Early Infant 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 macaques and the immunologic response following HIV-1 vaccination.

**Methods**

- Four groups each consisting of 5 neonatal rhesus monkeys were immunized on distinct immunization schedules:
  - Conventional
  - Co-Administration
  - Extended Interval
- Co-Administration

**Figure 1. Vaccine Schedule of Infant Rhesus Macaques.**

Conventional regimens received modified vaccinia Ankara virus (MVA) expressing HIV-1 env (IM) and MVA-SIVgag/pol (IM) at week 0, followed by MVA-SIVgag/pol and HIV-1 env protein (IM/W) at week 3, and 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-1 env protein concurrently at 0, 3, and 6 postnatal weeks. 3) The Protein Only regimen was dually immunized with MVA-SIVgag/pol and HIV-1 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-1 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 RNA 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 populations.

**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.

**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.

**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 V1Y2 and V3 binding with a disparate bacteria subset (blue boxes).

**Figure 5. Correlations of distinct Week 0 taxa vs. peak immunogenicity.**

A) Majority of correlations' p-value (not FDR) <0.007. Both correlations for FDR corrected and seen in panel 5B. B) Correlations of Megasphaera vs. A244, gp120 and MNA-gp120 met FDR (<0.06), among all correlations. Strong positive correlations observed.

**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**

Overall support for the HVRAD MIV01 study was provided by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health under Award #5P01AI117915. The content is solely the responsibility of the authors and does not necessarily reflect the official views of the National Institutes of Health.