Commensal Bacteria Within the Family Lachnospiraceae Robustly Inhibit HIV Infection

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Introduction

- The **gut microbiota** plays a vital role in host physiology and alters outcomes of numerous infectious diseases.
- HIV infection induces a dysbiosis in the gut microbiota, though it is not clear whether the gut microbiota—and **which specific microbes and mechanisms** —impacts susceptibility to HIV.

Identification of bacterial taxa that inhibit HIV

- We analyzed the gut microbiota of nursery-reared infant rhesus macaques orally challenged with SHIV, an NHP model for breastfeeding transmission of HIV[1] and utilized **microbe-phenotype triangulation**, a highly-specific platform we developed[2], to identify microbes related to protection (**Fig. 1**).

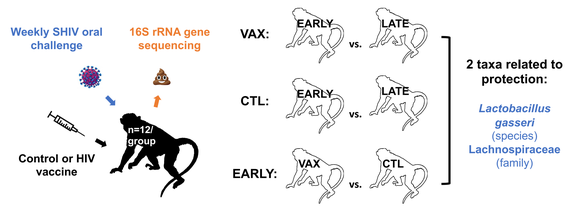


Figure 1. Schematic of the NHP study[1] and the microbe-phenotype triangulation approach[2] that associates two taxa with protection against HIV. Early and late infection groups were defined by both the experimental design and the distribution of time to acquisition of SHIV. DESeq2 was used to detect differentially abundant taxa for each pairwise comparison at the amplicon sequence variant level, with a focus on those taxa that are present in the first two but not the last.

- We acquired **human-derived isolates** of the bioinformatically identified taxa and showed they **robustly inhibit HIV** in TZM-bl cells. Two Lachnospiraceae isolates, *Clostridium immunis and Ruminococcus gnavus*, had the greatest inhibitory effect (**Fig. 2)**.
- Consistent with these results, HIV-naive and HEU children had greater fecal abundance of **Lachnospiraceae** compared to children perinatally acquired HIV, which suggests the NHP findings may generalize to humans (**Fig. 3**).

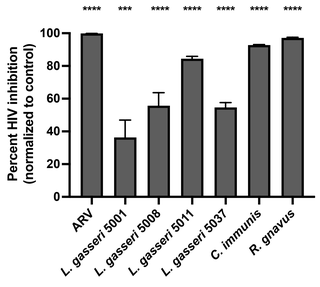


Figure 2. Percent HIV inhibition of the identified taxa *in vitro*. Asterisks above indicate significance level compared to the control bacterium using ANOVA corrected for multiple comparisons (Prism standards). Data plotted are representative of three independent experiments; error bars: SEM above mean (same for all figures unless noted otherwise).

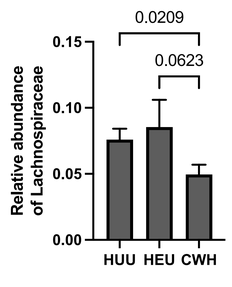


Figure 3. Relative abundance of the family Lachnospiraceae in fecal microbiota of a children cohort from Kenya. Age: 1-5 years, n=135. HUU: HIV unexposed uninfected; HEU: HIV exposed uninfected; CWH: children with HIV. Numbers above indicate p-values of Welch's ANOVA corrected for multiple comparisons.

We identified tryptophan-metabolizing commensals that inhibit HIV replication via the aryl hydrocarbon receptor, providing mechanistic insights into how the gut microbiota impacts susceptibility to HIV.

The tryptophan-metabolizing bacterial ArAT is critical for inhibiting HIV

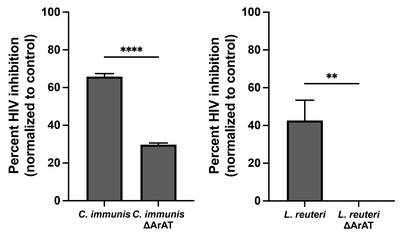


Figure 4. Percent HIV inhibition of wild type vs. ΔArAT mutant for *C. immunis* and *L. reuteri in vitro*. Our lab generated the isogeneic mutant for *C. immunis*, and acquired the *L. reuteri* strains externally[3]. Asterisks above indicate significance level from unpaired t-tests.

- Since tryptophan metabolism has been linked to HIV pathogenesis, we developed bacterial genetics for *C. immunis* and inactivated the **aromatic amino acid aminotransferase (ArAT) gene**, key to the microbially regulated tryptophan-indole pathway.
- *C. immunis* ΔArAT is **less effective** in inhibiting HIV, while another species, *Lactobacillus reuteri* also inhibits HIV infection via the ArAT (**Fig. 4**).
- In the NHP study, **metagenomics sequencing** of the same fecal samples also revealed a correlation between the ArAT gene level and time to SHIV acquisition (Spearman's rho=0.4, p=0.07).

Activation of the AhR pathway via an ArAT indole metabolite inhibits HIV

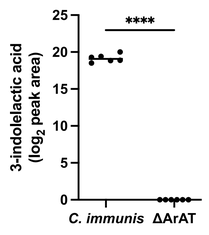


Figure 5. Mass spectrometric quantitation of 3-ILA produced by *C. immunis* and its ΔArAT mutant. Asterisks above indicate significance level from unpaired t-test.

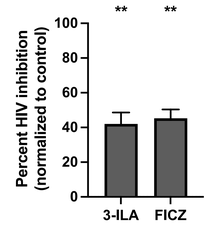


Figure 6. Percent HIV inhibition of 3-ILA and FICZ *in vitro*. Asterisks above indicate significance level compared to the control (DMSO) using ANOVA corrected for multiple comparisons.

- We and others confirmed the bacterial ArAT metabolizes tryptophan into **3-indolelactic acid (3-ILA)** in both *C. immunis* (**Fig. 5**) and *L. reuteri* [3], which is known to activate the **aryl hydrocarbon receptor (AhR)** [4].
- Purified 3-ILA and 6-formylindolo(3,2-b)carbazole (FICZ), another tryptophan derivative from UV radiation and **AhR agonist**, both inhibit HIV (**Fig. 6**).

The bacterial ArAT gene impacts RT and IN of HIV

- We used time-of-addition assays[5] to identify **steps in the HIV replication cycle** targeted by the ArAT gene downstream of AhR (**Fig. 7**). *C. immunis* inhibited entry, reverse transcription (RT), and integration (IN), while the ΔArAT mutant **lost the ability** to inhibit after entry.

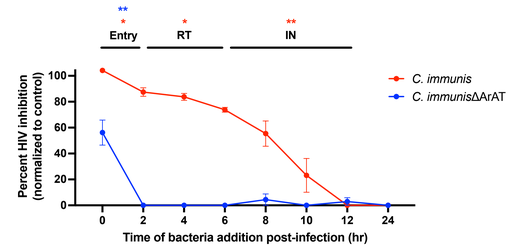


Figure 7. Percent HIV inhibition of *C. immunis* and its ΔArAT mutant relative to the hours of addition post HIV infection. Hours that HIV replication steps take place were determined by this assay using HIV drugs targeted for each specific step. Asterisks above indicate significance level for each step using Welch's ANOVA corrected for multiple comparisons.

Viral inhibition via bacterial ArAT is generalizable

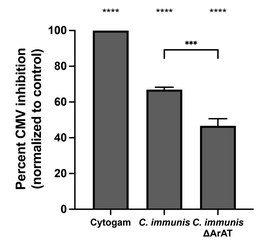


Figure 8. Percent CMV inhibition *in vitro*. Asterisks above indicate significance level using ANOVA corrected for multiple comparisons.

- The AhR is a **transcription factor** known to regulate immunity and affect viral infections[4].
- *C. immunis* also **inhibits CMV infection** via the ArAT gene in ARPE cells (**Fig. 8**), which further generalizes our finding.

Conclusion

- **Tryptophan metabolism by commensal bacteria inhibits HIV** and CMV replication via the bacterial ArAT gene and the AhR signaling pathway.
- Our finding provides **mechanistic insights** on the role of microbiome in HIV and adds to an emerging field of **commensal bacteria–virus interactions.**

References and Acknowledgements

1. Curtis AD 2nd, et al. Vaccine-Induced, High-Magnitude HIV Env-Specific Antibodies with Fc-Mediated Effector Functions Are Insufficient to Protect Infant Rhesus Macaques against Oral SHIV Infection. *mSphere*. 2022.
2. Surana NK, Kasper DL. Moving beyond microbiome-wide associations to causal microbe identification. *Nature* 2017.
3. Cervantes-Barragan L, et al. Lactobacillus reuteri induces gut intraepithelial CD4+CD8αα+ T cells. *Science* 2017.
4. Rothhammer V, Quintana FJ. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat Rev Immunol* 2019.
5. Daelemans D, el al. A time-of-drug addition approach to target identification of antiviral compounds. *Nat Protoc* 2011.

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