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Cutting Edge: High-Mobility Group Box 1 Preconditioning Protects against Liver Ischemia-Reperfusion Injury

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High mobility group box 1 (HMGB1) is a NF released extracellularly as a late mediator of lethality in sepsis and as an early mediator of inflammation following injury. Here we demonstrate that in contrast to the proinflammatory role of HMGB1, preconditioning with HMGB1 results in protection following hepatic ischemia/reperfusion (I/R). Pretreatment of mice with HMGB1 significantly decreased liver damage after I/R. The protection observed in mice pretreated with HMGB1 was associated with a higher expression of IL-1R-associated kinase-M, a negative regulator of TLR4 signaling, compared with controls. We thus explored the possibility that HMGB1 preconditioning was mediated through TLR4 activation. HMGB1 preconditioning failed to provide protection in TLR4 mutant (C3H/HeJ) mice, but successfully reduced damage in TLR4 wild-type (C3H/HeOuJ) mice. Our studies demonstrate that in contrast to the role of HMGB1 as an early mediator of inflammation and organ damage in hepatic I/R, HMGB1 preconditioning can be protective. The Journal of Immunology, 2006, 176: 7154–7158.

Ischemia/reperfusion (I/R) injury is a pathophysiologic process whereby hypoxic organ damage is accentuated following return of blood flow and oxygen delivery. Transient episodes of ischemia are encountered during solid organ transplantation, trauma, hypovolemic shock, and elective liver resection, when inflow occlusion or total vascular exclusion is used to minimize blood loss. The pathophysiology of liver I/R injury includes both direct cellular damage as the result of the ischemic insult as well as delayed dysfunc-

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4 Abbreviations used in this paper: I/R, ischemia/reperfusion; HMGB1, high-mobility group box 1; IRAK-M, IL-1R-associated kinase-M; sALT, serum alanine aminotransferase; rHMGB1, recombinant HMGB1.
Results and Discussion

Pretreatment with HMGB1 protects against liver I/R injury

To test our hypothesis that HMGB1 could precondition against organ injury following hepatic I/R, animals were given rHMGB1 at various concentrations 1 h before ischemia. Sixty minutes of warm hepatic ischemia followed by 6 h of reperfusion significantly increased sALT levels in control mice subjected to I/R. Pretreatment with 5 to 20 μg of rHMGB1 resulted in significant protection from hepatic injury in a dose-dependent manner (Fig. 1A). Liver histology confirmed the sALT estimation of liver damage (data not shown). Severe sinusoidal congestion and hepatocellular necrosis was present in liver tissue from control mice whereas minimal damage was noted in samples from HMGB1-treated mice.

Inflammatory cytokines, such as TNF and IL-6, have been shown to play key roles in the pathophysiology of hepatic I/R injury (12, 13). We measured serum levels of these cytokines after I/R. Compared with sham-treated animals, liver I/R in control animals resulted in increased levels of TNF and IL-6 (6 h after reperfusion; data not shown). However, animals pretreated with HMGB1 exhibited lower levels of serum TNF (Fig. 1B) and IL-6 (Fig. 1C) compared with control animals subjected to I/R.

FIGURE 1. Pretreatment with HMGB1 protects against liver I/R injury. A, Mice undergoing ischemia and 6 h of reperfusion were pretreated with rHMGB1 (2, 5, or 20 μg) or vehicle PBS i.v. 1 h before ischemia. Serum ALT levels were analyzed using ELISA kits from Biosource International. Data represent mean ± SE; n = 6 mice per group. *p < 0.05 vs mice subjected to I/R given vehicle PBS. B, Serum TNF and IL-6 (C) levels were measured following ischemia and 6 h of reperfusion in mice pretreated with 20 μg of HMGB1 or vehicle. Data represent mean ± SE; n = 6 mice per group. *p < 0.05 vs mice subjected to I/R given vehicle PBS. D, NF-κB activation during hepatic I/R injury was assessed. Mice undergoing ischemia and 1 h of reperfusion were pretreated with rHMGB1 Ab or vehicle PBS. Nuclear extracts were prepared from the ischemic livers and subjected to EMSA. Assay shown is representative of three experiments with similar results.
NF-κB is a transcription factor involved in signal transduction of a variety of extracellular stress stimuli. It is activated in the setting of hepatic I/R (14) and regulates both proinflammatory and protective responses in the liver (15–17). Using EMSA, we found increases in NF-κB DNA binding in the ischemic liver 1 h after reperfusion in control mice when compared with sham-treated animals (Fig. 1D). Mice pretreated with HMGB1 exhibited less NF-κB DNA binding activity. The specificity of the NF-κB bands were confirmed by cold competition in the presence of excess unlabeled NF-κB consensus motif. We previously performed supershift studies to determine that the NF-κB complex was a heterodimer composed of both p65 and p50 subunits (17). These results demonstrate that HMGB1 preconditioning can protect against warm hepatic I/R injury and that the protection is associated with a decrease in NF-κB activation and serum proinflammatory cytokine levels.

Protection with HMGB1 preconditioning in liver I/R associated with increase in IRAK-M expression

Recent in vitro and in vivo evidence suggest that TLR4 acts as a receptor to HMGB1 (3, 18, 19). The TLRs are one of the components by which the innate immune system senses the invasion of pathogenic microorganisms or tissue damage by recognizing specific molecular patterns that are present in microbial products (pathogen-associated molecular pattern molecules or PAMPs) or endogenous molecules released by damaged tissues (DAMPs) (20). Perhaps more than any of the other TLR family members, TLR4 sits at the interface of microbial and sterile inflammation by responding to both bacterial endotoxin and multiple other endogenous ligands, including hyaluronic acid (21), heparin sulfate (22), fibrinogen (23), HMGB1 (3, 18), and heat shock proteins (24).

Our recent studies suggest a central role for HMGB1 in the TLR4-dependent component associated with hepatocyte damage, and the resultant enhanced inflammation following hepatic I/R injury (3). We thus asked whether the mechanism of HMGB1 preconditioning involved down-regulation of TLR4 signaling. Upon TLR stimulation, multiple adaptor molecules are recruited to the TLR signaling complex. One molecule, IRAK-M, has been shown to be a negative regulator of TLR signaling (25). Thus, we examined the role of IRAK-M in HMGB1 preconditioning. Following 60 min of warm ischemia, IRAK-M protein expression was up-regulated in the liver of control mice (Fig. 2). However, mice pretreated with HMGB1 exhibited higher hepatic IRAK-M levels after reperfusion compared with control mice. Because IRAK-M expression was increased in preconditioned mice, we sought to determine whether TLR4 signaling was down-regulated in these protected mice. One of earliest events of TLR signaling involves the phosphorylation of the adaptor molecule IRAK-1 (26). Levels of phosphorylated IRAK-1 were increased in control mice after reperfusion (Fig. 2). In mice pretreated with HMGB1, phosphorylated IRAK-1 expressions were lower compared with control mice. Thus, the lower IRAK-1 phosphorylation in the livers of mice treated with HMGB1 was associated with increased hepatic IRAK-M expression after I/R.

HMGB1 preconditioning involves TLR4

Because the protection seen with HMGB1 preconditioning appeared to involve down-regulation of TLR4 signaling, we sought to determine whether TLR4 was required for the HMGB1-mediated preconditioning. TLR4 mutant (C3H/HeJ) mice and wild-type control (C3H/HeOuj) mice were subjected to liver I/R with or without HMGB1 pretreatment. In agreement with previous reports, TLR4 mutant mice were protected from hepatic I/R injury compared with wild-type mice (27) (Fig. 3A). Similar to that seen with C57BL/6 mice, HMGB1 treatment before the ischemic insult in TLR4 wild-type animals lead to protection from hepatic I/R damage (Fig. 3A). However, this protection was not seen in TLR4 mutant mice undergoing hepatic I/R. Whereas the liver damage in these mice is ~50% less than wild-type mice, it is still much greater than sham animals. HMGB1 pretreatment failed to reduce the damage in TLR4 mutant animals. We also examined the expression of serum TNF (Fig. 3B) and IL-6 (Fig. 3C) in the wild-type and mutant animals. Although TLR4 wild-type mice...
treated with HMGB1 exhibited decreased circulating TNF and IL-6 levels compared with control I/R animals, there was no difference in levels of these cytokines after I/R in TLR4 mutant mice treated with HMGB1 compared with mutant mice subjected to I/R but not receiving HMGB1.

Hepatic IRAK-M expression was also examined in both TLR4 mutant and wild-type mice (Fig. 4). Following 60 min of warm ischemia, wild-type mice pretreated with HMGB1 had higher expressions of hepatic IRAK-M after reperfusion compared with control mice. However, there was no significant difference in IRAK-M expression between TLR4 mutant animals treated with or without HMGB1. Of note, IRAK-M levels dropped to baseline levels by 6 h in the TLR4 mutant animals but remained elevated in the wild-type mice subjected to I/R. The up-regulation of IRAK-M in the TLR4 mutant animals indicates an early pathway for IRAK-M independent of TLR4, whereas the drop at 6 h indicates that the persistence is due to TLR4 activation.

**DISCUSSION**

**References**


