The Acute Inflammatory Response in Trauma / Hemorrhage and Traumatic Brain Injury: Current State and Emerging Prospects

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Abstract: Traumatic injury/hemorrhagic shock (T/HS) elicits an acute inflammatory response that may result in death. Inflammation describes a coordinated series of molecular, cellular, tissue, organ, and systemic responses that drive the pathology of various diseases including T/HS and traumatic brain injury (TBI). Inflammation is a finely tuned, dynamic, highly-regulated process that is not inherently detrimental, but rather required for immune surveillance, optimal post-injury tissue repair, and regeneration. The inflammatory response is driven by cytokines and chemokines and is partially propagated by damaged tissue-derived products (Damage-associated Molecular Patterns; DAMP’s). DAMPs perpetuate inflammation through the release of pro-inflammatory cytokines, but may also inhibit anti-inflammatory cytokines. Various animal models of T/HS in mice, rats, pigs, and non-human primates have been utilized in an attempt to move from bench to bedside. Novel approaches, including those from the field of systems biology, may yield therapeutic breakthroughs in T/HS and TBI in the near future.

Introduction

Traumatic injury, often accompanied by hemorrhagic shock (T/HS), continues to be the most common cause of death for young people and constitutes a significant source of morbidity and mortality for all ages [1,2,151]. Traumatic brain injury is the leading cause of death in the U.S. and Western Europe [147-150] and a budding epidemic throughout Asia and the Middle East [52]. Traumatic brain injury (TBI) is also a major cause of disability, with survivors acquiring long-term cognitive, motor, behavioural or speech-language disabilities [147]. The various forms of traumatic injury therefore represent a pandemic disease that affects every nation in the world without regard for economic development, racial or religious predominance, or political ideology; this disease is acute in onset and often results in chronic, debilitating health problems affecting far beyond the individual victims [1].

Further complicating the primary damage in acute trauma is the increased susceptibility to sepsis and Multiple Organ Dysfunction Syndrome (MODS), a poorly understood syndrome of sequential and gradual loss of organ function [3]. MODS is the most frequent cause of late deaths post-injury, accounting for substantial morbidity and mortality [4,5]. MODS is considered to be due, in part, to excessive or maladaptive activation of inflammatory pathways [6]. Organs such as the liver and the gut not only become damaged or dysfunctional from trauma-induced inflammation, but in turn further perpetuate this inflammatory vicious cycle [7,8,21]. Furthermore, patients admitted to the intensive care unit following trauma and hemorrhage often become susceptible to infection “second hit” further complicating attempts at immunomodulation early in the clinical course [9] (Fig. 1).

Trauma acts as a trigger of a complex cascade of post-traumatic events that can be divided into a hemodynamic, metabolic, neuro-endocrine and immune responses leading to a multifocal pathophysiological process [10]. However, inflammation is not in itself detrimental. It is in most cases a well-coordinated communication network operating at an intermediate time scale between neural and longer-term endocrine processes [11]. Inflammation is necessary for the removal or reduction of challenges to the organism and subsequent restoration of homeostasis [12].

However, hemorrhage and trauma, perhaps combined with failed attempts at therapy [13,14], can induce a dysregulated acute inflammatory response that affects several organ systems and sets in motion a vicious cycle of inflammation damage inflammation [12,15-18] driven by cytokines, chemokines, and products of damaged, dysfunctional, or stressed tissue (Fig. 2; see below).

Thus, though the inflammatory response is pivotal in clearing invading organisms and offending agents and promoting tissue repair, these same responses carried out under a set of extreme conditions can also compromise healthy tissue and further exacerbate inflammation [12,19].

A central question then is: how do we harness the beneficial effects of inflammation and allow proper lines of communication while simultaneously not allowing inflammation to exceed a threshold that becomes self-
sustaining? This review article will focus on the common inflammatory/immune responses to T/HS and TBI, and will aim to give an overview of both the current state of relevant translational/clinical research and several novel approaches being undertaken as trauma research moves from the bench to the bedside.

Figure 1 The ‘one-hit’ and ‘two-hit’ paradigm of traumatic injury. ‘One hit’ represents the initial, massive tissue injury and shock and SIRS along with remote organ injury. The ‘second hit’ refers to the less intense SIRS that normally resolves but leaves the patient vulnerable to a secondary inflammatory hit that can reactivate the SIRS and precipitate late MODS.

Trauma and the immune response from a clinical perspective

The pathophysiology of T/HS and TBI is now understood to consist of different phases that form a continuum [7,107]. Death from post-traumatic injury occurs in three phases. In the first phase, patients die immediately because of devastating trauma. In the second phase, which occurs during early resuscitation, death may be related to hypoxia or hypovolemia. In the third phase, days or weeks following injury, death may be due to general physical consequences of injury of which the dominant manifestations are adult respiratory distress syndrome (ARDS) and MODS [20]. In 1995, two models were proposed for the exaggerated immune inflammatory response [22], known colloquially as ‘one hit’ and ‘second hit’ phenomena. The ‘one hit’ model, which accounts for the initial, massive tissue injury and shock that gives rise to an intense systemic inflammatory response syndrome (SIRS) with remote organ injury [22]. The ‘second hit’ model indicates the initial, less intense SIRS that normally resolves but leaves the patient vulnerable to a secondary inflammatory hit that can reactivate the SIRS and precipitate late MODS [23] (Fig. 1)

In the case of TBI, primary brain injury consists primarily of unavoidable brain damage that occurs at the immediate moment of impact, resulting in the disruption of brain parenchyma and cerebral blood vessels. This injury is further classified into focal versus diffuse injury. A secondary brain injury develops in the minutes to months following the original insult, progressively contributing to worsened neurological impairment [153]. Death of resident cells of the central nervous system has traditionally been thought to take place in two phases: an early necrotic and an ongoing, long-term apoptotic phase [154,155].

Thirteen years after these two models regarding the pathophysiology of T/HS and TBI were proposed, the question arises of how the clinical community has benefited from these two theoretical models, with regard to decreasing patient mortality post-traumatic injury; we will attempt to address this thorny question in this review. We know that the post-traumatic inflammatory process occurs at multiple scales and involves the activation of signaling pathways that mobilize inflammatory cells, and stimulate the secretion of multiple, inflammatory mediators/biomarkers. The complexity of this response has stymied attempts at therapeutic modulation of trauma-induced inflammation, resulting in a dearth of therapeutic options, though, as we discuss below, novel approaches from the systems biology field may help in deciphering this complexity [11,89,108].

Figure 2 The inflammatory response to tissue injury. Traumatic injury signals various cell types to produce cytokines, chemokines, and DAMPs. In turn, DAMPs re-activate and further propagate the production of inflammatory mediators, setting in motion a positive feedback loop of inflammationÆdamageÆinflammation.

Figure 3 The spectrum of cytokines, chemokines, and DAMPs in T/HS and TBI. The inflammatory response generated in response to T/HS or TBI can be assessed by measuring a panoply of cytokines, chemokines, DAMPs, and ultimate markers of end-organ damage. Some of these biomarkers may also be candidates for therapeutic intervention.

Cytokines are a broad class of protein hormones that mediate inflammatory and immune responses in a complex, context-sensitive manner [12,88] (Fig. 3). Not surprisingly, cytokines play a major role in the body’s response to T/HS and TBI [107,109]. Major cytokines that participate in the response to trauma include tumor necrosis factor–alpha (TNF-α), interleukin-1 beta (IL-1β), IL-2, IL-6, IL-8 [20,24,25], IL-4 [26] and recently IL-18.
On the other hand, the cytokine IL-10 counteracts the effects of the pro-inflammatory cytokines IL-1, IL-6 and TNF-α in various contexts [28], including severe hemorrhagic shock [29]. Unlike septic shock, where the cascade of cytokines is well defined, the role of cytokines in trauma and hemorrhagic shock is not well elucidated, the experimental and clinical data are conflicting [7], and the response in humans (as opposed to animal models of T/HS) is still poorly understood [30]. Circulating levels of cytokines have been detected in animal models and in patients with severe sepsis, and these levels have some correlation with outcome [31]. Production of the free radical nitric oxide (NO), which is produced in inflammatory settings by the enzyme inducible NO synthase (iNOS) [110], was shown to be a central mediator of post-T/HS inflammation in mice [111]. In human trauma patients, circulating NO reaction products reflect the severity of injury during the first two hours after the traumatic insult, suggesting that increased NO production might play a role in the very early post injury period [48].

Among chemokines, Macrophage inflammatory protein-1 alpha (MIP-1α) appears to orchestrate both acute and chronic inflammatory host responses at the site of injury or infection, mainly by recruiting inflammatory cells [49,50]. Additionally, MIP-1α mediates an extensive repertoire of pro-inflammatory activities, including stimulating the secretion of TNF-α, IL-1, and IL-6 by peritoneal macrophages [91]. Studies in mice have shown that short-term manipulation of MIP-1α following T/HS might be advantageous for diminishing the inflammatory response and improving vital organ dysfunction. As in most cases of therapeutic immunomodulation, inhibition of MIP-1α is a two-edged sword, in this case an increased risk of late infection [49].

Monocyte chemoattractant protein (MCP-1), Macrophage Inflammatory Protein-1 beta (MIP-1β), Regulated on Activation Normal T Cell Expressed and Secreted (RANTES), Eotaxin, Interferon-inducible Protein 10 (IP-10), Monokine Induced by Gamma Interferon (MIG), and IL-8 are chemokines that may offer novel therapeutic or diagnostic targets for T/HS.

Pathogen-associated molecular patterns (PAMPs), damage-associated molecular patterns (DAMPs, also known as alarmins), and their receptors (e.g. Toll-like receptors [TLR]-2 and -4; Receptor for Advanced Glycation End products [RAGE]) represent a parallel and perhaps integrative [114] system that is turned on during infection as well as tissue injury, including T/HS [92] and perhaps also TBI [115].

Figure 4 A vision for the future of drug design for T/HS and TBI. The future of rational drug design for T/HS and TBI may involve the use of in silico (computer simulated) tools that would be based on a mechanistic understanding of the inflammatory response as well as pharmacokinetic and pharmacodynamic principles and used to determine the optimal properties, dosage, timing, and inclusion/exclusion criteria for a given drug candidate’s clinical trial. Key aspects of these simulations would be tested iteratively in cell culture experiments and pre-clinical animal models, streamlining the process (and reducing the time and cost) of clinical trial design and implementation.

Chemokines represent a class of cytokine-like immune modulators that are gaining attention as potential therapeutic targets for various inflammatory diseases [112,113] (Fig. 3). Chemokines are produced by a variety of immune cells (innate and adaptive immunity) such as macrophages, lymphocytes, neutrophils and dendritic cells that mediate various functions of these cells, including recruitment of other cells [90]. Chemokines have been the focus of intense study in relation to T/HS. The complex interaction between cytokines and chemokines may underlie the crucial role of these inflammatory modulators in the inflammatory process following T/HS and TBI [109], and in other disease setting such as tumors, infection, and autoimmune disease [49]. Indeed, chemokines initiate recruitment of peripheral leukocytes after TBI, and evidence now exists for their intra-cerebral production [153,156,157].

In an analogous fashion, DAMPs are produced by injured tissue and stimulate or propagate inflammation through the production of cytokines; in this way, DAMPs play an important role in the pro-inflammatory cascade of innate immunity [92,95] (Figs. 2 and 3). Molecules in this class of inflammatory mediators include High-mobility Group Box 1 (HMGB1), S100A and B, Uric acid, IL-1β, heat shock proteins, and a growing list of additional molecules (Fig. 3). HMGB1 is produced in diverse settings such as infection, trauma, ischemia, T/HS, and TBI, which may contribute to the pathogenesis of severe sepsis along with other early, classical pro-inflammatory cytokines such as TNF-α and IL-1β [94]. In animal studies, HMGB1 was shown to be a key mediator of inflammation in models of sterile injury, including hemorrhagic shock [99,100].

Serum HMGB1 concentrations were significantly increased 16-32 h after exposure to lipopolysaccharide, and systemic administration of HMGB1 was lethal [105]. Antibodies to HMGB1 were shown to be protective even in the setting of established septic shock in mice [117].

Animal models of T/HS and TBI

Traumatic hemorrhage can be a consequence of direct injury to blood vessels, with massive bleeding, or as a result of diffuse bleeding secondary to coagulopathy in...
vessels too small and too numerous for surgical management [51]. In the last few decades, the pathophysiology of the systemic response to T/HS has been studied extensively in an attempt to elucidate the hemodynamic mechanisms and immunological alterations associated with T/HS. However, translating these experimental findings into clinically applicable therapy has proven difficult, and investigators in this field are challenged by two sometimes mutually incompatible goals. Researchers desire to minimize the animal-to-animal variability and at the same time seek to simulate clinical conditions. Ideally, the experimental setup mimics the clinical situation associated with hemorrhagic shock in the trauma patient, while providing the controlled conditions that maximize reproducibility and standardization. There are three common variants of preclinical animal models, which all have their advantages and disadvantages. These experimental preparations are the uncontrolled hemorrhage model and the controlled hemorrhage model that is divided into two: the fixed pressure regimens, and the fixed volume models.

The model that best reflects the clinical setting is the uncontrolled hemorrhage model. Although the standardization and reproducibility of this model is poor, it can be combined with organ and tissue injury, and allows for assessment of compensatory mechanisms. On the other hand, controlled hemorrhage offers a much better management of the degree of shock induced. In fixed volume model, animals are bled to a fixed amount of blood, usually based on the weight of the animal. It is not as clinically relevant as the uncontrolled hemorrhage model, but one can achieve a reasonably good management of the degree of shock induced [32]. In a fixed pressure model, also called "Wiggers model", blood pressure is monitored and blood is removed or reinfused to achieve a fixed pressure [33]. In these models, the degree and duration of hypotension can be controlled by using a variable stress (blood loss) to maintain a constant level of response (blood level). However, the clinical comparability is poor and animals often need to be heparinized. Heparin has been shown to confound results in experimental models of hemorrhagic shock like release of catecholamine’s and alter cytokine levels [34,35]. Recent advances in computerized automation, however, raise the possibility that very precise hemorrhage can be carried out in both rats [118] and mice [119].

Animal models of TBI include both paradigms of focal injury such as closed cortical impact, fluid percussion, or stab wound injury [177], as well as models that involve diffuse injury that occurs from the tissue distortion, or shear, caused by inertial forces present at the moment of injury [177,178]. These are most commonly separated into four main pathologies: traumatic axonal injury (TAI), diffuse hypoxic brain damage, diffuse brain swelling and diffuse vascular injury, which seems to be the worst of the four [177-179]. In these animal models, IL-1β and TNF-α have been implicated as primary pro-inflammatory cytokines, while a potentially beneficial, anti-inflammatory role has been ascribed to IL-10. Interleukin-1β has been characterized extensively in animal models of TBI as a promoter of neuroinflammation [158,159]. The neuronal damage resulting from IL-1β release appears to be indirect, due to synergistic action with other pro-inflammatory cytokines such as TNF-α [160, 161]. Like IL-1β, TNF-α has been regarded as a purely pro-inflammatory cytokine in the short history of TBI research [153]. The time course of release of TNF-α has is remarkably consistent across experimental paradigms of focal TBI in rodents (closed cortical impact, fluid percussion, or stab wound injury), with detectable levels at 1 h post-injury, maximal concentration at 3-6 h, and a decline in release by 24 h within the brain [162,163]. In diffuse injury models, serum levels of TNF-α rise within 24 h with an absence of expression in brain tissue, suggesting that diffuse injury induces a different immune response [164]. Similar to TNF-α, IL-6 has shown to play a role in neuroinflammation that is detected by 1 h post-injury in animal models, followed by a peak concentration between 2 and 8 h [153,165,166]. On the anti-inflammatory side, experimental studies have demonstrated a beneficial effect of IL-10, with exogenous administration of this cytokine aiding neurological recovery and reducing pro-inflammatory cytokine expression [167].

**Human studies of T/HS**

Translational research aims to apply scientific discoveries in basic science into the clinical level hoping to provide measures that predict outcome and to decrease the mortality rate in humans [120]. In the setting of T/HS and TBI, initial efforts to understand the role of cytokines focused on post-traumatic blood levels and pharmacological therapy aimed at enhancing the protective cytokines and inhibiting the damaging cytokines are underway and have shown some improved survival rates in experimental animals [36]. Conclusions from these studies were that, at low concentrations, cytokines are important to the host response to trauma whereas in higher concentrations they are deleterious [37]. The best characterized and, apparently, earliest and most fundamental cytokine in the trauma-induced pro-inflammatory cascade is TNF-α. TNF-α triggers the production of other cytokines, which amplify and propagate the inflammatory response [96] where raised plasma TNF-α have been found in hemorrhagic shock patients [97,98]. TNF-α also participates in the generation of free radicals such as NO [110,121]. Clinical studies have demonstrated that levels of several inflammatory mediators, such as IL-6, IL-8 and IL-10, correlate closely with severity of injury and complication rates [101-104]. From the family of DAMPs, serum HMGB1 was significantly increased in patients with sepsis, and the highest concentrations were observed in samples from patients who died [106]. Recent studies in HS patients suggested that HMGB1 may be involved in the pathogenesis of human HS outcome [106], though further studies are needed to determine HMGB1 role in the inflammatory response to trauma. We have demonstrated recently that mean post-T/HS HMGB1 levels samples within the first 24 h were higher in non-survivors vs. survivors, and that these levels correlated with various indices of injury severity including Marshall Score, creatinine, and circulating liver transferases [122].

Various intrinsic factors such as age, gender, race, body temperature, resuscitation, and hypotensive period, among others, play a role in how the body responds to acute traumatic injury. In addition, aspects of the injury
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itself (assessed clinically as ISS score, Marshall score, lactate, and base deficit), as well as treatment with agents such as inotropes, are additional important variables that impact clinical outcomes. It is daunting to attempt to study this multitude of variables in the acute clinical setting, and thus they are often examined separately. For example, the effect of aging on the immune response to traumatic injury has been studied. The inflammatory response becomes radically altered during the process of aging [38-41]. Indeed, the two processes (inflammation and aging), have prompted some authors to coin the term “inflamm-aging” for this complex process [39]. However, the characteristics of the aged inflammatory response vary occasionally between rodents (the experimental animals typically used for studies of inflammation) and humans. Interestingly, inflammation in the aged is characterized by a confounding array of alterations in cytokine production rather than a clear-cut increase or decrease. Several studies in vitro have reported enhanced production of IL-6, TNF-α and IL-1β in elderly human peripheral blood mononuclear cells compared to younger controls after inflammatory stimulation [42]. In contrast, and illustrating the complex interplay of age and gender, spontaneous production of IL-8 by elderly males is lower than that produced by elderly females and young controls [43]. Furthermore, there is a lower degree of in vitro-stimulated production of the chemokines MIP-1α, RANTES and IL-8 by natural killer cells from elderly donors compared to younger ones [44]. Zhang et al [45] showed elevated serum levels of cytokines, including IFN-α, IL12p40 and TNF-α in aged compared with young mice. Others have also shown that LPS-induced cytokine production is increased in the serum of aged mice [46,47].

Studies focused on gender-specific differences in the response to traumatic injury in animal models suggest that this dimorphic response is, at least in part, based on the levels of estrogen, testosterone, or their derivatives [52-54]. In this respect AET (5-androstene-3B, 7B, 17B-triol) administered subcutaneously provided significant survival effect in a 40%-volume hemorrhage trauma model in rats. This was the first study to report the ability of AET to improve survival after traumatic shock [55]. A clinical study provided evidence for differences in the early cytokine response between females and males after injury, with males having persistently elevated IL-6 cytokine expression over time as compared to similarly injured females [56]. An alternative hypothesis states that X-linked genetic differences between males and females, independent of hormonal status, responsible for these gender-based differential outcomes after injury in humans [56,57,58]. These studies suggest a new avenue for T/HS research and interaction with the field of endocrinology.

Clinical studies in TBI have also linked cytokines to outcome. For example, IL-1α levels correlated with poor clinical outcome in either adult or pediatric population. Patients with elevated cerebrospinal fluid (CSF) levels of IL-1α tended to have significantly poorer Glasgow Outcome Scores [168,169]. TNF-α in both serum and CSF has been documented in clinical settings of patients with severe TBI [170]. Paradoxically, both neuroprotective and neurotoxic effects of TNF-α have been suggested in human TBI, in terms of the inverse relationship of TNF-α with the both pro-inflammatory IL-18 and the anti-inflammatory IL-10 [171,172]. IL-6 is the cytokine found in the highest concentration in human CSF [171]. Measurements in a TBI population displayed maximal levels of IL-6 in the CSF between 3 and 6 days, with a steady decline in release thereafter [173].

Evidence for the intrathecal production of anti-inflammatory cytokines in TBI patients also exists. For example, IL-10 was increased acutely within 24 h of injury, correlating with decreases in TNF-α. In addition, transforming growth factor-β1 (TGF-β1) was elevated in both CSF at day 1 and serum at 3 weeks post-injury [153,171,174]. Interestingly, serum levels of IL-10 were elevated in both the severely head injured, as well as those suffering polytrauma, potentially rendering this cytokine a nonspecific marker of TBI as well as pointing to common mechanisms of injury response in T/HS and TBI [169,175,176].

Systems biology approaches can shed insight into inflammation at the cellular, tissue, organ, and organism levels

The acute inflammatory response is generally recognized as a complex system, both in structure and behavior. Understanding and potentially manipulating the acute inflammatory response requires an extension beyond the traditional scientific paradigm of analysis via sequential reductionist experimentation. Accomplishing this task requires a formal, explicit means of synthesis, heretofore an intuitive process carried out in the mind of the researcher. The emerging scientific discipline of systems biology, encompassing the search for information relating to the behavior of many biological components interacting in unison and often embodied in “-omics” technologies (genomics, proteomics, metabolomics, etc.) holds promise with regard to gaining definitive insights into biological processes [123-132]. Both genomic [81,133-138] and proteomic [115,139-143] approaches have begun to yield insights into the mechanisms of the response to T/HS.

Computational simulations are often used to integrate genomic and proteomic information, and have been used extensively by researchers dealing with such complex dynamic systems as studied in many fields [59-62] but only recently in biology [63-67]. Both inflammation and associated processes (e.g. apoptosis and organ damage/dysfunction) have been studied at the molecular and cellular levels [68-71]. Given the central role of organ damage/dysfunction in acute illness [19], modeling at the tissue and organ level has also played an essential function, especially examining the issue of physiologic variability [73,74].

This type of modeling has been successful in yielding basic insights into acute inflammation [75-78] including quantitative insights into the biology underlying experimental paradigms of acute inflammation in animals [79-81]. A more recent concept has been that of “Translational Systems Biology” [11,72,82,108], which includes computational simulations of clinical trials [83-86], potential clinical diagnostics in the form of patient-specific models [144], streamlined usage of experimental animals [87], and rational device design [89]. Using these approaches, we have shed basic insights into the basic
interactions of trauma with hemorrhage in mice [81], and have already begun to crowd patient-specific, predictive simulations in human T/HS [145] and TBI [146].

Conclusion and future prospects

New knowledge derived from a rich set of studies in cells, animals, and humans, combined with computational methods that vividly communicate insights, promises to revitalize the way in which clinical studies and clinical practice in T/HS and TBI are being conducted. We are rapidly gaining a new understanding of the complex interactions between injury and the inflammatory response and vice versa, and these new insights will hopefully serve as the framework for improving patient care worldwide. We may envision a point at which an integrated, rational, and iterative program of simulated clinical trials, in vitro screening for new drug compounds, pre-clinical studies, and human clinical trials will lead to a raft of new therapeutic options for T/HS and TBI (Fig. 4).

This new frontier increasingly requires training not only in clinical medicine, but also in quantitative sciences, bioinformatics, and translational science. Moreover, this approach highlights the need for inter- and multidisciplinary teams. Finally, emphasis should be placed on applying this new methodology to the difficult, complex clinical scenarios of combined T/HS and TBI, and especially integrating additional factors such as age, gender, genetics, and co-morbidities. Despite the many challenges that remain, we are optimistic that a bright future lies ahead for the care of traumatic injury and critical illness.

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