# Mast cell-derived IL-6 is a critical mediator of posttraumatic inflammation and compromised fracture healing

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INTRODUCTION: The risk of fracture malunion increases in patients with multiple injuries and is closely linked to the severity of trauma (1). A major contributor to impaired bone healing is the excessive posttraumatic inflammatory response, initiated by the release of endogenous danger signals and a surge of inflammatory mediators (2,3). Among these, the cytokine interleukin-6 (IL-6) is considered a key driver of systemic inflammation due to its strong correlation with both injury severity and the development of complications (4). Recently, we identified mast cells, tissue-resident immune cells that are rapidly activated following tissue trauma, as contributors to trauma-induced inflammation and impaired fracture healing (5). Mast cells release various pro-inflammatory mediators, including IL-6, in response to fracture and tissue injury (6,7). In this study, we investigated whether mast cells represent a critical source of post-traumatically elevated IL-6 levels and thereby contribute to trauma-induced impairment of fracture healing.

METHODS: Twelve-week-old male mice with a mast cell-specific deletion of *IL-6* (*Mcpt5-Cre* x *B6.Il6$^{tm1.1Jho}$ flox)* and their Cre-negative littermates underwent femur osteotomy stabilized with an external fixator. To induce posttraumatic systemic inflammation, half of mice additionally received a thoracic trauma (Reg.No. 1518). Local and systemic immune responses were assessed at 3h and 24h post-trauma by analyzing inflammatory mediator concentrations in the blood (cytokine multiplex analysis, ELISA), lung inflammation (histology), the hepatic acute phase response (gene expression analysis), as well as immune cell populations in the fracture hematoma (flow cytometry). Bone healing was assessed on day 21 post-fracture using biomechanical testing, µCT analysis, and histomorphometry (n = 5-8/group). Statistics: ANOVA followed by Fisher's LSD post hoc test ($p < 0.05$).

RESULTS SECTION: In line with previous findings, littermate controls exhibited an increased inflammatory response following combined trauma compared to isolated fracture, as indicated by elevated blood levels of IL-6, monocyte chemoattractant protein-3, CC-chemokine ligand 11 (CCL11), and IL-12p70 (all $p < 0.05$ compared to isolated fracture) 3h post-trauma. Furthermore, control mice with combined injuries showed a higher lung damage score (isolated fracture vs. combined trauma: 33% vs. 60%) after 3h, along with an enhanced hepatic acute phase response, evidenced by increased gene expression of *Cxcl-1* ($p = 0.027$) and *Crp* ($p = 0.08$) at 24h compared to isolated fracture. Innate (macrophages, neutrophils) and adaptive (T and B lymphocytes) immune cell populations remained unchanged in controls 24h after combined trauma compared to isolated fracture. On day 21 post-fracture, controls with combined trauma demonstrated impaired fracture healing, characterized by significantly reduced bending stiffness ($p = 0.007$), fewer bridged cortices, and decreased newly formed bone ($p = 0.031$), while cartilage formation was significantly increased ($p = 0.025$) compared to isolated fracture. Notably, mast cell-specific deletion of IL-6 attenuated trauma-induced systemic inflammation at 3h, as confirmed by significant reductions in blood IL-6 ($p = 0.008$) and CCL11 ($p = 0.042$) levels compared to controls after trauma. In addition, mice with mast cell-specific IL-6 deletion exhibited less lung damage (60% vs. 17%) at 3h and reduced hepatic expression of the acute phase response gene *Cxcl-1* ($p = 0.013$) at 24h post-trauma. This deletion also led to reduced cytotoxic T cell numbers ($p = 0.008$) in the fracture hematoma of mice with combined trauma at 24h compared to controls. Importantly, mice with mast cell-specific IL-6 deletion were protected from trauma-induced impaired fracture healing, as indicated by significantly increased bone volume (1.0 vs. 1.6 mm³), callus size (6.7 vs. 8.9 mm³), and cortical bridging score ($p = 0.004$) compared to controls after trauma.

DISCUSSION: We demonstrated that the deletion of IL-6 in mast cells significantly attenuated posttraumatic systemic inflammation and the hepatic acute phase response. In addition, mast cell-specific IL-6 deletion mitigated lung damage and inflammation and led to a local reduction in cytotoxic T cell numbers at the fracture site. Given that an increased presence of $CD8^+$ T cytotoxic cells has been shown to negatively impact fracture healing (8), the reduced cytotoxic T cell infiltration may help to explain the improved healing outcomes observed 21 days post-injury in mice with mast cell-specific IL-6 deletion. As the study was only conducted in male mice, we will repeat the experiments in female mice to investigate sex differences. In conclusion, our findings identify mast cells as a critical source of IL-6 following trauma, contributing to increased posttraumatic inflammation and impaired fracture healing.

SIGNIFICANCE/CLINICAL RELEVANCE: Targeting mast cells and their mediators could represent a promising therapeutic approach for patients with multiple injuries who are at a high risk for fracture malunions.

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