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.

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.

**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.
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...
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 (ICU) 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


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