Role of Regulatory T-Cells in the Development of Post-Traumatic Osteoarthritis

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Background: Traumatic etiology is implicated in 12% of the cases of patients seeking care for osteoarthritis in the United States[1]. This type of arthritis, termed post traumatic osteoarthritis (PTOA), has been proposed to be mediated in part through an imbalance in inflammatory processes[2]. T-cells are an important mediators, and depending on the T cell subtype, they can either promote or inhibit inflammation. In murine models, athymic mice deficient in T-cells have been shown to develop more severe PTOA after intra-articular fracture (IAF) than control animals[3]. Conversely, MRL/MpJ mice, a "super healer" strain, have demonstrated accelerated and improved healing in a variety of injury models including PTOA after IAF[4]. Lesser severe osteoarthritis (OA) develops in MRL mice after IAF irrespective of fracture displacement. A subtype of these T-cells, called regulatory T-cells (T-regs), can suppress the activity of innate and adaptive immune cells including other T-cell subsets thereby reducing inflammation. T-regs have been shown to be a protective factor in several inflammatory diseases including rheumatoid arthritis[5], inflammatory bowel disease[6] and acute respiratory distress syndrome[7] to name a few. FOXP3 protein (transcription factor) and CD25 (IL-2 receptor) are expressed by T-regs and can be used as cell markers[8]. To assess the potential importance of T-regs in development of PTOA, we sought to quantify differences in T-reg population within the synovium in B6 (native) mice versus MRL-MpJ mice following IAF. We hypothesize that there would be a greater number T-regs (Foxp3+ and CD25+ receptor positive cells) and lower histologic synovial inflammation scores in the MRL mice when compared to the B6 mice.

Methods: Six experimental MRL/MpJ super healer mice (MRL) and six control c57BL/6 nude mice (B6) were each subjected to IAF, as previously described in Buchanan et. al. Mice were euthanized at 2 weeks at which point their hind limbs were harvested and their knees were prepared and analyzed for histology. The tissue was then subjected to dual immunohistochemical staining for Fox-P3 and CD25 markers to identify the regulatory T cells in the tissue. The number of positive cells was quantified using light microscopy and compared for statistical significance. Analysis of results was performed via Wilcoxon matched pairs. Synovitis Scores were quantified from H&E stained sections and analyzed with Kruskal-Wallis analysis.

Results: For the Foxp3 staining, there was no statistically significant increase in fracture (Fx) vs contralateral non-fractured (NonFx) limbs for either strain. Furthermore, B6 mice exhibited a trend of higher counts of Foxp3+ cells in synovium of both Fx and NonFx limbs compared to MRL. For the CD25 stain, the MRL fractured limbs had had higher counts of the CD25+ cells in synovium compared to contralateral NonFx limbs ($p=0.059$). There was no statistically significant difference in cells counts of NonFx or Fx limbs when comparing B6 to MRL mice. For the synovitis scores, there were no statistically significant correlations with Foxp3+ or CD25+ cell counts. B6 synovitis scores inversely correlated with Foxp3+ ($r_s=-0.55$, $p=0.257$) and MRL synovitis score inversely correlated with CD25+ ($r_s=-0.38$, $p=0.461$).

Conclusion: There was no significant difference observed in T-reg response or correlation with synovitis scores between B6 and MRL mice at 14 days following IAF . This suggests that differences in PTOA in the MRL may either: not be T-reg mediated, that time points other than 2 weeks post-injury may be more informative, or that the T-regs in synovial fluid or circulating in the blood may be more important than cells in the tissue. Further research is needed to determine the role of T-regs at more acute or chronic time points in the healing process. While we did see that there was a trend towards less synovitis in the MRL samples being correlated with a higher number of CD25+, this result was not significant. This suggests that T-regs may have a protective effect when it comes to severity of synovitis. Further, studies are needed to identify the role of T-regs in post-fracture inflammation and PTOA development.