Immunophenotyping to Characterize Immune Dysregulation in a Delayed Fracture Healing Murine Model

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Purpose: The immune system plays a critical role in bone fracture healing. As fracture healing progresses, analyzing the relative presence of different immune cells in key tissues can elucidate important factors in the immune response to fracture healing. Here, we employ spectral flow cytometry to determine the immunophenotype of the blood, bone marrow, and lymph nodes of wild type (WT) and delayed fracture healing, accelerated aging mice (Z24-/-) with tibia fractures. We hypothesize that there will be an influx of inflammatory- related immune cells at the day 3 post fracture time point and that Z24-/- mice would have increased senescence both before and after fracture.

Methods: Age matched Z24-/- and WT controls underwent right tibia fracture with intramedullary fixation. Mice were then sacrificed 0, 3, 14 and 21 days following fracture for serum collection and harvesting of ipsilateral and contralateral inguinal lymph nodes and tibia bone marrow. Samples were then analyzed using 32- panel spectral flow cytometry to quantify amounts of various immune cells.

Results: Both the bone marrow and lymph nodes of Z24-/- had decreased amounts of B cells and increased quantity of non-B cells when compared to WT mice 3 days post fracture. Z24-/- mice also had more granulocytes in the ipsilateral bone marrow but not in the contralateral bone marrow when compared to WT mice at the same time point. The serum of Z24-/- mice had fewer counts of some macrophage populations that were upregulated in WT mice at day 3 but did not have a diminished B cell repertoire similar to that seen in the bone marrow and lymph nodes. Z24-/- serum also showed increased C12FDG+ senescent cells pre-fracture 14 days post fracture.

Conclusion: This study employs a flow cytometry panel to investigate immune cell populations involved in the fracture repair immune response that are under-investigated in this context. Further, comparisons between WT and delayed fracture healing Z24-/- mice allows for characterization of the potentially impaired immune response that could contribute to delayed fracture healing.

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