Clinical and Immune Characteristics of ART-treated SIV/*P. fragile* Co-Infected Rhesus Macaques with Persistent Viremia

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BACKGROUND

- HIV and malaria, caused by infection with *Plasmodium* spp., are co-endemic in similar geographical locations[1].
- HIV and malaria are reciprocally antagonistic; co-infection exacerbates the risk and transmission of each disease[2-4].
- The gastrointestinal (GI) tract is a major HIV reservoir and *Plasmodium* spp. can sequester in the GI mucosa, both of which cause GI damage that associates with disease pathogenesis[5-8].
- The innate immune system is critical in the immune response against both HIV and *Plasmodium*[9,10].
- Neutrophils play both protective and detrimental roles in HIV and malaria[11-14], but their function in HIV/*Plasmodium* co-infection is not well understood.

The innate immune mechanisms by which P. fragile co-infection impacts SIV dynamics and accelerates disease progression, especially in the GI tract, is poorly understood.

HYPOTHESIS

P. fragile co-infection of ART-treated SIV+ RMs will result in persistently detectable SIV viremia and CD4+ T cell disruption, which will correspond with increased neutrophil frequency and inflammatory function.

METHODS

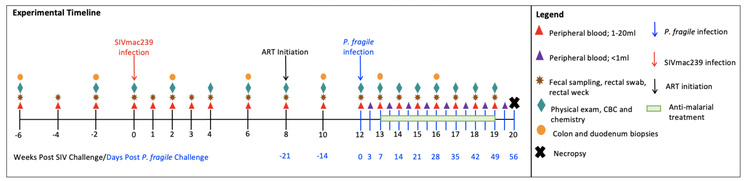


Figure 1. Experimental timeline depicting sample collection from adult male rhesus macaques (RM; n=6).

- SIVmac239: Intravenous (i.v.) inoculation; TCID50=50.
- Daily ART: Subcutaneous TDF/FTC/DTG; 5.1/30/2.5 mg/kg, respectively.
- *P. fragile*: i.v. inoculation; 20x10^6 infected erythrocytes.
- Anti-malarial drugs: quinine sulfate (150mg) or chloroquine (20mg/kg)

In all figures:

- Vertical dashed lines represent inoculation timepoints
- Dashed line above graph is indicative of the duration of ART
- Green dotted bar on the x-axis indicates the time period during which any RM received anti-malarial drugs.
- Baseline (BL) represents the median of data collected at weeks -6, -4, -2, and 0.
- Statistical significance back to BL: Geisser-Greenhouse correction and Dunnett's multiple comparisons test, with individual variances computed for each comparison. Multiplicity adjusted significant *P* values are shown above horizontal black bars.
- Statistical significance for AUC: Unpaired, two sample, parametric t-test using the False Discovery Rate (FDR) approach with a desired FDR of 1%.
- Statistical significance between groups: Mixed-effects analysis with the Geisser-Greenhouse correction and Sidak's multiple comparison test, with individual variances computed for each comparison. Multiplicity adjusted significant *P* values are shown above timepoints in pink.

RESULTS

P. fragile co-infection of ART-treated SIV+ RMs results in clinical hallmarks of malaria

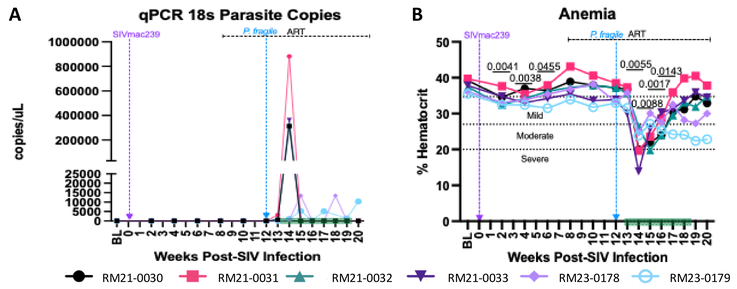


Figure 2. A) Quantification of *P. fragile* 18S DNA via quantitative polymerase chain reaction (qPCR). B) Anemia, as defined by % hematocrit (HCT) or the ratio of red blood cells to total blood, from complete blood counts.

RMs with above median VL during ART maintain greater viremia after *P. fragile* co-infection as compared to below median VL RMs

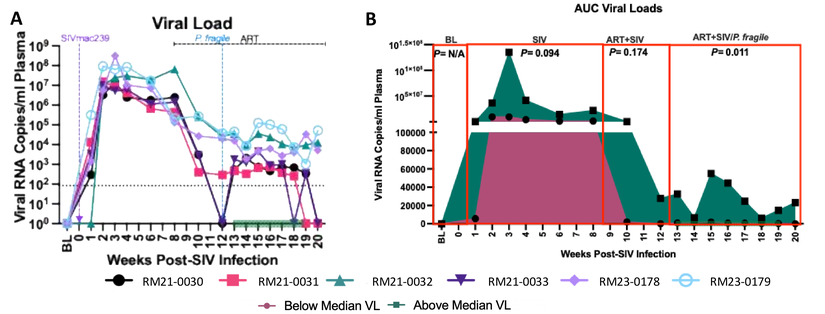


Figure 3. A) Plasma viral loads (VLs; RNA copies/ml). RMs were divided into above and below median VL groups based upon their median VLs at week 10 and week 12 p.i. B) VL area under the curve (AUC) in RMs with above or below median VL.

Above median VL RMs have greater CD4+ T-cell counts following *P. fragile* co-infection as compared to below median VL RMs

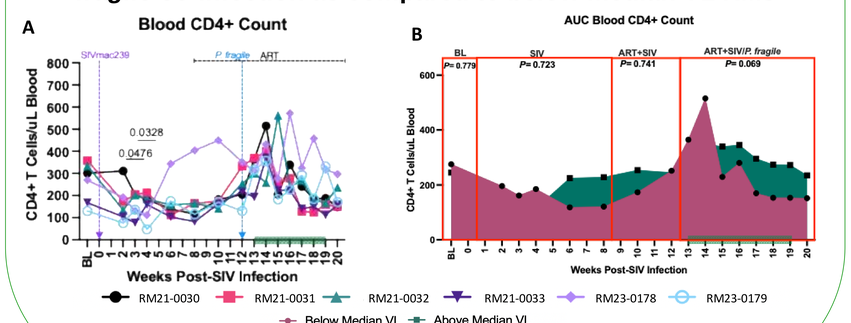


Figure 4. A) Absolute CD4+ T-cell counts per ul/blood. B) Absolute CD4+ T-cell count area under the curve (AUC) in RMs with above or below median VL.

RESULTS, CONTINUED

Above median VL RMs have significantly greater frequencies of neutrophil extracellular trap (NET)-forming neutrophils in the duodenum that associate with peripheral viremia

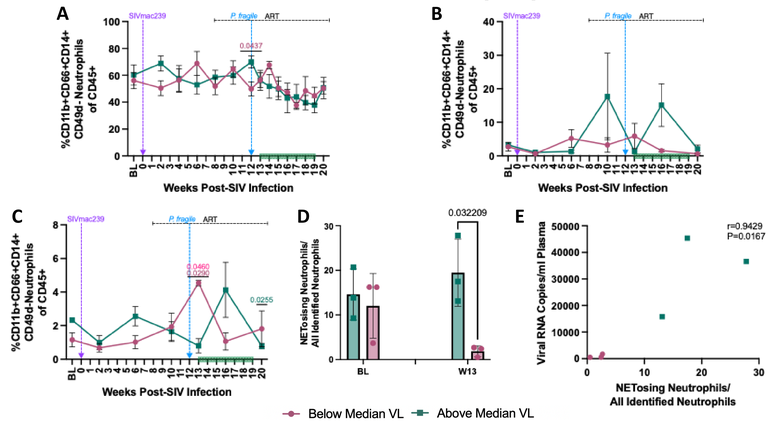


Figure 5. A) Blood, B) colon, and C) duodenum neutrophil frequencies identified as viable, CD45+ HLA-DR- CD11b+ CD66abce+ CD14+ CD49d- cells via flow cytometry in RMs with above and below median VLs. D) Proportion of MPO+CitH3+ neutrophils among total neutrophils in formalin-fixed paraffin embedded (FFPE) duodenum biopsies at BL and week 13 p.i. quantified via fluorescent immunohistochemistry (IHC). E) Correlation of peripheral VLs and the proportion of MPO+CitH3+ neutrophils among total neutrophils in duodenal biopsies.

SUMMARY AND CONCLUSIONS

- Our current data suggests that:
  - All ART-treated SIV+ RMs were successfully inoculated with *P. fragile*.
  - RMs with above median VL maintained significantly higher VLs following *P. fragile* co-infection as compared to RMs with below median VL.
  - Duodenum neutrophil frequencies were significantly higher following *P. fragile* co-infection in below median VL RMs as compared to above median VL RMs.
  - Despite lower total neutrophil frequencies via flow cytometry in the duodenum of above median VL RMs, these animals had greater frequencies of NET-forming neutrophils via fluorescent IHC following *P. fragile* co-infection.
  - The frequency of NET-forming neutrophils in the duodenum was significantly and positively associated with peripheral viremia.

Our data suggest that P. fragile-associated elevations in the frequency of NET-forming neutrophils in the GI tract could contribute to mucosal dysfunction and may subsequently influence viral replication.

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