Trauma-hemorrhage and dendritic cell functions: a critical review of splenic dendritic cell dysfunction following trauma-hemorrhage and therapeutic approach

T Kawasaki¹,¹, T Sata¹

Abstract

Introduction

Many studies demonstrated that trauma-hemorrhage induces marked alterations in various immune functions. The trauma-hemorrhage-induced immunosuppression is associated with an increased susceptibility to subsequent sepsis, organ failure and mortality. Previous studies demonstrated that trauma-hemorrhage induces immunosuppression in both innate immune systems and adaptive immune systems. Dendritic cell is the most potent antigen-presenting cell that initiates innate and adaptive immune response. The aim of this review was to discuss trauma-hemorrhage and dendritic cell functions.

Conclusion

Trauma-hemorrhage impairs splenic dendritic cell maturation. Suppressed TLR4 expression and MAPK activation contribute to the hyporesponsiveness of splenic dendritic cells following trauma-hemorrhage. 17β-oestradiol produces immunoprotective effects on splenic dendritic cells following trauma-hemorrhage. The immunomodulatory properties of 17β-oestradiol might be a potent therapeutic strategy for the treatment of depressed splenic dendritic cell functions following trauma-hemorrhage.

Discussion

The authors have referenced some of their own studies in this review. These referenced studies have been conducted in accordance with the Declaration of Helsinki (1964) and the protocols of these studies have been approved by the relevant ethics committees related to the institution in which they were performed. All human subjects, in these referenced studies, gave informed consent to participate in these studies. Animal care was also in accordance with the institution guidelines.

Does trauma-hemorrhage have any effects on splenic DC functions?

In the first part, we discuss the effect of trauma-hemorrhage on splenic DC functions. In a mice trauma-hemorrhage model, we demonstrated that the percentage of splenic DCs significantly decreased following trauma-hemorrhage compared with sham mice. The percentage of both Annexin V positive and PI negative cells (early phase of apoptosis) and Annexin V positive and PI positive cells (late phase of apoptosis) were significantly increased following trauma-hemorrhage. Our results demonstrate that apoptosis is a possible cause of splenic DC loss following trauma-hemorrhage. Previous reports also suggested that the loss of splenic DC occurs both in patients with sepsis and in mouse sepsis.
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antigen-presenting capacity. DCs isolated from trauma-hemorrhage mice showed a lower capacity to stimulate T-cell proliferation than those from sham controls. Trauma-hemorrhage depresses antigen-presenting function of DC.

We found that:

1. Trauma-hemorrhage-induced apoptosis of splenic DC.
2. The DC maturation marker, CD83 and MHC-II expression on splenic DC were suppressed following trauma-hemorrhage.
3. Trauma-hemorrhage suppressed the cytokine producing capacity of splenic DC.
4. Trauma-hemorrhage depressed the antigen-presenting function of splenic DC.
5. Trauma-hemorrhage impaired splenic DC maturation.

Does trauma-hemorrhage have any effect on toll-like receptor (TLR4) expression and mitogen-activated protein kinase (MAPK) activation of splenic DCs?

In the next part, we discuss how trauma-hemorrhage suppresses splenic DC functions. LPS is a potent activator of DCs and it induces the production of pro-inflammatory cytokines. TLR 4 has been shown to be essential for cellular responsiveness to LPS. After LPS stimulation, activated signals go down from the TLR4 to the MAPK/NF-kB signalling pathways. The phosphorylated MAPKs transduce their signals downstream and promote proinflammatory cytokine production. We hypothesised that trauma-hemorrhage alters TLR4 expression and MAPK activation of splenic DC.

We investigated the expression of TLR4 and the activation of three different kinds of MAPKs, p38, extracellular signal-regulated protein kinase (ERK) and stress-activated protein kinase/c-Jun NH2-terminal kinase (SAPK/JNK). LPS stimulation significantly increased the expression of the active phosphorylated form of p38 MAPK in cells from both sham and trauma-hemorrhage mice. The activated p38 MAPK-positive cells percentage after LPS stimulation was significantly decreased in the trauma-hemorrhage group. The level of phosphorylated p38 MAPK was significantly greater than that from traumahemorrhage DCs. Total p38 MAPK expression was not significantly different between sham and trauma-hemorrhage mice at any time points after LPS stimulation. As well as p38, LPS-stimulated phospho ERK activation was significantly suppressed following trauma-hemorrhage. The percentage of phospho ERK positive cells was also decreased following trauma-hemorrhage.

To determine whether trauma-hemorrhage influenced TLR4 gene expression, we used quantitative RT-PCR to assess changes in expression of TLR4 mRNA in purified splenic DCs from sham or trauma-hemorrhage.

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mice. A 30% decrease in TLR4 mRNA levels in splenic DCs from trauma-hemorrhage mice was observed compared with shams. To determine whether trauma-hemorrhage induces splenic DCs hyporesponsiveness to other TLR agonists, we examined the effect of trauma-hemorrhage on TLR2 ligand zymosan-induced cytokines production of splenic DCs, as well as TLR4 ligand LPS, TLR2 ligand zymosan-induced cytokines production of splenic DC. Trauma–hemorrhage also induced hyporesponsiveness of zymosan.

The hyporesponsiveness of splenic DCs is a likely factor in the immuno-suppression seen following trauma-hemorrhage. Trauma–hemorrhage alters the LPS-induced activation of the MAPK cascade that controls different aspects of the LPS hyporesponsiveness of splenic DCs following trauma-hemorrhage. Trauma–hemorrhage appears to induce decreased cross-talk between the MAPKs pathways, as well as uncoupling of some LPS response from the MAPK cascade. Hyporesponsiveness of splenic DCs was also found after stimulation with TLR2 agonist zymosan following trauma-hemorrhage. Thus, altered MAPK activation and signal transduction contributes to the development of the hyporesponsiveness of splenic DCs which is central in the development of immune complications following trauma-hemorrhage. However, it remains unclear whether the hyporesponsiveness of splenic DCs following trauma-hemorrhage would occur after stimulation with other TLR agonists such as TLR5 agonist flagellin and TLR9 agonist CpG. We found that:

1. Trauma-hemorrhage downregulated MAPK activation of splenic DC.
2. Surface expression of TLR4-MD-2 was also suppressed following trauma-hemorrhage.
3. These changes in MAPK activation and TLR4-MD-2 expression were associated with a suppressed ability of splenic DC to produce cytokines in response to LPS, a potent TLR4 ligand.
4. Suppressed TLR4 expression and MAPK activation were involved in splenic DC dysfunction following trauma-hemorrhage.

Do female sex steroids adversely or beneficially affect the depressed function of DC in males with trauma-hemorrhage?

In the final part of this review, we would like to discuss the therapeutic strategy for trauma-hemorrhage-induced immunosuppression. Previous studies demonstrated that IL-15, Fms-like tyrosine kinase-3 ligand (Flt3L), IL-28 and IL-29 enhance DC function.27–29 We focus on the effect of oestrogen, one of female sex hormones, on depressed splenic DC functions in this review.30–32

Bone reported a preponderance of morbidity and mortality from sepsis in males compared with females.33 McGowan et al. also reported a significantly higher incidence of bacteraemic infections in traumatised males than in females.34 A retrospective study incorporating 30,286 trauma victims with an injury severity score (ISS) >15 demonstrated a significantly higher incidence of pneumonia in males.35 Schrodler et al. have shown a significantly higher survival rate in women (74%) compared with men (31%) following the onset of sepsis.36 Similar gender-dimorphic findings have been demonstrated in experimental studies following severe blood loss and the induction of sepsis.37,38 Gender-specific immune response may be due to different effects and roles of sex hormones. Several studies were conducted in order to elucidate the effect of sex steroids on cell-mediated immune responses following trauma-hemorrhage. Male sex hormones play an important role in mediating immunosuppressive effects. Female sex hormones are immunoprotective.

Oestrogen is well known as the key regulator of cell growth, differentiation and function.39 To evaluate whether female sex steroids adversely or beneficially affect the depressed function of DC in males with trauma-hemorrhage, we treated male C3H/HeN trauma-hemorrhage mice with 17β-oestradiol (E2), oestrogen receptor (ER)-α agonist propyl pyrazoletriol (PPT) or ER-β agonist diarylpropionitrile (DPN) in the middle of resuscitation.40 Plasma IL-6, IL-10, TNF-α and MCP-1 concentrations were significantly increased 2 h following trauma-hemorrhage. Administration of E2 or PPT following trauma-hemorrhage attenuated the increase in cytokine concentration under those conditions. Administration of DPN also reduced the elevation of these cytokine levels, but the levels of plasma cytokines still remained significantly higher than that in trauma-hemorrhage mice treated with E2 or PPT. Plasma IL-12p70 and IFN-γ concentrations were not detectable in sham and trauma-hemorrhage groups. Administration of E2 normalised the percentage of both early phase and late phase of apoptotic cells under those conditions. Although PPT administration following trauma-hemorrhage also normalised the percentage of apoptotic cells, DPN administration did not affect the apoptotic rate under those conditions. These results are in accordance with a previous study that reported E2 increases the viability of splenic DC in vitro.40 Administration of E2 attenuated the suppression of CD40, CD83 and MHC-II expression under those conditions. PPT administration also normalised CD40, CD83 and MHC-II expression following trauma-hemorrhage; however, DPN treatment did not affect these expressions. Administration of E2 or PPT attenuated the suppressed antigen-presenting capacity of DCs under those conditions; however, ER-β agonist DPN did not improve DCs.
antigen-presenting function. Immature DCs have the ability to capture antigens by endocytosis or macropinocytosis; however, mature DCs lose this ability. A previous study demonstrated that E2 decreases the endocytosis of splenic DCs. Therefore, these results strongly suggest that E2 administration following trauma-hemorrhage restores the maturation of splenic DCs.

The predominant biological effects of E2 are mediated through ER-α and ER-β. These two receptors are differentially expressed in different tissues. To determine whether trauma-hemorrhage influenced ER-α and ER-β gene expression in splenic DC, we used quantitative RT-PCR to assess changes in expression of ER-α and ER-β mRNA in purified splenic DCs from sham or trauma-hemorrhage mice. Although trauma-hemorrhage did not significantly influence the expression of ER-α and ER-β mRNA in the splenic DC, the splenic DC expresses ER-α mRNA predominantly. Taken together, these results suggest that the salutary effects of E2 on splenic DC functions are mediated predominantly via ER-α.

We found that:

1. Administration of E2 was effective in normalising the cytokine production and antigen-presenting capacity of splenic DC.
2. E2 produced immunoprotective effects on splenic DC following trauma-hemorrhage.
3. ER-α agonist PPT also produced immunoprotective effects, whereas ER-β agonist DPN did not attenuate splenic DC functions.
4. The salutary effects of E2 on splenic DC functions were mediated predominantly via ER-α.
5. Splenic DC dysfunction following trauma-hemorrhage might be controlled by female sex steroid.

**Conclusion**

A major consequence of trauma-hemorrhage is the suppression of organ and immune cell functions. The findings reviewed in this article imply that trauma-hemorrhage impairs splenic DC maturation and oestrogen plays a decisive role in the depression or maintenance of DC functions following injury. The protective effects of oestrogen in restoring splenic DC function are through intracellular receptor, ER-α. Experimental studies clearly demonstrate that oestrogen and ER agonists are useful therapeutic adjuncts in protecting organ functions and improving outcome following trauma-hemorrhage.

However, in the clinical setting, there are conflicting reports whether gender dimorphic responses are evident following injury. The reasons for the lack of uniform results appear to be due to the fact that most studies do not take into consideration the hormonal status of the host at the time of injury. Thus, studies reporting protective effects of the female gender following injury could be due to patients having high oestrogen levels at the time of injury whereas those reporting a lack of protective effects in females could be due to low oestrogen levels. In view of this, it is important to carry out additional studies in which the hormonal status of the patient is measured as quickly as possible after injury and correlate sex steroid levels with the lack or prevalence of complications, circulating cytokine levels, incidence of organ dysfunction and failure and length of hospital stay.

**Abbreviations list**

DC, dendritic cell; DPN, diarylpropionitrile; E2, 17β-oestradiol; ER, oestrogen receptor; ERK, extracellular signal-regulated protein kinase; IL, interleukin; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex class; PPT, propyl pyrazolone; SAPK/JNK, stress-activated protein kinase/c-Jun NH2-terminal kinase; TLR, toll-like receptor; TNF, tumour necrosis factor.

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