Immune Characterization of the Dorsal Root Ganglia during Osteoarthritis

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Objective: Osteoarthritis (OA) is a painful disease of the synovial joints and the most common form of arthritis. OA is a major cause of chronic pain worldwide leading to significant years lived with disability and socioeconomic burden. While mechanical wear and tear is the canonical contributing factor to OA disease, it is now acknowledged that low-grade inflammation and the innate immune response significantly promotes OA disease progression. Further studies are needed to understand the neuro-immune interactions that are the forefront of OA pain. Specifically, macrophages are involved in the production of cytokines and chemokines that signal for inflammation and are likely to contribute to inflammatory pain. Here, we aimed to study macrophages and the innate immune response in the dorsal root ganglia (DRG) in mouse models of OA disease.

Methods: We utilized flow cytometry to quantify and measure expression of certain markers on immune cell populations in diseased OA animals compared to naïve animals. We also co-stained for neuronal markers to characterize the neuro-immune microenvironment within the DRG with or without OA disease. We examined neuro-immune populations in the destabilization of the medial meniscus (DMM) and partial meniscectomy (PMX) models of OA. We also studied CX3CR1-eGFP reporter mice to look at CX3CR1+ myeloid cell influx by immunofluorescence in OA or naïve mice.

Results: We optimized a protocol for isolation, digestion, and flow cytometric analysis of the mouse DRG in healthy and diseased conditions. On average, when using enzymatic digestion, we yielded about 1.5-2 million cells per mouse which includes 6 DRGs (L3-L5 lumbar spine) or ~250-300K cells per DRG. In addition, our protocol also yielded good viability (~80-90%) in each sample. We stained for immune cell markers (CD45, CD11b, Ly6C, Ly6G, F4/80, CD3, CD4, and CD8). Gated under live single cells, there were 1-13% total CD45+ leukocytes in the DRG, and naïve CD45+ cell frequencies varied with age. We observed significant changes in the immune cell populations and extent of immune infiltration the DRG in OA mice compared to naïve.

Conclusion: We now have an optimized protocol for flow cytometry analysis on mouse DRG. This technique will be applied to many important questions we seek to answer regarding OA pathology and chronic pain, specifically the neuro-immune communication that occurs during OA pathogenesis. For instance, we will be able to examine the infiltration and activity of macrophages at different time points as OA progresses and correlate these observations with pain behaviors in OA-diseased mice. Understanding the neuro-immune microenvironment within the DRG during OA will inform mechanisms of pain behavior and may lead to targeted therapies for patients suffering from OA in the future.