Post-traumatic Immunosuppression in Open Fractures and IL-12 Therapy

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Disclosures:

Introduction: Despite the advances in trauma research over the past decades, open fracture-associated infections still pose a major challenge and remain a leading cause of mortality and economic loss globally.[1] Trauma patients with open fractures are at high risk for infections due to possible post-traumatic immunosuppression.[2] Post-traumatic immunosuppression is characterized by decreased levels of pro-inflammatory cytokines like interleukin 12p70 (IL-12) which in turn hampers the innate immune system, the first line defense of the host. IL-12 promotes cell mediated immunity through the type 1 helper T cell (Th1) pathway and activates phagocytes such as macrophages and neutrophils to promptly and effectively kill bacteria and keep infections in check. However, during trauma, patients are prone to infections due to decreased levels of IL-12. In this study, the effect of trauma on the functions of macrophages and neutrophils were examined at post-surgery days 3 and 10 in a rat open femur fracture model. The role of IL-12 in minimizing the post-traumatic immunosuppression was also investigated at different concentrations. We hypothesized that open fracture will result in reduced bactericidal activities of immune cells (e.g. neutrophils, macrophages) and exogenous IL-12 will enhance the bactericidal activity of such immune cells against *S. aureus*.

Methods: Adult male Sprague-Dawley rats were randomly divided into 4 groups, each containing 6 rats. Two control groups underwent sham surgery and the other 2 groups underwent standardized femur fracture using a blunt trauma model.[3-7] Three and 10 days after the surgery, the rats were euthanized; macrophages and neutrophils were isolated from spleen and blood, respectively. After isolation, macrophages and neutrophils were cultured at a ratio of bacterium:neutrophil (or macrophage)=10:1 with *Staphylococcus aureus* (*S. aureus*) obtained from a patient’s chronic wound. After culturing for 1, 2, 4, and 24 hr, macrophage’s bactericidal and phagocytic activities were determined at 1, 2, and 4 hr. The bactericidal and phagocytic activities of neutrophils were evaluated at 30, 60, and 90 min since neutrophils are short lived. In addition, the macrophages from the fractured groups were treated with three different concentrations of IL-12 (25, 250, and 2500 pg/mL) and their bactericidal activities were analyzed and compared to the fractured only group.

Results: We found that the percent of neutrophils with phagocytized *S. aureus* in the fractured groups increased with increasing infection time from 30 to 60 to 90 min, and the ability of neutrophils to kill bacteria decreased with increasing infection time compared to the control in both the 3 day and 10 day groups (Fig. 1); data of the 3 day groups were not shown. A similar trend was observed for macrophages with an increased percent of macrophages with internalized bacteria and a decreased ability to kill in the fractured groups over 1, 2, and 4 hr compared to controls in both the 3 day and 10 day groups (Figs. 2 and 3), suggesting their decreased killing ability in the first 4 hr. However, in the 10 day group, an increased killing ability was observed in the fractured group at 24 hr (Fig. 3). Meanwhile, we found that IL-12 treatment led to less viable internalized bacteria compared to that in the fractured only group (Fig. 4). Of the three concentrations of IL-12 treatments, a 5% increased killing ability was exhibited in the fractured groups treated with 25 pg/mL and a 28% increased killing ability in the fractured groups treated with 250 and 2500 pg/mL.

Discussion: Trauma like open fractures have been found to result in diminished production of IL-12, reduced Th1 responses, decreased resistance to infection, and delayed healing. These findings are consistent with the observations that patients who have open fractures and those who have weakened immune systems are at greater risk of infection. Our data here indicated an increased percent of neutrophils and macrophages with phagocytized *S. aureus* and a decreased ability to kill bacteria as the infection time increased in the fractured groups compared to the controls in both the 3 day and 10 day groups. These results indicated that the neutrophils and macrophages from the groups subjected to blunt trauma have decreased bacterial killing ability, suggesting post-traumatic immunosuppression. Importantly, the IL-12 supplementation to the samples from the fractured group allowed increased bacterial killing by macrophages, and this finding suggested that IL-12 plays an important role in minimizing immunosuppression after open fractures and IL-12 administration may be an innovative approach to reduce open fracture-associated infections. In conclusion, we found decreased bacterial killing ability of neutrophils and macrophages in fractured groups due to post-traumatic immunosuppression. The optimal dose of IL-12 was 250 pg/mL which acted as a catalyst in boosting the killing ability of macrophages up to 28%.

Significance: Open fractures induce immunosuppression and decrease resistance to bacterial infection thereby leading to high infection rates; this study showed that IL-12 administration could enhance the bactericidal activities of immune cells like macrophages against *S. aureus* and may potentially reduce open fracture-associated infections.

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References:

![Graph](image)

**Fig. 1.** Percentage of neutrophils with internalized *S. aureus* vs. culturing time. Data are from day 10 groups.

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Fig. 2. Percentage of macrophages with internalized *S. aureus* vs. culturing time. Data are from day 10 groups.
Fig. 3. Live internalized S. aureus vs. post-infection incubation time. Data are from day 10 groups.
Fig. 4. Live internalized *S. aureus* vs. IL-12 concentration. Data are from day 10 groups.