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HMGB1 as a therapeutic target for sepsis: it's all in the timing!

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Abstract

Morbidity and mortality from severe sepsis remain high, despite decades of research and improvements in intensive care unit (ICU) care. There have been over 100 failed clinical trials of biological response modifiers aimed at single therapeutic targets, mostly to suppress the early pro-inflammatory responses. In the last decade, extracellular HMGB1 has emerged as a late mediator of sepsis in murine sepsis models, whose blockade improves mortality and has a wider therapeutic window than previous efforts. Although this review promulgates the use of HMGB1 inhibitor as a therapeutic target, it should be recognized that it may not be an optimal approach to the early systemic inflammatory response syndrome (SIRS) response and cytokine storm, but rather for those patients who survive their cytokine storm and present with a persistent inflammatory, immunosuppressive and catabolism response (PICS). With earlier implementation of evidence-based best care principles for treating sepsis, fewer patients are dying from early septic shock, and there is an endemic increase in sepsis survivors with dismal long-term outcomes. These patients have ongoing inflammatory processes that may well be driven by the late and continued release of HMGB1 and other damage-associated molecular patterns receptors (DAMPRs). HMGB1 therapeutics, whether antibodies or natural herbal approaches, may be one novel approach for targeting not the early, but the late persistent inflammation of sepsis survivors.

Keywords

HMGB1; sepsis therapeutics

HMGB1, originally studied for its intracellular role as a DNA binding protein facilitating gene transcription, was later found to have important extracellular functions that make it a critical late mediator in lethal systemic sepsis [1]. Extracellular HMGB1 is a pro-inflammatory cytokine that has been studied for over a decade and is believed to be a critical mediator of sepsis as it is released from activated macrophages and monocytes in response to endogenous and exogenous inflammatory signals [2]. Most recently, inflammasome activation has been found to play an essential role in the LPS-induced release of HMGB1 following endotoxemia [3]. HMGB1 is actively released from stimulated mononuclear cells and is passively released from necrotic, but not apoptotic, cells [4]. HMGB1 acts as an alarmin, or an endogenous danger signal, contributing to a potent immune response. It plays a pivotal role in activation of the innate immune response, by functioning as a chemokine facilitating movement of immune cells to sites of infection, as well as in functioning as a

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damage-associated molecular pattern (DAMP), activating other immune cells to secrete pro-inflammatory cytokines, thus propagating the immune response [5].

In *in vivo* models of sepsis, HMGB1 is found in the circulation 8 h after the onset of sepsis and peaks from 16 to 32 h [2]. In cecal ligation and puncture (CLP), the 'gold-standard' model of murine intra-abdominal sepsis, mice treated with anti-HMGB1 antibodies have a significant survival advantage over those undergoing CLP alone [6]. More importantly, Yang and colleagues demonstrated that the late administration of anti-HMGB1 antibodies, 24 h after the onset of sepsis in animals with clinical signs of shock, was able to 'rescue' these animals and improve mortality [6]. These studies provided insight into the wider therapeutic window and potential therapeutic uses of HMGB1 inhibitors for the treatment of sepsis [4]. In humans, HMGB1 was found to be a potent stimulator of cytokine release from human mononuclear cells, and it has been found in higher levels in human patients who die from sepsis and hemorrhagic shock [5].

In this issue of *Expert Opinion on Therapeutic Targets*, Wang and colleagues provide a concise overview of the various herbal-derived HMGB1 inhibiting agents that could be used in the treatment of lethal sepsis [7]. They report that epigallocatechin 3-gallate (EGCG), an herbal component found in green tea, can decrease cytoplasmic HMGB1 levels via stimulating autophagy in activated macrophages, along with decreasing circulating levels of IL-6 and KC, two reliable markers of sepsis lethality [8]. Similar to their seminal findings in which HMGB1 inhibitors 'rescued' mice from lethal sepsis [6], intraperitoneal and oral administration of EGCG 24 h after the onset of sepsis increased survival rates in mice from 53 to 82% and 16 to 44%, respectively [8]. Additionally, they found that EGCG enhanced bacterial clearance from the liver and the lung (Wang *et al.*, in press). Likewise, they report that carbenoxolone, a pannexin-1 channel blocker that regulates LPS-induced HMGB-1 release via inhibition of inflammasome-mediated activation of HMGB1 release, and TSN-SS, which facilitates endocytosis of extracellular HMGB1, both reduce HMGB1 levels locally and systemically (Wang *et al.*, in press). The group concluded that herbal components may be a viable therapeutic option in protecting patients from lethal sepsis, by inhibiting not only HMGB1, but other inflammatory targets as well.

Severe sepsis and septic shock remain significant causes of morbidity and mortality in modern ICUs, despite decades of successful research in murine models and improvements in supportive ICU care [9]. Although the blockade of HMGB1 has been successful in reducing mortality in murine models of sepsis [6], there is no current evidence that this will translate to human septic patients. The fact that it is a 'late' mediator of sepsis is appealing as early sepsis is often difficult to recognize, one of the many suspected reasons for the past failures of therapeutic agents for sepsis [10].

There have been well over 100 clinical trials that utilized various biological response modifiers acting to suppress and/or block the SIRS response [11,12], the majority of these having been enthusiastically successful in reducing mortality in murine models of abdominal sepsis [13]. The continual reliance on murine sepsis models has raised considerable controversy recently about the appropriateness of the models, given the known genomic differences in the inflammatory response between man and mouse [14], and the inability to

sustain a critically ill mouse with adequate cardiac and pulmonary support as we do human patients. Additionally, murine models of polymicrobial sepsis have generally failed to keep up with the advances in human critical care management, and are still trying to recapitulate the historic SIRS/CARS paradigm. Rather than inhibiting the function a single protein, cytokine or receptor, a successful therapeutic approach to sepsis is likely to require a multi-modal and individualized approach to treatment that more closely mimics the human condition. For instance, our group has previously shown that a 'genomic storm' occurs following severe injury [15], which consists of a massive dysregulation of the leukocyte transcriptome, and this response is likely similar following the onset of severe sepsis. Similarly, the challenge to research for sepsis therapeutics also revolves around the diversity of its response.

In fact, in a recent review, we have argued that the new challenge to the treatment of critically ill sepsis patients is not a targeted focus on either the SIRS or compensatory anti-inflammatory response syndrome (CARS) component alone, or on a single mediator of sepsis, but on a PICS phenotype that many critically ill septic patients manifest after their initial SIRS event [9]. Their clinical course is characterized by a persistent acute-phase response with ongoing protein catabolism (despite optimal nutritional support), poor wound healing, immunosuppression and recurrent infections with mild SIRS [9]. These patients (especially the elderly) are commonly discharged to long-term acute care and skilled nursing facilities and rarely rehabilitate to a functional life, and ongoing costs of care, disability, suffering and mortality are disturbingly high [16]. Therefore, we have argued that advances in early management including routine sepsis screening, oftentimes using computerized clinical decision support systems, with implementation of best practice standard operating procedures, combined with modern intensive care interventions are sufficient at reducing in-hospital mortality and organ failure, leaving behind increasing numbers of sepsis survivors [17]. It is the clinical management of these patients as they progress irreversibly toward indolent death that has become the most challenging problem for which therapeutics are currently unavailable.

Expert opinion

We agree with the authors that blockade of HMGB1 may indeed have a potential role in the treatment of the septic patient. But we argue that the primary application of this approach may not be in the early or even in the intermediate period of the SIRS response, or during the 'cytokine storm' that occurs in the first 12 – 72 h of a severe sepsis event. Single anti-inflammatory approaches in this early period have generally been a failure, and this may well be due to the inability to identify the correct patient population prospectively, as well as the redundancy of the early innate inflammatory response. In fact, the numbers of patients presenting with severe sepsis or septic shock are declining in those institutions that have standardized the recognition and management of these events. In addition, we are beginning to see fewer patients dying from early septic shock and multiple organ failure at those institutions that have implemented these protocols [17]. The likelihood that another anti-inflammatory approach, even one with a larger window of opportunity, can be successful during this early period is in our opinion unlikely. Clinical trials in early sepsis with anti-HMGB1 antibodies or immunoadhesins do not appear to be currently under serious

consideration due to these theoretical, as well as business concerns, as described by Wang *et al.* [7].

In contrast, recent research interest has tended to focus not on the early inflammatory response, but on the prolonged periods of immunosuppression that are seen in survivors of early sepsis. Therapeutic interventions like IFNγ, anti-PD-1/PDL-1 and GM-CSF have all been promulgated in these populations, mostly for their immune-stimulant properties. A 2014 review of ClinicalTrials.gov reveals 367 active clinical trials in sepsis, 2 studies examining IFNγ and 5 examining GM-CSF. That said, however, the underlying causes of this PICS response that we have described [9] remain unknown. In fact, we could speculate that the late immunosuppression in sepsis may be an integral component of the persistent inflammation. With adequate control of the invading bacteria, this persistent low grade inflammation may well be the result of endogenous alarmin production, secondary to mechanical interventions, low grade organ injury and muscle catabolism due to bed rest and inactivity. Continual low grade production of HMGB1 as one of several alarmins may be contributing to this persistent low grade inflammation and might be an appropriate target for intervention. Examining HMGB1 levels in observational clinical trials of post-sepsis patients with PICS would be a first step to understanding whether this alarmin contributes to this process.

Clearly, traditional approaches to the treatment of sepsis have not been successful, but early presentation of the disease has become a generally survivable event because of improved recognition and management. The future challenge is how to not only improve in-hospital survival and long-term outcomes, but also reduce hospital stays. This means treating the PICS syndrome and the understanding the processes that drive this process. Anti-HMGB1 approaches may be successful and warrant an interventional approach.

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Declaration of interest

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