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Hematopoietic stem progenitor cells prevent chronic stress-induced lymphocyte apoptosis

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ABSTRACT

Physical or psychological chronic stress can suppress the immune system. However, the mechanisms remain to be elucidated. We investigated the effect of hematopoietic stem-progenitor cells (HSPCs) on chronic stress-induced the alterations of immune responses. We demonstrate that HSPCs prevents stress-induced lymphocyte apoptosis. Moreover, we also demonstrate that the protective effect of HSPCs on stress-induced lymphocyte reduction exerts by steroid hormones. Furthermore, we reveal that chronic stress-induced T cell-mediated immune responses contributes to the protective effect of HSPCs. These results indicate that HPSCs might offer a novel therapeutic strategy against the deleterious effects of chronic stress on the immune system.

1. Introduction

Depending on the severity and duration, physical or psychological stress can enhance or suppress the immune system in both humans and animals (Yin et al., 2000; Hu et al., 2013; Hu et al., 2014; Cao et al., 2014). We and others have reported that acute restraint stress could significantly enhance delayed-type hypersensitivity reaction, while chronic stress could decrease immune function and enhance susceptibility to diseases (Dhabhar and McEwen, 1997; Yin et al., 2000; Shi et al., 2003. Li et al., 2011a; Smith et al., 2016). Acute stress and moderate stress such as routine exercise could increase immune responsiveness (Dhabhar and McEwen, 1999; Cao et al., 2014). However, chronic stress such as long-term emotional stress can reduce immune functions (Zorrilla et al., 2001; Reiche et al., 2004; Quan et al., 2001; Hawkley and Cacioppo, 2004; Zhang et al., 2008a). This effect is at least in part due to the reduction of lymphocytes (Zorrilla et al., 2001; Yin et al., 2000, Zhang et al., 2008b; Hu et al., 2014). Little progress, however, has been made in understanding chronic stress-induced immune suppression. To define the mechanisms of stress-induced immune responses and to design strategies for therapeutic intervention, we established an animal model for chronic restraint stress to determine the alterations of immune functions following stress. Our previous studies have defined that chronic restraint stress of mice induces reduction in lymphocyte numbers (Yin et al., 2000; Zhang et al., 2008b; Zhang et al., 2008c; Hu et al., 2014).

Hematopoietic stem cells (HSCs) are the stem cells that give rise to all the other blood cells through the process of haematopoiesis (Birbrair and Frenette, 2016). Hematopoietic stem-progenitor cells (HSPCs) can be readily isolated and expanded from a number of tissues, including bone marrow and umbilical cord blood (Brudecki et al., 2012; Fischer and Agrawal, 2013, Ito and Suda, 2014). Hematopoietic stem cells differentiate into multipotent progenitor cells expressing CD34 (Brudecki et al., 2012; Fischer and Agrawal, 2013, Ito and Suda, 2014; Cao et al., 2014). These cells have been shown to effectively treat various diseases, particularly those involving dysregulated immune responses, such as allergic diseases, genetic diseases, and autoimmune diseases (Brudecki et al., 2012; Fischer and Agrawal, 2013, Ito and Suda, 2014). Interestingly, our recent results shown that HSPCs modulates immune responses including levels of cytokine production (Brudecki et al., 2012). Therefore, a concept that prompted us to determine if HSPCs could prevent stress-induced immune suppression.

In the current study, we performed a mouse model of chronic restraint stress, one that has been widely used to investigate the effect of stress on the immune functions (Yin et al., 2000; Cao et al., 2014; Hu et al., 2014). We found that adoptive transfer of $CD34^+$ HSPCs protect lymphocytes from stress-induced apoptosis. Additionally, $CD34^+$ HSPCs ameliorate synthetic glucocorticoid dexamethasone-induced reduction in lymphocyte numbers. Furthermore, we found that $CD34^+$ HSPCs modulates T cell-mediated immune response following chronic stress. Thus, our studies provides a novel potential countermeasure against

Abbreviations: HSPCs, Hematopoietic stem progenitor cells; DTH, Delayed type hypersensitivity; DEX, Dexamethasone

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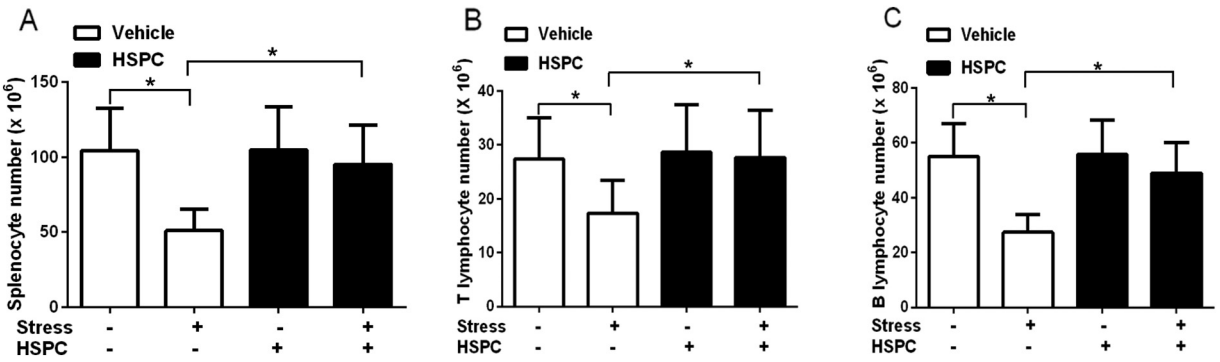

Fig. 1. CD34$^+$ HSPCs prevent chronic restraint stress-induced lymphocyte reduction. BALB/c male mice aged 7 weeks were intravenously injected with CD34$^+$ HSPCs (1×10^6 in 100 μl PBS, > 90% purity, i.v.) or vehicle (heat-killed 1×10^6 CD34$^+$ HSPCs in 100 μl PBS, i.v.) immediately before being subjected to 6 h restraint daily for 3 days. (A) Splenocyte numbers were counted with a hemocytomete. (B and C) Numbers of T lymphocytes and B lymphocytes were examined by multiplying total splenocyte number, by respective percentage determined by flow cytometry. Data are means ± SEM. N = 5. *$p < 0.01$.

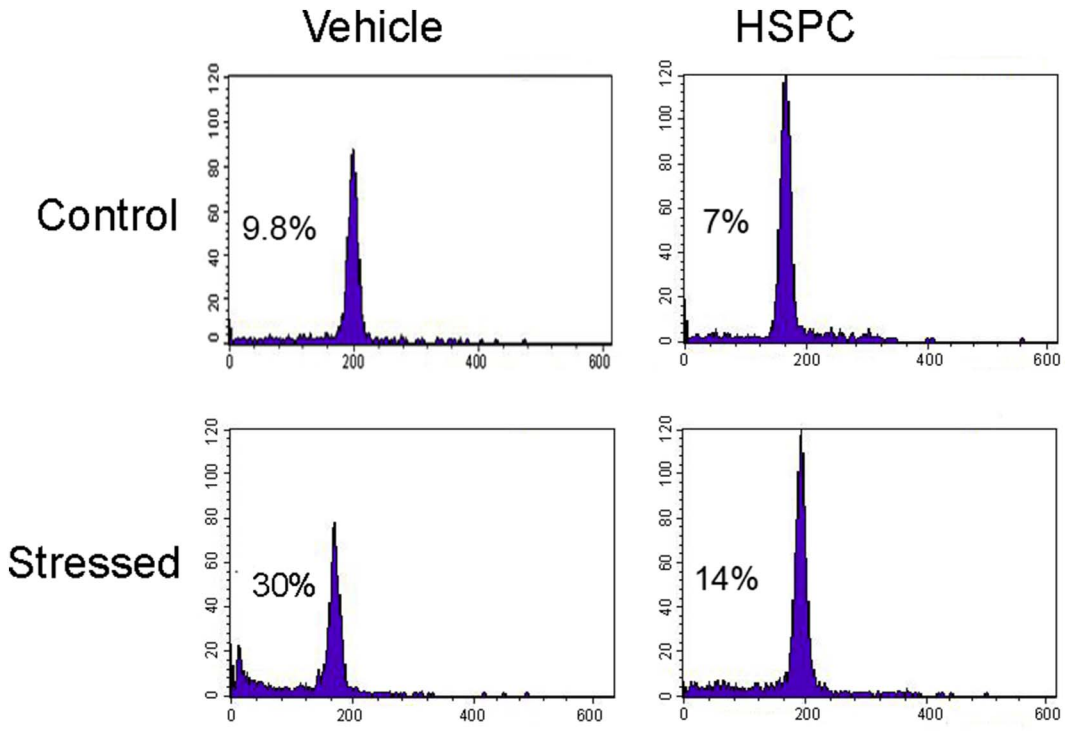

Fig. 2. CD34$^+$ HSPCs prevent splenocyte apoptosis induced by chronic restraint stress. BALB/c male mice aged 7 weeks were intravenously injected with CD34$^+$ HSPCs (1×10^6 in 100 μl PBS, > 90% purity, i.v.) or vehicle (heat-killed 1×10^6 CD34$^+$ HSPCs in 100 μl PBS, i.v.) immediately before being subjected to 12 h restraint daily. After 2 days of stress, splenocyte apoptosis was assessed by flow cytometry after staining with propidium iodide. These results are representative of three independent experiments.

stress-induced immune responses.

2. Materials and methods

2.1. Mice

BALB/c male mice were obtained from the Jackson Laboratory. All mice were maintained in the Division of Laboratory Animal Resources at East Tennessee State University (ETSU), a facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC). All aspects of the animal care and experimental protocols were approved by the ETSU Committee on Animal Care.

2.2. Physical restraint stress

Six- to seven-week-old male mice were subjected to an established chronic physical restraint protocol used in our laboratory as well as others (Yin et al., 2000; Zhang et al., 2008b; Hu et al., 2014; Cao et al., 2014). Briefly, mice were placed in a 50-ml conical centrifuge tube with multiple punctures to allow ventilation. Mice were held horizontally in the tubes for 12 h followed by a 12-h rest. During the rest period food and water were provided ad libitum. Control littermates were kept in their original cage and food and water were provided only during the 12 h rest. At 2 days after physical restraint, mice were sacrificed by CO_2 asphyxiation, and the spleens were harvested.

2.3. Isolation and injection of CD34$^+$ HSPCs of mice

The bone marrow cells were flushed out of the femurs and tibias with FBS-free RPMI 1640 medium under aseptic conditions (Brudecki et al., 2012). A single cell suspension was made by repeated pipetting and filtering through a 70-μm nylon strainer, followed by erythrocyte lysis. We isolated CD34$^+$ HSPCs using magnetic-assisted cell sorting as described by us (Brudecki et al., 2012). Bone marrow cells (2×10^7 cells) were incubated with biotinylated mouse anti-CD34 antibody