ELSEVIER

Blood 142 (2023) 4069–4070

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

503.CLONAL HEMATOPOIESIS, AGING AND INFLAMMATION

One-Carbon Metabolites Promote Systemic Inflammation, Gut Dysbiosis, and a Myeloid Lineage Differentiation Bias

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TET methylcytosine dioxygenase 2 (*TET2*) loss-of-function mutations induce a pre-malignant state known as clonal hematopoiesis of indeterminate potential (CHIP). CHIP occurs in approximately 10% of people over 65 years of age and confers a 10-fold greater risk of developing hematological malignancy. Several environmental factors, including radiation, sleep deprivation, atherosclerosis, and diet, have been associated with the expansion of pre-malignant clones in CHIP patients. It has previously been shown that hematopoietic *Tet2* deficiency in mice triggers a pro-inflammatory state with increased intestinal permeability, gut bacterial translocation, and accelerated clonal expansion. Gut microbes themselves can exert an influence on myeloid leukemia progression through synthesis of compounds including short-chain fatty acids (SCFAs), which promote intestinal barrier integrity. Dietary levels of one-carbon metabolites and cofactors have been found to alter gut microbial composition, affecting SCFA production and intestinal permeability, in disease-free adults. Given the connection between diet, SCFAs and gut permeability, we sought to determine the impact of dietary one-carbon metabolites on gut microbial composition and function in CHIP progression.

We performed competitive bone marrow transplantation assays with *Tet2*+/- cells in mice supplemented with altered one-carbon metabolites: a control diet, high or low methionine diets, high or low folate diets, and high or low vitamin B12 supplementation. Mice were treated for a total of 7 months before timed sacrifice. Altered supplementation of the one carbon metabolites tested did not influence the competitiveness of *Tet2*-deficient cells in peripheral blood, spleen or bone marrow, however, high vitamin B12 levels promoted a myeloid differentiation bias in the spleen and bone marrow. Plasma cytokine analysis also showed that high vitamin B12 supplementation caused increased circulating inflammatory cytokines (IL-1b, IL-23, IL-27, CCL3/4, CXCL2). Based on this strong inflammatory phenotype, we performed scRNA-sequencing of splenic *Tet2*+/- CD11b+ cells. These data confirmed a neutrophilic cell expansion in the spleens of vitamin B12-treated mice along with increased expression of innate inflammatory genes *S100a8/9* and *Lyz2* as well as *IL-1 b* and *Cxcl2* in the neutrophil clusters.

Gut microbial-dependent inflammation is known to drive *TET2*-dependent myeloproliferation, thus we collected fecal samples before timed sacrifice and performed shotgun sequencing. Vitamin B12 supplementation decreased alpha diversity and the composition of genera that produce butyrate, an SCFA known to promote intestinal barrier integrity. Metagenomic analysis revealed increased amino acid metabolism and fatty acid biosynthesis in the gut microbiome of vitamin B12-supplemented mice, along with decreased butanoate metabolism, suggesting an alteration in microbial function that could influence SCFA content. To test whether these alterations in SCFA-producers led to increased gut permeability, we performed fluorescence *in situ* hybridization (FISH) against bacterial ribosomal 16S in livers from our mouse cohorts and observed increased levels of bacterial dissemination in mice with vitamin B12 supplementation. Our findings suggest that vitamin B12 supplementation in mice exacerbates the myeloid lineage differentiation bias of *Tet2*+/- hematopoiesis, possibly due to decreased gut barrier integrity which contributes to a heightened innate inflammatory response. This study highlights the potential for micronutrients of one-carbon metabolism to influence CHIP progression through maintenance of proper gut microbial homeostasis.

Disclosures No relevant conflicts of interest to declare.

https://doi.org/10.1182/blood-2023-189726