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Original Article

Mouse endometrial stromal cells and progesterone inhibit the activation and regulate the differentiation and antibody secretion of mouse B cells

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Abstract: The regulation mechanism for the B cells in the female reproductive tract (FRT) is unclear now. The aim of this study was to analysis the effect of progesterone and mouse endometrium stromal cells (ESCs) on B cells and explore it roles in modulating B cells-mediated immune responses. We primary isolated mouse ESCs from endometrium of BALB/c mice and B cells from spleen cells of BALB/c mice, and then constructed these two kind of cells co-culture system, and treated with or without progesterone. We found that both treatment with progesterone and co-culture with ESCs reduced the expression of co-stimulatory molecules CD80 and CD86 on mouse B cells from spleen cells. In addition, the expression of CD138 (syndecan-1) on B cells was increased after co-culture with ESCs, however, progesterone could partly reduce this effect. Unlike progesterone, ESCs alone promoted the proliferation and stimulated the secretion level of antibodies IgG and IgA of B cells. Our current results progesterone and ESCs could inhibit the activation of B cells through deceasing CD80 and CD86 expression, regulated the differentiation status of B cells by up-regulating the expression of CD138 together, and might further inhibit the antigen presentation function of B cells, which is beneficial to the establishment of fertilization and pregnancy. In addition, ESCs also promoted the proliferation and antibody secretion, which might participate in the resisting infections during non pregnancy and pregnancy.

Keywords: Progesterone, endometrium stromal cells, B cells, the female reproductive tract, activation, antibody secretion

Introduction

The female reproductive tract (FRT) immune system is tightly regulated by cyclic changes in the sex hormones estradiol and progesterone to optimize stations for implantation. Progeste rone has been shown to affect immunity in rodents and humans, mainly at concentrations commensurate with pregnancy [1-3].

Progesterone is known to be essential for the establishment and maintenance of pregnancy including ovulation, uterine, and mammary gland development [4, 5]. It is indispensable in creating a suitable endometrial environment for implantation, and also for the maintenance of pregnancy. Successful pregnancy depends on an appropriate maternal immune response to the fetus. When the immune tolerance is weak, it is easy to progress into miscarriage. But the infection of FRT will come out because of the low level of immune response [2]. While it is reported that more than 30 different types of parasites, bacteria and viruses can infect FRT, especial in the phase of pregnancy [6]. Thus, an adequate balance between immune protection against pathogens and tolerance to allogeneic sperm and semi-allogeneic fetus results in successful reproduction [7].

As discussed elsewhere, changes in hormone levels during the menstrual cycle result in cyclical changes in FRT innate and adaptive immune responses, as well as the immune cell populations within the upper and lower tract [8]. Leukocytes comprise 6-20% of total cells and

are dispersed throughout the genital tract, both in the lower genital tract and upper genital tract with the uterine endometrial and fallopian tube having the highest proportion [7, 9, 10]. The major leukocyte component consists of T lymphocytes, including $CD3^+$ lymphocytes, which are present in all FRT tissues. Granulocytes are also present, particularly in the fallopian tubes. B lymphocytes and monocytes are dispersed throughout the FRT, but they are not as prominent as T lymphocytes and granulocytes [11, 12].

A number of studies have demonstrated that progesterone has immune suppressive properties [13]. Progesterone has been shown to have direct effects on T lymphocytes at concentrations consistent with pregnancy, suggesting that it may play a role in preventing maternal immune responses against fetal antigens [14]. It is also shown to increase the number of Langerhans cells in the human vaginal epithelium taking a role in antigen presentation [15]. In addition, the regulation of aggrecanases and immune factors by gonadal steroids in human endometrial stromal cells (ESCs) may play an important role during decidualization [16-18].

Lot of reports are about the innate immune cells such as macrophages, dendritic cells (DCs) and natural killer (NK) cells and gamma delta T cells (γδ T cells) and so on, which are influenced by progesterone in pregnancy and infections [1-3, 12]. However, little research is present to evaluate the effect of progesterone and ESCs on B cells. It is also unclear how B cell functions are regulated by progesterone and whether the hormonal effects on B cell function are direct achieved or mediated through ESCs. Therefore, we mimicked the environment of FRT by constructing B lymphocytes-ESCs coculture system, and observed their roles in modulating the biological function of B cells.

Material and methods

Primary tissues and cells

Female BALB/c adult mice aged 8-10 weeks old were purchased from Shanghai Center for Experimental Animal of Chinese Academy of Sciences and used for all the experiment. The mice were maintained under specific pathogen-free condition. All animal studies were conducted according to the institutional guidelines of Animal Care and Use Committee of fudan University.

Splenic Lymphocytes were isolated as previously described [19, 20]. Spleen was removed and dissociated by Nylon Netting (200 mesh, 75 μm), and the cells suspension were collected and washed with sterile PBS and the red blood cells were lysed by addition of 0.83% ammonium chlorine solution for 3 mins and then added 10 times volume 1×PBS to terminate the reaction. After centrifugation (300×g, 10 mins), the splenic cells were in resuspension with RPMI 1640 (Hyclone, Logan, UT, USA) containing 10% fetal bovine serum (FBS) (Gibco, USA) and 1% of penicillin-streptomycin-anfotericin (PSA) (sigma-Aldrich St. Louis, USA).

For isolation of ESCs from genital tract, vaginal and uterine horns (n=10) were removed and minced into 0.5 cm pieces, and were then digested with 1.37 mg/ml of collagenase I (sigma-Aldrich St. Louis, USA) in RPMI 1640 (Hyclone, USA). Digestion was accomplished at constantly shaking for 2.5h at 37°C. After digestion the cells were pooled by passing through 100, 300 and 400 meshes (150, 48, 38 μm), respectively. The cells collected from the filtrate were isolated by a 70%, 50%, 30% percoll (LGC-Biotecnologia, Cotia, Brazil) density centrifugation. Then we gathered the cells in 50% and 30% density, and washed with PBS. At last, the cells were cultured in DMEM (Dulbecco's modified Eagle's medium)/F-12 (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin and streptomycin in a humidified incubator at 37°C and 5% CO_2 for 30 mins to remove the fibroblast. Then we collected the un-stickable cells and were seeded in complete medium at 37°C and 5% CO_2.

Immunostaining

For immunocytochemical staining, the purified ESCs were fixed in 4% paraformaldehyde for 20 min at room temperature after being cultured for 48 hours, washed in PBS and permeabilized for 20 min in 0.1% Triton X-100-PBS. The cells were then incubated with 10% FBS in PBS for 30 min to reduce non-specific binding. Rabbit Anti-mouse vimentin monoclonal antibody (1:100 dilution; CST, USA) and CK7 (5 ug/ml, Abcam, USA) and isotope antibody were administrated overnight at 4°C. The cells were then

Table 1. Primer sequence of progesterone receptor

Gene name	Size (bp)	Primer sequences
PR	94	Sense: TCCCATTCAAGAAGAAAC
		Antisence: GTGAGGACAGGAAGAAGTA
β-actin	110	Sense: GAAGCTGTGCTATGTTGCTCTA
		Antisence: GGAGGAAGAGGATGCGGCA

incubated with a biotinylated secondary antibody for 30 mins at room temperature. Streptavidin-HRP was applied for another 30 min at room temperature. The cells were stained with DAB (Zhongshan Cambridge Company, Beijing, China) and counterstained with haematoxylin. The experiments were repeated three times.

B cells and T cells isolation from total splenic lymphocytes by MACS

Total splenic lymphocytes were incubated with microbeads of conjugated anti-mouse CD19 or anti-mouse CD3 mAb (Miltenyi Biotec), and then $CD19^+$ cells and $CD3^+$ cells were purified. Separation was performed with the AutoMACS instrument (Miltenyi Biotec).

Real-time PCR analysis

Total RNA was isolated from the purified B cells, T cells and splenic cells using the TRizol according to manufacturer's instructions (Invitrogen, Merelbeke, Belgium, USA). Reverse transcription of 1 μg RNA was performed by using RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, USA) following the manufacturer's protocol. Real-time quantitative PCR was performed by using SYBR® Premix Ex Taq TM II (Tli RNaseH Plus) (TaKaRa Biotechnology Co. LTD., Japan). For PCR 10 ng of the RT reaction were used in a 20 μl reaction using the ABI Prism 7900HT sequence detector system (Applied Biosystems Branch burgNJ, USA). Target genes were normalized to *β-actin* and quantified by using the comparative Ct (cycle threshold) assay. Gene expression was measured in triplicate with a good reproducibility and the average was calculated. The primer sequences were indicated in **Table 1** and were synthesized by Biosune Biotechnology Co., LTD. *β-actin* was applied as an internal control.

Treatment with progesterone and co-culture with ESCs: The isolated B cells (1×10^6 cells/well) was cultured with or without mouse ESCs (2×10^5 cells/well) in 24 wells plate, and incubated with progesterone at the different concentration (10^{-11}, 10^{-10}, 10^{-9} or 10^{-8} M) for 24h , 48h, 72h or 6d, the vehicle was added as the control.

Flow cytometry: After treatment with progesterone and co-culture with ESCs, the co-stimulatory molecules expression of CD80 and CD86 on B cells were evaluated by direct cell surface labeling. The cells of the every group were then washed twice and incubated at 4°C for 20 min with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse CD80, Phycoerythrin (PE)-conjugated rabbit anti-mouse CD86, Allophycocyanin (APC)-conjugated rabbit anti-mouse B220-CD45R, or the relevant isotype control (all from Biolegend, San Diego, USA). Then their positive percentage was detected by flow cytometry (FCM).

Cell proliferation/differentiation assays

For proliferation assay, a CFSE stock (10 mM in DMSO; Invitrogen, USA) stored at -20°C, was thawed and diluted in phosphate-buffered saline (PBS) to the desired working concentrations. In pilot experiments we tested different CFSE labeling conditions (final concentrations: 0.2, 0.5, 2 and 5 μM) to obtain a high cell viability and a broad CFSE signal measurement after progesterone treatment (data not shown). Then the desired working concentrations were used at 0.5 mM with enough fluorescence and lowest harm to the splenic cells. Then freshly purified splenic B cells were resuspended in PBS (0.1% BSA) at 1×10^6 cells/ml and incubated with CFSE (final concentration: 0.5 μM) for 10 min at 37°C in darkness with shaking several times every 3 mins. Cells were washed and resuspended in culture medium for 15 min to stabilize the CFSE staining. After a final wash step, cells were resuspended in 10% carbon adsorption FBS RPMI 1640 medium without phenol red at the indicated cell concentrations. Then these cells were collected, treated with progesterone and co-cultured with or without ESCs in darkness for 3 days. Then we collected the cells and centrifuged 1000 g for 5 min, resuspended with PBS, then labeled with Allophycocyanin (APC)-conjugated rabbit anti-mouse B220-CD45R at 4°C for 20 min. The proliferation of B cells was detected by flow cytometry.

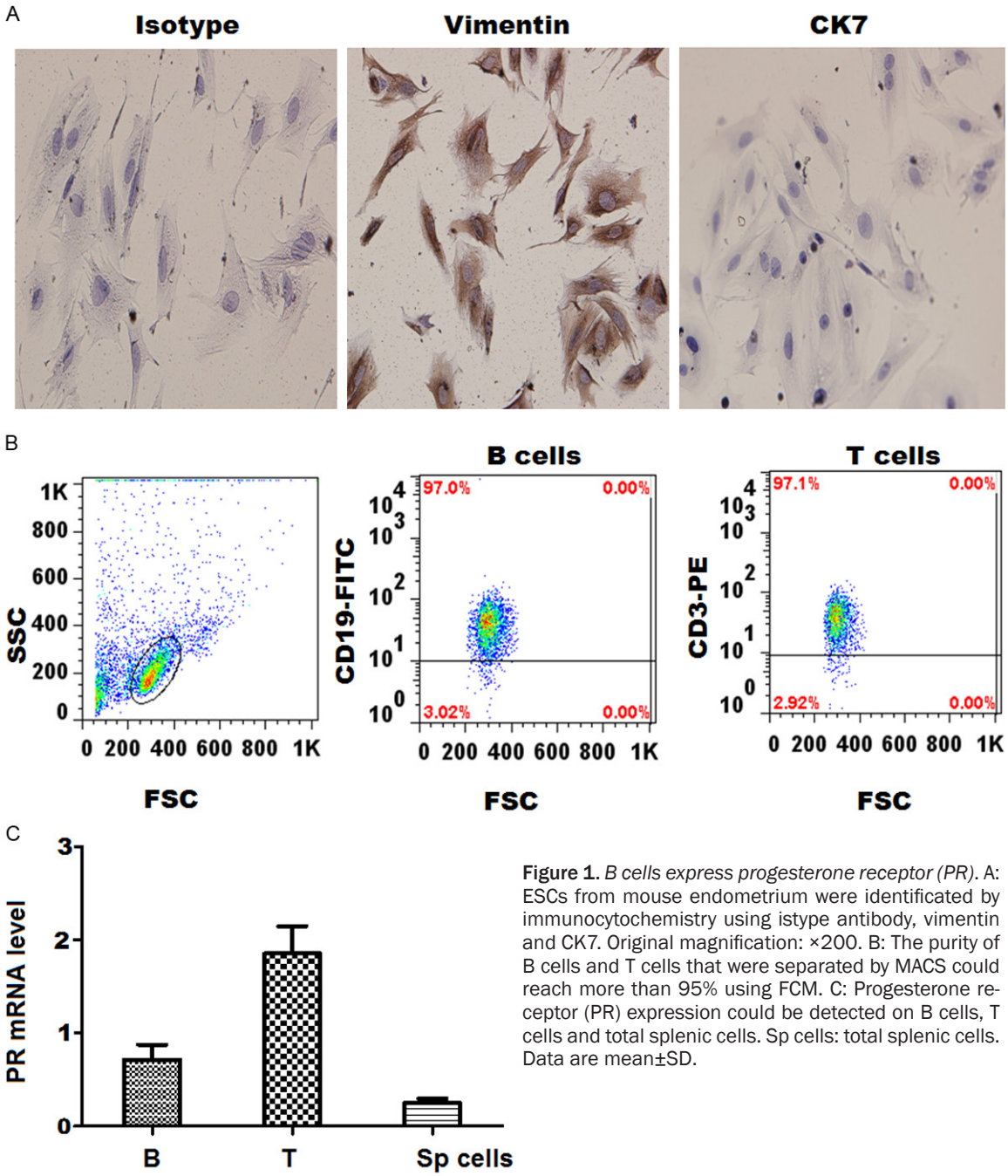

Figure 1. *B cells express progesterone receptor (PR).* A: ESCs from mouse endometrium were identificated by immunocytochemistry using istype antibody, vimentin and CK7. Original magnification: ×200. B: The purity of B cells and T cells that were separated by MACS could reach more than 95% using FCM. C: Progesterone receptor (PR) expression could be detected on B cells, T cells and total splenic cells. Sp cells: total splenic cells. Data are mean±SD.

For differentiation assay, after treatment with progesterone and co-culture with ESCs, the cells which were seeded in 10% carbon adsorption FBS RPMI 1640 medium without phenol red for 72h were collected and then were centrifuged 1000 g for 5 min. These cells were resuspended with PBS, and were labeled with CD45R/B220-APC and CD138-PE antibodies. Both the staining was performed at 4°C and away from light for 20 min. Then flow cytometry was used for analyzing the expression of CD138 on B cells.

Spontaneous antibody production assay

The cells were centrifuged at 1000 g for 10 min after cultured for 6 days, and the culture supernatants were collected and stored at -80°C until used. Enzyme-linked immunosorbent assay (ELISA) was performed to detect the total

IgG and IgA antibodies production according to the manufacturer's protocol (Icllab, America).

Statistical analysis

All data was presented as mean±SD. Data were analyzed by using Statistical Package for the Social Sciences software version 16. Statistical significance was determined by using the Student's t-test and one-way ANOVA. Differences were accepted as significance at $P<0.05$.

Results

B cells express progesterone receptor

At first, we isolated the primary ESCs from endometrium tissue of BALB/c mice. As shown in **Figure 1A**, we identified ESCs by immunocytochemical staining and found that the purity of Vimentin-positive and CK7-negative ESCs was beyond 98%. After using MACS to select B cells and T cells from spleen cells of BALB/c mice, we used immunofluorescence antibodies labeling for the $CD19^+$ and $CD3^+$ subsets, and confirmed that the purities of both $CD19^+$ and $CD3^+$ B cells isolated in our study were more than 95% (**Figure 1B**). Then progesterone receptor (PR) expression was detected by real-time PCR assay on B cells, T cells and splenic cells. The results showed that B cells, T cells and total splenic cells transcribed *PR* (**Figure 1C**).

Both progesterone and ESCs inhibit the expression of CD80 and CD86 on B cells

Humoral immune responses require B cell activation. The activated B cell undergoes Ab affinity maturation as well as class switch recombination (CSR) while these processes require activation-induced deaminase (AID) [21-23]. Moreover, it is reported that AID mRNA production in activated mouse splenic B cells can be reduced after progesterone treatment by inhibiting AID transcription [23]. So we want to study the intensive effect of progesterone on B cells. Co-stimulatory molecules CD80 and CD86 expression on B cells are important for B cells activation. Hence, we cultured B cells of BALB/c mice with or without primary mouse ESCs, and treated with different concentration of progesterone (10^{-11} M, 10^{-10} M, 10^{-9} M, or 10^{-8} M) for 24 or 48h, with vehicle as the control. In **Figure 2**, progesterone obviously reduced the level of CD80 and CD86 on B cells groups, especially at 10^{-9} or 10^{-8} M and culture for 48h ($P<0.001$) (**Figure 2A** right and **2B** right). Co-culture with ESCs also significantly decreased the expression of CD80 and CD86 on B cells ($P<0.01$ or $P<0.001$) (**Figure 2A** and **2B**). Interestingly, ESC and progesterone played a synergistic effect, further markedly reduced CD86 expression on B cells in the initial stage of culture (24h) ($P<0.01$ or $P<0.001$) (**Figure 2B** left). These findings indicated that both progesterone and ESCs might inhibit the activation of B cells through down-regulating the expression of CD80 and CD86.

Owing to the concentration of progesterone at 10^{-8} M was higher than its physiologic concentrations (about 10^{-10}-10^{-9} M), so the treatment concentration of progesterone was from 10^{-11} to 10^{-9} M in the later experiments.

The proliferation of B cells in splenocytes is promoted by ESCs not progesterone

Subsequently, we analyzed the effect of ESCs and progesterone on the proliferation. CFSE is a living cell fluorescent dye which the fluorescence intensity is attenuated by the division of cells. Before co-culture with or without mouse ESCs, and treatment with progesterone for 72h in darkness, B lymphocytes were labeled by CFSE with the final working concentration 0.5 μM. LPS from Escherichia coli (5 μg/ml) was added as positive control. Progesterone (0, 10^{-11}, 10^{-10}, 10^{-9} M) had not influenced the proliferation of B cells ($P>0.05$) (**Figure 3A** and **3B**). However, it can be dramatically promoted by ESCs (**Figure 3A**). These representative histograms of B cells proliferation were also shown in **Figure 3B** ($P<0.001$). Moreover, treatment with progesterone could not amplify the stimulating effect on the proliferation of B cells induced by ESCs.

Progesterone partly weakens the stimulatory effect on CD138 expression of B cells mediated by ESCs

Syndecan-1 (CD138) is an integral membrane heparin sulphate and chondroitin sulphate-containing proteoglycan. It can bind with extracellular matrix which may be helpful for cells retention in tissue. And it is predominantly expressed in epithelial cells, precursor B cells and plasma

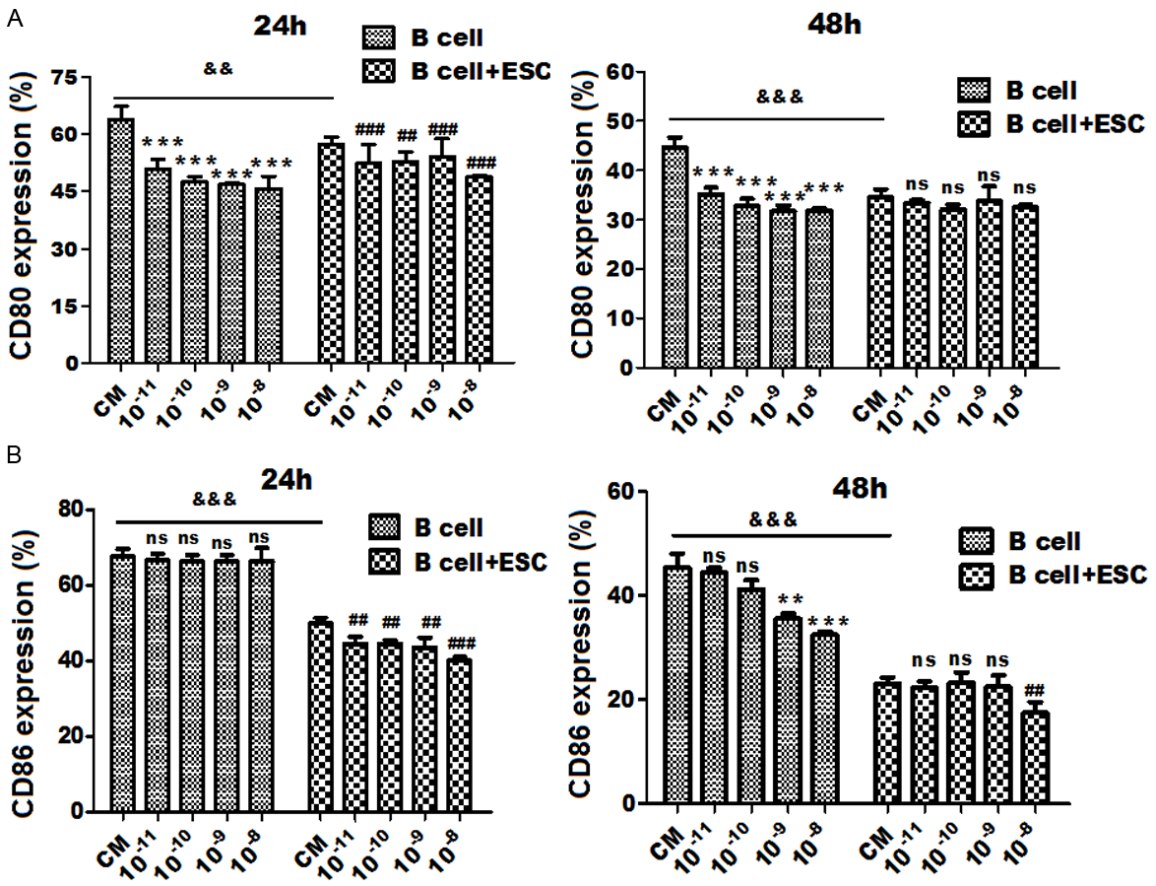

Figure 2. *Both Progesterone and ESCs inhibit the expression of CD80 and CD86 on B cells*. Splenic lymphocytes were incubated with progesterone at the different concentration (0, 10^{-11}, 10^{-10}, 10^{-9} or 10^{-8} M) and co-cultured with or without ESCs for 24h or 48h. Flow cytometry was performed to analysis the expression of CD80 (A) and CD86 (B) on B cells. Data are mean±SD. **P*<0.05, ***P*<0.01 or ****P*<0.001 compared to the control; $^{\#\#}P$<0.01 or $^{\#\#\#}P$<0.001 compared to co-culture group without progesterone treatment; $^{\&\&}P$<0.01 or $^{\&\&\&}P$<0.001 compared to B cells alone group.

cells, but not in mature B cells [20, 24, 25]. Hence, we wanted to know whether ESCs and progesterone regulated the differentiation of B cells through changing the expression of CD138. The B220/CD45R-APC positive cells were also set as B cells (**Figure 4A**). We found progesterone alone didn't changed the level of CD138 expression on B cells (*P*>0.05) (**Figure 4B** and **4C**). ESC itself and cooperated with progesterone significantly up-regulated the expression of CD138 (*P*<0.001) (**Figure 4B** and **4C**), however, the stimulatory effect could be partly reduced in B cell-ESC co-culture groups treated with progesterone (*P*<0.05 or *P*<0.001) (**Figure 4B** and **4C**). These results suggested that ESCs might repress the differentiation and maturity of B cells through increasing CD138 expression, and these effects could be partly reduced by progesterone.

ESCs promote the spontaneous antibody secretion of B cells

Finally, we further evaluated the antibody secretion level of B cells treated with progesterone and ESCs. Unlike progesterone, ESCs alone significantly promoted the secretion of the spontaneous antibodies IgG and IgA in the cultured supernatant of B cells (**Figure 5A** and **5B**), and these effect could not be influenced by progesterone.

Discussion

The female reproductive tract has a series of immune cells, and they play an important role in anti-infection immunity and embryonic genital planting. A variety of extra immune system factors, including hormones and none-immune

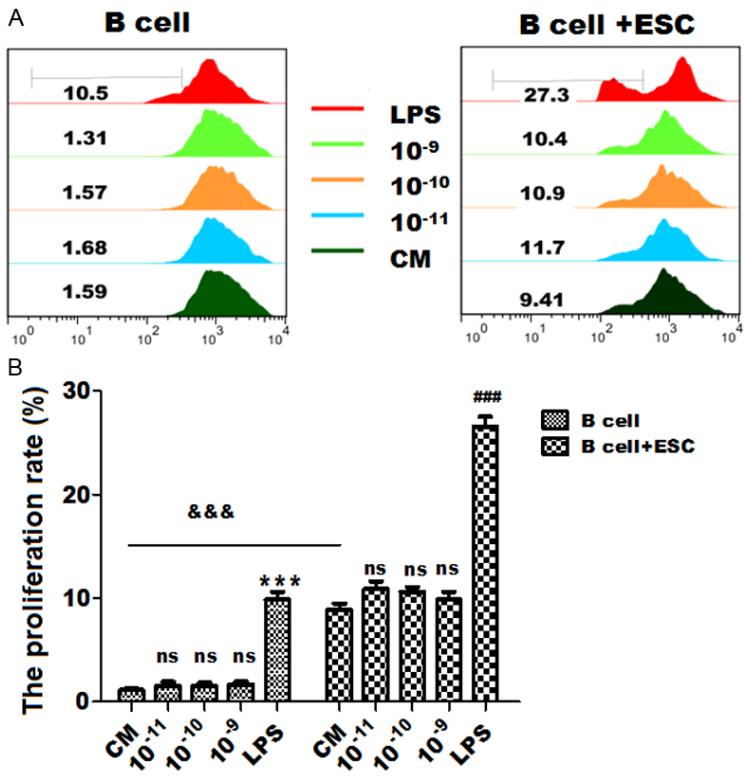

Figure 3. *The proliferation of B cells in splenocytes is promoted by ESCs not progesterone*. Lymphocytes labeled by CFSE with the final working concentration 0.5 μM were cultured in phenol red-free RPMI 1640 medium containing 10% charcoal-stripped fetal bovine serum, and then treated with progesterone at the different concentration (0, 10^{-11}, 10^{-10} or 10^{-9} M) and co-cultured with or without ESCs for72h in darkness. And then flow cytometry was used to analysis the proliferation of B cells. The proliferation histograms (A) and rate (B) of B cell was showed as the proliferation level of B cells. These CFSE histograms from one representative experiment were shown. LPS from Escherichia coli (5 μg/ml) was added as the positive control. Data are mean±SD. These pictures were representatives of three individual experiments. ***P<0.001 compared to the control; $^{###}P$<0.001 compared to co-culture group without progesterone treatment; $^{&&&}P$<0.001 compared to B cells alone group.

cells which are laying in FRT such as endometrial epithelial cell and ESCs also play a critical role in regulating maternal-fetal immunity [17, 26].

It has reported that the immune cells in the local FRT expressed the sex hormone receptors, which further explained that the female reproductive tract has the cross-talking between endocrine, reproductive epithelial, stromal cells and immune cells. Progesterone has been shown to affect immunity in rodents and humans pregnancy. These effects are primarily mediated via the progesterone receptor (PR), which acts as a transcription factor, although non-genomic effects of PR activation have been reported [2, 3, 27]. A randomized clinical trial of progesterone to prevent preterm delivery showed that the prolonged in vivo administration of progesterone minimized the ability of peripheral blood leukocytes to migrate into the uterus during parturition. It suggested that these are putative mechanisms by which progesterone might prevent preterm birth in women at high risk [28]. However, the regulation mechanism for the B cells in FRT is not clear. In our current study, we found mouse B cells also expressed PR. Therefore, we further evaluated the effects of progesterone ranges encompassing physiologic and pharmacologic concentrations and ESCs on B cells to determine whether progesterone and ESCs also played a role in modulating B cells-mediated immune responses.

Firstly, we found that both co-culture with ESCs and treatment with progesterone declined the expression of the co-stimulatory molecules CD80 and CD86 on mouse B cells. Moreover, there were a synergistic effect between ESCs and progesterone for down-regulating the expression of CD80 and CD86 and inactivating B cells. However, the mechanisms of this synergistic action need further research. CD138 (syndecan-1), is a plasma cell marker. B lymphocytes express and lose CD138 at specific stages of differentiation. In this work, we observed that culture with ESCs significantly increased the expression of CD138 on B cells, and this effect could be partly reduced by treatment with progesterone. These finding confirmed that combination of ESCs and progesterone inhibited the activation, reduced the external antigen presenting function including sperm by decreasing the expression of CD80 and CD86, and repressed the differentiation and

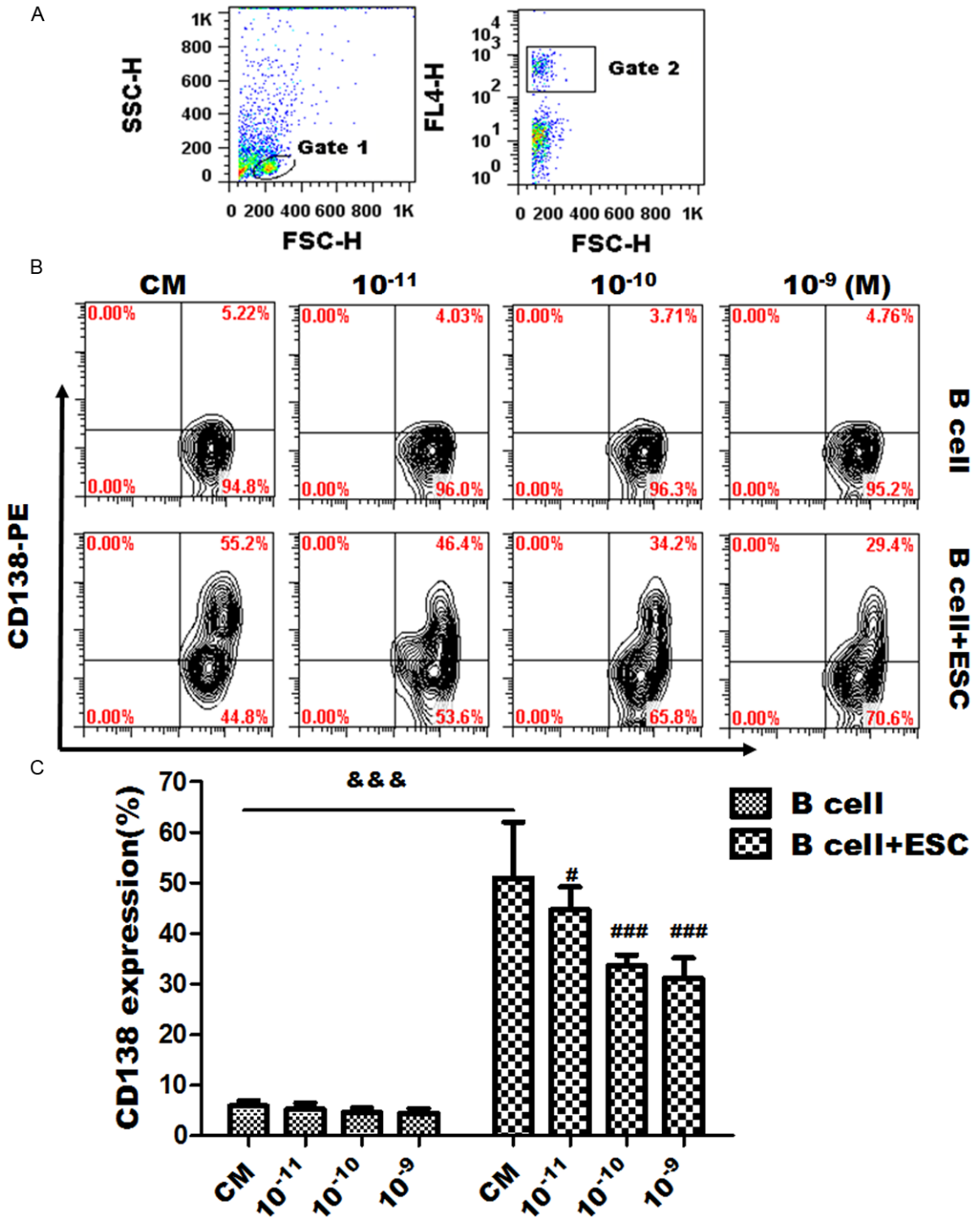

Figure 4. *Progesterone partly weakens the stimulatory effect on CD138 expression of B cells mediated by ESCs.* (A) The strategy of gating was choosing lymphocyte subsets for gate 1. Then FL4 (B220/CD45R-APC) positive cells were B cells (gate 2). After treatment with progesterone and co-culture with ESCs, the expression of CD138 on these B cells was detected by flow cytometry. The representative contours of the each group are shown (B). The histogram showed the differentiation molecule (CD138) of each group (C). Data are mean±SD. $^{\#}P<0.05$ or $^{\#\#\#}P<0.001$ compared to co-culture group without progesterone treatment; $^{\&\&\&}P<0.001$ compared to B cells alone group.

maturity of B cells by up-regulating CD138 expression. The coincident suppression on the immune response may be beneficial for fertilization.

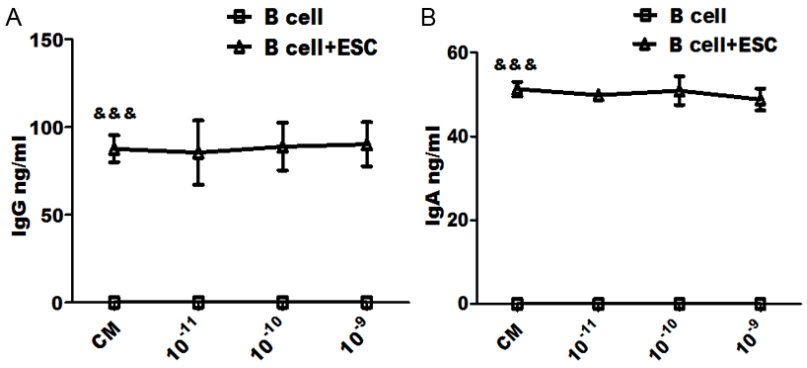

Figure 5. *ESCs promote the spontaneous antibody secretion of B cells.* After treatment with progesterone and co-culture with ESCs, the secretion level of IgG and IgA of B cells was evaluated by ELISA. Data are mean±SD. $^{\&\&\&}P<0.001$ compared to B cells alone group.

In rodents, the available data indicated that immune responses are suppressed in the upper genital tract and enhanced in the lower genital tract during progesterone-associated diestrus. The number and activity of DCs and MHC class II+ cells are minimal in the uterus and maximal in the vagina during diestrus [29]. The mucosa immune system primarily, if not exclusively, produces and secretes IgA and IgM [30]. Most typical external secretions contain secretory immunoglobulin A (S-IgA) as the dominant isotype. As we all know bathing the lining of the lower FRT are cervico-vagina fluids (CVF), composed of vaginal transudate, mucus, and epithelial cell secretions from the cervix, uterus, and fallopian tubes. CVF is known to restrict the infection of target cells by multiple pathogenic organisms including HIV and HSV-2 [31]. One study about the proteome of the human cervical-vaginal fluid (CVF) showed that CVF can be broadly considered a plasma transudate. Although the pattern of protein spots is very similar for all fluids analyzed, a relative overabundance of major plasma proteins are albumin, transferrin, immunoglobulins, apolipoproteins, alpha-1-acid glycoprotein 1, and calgranulins [32]. Some findings indicated that IgG and IgA in CVL can inhibit HIV infection and transmission to women [33]. Meanwhile, it is reported that immunoglobulins and antimicrobials could prevent viable pathogens from infecting the epithelial mucosa interspersed within mucin complexes at an aqueous phase [6]. The mucosal surface of FRT is the primary site of transmission for a plethora of sexually transmitted infections, including human immunodeficiency virus (HIV) [10]. So the antibodies secreted by B cells play an important role in defending the infection of FRT. The antibody response in the mucosa, specifically the genital tract, is characterized by binding and neutralizing IgG and IgA antibodies [10, 30].

In addition, the components of the reproductive tract milieu vary with specific stages of the menstrual cycle. For example, IgG and IgA in cervical mucus both decrease at ovulation but remain elevated during the proliferative and secretory phases of the cycle [34, 35]. Similarly, Mice were given OVA-Ag exposure either during the estrous (estrogen dominant) or diestrous (progesterone dominant) stage of the estrous cycle by vagina. The results shown that the magnitude of immune footpad swelling response was significantly reduced during estrus, while it had no change in diestrous stage. Serum Antibodies titers during estrus and diestrous had no significant change [29].

Then, we further studied the role of ESCs and progesterone in modulating the proliferation and functional antibody secretion, and found that unlike progesterone, ESCs co-culture not only obviously stimulated the proliferation of B cells, but also significantly increased the secretion level of the spontaneous antibodies IgG and IgA in the co-culture unit. Interestingly, these effects could not be changed by progesterone. The current data suggested that ESCs not progesterone may promote the ability of anti-infection of FRT through stimulating the proliferation and antibodies IgG and IgA production.

Collectively, our data showed that progesterone could directly inhibit the co-stimulatory molecules CD80, CD86 expression on B cells, and supress its antigen presentation, but it had no significant effect on its differentiation, proliferation and antibody secretion abilities. It suggested that progesterone in the reproductive tract mainly inhibited the antigen presentation function of B cells, and this might be conducive to the establishment of fertilization and preg-

nancy. While, besides inhibition on the activation and CD138 expression of B lymphocytes in co-culture group, ESCs could enhance the proliferation and antibody secretion capabilities of B lymphocytes. Meanwhile progesterone exerted its inhibition on B lymphocyte differentiation through ESCs. So both progesterone and ESCs took part in the process of depressing sperm antigen presentation mediated by immune cells, which restrained the following immune response and contributed to fertilization. Furthermore, the local mucosal ESCs significantly promoted the proliferation, differentiation of B lymphocytes, then enhanced mucosal immunity which resists microorganisms invade and prevented the genital tract infection. These results suggested that ESCs play a key regulatory role in the control of IgA and IgG spontaneous secretions. The increased antibodies in cervical-vagina fluids (CVF) might take part in the resisting infections during pregnancy. So ESCs are recognized as essential for the establishment and maintenance of pregnancy, as well as regulation of the B cells in the presence of a conceptus.

Under hormonal and the local environment of FRT, B cells participate in architectural changes that occur during the menstrual cycle. At the time of fertilization, antigen presentation throughout the female reproductive tract is dampened to optimize conditions for implantation and pregnancy, while the enhanced antibodies secretion can be helpful for defending infection.

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Disclosure of conflict of interest

None.

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