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Digit Amputation Level Influences Macrophage Polarization

INTRODUCTION: Macrophages (Mϕ) play a critical role in the healing process after physical trauma. Early after injury, Mϕs exhibit an inflammatory activation state which functions to create a sterile environment and promote angiogenesis. Inflammatory Mϕs transition to a resolution or "tissue repair" state in which they reduce inflammation and promote collagen deposition, bone mineralization, and myoblast differentiation[1,2]. Without the proper balance of inflammatory and tissue repair Mϕs, most injuries will not heal. Thus, Mϕs make promising therapeutic targets to improve wound healing in pathological conditions such as chronically open wounds. However, what controls the shift in Mϕs from inflammatory to wound resolution is not well understood.

METHODS: To better understand what drives this shift, we leverage a model of traumatic injury in which amputation through the bone and skin of a mouse digit tip (P3) results in complete regeneration of the tissue. A proximal amputation through the second phalanx (P2) results in bone callus and scar tissue formation. We first isolate secreted factors from the injury site by amputating the mouse digit at the P2 or P3 level. 16 digits per injury site are collected 10 days after injury and homogenized. The homogenate is filtered and total protein levels measured. To test the effects of the injury factors on Mϕs, marrow cells are collected from the femur and tibia of three CD1 outbred mice. Marrow cells are exposed to mCSF (L929 media)[5] and mature into naïve Mϕs (M0). M0s are then exposed to homogenates (1mL each) for 24 hours. To differentiate between Mϕ activation states, we measured changes in metabolic capacity. The Seahorse XF96 was used to measure glycolytic rate (higher in inflammatory Mϕs) and fatty acid oxidation (FAO; higher in resolution Mϕs)[6–8].

RESULTS/DISCUSSION: Our results show Mϕs exposed to P3 homogenate exhibited increased FAO. In contrast, P2 homogenate Mϕs did not change FAO. This implies that the local environment in regenerative injuries promotes a metabolic profile conducive to tissue repair. Mϕs exposed to both homogenates exhibited heightened extracellular acidification due to increased glycolysis, indicating increased glucose metabolism in response to injury-induced cues. However, it is noteworthy that P2 Mϕs displayed higher glycolytic rates compared to those exposed to P3. This implies that the P2 environment induces a shift towards glycolytic metabolism, suggesting a heightened inflammatory response. These results support our hypothesis that factors within the P2 nonregenerative environment polarize Mϕs toward a more inflammatory phenotype at least at 10 days after amputation. These results also suggest factors within the P3 environment at 10 days after amputation influence Mϕs to be more resolution-like.

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