Splenectomy Impairs Fracture Repair via Loss of Splenic Macrophages

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INTRODUCTION: Prior studies in trauma patients and in animals show that splenectomy delays fracture healing, implicating the spleen as a potential source of reparative cells. However, the mechanism causing this observation and potential methods to counteract the slower repair are unknown. There is a reservoir of macrophages in the spleen, and macrophage cells are critical mediators of skeletal repair. Previous work showed that the spleen is a reservoir for macrophages lineage capable of migrating to sites of injury. This study aimed to investigate the role of spleen-derived macrophages in fracture healing using a spleen transplantation model in mice.

METHODS:

We utilized a mouse in which macrophages are labeled with a fluorescent marker, and treatment with an otherwise inert drug will target macrophage cells for death. These MaFIA transgenic mice express green florescent protein (GFP) and a Fas-based suicide construct under the regulation of a promotor activated primarily in macrophage cells (*Csf1r* promoter). Spleens from MaFIA mice were transplanted into wild-type (non-transgenic or WT) recipients using microvascular anastomosis (Fig 1). After confirming transplant viability via contrast-enhanced and Doppler ultrasonography (Fig. 2), recipient mice underwent a mid-diaphyseal tibial fracture, followed by either a drug to selectively ablate donor-derived macrophages or vehicle as control. Mice that underwent splenectomy alone and mice that did not undergo a splenectomy were used as additional controls. There were 10 animals in each analysis group.

Flow cytometry was used to quantify GFP expressing macrophages at the fracture site and contralateral limb at 3 days post-fracture. Fracture healing was evaluated at 14 and 21 using histological analysis and micro-computed tomography (μCT).

RESULTS:

At 3 days post-fracture, GFP^+ macrophages constituted ~1% of live cells in fracture calluses and were also present at a much lower frequency in contralateral limbs (<0.01%), indicating systemic trafficking. Over 95% of GFP^+ cells were $CD11b^+$, and 20% co-expressed F4/80, confirming their macrophage identity. Histology at 14 dpf revealed increased fibrous tissue and decreased bone matrix in mice lacking spleen-derived macrophages and in mice subjected to a splenectomy. μCT analysis showed a significant reduction in bone volume fraction (BV/TV) and apparent mineral density at both 14 and 21 dpf in the macrophage depleted group and thr splenectomy group ($P<0.01$ for both). By 21 dpf, a marked increase in nonunion fractures was observed in macrophage-depleted mice (Fig 3). There was no difference between mice with a splenectomy and those who underwent a spleen transplant with macrophage depletion. In addition, there was no difference between mice without a splenectomy and those who underwent a spleen transplant without macrophage depletion.

DISCUSSION AND CONCLUSION:

Spleen-resident macrophages are actively recruited to fracture sites, where they support bone formation and modulate osteoblast activity. Their selective depletion results in impaired callus maturation, reduced bone volume, and increased nonunion, underscoring their essential role in successful fracture healing. These spleen macrophages come from a different embryonic source, and are an important cell type in fracture repair.

This study uncovers a critical and previously underappreciated systemic contribution of spleen-derived macrophages to bone repair. These findings offer new insight into the immunologic orchestration of fracture healing and suggest that targeting specific macrophage subsets could enhance outcomes in orthopedic trauma and delayed union. Furthermore, it explains why the pace of fracture healing is slow following a splenectomy, and provides a possible cell therapy approach to speed the pace of repair.

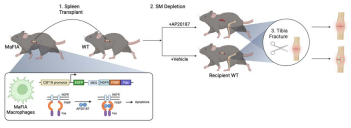

Fig. 1: Schematic of in vivo depletion of spleen macrophages in a fracture model

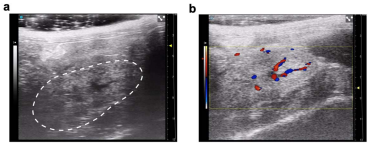

Fig 2. Spleen transplantation with venous and arterial anastomoses supports survival of the transplanted organ. A: Contrast-enhanced ultrasound imaging of the transplanted spleen one-week post-transplantation. **B**: Color Doppler ultrasound scans show sustained blood flow through the transplanted spleen.

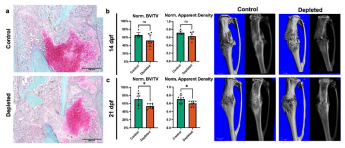

Fig. 3. Depletion of spleen macrophages impairs bone formation during fracture healing. A: Representative Safranin-O–stained sections of fracture calluses at 14 days after fracture from mice with intact spleen macrophages (Control) and mice with depleted spleen macrophages (Depleted). **b–c**: Left: Quantification of normalized bone volume fraction (BV/TV) and apparent density at 14 days after fracture (**b**) and 21 days after fracture (**c**). Right: Representative micro-CT images of fractured tibias from control and depleted groups at the corresponding timepoints (unpaired Student's t-test). *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001. Data are presented as mean ± standard deviation