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Inhibitory Effects of Progesterone Differ in Dendritic Cells from Female and Male Rodents

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Abstract

Background—Steroid hormones, such as progesterone, are known to have immunomodulatory effects. Our research group previously reported direct effects of progesterone on dendritic cells (DCs) from female rodents. Primarily affecting mature DC function, progesterone effects included inhibition of proinflammatory cytokine secretion, downregulation of cell surface marker (major histocompatibility complex class II, CD80) expression, and decreased T-cell proliferative capacity, and were likely mediated through progesterone receptor (PR) because the PR antagonist RU486 reversed these effects.

Objective—The goal of this study was to assess differences in response to progesterone by DCs from female and male rodents.

Methods—Using real-time reverse-transcriptase polymerase chain reaction, transcriptional expression of steroid hormone receptors was measured in immature bone marrow-derived DCs (BMDCs) from male and female rats. Expression of steroid hormone receptor protein was also assessed in these cells using flow cytometry and fluorescence microscopy. To evaluate functional differences between BMDCs from female and male rats in response to the steroid hormone progesterone, levels of secreted cytokines were measured using enzyme-linked immunosorbent assay.

Results—Higher numbers of immature BMDCs from males expressed glucocorticoid receptor (GR) and androgen receptor (AR) proteins compared with females (males vs females, mean [SD]: GR = 68.75 [7.27] vs 43.61 [13.97], $P = \text{NS}$; AR = 75.99 [15.38] vs 8.25 [1.88], $P = 0.002$), whereas higher numbers of immature BMDCs from females expressed PR protein compared with males (females vs males: PR = 74.19 [12.11] vs 14.14 [4.55], $P = 0.043$). These differences were not found at the level of transcription (females vs males: GR = 0.088 vs 0.073, $P = \text{NS}$; AR = 0.076 vs 0.069, $P = \text{NS}$; PR = 0.075 vs 0.065, $P = \text{NS}$). Compared with those from females, mature BMDCs from males produced higher quantities of cytokines (tumor necrosis factor- α [TNF- α], interleukin [IL]-1 β , IL-10) (females vs males: TNF- α = 920.0 [79.25] vs 1100.61 [107.97], $P = \text{NS}$; IL-1 β = 146.60 [38.04] vs 191.10 [10.47], $P = \text{NS}$; IL-10 = 167.25 [4.50] vs 206.15 [23.48], $P = \text{NS}$). Conversely, BMDCs from females were more sensitive to progesterone, as indicated by a more dramatic reduction in proinflammatory cytokine secretion (females vs males, highest concentration of progesterone: TNF- α = 268.94 [28.59] vs 589.91 [100.98], $P = 0.04$; IL-1 β = 119.50 [10.32] vs 154.35 [6.22], $P = \text{NS}$).

Conclusions—These findings suggest that progesterone effects on DCs in rodents may be more pronounced in females than in males, and this is likely due to differences in PR protein expression. Our observations may help elucidate disparities in the incidence and severity of autoimmune disorders between females and males, and the role specific steroid hormones play in regulating immune responses.

Keywords

steroid hormone receptors; immunomodulation; cytokines; gender differences

INTRODUCTION

Compared with males, females have a 2- to 10-fold higher incidence of autoimmune/inflammatory diseases.¹ This suggests a role for sex hormones, such as progesterone, which have been reported to modify immune responses and thus may contribute to autoimmune and other disease development.² The role of sex hormones in immunity has been extensively studied in the context of pregnancy, during which T-helper 1 (T_H1)-related auto-immune diseases (eg, rheumatoid arthritis, multiple sclerosis) tend to improve, whereas T_H2-related diseases (eg, systemic lupus erythematosus) tend to worsen.³ Dendritic cells (DCs) can drive a variety of immune responses, including T_H1, T_H2, and tolerogenic responses.⁴ Therefore, DCs may be a primary target of sex hormone actions in regulating immunity.

DCs are potent antigen-presenting cells important in both innate and adaptive immunity.⁵ These cells have 2 major functional states: immature for antigen processing and mature for antigen presentation. Immature DCs are able to efficiently process antigenic peptides and are believed to be prominent in initiating tolerogenic responses.^{6,7} Activation factors, such as the bacterial cell wall component lipopolysaccharide (LPS), are agents commonly used for promoting DC maturation. When stimulated, mature DCs are able to secrete copious amounts of cytokines that induce inflammatory immune responses, including tumor necrosis factor- α (TNF- α) and interleukin-1 β (IL-1 β).^{8,9} Mature DCs also express high levels of peptide in the context of major histocompatibility complex (MHC) molecules and costimulatory molecules, such as CD80 and CD86, on their surface. Expression of these molecules on DCs provide the appropriate signals to stimulate naive T lymphocytes and thereby direct potent T_H1 or T_H2 immune responses.^{4,10–12}

DCs are considered crucial to the amelioration of several disease states.^{13–15} They are able to initiate strong immune responses against pathogens^{16,17} and are actively being studied for use in vaccine development.^{18–20} In addition, these cells have been found to be important for modifying immune responses in a variety of autoimmune disorders.²¹ Because the incidence of autoimmune diseases has been reported to occur as much as 10-fold more frequently in females than in males, this would suggest a role for steroid hormones in regulating DC activity in these diseases.²

Progesterone is a steroid hormone primarily produced by granulosa cells in follicles and corpus luteum of the ovary.²² It is also produced in adrenal glands and other tissues, and serves as an intermediate to synthesis of all other steroid hormones. It is believed to be critical for ovulation²³ and activation of uterine cells,²⁴ and to be necessary in establishment and maintenance of pregnancy.^{22,25} Progesterone secretion is initiated by export of gonadotropin-releasing hormone (GnRH) from the hypothalamus that binds to GnRH receptors on cells of the anterior pituitary gland. This leads to the release of the gonadotropins (follicle-stimulating hormone and luteinizing hormone) that induce granulosa cell production of estrogen and progesterone, respectively, within the ovary.²⁶ Progesterone mediates its actions through binding to progesterone receptor (PR) in the cytoplasm of cells,

dimerizing and translocating to the nucleus to modify expression of PR-specific target genes.²⁷ Other steroid hormones, such as estrogen, may stimulate expression of PR in cells. This estrogen-induced increase in PR expression can serve to amplify effects of progesterone and act as a positive feedback mechanism when estrogen is used in combination.²⁸

Several studies have reported that progesterone also has immunomodulatory effects²⁹ and is generally immune suppressive. It has been shown to increase the number of Langerhans cells in human vaginal epithelium,³⁰ which may account for its role in contributing to women's susceptibility to HIV.³¹ Progesterone has also been found to have direct effects on T lymphocytes at concentrations consistent with pregnancy, suggesting a role in preventing maternal adaptive immune responses against fetal antigens.³² Our research group previously reported direct effects of progesterone on DCs from female rats and that treatment of bone marrow-derived DCs (BMDCs) with progesterone led to significant inhibition ($P < 0.05$) of proinflammatory cytokine (TNF- α , IL-1 β) secretion, downregulation of DC-associated activation markers (MHC class II, CD80), and a reduced capacity to stimulate proliferation of T lymphocytes.³³ These results were likely mediated through the receptor for progesterone, because the PR antagonist RU486 was able to reverse these effects. Similar results on DC function were observed using human chorionic gonadotropin, a placental hormone that induces production of progesterone.³⁴ Other groups have also reported immune-suppressive effects on macrophages after treatment with progesterone or progesterone metabolites, such as 5 α -3 α -tetrahydroprogesterone (THP).³⁵ However, there are currently no reports on the effects of THP on BMDC function.

The goal of this study was to evaluate differences in response to progesterone in DCs from female and male rodents.

METHODS

Animals

Female and male Fischer (F344/NHsd) rats, 8 to 11 weeks old, were obtained from Harlan Sprague Dawley, Inc. (Indianapolis, Indiana). Animals were maintained in pathogen-free facilities, and all procedures were performed using approved protocols in accordance with the National Institute of Mental Health's Animal Care and Use Committee.

Reagents

Recombinant granulocyte-macrophage colony-stimulating factor (rGM-CSF) and interleukin-4 (rIL-4) were obtained from PeproTech Inc. (Rocky Hill, New Jersey). Progesterone, fluorescein isothiocyanate (FITC)-conjugated dextran, propidium iodide, LPS from *Escherichia coli*, and RU486 (mifepristone) were purchased from Sigma-Aldrich Company (St. Louis, Missouri).

Antibodies

Purified antibodies that recognize and bind to amino acid residues 346–367 of the rat glucocorticoid receptor (GR) (100 μ g/mL, diluted 1:5000); amino acid residues 321 to 572 of the rat androgen receptor (AR) (100 μ g/mL, diluted 1:5000); and amino acid residues 533 to 547 of the rat PR (100 μ g/mL, diluted 1:5000) were purchased from Affinity Bioreagents, Inc. (Golden, Colorado). Phycoerythrin (PE)-conjugated antibodies to rat CD4 (Clone OX35; 0.1 mg/mL) and CD80 (Clone 3H5; 0.1 mg/mL), peridinin chlorophyll protein (PerCP)-conjugated antibodies to MHC class II RT1B (Clone OX6; 0.1 mg/mL), and FITC-labeled secondary antibodies recognizing mouse and rabbit antibodies were purchased from BD Biosciences (San Jose, California). FITC-conjugated or PE-conjugated antibodies to rat

CD11c (0.2 mg/mL) were obtained from eBioscience, Inc. (San Diego, California). Isotype control antibodies included the following: purified mouse immunoglobulin G (IgG) (PR control) (0.1 mg/mL, diluted 1:5000; BD Biosciences); purified rabbit IgG (GR, AR control) (1 mg/mL, diluted 1:50,000; R&D Systems Inc., Minneapolis, Minnesota); PE-conjugated mouse IgG1 (CD4, CD80 control) or PerCP-conjugated mouse IgG1 (RT1B control; 0.1 mg/mL; BD Biosciences); and Armenian hamster IgG (CD11c control; 0.2 mg/mL; eBioscience).

Isolation of Bone Marrow and Spleen Tissue

Animals were sacrificed by decapitation to obtain femurs, tibias, and spleen tissues in RPMI 1640 (Mediatech, Inc., Herndon, Virginia) containing 10% charcoal-stripped serum (CSS) (Biomed Corporation, Foster City, California), and 2% L-glutamine and 2% penicillin-streptomycin (both from Sigma-Aldrich), henceforth referred to as conditioned medium. CSS was used as a replacement for fetal bovine serum, because some components of serum have been shown to have hormone-mimicking properties. Muscle and connective tissue were removed from bones, and bone marrow cells were flushed out with IX PBS (pH 7.4). Bone marrow cells were passed through a cell strainer (70 μ m; BD Biosciences) to remove debris. Red blood cells of bone marrow and spleen tissue were lysed with ACK lysis buffer (BioWhittaker Inc., Walkersville, Maryland) containing ammonium hydroxide.

Generation of Bone Marrow-Derived Dendritic Cells

BMDCs were generated as previously described,³³ with slight modifications. Briefly, bone marrow cells (10×10^6) were plated in 6-well plates with conditioned medium supplemented with rGM-CSF (20 ng/mL) and rIL-4 (50 ng/mL) (both from PeproTech) at a total volume of 3 mL. On day 2, nonadherent cells were removed, and adherent cells were washed with IX PBS. Fresh conditioned medium with rGM-CSF and rIL-4 was added to each well. Cultures were fed with cytokines in conditioned medium (100 μ L total volume) on day 4. By day 5, semiadherent aggregates of cells had formed. Noted culturing conditions were used for all subsequent experiments.

Isolation of Splenic Dendritic Cells and T Lymphocytes

Single-cell suspensions of splenocytes were isolated using the Miltenyi Biotec magnetic bead-based cell isolation method, according to the manufacturer's instructions. Briefly, to obtain splenic DCs, cells were labeled with magnetic bead-conjugated antibodies recognizing rat MHC class II molecule RT1B and passed through a magnetic column to obtain cells that express this cell surface marker. RT1B⁺ cells were then labeled with bead-conjugated antibodies recognizing CD11c and passed through magnetic columns to isolate splenic DCs (CD11c⁺RT1B⁺). To obtain T lymphocytes, cells from spleens were labeled with magnetic bead-conjugated antibodies recognizing rat T-cell molecule OX52 and passed through a magnetic column for positive selection of splenic T lymphocytes. Cell isolation was verified by flow cytometry and found to have a purity of >89% for DCs (CD11c⁺) and T cells (CD3⁺).

RNA Isolation and Real-Time Reverse Transcriptase Polymerase Chain Reaction

Total RNA from magnetic bead-isolated or cultured cells was isolated using Trizol reagent (Invitrogen Corporation, Eugene, Oregon), and further purified through 2-propanol and graded ethanol. RNA was quantified using the NanoDrop Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware). cDNA was generated by reverse transcription using 200 ng of total RNA per sample and the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Inc., Hercules, California). Real-time reverse-transcriptase polymerase chain

reaction was performed with iQ5 Real-Time PCR Detection System using iQSYBR Green Supermix as a reporter dye (Bio-Rad).

The following primer sequences were used:

18S (Fwd - CCAGTAAGTGC GGTCATAAGC,
Rev - CCATCCAATCGGTAGTAGCGAC);
GR (Fwd - TGATGGGAATGACTTGGGC,
Rev - TTGGGAAACTCCTTCTCTGTCGGG);
AR (Fwd - ACCCTCCCATGGCACATTTT,
Rev - TTGGTTGGCACACAGCACAG);
PR-A+B (Fwd - CTTTGTTTCCTCTGCAAAAATTG,
Rev - GTATACACGTAAGGCTTTCAGAAGG).

Expression of the target gene was normalized with respect to 18S, which served as a control gene. Relative fold induction was determined using the $2^{-\Delta\Delta C_t}$ method, as previously reported.³³

Analysis of Hormone Receptor Expression

Immature cultured DCs (1×10^6 /tube) were collected into polystyrene Falcon tubes (BD Biosciences) and washed with fluorescence-activated cell sorter (FACS) buffer containing $1 \times$ PBS (Quality Biological, Inc., Gaithersburg, Maryland), 2% CSS (Biomedex), and 0.2% sodium azide (Sigma-Aldrich). Cells were centrifuged for 5 minutes at 2000 rpm followed by supernatant removal to prepare for cell labeling. Cells were mixed with 10 μ L FITC-labeled anti-rat CD11c (eBioscience) and 10 μ L PerCP-labeled anti-rat MHC Class II RT1B (BD Pharmingen, San Diego, California) on ice for ~20 minutes. Cells were washed with FACS buffer to remove excess antibody and centrifuged for 5 minutes at 2000 rpm. Supernatant was removed by decanting, and cells were then treated with Cytofix/Cytoperm solution (BD Biosciences) for 20 minutes on ice to permeabilize cells, followed by washing with Cytofix/Cytoperm buffer to remove excess solution and 5-minute centrifugation. Cells were mixed with 10 μ L of serum for 10 minutes on ice to prevent nonspecific binding of antibodies to intracellular proteins. Ten μ L of purified antibodies to rat GR, AR, or PR (all from Affinity Bioreagents) or appropriate isotype control were added to tubes for 10 minutes on ice. Fluorochrome-conjugated secondary antibody (goat anti-mouse or goat anti-rabbit) conjugated to PE was then added to each tube for an additional 10 minutes on ice. Experiments included analysis of 1 steroid hormone receptor expressed by cultured cells in each tube. Cells were collected into a BD FACSCalibur (BD Biosciences) and analyzed with FlowJo analysis software (Tree Star, Inc., Ashland, Oregon).

Hormone Treatment and Stimulation of Bone Marrow Cultures

Cells were treated (day 6) with varying doses of progesterone dissolved in ethanol. After a 2-hour hormone treatment, LPS from *E coli* (5 μ g/mL) was added to cultures. Cells not treated with LPS (remaining in immature state) were used as a control for maturation. Cells treated with ethanol alone (data not shown) or hormone alone served as a control for LPS treatment. Some LPS-stimulated cultures received the PR antagonist RU486 (10^{-8} M) to determine the role of the PR on the effects of progesterone. Cultures remained incubated up to 48 hours at 37°C/5% CO₂ to assess mature DC function.

Antigen Uptake

Immature DCs were cultured in temperature-sensitive, 96-well RepCell plates (CellSeed, Inc., Tokyo, Japan) after treatment with varying doses of progesterone and 100 ng/mL FITC-dextran for 2 hours at 37°C/5% CO₂. Treatment of cells without progesterone or with ethanol alone (data not shown) served as a control experiment for the effects of progesterone. Cells were refrigerated (4°C) for 30 minutes to facilitate cell detachment from temperature-sensitive plates and collected (1×10^6 /tube) into polystyrene Falcon tubes (BD Biosciences) for analysis of antigen uptake using a BD FACSCalibur (BD Biosciences) and FlowJo software (Tree Star).

Cytokine Analysis

After treatment with progesterone, RU486, LPS, or a combination, supernatants (140 μ L total volume per condition) were collected from cultured cells. TNF- α , IL-1 β , and IL-10 secretion was determined using the SearchLight multiplex array analysis service (Pierce Biotechnology, Inc., Woburn, Massachusetts). Previous time-course studies reported maximal cytokine production at 48 hours after LPS stimulation.³⁶

Statistical Analysis

For all statistical analyses, the level of significance was set at a probability of ≤ 0.05 to be considered significant. Data are presented as mean (SD) values. Analysis of variance and *t* tests were used to analyze data. Sample size for each set of experiments was determined as previously reported.³³

RESULTS

Expression of Steroid Hormone Receptors in Female and Male BMDCs

As shown in Figure 1, transcriptional expression of steroid hormone receptors (glucocorticoid, androgen, and progesterone) by immature DCs generated from bone marrow cells as well as DCs and T lymphocytes isolated from splenic tissues was observed in cells from both female and male rats ($n = 9$ each). No significant differences were noted in transcription of steroid hormone receptors by DCs between the sexes. Although splenic DCs displayed numerically higher levels of steroid hormone receptor transcription compared with BMDCs, this was not statistically significant. Similarly, no statistically significant differences were observed in transcriptional expression of steroid hormone receptors in T lymphocytes from females and males.

We also examined expression of steroid hormone receptor protein in immature BMDCs from female and male rats to identify possible differences between transcriptional and translational regulation of steroid hormone receptor expression in these cells. Higher proportions of immature BMDCs from males expressed GR and AR protein compared with immature BMDCs from females (males vs females, mean [SD]: GR = 68.75 [17.27] vs 43.61 [13.97], $P = \text{NS}$; AR = 75.99 [15.38] vs 8.25 [1.88], $P = 0.002$), whereas higher proportions of immature BMDCs from females expressed PR protein compared with immature BMDCs from males (females vs males: PR = 74.19 [12.11] vs 14.14 [4.55], $P = 0.043$), as measured by flow cytometry (Table I, $n = 10$ each; Figure 2A, $n = 7$ each). Using fluorescence microscopy, we observed that steroid hormone receptor expression was primarily found in the cytoplasm of cells (Figure 2B). Although higher numbers of immature BMDCs from males expressed GR, analysis of mean fluorescent intensity (assesses amount of protein or receptor expressed by an individual cell) indicated no significant difference in the amount of GR expressed by immature BMDCs from female and male rats (females vs males: GR = 194.14 [38.75] vs 227.57 [29.01], $P = \text{NS}$). However, immature BMDCs from males expressed higher levels of AR in their cytoplasm, and

immature BMDCs from females expressed significantly higher levels of PR in their cytoplasm (females vs males: AR = 112.11 [25.73] vs 358.52 [77.62], $P = \text{NS}$; PR = 386.75 [66.37] vs 125.99 [36.26], $P = 0.038$).

Antigen Uptake by Immature BMDCs from Male and Female Rats

Table II shows measurement of antigen uptake capacity by immature BMDCs from male and female rats after culture with FITC-dextran (100 ng/mL) and treatment with progesterone at varying concentrations, as assessed by flow cytometry ($n = 5$ each). Similar numbers of immature BMDCs from both sexes were able to take up antigenic peptide, although there was a slightly higher number of immature BMDCs from male rats that took up antigen (females vs males, mean [SD]: control/no treatment = 39.75 [4.23] vs 45.08 [2.95], $P = \text{NS}$). Treatment with progesterone did not alter antigen uptake ability by BMDCs from female or male rats. Similar numbers of immature BMDCs from female rats took up antigenic peptide with increasing concentrations of progesterone compared with untreated/control cultures (control vs progesterone-treated, highest dose: 39.75 [4.23] vs 45.05 [5.35], $P = \text{NS}$). A significantly higher number of immature BMDCs from male rats were also able to take up antigen with increasing doses of progesterone (control vs progesterone-treated, highest dose: 45.08 [2.95] vs 64.50 [5.31], $P = 0.036$). The number of immature BMDCs taking up antigen was statistically significant between female and male rat cultures only at the highest concentration of progesterone used.

Inhibition of Proinflammatory Cytokine Production

As shown in Figure 3 and Figure 4, we measured proinflammatory cytokine secretion (TNF- α , IL-1 β) by LPS-matured BMDCs from male and female rats using enzyme-linked immunosorbent assay ($n = 8$ each). Following LPS stimulation, higher concentrations of both TNF- α and IL-1 β were secreted by BMDCs from male rats than from female rats (females vs males, mean [SD]: TNF- α = 920.0 [79.25] vs 1100.61 [107.97], $P = \text{NS}$; IL-1 β = 146.60 [38.04] vs 191.10 [10.47], $P = \text{NS}$). We next examined effects of progesterone treatment on cytokine production by BMDCs from female and male rats. Although LPS-stimulated BMDCs from males secreted higher levels of TNF- α and IL-1 β compared with those from females, LPS-stimulated BMDCs from females exhibited greater sensitivity to progesterone treatment, indicated by a more dramatic decrease in cytokine production after treatment with progesterone (females vs males, highest concentration of progesterone: TNF- α = 268.94 [28.59] vs 589.91 [100.98], $P = 0.04$; IL-1 β = 119.50 [10.32] vs 154.35 [6.22], $P = \text{NS}$). Cells from female rats exhibited a dose-dependent reduction in secretion of both TNF- α (50% inhibitory concentration [IC₅₀] = 4.2×10^{-8}) and IL-1 β (IC₅₀ = 3.5×10^{-7}) that reached levels similar to those of the control (untreated) BMDCs. LPS-stimulated BMDCs from male rats treated with progesterone did not reach baseline concentrations at the doses of progesterone used; therefore, IC₅₀ values were not calculated. To determine whether effects of progesterone treatment on mature BMDC cytokine secretion were mediated through PR, we added the PR antagonist RU486 to LPS-stimulated BMDC cultures from female and male rats treated with progesterone. A reversal of the inhibitory effects of progesterone on LPS-stimulated BMDC secretion of TNF- α and IL-1 β secretion was noted after treatment with RU486 (females, highest concentration of progesterone vs highest concentration of progesterone with RU486: TNF- α , $P = 0.017$; IL-1 β , $P = 0.025$; males, highest concentration of progesterone vs highest concentration of progesterone with RU486: TNF- α , $P = \text{NS}$; IL-1 β , $P = \text{NS}$).

We also measured IL-10 (produced at elevated concentrations in T_H2-related autoimmune diseases such as systemic lupus erythematosus) as shown in Figure 5 ($n = 6$ each) and found LPS-stimulated BMDCs from male rats secreted slightly higher levels compared with LPS-stimulated BMDCs from female rats (females vs males, mean [SD]: IL-10 = 167.25 [4.50]

vs 206.15 [23.48], $P = \text{NS}$). We also examined IL-10 secretion by LPS-stimulated BMDCs from each group after treatment with progesterone and found that progesterone treatment of cells from female and male rats led to modest increases in IL-10 secretion that were not statistically significant.

DISCUSSION

In the current study, we investigated progesterone effects on BMDCs from female and male rats, and found that BMDCs from both groups expressed steroid hormone receptors. Therefore, steroid hormones may potentially have a direct effect on activity of these cells. Although BMDCs from female and male rats expressed PR, our observations indicated that BMDCs from female rats were significantly more sensitive to progesterone suppression compared with male rats. We also found significantly greater proportions of immature BMDCs expressing PR protein as well as significantly higher numbers of PR expressed per cell in BMDCs from females compared with those from males. This might account for the greater BMDC sensitivity to progesterone by female rats. However, other factors unrelated to progesterone and its receptors could also account for these differences.

Although sexual dimorphism has been reported in prepubertal rats in terms of insulin sensitivity,³⁷ blood–brain barrier permeability,³⁸ and epinephrine-induced expression of the β_2 -adrenergic receptor activity,³⁹ other studies evaluating inflammatory responses have identified gender differences only after the onset of puberty.^{1–3,40} Therefore, sex hormones may mediate differences in immune cell activity between females and males. Studies using humans have shown similar results, with more pronounced gender differences after the onset of puberty.^{27,31,41} In addition, women with polycystic ovarian syndrome (PCOS) and low progesterone levels have been found to have increased prevalence of autoimmune thyroiditis,⁴² suggesting a role for progesterone in regulating immunity. Auto-antibody production has also been reported in females with PCOS⁴³ and therefore might predispose these individuals to antibody-dependent autoimmune/inflammatory disorders, such as systemic lupus erythematosus.

We were not able to detect significant differences in transcriptional expression of steroid hormone receptors (GR, AR, PR) between immature BMDCs from female and male rats. We also examined expression of these receptors by freshly isolated DCs and T lymphocytes from spleens of female and male rats and found no significant differences. Therefore, we concluded these results were likely not due to culture conditions. We were able to identify differences in expression of steroid hormone receptor protein in BMDCs from females and males. Although not statistically significant, higher proportions of immature BMDCs from male rats expressed GR and AR compared with female rats. There were also significantly higher numbers of AR receptors expressed in the cytoplasm of these cells. Significantly higher proportions of immature BMDCs from females expressed PR compared with those from males, and the amount of PR in the cytoplasm of individual cells was also significantly higher. This discrepancy between gene and protein expression suggests that differences in steroid hormone receptor expression between immature BMDCs from female and male rats may occur at the level of translation.

We also assessed the functional capacity of BMDCs from female and male rats and found more dramatic differences between these groups after maturation. LPS-stimulated BMDCs from males secreted higher levels of the proinflammatory cytokines TNF- α and IL-1 β , as well as slightly higher concentrations of the T_H2 response-promoting and T_H1 response-inhibiting cytokine IL-10 compared with BMDCs from females. We treated cultured mature BMDCs with progesterone to investigate differences between female and male rats and found that BMDCs from females were more sensitive to progesterone suppression of

proinflammatory cytokine production compared with those from males. Inhibition of cytokine secretion was dose dependent, and these effects were likely mediated through PR, because treatment with the PR antagonist RU486 reversed the inhibitory effects of progesterone.

In contrast, progesterone had a less potent effect on the ability of immature BMDCs to take up antigen. Although not statistically significant, a slightly higher proportion of BMDCs from male rats were able to take up antigen compared with those from female rats. BMDCs from both groups treated with progesterone were able to maintain their antigen uptake capacity, and a statistically significant difference in antigen uptake was identified only at the highest concentration (pharmacologic dose). Because progesterone is a precursor molecule to the steroid hormone testosterone, it is possible that effects at the higher doses may be due to conversion to testosterone and its actions on AR, which were significantly higher in immature BMDCs from male rats. In addition to the effects of progesterone on immature BMDC ability to take up antigen, treatment of mature BMDCs with progesterone had little effect on production of IL-10 by these cells. The less dramatic effect of progesterone on immature BMDC antigen uptake and IL-10 production may be related to specific genes modified by PR when it functions as a transcription factor.

There are some limitations to this study. Hormone levels in females vary throughout the menstrual (human) and estrus (rodent) cycle. Therefore, it is possible that steroid hormone receptor levels change throughout the cycle as well. In addition, the specific mechanism by which progesterone mediates its effects on DC function was not assessed in this study. It is possible that progesterone could carry out its role through changes in expression of target genes such as TNF- α , a known target gene for PR. It is also possible that progesterone binding to its receptor (PR) could be modifying activity of other intracellular proteins, such as NF- κ B or other transcription factors. Moreover, progesterone is a metabolite of other steroid hormones and could be further processed into estrogens or androgens that are known to modulate immune cell function. Lastly, other factors may contribute to sex-specific differences in incidence of autoimmune diseases, including behavior and environmental exposures, which may also be important to consider when evaluating differences in progesterone effects on DCs from females and males.^{44,45}

CONCLUSIONS

Our findings indicate that progesterone had greater effects on mature BMDCs from female rats compared with male rats, especially as it related to inhibition of proinflammatory cytokine production. LPS-stimulated BMDCs from females were more sensitive to progesterone and experienced a more dramatic reduction in proinflammatory cytokine secretion with treatment. Progesterone treatment did not dramatically alter antigen uptake capacity of immature BMDCs from female or male rats and did not have a significant effect on T_H2 response promoting IL-10 secretion by mature BMDCs. This suggests progesterone may be important in shifting DC-mediated immunity away from T_H1 responses in females as well as during pregnancy and may play a prominent role in regulation of immune responses in females. Therefore, progesterone and PR agonists could be useful agents for ameliorating T_H1-related autoimmune/inflammatory disease in women. Conversely, reduced capacity to respond to progesterone, such as downregulation of PR or exposure to PR antagonists, could be a mechanism for dysregulation of immune responses in females and serve to increase susceptibility to autoimmune/inflammatory diseases.

It will be important to understand the mechanisms by which steroid hormones play a regulatory role in DC function, and how this relates to initiation or severity of autoimmune/inflammatory disease in women and men.

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Figure 1.

Transcriptional expression of steroid hormone receptor by immune cells from female and male rats ($n = 9$ each). Total RNA from cultured cells was isolated and analyzed for expression of the steroid hormone receptor genes for glucocorticoid (GR), androgen (AR), and progesterone (PR). Total RNA from CD11^c dendritic cells (DCs) and T lymphocytes was also isolated from splenic tissue for comparison to bone marrow–derived DCs (BMDCs). Values are presented as the mean (SD) of the relative fold induction of the specified gene.

Figure 2.

Expression of steroid hormone receptor protein by immature bone marrow–derived dendritic cells (BMDCs) from female and male rats ($n = 7$ each). (A) Representative histograms show proportions of immature BMDCs expressing receptors for glucocorticoid (GR), androgen (AR), and progesterone (PR). (B) Representative fluorescent micrographs show immature BMDC expression of steroid hormone receptor (red) and were counterstained (blue) with DAPI (4'-6-diamidino-2-phenylindole) to identify nuclei of cells.

Figure 3. Production of proinflammatory cytokine tumor necrosis factor- α by lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells from female and male rats ($n = 8$ each). Cells were stimulated to maturity with LPS and treated with progesterone or a combination of progesterone and the progesterone receptor antagonist RU486. Values are presented as mean (SD). * $p \leq 0.05$ versus LPS alone.

Figure 4. Production of proinflammatory cytokine interleukin-1 β by lipopolysaccharide (LPS)-stimulated bone marrow-derived dendritic cells from female and male rats ($n = 8$ each). Cells were stimulated to maturity with LPS and treated with progesterone or a combination of progesterone and the progesterone receptor antagonist RU486. Values are presented as mean (SD). * $p \leq 0.05$ versus LPS alone.

Figure 5. Production of T-helper 2 response-promoting cytokine interleukin-10 by lipopolysaccharide (LPS)-stimulated bone marrow–derived dendritic cells from female and male rats (n = 6 each). Cells were stimulated to maturity with LPS and treated with progesterone or a combination of progesterone and the progesterone receptor antagonist RU486. Values are presented as mean (SD).

Expression of steroid hormone receptor protein by bone marrow–derived dendritic cells from female and male rats (n = 10 each). Values shown include the mean (SD) proportion of total **CD11 c**⁺ cells expressing specified steroid hormone receptor and the mean fluorescent intensity (MFI) of fluorochrome-conjugated antibody, which indicates the number of receptors expressed by individual cells.

Table 1

Steroid Hormone Receptor	% Expression (Female)	% Expression (Male)	P	MFI (Female)	MFI (Male)	P
Glucocorticoid	43.61 (13.97)	68.75 (17.27)	0.471	194.14 (38.75)	227.57 (29.01)	0.896
Androgen	8.25 (1.88)	75.99 (15.38)	0.002	112.11 (25.73)	358.52 (77.62)	0.144
Progesterone	74.19 (12.11)	14.14 (4.55)	0.043	386.75 (66.37)	125.99 (36.26)	0.038

Table II

Effects of progesterone (Prg) on antigen uptake by bone marrow–derived dendritic cells (BMDCs) from male and female rats ($n = 5$ each). Data are presented as the mean (SD) proportion of CD11 c⁺ cells expressing fluoroisothiocyanate-conjugated dextran.

Condition	% BMOc Antigen Uptake (Female)	%BMOc Antigen Uptake (Male)	<i>P</i>
Control	39.75 (4.23)	45.08 (2.95)	0.48
Prg 10 ⁻¹¹	31.47 (1.12)	40.79 (1.97)	0.69
Prg 10 ⁻¹⁰	37.30 (4.32)	43.96 (3.08)	0.57
Prg 10 ⁻⁹	44.34 (3.43)	52.90 (1.79)	0.28
Prg 10 ⁻⁸	45.05 (5.35)	64.5 (5.31)	0.05