Digit Amputation Level Influences Macrophage Polarization

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INTRODUCTION: Macrophages play a critical role in the body's healing process after physical trauma to skin, bone and muscle. Early after injury, macrophages exhibit an inflammatory activation state. These inflammatory macrophages create a sterile environment, clean up tissue debris, and recruit new blood vessels into the healing tissue. Inflammatory macrophages then transition to a resolution or "tissue repair" state in which they reduce inflammation and promote collagen deposition by fibroblasts, bone mineralization by osteoblasts and myoblast differentiation in muscle[1,2]. Without the proper balance of inflammatory and tissue repair macrophages, skin, bone and muscle injuries will not heal. Thus, macrophages orchestrate the healing response across the body, and make promising therapeutic targets to improve wound healing in pathological conditions such as non-union fractures, volumetric muscle loss, and chronically open wounds. However, what controls the shift in macrophages from inflammatory to wound healing is not well understood.

To better understand what drives a shift in macrophage activation state, 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. In comparison, a more proximal amputation through the mouse second phalangeal element (P2) results in unpatterned bone callus and scar tissue formation. These two injuries give us a comparison of regeneration and scar formation within the same animal and is ideal for the comparison of studies that investigate the impact of the local environment on macrophage activation state. Previous studies suggest macrophages in regenerative tissue secrete lower levels of cytokines and are less inflammatory than macrophages in a non-regenerating injury[3,4]. We additionally observe macrophages in P2 at 10 days after amputation express higher levels of inflammatory cytokines compared to macrophages in the P3 injury 10 days after amputation. We therefore hypothesize that macrophages exposed to P2 injury will polarize to a more inflammatory state compared to macrophages exposed to a P3 injury. To better understand how the environment impacts macrophage activation state, we expose macrophages in vitro to homogenate collected from the P2 and P3 injury sites at 10 days after amputation and measure changes in inflammatory markers.

METHODS: We first isolate secreted factors from the injury site, by amputating the mouse digit at the P2 (non-regenerative) or P3 (regenerative) level. 16 digits per injury site are collected ten days after injury and homogenized. The homogenate is filtered and total protein levels measured. To test the effects of the injury factors on macrophages, marrow cells are collected from the femur and tibia of three CD1 outbred mice. Marrow cells are exposed to mCSF (L929 media)[5] and allowed to mature into naïve macrophages (M0). Naïve bone marrow derived macrophages are then exposed to filtered homogenates (1mL each) from the P2 or P3 injury site for 24 hours. Each experimental condition was repeated on bone marrow cells from n=3 mice. All animal experiments were conducted with approval of IACUC at LSUHSC. To differentiate between macrophage activation states (inflammatory and resolution populations), we measured changes in metabolic capacity. The Seahorse XF96 was used to measure glycolytic rate (higher in inflammatory macrophages) and fatty acid oxidation (higher in resolution macrophages)[6–8].

RESULTS/DISCUSSION: Our results show macrophages exposed to the homogenate from regenerative injury sites (P3) exhibited an increase in fatty acid oxidation (FAO), suggesting a metabolic shift associated with tissue repair. In contrast, macrophages exposed to homogenate from non-regenerative injury sites (P2) did not display a change in FAO. These observations imply that the local environment in regenerative injuries promotes a metabolic profile conducive to tissue repair, possibly by providing energy sources that support resolution-like macrophage functions. Furthermore, macrophages exposed to both P3 and P2 homogenates exhibited heightened extracellular acidification due to increased glycolysis, indicating increased glucose metabolism in response to injury-induced cues. However, it is noteworthy that macrophages exposed to P2 homogenate displayed even higher glycolytic rates compared to those exposed to P3 homogenate. This finding implies that the P2 non-regenerative environment induces a more pronounced shift towards glycolytic metabolism in macrophages, suggesting a potentially heightened inflammatory response. These results support our hypothesis that factors within the P2 non-regenerative environment polarize macrophages 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 macrophages to be more resolution-like.

Overall, our experimental results provide compelling evidence to support our hypothesis that the local environment plays a pivotal role in driving macrophage polarization towards inflammatory or resolution-like states. At 10 days post-amputation, the P2 environment appears to skew macrophages towards an inflammatory phenotype, characterized by both elevated glycolytic metabolism and heightened expression of inflammatory cytokines. In contrast, the P3 environment promotes macrophages to adopt a resolution-like phenotype, marked by increased fatty acid oxidation and a potential dampening of inflammatory cytokine expression. These findings underscore the significance of the wound microenvironment in shaping macrophage activation states and subsequently influencing the overall wound healing process. Further investigations into the specific molecular cues within the P2 and P3 environments that orchestrate these macrophage responses are warranted. Understanding these cues could provide valuable targets for therapeutic interventions aimed at promoting favorable macrophage activation states to enhance wound healing in various pathological conditions.

SIGNIFICANCE/CLINICAL RELEVANCE: This study's findings highlight the pivotal role of the local wound microenvironment in driving macrophage activation states during the healing process. These insights into the metabolic and cytokine expression differences between regenerative and non-regenerative injury sites hold promise for developing targeted interventions to enhance wound healing outcomes in conditions such as non-union fractures, volumetric muscle loss, and chronic wounds.

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