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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.

Graphical abstract

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Keywords

Stress; Lymphocytes; Hematopoietic Stem Progenitor Cells; Immune Response

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 et al. 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 et al., 2013, Ito et al., 2014). Hematopoietic stem cells differentiate into multipotent progenitor cells expressing CD34 (Brudecki et al., 2012; Fischer et al., 2013, Ito et al., 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 et al., 2013, Ito et al., 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 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 CO₂ 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 (eBioscience, CA) for 20 min at 4°C. After being washed, cells were incubated with antibiotin magnetic beads (Miltenyi Biotech, CA) for 20 min at 4°C and subsequently applied to a Milteny selection (MS) column for positive selection of CD34⁺HSPCs according to the manufacturer's instructions. The purity of the CD34⁺HSPCs was higher than 90% by flow cytometric analysis.

For injection of CD34⁺HSPCs into mice, we washed isolated CD34⁺HSPCs and resuspended in sterile PBS, male mice aged 6–7-week old were intravenously injected with CD34⁺HSPCs (1×10^6 in 100 µl PBS, > 90% purity) or vehicle (heat-killed 1×10^6 CD34⁺HSPCs in 100 µl PBS) immediately before being subjected to 12 h restraint daily.

2.4. DNA content analysis

Cellular DNA content was analyzed by flow cytometry of propidium iodide-stained cells as described previously by us (Yin et al., 1999a; Yin et al., 1999b; Yin et al., 2000). Briefly, splenocytes were fixed with 70% ethanol for 30 min at 4°C, followed by two washes with 1×PBS. The fixed splenocytes were then incubated in PBS containing propidium iodide (Sigma) at 50 µg/ml and RNase (Boehringer Mannheim, Indianapolis, IN) at 0.1 mg/ml at room temperature for 30 min. DNA content was determined by flow cytometry on FACScan (BD Biosciences). Apoptotic cells were shown as a hypodiploid peak.

2.5. Enzyme linked immunosorbent assay (ELISA) for cytokines

ELISA was described previously (Hu et al., 2013; Hu et al. 2014). Briefly, splenic lymphocytes were adjusted to a final concentration of 5×10^5 cells/ml in 96-well plates. Splenocytes were cultured with anti-CD3/anti-CD28 antibodies for 48 h for IL-2 and IL-4 production using a Quantikine Mouse ELISA kit (R&D Systems, MN). Splenic CD4⁺ T cells were negatively selected by using the MagCelect™ mouse CD4⁺ T cell isolation kit (R&D Systems, Minneapolis, MN) (Hu et al., 2013; Hu et al. 2014). The splenic CD4⁺ T cells cultured with anti-CD3/anti-CD28 antibodies for 48 h for IL-17 production by ELISA.

2.6. Induction of delayed type hypersensitivity (DTH)

DTH was induced as described in our previous studies (Li et al., 2011b). Briefly, seven to eight-week-old mice were immunized with ovalbumin (OVA, s.c. injection of 100 µg) in complete Freund's adjuvant (CFA) on the first day of stress and follow up with a boost with OVA (s.c. injection of 20 µg) in incomplete Freund's adjuvant (IFA) on the seventh day. Mice were subjected to a 12 h physical restraint daily for 3 days. DTH (hind footpad) of all groups were measured on day 10 in a blinded fashion using a caliper meter.

2.7. Statistical analysis

The results were presented as Means and SEs. The data were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni tests to determine where differences among groups existed. A value of $p < 0.05$ was considered statistically significant.

3. Results

3.1. CD34⁺HSPCs prevent chronic restraint stress-induced lymphocyte reduction

Our previous studies have shown that chronic stress promotes a significant depletion in lymphocyte numbers (Yin et al., 2000; Hu et al., 2014; Li et al., 2011a). Recent studies from us and others have shown that HSPCs can modulate the immune response when exposed to a microenvironment with minimal inflammation (Brudecki et al. 2012; Nagai et al., 2006). We asked whether HSPCs contributes to chronic restraint stress-induced lymphocyte reduction. When mice were adoptive transferred CD34⁺HSPCs (> 90% purity) by intravenous injection immediately prior to restraint stress, splenocyte numbers were largely preserved (Fig. 1A), demonstrating that CD34⁺HSPCs prevent splenocyte reduction induced by chronic stress. Both T lymphocytes and B lymphocytes were attenuated by chronic stress, and these effects were reversed in CD34⁺HSPC-treated mice (Fig. 1B, C). Therefore, HSPCs prevent lymphocyte reduction induced by chronic stress.

3.2. CD34⁺HSPCs prevent splenocyte apoptosis induced by chronic restraint stress

We have shown that the reduction of lymphocytes induced by stress is mediated by apoptotic cell mechanism (Yin et al., 2000; Hu et al., 2014). We evaluated apoptotic cells of splenocytes using flow cytometric analysis. We found that a significant number of splenocytes of stressed mice were undergoing apoptosis, whereas only a few apoptotic cells were detected in the splenocytes of stressed mice with HSPC pretreated (Fig. 2). Thus, HSPCs prevent chronic stress-induced splenocyte apoptosis.

3.3. CD34⁺HSPCs ameliorate synthetic glucocorticoid dexamethasone-induced reduction in splenocyte numbers

We then examined the effect of HSPCs on steroid hormone-induced alteration of splenocyte numbers, BALB/c male mice were administered intraperitoneal injection of the synthetic glucocorticoid dexamethasone (DEX) and intravenous injection of CD34⁺HSPCs or vehicle. We showed that dexamethasone significantly decreased the number of splenocytes (Fig. 3). Interestingly, this dexamethasone-induced splenocyte depletion was ameliorated by CD34⁺HSPCs. These results suggest that the protective effect of HSPCs on chronic restraint stress-induced splenocyte reduction exerts by alleviating the effect of the steroid hormones.

3.4. CD34⁺HSPCs attenuate restraint stress-induced suppression of delayed type hypersensitivity response

We and others have reported that delayed type hypersensitivity (DTH) responses are antigen-specific T cell-mediated immune reactions (Dhabhar and McEwen, 1997; Li et al., 2011b). DTH lesions are not easy to evaluate objectively in the mouse. They are usually evaluated by measurement of foot pad thickness or increased ear thickness after challenge with antigen.

We and others have previously established that chronic restraint stress suppressed the DTH response (Dhabhar and McEwen, 1997; Li et al., 2011b). DTH responses are antigen-specific T cell-mediated immune reactions (Dhabhar and McEwen, 1997; Li et al., 2011b). We then determined the effect CD34⁺HSPCs on DTH response following restraint stress. As shown in Fig. 4, stress dramatically decreased footpad thickness in control mice. Interestingly, restraint stress failed to induce a decrease in footpad thickness in CD34⁺HSPC-treated mice. These data suggest that HSPCs plays an important role in T cell-mediated immunity during stress. However, we do not know the mechanisms by which HSPCs contribute to T cell-mediated immunity following stress.

3.5. Effect of CD34⁺ HSPCs on T cell-mediated immune response following chronic stress

Our previous studies reported that chronic stress induces the alternations of cytokine levels (Yin et al., 1999b; Hu et al., 2013; Hu et al., 2014). We and others have shown that chronic stress induces immune suppression through alterations of the production of Th1 IL-2 and Th2 IL-4. To examine the effect of CD34⁺ HSPCs on chronic stress-mediated cytokines, we first administered stressed or unstressed control mice with or without CD34⁺ HSPCs and then isolated CD4⁺ T cells from spleens. Our studies showed that CD34⁺ HSPCs significantly attenuated chronic stress-induced alterations of Th1 IL-2 and Th2 IL-4 levels (Fig. 5A, B), suggesting HSPCs inhibit chronic stress-induced immune suppression. We next determined the level of Th17 cytokine IL-17, a relatively new cytokine. We found that CD34⁺ HSPCs markedly inhibited stress-enhanced production of IL-17 (Fig. 5). Taken together, these data suggest that CD34⁺ HSPCs block T cell-mediated immune responses induced by chronic stress.

4. Discussion

Psychological and physical stress is a known risk factor for many human diseases, such as autoimmune diseases and cancer (Reiche et al., 2004; Shi et al., 2003; Cao et al., 2007; Hu et al., 2014). Psychological and physical stressors influence cytokine responses (Stone and Bovbjerg, 1994; Wilder, 1995; Hu et al., 2014). Several investigations with different model systems have defined that stress could either enhance or reduce immune functions (Ader and Cohen, 1993; Cao et al., 2007; Hu et al., 2014; Smith et al. 2016). It has been established that is generally believed that acute and moderate stress could enhance immune function, while chronic stress often results in reduction of immune function and increase in the susceptibility to diseases (Dhabhar and McEwen, 1997; Shi et al., 2003, Hu et al., 2014). The immunological consequences of stress by which stress compromises the immune system are critical areas of study. However, the mechanisms associated with deleterious immune responses in stress remain to be defined.

Our previous studies have defined that chronic restraint stress of mice induces lymphocyte reduction (Yin et al., 2000; Zhang et al., 2008b; Zhang et al., 2008c; Hu et al., 2014). HSPCs have great potential for treatment of various diseases, including allergic diseases, and autoimmune diseases (Brudecki et al. 2012; Fischer et al., 2013, Ito et al., 2014). We then asked whether HSPCs played a role in the loss of lymphocytes resulting from chronic restraint stress. We found that HSPCs can actually prevent chronic stress-induced

lymphocyte reduction (Fig. 1). Furthermore, we found that chronic restraint stress of mice induces lymphocyte reduction through a cell apoptotic mechanism (Fig. 2). Additionally, the protective effect of HSPCs on chronic restraint stress-induced lymphocyte reduction exerts by alleviating the effect of the steroid hormones (Fig. 3).

We and others have shown that chronic stress regulates immune responses (Dhabhar and McEwen, 1997; Shi et al., 2003, Zhang et al., 2008b; Hu et al., 2014). However, the mechanisms by which stress affects the immune responses remain to be elucidated. In the current study, we extended these findings to demonstrate that HSPCs reduce chronic restraint stress-induced suppression of delayed type hypersensitivity response (Fig. 4). Importantly, we found that HSPCs significantly attenuated chronic stress-induced alterations of Th1 IL-2 and Th2 IL-4 levels (Fig. 5), suggesting HSPCs inhibit chronic stress-induced immune suppression. Furthermore, we demonstrates HSPCs significantly reduced stress-increased IL-17 production (Fig. 5), suggesting that HSPCs attenuate T cell-mediated immune responses induced by chronic stress.

In summary, to the best of our knowledge, this study is the first report to reveal that HSPCs are capable of preventing chronic restraint stress-induced lymphocyte apoptosis (Figs. 1–2). We also have revealed that T cell-mediated immune responses induced by chronic stress contribute to the protective effect of HSPCs (Figs. 4–5). Nevertheless, the cellular and molecular mechanisms underlying the protective effect of HSPCs need to be further explored. We believe that investigation of the cellular and molecular mechanisms underlying T cell-mediated the protection from chronic stress-induced lymphocyte apoptosis by HSPCs could provide new targets for the development of novel anti-immune dysfunction medications.

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Abbreviations

HSPCs	Hematopoietic stem progenitor cells
DTH	delayed type hypersensitivity
DEX	dexamethasone

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Research highlights

- ▶ Define the effect of HSPCs on chronic stress-induced immune responses.
- ▶ HSPCs prevents lymphocyte apoptosis induced by chronic stress in mice.
- ▶ The protective effect of HSPCs on lymphocyte reduction exerts by steroid hormones.
- ▶ T cell-mediated immune responses contributes to the protective effect of HSPCs.

Figure 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 PBS, > 90% purity, i.v.) or vehicle (heat-killed 1×10^6 CD34⁺HSPCs in 100 μ l PBS, i.v.) immediately before being subjected to 6 h restraint daily for 3 days. **(A)** Splenocyte numbers were counted with a hemocytometer. **(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 $\pm$ SEM. N = 5. * $p < 0.01$.

Figure 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 μ l PBS, > 90% purity, i.v.) or vehicle (heat-killed 1×10^6 CD34⁺HSPCs in 100 μ l 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.

Figure 3.

CD34⁺HSPCs prevent dexamethasone (DEX)-induced splenocyte reduction. BALB/c male mice aged 7–8 weeks were treated with DEX (5 mg/ kg. i.p.) and vehicle (heat-killed 1×10^6 CD34⁺HSPCs in 100 μ l PBS, i.v.) or CD34⁺HSPCs (1×10^6 in 100 μ l PBS, i.v.). At 2 days after injection, splenocyte numbers were determined with a hemocytomete. Data are means $\pm$ SEM. N= 5. * p < 0.01, ** p < 0.01.

Figure 4.

CD34⁺HSPCs attenuate stress-induced suppression of DTH response. BALB/c male mice aged 7 weeks were immunized with OVA (100 µg. s.c.) in complete Freund's adjuvant (CFA) on the first day of stress and follow up with a boost with OVA (20 µg. s.c.) in incomplete Freund's adjuvant (IFA) on the seventh day. The mice were injected with CD34⁺HSPCs (1×10⁶ in 100 µl PBS, > 90% purity, i.v.) or vehicle (heat-killed 1×10⁶ CD34⁺HSPCs in 100 µl PBS, i.v.) immediately before being subjected to a 6-h restraint daily for 3 days. DTH (hind footpad) of all groups were measured on day 10 in a blinded fashion using a calliper meter. Data are means ± SEM. N = 6. **p* < 0.01.

Figure 5.

CD34⁺HSPCs prevent restraint stress-induced T cell-mediated immune response. BALB/c male mice aged 7 weeks were intravenously injected with CD34⁺HSPCs (1×10^6 in 100 μ l PBS, > 90% purity, i.v.) or vehicle (heat-killed 1×10^6 CD34⁺HSPCs in 100 μ l PBS, i.v.) immediately before being subjected to a 6-h restraint. (**A and B**) Splenocytes were isolated and cultured with anti-CD3/anti-CD28 antibodies for 48 h. Levels of IL-2 and IL-4 in the supernatants was determined by ELISA. (**C**) Splenic CD4⁺ T cells were isolated and cultured with anti-CD3/anti-CD28 antibodies for 48 h. IL-17 production in the supernatants was determined by ELISA. Data are means $\pm$ SEM. N = 3. * p < 0.01.