Natural Trained Immune Mice Develop Primed Innate Immune Signaling Responses

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Background and Introduction

- Specific Pathogen Free (SPF) laboratory mice are widely used for mechanistic immunology research because 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
- Research using **"natural" immune mice** is not feasible at many institutions
- Multigenerational natural immune inbred and outbred mouse lines (C57BL/6, BALB/c, and CD-1) were established for immunology research
- *We hypothesize that natural trained immune mice will provide a novel resource to help better translate mouse immunology and disease models to humans*

Methods

- Blood samples were prepared from BALB/c (inbred) and CD1 (outbred) SPF mice or naturel trained immune
- 0.2 mL of whole blood was incubated with 5µg/mL of *E. coli* lipopolysaccharide (LPS, O26:B6) or no stimuli for 10 minutes
- Proteomic stabilizer buffer (Smart Tube, Inc) was added, then cells were frozen at -80°C
- Samples were thawed and prepared for batched CyTOF staining with a 31-marker antibody panel (7 phospho-protein markers and 24 phenotyping markers)
- CyTOF staining data was analyzed using our OMIQ analysis platform for computational clustering, dimensional reduction, and statistics

Results

- Neutrophils (PMNs), monocytes, and Tregs were more abundant in natural immune mice vs. SPF mice, while B cells were less abundant
- LPS stimulation induced signaling responses by PMNs, monocytes, B cells, and NK cells and monocytes within 10 minutes
- BALB/c and CD1 natural immune mice demonstrated significantly higher LPS-induced phospho-p38 MAPK signaling by PMNs and phospho-STAT1 signaling by monocytes than the SPF mice
- Monocytes from outbred CD1 natural immune mice also showed "primed" P-S6-Ribo and P-CREB signaling responses to LPS stimulation

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 innate immune cell phenotypic changes indicative of trained immunity with enhanced innate immune cell signaling responses to TLR4 stimulation by LPS
3. Natural immune mice will contribute "transitional translational" insights into basic immunological mechanisms by providing better pre-clinical model platforms for human disease processes
4. Future studies will compare injury, bacterial infection, and vaccine responses in matched normal immune and SPF mouse models
5. The establishment of a natural immune mouse facility with adjacent research space provides future opportunities for research collaborations and fee-for-service access by the immunology and other research community

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Natural immune mice show immune phenotypes consistent with acquired trained immunity and will provide new opportunities for "transitional translational" research addressing human diseases

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FIGURES AND TABLES

Table 1: Phospho-Signaling CyTOF Ab Panel

Marker	Metal
T-bet	*111Cd*
CD4	*114Cd*
CD45	*115In*
CD44	*141Pr*
CD11b	*142Nd*
p-NF-k β P65 (S536)	*148Nd*
PD-L1	*149Sm*
AKT	*150Nd*
Ly6C	*151Eu*
CD3	*152Sm*
TLR-4	*154Sm*
CD68	*155Gd*
CD19	*156Gd*
Ly6G	*158Gd*
CD206	*159Tb*
p-Stat1 (Y701)	*160Gd*
Arg-1	*161Dy*
FoxP3	*162Dy*
NK1.1	*163Dy*
CD8	*164Dy*
TCRgd	*165Ho*
p-p38 MAPK (T180/Y182)	*166Er*
CD25	*167Er*
p-AKT (S473)	*168Er*
p-stat3 (Y705)	*169Tm*
Siglec-F	*170Er*
CD49b	*171Yb*
KLRG-1	*172Yb*
I-A/I-E	*174Yb*
p-S6 Ribo (S235/236)	*175Lu*
p-CREB (S133)	*176Yb*

Figure 1: Blood Immune Cell Subset ID by CyTOF

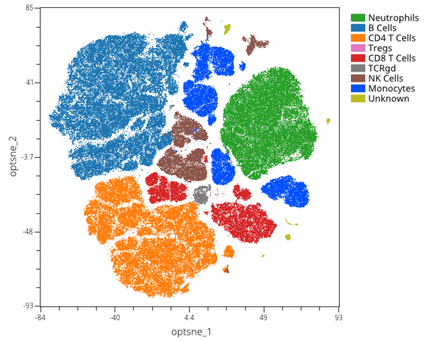

Figure 2: Blood Immune Cell Subset Differences Between Natural and SPF Mice

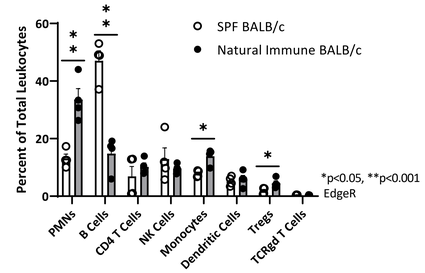

Figure 3: OptSNE Whole Blood CyTOF Phospho-Signaling Plots

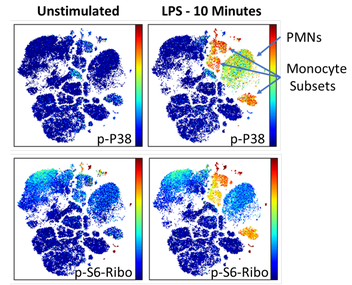

Figure 4: Phospho-Signaling Profile of Blood Immune Cell Subsets

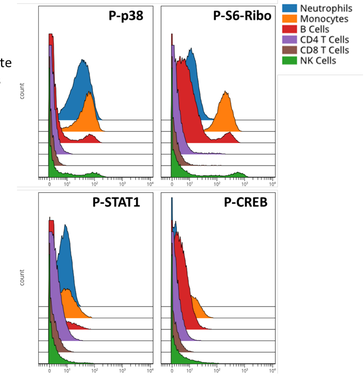

Figure 5: LPS Signaling Differences Between SPF and Natural Immune Mice: "Primed" Neutrophil and Monocyte Signaling in Natural Immune Mice