effect is abrogated (Figure 2).

Thus far, we have shown that there is a significant and persistent hypercatecholamine state after severe injury. Our *in vitro* analyses support the notion that erythropoiesis is also modulated by the adrenergic system. Our research discovered a dichotomy. On one hand there is a hypercatecholamine period after severe injury that is characterized by overt bone marrow dysfunction, seen as anemia and leucopenia. Yet, on the other hand, our data shows that the addition of adrenergic agents to erythroid precursors *in vitro* leads to an overwhelming pro-erythropoietic stimulus. Future studies will focus on the mechanisms that account for the paradox of adrenergic stimulation observed in trauma patients as opposed to the *in vitro* effects on bone marrow cultures. There may be a direct correlation between the magnitude and duration of the stress response seen following injury and resultant erythropoietic dysfunction. Perhaps with the use of adrenergic antagonists, the adrenergic modulation of erythropoiesis may be altered.

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# Preventing inflammatory complications of shock and trauma

*by* **Carl J. Hauser**

Trauma causes more than 140,000 deaths per year in the U.S. and is the leading cause of death between birth and age 45. From birth to age 36, death from trauma exceeds all other causes of death combined. More than 50,000 preventable deaths occur per year when trauma, hemorrhage, sepsis or shock triggers the systemic inflammatory response syndrome, or SIRS. SIRS, however, is simply a convenient clinical descriptor. In fact, after injury, the organism is exposed to a wide variety of non-specific initiators of inflammation such as ischemia/reperfusion, molecular "danger signals," and non-apoptotic cell death. These events can lead to a complex and sometimes massive activation of innate immunity. Circulating neutrophils (polymorphonuclear leukocytes) are the dominant effector cells of the innate immune response to injury. Thus after injury, when ischemia, reperfusion, sepsis or other insults activate neutrophils, the result can be an immune attack on vital organs. This is most commonly clinically manifested as the acute respiratory distress syndrome or as multiple organ failure.

During the last seven years my laboratory has focused on the translational biology of neutrophil inflammation after injury. I began by studying a group of G-protein coupled (GPC) white blood cell chemoattractants called chemokines. Chemokines were known to exist in high concentration in clinical plasma samples of injured patients. The concentrations of chemokines in

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plasma after trauma were known to be related to patient prognosis, even though those concentrations were sub-threshold for neutrophil activation. We subsequently studied fluids from fractures and soft-tissue injuries and from the abdominal cavities of injured patients, and found that the chemoattractants of concern in the blood were typically present at more than 100-fold higher concentrations in these fluids than they were in the bloodstream. Moreover, we found that these immune mediators could be released into the blood in high (activating) concentrations at times when such tissue reservoirs of chemoattractants were manipulated during surgery. Although the plasma mediator concentrations did not stay in their activating ranges for long, it became clear that their transient release had profound and long-lasting effects upon patients' neutrophils and their immune systems, making the patients susceptible both to organ injury and to bacterial infection.

We therefore began to study the effects of the release of chemokines from tissue injury sites on subsequent neutrophil activity both *in vitro* and in clinical samples. All GPC neutrophil chemoattractants release cell calcium stores after receptor occupation via the classical Phospholipase C/inositol triphosphate (InsP3) pathway. We therefore also started to focus on cell calcium measurements as "real-time" markers for neutrophil responses to chemokines. Subsequently, we showed that inflammatory mediator release in response to trauma appears to modify neutrophil responses to GPC inflammatory mediators in several major ways. First, during the process of systemic neutrophil activation by clinical plasma concentrations of GPC agonists, the surface expression of neutrophils' high-affinity receptors for chemokines and leukotrienes (CXCR1 and BLT1 respectively) is markedly down-regulated. The loss of these receptors leaves neutrophils deficient in their ability to migrate toward distant inflammatory sites. The same events, however, simultaneously prime the cells for non-specific attack should they encounter activated host 'bystander' tissues. Such changes in neutrophil responses can be associated both with subsequent ARDS and infective events. These findings were published in the *Journal of Trauma* as well as the *Journal of Immunology*. During those studies we also noted that activation of neutrophils radically changes the dynamics of post-receptor neutrophil calcium mobilization. Such changes in calcium mobilization alter stimulus-response coupling and functional responses (like chemotaxis or respiratory burst) to GPC receptors that are not down-regulated, but that still signal by increasing cell calcium concentration. We subsequently showed that these crucial aberrations of neutrophil calcium signaling reflect changes in the late or "store-operated" phase of calcium entry (SOC) into the cell.

The mechanisms of SOC are incompletely understood, but SOC is the dominant mechanism of cell calcium mobilization in the neutrophil. Going deeper into the molecular mechanisms involved, we showed that SOC occurs through a complex system of calcium entry channels composed of "Transient Receptor Potential" proteins. More recently, we showed that the sphingolipid metabolite sphingosine 1-phosphate (S1P) acts as a second messenger in neutrophils, linking agonist-initiated depletion of calcium stores to cell membrane channel opening, and thus eliciting SOC. These studies appeared in the *Journal of Immunology* and in the *Journal of Biological Chemistry.* Our most recent studies suggest that related lysophospholipids probably act much like S1P.

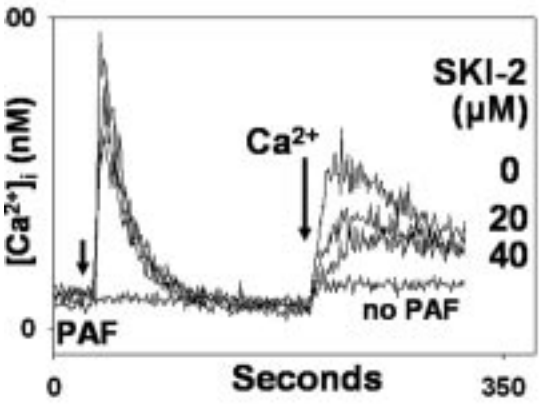


*Figure 1: Sphingosine kinase (SK) inhibitors do not interfere with $Ca^{2+}$ store depletion responses to the chemoattractant platelet activating factor (PAF) in human neutrophils studied in calcium free media. When calcium is re-added to the medium we see that SK inhibition inhibits SOC in a dose-responsive fashion.*

Since calcium entry was clearly a key regulator of neutrophil activity and was abnormally regulated after injury, these findings seemed to have potential implications for the development of strategies to modulate neutrophil-mediated inflammation after shock and trauma. In recent work, therefore, we have gone back to *in vivo* animal models to investigate the hypothesis that inhibition of S1P-mediated calcium entry after trauma and shock might be used to modulate neutrophil-mediated inflammation and related organ failure. Animal studies done so far strongly support this concept.

My future plans are therefore to study the inhibition of S1P/SOC pathways as a potentially practical strategy for reducing neutrophil activation and organ failure after injury in larger animals and, eventually, in patients. If successful, these efforts could lead to significant reductions in the overall morbidity and mortality of trauma. My studies during the past seven years were supported initially by the NJMS Department of Surgery and by the Foundation of UMDNJ. Subsequently, my laboratory was funded by the National Institutes of Health.

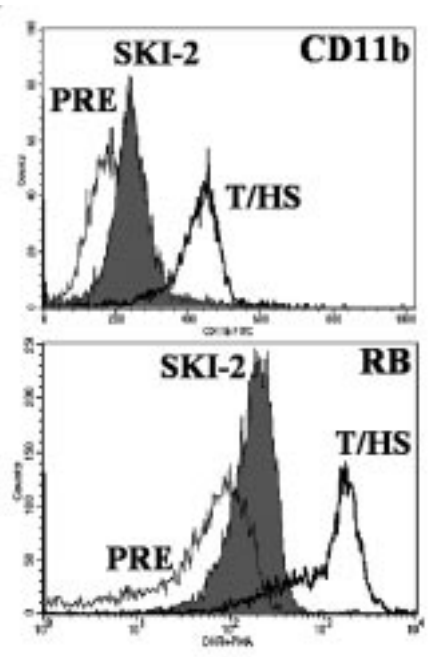


*Figure 2: Sphingosine kinase inhibition suppresses neutrophil activation* in vivo *in rats subjected to trauma and hemorrhagic shock (T/HS). Whole blood flow-cytometric assays show that SK inhibition (SKI-2) prevents neutrophil surface expression of key integrin adhesion molecules (CD11b) after T/HS to a pre-shock (PRE) level. Inhibition of SK also prevented T/HS priming of the neutrophil respiratory burst (RB) in response to phorbol esters.*

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