Immune Dysregulation in a Rat Model of Infected Femoral Segmental Bone Defect

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Purpose: Open contaminated fractures are a clinical challenge. Morbidity includes impaired bone healing, infected nonunion, osteomyelitis, and associated hardware failure. In the setting of trauma and infection, it has been proposed that immune dysregulation can further impair healing. The purpose of this study was twofold: to establish a clinically relevant model of infected segmental bone loss, and to investigate the effects on immune response.

Methods: 13-week-old rats underwent unilateral noncritical femur bone defects surgery with plate stabilization. Animals were placed in 1 of 3 groups: naïve (no surgery, n = 2), control (segmental defect only, n = 2), and infection (segmental defect + infection, n = 4). The infection group received a gelatin sponge soaked with luciferase expressing *Staphylococcus aureus* (Xen 29; 10^4 CFU). Bacterial growth was monitored by bioluminescence. Radiographic imaging was performed every 2 weeks. The end point was at 12 weeks, but animals were euthanized if hardware failure was observed. Bacterial contamination confirmed by wound culture and systemic immune response was measured by serum ELISA (enzyme-linked immunosorbent assay), collected upon euthanasia. Luminex data were analyzed by D-PLSR modeling in MATLAB with the partial least squares algorithm by Cleiton Nunes. MicroCT scans were performed to quantify bone bridging and periosteal growth.

Results: Bioluminescent signal appeared in infection animals at day 3 post-surgery and was present up to day 7. In the infection group, serial radiographs showed no bridging at any time point, with 3 of 4 with hardware failure. Inspection of the thighs of euthanized infected animals demonstrated deep purulence. Bacterial cultures were positive for the infection group and negative for the controls. Infected rats had increased expression of immune suppressive cytokines (interleukin [IL]-10) and reduced concentration of inflammatory cytokines (tumor necrosis factor [TNF]-α, IL-1). Flow cytometry showed increased myeloid-derived suppressor cells (MDSCs) and Tregs population in spleens from infected animals.

Conclusion: We present a novel clinically relevant infected rat segmental bone defect model with associated hardware failure. Serum samples from infected animals possessed lower concentrations of key proinflammatory cytokines and an increase in immunosuppressive cell populations (eg, MDSCs) in the spleen, suggesting chronic systemic immune dysregulation due to the infection. While these preliminary results are promising, further work is ongoing to perform histology, increase sample sizes, and optimize control groups.