Modulating Tregs controls the infection response following traumatic injury

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Treg enrichment in a mouse injury model restores immune responses and survival to bacterial infection by modulating inflammation and organ injury

Background:

- Opportunistic infections are a common complication of traumatic injuries and is one of the leading causes of morbidity and mortality.
- This complication is in part due to immune dysfunction following injury and/or an imbalance in inflammatory and counter inflammatory control mechanisms.
- CD4+ regulatory T cells (Tregs) are responsive to injury and expand
- Tregs may play a role in controlling immune homeostasis following severe injury.
- In humans, DR3 regulates inflammation and immunity through controlling development of effector T cells and differentiation of myeloid subsets

Methods:

- Treg populations in FoxP3 DTR and C57BL/6 mice were depleted by Diphtheria toxin (DT) at 40 ng/g or expanded using a Death Receptor 3 (DR3) agonist antibody at 10 µg/g
- DR3 treatment and DT treatments were administered 2 hour and 24 hours after burn injury
- Mice given anesthesia and underwent 25% body surface scald burn injury by exposure to 90 °C water for 9 seconds
- Number and immune phenotype of Treg and myeloid cells were assessed via flow cytometry 72 hours after burn injury
- Two days following injury, mice were inoculated intranasally with *P. aeruginosa* (0.8-1.6 ×10^6 CFU). Survival was monitored for 7 days
- Bacterial clearance, organ injury (TUNEL assay), and plasma cytokines concentrations were measured at 24 hours after infection
- Numbers and immune cell phenotypes in the blood, lymph nodes, and spleen were measured by mass cytometry (CyTOF) at 48 hours after burn injury

Results:

- Modulating Tregs by depletion or expansion did not influence the infection survival of uninjured mice.
- Treg depletion led to decreased survivability in injured mice, while expansion of Tregs enhanced survival.
- Treg depletion showed a significant reduction in the ability to clear bacteria from the lungs
- Treg enrichment did affect bacterial clearance compared to PBS treated mice
- Organ injury levels were highest in burn injured Treg depleted mice, while organ injury was reduced in injured mice treated with anti-DR3 antibody
- Burn injury markedly reduced systemic cytokine levels in response to infection
- Treg expansion partially restored the cytokine response to infection, specifically levels of IL-6, IL-12p70, TNF-α, MCP-1, IFN-γ, G-CSF, and KC were increased, while IL-17 and IL-18 levels remained low
- Changes to the immune landscape as measured by CyTOF
  - **Blood;** Treg enrichment increased neutrophils, B cells. Treg depletion increased neutrophils, but decreased B cells, CD4 T cells, DCs, and eosinophils.
  - **Lymph node;** Treg enrichment increased B cells, and decreased CD4 T cells, CD8 T cells, GD T cells, NK cells, and M1 macrophages. Treg depletion increased in the B cell population, and a decrease in CD4 T cells.
  - **Spleen;** Treg enrichment increased B cells, and decreased CD4 T cells. Treg depletion increased B cells, and decreased CD4 T cells.

Conclusions:

1. **Tregs play a central role in controlling immune homeostasis following severe injury and increases the tolerance to bacterial infections and sepsis**
2. **Tregs modulate the inflammatory and organ injury response to infection**
3. **Future therapeutics targets in human trauma should consider modulating Tregs as a strategy**

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Figure 1: Secondary Bacterial Infection Survival Curve

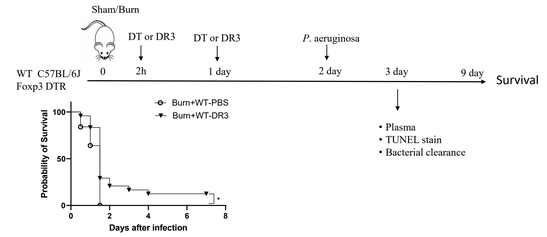

Figure 2: Organ Injury in Kidney

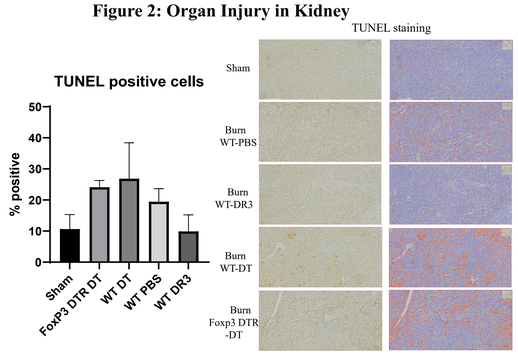

Figure 3: Plasma Cytokines at 24 Hours Post Infection

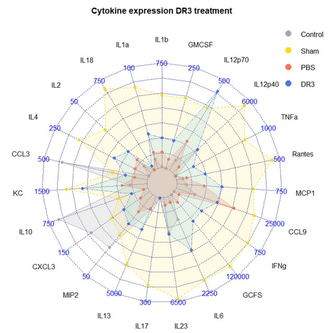

Figure 4: Lung Bacterial Clearance at 24 Hours Post Infection

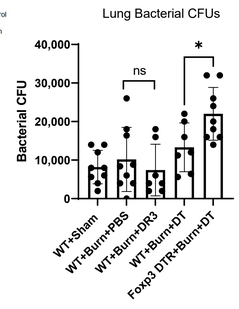

Figure 5: DR3 Induced Changes in Treg and Myeloid Subset Percentages by Flow Cytometry at 3 Days After injury

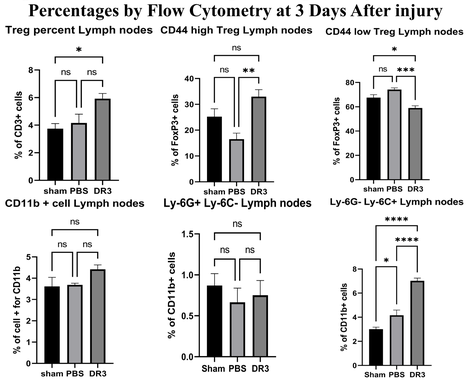

Figure 6: Cell Subset Differences 48 Hours Post Injury by CyTOF