Fecal microvesicles differentially influence translocating bacterial taxa after progressive SIV infection.

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Introduction

- Microbial translocation contributes to persistent inflammation in both treated and untreated HIV infection.
- Although translocation is due in part to a disintegration of the intestinal epithelial barrier, there is a bias towards the translocation of Proteobacteria, suggesting that translocation is not stochastic.
- In murine models, epithelial-derived microvesicles (MVs) have been shown to influence bacterial gene expression and growth in a cargo-dependent manner.

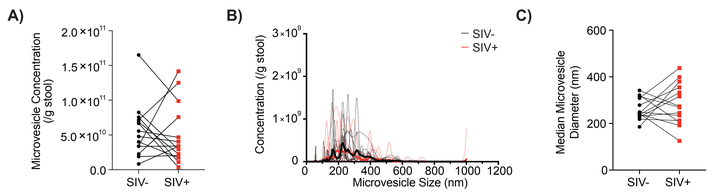
We hypothesize that intestinal epithelial MVs biologically differ after progressive SIV infection and that altered miRNA and/or antimicrobial peptide (AMP) content may contribute to biased microbial translocation.

Methods

- Samples were collected from rhesus macaques (Macaca mulatta) or sooty mangabeys (Cercocebus atys). SIV-infected macaques were experimentally infected with SIVmac239 whereas SIV-infected mangabeys naturally acquired SIVsm. Samples accessed for this study were collected during the chronic phase of infection (> day 150 post-infection) unless noted.
- Feces were collected fresh from each animal by inserting a sterile swab into the rectum and "swirling" to collect available sample. Collected feces were snap frozen and stored at -80˚C until accessed.
- MVs were isolated from feces utilizing the ExoEasy kit (Qiagen).
- MVs were quantified and characterized by Nanosight (Malvern Panalytical) and standardized to $3x10^{10}$ particles/mL.
- Microvesicular miRNA was isolated using the mirVana (Thermo) kit.
- miRNA was quantified by qRT-PCR, utilizing TaqMan Array Human MicroRNA Cards or individual TaqMan Advanced miRNA Assays with spike-in control (**Figure 2**).
- ELISAs were custom designed, utilizing human-reactive, commercially available antibodies (**Figure 3**).
- Translocating bacteria were cultured from liver, spleen, and mesenteric lymph nodes of SIV-infected macaques (**Figure 4**). Tissue biopsies were collected in (aerobic) RPMI and Anaerobic Media and manually homogenized. Homogenates were plated aerobically on Brain Heart Infusion, Eosin Methylene Blue, Trypticase Soy Agar (TSA) +Tween-80, and TSA+5% Sheep's Blood media and anaerobically on Brucella Blood or CDC Blood media. Bacterial isolates were grown for 1-7 days, streaked for pure colonies, identified by MALDI-TOF or 16S rRNA sequencing, and stored as glycerol stocks at -80ºC until accessed.
- Bacteria were co-cultured with MVs and bacterial growth measured by spectrophotometer (**Figure 5**). Bacterial cultures were aliquoted into individual wells with MVs and growth measured at OD600 in through stationary phase.

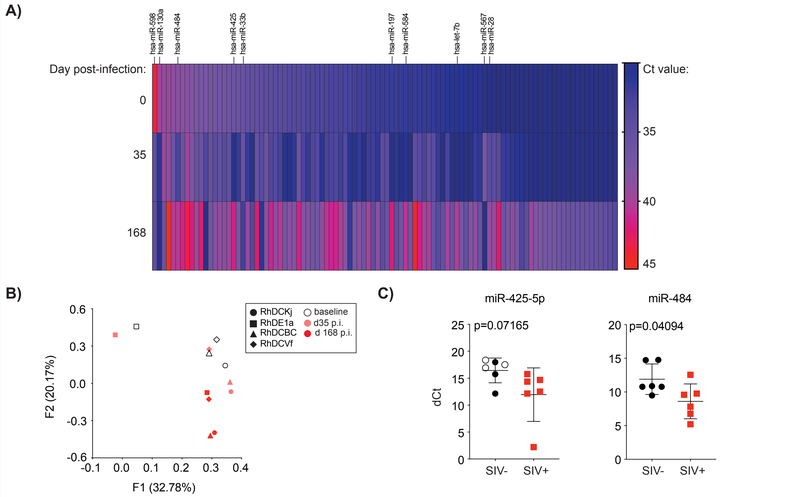
Results

Figure 1. Fecal microvesicle load and distribution is comparable after progressive SIV infection.
- Fecal microvesicle concentration and diameter distribution are similar after SIV infection in macaques.



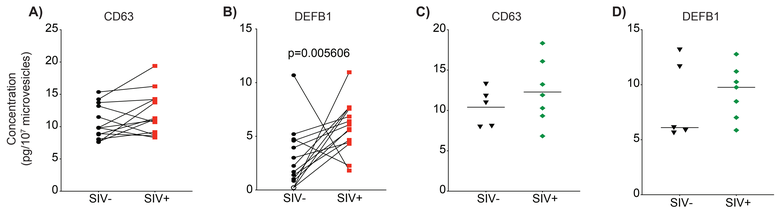
A) Mean concentration of MVs per gram of stool in SIV-uninfected and -infected rhesus macaques. **B)** Distribution and concentration of fecal MV diameter in SIV-uninfected and -infected rhesus macaques. Dim lines represent individual distributions, heavy lines represent median distributions. **C)** Median diameter of fecal MVs in SIV-uninfected and -infected rhesus macaques.

Figure 2. Intestinal microvesicles from SIV-infected rhesus macaques display increased host miRNA expression.
- Differences from baseline are apparent during acute and chronic infection.
- Chronic infection samples show clustering distinct from baseline and acute samples.
- miR-484 and miR-425-5p are higher in post-SIV intestinal MVs.



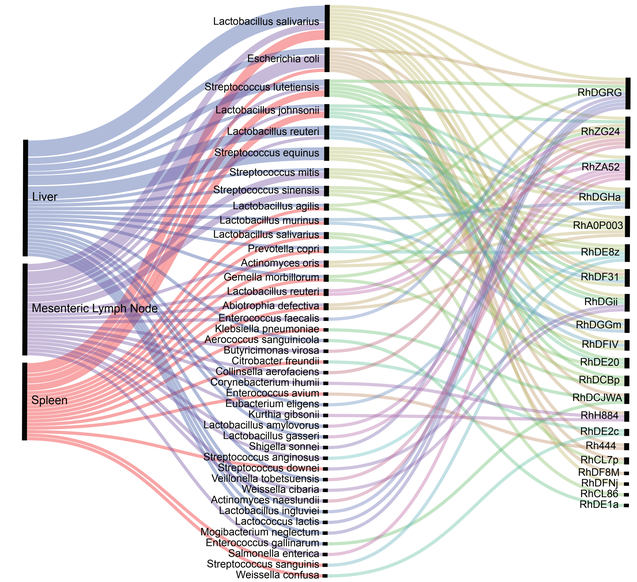
A) Heatmap displaying longitudinal host miRNA expression from macaque fecal MVs isolated at baseline and days 35 and 168 post-SIV. Data ordered from highest to lowest expression (Ct) value at baseline for miRNAs where Ct values above threshold were obtained from more than one animal at more than one timepoint. **B)** Principle component analysis considering animal, day post-infection, and Ct values of miRNA selected as in **A**. **C)** qRT-PCR results of two differentially regulated miRNAs identified in **A**. Values are normalized to spike-in and represent the mean dCT of individual, unpaired samples in triplicate. Ct values below threshold were normalized to 45 and are represented by open symbols. Significance in **C** assessed by unpaired, two-way t-test.

Figure 3. bDEF1 is significantly in within intestinal microvesicles after progressive SIV infection.
- Expression of tetraspanin and exosomal marker CD63 is preserved after SIV infection.
- Perturbed bDEF1 expression is unique among MV isolated after progressive SIV infection.



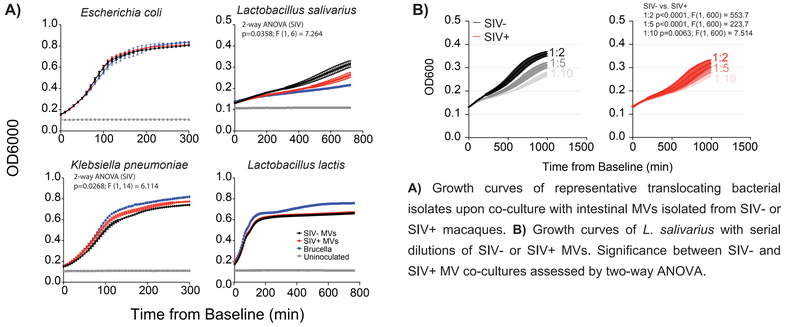
Concentrations of exosomal marker CD63 and antimicrobial peptide beta-defensin 1 (DEFB1) in fecal MVs from SIV- and SIV+ paired macaques (**A+B**) or unpaired mangabeys (**C+D**). Absorbance below the limit of detection (LOD) of each assay normalized to the LOD (open symbols). Significance assessed by paired (**A+B**) or unpaired (**C+D**) two-way t-test.

Figure 4. Translocating bacterial taxa in SIV-infected macaques are phylogenetically disparate.
- Isolated, translocating taxa span multiple taxa and bacterial classifications.
- Commonly identified taxa show the capacity for systemic dissemination.



Alluvial diagram linking the tissue and animal origin of all isolated bacterial species.

Figure 5. Translocating bacteria show differential sensitivity to MV co-culture.
- Individual bacterial isolates display differing sensitivities upon co-culture with intestinal MVs.
- *L. salivarius* and *K. pneumoniae* show unique sensitivity to co-culture with MVs from SIV+ macaques.



A) Growth curves of representative translocating bacterial isolates upon co-culture with intestinal MVs isolated from SIV- or SIV+ macaques. **B)** Growth curves of *L. salivarius* with serial dilutions of SIV- or SIV+ MVs. Significance between SIV- and SIV+ MV co-cultures assessed by two-way ANOVA.

Conclusions and Future Directions

- Fecal MV cargo differs between SIV- and SIV+ macaques, with MVs from SIV+ macaques displaying increased miR-484 and increased DEFB1.
- *L. salivarius* displays a high propensity for translocation and is uniquely responsive to MVs from SIV+ animals.
- Translocating bacteria display differing sensitivities to co-culture with intestinal MVs.

During lentiviral infections, unique biological signatures of intestinal MVs may promote the translocation of specific bacterial species. Understanding the mechanisms that promote the translocation of specific taxa may lead to the development of unique anti-microbial therapeutics.