Introduction:

Post-traumatic immune dysfunction can be a lethal complication in injured patients and the contribution of both hemorrhagic shock and soft tissue injury in cell mediated immune suppression has been identified. However, the role of severe skeletal trauma has not been clearly evaluated owing to complications in current murine fracture models that restrict the late term study of the post-traumatic immune response. To assess the resulting consequences of severe tissue injury on immune dysfunction, we developed a novel pseudofracture model which simulates the systemic and end organ responses observed following bilateral femur fracture. We hypothesized that skeletal injury would significantly contribute to late term inflammatory responses and we have found a significant delayed post-traumatic immune dysfunction in this model.

Methods:

Male C57/BL6 mice (n=4-8), weighing 20-30g, 7-10 weeks old, were subjected to pseudofracture (crushed bone solution injection and soft tissue injury to the thigh musculature bilaterally) and allowed freedom of movement as anaesthesia subsided. Bone solution preparation involved harvesting bilateral femur and tibia from age-matched syngeneic donor mice, crushing and suspending the bones in 2ml of phosphate-buffered saline. A volume of 0.15mL of this bone solution was then injected into the crush-injured thigh muscles bilaterally.

Mice were also subjected to pseudofracture (PF) in combination with hemorrhagic shock/resuscitation (HS/R) as a multiple injury model. A two and half hour pressure-controlled hemorrhage via femoral artery cannulation to a mean arterial pressure of 30mmHg was followed by resuscitation with Lactated Ringer’s to three times the shed blood volume. Control mice had no experimental manipulation; Sham mice underwent femoral artery cannulation and immobilization for two and half hours.

Measured markers of systemic inflammation were plasma cytokines Interleukin(IL)-6 and IL-10, measured through ELISA. Markers of remote organ dysfunction were aspartate aminotransferase, alanine aminotransferase, hepatic NF-kB activation, pulmonary myeloperoxidase and bronchoalveolar lavage (BAL) to serum protein concentration, and hepatic ALT and AST.

The mice were sacrificed at several time points: 6, 12, 24, 48, 72 hours in order to evaluate the proportionate responses of each measured immune marker after trauma.

Results:

Systemic inflammation:

Plasma Interleukin-6 levels showed a significant (p<0.05) maximal increase at 6hours after pseudofracture (IL-6:177 ±7pg/mL) in comparison with controls, decreasing thereafter. The addition of HS/R further increased the IL-6 measurements at all time points in a parallel pattern, with a significant increase within the first 24hours and slowly decreasing out to 72hours (IL-6: 237 ±27 pg/mL at 6hrs).

Plasma IL-10 levels showed a maximal increase within the first 24hours after pseudofracture (IL-10: 94 ±8pg/mL at 24hours), and a similar pattern after combined pseudofracture and shock, though with further elevated values (IL-10: 129 ±38pg/mL at 24hrs).

End organ damage:

Liver enzymes showed a significant (p<0.05) increase early within 24hours, with a maximum at 6hours (AST: 455 ±66 U/I at 6hrs, ALT: 118 ±7 U/I at 6hrs), that slowly returned to baseline by 72 hours in the PF alone groups. The combination of PF with HS/R showed significantly higher results (AST: 113 ±181U/I at 24hrs, ALT: 311 ±57U/I at 6hours) at the first 3 time points then a similar decrease. Hepatic NF-kB measurement showed maximal activation at 24hours, with pseudofracture alone and in combination with HS/R.

Pulmonary myeloperoxidase showed a steady increase over time to significantly elevated values at 48hours and maximum at 72hours (3126 ±497ng/mL in PF+HS/R), in both PF and the combined PF+HS/R. However, the BAL – serum protein ratio did not show a significant trend over the 3 day period.

Immune dysfunction:

A significant (p<0.05) decrease (23,886 ±1,880c.p.m.) in splenocyte proliferation post-pseudofracture was seen at 48hours in comparison to controls (49,229 ±5,996c.p.m.). The 24 and 72 hour time points showed substantial decreases in measured proliferation that was not present at the earlier time points.

The combined pseudo-fracture and hemorrhagic shock groups also had significantly (p<0.05) decreased (19,453 ±771c.p.m.) splenocyte proliferation at the 48 hour time point in comparison to shams.

Splenocyte release of Th1 cytokines was significantly (p<0.05) decreased in the injured mice at the 24, 48 and 72 hour time points (IFN-g: 112.7 ±89.9 pg/mL at 48hrs after PF, IL-2: 114.3 ±20.2 pg/mL at 48hrs after PF) in comparison with controls (IFN-g: 654.8 ±140.5 pg/mL, IL-2: 250.2 ±45.2 pg/mL). Extremely low IL-10 levels were measured in all groups.

Splenocytes from mice that underwent pseudofracture in combination with shock had a significantly (p<0.05) decreased release of both Th1 cytokines from the 6hour time point (IFN-g: 347.3 ±50.9 pg/mL, IL-2: 189.5 ±25.1 pg/mL) that decreased significantly further to the lowest value at 48hours (IFN-g: 86.5 ±33.4pg/mL, IL-2: 109.1 ±10.8 pg/mL).

Discussion:

Characterization of the immune response in our murine model of severe skeletal injury has shown induction of an early systemic inflammatory response typically observed following long bone fractures, but also the delayed immune dysfunction typical of severe injury models.

Combination with hemorrhagic shock/resuscitation resulted in a more exaggerated immune response in a similar pattern to the pseudofracture alone, and is a proportionate representation of a multiple injury model.

Our findings in this model will allow detailed future investigations into this delayed post-traumatic immune dysfunction through the availability of many transgenic and knock-out mouse strains, with the potential for identification of therapeutic targets that may limit the immunosuppression.

References: