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Title: Characterizing the local immune environment in a murine model of periprosthetic joint infection

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Background: Periprosthetic joint infection (PJI) is a devastating complication of endoprosthetic reconstruction in orthopaedic oncology patients. The formation of a local immunosuppressive immune microenvironment (IME) may drive the development of chronic infection in PJI. Recent investigations suggest that upregulation of immune checkpoint pathways, and recruitment of myeloid-derived suppressor cells (MDSCs) may dampen the local immune response to bone and soft tissue Staphylococcal infection. To identify novel immunopotentiating agents, it is critical to elucidate the cellular mechanisms that promote immunosuppression in PJI. In this study, we endeavor to determine the molecular and cellular changes that occur throughout time in a murine model of PJI.

Questions/Purposes: In a murine model of knee arthroplasty, how do relevant cytokines and local cell populations change over time? Are immune checkpoint markers or MDSCs upregulated in infected animals? What is the predominant immune response in this PJI model?

Methods: An established mouse model of PJI was utilized. A titanium pin was implanted retrograde into a mouse femur and inoculated with 10\textsuperscript{5} CFUs of S. aureus (Xen36) or sterile saline (sham). A further negative control included mice that did not undergo surgery. On post-operative days (PODs) 1, 3, 7, 14, 21, and 35, periarticular tissue was homogenized, normalized, and analyzed for cytokine production using a 32-plex Luminex cytokine array. In a parallel experiment, infected, sterile, and non-operative femurs were isolated, implants were removed, and peri-implant intramedullary IME cells were extracted for flow cytometry analysis using high-speed centrifugation. Flow cytometry analyses was used to quantify innate and adaptive immune populations, and co-expression of relevant immunosuppressive surface markers (PD-1, PD-L1 and CSF-1R).

Results: 14/32 cytokines were significantly elevated in the peri-articular tissue of infected mice vs. sterile controls. Cytokines that peaked on PODs 1 or 3 include G-CSF (POD1: 4.2 vs. 65.3 pg/ml, p = 0.003), CCL-3 (POD1: 4.8 vs. 37.3 pg/ml, p = 0.02), CXCL-1 (POD1: 51.6 vs. 480.6 pg/ml, p = 0.001), CXCL-2 (POD1: 116.3 vs. 1452 pg/ml, p = 0.008), and IL-2 (POD3: 2.9 vs. 10.3 pg/ml, p = 0.003). Cytokines that peak on PODs 7 or 14 include IL-1\alpha (POD14: 9.3 vs. 34.5 pg/ml, p = 0.01), IL-6 (POD7: 9.8 vs. 221 pg/ml, p = 0.005), IL-17 (POD7: 0.03 vs. 4.3 pg/ml, p = 0.001), RANTES (POD14: 3.1 vs. 14.4 pg/ml, p = 0.008), IL-1\beta (POD7: 0.5 vs. 8.6 pg/ml, p = 0.0002), CCL-4 (POD7: 19.35 vs. 115.9 pg/ml, p = 0.01), CXCL-9 (POD14: 255.7 vs. 10527 pg/ml, p = 0.004), and CXCL-10 (POD14: 94.4 vs. 594.8 pg/ml, p = 0.04). On flow cytometry, in both sterile and infected groups neutrophil recruitment (POD3) was followed by F4/80\textsuperscript{+} macrophage recruitment by POD7. PD-L1\textsuperscript{+}CSF-1R\textsuperscript{+} F4/80\textsuperscript{+} macrophages and monocytic MDSCs (M-MDSCs, CD11b\textsuperscript{+}Ly6C\textsuperscript{hi}Ly6G\textsuperscript{lo}/CD45\textsuperscript{hi}) were significantly elevated in PJI samples on POD7 (29.6\% vs. 45.8\%, p = 0.007 and 13.1\% vs. 18.9\%, p = 0.0006, respectively). PD-L1\textsuperscript{+} immune cells (as a percentage of CD45\textsuperscript{+} cells) were significantly elevated in both sterile control (titanium pin) and infected mice compared to non-operative control (POD7: 4.0\% vs. 15.1\%, p < 0.001, and 4.0\% vs. 21.1\%, p <0.001), and were significantly elevated in infected mice compared to sterile control at POD7 (15.1\% vs. 21.1\%, p = 0.002).

Conclusions: In a murine model of PJI, the early cytokine response corresponds with an acute monocyte and neutrophil recruitment. As inflammation secondary to PJI persists, the cytokine milieu further corresponds with cellular immunity in a Th1/Th17 dominated manner, despite a low proportion of T-cells. This native immune response fails to eradicate S. aureus PJI. In this model, chronic S. aureus PJI promotes the recruitment of MDSCs and...
immunosuppressive PD-L1+ CSF-1R+ macrophages (F4/80+). Immunosuppressive polarization may be driven by both pin implantation and PJI, resulting in MDSC recruitment and immune checkpoint activation.