The Immune Response Against *Staphylococcus aureus* Infections with Different Susceptibility Profiles

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INTRODUCTION: Our goal is designing drug delivery devices for antibiotics based on the risk of infection to decrease the incidence of periprosthetic infection. Our study focuses on evaluating the differences in the immune response to a laboratory strain (ATCC 12600) susceptible to local antibiotics and a multi-drug resistant clinical strain (L1101) of *Staphylococcus aureus*.

METHODS: Under the approved MGH IACUC protocol 2021N000127, we implanted stainless steel plates (10x3x1 mm) subcutaneously on the dorsum of 75 Sprague Dawley rats (n=6/animal). These rats were divided into distinct groups, each receiving varying bacterial inoculants: 10^8 CFU ATCC 12600 (n=30) and 10^8 CFU L1101 (n=30). A non-infected control group (NIC, n=15) was included. Sacrifices were scheduled on postoperative days (POD) 1, 3, 7, or 21. We evaluated the inflammatory response by determining the plasma α-2-macroglobulin (a2M) concentration through ELISA (Abcam ab157730), assessed tissue TNF-α gene expression via RT-PCR, measured tissue T-NF-α and IL-6 protein levels through immunofluorescent staining, and determined tissue capsule thickness using H&E staining. We extended our analysis to include tissue gene expression of IL-12, IL-6, IFN-γ, macrophage colony-stimulating factor (MCSF-1), various T-cell markers, IL-17, VEGFα, matrix metalloproteinase-1 (MMP1), MMP3, and MMP13 using RT-PCR. We visualized bacteria in skin tissue via Brown and Brenn staining. Statistical significance was assessed using t-test analysis.

RESULTS SECTION: Immune cell recruitment was assessed using expression levels of MCSF-1 and T-cell markers (CD4, CD5, CD6, CD8), which were significantly increased. Among gene expressions, the 12600 group had lower MMP-1 on POD 7 than the L1101 group, but higher MMP13 on POD 3. Conversely, L1101 group showed reduced MCSF-1 and VEGFα levels on POD 3 compared to the 12600 group. All infected groups exhibited downregulated VEGFα-CXCR pathway, indicating hindered wound healing by POD 1. Regarding cell-mediated inflammation, the CD4-IL-1β-IL17 pathway was implicated, reflected in lower CD4 and IL-1β gene expressions on POD 3 in the 12600 group, while L1101 showed elevated IL-17 levels on day 1 (**Figure 1**). Cell-mediated immunity was mediated through the CD4-IL12-IFN-gamma pathway, as L1101 group showed lower IL12b at POD 3 and decreased IFN-gamma levels by POD 21 compared to 12600 (**Figure 2**). Collectively, these patterns suggest L1101 infection displayed delayed onset but increased aggressiveness compared to 12600, particularly in early stages via inflammatory cytokines and T-cell markers. Brown and Brenn staining consistently displayed stronger bacteria staining for L1101 than 12600 across all time points (**Figure 3**).

DISCUSSION: This study sheds light on the interaction between Staphylococcus aureus strains with differing susceptibility and the host's defense mechanisms. The increased expression of MCSF-1 and T-cell markers indicates immune activation, with varying profiles implying diverse immune strategies. Strain-specific expressions of MMP-1 and MMP13 point to distinct tissue remodeling dynamics. Notably, lower MCSF-1 and VEGFα levels in the L1101 group on POD 3 suggest altered tissue repair processes. Shared downregulation of the VEGFα-CXCR pathway across infections indicates compromised early wound healing. A CD4-IL-1β-IL17 inflammatory pathway was evident, with suppressed CD4 and IL-1β in the 12600 group on day 3 and early adaptive response in L1101 through high IL-17 levels on day 1. Similarly, CD4-IL12-IFN-gamma immunity was observed, with L1101 showing delayed but robust responses, manifested by decreased IL12b and IFN-gamma levels. The more intense bacterial infection caused by L1101, substantiated by consistent bacteria staining, was responded to by delayed yet aggressive inflammation.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is significant for understanding the immune responses elicited by distinct strains of Staphylococcus aureus and their implications for medical-device-associated infections. The differences in gene expression patterns, especially those related to cytokines and immune cell markers, highlight potential targets for therapeutic interventions. The observed variations in bacterial staining and immune response kinetics underline the need for tailored treatment approaches based on the specific infecting strain. Ultimately, this study contributes to the foundation for designing more effective strategies for treating medical-device-associated infections and improving patient outcomes.

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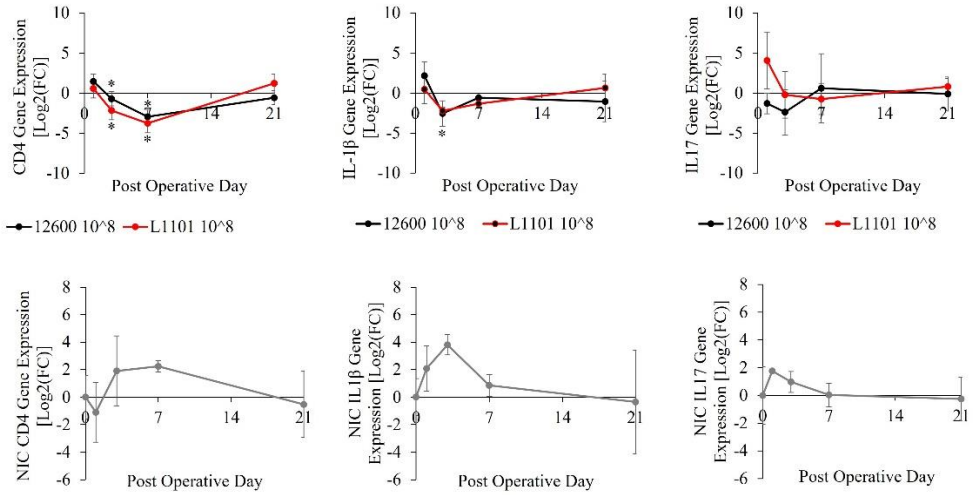

Figure 1. Cell-mediated inflammation, gene expression on POD 1, 3, 7 and 21. Error bars are ±1 SD. (*p < 0.05 vs. day 1).

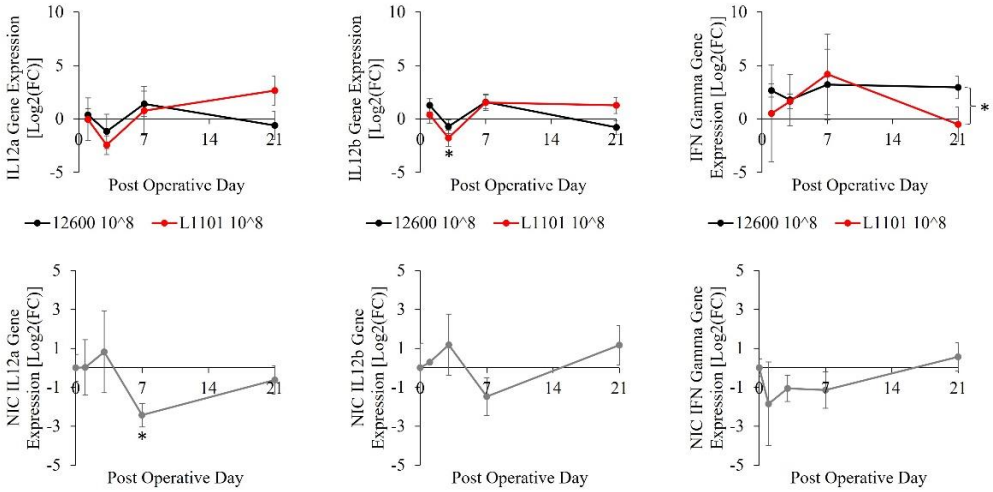

Figure 2. Cell-mediated immunity, gene expression on POD 1, 3, 7 and 21. Error bars are ±1 SD. (*p < 0.05 between groups where indicated or vs. day 1; NIC day 7 vs. day 0).

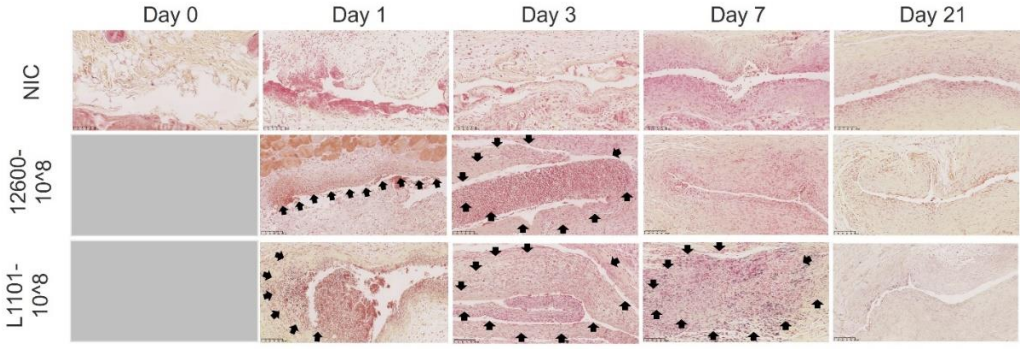

Figure 3. Bacteria analysis via Brown and Brenn staining on POD 1, 3, 7, and 21 (Scale bar = 100 µm).