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Mesenchymal stem cells prevent restraint stress-induced lymphocyte depletion via interleukin-4

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ABSTRACT

Chronic stress has dramatic impacts on the immune system and consequently contributes to the onset and progression of a variety of diseases, including cancer, immune disorders, and infections. Recent studies in animals and humans have demonstrated that mesenchymal stem cells (MSCs) significantly modulate the immune system. Here we show that administration of MSCs *in vivo* prevents lymphocyte depletion induced by physical restraint stress (12:12-h stress–rest, 2 repetitions) in mice. This effect was found to be exerted not through modulation of glucocorticoid levels in the circulation, but rather through direct effects on lymphocyte apoptosis. By testing various possible protective mechanisms, we found that IL-4 provides a strong anti-apoptosis signal to lymphocytes in the presence of dexamethasone. When neutralizing antibody against IL-4 was co-administered with MSCs to restraint-stressed mice, the protective effect of MSCs was diminished. Furthermore, in mice deficient in STAT6, a key molecule in IL-4 receptor-mediated signaling, MSCs had no effect on restraint stress-induced lymphocyte depletion. Additionally, MSCs administered to stressed mice promoted IL-4 production by splenocytes. This study reveals that MSCs can effectively prevent stress-induced lymphocyte apoptosis in an IL-4-dependent manner and provides novel information for the development of countermeasures against the deleterious effects of stress on the immune system.

1. Introduction

Epidemiological studies and empirical evidence have long suggested that stress takes a toll on human health (Chrousos, 2009; Segerstrom and Miller, 2004). On one hand, stress has a direct role in promoting disease progression. Stress has been shown to down-regulate p53 protein expression via glucocorticoid production and thereby contributes to tumorigenesis (Feng et al., 2012). On the other hand, a great deal of attention has been focused on the indirect effects of stress, particularly on the immune system. Modulation of immune responses can critically allow the onset and progression of a broad range of diseases, such as viral infections, wound healing, and cancer (Glaser and Kiecolt-Glaser, 2005). Our understanding of the contribution of stress to the disease pathogenesis has advanced significantly only in the last few years. Potential countermeasures against stress-induced immunosuppression still largely await development. Given its effects on the onset and progression of various diseases (Chrousos, 2009), a detailed understanding of the molecular mechanisms underlying stress-induced changes in the immune system is critically important.

Mesenchymal stem cells (MSCs) are pluripotent adult stem cells that can be readily isolated and expanded from a number of tissues, including bone marrow, fat tissue, Wharton's jelly of the umbilical cord, and amniotic fluid (Shi et al., 2012). These cells have been shown to effectively treat various diseases, particularly those involving dysregulated immune responses, such as multiple sclerosis, graft-versus-host disease, type I diabetes, rheumatoid arthritis, and lupus (Uccelli et al., 2008). We have reported that the therapeutic effects of MSCs are largely exerted through modulation of the immune system. We have shown that upon exposure to various pro-

Abbreviations: MSCs, mesenchymal stem cells; IL-4, interleukin-4; DEX, dexamethasone; spl, splenocyte; IFN-γ, interferon-gamma; TNF-α, tumor necrosis factor-alpha; IL-1α, interleukin-1-alpha; IL-1β, interleukin-1-beta; ELISA, enzyme-linked immunosorbent assay.

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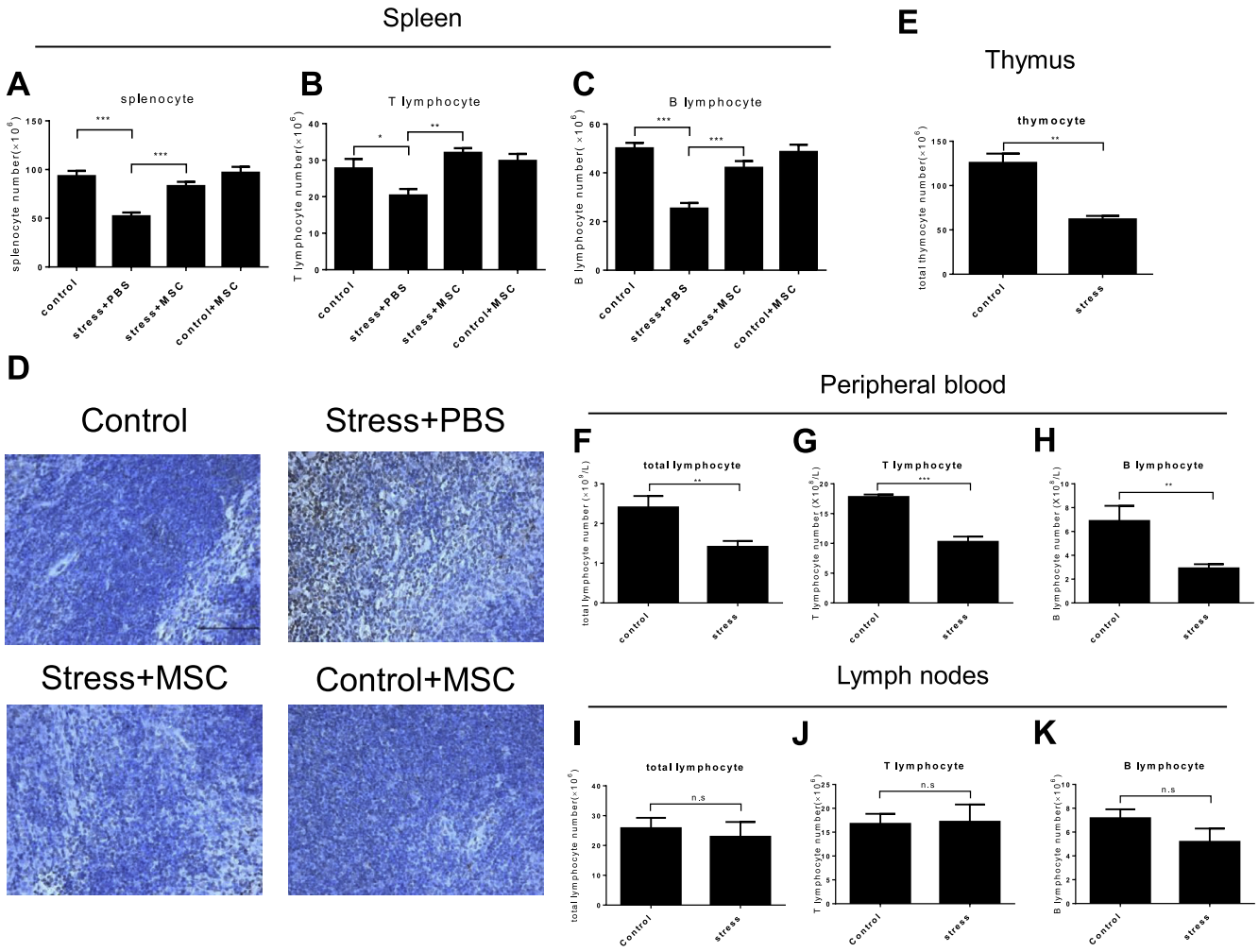

Fig. 1. MSCs prevent restraint stress-induced lymphocyte depletion. BALB/c male mice (8–10 weeks old) were intravenously injected with MSCs (1×10^6) in 200 μl PBS or vehicle alone immediately before being subjected to restraint stress. (A) Total cells in each spleen were counted. (B and C) Numbers of T lymphocytes and B lymphocytes were determined by multiplying total splenocyte number, by respective percentage determined by flow cytometry. (D) *In vivo* apoptosis of splenocytes was evaluated by TUNEL staining after of the 2-day stress regime. Scale bar: 115 μm. (E) Total cell numbers in each thymus was determined. (F–H) Total lymphocytes, T lymphocytes, and B lymphocytes in peripheral blood were determined as in (A–C). (I–K) Total lymphocytes, T lymphocytes and B lymphocytes in lymph nodes were determined as in (A–C). Data are presented as mean ± SEM; $n = 4$. Treatment groups were compared for statistical significance by one way analysis of variance (ANOVA) followed by Newman–Keuls post-hoc test: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

inflammatory cytokine combinations, specifically IFN-γ plus TNF-α, IFN-γ plus IL-1α, or IFN-γ plus IL-1β, MSCs produce nitric oxide and chemokines whose concerted action results in immunosuppression (Ren et al., 2008). Intriguingly, our most recent studies found that MSCs can also actually enhance the immune response when exposed to a microenvironment with minimal inflammation (Li et al., 2012). Therefore, depending on the severity of an inflammatory response, MSCs may modulate the immune response to an appropriate level, a concept that prompted us to investigate whether MSCs could prevent stress-induced immunosuppression.

In the present study, we employed a mouse model of restraint stress, one that has been widely used to examine the impact of stress on the immune system (Wang et al., 2007; Yin et al., 2000). We found that *in vivo* administration of MSCs prevented lymphocyte depletion in mice subjected to restraint stress. By using neutralizing antibodies and genetically-modified mice, we found that protection by MSCs is dependent on interleukin-4 (IL-4). Additionally, when administered to restraint-stressed mice, MSCs promoted the production of IL-4 from splenic cells. Therefore, our study reveals a novel potential countermeasure against stress-induced lymphocyte depletion.

2. Materials and methods

2.1. Mice

Male BALB/c mice, 8–10 weeks of age, were purchased from the Shanghai Laboratory Animal Center of Chinese Academy of Sciences (Shanghai, China). Stat6-deficient mice and T-bet-deficient mice were from Jackson Laboratory (Bar Harbor, ME, USA). All mice were housed in the animal facility of Shanghai Jiao Tong University School of Medicine, utilizing a 12 h/12 h light-dark cycle. Four mice were housed per cage. All experimental procedures were in accordance with guidelines of the Institutional Animal Care and Use Committee of the Institute of Health Sciences, Shanghai Institutes for Biological Sciences of the Chinese Academy of Sciences.

2.2. Reagents

Recombinant mouse interleukin-4, interleukin-6, interleukin-7, and interleukin-15 were from R&D systems (Minneapolis, MN, USA). Neutralizing antibody against interleukin-4 was produced by Harlan laboratory (Indianapolis, IN, USA). Dexamethasone and