Hematopoietic stem cells engage a transient control mechanism that limits their ability to respond to repeated inflammatory stimulation

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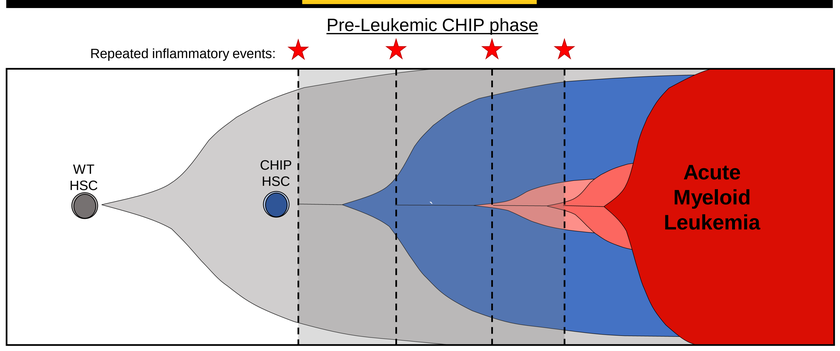
Introduction

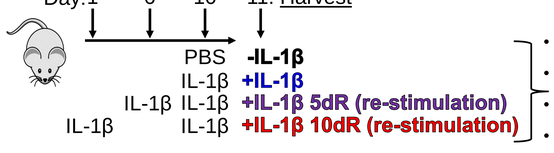


- Clonal hematopoiesis of indeterminate potential (CHIP) is a pre-leukemic state characterized by the clonal expansion of hematopoietic stem cells (HSC) in the bone marrow (BM)
- Hematopoietic progenitors acquire loss of function mutations most commonly in epigenetic modifiers (*Tet2, Dnmt3a, Asxl1*) leading to their clonal expansion.
- HSC with CHIP mutations are found in healthy individuals, but only individuals exposed to repeated inflammatory events develop any form of pathology
- The mechanism(s) behind the selective expansion of CHIP clones is not understood

We hypothesize normal HSC become refractory to cell cycle entry during repeated inflammatory episodes, limiting expansion of hematopoietic progenitors

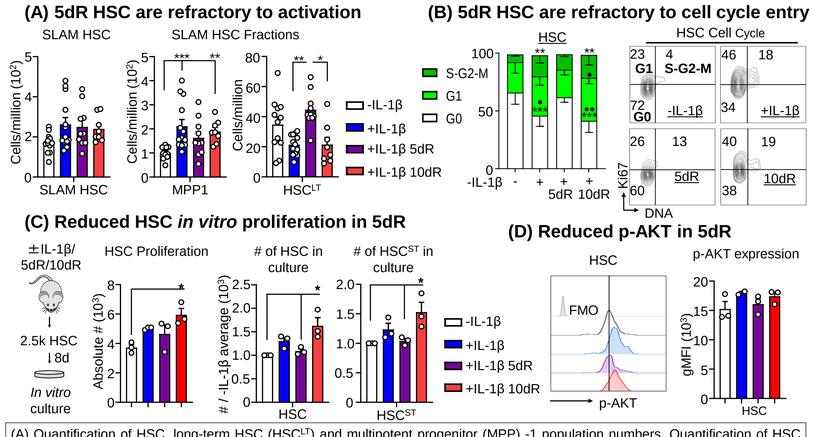
Results

1 Mouse model of repeated inflammatory stimulation



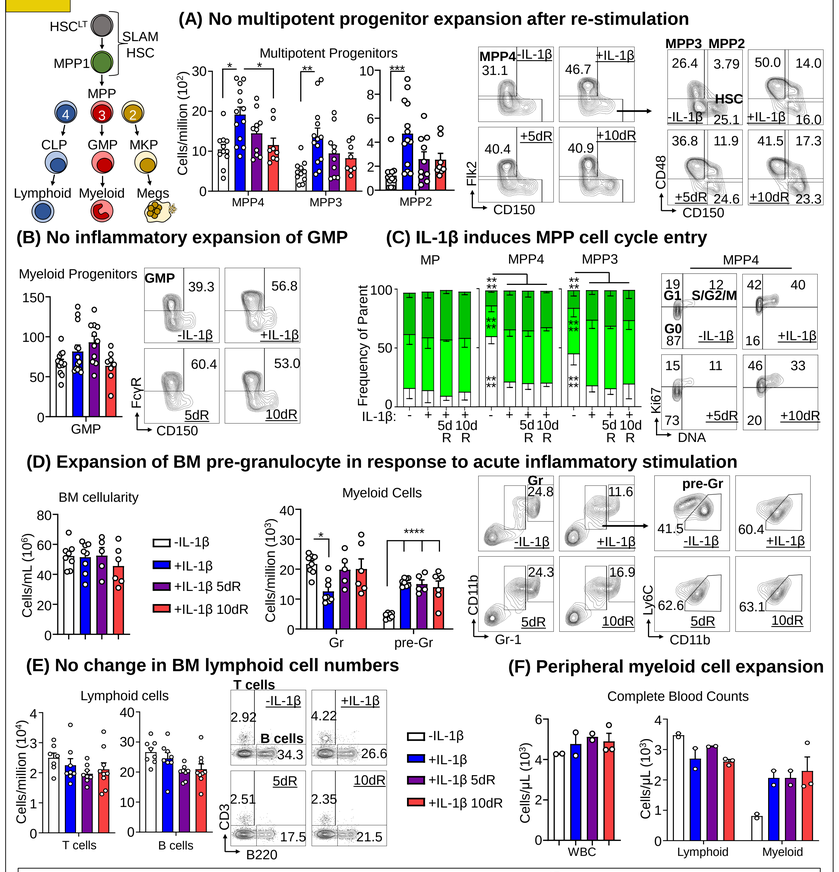
- Cell Cycle analysis
- FACS immunophenotyping
- *In vitro* cell culture
- Seahorse metabolic assay

2 5dR HSC are refractory to inflammatory stimulation



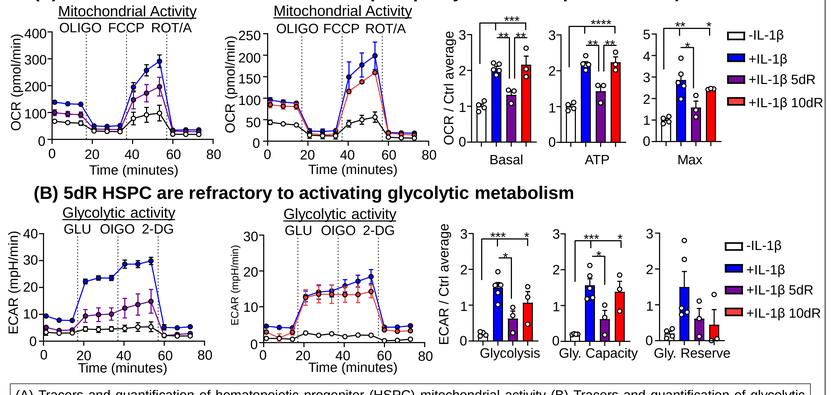
(A) Quantification of HSC, long-term HSC (HSC^{LT}) and multipotent progenitor (MPP) -1 population numbers. Quantification of HSC cell cycle characteristics with representative FACS plots. Quantification of *in vitro* HSC culture total cell number as well as number of HSC and short-term HSC (HSCST) numbers remaining at the end of the culture period; Representative histogram and quantification of HSC p-AKT FACS staining.; n= 5-8; *p<0.05, **p<0.01, ***p<0.001. * compared to control, • compared to 5d re-stim.; Statistical significance determined by one-way ANOVA.

3 Hematopoietic progenitors maintain supply of mature cells



(A) Model of hematopoiesis; Hematopoietic stem cells (HSC), long-term HSC (HSC^{LT}), multipotent progenitor (MPP), common lymphoid progenitor (CLP), granulocyte/monocyte progenitor (GMP), megakaryocyte progenitor (MkP), megakaryocyte (Megs). Quantification of MPP population numbers and example FACS plots; (B) Quantification of GMP and example FACS plots. (C) Quantification of myeloid progenitor (MP) and MPP cell cycle characteristics with example FACS plot of MPP4. (D) Quantification of total BM cellularity and mature myeloid cell numbers with example FACS plots; granulocytes (GR) pre-granulocytes (pre-Gr). (E) Quantification of mature lymphoid cell numbers with example FACS plots. (F) Quantification of peripheral blood complete blood counts (CBC); white blood cells (WBC). n = 5-8 for immunophenotyping, n= 2-3 for central blood counts, *p<0.05, **p<0.01, ; Statistical significance determined by Kruskal-Wallis.

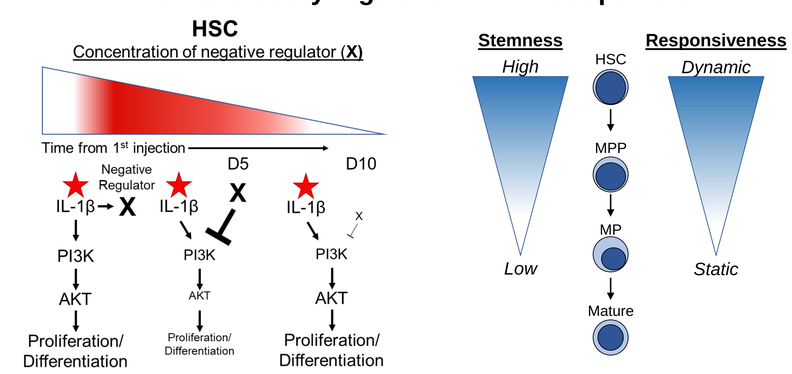
4 HSPC cellular metabolism reflects HSC refractory period



(A) Tracers and quantification of hematopoietic progenitor (HSPC) mitochondrial activity (B) Tracers and quantification of glycolytic activity in control, one day IL-1β, day five IL-1β re-stimulated, and day 10 IL-1β re-stimulated; n = 7-10. 150k magnetically sorted HSPC were plated following a four-hour incubation period in a tissue culture incubator; n = 3 for chronic, 5d re-stimulation, and 10d re-stimulation. *p<0.05, **p<0.01, ***p<0.001. * compared to control.; Statistical significance determined by one-way ANOVA

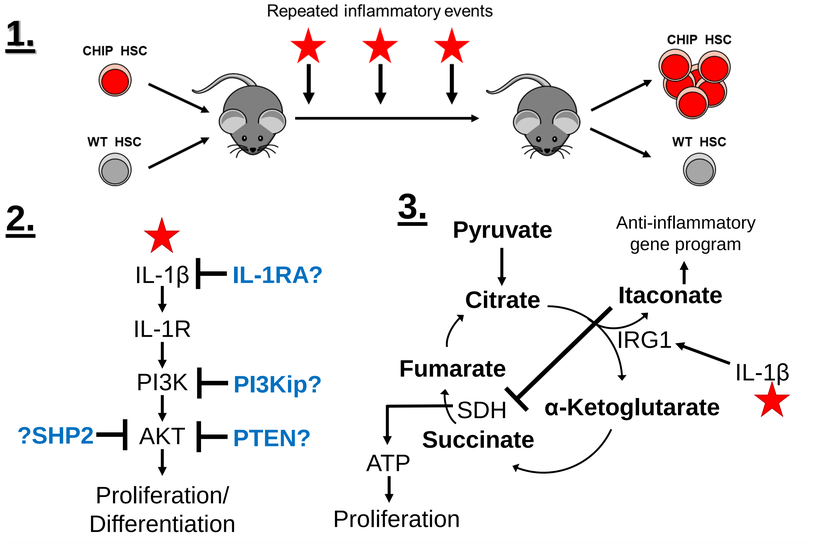
Conclusions

Inflammatory regulation of hematopoiesis



- HSC are transiently refractory to proliferation by limiting AKT phosphorylation.
- MPP consistently enter the cell cycle in response to IL-1β stimulation.
- MP do not change cell cycle state or numbers during inflammatory stimulation.
- There is increased mature cell myelopoiesis in the re-stimulated groups.
- HSPC oxidative and glycolytic metabolism correlates with HSC activity and phenotype.
- The cooperative regulation of HSPC proliferation among the hematopoietic hierarchy ensures a constant supply of mature immune cells, while also limiting the activity of HSC to inhibit aberrant cellular expansion as well as proliferative aging.
- The refractory phenotype of HSC during short term inflammatory re-stimulation could lead to a competitive disadvantage during CHIP that would allow transformed clones to preferentially expand

Future Directions



1) Future analysis of a competitive transplant between CHIP and WT HSC in the setting of repeated inflammatory events will determine if the CHIP clones do not become refractory which would result in a competitive advantage compared to WT clones.
2) We will further interrogate modulation of the IL-1β signaling pathway in HSC, and specifically negative regulators of the PI3K Pathway.
3) We will investigate if the HSC refractory period is being induced by the metabolite itaconate which limits the TCA cycle and engages an anti-inflammatory gene program.
HSC function in a manner that causes HSC to become refractory to inflammatory activation.
4) Finally, we will investigate changes to metabolism directly in HSC during inflammatory (re)stimulation using the fluorescence lifetime imaging microscopy (FLIM) assay