Early Infancy Gut Microbiota Predicts the Quality of Vaccine-Induced Antibody Responses in Rhesus Macaques

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Introduction

- We remain without a highly-effective HIV vaccine or novel strategies for pediatric HIV-1 prevention that move beyond ART-based therapy.
- Evidence is emerging in a number of vaccine settings that commensal microbiota are linked to vaccine-elicited immune responses.
- The gut microbiota is most plastic during infancy, with the transition from a relatively sterile environment in utero to one of constant exposure to pathogenic and nonpathogenic microbial organisms .
- A successful HIV-1 vaccine may need to harness the unique landscape of the pediatric immune system by early immunization with concurrent rational manipulation of the microbiota to enhance vaccine efficacy.

Study goal:

- To define the relationship between the developing microbiota in infant rhesus monkeys and the immunologic response following HIV-1 vaccination.

Methods

- Four groups each consisting of 5 neonatal Rhesus monkeys were immunized on distinct immunization schedules:

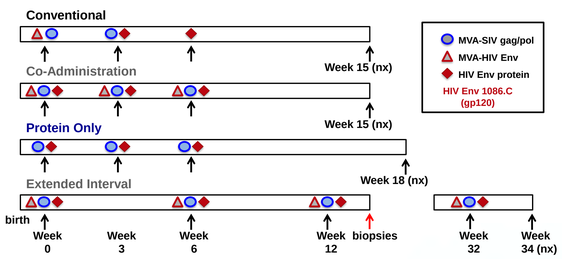


Figure 1. Vaccine Schedule of Infant Rhesus Macaques. 1) *Conventional* regimen animals were given modified vaccinia Ankara virus (MVA) expressing HIV-env (IM) and MVA-SIVgag/pol (IM) at week 0, followed by MVA-SIVgag/pol and HIV-env protein (IM/IN) at week 3, then administered a final dose of HIV-env protein at week 6; 2) the *Co-Administration* group received MVA-SIVgag/pol, MVA-HIV-env, and HIV-env protein concurrently at 0, 3, and 6 postnatal weeks; 3) the *Protein Only* regimen were dually immunized with MVA-SIVgag/pol and HIV-env protein at 0, 3 and 6 postnatal weeks, and 4) similar to group 2, the *Extended Interval* regimen received a co-administration of MVA-SIVgag/pol, MVA-HIV-env, and HIV-env protein over an extended time course of 0, 6, 12 and 32 postnatal weeks.

- Envelope-specific binding responses were measured by ELISA; functional antibody responses were measured by neutralization by TZMbL assay, and ADCC using the GranToxiLux assay with gp120-coated target cells.
- Phylogenetic profiling of infant microbiomes was conducted by extracting 16S ribosomal RNA from stool samples. The variable region 4 (V4) of 16S rRNA was amplified and amplicons sequenced using the Illumina MiSeq platform.
- 16S rRNA reads were quality filtered, demultiplexed, and clustered into operational taxonomic units (OTUs) using vsearch. A subsequent diversity analysis was performed with *QIIME*, *LASSO* regression, PCA, and other machine based learning algorithms.
- The immune responses to the vaccine regimens were correlated to corresponding microbiome population.

Results

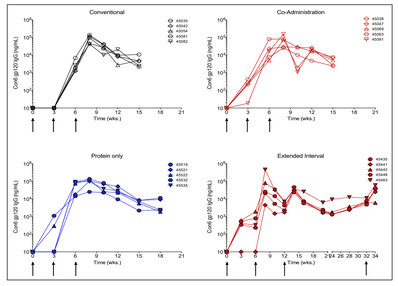


Figure 2. Infant monkey vaccine-elicited plasma gp120-specific IgG kinetics. Kinetics of the HIV-1 envelope-specific IgG response was slightly more rapid in the "Protein Only" regimen, but the maximum responses did not differ between the groups. Response persisted in the "Extended Interval" group.

Data by: Erika Kunz

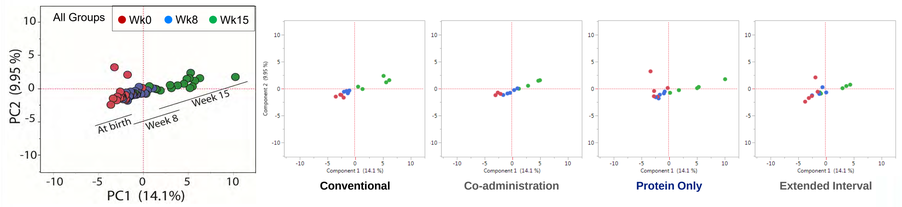


Figure 3: Microbial diversity by vaccine group at the genera level, over time. Diversity is variable within the vaccine groups, though there is a trend of increased diversification as the infants age.

Data by: Amir Ardeshir

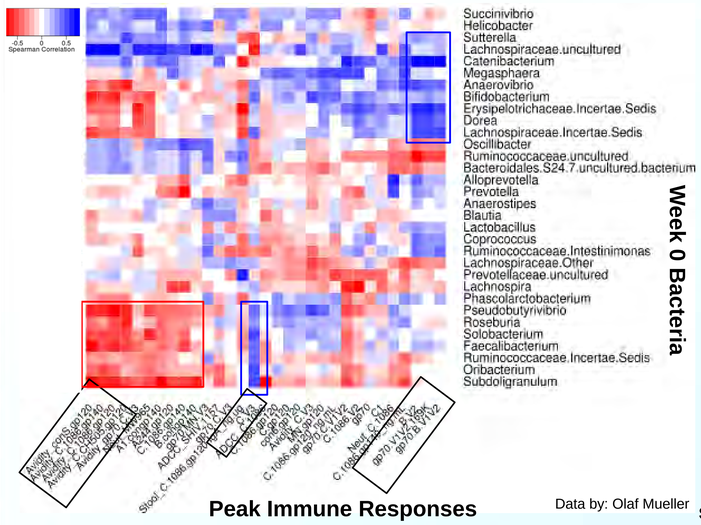


Figure 4. Correlations between week 0 bacteria taxa and antibody measures from the peak time point (2 weeks after the 3rd vaccine) showed a strong, inverse correlation between the avidity immunologic data and a subset of bacteria (red box). There was also a strong, positive correlation between the V1V2 and V3 binding with a disparate bacteria subset (blue boxes).

Data by: Olaf Mueller

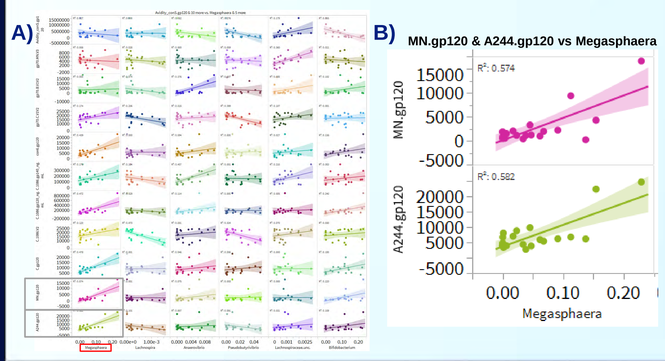
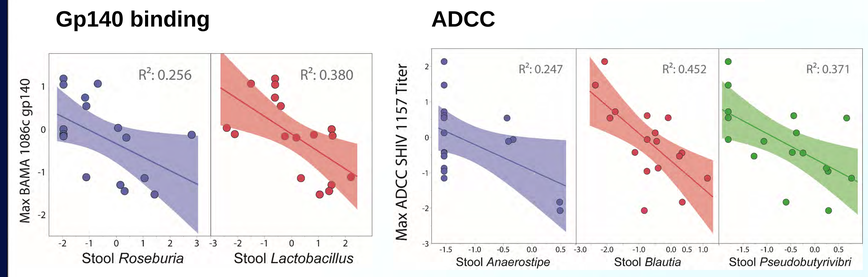


Figure 5. Correlations of distinct Week 0 taxa vs. peak immunogenicity. A) Majority of correlations' p-value (not FDR) <0.007. Boxed correlations are FDR corrected and seen in panel 5B. B) Correlations of *Megasphaera* vs. A244.gp120 and MN.GP120 met FDR (<0.06), among all correlations. Strong positive correlations observed.

Results



Adaptive LASSO:
R-square training set (70%): 0.61
R-square validation set (30%): 0.41

Adaptive LASSO:
R-square training set (70%): 0.43
R-square validation set (30%): 0.25

Figure 6: LASSO (least absolute shrinkage and selection operator) regression identifies week 0 bacterial taxa negatively associated with Env gp140-binding and ADCC responses from the peak immune response time point. Variables are transformed using Johnson transformation.

Data by: Amir Ardeshir

Conclusions

- The kinetics of the HIV-1 envelope-specific IgG response were similar, and the maximum responses did not differ between the groups.
- Microbiota diversity indexes indicated greater bacterial diversity at week 15/18 compared to week 0 or week 8.
- The magnitude of the vaccine-elicited gp120-specific IgG responses positively correlated with the frequency of the stool bacteria Megasphaera present at birth.
- The frequency of Lactobacillus and Roseburia in stool bacteria at birth was negatively associated with vaccine-elicited gp140-specific binding IgG responses whereas Anaerostipes, Blautia, and Pseudobutyrivibrio was negatively associated with ADCC responses.
- These exploratory data suggest that in infant rhesus macaques, certain intestinal microbiota is associated with HIV Env-elicited immune responses.

Future Directions

- An additional cohort of infant macaques' stool and saliva will be analyzed using the same methods, validating this data set.
- Subsequent investigations will seek to identify specific taxa that enhance Env-elicited immunity, thus facilitating rational manipulation of the microbiome using probiotics to enhance potentially-protective immune responses following HIV-1 immunization.

Acknowledgements

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