LT-HSC iduce a heritable trained immunity program in response to Pristane

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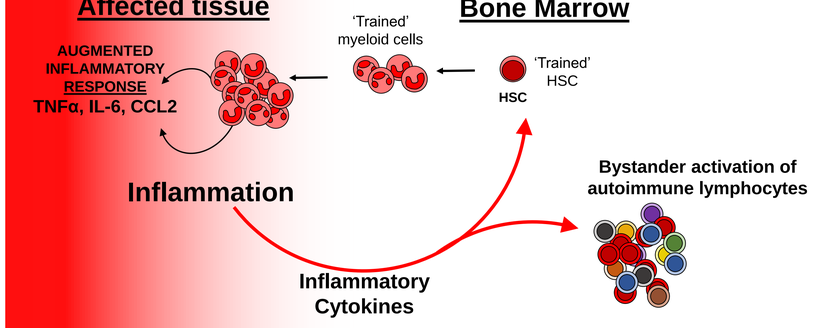
Introduction



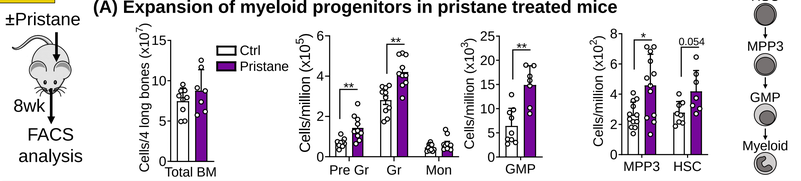
- Auto-immune disorders develop when adaptive lymphocytes initiate an immune response against self antigens, however the etiology behind their activation remains elusive.
- One possible mechanism is via bystander activation, which occurs during amplified and prolonged inflammatory cytokine stimulation.
- Trained Immunity is a myeloid cell phenotype that leads to augmented inflammatory cytokine release due to prior stimulation, and hematopoietic stem cells (HSC) have been shown to activate this heritable program.

We hypothesize that HSC are the reservoir for hyper-inflammatory 'trained' myeloid cells, and consequently are the root of auto-immune disorder development and relapse

Results

1 Pristane exposure drives myeloid expansion

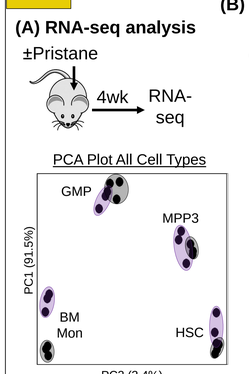
(A) Expansion of myeloid progenitors in pristane treated mice



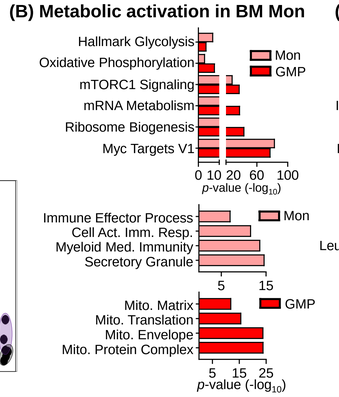
(A) Quantification of bone marrow cellularity, pre-granulocyte (Pre Gr), Granulocyte (Gr), Monocyte (Mon), granulocyte-monocyte progenitor (GMP), multipotent progenitor (MPP) -3, and hematopoietic stem cell (HSC) numbers; n= 8-9; *p<0.05, **p<0.01, compared to control; Statistical significance determined by Mann-Whitney U-test.

2 Pristane induces defense response program in HSC

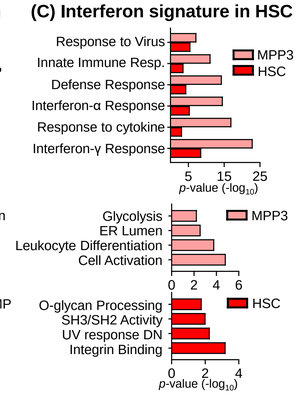
(A) RNA-seq analysis



(B) Metabolic activation in BM Mon

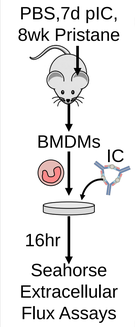


(C) Interferon signature in HSC

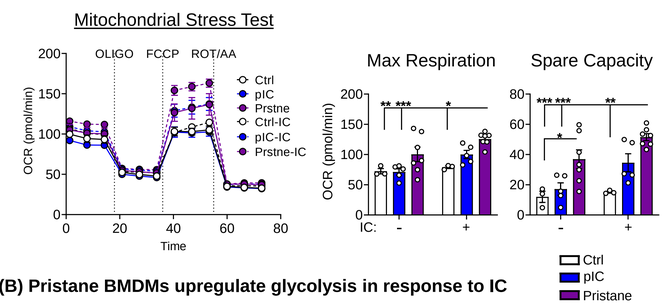


(A) Mouse model for RNA-seq and principal component analysis (PCA) of all cell types, BM-Mon, GMP, MPP-3, HSC. (B) Over representation analysis (ORA) of significantly upregulated (padj.<0.05) genes in BM-Mon and GMP with significantly altered terms (p<0.05). (C) ORA of significantly upregulated (padj.<0.05) genes in MPP3 and HSC with significantly altered terms (p<0.05); n= 3

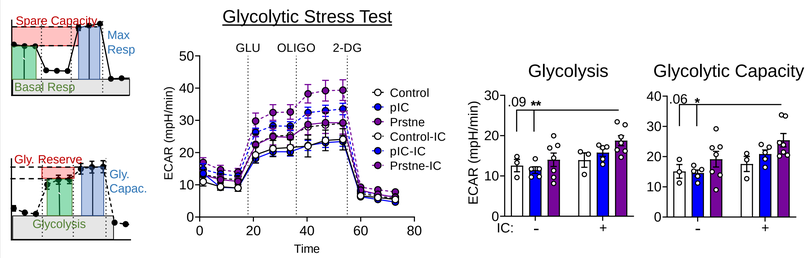
3 Pristane inflammation increases myeloid cell metabolism



(A) Pristane BMDMs upregulate OxPhos in response to immune-complexes (IC)



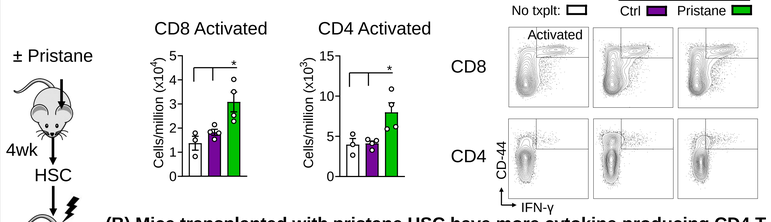
(B) Pristane BMDMs upregulate glycolysis in response to IC



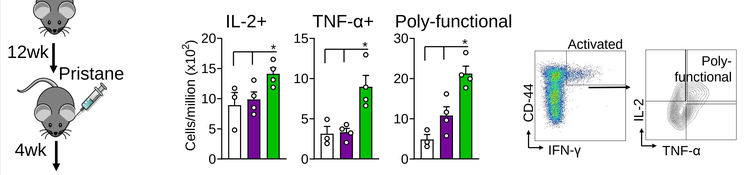
Mouse model of bone marrow derived macrophage (BMDM) stimulation with immune complexes (IC) and example graphs of Seahorse mitochondrial stress test and glycolytic stress test showing test parameters. (A) Tracer of mitochondrial stress test and quantification of maximal respiration and spare capacity. (B) Tracer of glycolytic stress test and quantification of glycolysis and glycolytic capacity. *p<0.05, **p<0.01, ***p<0.001 significance determined by two-way Anova with Tukey's post test; n= 3-7.

4 Increased T cell activity in pristane-HSC transplanted mice

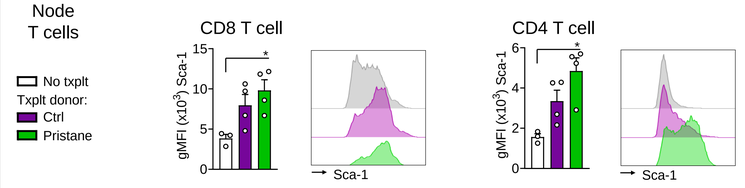
(A) Increased numbers of activated T cells in mice transplanted with pristane HSC



(B) Mice transplanted with pristane HSC have more cytokine producing CD4 T cells



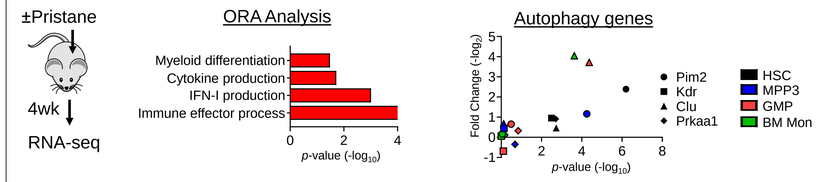
(C) T cells from mice transplanted with pristane HSC have elevated Sca-1 expression



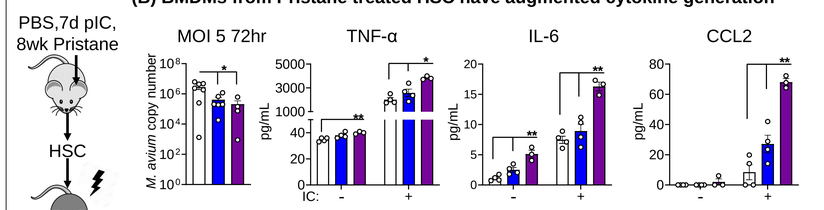
Mouse model for HSC training driving inflammatory activation transplant: 2000 HSC from 4wk pristane mice were transplanted into busulfan conditioned mice, 12wks later all recipient mice received pristane treatment, and 4wks later lymph nodes were harvested for T cell analysis. (A) Quantification of the number of CD-44+IFN-γ+ CD8 and CD4 activated T cells. (B) Quantification of the number of cytokine secreting CD4 T cells after *in vitro* stimulation with PMA ionomycin. (C) Expression levels of interferon sensitive gene Sca-1 on CD8 and CD4 T cells. Significance determined by one-way Anova, n=3-4. *p<0.05

5 BMDMs inherit HSC gene program

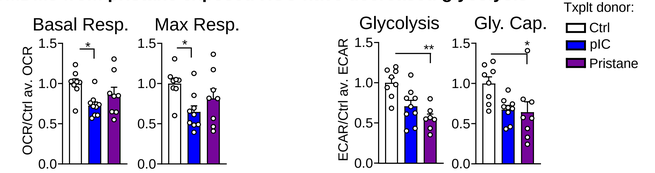
(A) HSC activate an autophagy gene program



(B) BMDMs from Pristane treated HSC have augmented cytokine generation



(C) BMDMs from pristane exposed HSC have decreased glycolysis



Mouse model: 4wk Pristane treatment RNA-seq analysis (A) Left: Quantification of over representation analysis of significantly up-regulated genes in HSC; right: quantification of fold change and p-value for genes involved with activation and regulation of autophagy n=3. Mouse model: 500 HSC were transplanted into lethally irradiated congenic recipients, 16 weeks later BMDMs were generated and tested in functional assays. (B) Results from 72hr *Mycobacterium avium* killing assay with BMDMs; left: *M. avium* copy numbers, right: cytokine concentrations in the supernatant; significance determined by one-way Anova, n=3-7. (C) Seahorse extracellular flux mitochondrial stress test and glycolytic stress test; Quantification of basal respiration, maximal respiration, glycolysis and glycolytic; significance determined by Kruskal-Wallace test, n=8-9. *p<0.05, **p0.01

Conclusions

- Pristane inflammation induces alternative programming in hematopoietic cells, with BM Mon and GMP inducing cellular and metabolic activation, while MPP3 and HSC are activating a defense response.
- BMDMs from pristane mice showed increased metabolic activation in response to IC stimulation.
- Pristane mice mice transplanted with pristane-HSC, have increased numbers of cytokine producing T cells and show increased Sca-1 expression
- In response to pristane treatment, HSC are generating a heritable program that leads to augmented pathogen killing and inflammatory cytokine release.

Future Directions

1) We will use LiCAT-seq analysis to investigate the epigenome and transcriptome of pristane-HSC-derived cells to uncover mechanisms behind the heritable "trained immunity" program.

2) Further, by using metabolic inhibitors of glycolysis and the TCA cycle in the setting of immune stimulation, we will determine if metabolic activation underlies myeloid cell hyper-responsiveness.

3) Next, we will Investigate if human systemic lupus erythematosus patient myeloid cells exhibit a similar hyper-responsive "trained immunity" phenotype.

4) Lastly, we will be able to determine the role HSC have in auto-immune disease development by transplanting pristane-HSC, then inducing Lupus in recipient mice, and assessing disease development and severity.