17β-estradiol contributes to the accumulation of myeloid-derived suppressor cells in blood by promoting TNF-α secretion

Guanjun Dong, Ming You, Hongye Fan, Jianjian Ji, Liang Ding, Pengfei Li, and Yayi Hou

Abstract

Estrogens are strongly implicated in gender differences in immune responses by influencing the development and activation of immune cells. Recent studies have shown that myeloid-derived suppressor cells (MDSCs), derived from CD11b+Gr-1+ myeloid cells under pathological conditions, play vital roles in modulating immune responses. However, it is still unknown the effects of estrogens on MDSCs. In the present study, we investigated the effects and mechanisms of estrogens on regulating the accumulation of MDSCs. It was found that, compared with male patients with systemic lupus erythematosus (SLE), female patients with SLE showed a higher frequency of MDSCs in peripheral blood mononuclear cells and a higher level of tumor necrosis factor α (TNF-α) in serum. Notably, estradiol level in the serum of female patients with SLE was positively correlated with the frequency of MDSCs. Moreover, 17β-estradiol could promote TNF-α-induced accumulation of MDSCs in vivo by increasing the fundamental frequency of CD11b+Gr-1+ cells. Furthermore, 17β-estradiol promoted the secretion of TNF-α in vivo, which contributed to the increase of the frequency of CD11b+Gr-1+ cells. In addition, it was also found that female mice showed a higher frequency of CD11b+Gr-1+ cells and a higher TNF-α level in blood than the age-matched male mice. These data indicate that 17β-estradiol contributes to the accumulation of MDSCs in blood by promoting TNF-α secretion, which increases the fundamental frequency of CD11b+Gr-1+ cells. Our findings provide a new insight into the mechanism of gender difference in the prevalence of inflammation and autoimmune diseases.

Key words: gender difference, estrogen, myeloid-derived suppressor cells, CD11b+Gr-1+ cells, tumor necrosis factor α

Introduction

Gender differences are universal in health and in the progression of disease, as well as in medical and pharmacological research. Clinical and experimental researches have demonstrated naturally occurring gender differences in immune responses [1,2]. Interestingly, our and others’ studies have shown that cells of males and females display several different features and behaviors [3–5]. For example, women had higher levels of CD4+ and CD8+ T cell activation and inflammation-associated gene expression [5]. Lymphocyte subset enumeration revealed higher B cells in females [6]. Although it has been
well known that immune responses are sexually dimorphic [7], the mechanisms responsible for the relationship of gender difference in immunity remain poorly understood.

Multiple studies have demonstrated significant gender differences in the prevalence of inflammatory and autoimmune diseases [8–10]. For example, systemic lupus erythematosus (SLE), an autoimmune disease characterized by the production of unusual antibodies and abnormal activation of B and T cells, develops at a female-to-male ratio of 9:1 [11]. Now, it has been well accepted that estrogens are strongly implicated in the gender differences in the prevalence of inflammatory and autoimmune diseases by mediating the development and activation of immune cells, such as B cells, T cells, and dendritic cells [12–14].

Myeloid-derived suppressor cells (MDSCs) are an abnormal accumulation of different stages of myeloid progenitor cells and immature myeloid cells (IMCs) under some pathological conditions, such as cancer, inflammatory disorders, and infections [15–17]. It can broadly be characterized as HLA-DR−CD11b+CD14+CD33+ in humans [18,19] and CD11b+Gr-1+ in mice [20,21]. In healthy mice, CD11b+Gr-1+ cells are composed of IMCs at the early stages of differentiation, including macrophages, granulocytes, and dendritic cells [22,23]. In pathological conditions, a partial block in the differentiation of IMCs into mature myeloid cells results in the expansion of this population. Importantly, these cells are collectively known as MDSCs because these activated cells possess immune suppressive activity [22]. Accumulating studies have shown that MDSCs possess broad and potent immune suppressive capacity by inhibiting both adaptive and innate immunity in a context-dependent manner [24,25]. The protective role of MDSCs against inflammation and autoimmunity has been widely observed in various pathophysiological conditions [26–28]. However, several recent studies have shown that MDSCs play a proinflammatory role in the pathogenesis of experimental allergic encephalitis (EAE) and rheumatoid arthritis (RA) by promoting Th17 cells differentiation [29–31], suggesting that the roles of MDSCs in inflammatory and autoimmune diseases remain controversial.

A recent study has shown that male (NZB × NZW) F1 mice harbor elevated levels of Gr-1abhCD11b+ MDSCs in spleen and bone marrow when compared with age-matched female (NZB × NZW) F1 mice [32]. Interestingly, the elevated level of Gr-1abhCD11b+ MDSCs in male mice was due to the male sex hormones, indicating that sex hormones are involved in the regulation of MDSC accumulation. However, there is no report about the effects of estrogens on MDSC accumulation.

Tumor necrosis factor-alpha (TNF-α) is a key inflammatory cytokine involved in the pathogenesis of SLE [33,34]. Much evidence has demonstrated that TNF-α can drive the pathological conditions of MDSCs [35,36]. Given that estrogens can alter the transcription of inflammatory genes by inhibiting NF-κB activity and recruiting steroid receptor cofactors that act as transcriptional repressors [37–39], we supposed that TNF-α may act as a link between estrogens and the accumulation of MDSCs.

To test our hypothesis, we investigated the possible involvement of estrogens in regulating the accumulation of MDSCs. Here, we reported for the first time that female patients with SLE showed a higher frequency of MDSCs in peripheral blood mononuclear cells (PBMCs) than male patients. Intriguingly, 17β-estradiol could promote TNF-α-induced accumulation of MDSCs in vivo by increasing the fundamental frequency of CD11b+Gr-1+ cells. Furthermore, 17β-estradiol could promote the secretion of TNF-α in vivo, which can increase the fundamental frequency of CD11b+Gr-1+ cells. Our results demonstrate a critical role of 17β-estradiol in regulating the accumulation of MDSCs. Our data provide a new insight into the pathogenic mechanism of estrogens in the pathogenesis of inflammation and autoimmune diseases.

**Materials and Methods**

**Patients and healthy donors’ information**

The study protocol was approved by the Research Ethics Committee of Nanjing University (Nanjing, China). After obtaining written informed consent from each SLE patient and healthy donor, peripheral blood samples were obtained from SLE patients and healthy donors. All SLE patients were diagnosed according to the criteria set out by American College of Rheumatology revised criteria in 1997. Whole bloods of healthy subjects with the age of 28 ± 6 years and SLE patients with the age of 28 ± 8 years were recruited. All patients were on treatment, using standard protocols incorporating glucocorticoids and immunosuppressive agents (e.g. cyclophosphamide and azathioprine). Although patients were on a variety of disease-modifying agents, patients on high-dose immunosuppressive therapies or steroids were excluded from the study. Patients with overlap syndrome were also excluded from the study. Disease activity was evaluated using the SLE Disease Activity Index (SLEDAI) and a cutoff of ≥8 was used to define active disease.

**Isolation of human PBMCs**

Human PBMCs were separated from plasma by Ficoll centrifugation (Lymphoprep; Nycomed, Oslo, Norway) according to the standard procedures.

**Animals**

Female and male C57BL/6 mice, 6–8 weeks old, were obtained from Model Animal Research Center at Nanjing University. Female C57BL/6 mice, ovariectomized at 4 weeks old, were also obtained from Model Animal Research Center at Nanjing University. All mice were maintained under specific pathogen-free conditions. All experiments were conducted in accordance with institutional guidelines for animal care and used based on the guide for the Animal Care Committee at Nanjing University.

**Generation of mouse MDSCs in vitro**

Bone marrow cells were planted into dishes using RPMI 1640 medium (Gibco, Carlsbad, USA) supplemented with 2 mM L-glutamine, 10 mM HEPES, 20 mM 2-ME, 150 U/ml streptomycin, 200 U/ml penicillin, and 10% fetal bovine serum (FBS), and stimulated with combinations of GM-CSF (40 ng/ml) and IL-6 (40 ng/ml). The cultures were maintained at 37°C in 5% CO2-humidified atmosphere in 24-well plates. Medium was changed on the third day. The proportion of CD11b+Gr-1+ MDSCs was analyzed by flow cytometry on the sixth day.

**Mouse ovariectomy and 17β-estradiol exposure**

Thirty-six C57BL/6 female mice (4 weeks old) were randomly divided into three groups: sham mice (n = 13), ovariectomy (OVX) + placebo mice (n = 13), and OVX + 17β-estradiol mice (n = 13). Mice were surgically ovariectomized or sham-operated (n = 13) after anesthesia using sodium pentobarbital. All animals were well owed to recover for 2 weeks and then received daily s.c. injections of 17β-estradiol (100 µg/kg/day; Sigma, St Louis, USA) or phosphate-buffered saline.
(PBS) every morning for 4 weeks. Mice were euthanized at 24 h after receiving their last injection.

**TNF-α or TNF-α antagonist etanercept treatment**

The TNF-α (0.01 mg/dose; PeproTech, Rocky Hill, USA) or TNF-α antagonist etanercept (0.5 mg/dose; Wyeth, New York, USA) was administered daily by systemic i.p. injection for 7 days before cell harvest. Control mice were injected with PBS.

**Flow cytometry assay**

For phenotype staining, cells were washed twice with PBS containing 1% FBS and 0.1% NaN₃. Then, cells were incubated for 30 min at 4°C with anti-human CD14-PE-Cy5.5 antibody, anti-human CD33-PE antibody, anti-human CD11b-APC antibody, anti-mouse Ly6G-PE antibody, or anti-mouse CD11b-APC antibody (eBioscience, San Diego, USA) according to the standard procedure. After being washed twice with PBS, the cells were analyzed by FACS Calibur (Becton Dickinson, Pasadena, USA). An isotype control was used for each antibody. For the analysis of cell apoptosis, cells were washed twice with PBS containing 1% FBS and 0.1% NaN₃, and then re-suspended in 1X binding buffer (0.01 M Hepes/NaOH, pH 7.4, containing 0.14 M NaCl, and 2.5 mM CaCl₂). The cells were incubated for 3 min at room temperature in the dark with Annexin V and subsequently incubated with PI for 10 min at room temperature in the dark. Then, the cells were analyzed by FACS Calibur (Becton Dickinson). Data analysis was performed using the FlowJo (TreeStar, San Carlos, USA).

**Quantitative real-time PCR analysis**

Total RNA was extracted using Trizol (Invitrogen, Carlsbad, USA) according to the manufacturer’s instructions. Quantitative real-time PCR (qPCR) assays of mRNA were carried out on a StepOne Plus real-time PCR system or ABI Vii 7 detection system (Applied Biosystems, Foster City, USA) using SYBR Green PCR Master Mix. The reagents and procedures were performed as described by the manufacturer. Total RNA was extracted using Trizol (Invitrogen, Carlsbad, USA) according to the standard procedure. After being washed twice with PBS, the cells were analyzed by FACS Calibur (Becton Dickinson). An isotype control was used for each antibody. For the analysis of cell apoptosis, cells were washed twice with PBS containing 1% FBS and 0.1% NaN₃, and then re-suspended in 1X binding buffer (0.01 M Hepes/NaOH, pH 7.4, containing 0.14 M NaCl, and 2.5 mM CaCl₂). The cells were incubated for 3 min at room temperature in the dark with Annexin V and subsequently incubated with PI for 10 min at room temperature in the dark. Then, the cells were analyzed by FACS Calibur (Becton Dickinson). Data analysis was performed using the FlowJo (TreeStar, San Carlos, USA).

**Table 1. Primer sequences used in the qPCR**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer (5′–3′)</th>
<th>Reverse primer (5′–3′)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HUMAN-TNF-α</td>
<td>CCTCTCTCTAATTCCACCGCTCTTG</td>
<td>GAGGACCTGGGAGTAGATGAG</td>
</tr>
<tr>
<td>HUMAN-GAPDH</td>
<td>AGAAGGCTCTGGGTCATTG</td>
<td>AGGGGCCATTCCAGACGCTTC</td>
</tr>
<tr>
<td>MOUSE-TNF-α</td>
<td>TCGAGTGACAAGGCTGCAGC</td>
<td>CTACGAGATCTCCGAGACGTC</td>
</tr>
<tr>
<td>MOUSE-GAPDH</td>
<td>AACGACCCCTTCATGAGC</td>
<td>TCCAGGACATATTCAGCAC</td>
</tr>
</tbody>
</table>
with SLE (0.93% ± 0.077% vs. 0.55% ± 0.07%, P < 0.05). Moreover, the percentage of MDSCs in PBMCs was positively correlated with estradiol level in serum from female patients with SLE (Fig. 1C) as well as the SLEDAI (Fig. 1D). These results demonstrate that female patients with SLE show a higher level of accumulation of MDSCs in blood than male patients with SLE.

Estrogen and TNF-α are related to the gender difference in accumulation of MDSCs in SLE

As TNF-α has been shown to play a critical role in driving the accumulation of MDSCs [35,36], we analyzed TNF-α levels in serum from patients with SLE and healthy donors. As shown in Fig. 2A, the levels of TNF-α in serum of female and male patients were 77.48 ± 7.23 pg/ml and 49.6 ± 4.47 pg/ml, respectively. The levels of TNF-α in serum of female and male healthy donors were 22.68 ± 2.25 pg/ml and 17.72 ± 1.56 pg/ml, respectively. These data indicated that the level of TNF-α was significantly higher in serum from patients with SLE than that in healthy donors. Intriguingly, female patients with SLE showed a significantly higher level of TNF-α in serum than male patients with SLE (77.48 ± 7.23 pg/ml vs. 49.6 ± 4.47 pg/ml, P < 0.05). Moreover, the level of TNF-α in serum from female patients with SLE was positively correlated with the percentage of MDSCs in PBMCs from female patients with SLE (Fig. 2B), as well as estradiol level in serum (Fig. 2C). All these results demonstrate that estradiol and TNF-α are related to the gender difference in the accumulation of MDSCs in PBMCs from patients with SLE.

17β-estradiol augments TNF-α-induced accumulation of MDSCs in mice

To further investigate whether estrogens can promote the accumulation of MDSCs, in vivo study was conducted in mice. Ovariectomy was performed on 4-week-old female mice. After recovered for 1 week, these ovariectomized mice were treated with 17β-estradiol or placebo for 2 weeks prior to treatment with TNF-α for 7 days. As shown in Fig. 3A,B, TNF-α could significantly induce the accumulation of MDSCs in blood in 17β-estradiol-treated ovariectomized mice (12.74% ± 1.45%), placebo-treated ovariectomized mice (5.48% ± 0.87%), and age-matched male mice (6.03% ± 1.52%). Interestingly, TNF-α induced a higher frequency of MDSCs in 17β-estradiol-treated ovariectomized mice than those in placebo-treated ovariectomized mice as well as age-matched male mice. These data indicate that 17β-estradiol augments the TNF-α-induced accumulation of MDSCs in blood in vivo.

In addition, we also investigated the effects of 17β-estradiol and TNF-α on proliferation and apoptosis of MDSCs. Murine bone marrow-derived MDSCs were stimulated with different concentrations of 17β-estradiol (1, 10, and 100 nM) or TNF-α (1, 10, and 100 ng/ml) for 48 h. As shown in Fig. 3C,D, 17β-estradiol and TNF-α had no effect on the proliferation of MDSCs. Although 17β-estradiol had no effect on apoptosis of MDSCs, TNF-α could significantly inhibit the apoptosis of MDSCs (Fig. 3E,F).

17β-estradiol increases the fundamental frequency of CD11b+Gr-1+ cells in vivo

We further investigated how 17β-estradiol augments TNF-α-induced accumulation of MDSCs in mice. Strikingly, we found an interesting phenomenon from the results of Fig. 3A,B that 17β-estradiol-treated ovariectomized mice showed a higher fundamental frequency of CD11b+Gr-1+ cells in PBMCs than placebo-treated ovariectomized mice and age-matched male mice. To further confirm it, ovariectomy or sham-operation was performed on 4-week-old female mice. The ovariectomized mice were treated with placebo (n = 13) or 17β-estradiol (n = 13). Two weeks later, these mice were euthanized, and the percentages of CD11b+Gr-1+ cells in PBMCs, spleen, and bone marrow were analyzed. Strikingly, ovariectomized mice showed a lower frequency of CD11b+Gr-1+ cells (1.52% ± 0.14%) in PBMCs.
than sham female mice (3.38% ± 0.16%), whereas treatment with 17β-estradiol could elevate the frequency of CD11b+Gr-1+ cells in PBMCs from 17β-estradiol-treated ovariectomized mice (3.25% ± 0.14%) (Fig. 4A,B). Interestingly, the frequencies of CD11b+Gr-1+ cells in the spleen of 17β-estradiol-treated ovariectomized mice, placebo-treated ovariectomized mice, and age-matched male mice were 2.4% ± 0.11%, 2.15% ± 0.13%, and 2.25% ± 0.09%, respectively (Fig. 4C,D). The frequencies of CD11b+Gr1+ cells in bone marrow of 17β-estradiol-treated ovariectomized mice, placebo-treated ovariectomized mice, and age-matched male mice were 47.73% ± 0.59%, 47.94% ± 0.71%, and 48.27% ± 0.8%, respectively (Fig. 4E,F). OVX and treatment with 17β-estradiol have no
effect on the frequencies of CD11b+Gr-1+ cells in spleen and bone marrow. These data demonstrate that 17β-estradiol contributes to the increase of the frequency of CD11b+Gr-1+ cells in blood.

Gender difference in the fundamental frequency of CD11b+Gr-1+ cells in mice

Then, we investigated the fundamental frequency of CD11b+Gr-1+ cells in PBMCs, spleen, and bone marrow from female mice and age-matched male mice. As shown in Fig. 5A,B, the percentage of CD11b+Gr-1+ cells was significantly higher in PBMCs from female mice than in PBMCs from age-matched male mice (2.97% ± 0.23% vs. 2.13% ± 0.13%, P < 0.01). Interestingly, female mice showed significantly reduced percentages of CD11b+Gr-1+ cells in spleen (2.2% ± 0.11%) (Fig. 5C,D) and bone marrow (46.42% ± 1.21%) (Fig. 5E,F) compared with age-matched male mice (3.55% ± 0.29% and 56.77% ± 0.83%, respectively). These data demonstrate a remarkable gender difference in the frequency of CD11b+Gr-1+ cells in PBMCs, spleen, and bone marrow from mice.

17β-estradiol increases the frequency of CD11b+Gr-1+ cells in blood by promoting TNF-α secretion

TNF-α is a proinflammatory, multifunctional, and immunomodulating cytokine, which has been shown to drive MDSC accumulation [35,36]. To investigate whether TNF-α can affect the frequency of CD11b+Gr-1+ cells in blood from mice, in vivo study was conducted. TNF-α antagonist etanercept or PBS was injected into sham female mice, 17β-estradiol-treated OVX mice, placebo-treated OVX mice, and age-matched male mice for a week. Then, the frequencies of CD11b+Gr-1+ cells in PBMCs from these mice were detected. As shown in Fig. 6A and Supplementary Fig. S1, etanercept could significantly reduce the frequency of CD11b+Gr-1+ cells in PBMCs in sham female mice (from 3.06% ± 0.17% to 1.13% ± 0.19%, P < 0.001), placebo-treated OVX mice (from 1.72% ± 0.24% to 0.88% ± 0.22%, P < 0.05), 17β-estradiol-treated OVX mice (from 3.13% ± 0.41% to 1.1% ± 0.15%, P < 0.001), and age-matched male mice (from 1.79% ± 0.27% to 0.89% ± 0.25%, P < 0.05). Intriguingly, 17β-estradiol-treated OVX mice showed a higher frequency of CD11b+Gr-1+ cells than placebo-treated OVX mice, whereas etanercept could eliminate 17β-estradiol-induced increase of the frequency of CD11b+Gr1+ cells. These data suggest that TNF-α plays a critical role in 17β-estradiol-induced increase of the frequency of CD11b+Gr1+ cells in blood in vivo.

Then, we detected the influence of 17β-estradiol on TNF-α secretion. As shown in Fig. 6B, TNF-α level in serum from female mice was higher than that in age-matched male mice (34.63 ± 1.67 pg/ml vs. 27.53 ± 1.16 pg/ml, P < 0.01). Moreover, the mRNA level of TNF-α in PBMCs from female mice was also higher than that in age-matched
male mice ($P<0.01$) (Fig. 6C). Notably, the level of TNF-α in serum was positively correlated with the percentage of CD11b$^+$Gr-1$^+$ cells in PBMCs from female and age-matched male mice (Fig. 6D,E).

We further analyzed the levels of TNF-α in serum and PBMCs from sham female mice, placebo-treated ovariectomized mice, and 17β-estradiol-treated ovariectomized mice. As shown in Fig. 6F, placebo-treated ovariectomized mice showed reduced TNF-α level in serum (25.48 ± 1.24 pg/ml) compared with sham female mice (36.26 ± 1.23 pg/ml), and 17β-estradiol treatment could elevate the TNF-α level in serum from 17β-estradiol-treated ovariectomized mice (34.82 ± 0.85 pg/ml). Moreover, placebo-treated ovariectomized mice showed reduced mRNA level of TNF-α in PBMCs compared with sham female mice, and hormone reconstitution could elevate the mRNA level of TNF-α in serum from 17β-estradiol-treated ovariectomized mice (Fig. 6G). Notably, the level of TNF-α in serum was positively correlated with the percentage of CD11b$^+$Gr-1$^+$ cells in PBMCs from placebo-treated and 17β-estradiol-treated ovariectomized mice (Fig. 6H,I).

In addition, we also investigated the levels of TNF-α in serum and PBMCs from pre-menopausal women, age-matched men, and post-menopausal women. As shown in Fig. 6J, the level of TNF-α in serum from pre-menopausal women (18.16 ± 1.42 pg/ml) was higher than those from age-matched men (10.94 ± 0.92 pg/ml) and post-menopausal women (11.89 ± 0.94 pg/ml). Moreover, the mRNA level of TNF-α in PBMCs from pre-menopausal women was also higher than those from age-matched men as well as post-menopausal women (Fig. 6K). Altogether, these data indicate that 17β-estradiol contributes to accumulation of MDSCs in PBMCs by promoting the secretion of TNF-α.

**Discussion**

SLE predominantly affects female population. It has been known that the estrogen has a promoting function in the pathogenesis of SLE [40]. However, the mechanism by which estrogen exert pathogenic effects on the immune system is still not well understood. In the present study, we investigated the effects of estrogen on MDSC accumulation. Manipulation of estrogen levels through ovariectomy significantly influenced TNF-α-induced accumulation of MDSCs in vivo, suggesting that estrogen may exert pathogenic effects on the pathogenesis of autoimmune diseases through influencing the accumulation of MDSCs. Our findings provide a new insight into the...
pathogenic function of estrogens in the pathogenesis of inflammation and autoimmune diseases.

Although accumulation of MDSCs has also been reported in many inflammatory conditions [26–28], it remains unknown whether accumulation of MDSCs occurs in patients with SLE. Here, we reported for the first time that the frequency of MDSCs was significantly higher in PBMCs from patients with SLE than that from healthy donors. Strikingly, female patients with SLE showed a higher frequency of MDSCs in PBMCs than male patients. Although transplantation of MDSCs can improve the condition of several autoimmune diseases in animal models, several recently published studies have shown that MDSCs play a proinflammatory role in the pathogenesis of EAE and RA by promoting T helper 17 cell differentiation [29–31]. Notably, CD11b+Gr-1+ MDSCs are a complex mixture of myeloid phenotypes. Moreover, MDSCs morphologically and functionally differ in various tissues under different inflammatory conditions [9]. Even within the same inflammatory process, phenotypic differences in MDSCs occur over time [10]. Taken together, the phenotypes within MDSCs change in the process of inflammatory progresses [10]. This might account for their diverse immunological activities under different inflammatory conditions. Therefore, more studies are needed to assess the role of MDSCs in the pathogenesis of autoimmune diseases.

Notably, we found that 17β-estradiol promotes TNF-α-induced accumulation of MDSCs in mice. As is known, the severity and incidence of innate immune conditions, such as sepsis and post-surgery infections, are profoundly less in women than in age-matched men [40,41]. Given that MDSCs play a vital role in regulating immune responses and pathogenesis of many inflammatory and autoimmune diseases [15–17], we propose that estrogen may participate in the sex-based difference in the pathogenesis of infectious diseases through promoting accumulation of MDSCs under inflammatory conditions. Thus, more studies are needed to reveal the role of MDSCs in the sex-based difference in the pathogenesis of infectious diseases.

TNF-α is implicated in the pathogenesis and development of autoimmune diseases [33,34,42]. Several FDA-approved TNF-α antagonists have been used for the treatment of various chronic pathologies, such as RA, psoriasis, type II diabetes, Crohn’s disease, and cancer [43]. Notably, etanercept, one of these antagonists, has
been shown to be effective in treating patients with several diseases [44,45]. However, the mechanism by which TNF-α and its corresponding antagonists regulate the host’s immune system remains unclear. Interestingly, it was found that etanercept could significantly reduce the fundamental frequency of CD11b+Gr-1- cells in PBMCs from C57BL/6 mice, suggesting that etanercept might relieve disease conditions of autoimmune diseases through reducing the fundamental frequency of CD11b+Gr-1- cells in vivo.

In addition, we investigated the effect of 17β-estradiol and TNF-α on the proliferation and apoptosis of MDSCs. Murine bone marrow-derived MDSCs were stimulated with different concentrations of 17β-estradiol or TNF-α for 48 h. As shown in Fig. 3C,D, 17β-estradiol and TNF-α had no effect on the proliferation of MDSCs. Although 17β-estradiol had no effect on apoptosis of MDSCs, TNF-α could significantly inhibit the apoptosis of MDSCs. Given that TNF-α plays a critical role in driving the accumulation of MDSCs [33,36] and enhanced apoptosis is responsible for reduced MDSC numbers in TNFR-/- mice [36], more studies are needed to reveal the mechanism through which TNF-α inhibits apoptosis of MDSCs.

Although it is well accepted that estrogens contribute to the pathogenesis of SLE, the target of regulation and the specific mechanism of immune response mediated by estrogens remain unidentified. Notably, we found that 17β-estradiol could increase the frequency of CD11b+Gr-1- cells in PBMCs from normal mice. We also found that the percentages of CD11b+Gr-1- cells were significantly lower in spleen and bone marrow from female mice than that in age-matched male mice. These data suggest that sex hormones may have different function on immune cells in different tissues and organs.

Previous studies have shown that estrogens can alter the transcription of inflammatory genes [37–39]. More importantly, treatment with estrogens increases the frequency and numbers of TNF-α-producing CD8+ T cells in mice [46]. Consistently, we found that estrogens could promote the secretion of TNF-α in vivo. As is known, TNF-α is a key inflammatory cytokine involved in the pathogenesis of SLE [33,34] and estrogens contribute to the pathogenesis of SLE [12–14]. Thus, estrogens may contribute to the pathogenesis of SLE through promoting the secretion of TNF-α. More studies are needed to reveal the mechanism through which estrogens affect TNF-α secretion.

In summary, we demonstrate for the first time that 17β-estradiol promotes the secretion of TNF-α in vivo and contributes the accumulation of MDSCs in blood. This study may help us to understand the role of estrogens in the progression and development of inflammatory and autoimmune diseases. Further investigation into the related signaling pathways and potential mechanisms underlying the effects of estrogens are required to fully understand the pathogenic function of estrogens.

Supplementary Data
Supplementary data is available at ABBS online.

Funding
This work was supported by the grants from the National Natural Science Foundation of China (No. 31370899) and the Ministry of Science and China Postdoctoral Science Foundation (No. 2013M531331).

References


