Synergistic Immunomodulatory Effects of Transferred M2 Macrophages and IL4-releasing Microparticles in Murine Hindlimb Ischemia

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Introduction: Immunomodulatory biomaterials are a great tool to influence the body's healing capacity by providing external stimuli. Macrophages are an ideal cell type to be targeted, because they play large roles in both inflammation as M1 macrophages, and in wound healing/resolution as M2 macrophages. M1 macrophages are thought to promote angiogenesis, while various M2 phenotypes support and stabilize newly formed vasculature. A potent stimulator of the M2 macrophage phenotype is interleukin-4 (IL-4). When targeting this cell type, care must be given to ensure that immunomodulatory treatment is optimized, to promote the proper phenotype. In this study, our strategy was to inject M2 macrophages into an ischemic injury in mice to resolve inflammation. IL4-releasing polylactic-co-glycolic (PLGA) microparticles were included to maintain the phenotype of the injected macrophages in the inflammatory injury microenvironment and additionally modulate host macrophages' phenotype. We hypothesized that the inclusion of macrophages along with treatment would be synergistic to creating and maintaining an M2-promoting environment.

Methods: Mice underwent surgery in which the femoral artery and vein were unilaterally ligated to create a model of hindlimb ischemia (HLI). This surgery generated a wound environment in the gastrocnemius muscle that was highly inflamed. Injured muscles were then injected 3 days later with a treatment of phosphate buffered saline (PBS), 0.5mg of IL-4 loaded (40ng IL-4 /mg PLGA) PLGA microparticles (20-30um in diameter), or 0.5mg of IL-4-loaded PLGA microparticles co-injected with $5x10^5$ green fluorescent protein (GFP)-expressing M2a-polarized bone marrow-derived macrophages. 7 days after surgery, the mice were sacrificed, and gastrocnemius muscle harvested. The muscle was digested and stained with flow cytometry markers specific to macrophages and their phenotypes as well as a live/dead marker to gate out dead cells. All animal studies were approved by the Drexel Institutional Animal Use and Care Committee.

Results: Mice treated with the IL-4 loaded microparticles and IL-4 microparticles with macrophages both caused increased expression of ARG1, an M2 marker, in host macrophages. Interestingly, the IL-4 microparticle treatment had no effect on CD206 expression, another broadly used M2a marker. Delivery of IL-4 microparticles reduced macrophage expression of C-X-C chemokine receptor type 4 (CXCR4), compared to PBS, which was further reduced when M2 macrophages were co-injected with the microparticles. The IL-4 microparticle treatment also significantly inhibited the expression of M1 marker CD86, and co-injection of macrophages further increased this effect. Though the co-injection group was more effective in inhibiting inflammation than IL-4 microparticles alone, it is quite intriguing that the population of recovered GFP macrophages was extremely small, at around 0.5% of the F4/80+ macrophages in the muscle.

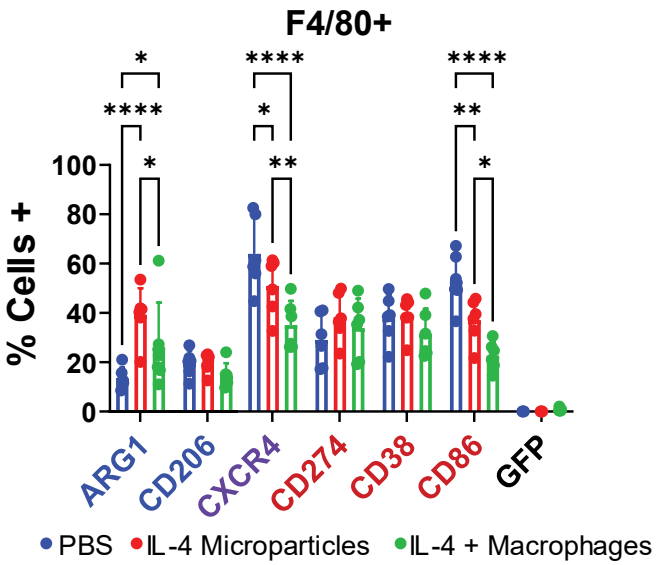

Figure 1. Flow cytometric analysis of macrophages harvested from ischemic gastrocnemius muscle. Macrophages were stained with M2 markers (ARG1 and CD206, in blue), hybrid M1M2 marker (CXCR4, in purple) and M1 markers (CD274, CD38, and CD86, in red).

Conclusion: The reduction of CXCR4 is interesting as CXCR4 inhibition shown to reduce production of inflammatory cytokines such as interleukin-6 and tumor necrosis factor alpha **(**Tian, X. Cell Biosci 2019; 9;55). The GFP macrophages injected into the wound site may have migrated out of the muscle site over the period of 4 days between injection and tissue harvest. It is also possible that the high shear stress resulting from injection caused macrophage cell apoptosis. Phagocytosis of apoptotic cells has been shown to inhibit inflammasome activity in macrophages, which could explain why some of the inflammatory markers, such as CD86, were reduced (Benoit ME, J Immunol. 2012 ;188;11). This research shows that co-injection of macrophages can help immunomodulatory biomaterials to reduce inflammation, while still promoting an M2 phenotype.

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