# The Role of IL-10 in a Model of Biofilm in Periprosthetic Joint Infection

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**Disclosures:** None

INTRODUCTION: The formation of biofilm on prosthetic implants after surgery is associated with the refractoriness of periprosthetic joint infections (PJI) to antibiotic therapy. Biofilm allows the bacteria to evade phagocytosis and the infection to persist until the prosthetic is surgically removed. *S. aureus*, the most common pathogen in PJI, can circumvent immune responses in part by suppressing neutrophil and monocyte function. IL-10 is a potent suppressor of the inflammatory response and monocyte activation, thus blocking IL-10 may potentiate the inflammatory and anti-bacterial functions of monocytes. We hypothesize that when IL-10 is blocked in an in vitro biofilm system, inflammatory activation of monocytes and their expression of pro-inflammatory genes should be upregulated and promote bacterial clearance.

METHODS: Xen 36 *S. aureus* was cultured in RPMI with 10% FBS until reaching an OD of 0.5, corresponding to $5x10^8$ colony-forming units. In a tissue culture plate, a 1:100 dilution of *S. aureus* was grown for 96 h, during which the media was changed every 24 h and planktonic *S. aureus* was removed. At 96 h, the biofilm was treated with 50 ug/mL gentamicin overnight to kill the remaining planktonic bacteria in the biofilm system. CD14+ monocytes were extracted from whole human blood via Lymphoprep separation and MACS magnetic beads. CD14+ monocytes were suspended in RPMI with 10% FBS, 5 ug/mL of gentamicin (to prevent the emergence of planktonic bacteria), and 20 ng/mL monocyte-colony stimulating factor. CD14+ monocytes were then plated onto tissue culture plates and cultured overnight. The 50 ug/mL gentamicin was removed from the biofilm system immediately prior to seeding of the monocytes. $1x10^6$ CD 14+ monocytes were then seeded in 5 ug/mL gentamicin RPMI 10% FBS media ± IL-10 blockade, 50 ug/ml gentamicin-treated 96 h biofilm ± IL-10 blockade, or 25 ng/mL Pam3Cys (an agonist of TLR2 that is activated by *Staph* and serves as a positive control) ± IL-10 blockade. The IL-10 blockade consisted of 10 ug/ml anti-IL-10 and 10 ug/ml anti-IL-10R antibodies added to the system at the same time as the CD14+ monocytes. At 6 h after plating, cells were collected for qPCR analysis and gene expression was quantified. Statistical analysis was performed using a repeated measures two-way ANOVA with Sidak post-hoc test. This study was approved by the IRB and consisted of a series of four experiments utilizing the blood of 10 healthy donors.

RESULTS SECTION: The exposure of monocytes to biofilm in the absence of planktonic bacteria upregulated the expression of anti-inflammatory IL-10 (Fig 1). The exposure of monocytes to biofilm also upregulated the expression of the inflammatory genes TNF and IL-6. IL-10 blockade resulted in a significant superinduction of TNF and IL-6 (Fig 2) compared to untreated controls, showing the biological importance of biofilm-mediated induction of the IL-10 inhibitory pathway. Interestingly, in addition to IL-10, biofilm induced the expression of various other anti-inflammatory genes, including PD-L1 (which inhibits T cells) and IL1RN (which suppresses IL-1). In contrast to the superinduction of inflammatory genes, the expression of these anti-inflammatory genes was attenuated after IL-10 blockade (Fig 3).

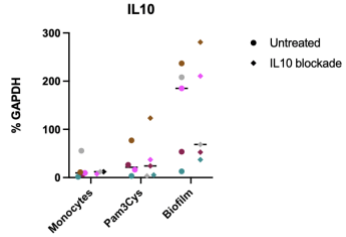


**Figure 1. IL-10 is highly induced in a biofilm system**. qPCR analysis displays higher IL-10 induction in monocytes exposed to biofilm in comparison to untreated monocytes.

DISCUSSION: In our invitro biofilm model monocytes displayed the ability to induce inflammatory cytokines; however, this response was increased by the blockade of IL-10. Biofilm induced a broader anti-inflammatory gene response, including PD-L1 and IL1RN in a manner partially dependent on IL-10. Targeting PD-L1 is effective in immune checkpoint blockade (ICB)-mediated activation of immune responses against tumors. These results suggest that ICB may also be a feasible strategy to suppress PJI. Future studies should be directed towards elucidating if therapies that potentiate the pro-inflammatory activation of monocytes are sufficient to overcome the protective factors of *S. aureus* biofilm in vivo.

SIGNIFICANCE/CLINICAL RELEVANCE: This study is clinically significant because it suggests that the administration of IL-10 antibodies can aid in the resolution of PJIs by inducing a stronger inflammatory response. Using antibodies to treat PJIs is less invasive than the current treatment for PJIs and could lead to improved clinical outcomes.

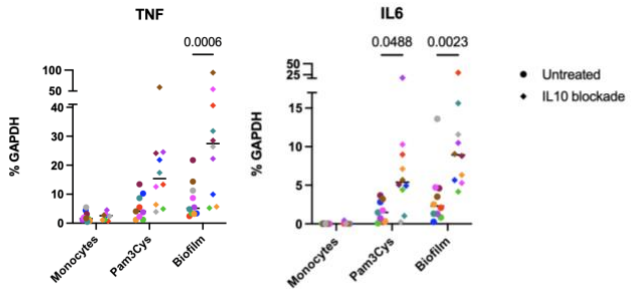


**Figure 2. Inflammatory genes are more greatly induced when IL-10 is blocked.** qPCR analysis revealed monocyte exposure to biofilm upregulates TNF and IL-6 compared to untreated monocytes and these inflammatory genes are further upregulated in a biofilm system with IL-10 blockade.

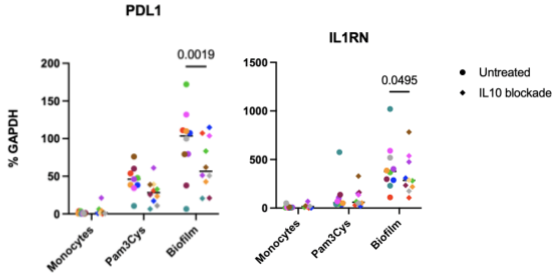


**Figure 3. Anti-inflammatory genes are downregulated when IL-10 is blocked.** qPCR analysis displayed decreased induction of PD-L1 and IL1RN in a biofilm system with an IL-10 blockade compared to an untreated biofilm system.