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Severe Anemia Causes Chronic Hypoxia and Alters Immune Cell Atlas in Liver of Murine Neonates: A Single Cell Approach

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Background: Neonatal anemia is nearly universal in preterm infants and is associated with increased morbidity and mortality worldwide. We have recently described that the anemia associated "leaky gut" phenotype that leads to monocyte infiltration in intestine and RBC-transfusion activates these cells, resulting in necrotizing enterocolitis (NEC).

Significance of the Problem: Charting the immune cell atlas in neonatal liver during anemia will define the monocyte populations that causing NEC.

Hypothesis, Problem OR Question: Phlebotomy-induced anemia facilitates chronic hypoxia that may alter immune cells atlas in neonatal hematopoietic organ like liver. In this study, we are investigating the effects of phlebotomy-induced severe anemia in the liver and its immunological responses by single cell RNA sequence analysis.

Methods: C57BL/6 mouse pups were studied in 2 groups (n=6 each): (1) naïve controls; (2) severely anemic (hct 20-24%). Pups were rendered severely anemic by facial vein phlebotomies on P2, 4, 6, 8, and 10. On P11, the liver tissue was extracted and used for single-cell RNA sequence analysis and flow cytometry validation. For the scRNA-seq experiments, liver tissues from control and severe anemic groups (n=6/each group) were processed through all steps to generate stable cDNA libraries (!0x Genomics), sequenced on a NextSeq 500 (Illumina) and analyzed. Single cell suspensions from liver tissue were stained with antibodies against CD45, CD11b, F4/80, Ly6C, Ly6G, CD11c, CD3, NKG7, B220, GYPA and CD15. Then, these cells were acquired by BD LSRII-flow cytometry followed by FlowJo for gating the monocyte, macrophage, neutrophils, dendritic cells, B-cells, T-cells, NK cells, erythroid cells and granulocytes.

Results: We have found that 8-type of immune cells clusters (dendritic cells, T-cells, NK-cells, B-cells, neutrophils, non-inflammatory macrophages and inflammatory macrophages with monocyte phenotype) and 4-type of non-immune cells clusters (neuronal cells, astrocytes, hepatocytes and stellate cells) have categorized into overall 12 clusters in liver single cell suspensions from control and anemic liver, gene expression atlas confirmed the significant enrichment of inflammatory macrophages, neutrophils and erythroid cells in anemic liver compared to control. A significant change in cell counts of inflammatory macrophages (1939±798 in anemia vs 716±53.32 in control; *p<0.1), erythroid cells (3784±549 in anemia vs 1188±354 in control; *p<0.01) and neutrophils (1226±250 in anemia vs 854±73 in control; *p<0.1) were found in anemic liver than control. Differential Gene Expression (DGE) analysis showed that these enriched cells displayed inflammatory phenotype, including trem1, IL1α, IL1β and S100A8. In consistent with scRNA seq analysis, flow cytometry analysis showed higher frequencies (%) of granulocytes with increased number of monocytes and neutrophils in severe anemia compared to controls, also the GYPA+ erythroid cells were significantly increased in anemic liver, showing that anemia-associated hypoxia skews the hematopoietic system toward emergency myelopoiesis and leads to stressed erythropoiesis respectively.

Conclusions: Severe anemia-associated chronic hypoxia state in the liver alters the immune cell atlas with increased production of inflammatory macrophage with enrichment of inflammatory phenotype of monocytes and triggered a brisk erythropoiesis.