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17β-Estradiol's salutary effects on splenic dendritic cell functions following trauma–hemorrhage are mediated via estrogen receptor-α

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Abstract

Although 17β-estradiol administration following trauma–hemorrhage attenuates Kupffer cell, splenic and peritoneal macrophage functions, it remains unknown whether 17β-estradiol has any salutary effects on splenic dendritic cell (DC) functions and if so, whether such effects are mediated via the estrogen receptors (ER). We hypothesized that 17β-estradiol administration following trauma–hemorrhage has salutary effects on splenic DC functions. Male C3H/HeN (6–8 weeks) mice were randomly assigned to sham operation or trauma–hemorrhage. Trauma–hemorrhage was induced by midline laparotomy and ∼90 min of hemorrhagic shock (blood pressure [BP] 35 mmHg), followed by fluid resuscitation (4× the shed blood volume in the form of Ringer's lactate). Estrogen receptor (ER)-α agonist propyl pyrazole triol (PPT; 5 μg/kg), ER-β agonist diarylpropionitrile (DPN; 5 μg/kg), 17β-estradiol (50 μg/kg), or vehicle (10% DMSO) was injected subcutaneously during resuscitation. Two hours later, the mice were sacrificed, splenic DCs were isolated and the changes in their apoptosis, co-stimulating factors and MHC class II expression, ability to produce cytokines, and antigen presentation capacity were measured. Apoptosis of splenic DC increased following trauma–hemorrhage; however, 17β-estradiol administration after trauma–hemorrhage normalized the rate of apoptosis. Moreover, splenic DC cytokines production, co-stimulating factors and MHC class II expression, and antigen presentation capacity were significantly decreased following trauma–hemorrhage; however, 17β-estradiol as well as PPT also prevented these depressions. In contrast, DPN did not attenuate splenic DC functions following trauma–hemorrhage. Since PPT administration following trauma–hemorrhage was more effective in normalizing splenic DC functions than DPN, the salutary effects of 17β-estradiol on splenic DC functions are mediated predominantly via ER-α.

Keywords: Shock; Trauma; Hemorrhage; Splenic dendritic cell; Estradiol; Estrogen receptors

1. Introduction

Many studies have demonstrated that trauma–hemorrhage induces marked alterations in various immune functions (Meldrum et al., 1997; Murphy et al., 2004, 2005; Noel et al., 2005; Shukla et al., 2004; Zuckerbraun et al., 2005). Severe hemorrhage followed by adequate resuscitation with blood and crystalloid solutions is known to produce depression of immune system, such as lymphocyte functions and macrophage functions (Chaudry and Ayala, 1993). Traumatic injury due to burn or soft tissue damage is also known to induce a profound depression of cell-mediated and humoral immunity (Frink et al., 2007; Purcell et al., 2006). The trauma/injury-induced immunosuppression is also associated with an increased susceptibility to subsequent sepsis, organ failure and mortality (Baue, 2005, 2006; Menges et al., 1999; Ulloa and Tracey, 2005).

Previous studies have demonstrated gender dimorphism in both immunological and physiological responses following trauma–hemorrhage (Choudhry et al., 2005; Coyle et al., 2006; Frink et al., 2007). In general, these studies have concluded that males are susceptible to the deleterious effects of hemorrhage shock, whereas proestrus females, with elevated systemic estrogen levels, are protected. With regard to the immune system, proestrus female mice showed normal immune

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response; however, male mice have markedly altered immune responses following trauma–hemorrhage (Angele et al., 2000). In addition, male animals treated with 17β-estradiol following trauma–hemorrhage display an immunological response that closely mimics that of proestrus females (Suzuki et al., 2007; Yu et al., 2006). Studies have also demonstrated that male sex steroid appear to be responsible for producing the depression in cell and organ functions following trauma–hemorrhage (Angele et al., 1999; Shimizu et al., 2007). These studies therefore suggest that male and female sex steroids have opposite effects on immune functions following trauma–hemorrhage.

Dendritic cells (DCs) are the most potent antigen-presenting cells (APC) that are intimately involved in the initiation of innate and adaptive immunity (Liu, 2001; Palucka and Banchereau, 1999; Steinman and Hemmi, 2006). Immature DCs are strategically located in tissues that represent pathogen entry routes. After microbial invasion and tissue destruction, immature DCs become activated and undergo maturation. Mature DCs reduce endocytic and phagocytic capacity, but they acquire an antigen-presenting capacity and migratory functions that allow antigen-loaded DCs to move to the T cell areas or lymphoid tissues. Mature DCs also express high levels of MHC class II and co-stimulatory molecules, including CD40, CD80, CD83 and CD86, on their surface. They also have the ability to activate both T helper 1 (Th1) and Th2 cell responses. Our previous studies have shown that trauma–hemorrhage causes a decrease in splenic DCs maturation (Kawasaki et al., 2006). Although treatment with 17β-estradiol following trauma–hemorrhage alters the circulating plasma cytokine levels and improves lymphocyte and macrophage immune functions, it remains unknown whether 17β-estradiol also modulates splenic DC function under those conditions. The aim of this study, therefore, was to investigate the effect of 17β-estradiol on splenic DC functions following trauma–hemorrhage. In addition, using estrogen receptor (ER) specific agonist, we investigated which of the two estrogen receptors is more critical in mediating the effects of 17β-estradiol on splenic DC.

2. Materials and methods

2.1. Mice

Male C3H/HeN mice (Charles River Laboratories, Wilmington, MA) 6–8 weeks old and weighing 20–25 g, were used in the experiments. These mice were allowed to acclimatize in the animal facility for 1 week before the experiments. All animal experiments were conducted in accordance with guidelines set forth in the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals* by the National Institutes of Health (NIH) and approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham.

2.2. Trauma–hemorrhage

Animals were fasted overnight but allowed water *ad lib*. They were anesthetized with isoflurane (Attane; Minrad, Buffalo, NY) and restrained in supine position. A 2.0-cm midline laparotomy (i.e., induction of soft tissue trauma) was performed and then closed aseptically in two layers using 6-0 Ethilon™ sutures (Ethicon, Somerville, NJ). Subsequently, both femoral arteries were aseptically catheterized with polyethylene-10 tubing (Clay-Adams, Parsippany, NJ) and the animals were allowed to awaken. Blood pressure was monitored continuously through one of the femoral artery catheters using a blood pressure analyzer (Digi-Med BPA-190; Micro-Med, Louisville, KY). Upon awakening, the animals were bled through the other catheter to a mean arterial pressure (MAP) of 35 ± 5 mmHg that was maintained for 90 min. At the end of that period, animals were resuscitated with four times the shed blood volume in the form of lactated Ringer's solution over 30 min. Fifteen minutes before the end of the resuscitation, the mice received estrogen receptor (ER)-α agonist propyl pyrazole triol (PPT; 5 μg/kg BW), ER-β agonist diarylpropionitrile (DPN; 5 μg/kg BW), 17β-estradiol (50 μg/kg BW), or an equal volume of the vehicle (∼0.02 ml, 10% dimethyl sulfoxide; DMSO) subcutaneously (Suzuki et al., 2007; Yu et al., 2006). Lidocaine was applied to the groin incision sites, the catheters were removed, the vessels were ligated, and the incisions were then closed. Sham-treated animals underwent the same anesthetic and surgical procedures, but neither hemorrhage nor fluid resuscitation was performed. The animals were anesthetized by isoflurane inhalation 2 h following trauma–hemorrhage and resuscitation, and blood and spleen were collected for analysis.

2.3. Plasma collection and storage

Blood was obtained by cardiac puncture and centrifuged at $400 \times g$ for 10 min at 4 °C. Plasma was immediately frozen and stored at −80 °C until analyzed.

2.4. Quantitation of plasma estrogen levels

Plasma estradiol levels were determined by EIA kit (Cayman Chemical, Ann Arbor, MI).

2.5. Isolation of splenic dendritic cells and flow cytometric analysis

Spleens were digested by Liberase CI (Roche, Indianapolis, IN) and teased apart by repeated pipetting in PBS containing 5% FCS and 5 mM EDTA. The red blood cells were osmotically lysed and splenocytes were blocked with 1 μg/ml Fc block (clone: 93) antibody for 15 min on ice. Cell suspensions were enriched with anti-CD11c magnetic beads and positive selection columns MS+ according to manufacturer's instructions (Miltenyi Biotec, Auburn, CA). Flow cytometric analysis demonstrated that cells contained >90% CD11c-positive cells. Cell suspensions were stained with antibody against surface markers MHC II (clone: M5/114.15.2), CD40 (clone: 1C10), CD80 (clone: 16-10A1), CD83 (clone: Michel-17), or CD86 (clone: GL1) for DCs (eBioscience, San Diego, CA). Cells were acquired using a BD LSRII™ (BD Biosciences, San Diego, CA), and 10,000 events were collected for analysis.