Developing a Minimally Invasive Cell-Based Model to Predict Response to Major Trauma

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Background

The physiologic response to injury is heavily influenced by the immune system. The complexities of the immunologic response to injury are becoming increasingly understood as researchers have leveraged computational methods that allow temporal and spatial coordination of immune mediator orchestration to be quantified. Recently, early differences in immunologic orchestration have been shown to stratify individual tolerance to injury. Specifically, there are subsets of trauma patients that are either sensitive or tolerant to hemorrhage that demonstrated notably different early immunologic orchestration of mediators from clusters of cytokines that are primarily tissue protective or pro/anti-inflammatory.   These differentiating networks of mediators formed and dissipated over the initial 72 hours after injury clearly demonstrating that the immunologic response to injury is an acute dynamic event that has pathomechanistic relevance to outcomes after injury. Additionally, it is distinctly possible that individualized differences in immune response may determine tolerance/sensitivity to injury.

The differential immunologic response to trauma represents an opportunity to discover specific factors that may be predictive of a patients' response to traumatic injury and subsequent hemorrhagic shock. Accordingly, we have embarked on a line of experimentation to explore potential precision approaches based on individual immunologic response to injury. Here we report our initial experimental findings in conceptual model development with the ultimate goal of developing minimally invasive/non-injurious testing that will accurately identify individual tolerance to hemorrhage and injury. In this experiment, an in-vitro cell-based assay was designed to mimic traumatic injury. Specifically, we tested the

Kayla Gates (she/her/hers) is a third year medical student currently interested in orthopedic surgery because she is drawn towards the idea of seeing immediate improved quality of life for patients. "I think being able to directly increase functionality for patients would be extremely rewarding. incredible learning experience." "My most important take away from this research experience is an appreciation for how different body systems can be so intricately linked. I've enjoyed studying the connections between the immune system and the musculoskeletal system, and I've gained a broader understanding of the inflammatory response to trauma."

Rebecca Nunge is a third year medical student who is currently undecided in her field of interest. "It has been a very thought-provoking and valuable experience to participate in a research project with such an interesting research question about the connection between trauma and immunologic responses. I have enjoyed working on a multidisciplinary team and learning from the mentors we have on this project."

immunologic response in murine splenocytes to a simulated hypoxic injury, a simulated mechanical injury and a simulated open wounding injury. The development of a reliable cell-based model will allow investigation to determine correspondence and relevance between cell-based responses to non-traumatic injury and in vivo immunologic response to trauma, with the overall goal of developing a reliable test to predict response to traumatic injury in humans.

Methods

In-vitro cellular responses of murine splenocytes are reflective of peripheral blood cell responses and were used for pilot experiments. Splenocytes from C57BL/6 (B6) BALB/c or CH3/HeJ strains mice were used with stimuli that mimic traumatic injury using chemical (hypoxia or sepsis) or mechanical (shear stress) stimuli that might from an open wounding type of injury. Hypoxia was simulated by subjecting cell cultures to hydrogen peroxide. Sepsis was simulated by

subjecting cells to lipopolysaccharide (LPS). Some culture conditions included several individual cytokines associated with acute inflammation and external pathogens (interleukin (IL)-6, IL-1β, IL-33), the damage molecule high mobility group box protein (HMGB)-1, or combinations of LPS and the cytokines. Following treatment, cDNA was prepared and used for qPCR amplification of TNFα, HIF1α, and BAX to assess inflammation, hypoxia, and apoptosis, respectively. Multiplex analysis of IL-21, IL-4, IL-22, IL-5, and IL-10 expression was performed from culture supernatant collected at 24 hours after stimulation. Flow cytometry was performed to assess proliferation of immune cells following treatment. ELISA was conducted to quantify production of the cytokine IL-9 that occurred following splenocyte stimulation.

Results

Analysis of C57BL/6 splenocyte viability show that any combination of cytokines or LPS did not impact cell survival, while hydrogen peroxide reduced survival significantly in each treatment group. From the qPCR data, LPS generated a 4x increase in TNFα expression relative to control, while cytokine treatment yielded no expression changes. Treatment with LPS + cytokines closely resembled the LPS treatment group. LPS treatment reduced expression of HIF1α, while hydrogen peroxide increased expression of HIF1α. The addition of cytokines reduced expression of HIF1α in groups that were treated with both hydrogen peroxide and cytokines. ELISA analysis of the proinflammatory cytokine IL-9 indicated increased production of IL-9 following treatment with LPS + cytokines.

In the second experiment, the model was applied to three different strains of mice in order to gauge differences in the immune response to the same cellular stress. Multiplex analysis showed no significant changes in IL-4 or IL-21 expression in any of the strains. C3H mice showed no response to LPS, which was expected due to LPS resistance in these strains. In the B6 and BALB mice, IL-10 was induced by LPS treatment. BALB mice also showed increased expression of IL-5 and IL-22 in response to mechanical stress, while the other strains showed no response. IL-10 expression was not induced by mechanical injury in any strain. Flow cytometry analysis was used to assess immune cell

response to stimuli. Both B6 and C3H mice showed increased percentages of CD4 and CD8 cells in response to mechanical stimulus, LPS, and LPS + cytokine treatment relative to control. Macrophage levels were more elevated in B6 mice in response to mechanical stimulus, whereas levels decreased in the C3H mice.

Discussion

The overall goal of this line of investigation is to develop minimally invasive and non-injurious testing that can be used to determine individual tolerance/sensitivity to trauma and hemorrhage. These pilot studies were used to determine how immune cells can be isolated and stimulated to mimic injury. Splenocytes were used as they encompass a broad cross-section of white blood cells. Clear inter-strain differences were evident between the B6, BALB and C3H mice. Hypoxia stimuli consistently resulted in roughly a 50% loss of cell viability and accordingly may not be a viable strategy. The greatest effects were encountered with LPS +/- addition of stimulating cytokines. We measured changes in five of six cytokines in B6 mice and four of six BALB mice involving reparative cytokines (IL-21 and 22), anti-inflammatory cytokines (IL-10) and in type 2 cytokines (IL-4 and IL-5). Accordingly, these strains and stimulation methods will be expanded to determine effects on production on a broader panel of cytokines. In addition, computational methods will be leveraged on the next experiment to determine in-vitro effects on immunologic mediator orchestration to account for time-dependent mediator networks and spatial networks of mediators.

Moving forward, these experiments will be repeated to reproduce our findings and improve our ability to distinguish between varying immune responses. Results will then be paired with studies examining the responses to traumatic injury among these and other strains. The overall goal of this project is to accurately predict the response to an in-vivo injury using an in-vitro non traumatic stimulus. Findings from this project will enable the development of a clinical test that accurately predicts immunologic response to trauma and stratify individual tolerance to hemorrhage and injury.