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- Pathophysiology of Post-Injury Infection and Organ Failure
- Risk for and Consequences of ICU-related Infections

FUNDING
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Severe traumatic injury results in biochemical and physiological changes that can lead to nosocomial infection (pneumonia, wound infections, etc.) and remote organ (lung, kidney, liver) failure. In patients who survive beyond the initial few hours after injury, infection and organ failure (MODS; multiple organ dysfunction syndrome) are leading causes of death and contribute to prolonged and resource-intensive hospital stays. Our understanding of the biology of these complications is incomplete. We understand many of the clinical factors that predict who will develop post-traumatic infection and organ failure and know that a number of inflammatory markers are increased after injury and that many of these identify patients who progress to organ failure. For example, and as shown in Figure 1, severely injured patients who develop organ failure (predominantly lung and kidney failure) have greater elevations in circulating interleukin(IL)-6 in the first 24 hours after injury. This is one circulating marker that reflects the systemic physiological response that sets the stage for remote organ failure and infections.

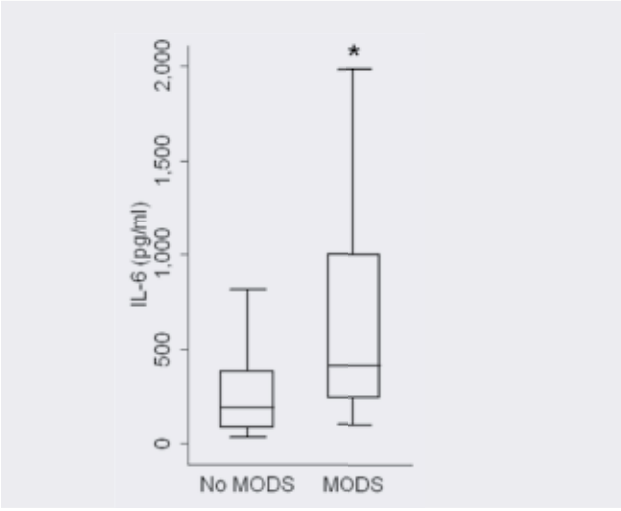

FIGURE 1: Plasma IL-6 and multiple organ dysfunction
Plasma IL-6, measured within 24 hours of injury, is associated with an increased risk for subsequent multiple organ dysfunction syndrome (MODS). This finding was validated in a second cohort of patients. An IL-6 concentration > 350 pg/ml was highly predictive of MODS and prolonged hospitalization. These data are from reference 7.

However, there are many gaps in our understanding of how early changes after injury contribute to later complications. Furthermore, other factors, independent of the injuries, doubtless contribute to these complications. Delays and other variation in treatment are likely detrimental, pre-existing health status can increase the risk for infections and organ failure, and individual genetic differences might influence a person's risk for complications and ability to recover from them once they develop.

Sequencing the human genome has led to a greater understanding of its structure (DNA sequence variation) and function (mRNA expression and protein translation) in new and comprehensive, "genome-wide" ways. Our program incorporates new methods and technology into understanding post-traumatic infection. First, we are studying the genetic basis for variation (single nucleotide polymorphisms; SNPs) in inflammatory responses and how these differences influence the clinical course of sepsis. Second, and based upon our clinical and experimental observations, we are studying pathways that have traditionally been not considered "inflammation-related", but appear to affect the human inflammatory and innate immune responses. Finally, we are also studying how established treatments might influence outcome in ways that have previously been overlooked. For example, we have examined the role of blood transfusion in the development of post-traumatic infection and are also re-examining infectious complications after injury to try to understand their connection to altered biology.

Identification of genetic variation associated with post-traumatic ventilator-associated pneumonia (VAP)

Genetic variation in the innate immune response contributes to the marked variation seen in the risk of and outcome from a number of infectious diseases. Epidemiologic studies have demonstrated a strong familial association with death from infectious disease in general and, more specifically, an association between a familial "anti-inflammatory" response and death from meningococcal sepsis. The role of specific genetic differences in conferring risk is less certain, with many examples of discordant observations regarding numerous genetic variants. Examples of conflicting observations have primarily concerned single nucleotide polymorphisms (SNPs) in genes involved in the innate immune response, such as tumor necrosis factor–alpha (TNF-α), toll-like receptor 4 (TLR4) lipopolysaccharide binding protein (LBP), and others.

Genome-wide approaches to identifying associations with common diseases (diabetes, hyperlipidemia, etc) have generally replaced studies focused on small numbers of candidate polymorphisms. We have developed a large DNA bank that is linked to clinical data for over 3500 injured patients and have studied specific SNPs and inflammation-related genes, and have now begun to identify genome-wide associations (a genome-wide association study, GWAS) with infectious outcomes after injury. We have recently completed the first phase of a GWAS aimed to identify genetic variation associated with VAP after injury.

Understanding the role of MAPK phosphatase (MKP-1/DUSP1) as a potential mediator of epinephrine induced immune suppression

Cyclic AMP (cAMP) is a prototypic intracellular second messenger with many effects. It is a common pathway for a number of extracellular signaling molecules that signal through G-protein coupled receptors. Epinephrine is one such signaling molecule that increases levels of cAMP via the G-protein-coupled β-adrenergic receptor. Sympathetic activation with local and systemic release of adrenergic mediators such as epinephrine is an important component of the immediate stress response that leads to increased intracellular cAMP in cells expressing the β-adrenergic receptor. Data indicate that stimulation of β2 adrenergic receptors (β2AR) increases intracellular cAMP and decreases production of pro-inflammatory cytokines, such as TNF-α, while increasing production of others, such as the anti-inflammatory cytokine IL-10. These changes in the balance of inflammatory responses may have important implications

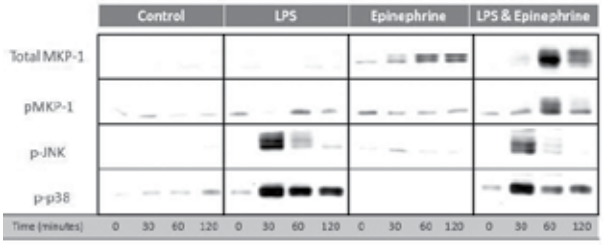

Figure 2: Effects of epinephrine on endotoxin-induced kinase activation
Legend: This figure is an image of an immunoblot for protein, and demonstrates that monocytes exposed to epinephrine have higher levels of total MKP-1 and phosphorylated MKP-1 beginning at 30 minutes after stimulation compared with endotoxin alone. This elevation in MKP-1 corresponds to a more rapid decrease in phosphorylated p38 and JNK.

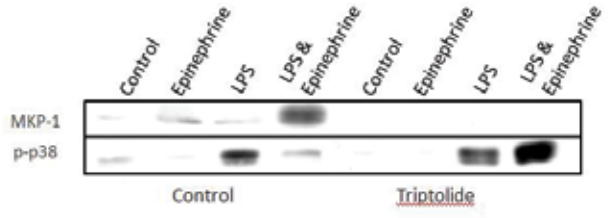

Figure 3: Inhibition of MKP-1 restores MAPK phosphorylation
This figure is an immunoblot for protein after exposure of monocytes to an MKP-1 inhibitor (triptolide). It demonstrates that complete blocking (removal) of MKP-1 protein restores p38 phosphorylation; eliminating the suppression due to epinephrine.

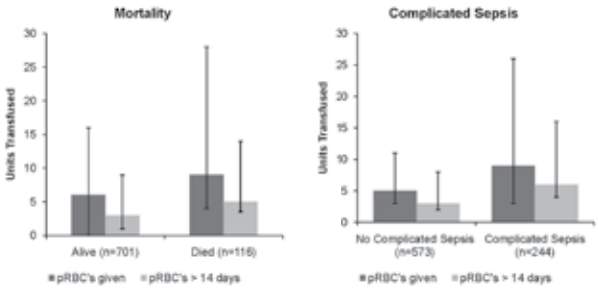

Figure 4: Mortality and complicated sepsis after blood transfusion
This figure illustrates the results of our study examining the relationship between the transfusion of packed red blood cells and outcomes after injury. As shown on the left, the patients who died received more units of blood in total than the patients who lived. The amount of older blood (storage > 14 days) transfused was somewhat higher in the patients who died. The observed association of the amount of older blood transfused and the development of severe infections (complicated sepsis) was strong. Even after adjusting for the total amount of blood transfused, patients who developed complicated sepsis received significantly more units of older blood. These data are from reference 6.

Our program is focused on developing new knowledge in the field of innate immunity and inflammation in the context of severe traumatic injury. We also aim to learn how we can better apply existing therapies to critically ill patients. We use these objectives and research areas as the foundation on which to educate and train future investigators in biological sciences.

for an individual's ability to respond to infection during times of stress, such as acute traumatic injury. The intracellular mechanisms leading to these effects, particularly to the suppression of TNF-α release, are unclear.

Using Affymetrix GeneChips, and applying Ingenuity Network Analysis, we have identified a potential role for the MAP kinase phosphatase MKP-1 (also known as DUSP1). In a human monocyte cell line, we have also observed total MKP-1 protein and phosphorylated MKP-1 to increase rapidly in response to LPS stimulation, epinephrine stimulation and, most strikingly, combined stimulation to both agents. Additional experiments have examined both phosphorylation and subsequent dephosphorylation of p38 and JNK, intracellular signaling molecules important to inflammatory activity. We observed that initial MAPK activation was not influenced by β-adrenergic stimulation but that MAPK **deactivation or dephosphorylation** was. Results of these experiments are shown in Figure 2. Our most recent observations seem to confirm a role for MKP-1 in β-adrenergic mediated immune suppression. By pharmacological inhibition of MKP-1 we have shown a restoration of p38 and JNK phosphorylation (Figure 3).

Critically ill patients have early elevations in circulating epinephrine and are often treated with adrenergic and anti-adrenergic agents. Our data suggest that these agents, whether endogenous or administered, have important effects on innate immune function. This may represent an opportunity to manipulate the inflammatory response and reduce the incidence of post-traumatic infection.

Measuring the influence of duration of blood storage on infectious complications in injured patients receiving transfusions

The transfusion of allogeneic red blood cells (PRBCs) is life-saving but also known to suppress immunity and influence outcomes in critically ill and injured patients. The influence of blood on the risk of infection and death may, in part, be related to the duration of storage. We investigated whether there was a relationship between the duration of blood storage and outcomes in trauma patients who received transfusions.

We studied 820 patients who were transfused at least 1 unit of blood within 24 hours of injury. Patients who died (n = 117) received more units of older blood than those who lived (5 units [interquartile range, IQR 2-9] versus 3 units [IQR 2-6], $p < 0.001$). Patients with complicated sepsis (n = 244) received a greater volume of older blood than those without complicated sepsis (6 units [IQR 2-10] versus 3 units [IQR 1-5], $p < 0.001$). These data are shown graphically in the right pane of Figure 4. After adjusting for clinical factors, including the total amount of blood transfused, patients receiving ≥ 7 units of older blood had a higher risk of complicated sepsis than patients receiving 1 or fewer units (odds ratio, OR = 1.9, $p = 0.03$).

Our observations have demonstrated that the risk for complicated sepsis and death in trauma victims who are transfused blood is high and that the amount of older blood transfused is associated with complicated sepsis. In addition to avoiding unnecessary transfusions, our data suggest it is important to avoid transfusing multiple units of older blood.

Understanding the potential manifestations of alterations in innate immunity after traumatic injury

In conjunction with our studies of the influences of genetic differences on innate immune responses and clinical outcomes of critically ill patients, we have examined the responses to and outcomes from severe nosocomial infections. Ventilator-associated pneumonia (VAP) is the most common nosocomial infection in critically ill patients, and is considered an important cause of prolonged hospitalization and mortality. With timely diagnosis and appropriate antimicrobial treatment, patients with VAP typically survive. This is particularly true in trauma victims who are generally young and have few co-morbid conditions. However, bacteremia in conjunction with pneumonia may

reflect a failure of the host's innate immune response, and may be a harbinger for death. To examine this possibility, we have studied the clinical course of 544 trauma patients who developed VAP.

Of 554 patients with VAP, 74 (14%) patients had associated bacteremia, and 480 (86%) had VAP alone. Nineteen of 74 patients (26%) with bacteremia died. Patients with VAP alone had a much lower case-fatality rate of 12% (56/480 patients). The unadjusted relative risk for death associated with bacteremia was 2.2 (95% confidence interval, CI = 1.4 – 3.5).

Patients with bacteremia also spent a longer period of time in the intensive care unit (IUC) than patients with VAP alone. The median ICU length of stay for patients with bacteremia was 25 (16 – 41) days compared to 17 (12 – 25) days for patients with VAP without bacteremia. We also observed that patients with bacteremia who died were more likely to die as a consequence of infection and remote organ failure, and they had a more protracted stay in the ICU before dying (median stay of 26 versus 15 days, p = 0.005). As a cause of death, multiple organ failure secondary to infection was more common in patients with bacteremia than in patients without bacteremia (13/18, [72%] versus 15/48 [31%], p = 0.003).

Our observations suggest that bacteremia in association with VAP identifies a sub-group of patients with a substantial risk of dying in the ICU. In most cases, death occurs after a lengthy ICU stay that is punctuated by repeated infections and culminates in progressive organ failure. There appears to be a window of time where we may be able to intervene and prevent this progressive deterioration and death.

Summary

Our research program aims to understand the genetics and biology of critical illness, particularly in severely injured patients. We hope to be able to use our knowledge of host genetic influences on infection risk and outcomes, to enable us to focus and test better treatments. In order to find better ways to treat critically ill patients, we must understand both the clinical risks and the pathophysiological basis of the important complications.

RELATED PUBLICATIONS

1. Sena MJ, Utter GH, Cuschieri J, Maier RV, Tompkins RG, Harbrecht BG, Moore EE, O'Keefe GE. Early supplemental parenteral nutrition is associated with increased infectious complications in critically ill trauma patients. *J Am Coll Surg* 207:459-467, 2008.
2. Dissanaike S, Pham T, Shalhub S, Warner K, Hennessy L, Moore EE, Maier RV, O'Keefe GE, Cuschieri J. Effect of immediate enteral feeding on trauma patients with an open abdomen: protection from nosocomial infections. *J Am Coll Surg* 207:690-697, 2008.
3. O'Keefe GE, Shelton M, Cuschieri J, Moore EE, Lowry S, Harbrecht B, Maier RV. Inflammation and the Host Response to Injury, a Large-Scale Collaborative Project: Patient-Oriented Research Core – Standard Operating Procedures for Clinical Care. VIII. Nutritional Support of the Trauma Patient. *J Trauma* 65:1520-1528, 2008.
4. Shalub S, Junker CD, Imahara S, Mindrinos M, Dissanaike S, O'Keefe GE. Variation in the TLR4 gene influences the risk of organ failure and shock post-trauma: a cohort study. *J Trauma* 66: 115-123, 2009.
5. Ko DC, Shukla KP, Fong C, Wasnick M, Brittnacher MJ, Wurfel MM, Holden TD, O'Keefe GE, Van Yserloo B, Akey JM, Miller SI. A genome-wide in vitro bacterial-infection screen reveals human variation in the host response associated with inflammatory disease. *Am J Hum Genet* 85:214-227, 2009.
6. Hassan M, Warner K, Cuschieri J, Nester T, O'Keefe GE. The age of transfused packed red blood cells does not influence the risk and severity of sepsis and multiple organ failure after trauma. *Shock* 35:3-8, 2011.
7. Cuschieri J, Bulger E, Schaeffer V, Sakr S, Nathens AB, Hennessy L, Minei J, Moore EE, O'Keefe G, Sperry J, Remick D, Tompkins R, Maier RV; Inflammation and the Host Response to Injury Collaborative Research Program. Early elevation in random plasma Il-6 following severe injury is associated with development of organ failure. *Shock* 34:346-351, 2010.

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