The role of the intestinal microbiome in rotavirus vaccine immunogenicity
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Chapter 8

Summary
SUMMARY

Rotavirus (RV) is one of the leading causes of serious gastroenteritis and diarrheal deaths in children under the age of five across the globe (Tate et al., 2016; Walker et al., 2013). Rotavirus vaccines (RVV) protect infants, reducing rotavirus disease, diarrheal outpatient visits, hospitalizations, and deaths following introduction into a national immunization program. However, the vaccines have a significantly diminished effectiveness in poor settings where the majority of rotavirus deaths occur (Jonesteller et al., 2017). Identifying modifiable etiologies of this effectiveness gap in order to reduce rotavirus hospitalizations and deaths is therefore a global public health priority. The central aim of this thesis is to interrogate the hypothesis that the intestinal microbiome modulates RVV immunogenicity and thereby contributes to the diminished RVV efficacy observed in developing country settings.

Part I - Background

In order to provide a context for why the microbiome may be relevant to RVV immunogenicity, Chapter One provides a brief background of the epidemiology and pathophysiology of rotavirus as well as a summary of the efficacy and effectiveness of rotavirus vaccines that are currently in use. An outline is given of the major factors already known to correlate with diminished RVV efficacy in developing countries and an overview of which strategies to improve RVV protection have already been tested. Finally, 4 categories of supporting evidence are provided for the hypothesis that the intestinal microbiome contributes to RVV immunogenicity namely: the intestinal microbiome differs by geography across all ages; that the intestinal microbiome shapes and regulates infant immunity; that enteric viruses are known to interact with the bacterial microbiome; and finally, that the intestinal microbiome can alter vaccine responses in mouse models.

Chapter Two takes a broader view of the intestinal microbiome, and is an appraisal of its relevance to infectious diseases in general. The increasing accessibility of next-generation sequencing has rapidly expanded research on the intestinal microbiome, however the majority of research remains correlative and therefore speculative. Chapter Two offers examples of research that has bridged the gap from correlation to causation and therefore become relevant to infectious disease practitioners in general. These examples are valuable comparators for the claims of causality laid out in this thesis. The chapter also gives a more in depth description of the intestinal microbiome and examples of the ways the intestinal microbiome can shape early immune development and influence enteric pathogen
infections, and of the risks of microbial perturbations in vulnerable hosts. Each of these roles has applicability for a potential interaction between the intestinal microbiome and RVVs.

Part II – Correlations of microbiome composition and RVV immunogenicity

The second part of this thesis is concerned with establishing a correlation between intestinal microbiome composition and RVV immunogenicity. A more limited hypothesis is posited, namely, does the intestinal microbiome composition differ significantly between infants who do and do not seroconvert following RVV. The highest RV mortality and lowest RV efficacy are in sub Saharan Africa and Asia (Jonesteller et al., 2017; Tate et al., 2016). Therefore, this hypothesis was tested in two parallel studies in settings with relevance to RVV performance – an urban slum in Karachi, Pakistan (Chapter Three) and a rural area in Navrongo in northern Ghana (Chapter Four). The two studies employed a matched case-control study design, comparing the pre-vaccination fecal microbiome between infants with (case) and without (control) seroconversion following Rotarix™ (RV1) vaccination. Infants were matched for known confounders of RV seroconversion and intestinal microbiome composition including RV season, age, mode of delivery, feeding practices, and malnutrition. In both settings, the intestinal microbiome composition differed significantly between RVV responders and non-responders.

The study in Pakistan in Chapter Three was limited by sample size, as only 10 of 66 infants seroconverted. Nevertheless, Proteobacteria, specifically Gamma-proteobacteria from the Enterobacteriacea family were enriched in RVV responders. Pakistani infants were then matched with healthy Dutch infants assumed to have normal RVV seroconversion, and a linear association between Enterobacteriacea and RVV response was maintained with the highest abundance of Enterobacteriacea in Dutch children and the lowest in Pakistani non-responders. Chapter Four describes the parallel study in Ghana, which was larger, with 39 paired responders and non-responders. Microbiome differences between responders and non-responders were more pronounced and distinct from Pakistani infants. In Ghana, the differences were driven primarily by an increased abundance of the Bacteroidetes phylum in the non-responder infants and an increased abundance of bacteria related to Streptococcus bovis in the responder infants. These associations were confirmed in a comparison with 154 matched Dutch infants, and Dutch infants’ microbiota overall were significantly more similar to Ghanaian responder than to non-responders. These two independent yet parallel studies with synchronized methodology demonstrate that significant, albeit geography-
dependent, correlations between microbiome composition and RVV response exist.

Chapter Five is a more in-depth look at the cohort of Ghanaian infants described in Chapter Four. It tests whether correlations in microbiome composition and RVV response extend beyond the pre-vaccination time point. It also presents preliminary data on correlations between intestinal virome and mycobiome composition and RVV immunogenicity. Bacterial microbiome composition emerges as the most strongly correlated with RVV seroconversion at the first vaccination dose. A high abundance of Bacteroidetes and the presence of specific Bacteroidetes taxa prior to the first dose of vaccine correlate with low RVV seroconversion. In addition, a Proteobacteria bloom following the first vaccination correlates with seroconversion, suggesting that Proteobacteria expansion either supports RVV replication or is a result of effective RVV replication and disruption. Broader characterization of other kingdoms also suggests novel microbiome differences between RVV responders and non-responders. Fungal ITS sequencing suggest that a higher abundance of fungi as well as an increased fungal diversity at the first dose of vaccination correlated with decreased RVV seroconversion. Finally, preliminary evaluation of the virome revealed an incredible richness in eukaryotic viruses and phage in these infants and several correlative visual trends. This study provides an exceptionally dense and deep description of developing country infants’ microbiome between 6 and 15 weeks of age, a time-window critical for vaccine-induced immunity, and underscores potential interactions between bacterial, fungal and viral communities in determining RVV seroconversion.

Part III - From correlation to causation
Statistically significant correlations between microbiome composition and RVV immunogenicity are not sufficient proof that the intestinal microbiome composition has a causal role in determining RVV immunogenicity. It is important to establish this causal role for the intestinal microbiome in RVV immunogenicity prior to placing infants at risk and devoting public health resources in altering the infant microbiota. The third part of this thesis therefore tackles the imperative to move from intriguing association studies to establishment of causation and ultimately mechanism. The thesis describes two strategies to establish causation between the intestinal microbiome and RVV immunogenicity: a rotavirus mouse model (Chapter Six) and a human volunteer study (Chapter Seven).

Six describes the establishment of a novel murine model that can be used to interrogate microbiome-rotavirus interactions. Genetically-identical mothers and
pups from three vendors (Charles River (CR), Taconic (Tac) and Envigo (Env)) were infected with the homologous rotavirus EDIM. Mice from CR had delayed and significantly higher RV Ag shedding as well as increased RV-specific IgA and IgG when compared to mice from both Tac and Env. Microbiome compositions differed significantly per vendor. Mice from CR had both a unique increase in alpha diversity between 0 and 10 days post-infection and a higher abundance of the genera Turicibacter, Faecalibaculum, and Bifidobacterium distinguishing them from both Tac and Env over time. Thus, CR mice had both a highly immunogenic phenotype and distinct microbiome, suggesting that an immunogenic RV phenotype correlates with a vendor-specific microbiome. This novel murine model reinforces the correlation between RVV immunogenicity and microbiome composition as seen in Chapters Three to Five. Yet it also provides a much-needed in vivo platform on which to test if alteration of the intestinal microbiome alters RVV immune responses and potential mechanisms or RV-microbiome interactions. The potential of this platform to elucidate mechanism is discussed in Future Directions, Chapter Nine.

Finally, Chapter 7 describes a proof-of-principle clinical trial in adult human volunteers. The study hypothesized that if the intestinal microbiome causally influences RVV immunogenicity, then alteration of the intestinal microbiome should alter RVV immunogenicity. The study was designed as an open-label randomized control trial that enrolled healthy men aged 18-35 in the Netherlands. The study aimed to maximally suppress the bacterial microbiome by using a broad-spectrum antibiotic regimen and also alter the microbiome to resemble immunogenic phenotypes in Pakistan (increased Proteobacteria) and Ghana (diminished Bacteroidetes) by using oral vancomycin. Subjects were therefore randomized to one of three arms: 7-days broad-spectrum (oral vancomycin, ciprofloxacin, and metronidazole), narrow-spectrum (oral vancomycin), or no antibiotics. Subjects were then vaccinated with RVV (Rotarix™), polysaccharide-pneumococcal (Pneumo 23), and tetanus-toxoid vaccine. The primary study endpoint was difference in 28 days-post-vaccination anti-RV IgA height. Secondary endpoints were proportion of volunteers with day 7 anti-RV IgA boosting (≥2-fold increase), absolute and proportion of RV-antigen shedding, anti-RV, pneumococcal, and anti-tetanus IgG and correlations between microbiome and outcomes. 21 volunteers per study arm (63 in total) completed the study per-protocol. Baseline anti-RV IgA was high in all groups. There were no 28-day anti-RV IgA differences but anti-RV IgA boosting was significantly higher in the narrow-spectrum group (8/21 vs. 1/21 each, RR=0.125, 95% CI 0.02-0.67, p=0.021). Higher proportions of volunteers shed RV in narrow and broad-spectrum groups (8/21 each vs. 1/21, RR 8, 95%
CI 1.5-47.4, p=0.02) and narrow-spectrum antibiotics had significantly higher OD-shedding overall (p= 0.0027). There were no differences in anti-RV, tetanus or pneumococcal total IgG. Both antibiotic treatments decreased Bacteroidetes; vancomycin alone reduced Firmicutes and expanded Proteobacteria abundance. This study therefore demonstrates that targeted alteration of the intestinal microbiota boosts rotavirus vaccine response in healthy adults. The results fulfill the proof of principle that the intestinal microbiome has a causative role in determining RVV immunogenicity and thereby suggest microbiome manipulation could improve rotavirus vaccine immunogenicity in developing countries.

Part IV - From causation to intervention

In summary, rotavirus causes life-threatening gastroenteritis in infants across the globe. RVV designed to protect infants against the disease have diminished effectiveness in developing countries with the highest burden of rotavirus mortality. This thesis hypothesizes that one of the factors determining the diminished RVV effectiveness in developing countries is the intestinal microbiome composition. This thesis establishes that there are geographic-specific but nonetheless significant correlations between the intestinal microbiome composition and RVV immunogenicity in developing country settings where RVVs have diminished effectiveness. The thesis outlines a novel murine model that could be a platform upon which to test the effect of microbiome modulation on RVV immunogenicity. Finally, the thesis demonstrates that prospective alteration of the intestinal microbiome in an adult volunteer model is capable of altering RVV immunogenicity. Chapter Nine, Discussion and Future Directions, speculates how these findings could be used as a basis for determining mechanisms behind microbiome, RVV, and host interactions and ultimately, harnessing the potential of the microbiome for improving RVV immunogenicity for developing country infants.
REFERENCES

