illumina MiSeq was used for sequencing and QIIME was used for data processing and analysis. OTUs were picked at 97% similarity against a reference database constructed from Greengenes.

Taxonomic profiles, phylogenetic relatedness, differential abundance testing and diversity metrics were measured and tested statistically using QIIME, R and Biobakery tools4.

Stool pellets were homogenized by bead beating and centrifuged to remove large particles.

Stool bacteria in the supernatants were washed and stained with PE-conjugated Anti-human IgA. Samples were incubated with Anti-PE magnetic Activated Cell Sorting (MACS) beads and sorted for enrichment prior to bacterial cell sorting.

A fourfold increase in titer is classified as a seroconversion response to vaccination, and titers were measured against both Inaba and Ogawa serotypes.

Diversity measures

After controlling for relatedness among participants, gut microbial diversity by alpha, beta, Bray-Curtis and Shannon diversity were not significantly changed between baseline and timepoints after vaccination.

Results

Vibriocidal titer magnitude and kinetics were used to classify participants responses to vaccination (table 1).

By Day 7 after vaccination, 82 of 83 participants had seroconverted, defined as a fourfold or greater increase in vibriocidal titer. 21 of 82 (26%) participants seroconverted day 3 after vaccination.

Differential abundances

Figure 4. Rate of seroconversion was faster in participants with increased bacteria from the family Ruminococcaceae (multivariate analysis using linear models, q value 0.04). Relationships with other phylogenetic groups were not significant.

Conclusion

• Gut microbial diversity is not altered by oral cholera vaccination.
• Specific microbiome profiles are correlated with the rate of seroconversion after receiving the vaccine.
• Our results add to new evidence that rare microbial groups may have a role in impacting immune responses.
• IgA coating of gut bacteria does not change with vaccination.
• Next steps in this work include identifying stool bacteria that are IgA coated at the time of vaccination and correlation of this result with amplitude and rapidity of immune responses.

References