Isolation of Translocating Bacteria in Progressive SIV Infection of Rhesus Macaques

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BACKGROUND

- Microbial translocation is commonly observed in HIV-infected humans and is a significant contributor to inflammation.
- In SIV-infected Asian macaques, translocation has been demonstrated to occur across the gastrointestinal barrier; however, translocating bacterial taxa are not representative of the gut microbiota, with Proteobacteria appearing to preferentially translocate.
- To fully characterize translocating bacterial populations, we isolated systemically translocating bacteria from chronically SIV-infected macaques and identified them subsequent to live culture.

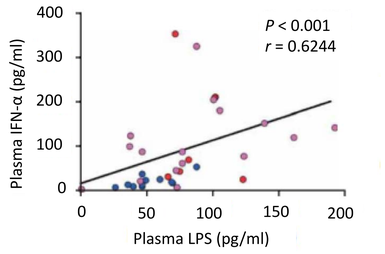


Figure 1. Microbial translocation as a contributor to immune activation
Positive correlation between LPS and IFN-α plasma levels, in cohorts of subjects chronically infected with HIV and individuals who have progressed to AIDS[1].

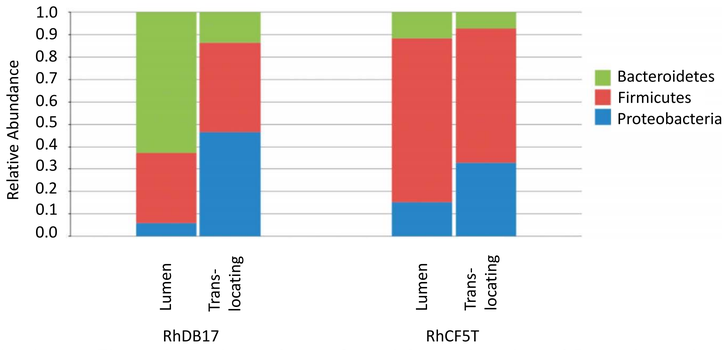


Figure 2. Evidence for preferential translocation of Proteobacteria from the colon
Relative abundance of Bacteroidetes, Firmicutes, and Proteobacteria in the lumen and sites of translocation in the lamina propria of SIV-infected rhesus macaques by qPCR[2].

EXPERIMENTAL GOAL

To isolate bacteria that are preferentially translocating from the gut to other bodily sites in rhesus macaques (*Macaca mulatta*) undergoing progressive SIV infection; identify and preserve isolates for future experiments.

METHODS

- Liver, mesenteric lymph node (MLN), and spleen samples were taken aerobically and anaerobically during necropsy from seventeen, chronically SIV-infected rhesus macaques.
- Swabs were used on the chest/abdomen of necropsied macaques to sample the skin microbiome as a control for potential contamination. Samples from uninfected rhesus macaques are not available as a control at this time.
- Tissue samples were mechanically homogenized and plated on: a) Brain Heart Infusion (BHI), b) Eosin Methylene Blue (EMB), c) Trypticase Soy Agar (TSA)+Tween 80, and d) TSA+5% Sheep's Blood media under aerobic conditions, and e) Brucella Blood and f) CDC Blood media under anaerobic conditions.
- Isolates were grown at 37ºC for 1-7 days, depending on observed density. Colonies were streaked for pure cultures and identified using MALDI-TOF and 16S rDNA sequencing.
- Isolates found to be present in both the tissue and skin microbiomes of the same animal were eliminated from consideration.
- Relevance of a species to the study was determined by whether or not the isolate was a normal resident of the digestive tract, with our focus being on species that are intestinal residents (other species have been isolated, but excluded based on this criteria).

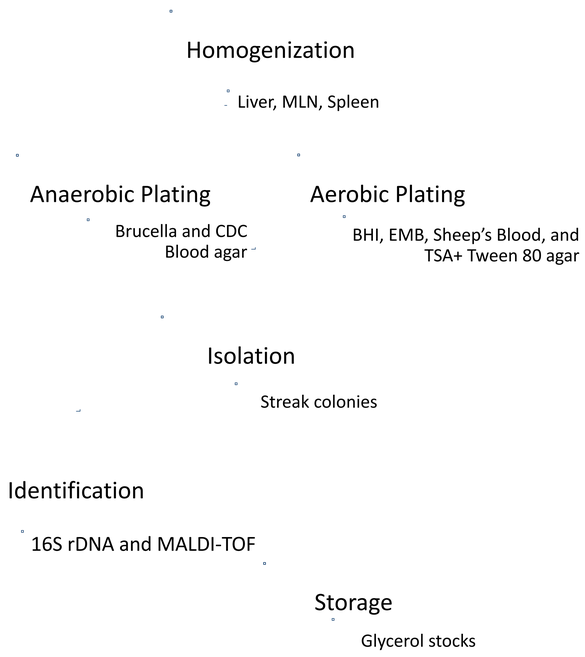


Figure 3. Schematic setup for isolation protocol.
Tissues are homogenized, then spread plated. Colonies are streaked from spread plates for pure cultures, which are used for identification and long term storage preparation.

RESULTS

Twenty-Eight Relevant Species Isolated and Identified

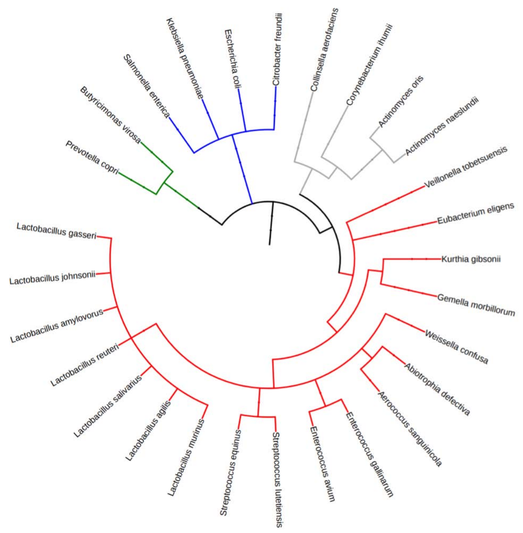


Figure 4. Phylogenetic distribution of identified isolates.
Phylogenetic tree displaying isolates by 16S rDNA sequence relatedness. Branches are colored by Phylum of the included bacteria: Bacteroidetes in green (2/28), Proteobacteria in blue (4/28), Actinobacteria in grey (4/28), and Firmicutes in red (18/28). Of particular interest are the species of *Lactobacillus salivarius* and *Escherichia coli*, which have been found to be translocating in five and four animals, respectively. A potential novel species from the *Clostridium* genus has also been isolated, but is still under investigation.

Majority of Translocating Bacteria are from the Phylum Firmicutes

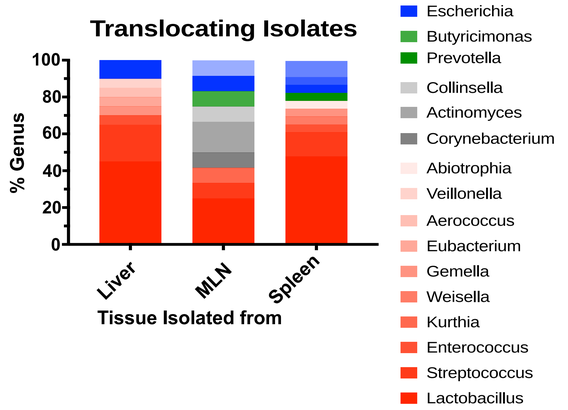


Figure 5. Genera abundance of relevant species isolated.
Distribution plot of relative abundances of isolated taxa based on genus level classification and tissue source. For tallying genera, if a species was found in more than one animal, it received a point for each animal it was found in. Genera from Bacteroidetes are in shades of green, Proteobacteria in shades of blue, Actinobacteria in shades of grey, and Firmicutes in shades of red. Contrary to expectation, species from the Firmicutes phylum make up the majority of isolated translocating bacteria, not Proteobacteria.

Future Directions

•Although bacterial taxa have been shown to translocate across the gastrointestinal epithelium, translocating taxa do not mirror the composition of the gastrointestinal microbiome, suggesting that translocating bacterial taxa are enriched for motile taxa or are functionally altered.

•We are currently preparing samples for a comparative proteomics study between translocating and non-translocating bacterial species, in an effort to identify proteins that may be aiding in translocation in the context of progressive SIV infection.

•In order to confirm localization of bacteria in tissues, we are planning on performing fluorescence in situ hybridization targeting 16S rRNA on preserved tissues.

•We intend to grow these isolates in the presence of sorted immune cell populations from SIV-uninfected and -infected macaques in order to measure microbial growth and motility to determine how infected host immunity contributes to biased translocation.

ACKNOWLEDGEMENTS

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REFERENCES

1. Brenchley JM, Price DA, Schacker TW, et al.: Microbial translocation is a cause of systemic immune activation in chronic HIV infection. Nat Med 2006;12(12):1365–1371.
2. Z. Klase et al. Dysbiotic bacteria translocate in progressive SIV infection. Mucosal Immunol, 8 (2015), pp. 1009-1020