Gut Microbiome Dysbiosis and Chronic Lymphocytic Leukemia Pathogenesis in the Eμ-TCL1 mouse model

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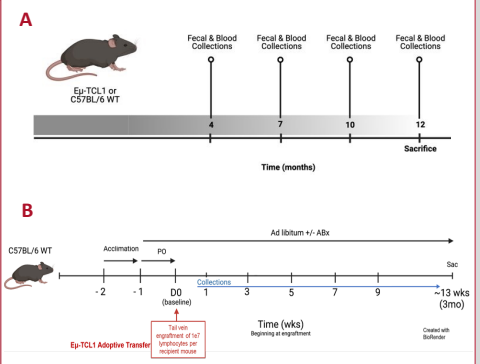
Introduction

- Chronic lymphocytic leukemia (CLL) is an incurable disease of aging, disproportionately affecting the elderly and representing the most common adult leukemia in the United States.
- The pathogenesis of CLL is related to activation of B-cell receptor (BCR) signaling where microbial antigens are known to be key stimulators to drive CLL B-cell proliferation and disease progression.
- Here we describe initial microbiome profiling and association of a dysbiotic microbiome to CLL pathogenesis using a transgenic and adoptive transfer mouse model of CLL.

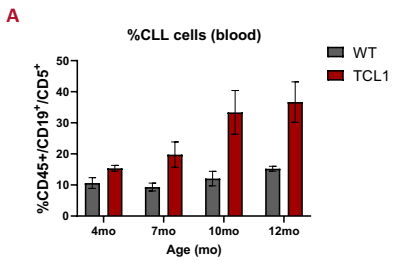
Experimental Design

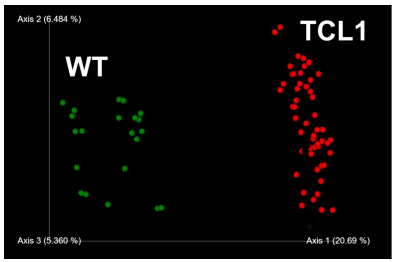


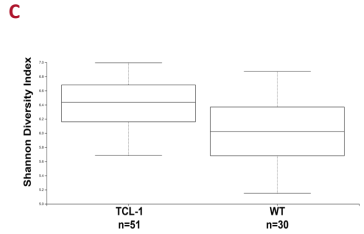
(A) Transgenic E*μ*-TCL1 mice (a murine CLL model) and wild type B6 (WT B6) mice were housed in separate cages and monitored with serial sample collection (fecal pellets and blood) over 1 year. At the end of study, mice were sacrificed, and tissues including spleen, intestines, and liver were collected.

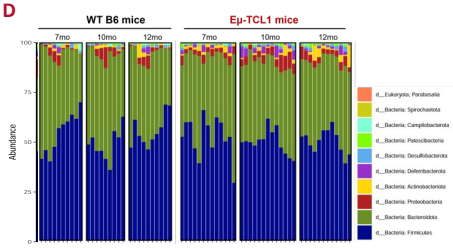
(B) C57BL/6 WT mice were split into 2 groups . Group 1 received a high dose antibiotic (ABx) regimen for five days consisting of vancomycin, neomycin, metronidazole, and amphtericin B followed by continued ABx therapy at lower amounts in drinking water. Group 2 did not receive antibiotics. Mice next underwent leukemia engraftment from advanced-disease E*μ*-TCL1 mice and were followed for ~13 weeks with serial blood and fecal pellet sampling. Fecal samples underwent DNA extraction and 16S rRNA gene sequencing using V3/4 primers on the Illumina Miseq platform

Eμ-TCL1 Mice Harbor a Unique and Dysbiotic Gut Microbiome



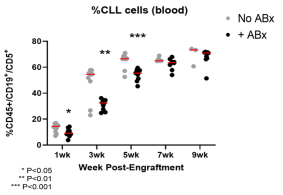


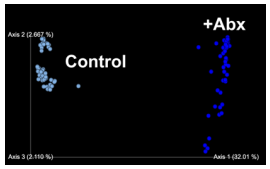


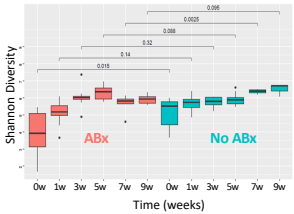


(A) CLL burden in peripheral blood by flow cytometry. **(B)** PCOA-plot depicting Jaccard dissimilarity as a measure of high Beta-diversity between TCL-1 and age-matched WT mice. **(C)** Alpha diversity measured by Shannon diversity index demonstrates increased alpha diversity in TCL-1 mice compared to control WT. **(D)** Relative abundance of key phyla between TCL-1 and WT identifies a dysbiotic community enriched for *Proteobacteria, Actinobacteriota, and Deferribacterota* phyla within the TCL1 cohort.

Antibiotic Ablation Impacts CLL Pathogenesis and Forms Unique Gut Microbial Communities

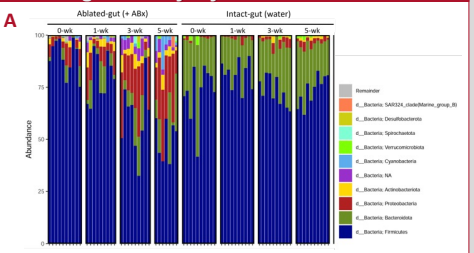


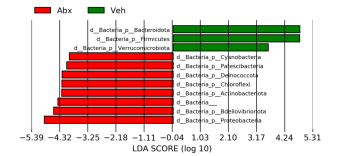


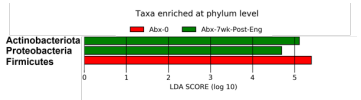


A) CLL burden in peripheral blood over time according to antibiotic (ABx) exposure. **B)**) PCOA-plot depicting Jaccard dissimilarlity as a measure of high beta-diversity between the ABx and no ABx group. **C)** Alpha diversity changes over time according to antibiotic exposure (red) versus not (blue).

Antibiotic Treatment Produces a Progressively Dysbiotic Microbiome







(A) Relative abundance table at the phylum level depicts progressive changes to gut microbial populations in antibiotic-treated TCL-1 mice (+ABx) compared to untreated TCL-1 mice (intact gut). **(B)** Linear discriminant analysis Effect Size (LEfSe) analysis of key phyla differences between the groups highlights increasing population of *Proteobacteria* indicative of progressive dysbiosis. **(C)** LEfSe analysis in the antibiotic treatment group over time identifies an increase in *Proteobacteria* and *Actinobacteriota* phyla similar to the transgenic model (red time 0, green ABx at 7 weeks post-engraftment).

Conclusions and Future Directions

- CLL progression in transgenic Eμ-TCL1 mice is associated with a unique and dysbiotic microbiome compared to healthy controls.
- Antibiotic-mediated ablation of the gut microbiome initially delays CLL progression, which increases over time in association with a progressive dysbiotic increase in *Proteobacteria* and *Actinobacteriota* phyla.
- *Proteobacteria* and *Actinobacteriota* were similarly identified in the separate transgenic Eμ-TCL1 mice, suggesting a potential relationship between these taxa and CLL progression.
- Samples from CLL patients are being actively collected (n=30/50) to translate findings from the mouse model.

Acknowledgements

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