Untamed Immunity: Using "Dirty" Mice as a New Paradigm for Translatable Trauma Immunology Research

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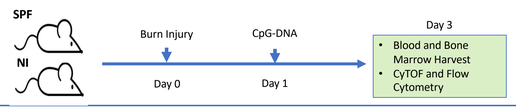
Background and Introduction

- Trauma induces an immune response resulting in an increased susceptibility to infections
- Specific Pathogen Free (SPF) laboratory mice are widely used for mechanistic immunology research because they can be safely housed to maintain immune system homogeneity
- Wild or pet shop mice develop trained or educated immune systems that more closely resemble humans
- Multigenerational natural immune (NI) inbred and outbred mouse lines (C57BL/6, BALB/c, and CD-1) were established for immunology research
- CpG-DNA is a TLR-9 agonist shown to improve immune function and recovery in SPF mice
- *Hypothesis 1: natural trained immune mice will provide a novel resource to help better translate mouse immunology and trauma disease models to humans*
- *Hypothesis 2: CpG-DNA will improve immune function following traumatic injury in SPF and NI mice*

Methods

- 38 SPF and 20 NI C57BL/6 mice underwent sham or burn injury under anesthesia
- Mice were injected with CpG-DNA or GpC-DNA (control) 24 hours post-injury
- Blood and bone marrow cells were prepared 48 hours post treatment for phenotyping by CyTOF mass cytometry or flow cytometry
- CyTOF and flow staining data was analyzed using our OMIQ analysis platform for computational clustering, dimensional reduction, and statistics



Results

- Neutrophils and monocytes were more abundant in NI vs. SPF mice, while NK cells were less abundant
- Burn injury caused a decrease in CD4+ and CD8+ T cells, NK cells, and B cells in SPF mice
- In the bone marrow, NI mice had higher Mesenchymal Stromal Cells (MSCs), Short-Term Repopulating Hematopoietic Stem Cells (ST-HSCs), Multi-Potent Progenitor Cells (MPPs), and Common Lymphoid Progenitor Cells (CLPs)
- Burn injury increased lineage(-)Sca-1(+)c-Kit(-) Cells (LSKs) and lineage(-)c-Kit(+)Sca-1(-) (LKs) in SPF mice ($p<0.001$), and Megakaryocyte-Erythrocyte Progenitor Cells (MEPs) and CLPs for both SPF and NI mice ($p<0.006$)
- CpG-DNA increased Long-Term Repopulating Hematopoietic Stem Cells (LT-HSCs) in both species ($p<0.004$) and decreased ST-HSCs in NI mice ($p<0.05$)

Natural immune mice show immune phenotypes consistent with acquired trained immunity and will provide new opportunities for "transitional translational" research addressing traumatic injury in humans

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Figures and Tables

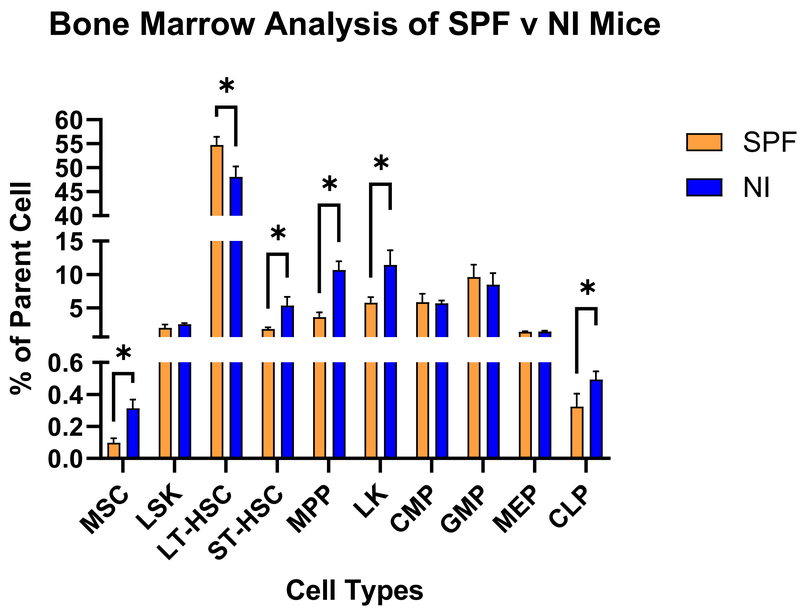

Figure 1. Bone marrow analysis of SPF and NI sham mice by percent of parent cell. MSC=Mesenchymal Stromal Cells; LSK=lineage(-)Sca-1(+)c-Kit(-) Cells; LT-HSC=Long-Term Repopulating Hematopoietic Stem Cells; ST-HSC=Short-Term Repopulating Hematopoietic Stem Cells; MPP=Multi-Potent Progenitor Cells; LK=lineage(-)c-Kit(+)Sca-1(-); CMP=Common Myeloid Progenitor Cells; GMP=Granulocyte-Monocyte Progenitor Cells; MEP=Megakaryocyte-Erythrocyte Progenitor Cells; CLP=Common Lymphoid Progenitor Cells

Figures and Tables Continued

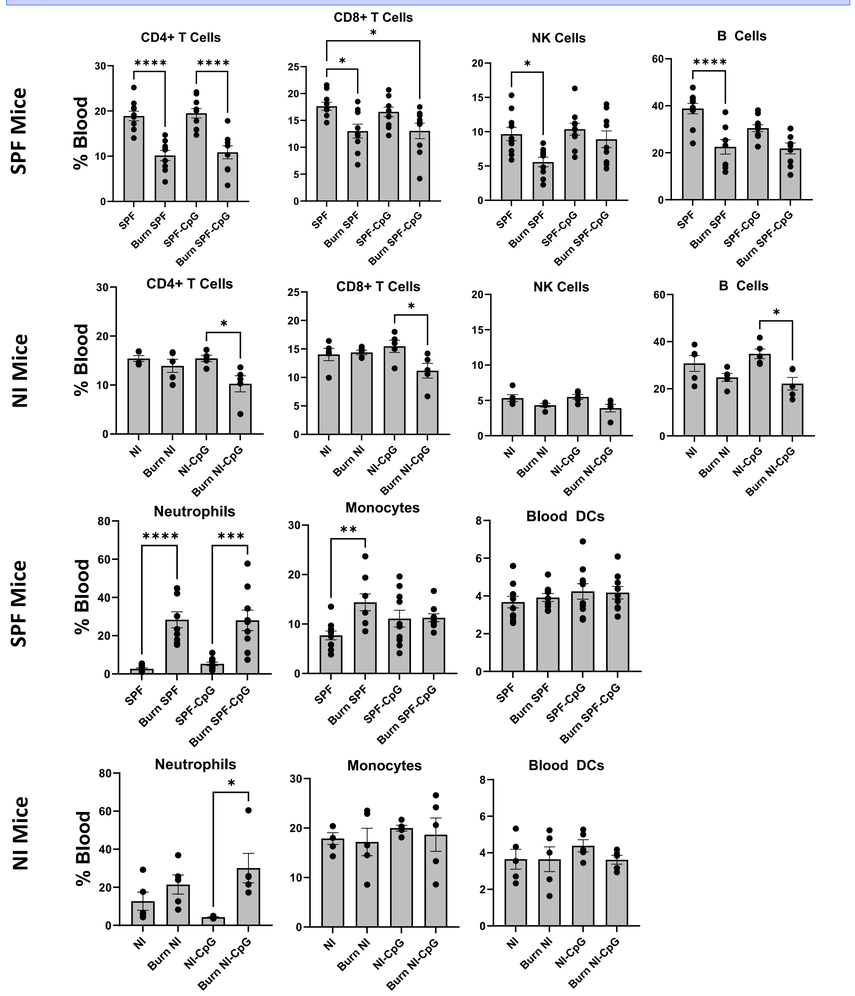

Figure 2. (Above) Peripheral blood immune cell subtypes in SPF and NI mice with and without CpG with sham versus burn injury

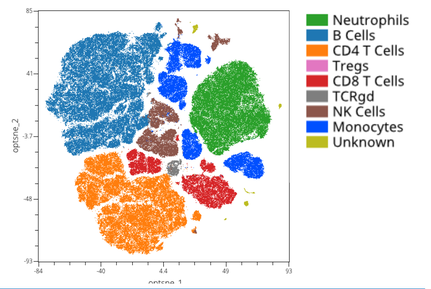

Figure 3. (Right) Blood Immune Cell Subset ID by CyTOF

Conclusions and Future Directions

1. Multi-generational establishment of natural immune mice from commonly used SPF inbred and outbred mice is feasible and results in development of a naturally-acquired immune system landscape.
2. Natural immune mice demonstrate immune cell phenotypic changes indicative of trained immunity with more robust stem and progenitor cell populations and less dramatic cell loss with burn injury.
3. CpG-DNA treatment showed increases in hematopoiesis in NI mice moving this preclinical research closer to human translation.
4. Natural immune mice will contribute "transitional translational" insights into basic immunological mechanisms by providing better pre-clinical model platforms for human disease processes.
5. Future studies will compare bacterial infection and vaccine responses in matched NI and SPF mouse models.