Modulating Endogenous CD4+ T cell Restoration Following Sepsis

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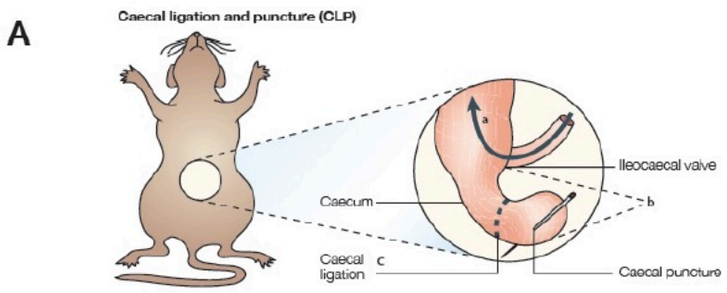
Background

The phenomenon of septicemia often leaves surviving patients with detrimental immunosuppressive sequelae resulting in nosocomial burden. These secondary infections following the acute phase of sepsis kill more than 200,000 hospitalized patients annually[1]. Although survivors restore total lymphocyte cell counts after several weeks, the efficacy of the adaptive immune system is impaired. A significant influence on this impairment can be contributed to the changing profile of the CD4+ T cell population in a given system. While definitive causative agents have not been identified, reactive oxygen species and metabolic stress are candidates when considering contributors to this lymphopenic state. The surviving CD4+ populations undergo homeostatic proliferation to restore bulk numbers, however, the diversity in the repertoire of TCRs can decrease dramatically. Additionally, remaining cells can enter an anergic-like state where effector functions and phenotype plasticity can be limited. With newly acquired deficits in CD4+ responses, patients are left susceptible to a variety of bacterial and viral infections[2]. Many interleukins are critical for T cell development and proliferation. IL-2 has already been implemented in treating patients with severely compromised T cell function. These cytokines may also have the potential to assist T cells reboundiing from a septic environment.

Hypotheses

1) Cytokine therapy (e.g. IL-2 or IL-7) after sepsis augments CD4+ T cell recovery and function.
2) CD4+ T cells reactive to intestinal flora expand following sepsis.

Methods



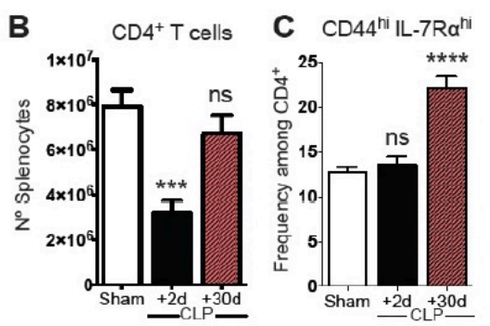


Fig 1. Cecal Ligation and Puncture Model induces a transient state of lymphopenia recovered by peripheral mechanisms of proliferation. A) Animated graphic representing CLP surgery. Anesthetized mice underwent a mid-line laparotomy to locate, ligate, and puncture the distal one-third (~1 cm) of the cecum. **B)** Absolute counts of CD4 T cells in the spleen by day 2 and 30 following CLP surgery. **C)** Day 30 mice develop increased frequency of memory phenotype and upregulated IL-7Rα, indicative of homeostatic proliferation.

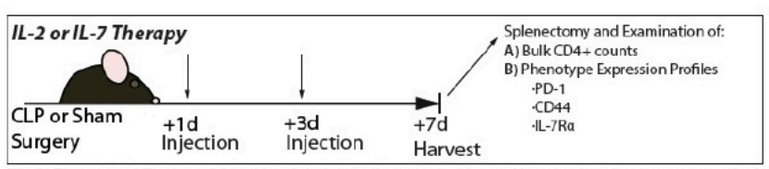


Fig 2. Experiment Design of Cytokine Administration. Interleukin peptides complexed with cognate antibody have been shown to significantly increase half-life while reducing adverse effects systemically[3]. IL-2: S4-B6 (anti-mIL-2 ab) and IL-7: M25 (anti-mIL-7 ab) complexes were given as retro-orbital injections on days 1 and 3 post-CLP surgery before harvesting the spleen on day 7. Samples were stained with anti-PD-1, anti-CD44, and anti-IL-7Rα among others for flow cytometry analysis.

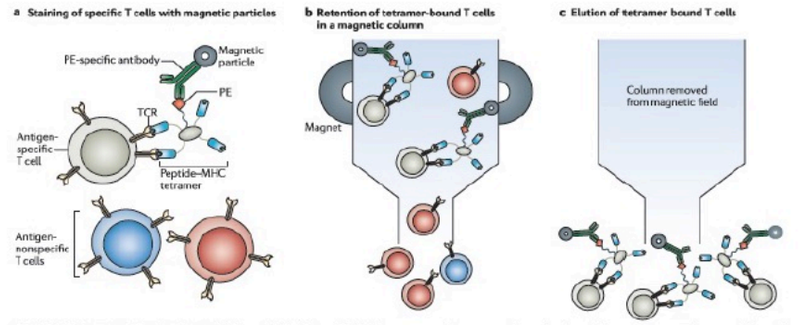


Fig 3. Tetramer Enrichment. Four MHC Class-II: Antigen complexes conjugated to a fluorochrome-tagged streptavidin core selectively bind naive antigen-specific cells with TCRs complementary to the antigen. Samples stained with 2W and NP311 MHC Class-II tetramers were conjugated with Miltenyi magnetic micro beads and applied to MACS columns to enrich the sample with tetramer positive cells.

Cytokine Therapy

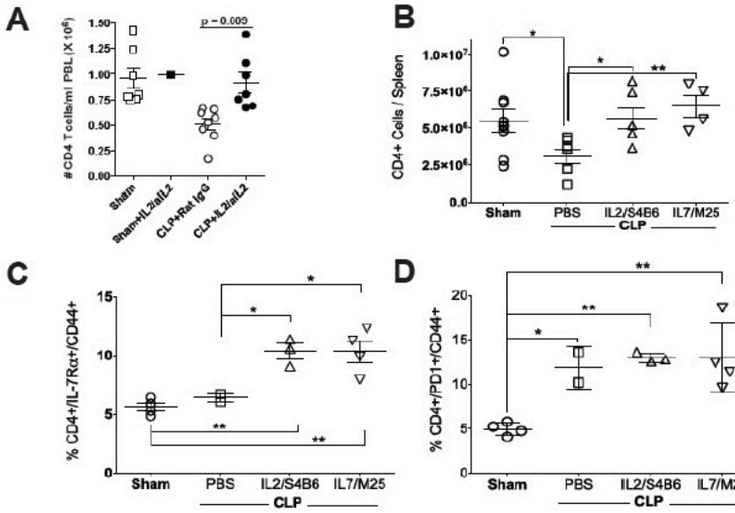


Fig 4. Naive CD4+ responses to treatment with IL-2 and IL-7 cytokine complexes. A) Bulk PBL CD4+ counts increase with IL-2:anti-IL-2 treatment following sepsis. **B)** Bulk CD4+ splenocytes recover similarly after IL-2: anti-IL-2 or IL-7: anti-IL-7 therapy. **C)** Treatment with either cytokine increases the percentage of IL-7Rα upregulated CD4+T cells. **D)** All CLP groups have similar and elevated frequency of PD-1^{hi} expression compared to shams.

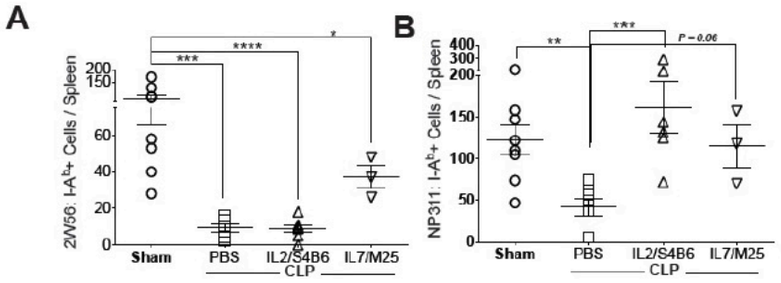
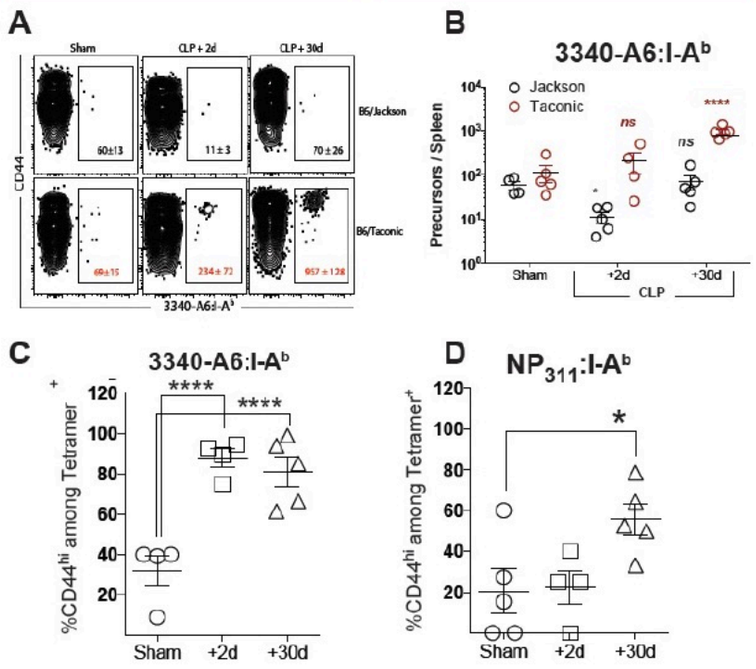


Fig 5. 2W and NP311 Tetramer+ T cell counts among cytokine complex treatment groups. A) Improved recovery of 2W-specific CD4+ cells with IL-7: anti-IL-7 treatment trends toward significance. **B)** NP311-specific CD4+ cells proliferated significantly with IL-2: anti-IL-2 treatment, with a trend towards significance in the IL-7: anti-IL-7 group.

Gut Flora Antigen Stimulation



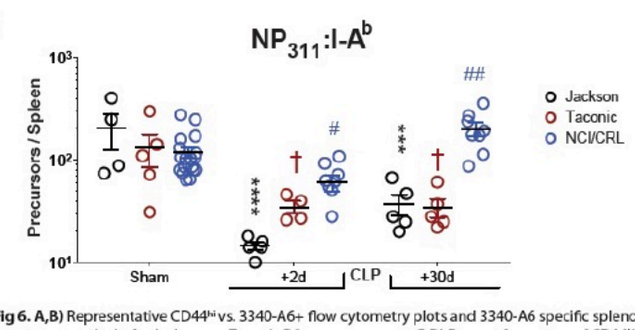


Fig 6. A,B) Representative CD44^{hi} vs. 3340-A6+ flow cytometry plots and 3340-A6 specific splenocyte counts, respectively, for Jackson vs. Taconic B6 surgery groups. **C,D)** Percent frequency of CD44^{hi} cells among 3340-A6 and NP311 clones, respectively, for taconic surgery groups. **E)** Number of NP311-specific clones among Jackson, Taconic, and NCI vendors following surgery.

Summary of Findings

1) **IL-2 and IL-7 complex treatments enhance numerical recovery of bulk CD4+ T cells.**
2) **Enhancement of antigen-specific CD4+ splenocyte recovery from sepsis using interleukin therapy is cytokine selective.**
3) **Variability in gut microbiota influences the recovering capacity of antigen-specific T cell populations after sepsis.**

Acknowledgements and References

1. Griffith Laboratory
2. The Center for Immunology, University of Minnesota
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4. Dan Mueller, M.D.
5. V.A. Hospital, Minneapolis, MN

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