The discovery of novel molecular signatures and cellular populations that drive chronic infection in a murine model of periprosthetic joint infection

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INTRODUCTION:

Periprosthetic joint infection (PJI) is a devastating complication of arthroplasty. We have found that chronic smoldering inflammation generated within the peri-implant immune microenvironment (IME) promotes immunosuppression through similar inflammatory and metabolic mechanisms employed by tumors within the tumor microenvironment (TME). In this work, we sought to define the peri-implant IME with the intention of identifying candidate immunotherapeutics.

METHODS:

An established murine model of PJI was utilized. On various post-operative days (PODs), periarticular tissue was homogenized and peri-implant IME cells were isolated for 32-plex cytokine array (Luminex), flow cytometry analyses, and multiplex transcriptome and protein immune profiling in single cells (CITE-seq). This data was analyzed utilizing a novel tensor method to algorithmically identify key findings within our data set. Lastly, an *in vivo* efficacy pilot was performed utilizing FDA approved immunotherapies (Figure 1A). These findings were subsequently analyzed for biologic significance through extensive literature review.

RESULTS:

Luminex and flow cytometry tensor analysis identified three components that represent patterns in the data. Component 1 observed in sterile animals revealed M-CSF-mediated recruitment of macrophages and myeloid derived suppressor cells (MDSCs) that co-express immunosuppressive markers (Figure 2A). Component 2 observed in infected animals revealed acute inflammatory response characterized by an upregulation of cytokines that promote the NF-κB and STAT3 pathways (Figure 2B). Component 3, observed in sub-acute and chronically infected samples, demonstrated STAT1 signaling and alternatively activated macrophages (M2) and MDSCs (Figure 2C). CITE-seq analysis on POD7 demonstrated increased expression of THBS1, Gpr27, C1qb, ApoE, Lgmn, AIF1, and decreased expression of IL-1β, Fgl2, CD7, and Vcan (Figure 3C). CSF-1R immunotherapy reduced tissue bacterial burden by 1.5 log colony forming units (CFUs) (Figure 1C).

DISCUSSION AND CONCLUSION:

Sterile surgery induced healing via pro-phagocytic, poor antigen presenting phagocytes recruited by M-CSF. There was early activation of the NF-κB and STAT3 pathways via IL-1β and IL-6. As inflammation persisted on POD7, STAT1 signaling was predominant and driven by IFN-γ inducible cytokines MIG and IP-10. CITE-seq analysis on POD7 revealed gene expression consistent with unresolvable inflammation and immune checkpoints typified by C1q-ApoE and THBS1. ApoE is a lipoprotein that complexes with C1q, the first component of the classical complement cascade (CCC), to prevent its activation. THBS1 is a pleiomorphic cytokine that induces smoldering inflammation via STAT3 signaling, driven by NF-κB and TGF-β activation which secrete IL-6. However, THBS1 dampens inflammation via multiple parallel processes that induce fibrosis and apoptosis in immune cells, inhibit angiogenesis, inhibit vasodilation and chemotaxis, and inhibit T cell activity via dendritic cell tolerance and Treg cell recruitment. These “breaks” on the immune system appear to inhibit the clearance of persistent PJI. In addition, novel metabolic perturbations unique to PJI were observed including increased cysteine proteases (Lgmn) and changes in insulin metabolism (Gpr27). Finally, AIF1 induces a suppressive M2 macrophage phenotype driven by CSF1. This may explain the reduction in tissue infectious burden observed in the $CSF\text{-}1R_i$ group. This study identifies multiple potential immunotherapeutics: inhibitors of lipoproteins (ApoE), THBS1, TGF-β, CSF-1R or STAT3 and their bi-directional effectors.

Figure 1: Immunotherapy efficacy trial utilizing FDA approved therapies anti-PDL1 and CSF1R

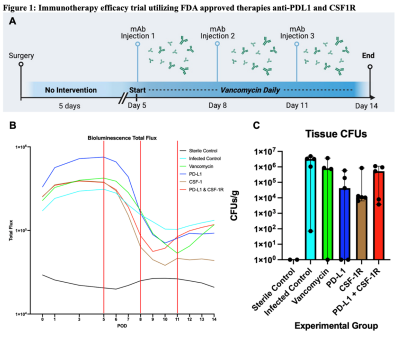

Figure 2: Tensor analysis identifying three distinct components that integrate and characterize Luminex and flow cytometry data

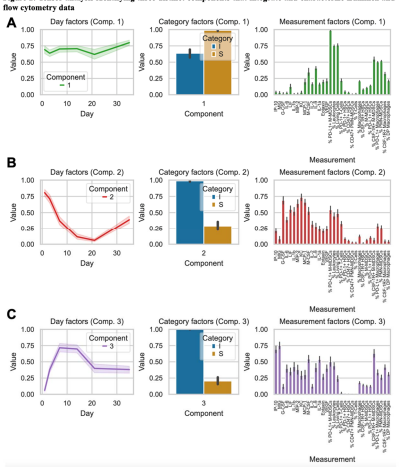

Figure 3: Tensor analysis of CITE-seq data highlighting components 22 and 48