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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Chronic Alcohol Exposure Causes Myeloid-Biased Hematopoiesis Via Double-Strand RNA Sensors and Type 1 Interferon Response

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Excessive alcohol use is well-known to cause cytopenias and bone marrow (BM) suppression. However, the mechanisms by which alcohol suppresses BM are poorly understood. Moreover, even though the prevalence of moderate drinking is high worldwide, the effects of chronic moderate alcohol consumption on the human hematopoietic system remain unknown. To fill this knowledge gap, we sought to investigate the effects of chronic moderate alcohol consumption on human hematopoiesis using cell-based and animal models.

To examine the effects of alcohol on hematopoietic cells *in vitro*, we tested varying doses of alcohol (0-100 mM) on four leukemia cell lines (Jurkat, MOLM13, MV-4-11, and THP1) and measured population doubling over 6 days. Population doubling levels significantly decreased in a dose-dependent manner in all cell lines tested. We also measured mitochondrial (mt) ROS by MitoSOX-Red flow cytometry in Jurkat, which showed a dose-dependent increase of mtROS (trends only at 10, 25 mM; significantly increased at 50, 100 mM alcohol) compared to untreated. These findings confirm that alcohol negatively affects cell growth and increases mtROS in a physiologic range of doses.

To determine the effects of alcohol on human hematopoietic stem progenitor cells (HSPCs) *in vitro*, we treated human BM CD34+ cells with 0 vs. 50 mM alcohol for 48 hours and performed bulk RNA-sequencing in triplicate. Gene Set Enrichment Analysis (GSEA) revealed that multiple pathways related to platelet activation and coagulation were enriched in alcohol-treated HSPCs, suggesting the induction of inflammation and/or platelet-biased differentiation by alcohol.

To assess the effects of chronic moderate alcohol consumption on HSPCs *in vivo*, we employed a xenotransplant model. In brief, human BM CD34+ cells were transplanted to 5-to-6-week-old female NBSGW mice. We confirmed their engraftment by bone marrow aspiration at six weeks post-transplant, and then they were randomly assigned to the alcohol vs. control groups. After 2 weeks of adjustment, mice were fed a liquid diet (their only source of water and food) containing alcohol 5% vol/vol vs. iso-caloric control diet for 8 weeks. At 4 and 8 weeks, the alcohol-fed group showed significant myeloid bias, reaching greater than 3 times higher myeloid-to-lymphoid ratio than the control group at 8 weeks, while overall engraftment levels were not different. We will report the results of the secondary transplantation of this experiment at the annual meeting.

After 8-week alcohol feeding, sorted human CD45+CD34+ cells from mouse BM were subject to 10x Genomics Chromium X 3′ HT single-cell RNA-sequencing (scRNA-seq). Complementing the flow data, our transcriptomic data portrayed stark differences between the two groups. Over 50% of cells in the alcohol group comprised granulocyte progenitors, with B cell progenitors representing less than 25%. Inversely, control group data revealed approximately 30% granulocyte progenitors and 55% B cell progenitors, confirming the myeloid bias of HSPC caused by alcohol.

Examining differentially expressed genes and GSEA highlighted a significant upregulation of genes associated with the response to virus and type 1 interferon (IFN-1) signature across all HSPC types. A closer look into the gene set showed increased expression of genes tied to cytosolic double-strand (ds) RNA sensing pathways and subsequent interferon type 1 response. Specifically, dsRNA sensors like MDA5, RIG1, PKR, and OAS1-3, as well as interferon regulatory or responsive genes, such as IRF7, ISG15, IFI6, IFI44L, MX1, and STAT1, were notably increased in HSCs and most progenitor populations. Unexpectedly, alcohol-exposed HSCs tended to be in the G0/G1 phase rather than the S or M phase compared to their counterpart upon cell cycle analysis using scRNA-seq data. This may be due to the activation of the PKR pathway, a known cell cycle inhibitor in mouse HSCs.

In summary, our findings demonstrate that chronic moderate alcohol consumption can significantly reshape the hematopoietic system through myeloid-biased differentiation and potentiate inflammation by activating the cytosolic dsRNA sensing pathways and IFN-1 response.

Disclosures No relevant conflicts of interest to declare.

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