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Toll-like receptor activation as a biomarker in traumatically injured patients

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ABSTRACT

Background: Surgical insult and trauma have been shown to cause dysregulation of the immune and inflammatory responses. Interaction of damage-associated molecular patterns (DAMPs) with toll-like receptors (TLRs) initiates innate immune response and systemic inflammatory responses. Given that surgical patients produce high levels of circulating damage-associated molecular patterns, we hypothesized that plasma-activated TLR activity would be correlated to injury status and could be used to predict pathological conditions involving tissue injury.

Methods: An observational study was performed using samples from a single-institution prospective tissue and data repository from a Level-1 trauma center. *In vitro* TLR 2, 3, 4, and 9 activation was determined in a TLR reporter assay after isolation of plasma from peripheral blood. We determined correlations between plasma-activated TLR activity and clinical course measures of severity.

Results: Eighteen patients were enrolled (median Injury Severity Score 15 [interquartile range 10, 23.5]). Trauma resulted in significant elevation in circulation high mobility group box 1 as well as increase of plasma-activated TLR activation (2.8-5.4-fold) compared to healthy controls. There was no correlation between circulating high mobility group box 1 and trauma morbidity; however, the plasma-activated TLR activity was correlated with acute physiology and chronic health evaluation II scores (R square = 0.24-0.38, $P < 0.05$). Patients who received blood products demonstrated significant increases in the levels of plasma-activated TLRs 2, 3, 4, and 9 and had a trend toward developing systemic inflammatory response syndrome.

Conclusions: Further studies examining TLR modulation and signaling in surgical patients may assist in predictive risk modeling and reduction in morbidity and mortality.

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Introduction

Despite significant improvements in injury prevention and emergency response, injury-related death and morbidity continues to increase both in the United States and worldwide.[1] In addition, trauma patients who survive prehospital transport are at risk for sepsis and multiorgan dysfunction once hospitalized. Trauma induces significant immune consequences at local and remote organ sites, as well as systemic circulatory changes.[2-6] The endogenous response to injury is further exacerbated by hemorrhage, ischemia, and reperfusion in combination with resuscitative efforts. The inflammatory response is driven by cells and mediators of the innate and adaptive immune system.[7,8] The immune response to injury is characterized clinically by the systemic inflammatory response syndrome (SIRS), whereby inflammation is driven by the systemic release of endogenous innate immune stimulators, such as damage-associated molecular patterns (DAMPs) from injured tissues.

Because of the profound inflammatory response to tissue injury, there has been significant interest in elucidating immune mechanisms that determine recovery after surgical trauma. Trauma patients produce a host of danger signals and high DAMP levels that may heighten or maintain the overactivation of the trauma patient's immune system. DAMPs are recognized by various innate immune receptors, for example, toll-like receptors (TLRs). At least 13 TLRs are expressed in mammalian cells. Each TLR recognizes a particular molecular pattern presented in DAMPs. For instance, TLRs 2 and 4 sense nuclear proteins (e.g., high mobility group box 1 [HMGB1] and histone) and polysaccharides (e.g., heparan sulfate and hyaluronan), whereas TLRs 3, 7, 8, and 9 sense nucleic acids (e.g., DNA and RNA). On binding to DAMPs, TLRs trigger intracellular signaling cascades that lead to the expression of inflammation-associated genes, such as cytokines and chemokines, which subsequently modulate inflammation and innate and adaptive immune responses. Thus, the levels of circulating DAMPs and their downstream inflammatory and immune signatures have been developed as potential prediction markers of clinical outcomes of trauma patients.[4,8-13] Because of diversity and redundancy, the measurement of single or dual DAMP levels in the blood may not accurately reflect the true extent of inflammation and immune stimulation in patients with traumatic injuries. Given that TLRs are predominant innate immune receptors that recognize DAMPs, determination of plasma-activated TLR signaling intensity will suggest the true levels of total DAMPs in the blood of trauma patients. Herein, we hypothesized that plasma-activated TLR activity would be correlated to injury status and pathological conditions involving tissue injury.

Material and methods

Patient blood samples

The use of human blood samples was approved by the Institutional Review Board of Duke University Medical Center. Patients with injury or illness requiring surgical care or treatment in a critical care or emergency setting older than age 18 y were included. Patient samples were obtained on or near admission date. Samples were obtained at approximately 48 h intervals throughout the clinical course of the patient. Plasma from sodium citrated blood was collected from patients with polytrauma at various time points and normal healthy volunteers (Table 1).

Cell culture

Human melanoma cell line WM266-4 (ATCC, Manassas, VA) was maintained in Eagle's Minimum Essential Medium supplemented with 10% fetal bovine serum, 1X nonessential amino acid, and 1 mM sodium pyruvate (all from Invitrogen, Carlsbad). TLR reporter cell lines, HEK-Blue hTLR2, HEK-Blue hTLR3, HEK-Blue hTLR4, and HEK-Blue hTLR9 cells (all from InvivoGen, San Diego) stably express an NF-κB/AP-1-inducible secreted embryonic alkaline phosphatase (SEAP) and corresponding TLR, and these cells were maintained following the manufacturer's instructions.

Generation of DAMPs

To generate sonication-induced cell death, 2×10^6 WM266-4 cells in 1 mL of complete culture media were sonicated for 1.5 min with Branson Sonifier 250 (Branson Ultrasonics, Danbury). Live cells and cell debris were removed by centrifugation for 5 min at 1200 RPM and 3200 RPM. DAMP-containing supernatants were collected and stored at −80°C until use.

Table 1 – Trauma patient characteristics

	Patient characteristics median	Interquartile range
Age	39.5	(25.25-61.5)
GCS	15	(15-15)
ISS	15	(10-23.5)
Initial APACHE II	5	(2-9.25)
Peak APACHE II	9	(3.5-13)
Length of stay (d)	6	(4.75-11)
ICU length of stay (d)	3	(0-6.75)
	Demographics	n
Gender		
Male	56%	(10/18)
Female	44%	(8/18)
Mechanism		
Blunt	94%	(17/18)
Penetrating	6%	(1/18)
SIRS incidence	61%	(11/18)
Transfusion	28%	(5/18)
Mortality	0.11	(2/18)