Original Article

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Effects of Bu-Shen-Ning-Xin Decoction on immune cells of the spleen and bone marrow in ovariectomized mice

Xuemin Qiu[1,2,3,4], Yuyan Gui[1,2,3,4], Na Zhang[1,2,3,4], Yingping Xu[1,2,3,4], Dajin Li[1,2,3,4,*], Ling Wang[1,2,3,4,*]

[1] *Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China;*
[2] *Shanghai Key Laboratory of Female Reproductive Endocrine Related Diseases, Shanghai, China;*
[3] *Laboratory for Reproductive Immunology, Hospital & Institute of Obstetrics and Gynecology, IBS, Fudan University Shanghai Medical College, Shanghai, China;*
[4] *The Academy of Integrative Medicine of Fudan University, Shanghai, China.*

Summary **Osteoimmunology is a new discipline that focuses on the interaction between the bones and the immune system. Immune cells play an important role in bone metabolism. The aim of this study was to illustrate the effect of Bu-Shen-Ning-Xin Decoction (BSNXD) on lymphocytes in the spleen and bone marrow to explore the potential role on the bone. C57BL/6 mice were divided into four groups: sham, ovariectomized (OVX), OVX+BSNXD, and OVX+ estrogen. The sham and OVX groups were treated with saline, the OVX+BSNXD group was treated with BSNXD, and the OVX+ estrogen group was treated with estrogen. After mice were sacrificed, the spleens and bones were collected, and the lymphocytes in the spleen and bone marrow were analyzed. We found that BSNXD lessened the extent of the increase of $CD4^+$ and $CD8^+$ T cells by ovariectomy. BSNXD increased the numbers of CTLA-4^+ regulatory T cells (Tregs), but had no effect on $Foxp3^+$ Tregs, which is a different finding than the OVX+ estrogen group. BSNXD decreased the proportion of $CD19^+$ and $B220^+$ B cells in the spleen and bone marrow. In contrast, these numbers were both increased in the OVX group. BSNXD had no influence on the percentage of γδ T cells. However, it increased the proportion of NK cells in the spleen and bone marrow. BSNXD lessened the extent of the increase of monocytes by ovariectomy. *In vitro* experiment, we found Tregs can decrease osteoclastogenesis when co-cultured with osteoclast precursor cells. This study suggests that BSNXD changes the immune environment and immune cells have a role in bone metabolism in OVX mice.**

Keywords: Traditional Chinese medicine, ovariectomy, T cells, regulatory T cells

1. Introduction

Postmenopausal osteoporosis (PMO) occurs after 5-7 years of menopause. Estrogen deficiency is the primary reason for the rapid and sustained increase in the rate of bone loss. Estrogen replacement therapy (ERT) is a widely used therapy to treat PMO. Many studies have suggested that estrogen can act directly on bone cells, lymphocytes, and may influence bone metabolism through immunoregulatory and anti-inflammatory activities (*1-6*). In our previous study, we found estrogen enhances the function of $CD4^+CD25^+Foxp3^+$ regulatory T cells (Tregs), which can suppress osteoclast differentiation and bone resorption *in vitro* (*7*). In the present study, estrogen was used as a positive control.

Bu-Shen-Ning-Xin Decoction (BSNXD) is composed of traditional Chinese medicinal compounds that are used to treat women with PMO in clinical, and the composition of prescription, the main effective components, function of BSNXD was introduced in Table 1 (*8-18*). We have done many researches on the effects of BSNXD on bone, previously (*19,20*). BSNXD ameliorated the osteoporotic phenotype of ovariectomized mice without affecting the serum

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*Address correspondence to:
Dr. Ling Wang and Dr. Dajin Li, Laboratory for Reproductive Immunology, Hospital and Institute of Obstetrics and Gynecology, Fudan University Shanghai Medical College, No.413, Zhaozhou Road, Shanghai 200011, China.
E-mail: dr.wangling@vip.163.com (Wang L), djli@shmu.edu.cn (Li DJ)

Table 1. The composition of herbal formula Bu-Shen-Ning-Xin Decoction (BSNXD)

Pinyin Name	Common name	Latin name	Content	Main constituents	Biological activity
Gan di huang/ Sheng di huang	Dried Rehmannia Root	Radix Rehmanniae Exsiccata	15 g	iridoid glycosides, monosaccharides, amino acids	Immune regulation, promoting adrenal cortex and sex glands functioning, stimulating bone marrow, enhancing blood coagulation process, improving kidney and heart functioning, diuresis, lowering blood pressure, inhibiting gastric secretions, anti-tumor, inhibiting epithelial cell proliferation, anti-oxidative and anti-aging (*8*).
Zhi mu	Common Anemarrhena Rhizome	*Anemarrhena asphodeloides* Bunge	15 g	xanthones, steroidal compound	Resistant to microbial action, sympathetic – adrenal function, fall blood sugar function, antipyretic, antitumor (*9*).
Huang bo	Bark of Chinese Corktree	*Phellodendron amurense* Rupr	9 g	berberine, jatrorrhizine, magnoflorine, phellodendrine	Antibacterial, antifungal, suppression of cough, lower blood pressure, trichomonad resistance, anti-hepatitis, anti-ulcer, immunomodulatory (*10*).
Gou qi zi	Barbary Wolfberry Fruit	Fructus Lycii barbari	15 g	carotenoids, flavonoids, polysaccharides	Immunomodulatory, anti-oxidant, anti-stress, neuro-protective, anti-tumor, liver/eyes/male-fertility/glycemia level and hyperlipidemia/blood pressure effects (*11-13*).
Chang pu	Rhizoma Acori Tatarinowii	Acorus tatarinowii	12 g	cal-amendiol, acorenone, shyobunone, acorone, acoragermacrone, acolamone, isoacolamone	Antifungal, central nervous system/cardiovascular system/digestive system effects (*14*).
Xian ling pi/ Yin yang huo	Shorthorned Epimedium	*Epimedium brevicornum* Maxim	12 g	icariin, total flavonoids of epimedium (TFE)	Sexual function/cardiovascular system/ immune function/respiratory system effect, anti-virus (*15*).
Suan zao ren	Spina Date Seed	*Ziziphus jujuba* Mill. var. *spinosa*	9 g	jujuboside, betulinic acid, ascorbic acid	Sedative and hypnotic, radioprotective, protection from anoxia and reoxygenation damage, central nervous system, protective effects on cardiac cells, anti-neoplastic, enhancing immunity, protective against ischemic cerebral damages, tonify liver, sedative heart, restrain sweat, generate saliva (*16*).
Bu gu zhi	Malaytea Scurfpea Fruit	*Psoralea corylifolia* Linn.	12 g	volatile oil, coumarin, flavones, monoterpene phenols, lipid compounds, resins, stigmasteroids	Cardiovascular system/white blood cells/skin conditions effects, anti-cancer, estrogen-like effects (*17*).
Ze xie	Oriental Waterplantain Rhizome	*Alisma plantago-aquatica* Linn	12 g	alisol, alisol A monoacetate, alisol B monoacetate, alisol C monoacetate, alismol, alismoxide	Lipid- lowering, liver protection, cardiovascular/ diuretic/lipid metabolism effect (*18*).

estrogen concentration or uterus (*21*); BSNXD modulates mesenchymal stem cell differentiation into osteoblasts (*22*); BSNXD inhibits osteoclastogenesis by abrogating the RANKL-induced NFATc1 and NF-κB signaling pathways through selective estrogen receptors (*23*); BSNXD suppresses osteoclastogenesis *via* increasing dehydroepiandrosterone to prevent postmenopausal osteoporosis (*24*). However, how BSNXD affects immune cells and whether immune cells participate in the effect of BSNXD on bone metabolism is still unclear.

The interaction between immune cells and bone has attracted many attentions, and osteoimmunology is a new discipline that focuses on the influence of immune cells on bone cell function. In the previous work, though we have gotten some development on bone metabolism, but we know little about immune cells on the process of BSNXD treatment. Many immune cells were found can influence bone metabolism, for example, Receptor activator of NF-kB ligand (RANKL), a tumor necrosis factor (TNF) family member that is expressed on activated T cells, is one of the key differentiation and survival factors for osteoclasts and provides a potential link between normal immune responses and bone metabolism (*25*). In the present study, we detected the changes of T cells, regulatory T cells, B cells, and even innate immune cells, such as NK cells, γδ T cells, and monocytes to explore whether immune cells participate in the effect of BSNXD on bone metabolism.

2. Materials and Methods

2.1. *Media and reagents*

Fetal bovine serum (FBS) and phenol red-free minimum essential media (MEM) were purchased from Gibco (Grand Island, NY, USA). Minimum essential media and 17-β-estradiol (E2) were purchased from Sigma-Aldrich

Co (Saint Louis, MO, USA). Flow cytometry antibodies fluorescein isothiocyanate (FITC)-conjugated anti-CD4, PE-conjugated anti-CD19/CD14/CD62L/B220/Foxp3/ anti-cytotoxic T lymphocyte antigen-4 (anti-CTLA-4), APC-conjugated anti-CD25, and their corresponding isotypes were obtained from eBioscience (San Diego, CA, USA). E2 and Leukocyte Acid Phosphatase Kit were purchased from Sigma-Aldrich Co (Saint Louis, MO, USA). Cell strainers were purchased from Becton Dickinson Labware, Franklin Lakes, NJ, USA). Regulatory T cell Isolation Kits were from Miltenyi Biotec (Bergisch Gladbach, Germany). The M-CSF was supplied by R&D Systems (Minneapolis, MN, USA), and RANKL was obtained from Peprotech (Rocky Hill, NJ, USA).

2.2. *BSNXD and BSNXD serum preparation*

BSNXD was obtained from the pharmacy of the Hospital of Obstetrics and Gynecology, Fudan University, Shanghai, China. BSNXD was formulated in accordance with traditional Chinese medicine theory and the clinical experience of the authors. BSNXD crude herbs were taken together, and then dissolved in double distilled water. BSNXD solution was intragastric administration to C57BL/6 mice, 7 days later, mice were sacrificed and heart blood was collected. Heart blood was solidification under the normal temperature for a half hour, and blood serum was collected for future use.

2.3. *Mice*

C57BL/6 mice (6-8 weeks) were purchased from the Laboratory Animal Facility of the Chinese Academy of Sciences (Shanghai, China). Housing and handling was in accordance with the guidelines of the Chinese Council for Animal Care. The mice were divided into 4 groups and treated by oral administration: sham group treated with saline (n = 15), OVX (ovariectomized) group treated with saline (n = 15), OVX+BSNXD group treated with BSNXD (total raw herbs 1 g/mL, n = 15), and OVX+ estrogen group treated with 17-β-estradiol (100 μg/kg/day orally, n = 15). After 12 weeks, the spleens and bones were collected for flow cytometry analysis of splenocytes and cells in the bone marrow.

2.4. *Flow cytometry analysis*

Splenocytes and bone marrow cells (1×10^6) were incubated for 30 minutes on ice with the indicated antibodies, washed, and resuspended in PBS containing 1% bovine serum albumin and 0.1% sodium azide. The non-specific signal was estimated by incubation with rat FITC- and PE-conjugated IgG isotype controls. Labeled cells were analyzed with a flow cytometer (Becton Dickinson, Palo Alto, CA, USA). Data are expressed as the percentage of positive cells.

2.5. *Regulatory T cell's separation and purification*

Spleens were harvested from mice 6 to 8 weeks old, gently cut into small pieces and passed through cell strainers. Red blood cells were lysed. To purify $CD4^+CD25^+$ T cells, cells were labelled with magnetic-activated cell sorting (MACS) beads by CD4 negative selection and CD25 positive selection. $CD4^+CD25^+$ T cells were selected on an LS column in a magnetic field, and flushed out by a plunger. The purity of the $CD4^+CD25^+$ T cells population varied from 90% to 95%.

2.6. *Osteoclast culture and TRAP staining in vitro*

Bone marrow-derived monocyte/macrophage precursor cells (BMMs) of 10-week-old mouse femurs were cultured in MEM without phenol red supplemented with FBS in the presence of 10 ng/mL M-CSF for 2 days and then differentiated into osteoclasts using 50 ng/ml RANKL and 10 ng/mL M-CSF for 3 days.

To estimate the effect of Tregs on osteoclastogenesis *in vitro*, the cells were exposed to BSNXD (10% serum) or estrogen (10^{-9} M) or solvent control with or without Tregs in presence of RANKL stimulation for 72 hours.

Then, osteoclastogenesis was calculated by TRAP staining by means of the Leukocyte Acid Phosphatase Kit. TRAP-positive multinucleated cells ($TRAP^+$ MNCs; more than five nuclei) were counted under microscope. Results from at least six independent experiments are shown.

2.7. *Statistical analysis*

All values are expressed as the mean ± standard error of the mean (S.E.M.). Data were analyzed by using SPSS and the variance was evaluated by using one-way analyses of variance (ANOVA). $p < 0.05$ was considered statistically significant.

3. Results

3.1. *BSNXD improves the proportion of T cells in the spleen of ovariectomized mice*

Activated T cells participate in osteoclast maturation by secreting RANKL. In this study, we assessed the proportion of $CD4^+$ and $CD8^+$ T cell subsets. We found that both $CD4^+$ and $CD8^+$ T cells were increased in the OVX group, but the cells were both decreased in the OVX+BSNXD and OVX+ estrogen groups ($p < 0.05$, Figure 1).

3.2. *BSNXD increases CTLA-4$^+$ Tregs in the spleen of ovariectomized mice*

$Foxp3^+$ Tregs are a T cell subset that inhibits immune responses. We assessed the percentage of $Foxp3^+$ and

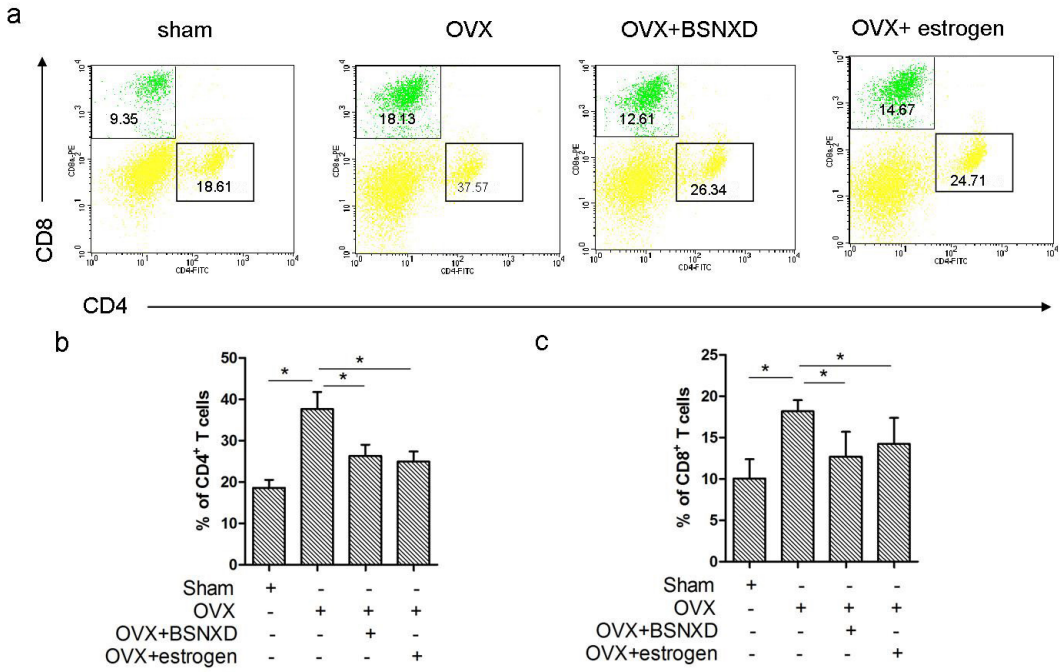

Figure 1. Proportion of T cells in the spleen of ovariectomized mice. (a) Flow cytometry of $CD4^+$ and $CD8^+$ T cells from each group. **(b, c)** Frequency of $CD4^+$ and $CD8^+$ T cells in mice as in **a**. $^*p < 0.05$. Data are representative of at least three experiments.

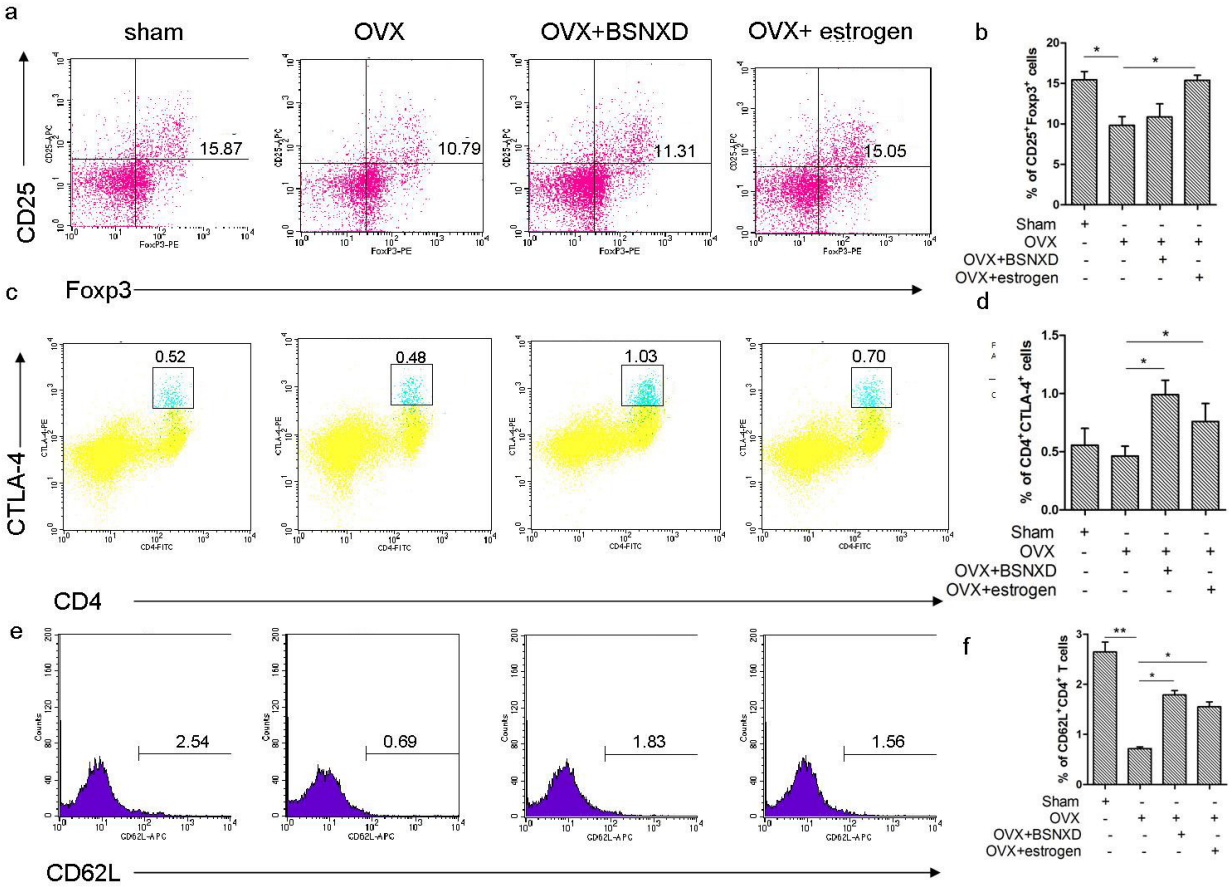

Figure 2. Regulatory T cells in the OVX mice. (a) Expression of CD25 and Foxp3 on $CD4^+CD25^+$ T cells from each group. **(b)** Frequency of $CD4^+Foxp3^+$ T cells in mice as in **a**. **(c)** Expression of CD4 and CTLA-4 on $CD4^+CD25^+$ T cells from each group. **(d)** Frequency of $CD4^+$ $CTLA\text{-}4^+$ T cells in mice as in **c**. **(e)** Analysis of CD62L expression on $CD4^+$ T cells from each group. **(f)** Frequency of $CD4^+$ $CD62L^+$ T cells in mice as in **e**. $^*p < 0.05$. Data are representative of at least three experiments.

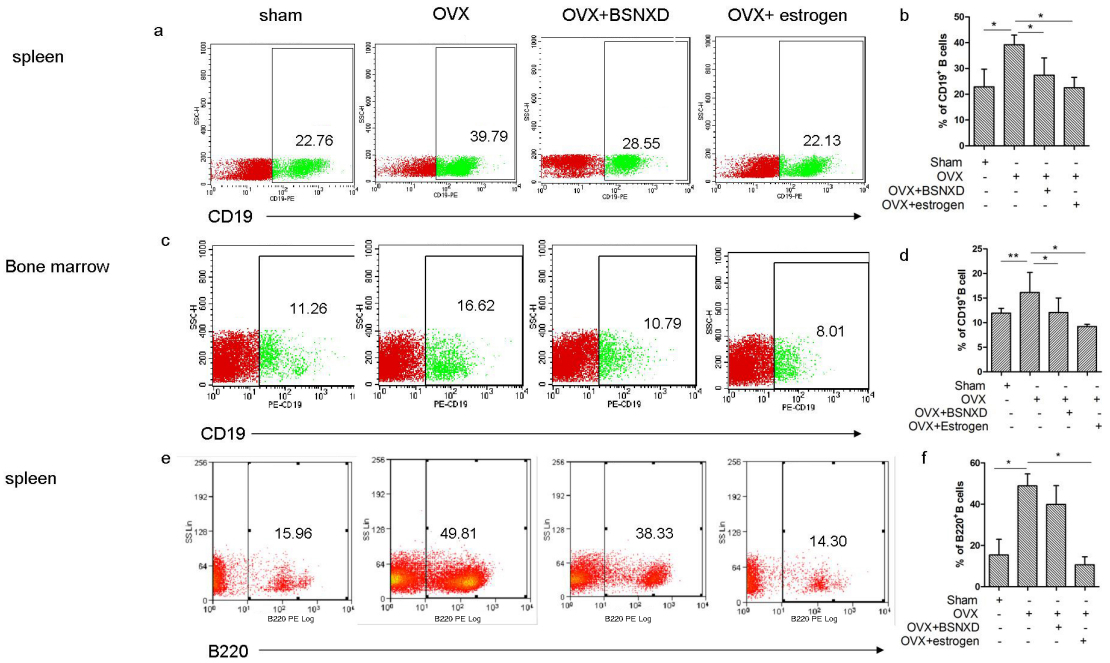

Figure 3. Proportion of B cells in the spleen and bone marrow of OVX mice. (a) Expression of CD19 on lymphocytes from each group. **(b)** Frequency of $CD19^+$ B cells in mice as in **a**. **(c)** Expression of CD19 on bone marrow lymphocytes from each group. **(d)** Frequency of $CD19^+$ B cells in the bone marrow of mice as in **c**. **(e)** Expression of B220 on splenocytes from each group. **(f)** Frequency of $B220^+$ B cells in mice as in **e**. *$p < 0.05$. Data are representative of at least three experiments.

$CTLA\text{-}4^+$ Tregs. We found that $Foxp3^+$ Tregs were decreased in the OVX group, but there was no change in the percentage of $CTLA\text{-}4^+$ Tregs compared with the sham group. Compared with the OVX group, $CTLA\text{-}4^+$ Tregs were increased, but $Foxp3^+$ Tregs did not change in the OVX+BSNXD group ($p < 0.05$). Compared with the OVX group, both $CTLA4^+$ and $Foxp3^+$ Tregs were increased in the OVX+ estrogen group ($p < 0.05$, Figure 2a, b, c, d).

CD62L (L-selectin) is a cell adhesion molecule expressed on lymphocytes and acts as a "homing receptor". CD62L is also expressed on central memory T cells that have encountered antigen to enable them to localize to secondary lymphoid organs. Compared with the sham group, CD62L was decreased in the OVX group, while it was increased after treatment with BSNXD or estrogen ($p < 0.05$, Figure 2e, f).

3.3. *BSNXD decreases the proportion of B cells in the spleen and bone marrow*

B cells produce RANKL and OPG, which increases after T-cell stimulation, and they are the major source of OPG in the bone marrow (*26*). Compared with the sham group, both $CD19^+$ and $B220^+$ B cells in the spleen were increased in the OVX group, but the cells decreased after BSNXD and estrogen treatments ($p < 0.05$, Figure 3a, b, e, f). The same phenomenon was found in the lymphocytes of the bone marrow; BSNXD and estrogen treatments lessened the extent of the increase by ovariectomy ($p < 0.05$, Figure 3c, d).

3.4. *BSNXD has no effect on γδ T cells in the spleen and bone marrow*

To analyze the influence of γδ T cells on the bone (*27*), we assessed the percentage of γδ T cells in the spleen. Compared with the sham group, splenic γδ T cells were decreased in the OVX group, but the percentage did not change in the OVX+BSNXD and OVX+ estrogen groups ($p > 0.05$, Figure 4a, b).

3.5. *BSNXD increases NK cell numbers in the spleen and bone marrow*

Natural killer (NK) cells are bone marrow-derived cells that play a crucial role in the immune defense against viral infections. NK cells trigger osteoclast apoptosis in a dose-dependent manner that results in decreased bone erosion (*28*). Compared with the sham group, the percentage of splenic NK cells decreased in the OVX group, but the percentage was increased in the OVX+BSNXD and OVX+ estrogen groups ($p < 0.05$, Figure 4c, d). Though there was no difference in the numbers of bone marrow lymphocytes in the sham and OVX groups, BSNXD and estrogen treatments increased the proportion of NK cells ($p < 0.05$, Figure 4e, f).

3.6. *BSNXD decreases the proportion of mononuclear cells in the spleen and bone marrow*

Osteoclasts originate from mononuclear cells and can be induced by M-CSF and RANKL. The proportion of

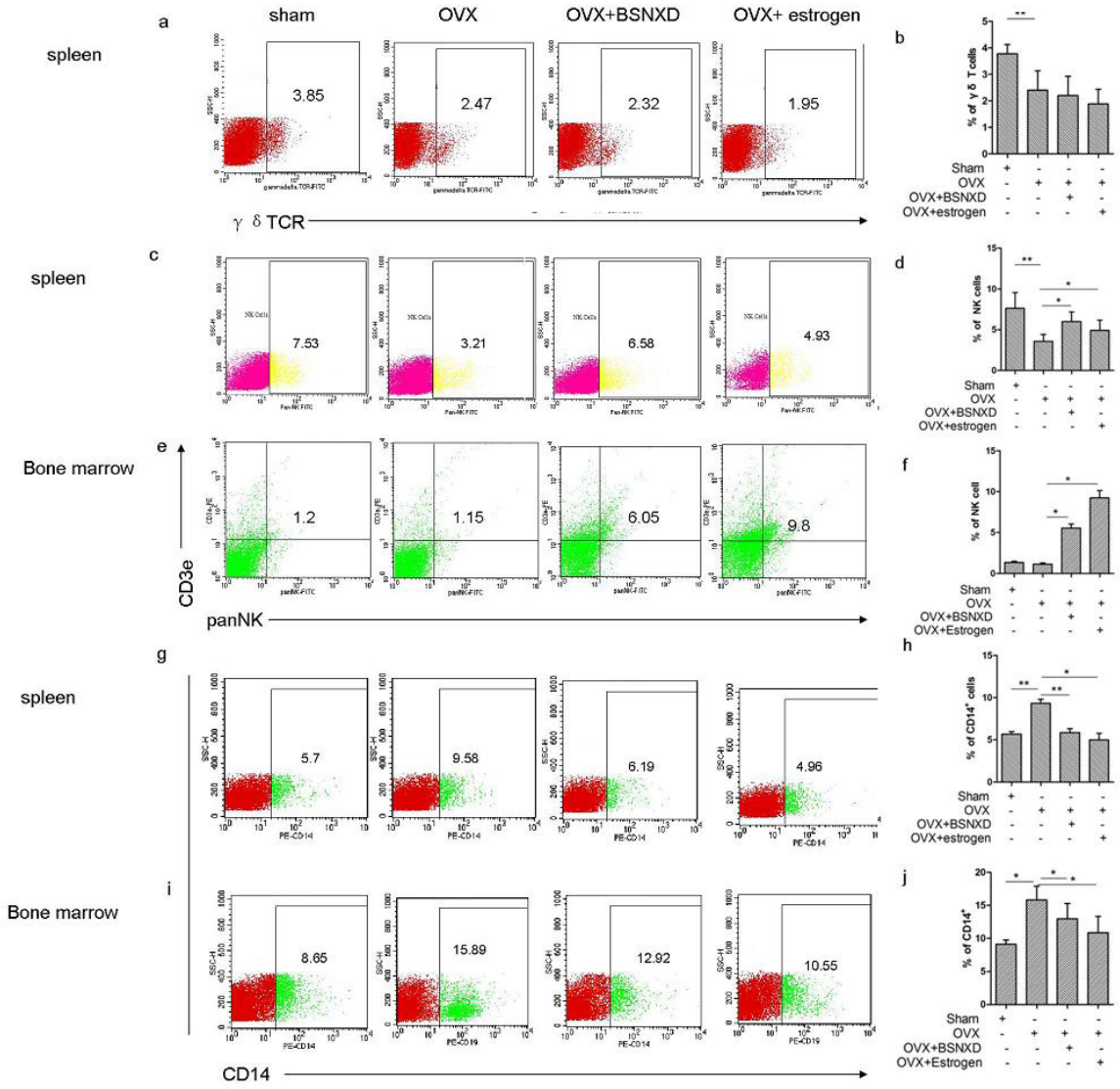

Figure 4. Innate immune cells in the spleen and bone marrow of ovariectomized mice. (a) Analysis of the γδ TCR on lymphocytes from the spleen of each group. **(b)** Frequency of γδ T cells in mice as in **a**. **(c)** Analysis of CD3e and panNK on splenocytes from each group. **(d)** Frequency of $CD3e^+$ $panNK^+$ NK cells in mice as in **c**. **(e)** Analysis of CD3e and panNK on bone marrow lymphocytes from each group. **(f)** Frequency of $CD3e^+$ $panNK^+$ NK cells in the bone marrow of mice as in **e**. **(g)** Expression of CD14 on lymphocytes from the spleen of each group. **(h)** Frequency of $CD14^+$ monocytes in mice as in **g**. **(i)** Expression of CD14 on bone marrow lymphocytes from each group. **(j)** Frequency of $CD14^+$ monocytes in the bone marrow of mice as in **i**. $*p < 0.05$. Data are representative of at least three experiments.

$CD14^+$ cells was increased in the OVX group suggesting that there was an increase in osteoclast production. However, it was decreased in the OVX+BSNXD and OVX+ estrogen groups ($p < 0.05$, Figure 4g, h). Similarly, in the bone marrow, the proportion of $CD14^+$ cells was decreased after BSNXD or estrogen treatments ($p < 0.05$, Figure 4i, j).

3.7. *Tregs decreases osteoclastogenesis*

In order to explore the role of Tregs on osteoclast differentiation, we cultured bone marrow monocytes with or without Tregs, and estrogen was also used as a positive control. Compared with control group, BSNXD serum (10%) and estrogen treatment decreased $TRAP^+$ multinucleate cells (MNC), and there were less $TRAP^+$ MNC when Tregs were added to cell culture system. ($p < 0.05$). There was no difference in osteoclastogenesis between BSNXD serum group and estrogen group ($p > 0.05$, Figure 5).

4. Discussion

Immune cells participate in many physiological activities and their role on the bone has attracted much attention. In this study, we analyzed the changes of the percentage of immune cells in the spleen and bone marrow after BSNXD treatment using estrogen as

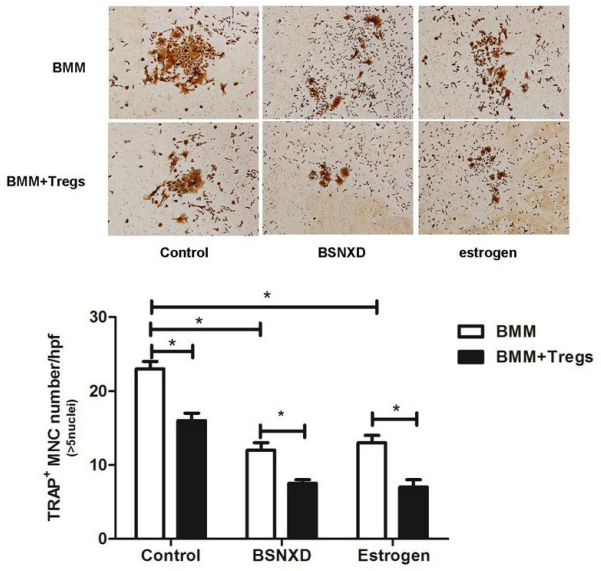

Figure 5. Effect of Tregs on osteoclast differentiation. $CD4^+CD25^+$ regulatory T cells were collected through magnetic cell sorting (MACS) by CD4 negative selection and CD25 positive selection and then added to the bone marrow monocyte monoculture system with or without BSNXD serum/estrogen. 3days later, osteoclastogenesis was calcuated by TRAP staining. Brown: osteoclast. *$p < 0.05$, Data are representative of at least three experiments.

the positive control. We found that both BSNXD and estrogen treatments affected the proportion of immune cells *in vivo*.

4.1. *The activation of $CD4^+$ and $CD8^+$ T cells and osteoporosis*

T cells produce a number of mediators that influence osteoclast differentiation. Activated T cells produce factors, including RANKL, that strongly stimulate osteolysis, consistent with the association between inflammation and bone loss. However, T cells (particularly naive T cells) also mediate anti-osteoclastogenic activities. T cells can be divided into two major functional compartments: cytotoxic T cells (CTLs) that express CD8 and T helper cells that express CD4. Based on the importance of T cells on the bone, we assessed the proportion of T cells in the spleen. Lymphocytes express estrogen receptors, and in humans, estrogen loss is reported to cause a decline in T cell subsets. Conversely, ovariectomy increases T cell activation and TNF secretion in mice (*29-32*). In our study, we found that ovariectomy increased the proportion of T cells, and BSNXD treatment can reverse this effect. We found an increase in the proportion of $CD8^+$ T cells in the OVX group, which is consistent with the status prior to surgery. Interestingly, Pietschmann *et al*. found that $CD8^+$ T cells bearing markers suggestive of cell senescence are significantly increased in elderly patients with osteoporotic fractures (*33*). Some investigators focused on the CD4/CD8 T cell ratio, which is significantly higher in osteoporotic patients. The total lymphocyte and T cell counts are unchanged compared with controls, and young normal control subjects have CD4/CD8 ratios that are similar to the elderly non-osteoporotic subjects (*34*).

4.2. *Protection of Tregs on bone*

Tregs suppress immune responses to maintain immune homeostasis and tolerance towards self-antigens (*35*). Tregs reduce joint destruction in an arthritis model by elevating the production of osteoclast-inhibiting cytokines. Tregs directly inhibit osteoclast formation through the transmembrane protein CTLA-4. Further studies on CTLA-4 activity can help explain the direct influence of Tregs on osteoclasts (*36*). In the present study, ovariectomy induced a decrease in $CTLA\text{-}4^+$ Tregs. However, after BSNXD and estrogen treatments, $CTLA\text{-}4^+$ Tregs were increased, suggesting a potential inhibitory role of BSNXD and estrogen on osteoclast maturation. Foxp3 is the transcriptional factor for Tregs and many studies have been conducted on the interaction between $Foxp3^+$ Tregs and the bone (*37,38*). In this study, we found that estrogen increased the numbers of $Foxp3^+$ Tregs. In contrast, BSNXD had no effect on the numbers of $Foxp3^+$ Tregs, suggesting that BSNXD and estrogen act through different pathways.

CD62L retains cells in the lymph nodes and its downregulation facilitates the emigration of effector T cells. In this present study, ovariectomy induced a decrease in CD62L. However, BSNXD treatment increased CD62L, suggesting that T cell activation was inhibited, which is consistent with the data shown in Figure 1. BSNXD inhibited T cell activation by increasing the proportion of $CTLA\text{-}4^+$ Tregs.

4.3. *The controversial role of B cells on bone*

Ovariectomy, which causes significant bone loss, increases $B220^+$ pre-B cell numbers, and estrogen deficiency is associated with an increase in bone marrow $B220^+$ B cells, which is not found in humans (*39*). In fact, in human males made hypogonadal and selectively replaced with either estrogen or testosterone, estrogen is associated with an increase in the percentage of bone marrow $CD19^+$ B cells (*40-43*). In our study, we found that ovariectomy induced an increase in $CD19^+$ and $B220^+$ B cells, but BSNXD treatment decreased B cell numbers in the BSNXD+OVX group. Though, the influence of B cells on the bone is controversial, their abundance in the bone marrow suggests they have some influence on the bone mass.

4.4. *The participation of γδ T cells, NK cells, and monocytes on bone metabolism*

The T cell-derived proinflammatory cytokines tumor necrosis factor (TNF)-α and interleukin (IL)-17 trigger

bone erosion by increasing the stimulation of osteoclast formation and activity. Recent studies in animal models of rheumatoid arthritis (RA) have implicated that γδ T cells are the major producers of pathogenic IL-17, and activated γδ T cells inhibit osteoclast differentiation and resorptive activity *in vitro* (*27*). Though we found a decrease in γδ T cells in the OVX group, we did not observe a change after BSNXD and estrogen treatments.

Activated NK cells are present in inflammatory sites associated with enhanced bone erosion. NK cells may participate in inducing apoptosis of osteoclasts that have matured and attached to the bone, which decreases bone resorption (*44-46*). Osteoclast precursors are derived from multipotent precursors of the monocyte-macrophage lineage (*47,48*). BSNXD and estrogen treatments increased the proportion of NK cells and monocytes in the OVX group.

4.5. *The difference between BSNXD and estrogen*

In the present study, we compared BSNXD with estrogen, and we found they are similar in most of effects on immune cells, but there are also some differences. The most interesting thing is the difference on the expression of Foxp3, CTLA-4 in Treg cells, of which BSNXD increases CTLA-4 expression, but estrogen increases the Foxp3 expression, suggesting a different modulation on regulatory T cells. *In vitro* experiment, Tregs decreased osteoclastogenesis, especially in BSNXD serum and estrogen group. In previous research, we also found there are some differences between BSNXD and estrogen on the effect of regulating mesenchymal stem cell differentiation: BSNXD increases MSCs differentiation to osteoblast and decreases MSCs differentiation to adipocyte; while, estrogen increases MSCs differentiation to osteoblast without affecting adipocyte differentiation (*49*).

5. Conclusion

In conclusion, the present study showed that BSNXD regulates the proportion of immune cells in the spleen and bone marrow. Regulatory T cells participate in the process of bone protecting. These findings place osteoimmunology in a position of unique clinical significance. BSNXD plays a different role from estrogen on immune cells to regulate the bone metabolism of OVX mice. The link between ovariectomy-induced bone loss and the change in the proportion of immune cells remains to be clarified.

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