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POSTER ABSTRACTS

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

Mitigating Oxidative Stress Promotes Quiescence of Hematopoietic Stem Cells from Concurrent TLR4 Activation and IL-10R Blockade Mediated Inflammation

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Inflammatory stress provides a clonogenic advantage towards CHIP mutant hematopoietic stem and progenitor cells (HSPCs) via different mechanisms including resistance to apoptosis, enhanced self-renewal and limited generation of reactive oxygen species. Reactive oxygen species (ROS) play a causative role in hematopoietic stem cell (HSC) dysfunction due to exit from quiescence and replication stress-induced stem cell exhaustion. Oxidative stress mediated decline in the fitness of WT hematopoietic stem cells (HSCs) may allow mutant HSCs to gain a selective advantage. Therefore, protection of WT HSCs from ROS may maintain their fitness, and prevent the selective expansion of mutant cells.

To test whether the anti-oxidant N-Acetylcysteine (N-AC) protects HSCs from chronic inflammatory stress, we mice were challenged daily with lipopolysaccharide (LPS) (5μg, intraperitoneal) or saline control for 30 days while their drinking water was supplemented with N-AC at 2mg/ml or untreated regular water. At day 30, bone marrow was harvested for competitive repopulation assays using equal numbers of whole bone marrow cells from each competitor (Figure 1A). As expected, bone marrow from mice exposed to 30 days of chronic LPS on regular water showed inferior reconstitution compared to saline exposed mice. However, bone marrow from mice exposed to 30 days of LPS and maintained on N-AC supplemented water performed equivalently in competitive transplants as saline exposed mice. To demonstrate that this functional defect of HSCs stemming from chronic LPS treatment is dependent on increased HSC divisions, we challenged TetOP-H2B-GFP mice with LPS for 30 days and assessed H2B-GFP label retention in immunophenotypically defined long-term HSCs. Progenitor populations in the bone marrow demonstrated low levels of H2B-GFP expression while LT-HSCs showed high GFP signal indicating low turnover. Meanwhile, LPS treatment led to increased cell divisions in LT-HSCs compared to saline treated mice.

We hypothesized that N-AC protects HSCs via reducing entry into cell cycle in response to inflammatory stimuli, which is ROS dependent. We assessed the impact of N-AC treatment on ROS in HSCs and its correlation with cell cycling. To induce acute inflammation, wildtype (WT) mice were injected with LPS (5μg) and IL-10R blocking antibody (0.1mg). IL-10R blocking antibody was used in conjunction with LPS because we have previously found that IL-10R blockade extends LPS induced HSC proliferation. Intracellular ROS was determined using the ROS sensitive dye, CM-H $_2$DCFDA (DCF). The DCF signal was increased in HSCs (Lin $^-$, c-Kit $^+$, Sca1 $^+$, CD150 $^+$, CD48 $^-$) in response to TLR4 activation by LPS and the addition of αIL-10R further augmented this increase in ROS (Figure 1B). Pre-treatment with N-AC significantly reduced the increase in ROS mediated by LPS alone. Cell cycle status was determined by intracellular Ki67 staining of HSCs. LPS significantly increased HSC proliferation as determined by increased percentage of cells in the G1 phase of the cell cycle while IL-10R blockade further augmented HSC cycling. Mice pre-treated with N-AC did not show significant increase in HSC proliferation indicating protection from ROS mediated exit from quiescence.

In conclusion, incorporation of N-AC into drinking water protected WT HSCs from inflammation induced exhaustion and promoted persistence of quiescence in HSCs. N-AC could potentially be utilized as a preventative method to preserve fitness of HSCs, particularly in those with chronic inflammatory stress. Moreover, preservation of HSC fitness should be evaluated for its ability to prevent emergence of clonal hematopoiesis.

Disclosures Fleischman: *Pharmaessentia, CTI:* Speakers Bureau; *GSK, Incyte, CTI:* Consultancy.

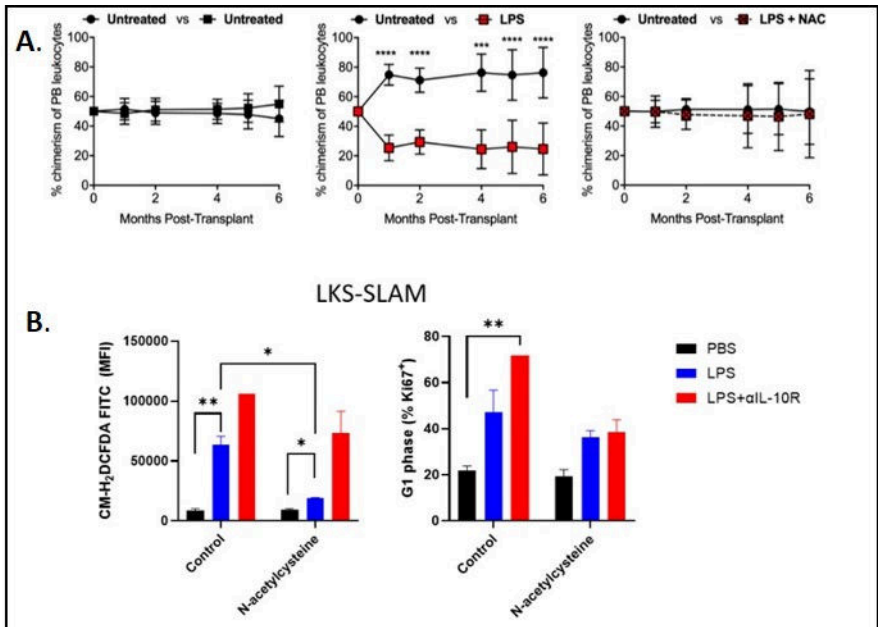

Figure 1. (A) N-AC protects from inflammation induced HSC exhaustion. Donor mice maintained on either regular water or N-AC (2mg/ml) drinking water were injected intraperitoneally with either saline or 5μg LPS daily for 30 days. Bone marrow was harvested and whole bone marrow cells were used for competitive transplants into lethally irradiated hosts. Peripheral blood chimerism (using CD45.1/2 staining to identify donor) was followed for 6 months. (B) LPS (5μg) and LPS+αIL-10R (0.1mg) were injected to induce acute inflammation and determine oxidative stress (left) and cycling (right) in LT-HSCs in the presence of N-AC.

Figure 1

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