Toll-like receptor signaling in the host response to insulin infusion cannulas

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Introduction: Insulin infusion sets (IIS) are critical components of insulin pump systems, and are used to deliver insulin to the subcutaneous fat via poly(tetrafluoroethylene) (PTFE) or stainless steel cannulas. Despite advances in pump and IIS design, the insulin infusion cannula remains the weakest link in the insulin pump system. Currently, patients are recommended to replace their IIS every 2-3 days, because insulin adsorption becomes more variable and glycemic control deteriorates over time. Furthermore, IIS cannulas frequently require early, unplanned replacement due to unexplained hyperglycemia, in which bolus insulin injections fails lower blood glucose as expected. While the mechanisms that contribute to the variable and unreliable insulin adsorption at infusion sites are poorly understood, the inflammatory response to the inserted cannula is thought to play an important role. Every time a cannula is inserted into the skin, it damages the surrounding tissue and causes an acute inflammatory response that is exacerbated by the persistent presence of the cannula. This form of an acute foreign body reaction is initiated by the adsorption of molecules derived from blood and damaged cells onto the cannula surface, and the accumulation of leukocytes at the implant site. Toll-like receptors (TLRs) play a critical role in initiating sterile inflammatory responses by recognizing damage-associated molecular patterns (DAMPs) that are released upon tissue injury and cell death. Most TLRs signal via a MyD88-dependent pathway to activate NF-κB and AP-1 transcription factors, which regulate the expression of many pro-inflammatory cytokine and chemokines. We hypothesize that TLRs contribute to the inflammatory response to IIS cannulas by binding DAMPs that are created at the infusion site and adsorbed to the surface of the biomaterial, and that the TLR signaling pathway represents a therapeutic target for modulating the acute biomaterial host response to IIS cannulas.

Experimental methods: Bone marrow cells were isolated from C57Bl6 (wild-type, WT), TLR2-deficient ($TLR2^{-/-}$) and MyD88-deficient ($MyD88^{-/-}$) mice and plated in macrophage differentiation media (RPMI 1640 supplemented with 10% FBS, 20% L929 conditioned media and 50 µg/mL gentamicin) for 10 days to obtain differentiated bone marrow derived macrophages (BMDMs). Macrophage purity ($F4/80^{+}CD11b^{+}$) was confirmed using flow cytometry. Mouse 3T3 fibroblast lysate was made by freeze-thaw cycling (3x) and used as an *in vitro* source of cell-derived DAMPs. Protein solutions of 10% mouse plasma (control) or 10% (w/w) lysate in plasma were adsorbed to fluorinated PTFE (fPTFE) coated well plates for 60 minutes. BMDMs were cultured on pre-adsorbed fPTFE surfaces for 24 hours, and Pam3CSK4 (TLR2 ligand, 150 ng/mL, Invivogen) was used as a positive control. The concentration of pro-inflammatory chemokines and cytokines in the supernatant of BMDMs cultured on fPTFE for 24 hours were analyzed using bead-based assays (LegendPlex, Biolegend), according to manufacturer directions, and analyzed using a flow cytometer.

Results and discussions: Our previous work using an NF-κB reporter macrophage cell line showed that adsorbed DAMPs potently activated macrophages on a variety of biomaterial surfaces, and that this response was largely TLR2-dependent[1]. Here, we assessed protein expression of wild-type, TLR2-deficient and MyD88-deficient BMDMs in response to adsorbed lysate on model fPTFE surfaces. Secretion of pro-inflammatory cytokines IL-1β, IL-6, CCL5, TNF-α and CXCL1 were noticeably increased in wild-type BMDMs exposed to adsorbed lysate compared to plasma, as was the expression of anti-inflammatory cytokine IL-10 (Figure 1). In $TLR2^{-/-}$ and $MyD88^{-/-}$ macrophages, the expression of these cytokines was reduced compared to that of wild-type macrophages, indicating that acute expression and secretion of these cytokines are regulated by MyD88-dependent TLR2 signaling.

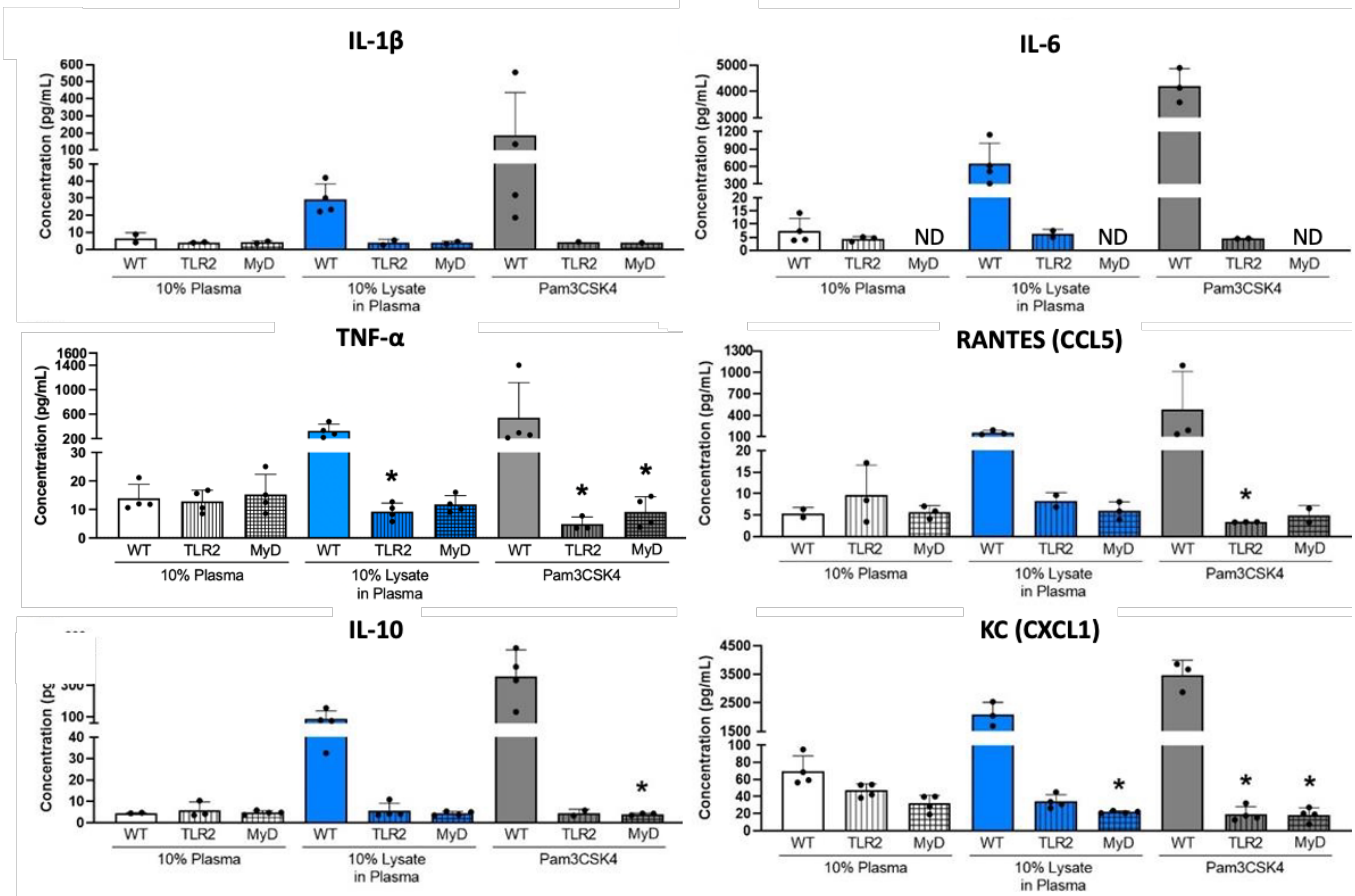

*Figure 1. Secretion of cytokines by WT, TLR2-/-, and MyD88-/- BMDMs cultured on fPTFE for 24 hours. Results are displayed as mean ± SD, with individual points showing the mean concentration of each experiment. WT = wildtype, TLR2 = TLR2-/-, MyD = MyD88-/-. * $p < 0.05$ compared to WT within same condition.*

Conclusions: Using our "tissue damage" protein adsorption model that combines cell lysate (in vitro source of DAMPs) with plasma, we have demonstrated that DAMP-containing adsorbates on model fPTFE surfaces potently increased secretion of chemokines and cytokines in primary wild-type mouse macrophages, compared to adsorbed plasma alone. In TLR2-deficient and MyD88-deficient macrophages, chemokine and cytokine secretion was noticeably reduced for all conditions compared to wild-type, suggesting macrophage activation on DAMP-adsorbed PTFE surfaces was mediated by MyD88-dependent TLR2 signaling. These *in vitro* results support our hypothesis that MyD88, and specifically TLR2, play important roles in the acute inflammatory response to PTFE cannulas used in insulin pump therapy. We also conclude that the similar cytokine expression profile of the TLR2- and MyD88-deficient macrophages suggests that TLR2 may represent a more selective target for modulating macrophage activation in the presence of biomaterial-adsorbed DAMPs, whereas MyD88 inhibition strategies would more broadly affect additional TLRs and the IL-1 receptor signaling cascades. Future work will investigate the contribution of TLR signalling in the host response to implanted PTFE cannulas in adipose tissue.

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References: [1] L.A. McKiel & L.E. Fitzpatrick (2018) *ACS Biomater Sci Eng* 4(11):3792-3801.