Transcriptomic Perturbations in the Local Innate Immune Response Caused by Staphylococcus Aureus Infected Burn Wounds

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INTRODUCTION

Immune activation, particularly that which results in inflammation, is critical in determining wound healing success as well as physiologic status. This is especially the case in burn-injured tissue. Infection complicates these pathways, particularly if the pathogen produces virulence factors. Determining the innate host response of a burn wound infection at a local, molecular level may help elucidate novel therapeutic targets, potentially topical in nature. This investigation may also lead to identification of a host biomarker signature for determining pathogenicity and presence of bacterial proteins including virulence factors.

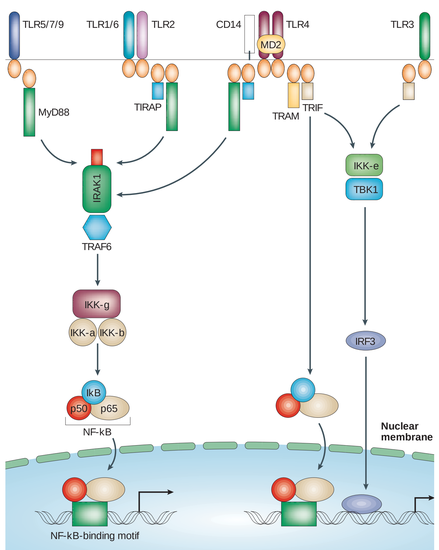

Figure 1: TLR signaling cascade

Figure Reference: Akira, Shizuo, and Kiyoshi Takeda. "TOLL-LIKE RECEPTOR SIGNALLING."*Nature Reviews Immunology* 4 (2004): 499-511. Web.

METHODS

Sprague Dawley rats received burn injuries (2, 2cm x 2cm), that were then inoculated with a virulence factor-producing strain of MRSA or media alone (burn/negative control). A subset of the infected animals received antibiotic treatment, while one group did not (MRSA/positive control). Wound biopsies were obtained daily for 10 days. Half of the biopsies were used to determine bacterial colonization, and the other half were processed for nucleic acid isolation. Isolated RNA was used in an 84-gene antibacterial response PCR Array to quantify gene expression. Real time RT-PCR was then performed to examine differentially regulated genes of interest.

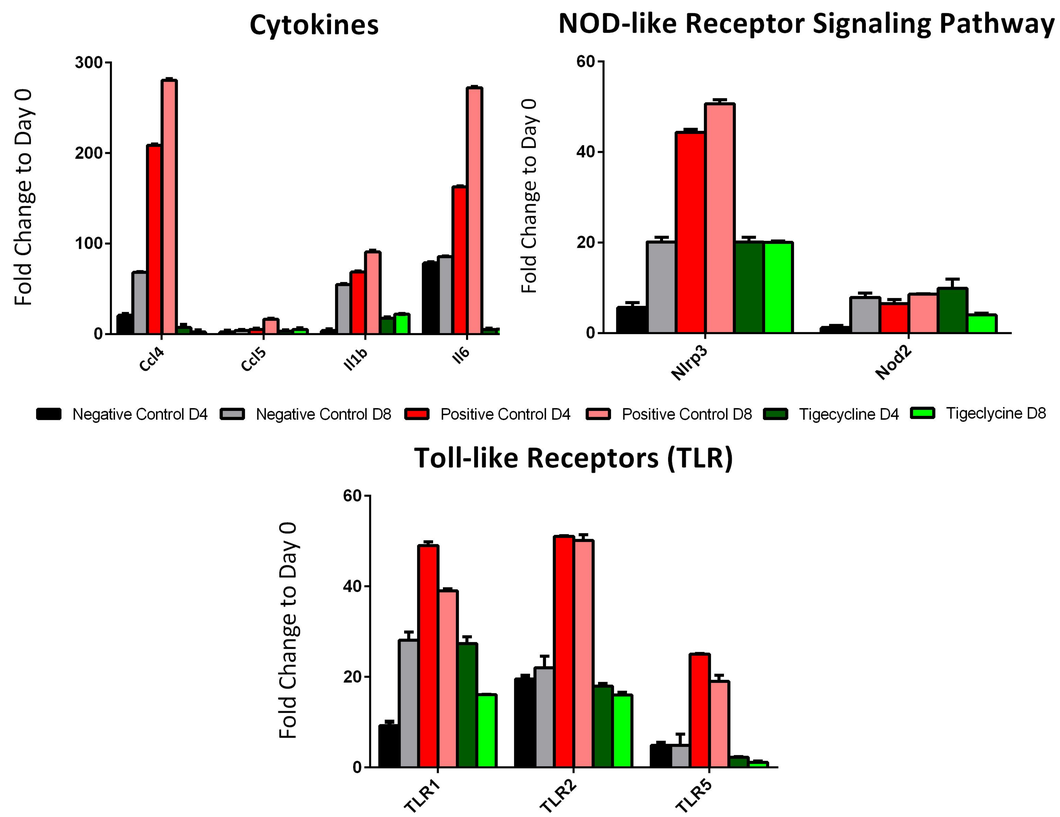

Figure 2: PCR microarray data showing differential mRNA expression of Nod-like receptor signaling pathway, downstream cytokines, and Toll-like receptors based on treatment.

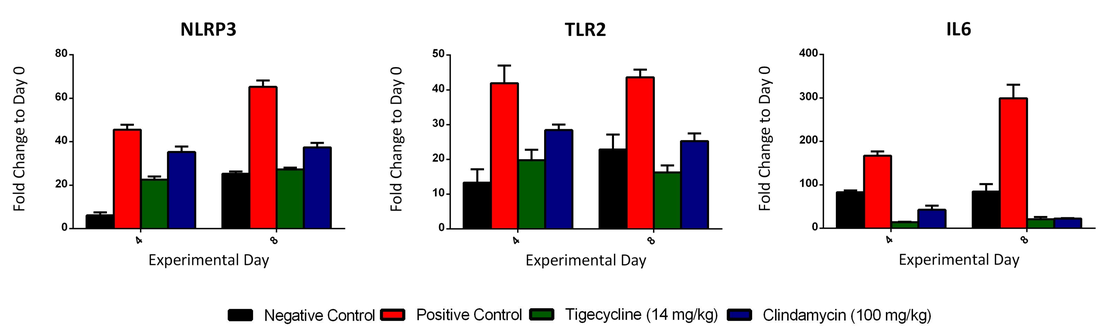

Figure 3: Confirmatory RT qPCR showing differential mRNA expression of NLRP3, TLR2, and IL6 based on treatment

RESULTS

- MRSA was absent in sham animals, but was present as an invasive infection by day 5 in infected, untreated (positive control) animals. Antibiotic treated animals demonstrated significantly lower levels of colonization than positive controls (Fig. 4; $p<0.01$).
- Functional gene groups most impacted by infection were pathogen recognition and signaling, and inflammatory response.
- Transcript levels of genes involved in pathogen recognition, such as TLR2 ($p<0.001$), TLR5, and NLRP3 ($p<0.0001$), were significantly unregulated in positive control animals as compared to negative control (burn only) animals (Fig. 2 and 3).
- Cytokines, such as IL-1B and IL6 ($p<0.001$), were differentially regulated between groups (Fig. 2 and 3).
- Antibiotic treatment modulated the upregulation associated with infection by bringing the same gene expression levels closer to burn only levels (Figure 2 and 3)
- While burn only animals demonstrated lower fold changes in expression of many of the innate immune response genes, an increasing trend from baseline was still observed over time

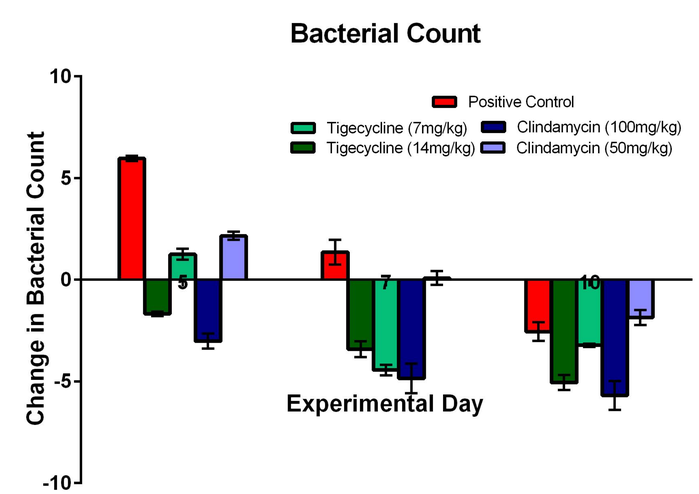

Figure 4: Bacterial count, displayed as a fold change vs. Day 2, based on treatment

CONCLUSION

Significant insight into host response to injury and infection can be obtained from molecular examination of injured tissue. Further studies will investigate sham model up-regulation of innate immunity functional genes.