ELSEVIER

Blood 142 (2023) 4630–4632

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Progressive Gut Microbiome Dysbiosis Is Associated with Chronic Lymphocytic Leukemia Pathogenesis and Development in the Eμ-TCL1 Mouse Model

Sydney A Skupa, BS[1], *Audrey L Smith, BS*[1], *Alexandria P Eiken, BS*[1], *Elizabeth A Schmitz, BS*[1], *Kathryn M Cooper, PhD*[2], *Christopher R D'Angelo, MD*[3], *Dalia El-Gamal, PhDMSc*[4]

[1] Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE
[2] School of Interdisciplinary Informatics, University of Nebraska at Omaha, Omaha, NE
[3] Fred and Pamela Buffett Cancer Center/Division of Hematology and Oncology/Department of Internal Medicine, University of Nebraska Medical Center, Bennington, NE
[4] Eppley Cancer Institute, University of Nebraska Medical Center, Omaha, NE

Introduction: The gut microbiome is home to a community of microorganisms such as bacteria, fungi, viruses, archaea, and protozoa. Maintaining a balance between "favorable" and "unfavorable" bacteria within the gut microbiome is essential for homeostatic physiological processes including proper immune system development, regulation of metabolic pathways, protection against pathogens, and gut epithelium integrity. Chronic lymphocytic leukemia (CLL) is a mature B-cell malignancy characterized by the accumulation of $CD5^+$ B-cells in the blood, bone marrow, and secondary lymphoid tissues. The pathogenesis of CLL is related to the activation of critical survival pathways such as B-cell and toll-like receptor signaling pathways, where microbial antigens are known to be key stimulators driving CLL B-cell proliferation. In this study, we investigated how the gut microbiota composition contributes and correlates to CLL disease progression.
Methods: Using a well-established murine model representative of CLL development in humans, we profiled the gut microbiota of transgenic Eμ-TCL1 mice and age-matched wild-type B6 (WT B6) mice over the course of 12 months (n = 7-10/group). Fecal pellets were collected aseptically from study mice at four time points (4, 7, 10, and 12 months) for DNA extraction using the QIAamp® PowerFecal® Pro DNA Kit. To characterize the gut microflora, we utilized 16S rRNA sequencing using the Illumina 16S metagenomics protocol. Bacterial taxonomy was assigned and analyzed using the QIIME2™ platform. Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis was employed to identify differentially expressed bacterial taxa between cohorts. Beta diversity indices were used to evaluate intersample differences in the fecal microbiota. Mice were monitored for peripheral expansion of leukemia cells (%$CD45^+$/$CD19^+$/$CD5^+$ cells) via flow cytometry. Next, we evaluated the microbiome in an aggressive model of CLL. Utilizing the Eμ-TCL1 adoptive transfer murine model, similar methods were adopted to profile the gut microbiome over the course of 7 weeks. Lastly, to evaluate the contribution of the gut microbiota to CLL development, we employed an antibiotic-mediated gut microflora ablation to the adoptive transfer Eμ-TCL1 model, with leukemia expansion monitored post-engraftment on a weekly basis.
Results: A unique microbiome signature was identified in both the indolent and aggressive model of CLL. Evaluation of beta diversity, using Bray-Curtis distance metrics, confirmed distinct differences in the gut microbial communities between Eμ-TCL1 and WT B6 cohorts. At the phylum level, the gut microbiota of the Eμ-TCL1 and WT B6 cohorts were similarly dominated by *Firmicutes* and *Bacteroidota*. However, *Proteobacteria Deferribacterota* and *Desulfobacterota* were more abundant in the Eμ-TCL1 mice over 12 months as compared to age-matched WT B6. Abundance of *Actinobacteriota* increased specifically at 12 months in the Eμ-TCL1 mice. Additionally, LEfSe analysis showed enrichment of certain phyla in the Eμ-TCL1 mice at 4 months, 10 months, and 12 months. Presence of these bacteria paralleled with expansion of disease. In the aggressive model of CLL, beta diversity confirmed distinct differences in gut microbial communities as CLL disease progressed. Moreover, certain phyla were enriched in leukemic mice at 7 weeks post-engraftment including *Proteobacteria* and *Actinobacteriota*. Lastly, in the antibiotic-mediated gut flora ablation model, a delay of disease onset was reflected in mice with ablated gut microbiomes compared to mice with intact gut microbiomes, resulting in prolonged overall survival.
Conclusion: We provide novel results associating the gut microbiome with the initiation and progression of CLL disease. In both the indolent and aggressive CLL disease models, we showed that there is a distinct gut microbiome as evident by beta diversity. A dysbiotic signature represented by increasing *Proteobacteria* abundance was similarly identified in both models.

Proteobacteria are known to be evident in diseases characterized by inflammation. These findings support future studies to determine a potential causal mechanism between gut microbiota changes and CLL pathogenesis.

Disclosures D'Angelo: *Fate Therapeutics:* Research Funding; *Seagen:* Other: advisory board meetings; *Bristol Myers Squibb:* Other: advisory board meetings, Research Funding; *Abbvie:* Consultancy, Other: advisory board; *Ono Pharmaceutical:* Other: advisory board meeting, Research Funding.

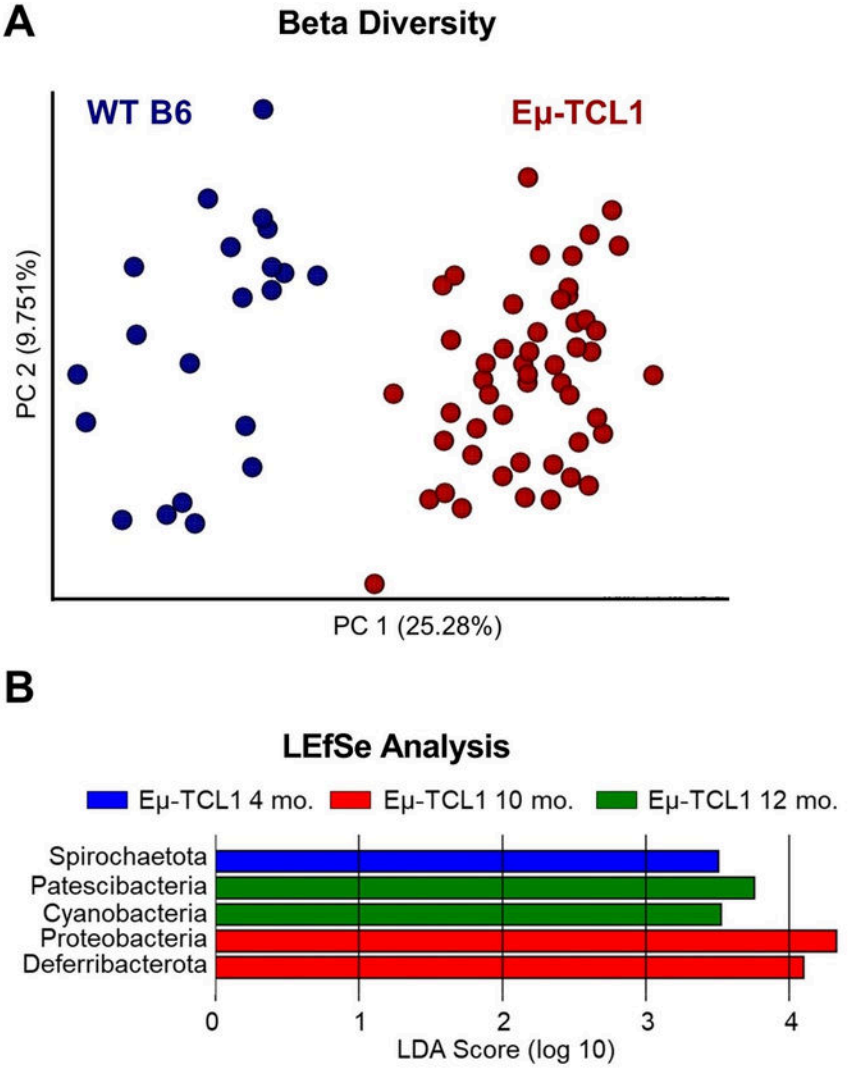

Figure 1. Association between the gut microbiome and CLL progression. (A) Principal coordinate analysis (PCoA) plots depicting gut microbiota composition differences (Bray-Curtis distance metrics) between transgenic Eμ-TCL1 mice and age-matched control WT B6 mice. **(B)** LEfSe analysis demonstrating enrichment of specific bacterial taxa at the phylum level in the transgenic Eμ-TCL1 mice and control WT B6 mice over the course of 12 months.

Figure 1

https://doi.org/10.1182/blood-2023-189606