Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone


Introduction

From conception to senescence, women have a significant survival advantage.1 Men are more susceptible to sepsis and subsequent morbidity and mortality than women of reproductive age.2,4 The incidence of sepsis in postmenopausal women increases to levels almost equal to those seen in age-matched men.5,6 The exact mechanism mediating this sexual dimorphism is unclear. However, female sex hormones have been implicated because they modulate the immune system under normal and stress conditions.7 Following trauma or hemorrhage, female mice maintain their immune function, whereas male mice have significantly depressed responses.8 Estradiol has been shown to be protective in organ ischemia-reperfusion injury and shock by preventing androgen-induced immunosuppression in male animals.9-12 Thus, female sex hormones may be a useful adjunct in preventing trauma-induced immunosuppression and the associated increased susceptibility to sepsis.13 Estrogen treatment, testosterone depletion, and testosterone antagonism of nitric oxide synthase activity,15 and interference with the apoptotic process in a variety of cell systems.

Altered neutrophil apoptosis has been implicated in the pathogenesis of several inflammatory conditions.16,17 Excessively delayed neutrophil apoptosis is associated with the systemic inflammatory response syndrome (SIRS). This syndrome is also characterized by activated neutrophils with increased production of reactive oxygen intermediates (ROIs) and associated tissue damage, which may result in multiple organ failure.2,18

Previous studies have confirmed that neutrophils from women at term pregnancy have a significant delay in apoptosis,19 which may contribute to their physiologic neutrophilia.20 Progesterone and estradiol levels rise throughout pregnancy and may peak at a 100-fold higher than normal nonpregnant values.21 In view of these findings, the aggravated morbidity experienced by the postmenopausal woman, and the widespread use of natural or synthetic ligands of steroid receptors in fertility control and cancer endocrine therapy, we further investigated the impact of female hormones on neutrophil survival and function. We hypothesized that female sex steroids may mediate the female immunologic advantage by altering neutrophil survival and function. The objectives of this study were to investigate the effects of estradiol and progesterone on normal male and female neutrophil apoptosis and function and the mechanisms involved in these processes.

Materials and methods

Reagents and antibodies

Dulbecco modified Eagle medium (DMEM), penicillin, streptomycin solution, L-glutamine, and fetal calf serum (FCS) were from Gibco Life Technologies (Paisley, United Kingdom); dextran T-500 and Ficol from

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Pharmacia (Buckinghamshire, United Kingdom); E-Lyse from Cardinal Associates (Santa Fe, NM); pro and active caspase 9 antibody from Becton Dickinson (Cambridge, United Kingdom); pro and active caspase 3 antibody from Santa Cruz Biotechnology (Santa Cruz, CA); and dihydro- 
rodamine 123 (DHR) from Molecular Probes (Eugene, OR), CD11b (LeuTM-15) phycoerythrin (PE)-labeled monoclonal antibody (mAb) was from Becton Dickinson (San Jose, CA); monoclonal anti-β-FAS (CD95) fluorescein from R&D Systems (Minneapolis, MN); monoclonal agonistic 
Fas (CD95) antibody (CH-11) from Immunotech (Marseille, France); caspase 3 substrate Ac-DEVD-AMC and caspase 9 substrate Ac-LEHD- 
AMC from Biomol Laboratories (Plymouth Meeting, PA); rabbit antiman- 
gese superoxide dismutase (MnSOD) polyclonal antibody from Stress- 
gen (Victoria, BC, Canada); ICI 182 780 (Faslodex) from Tocris (Bristol, 
United Kingdom); charcoal dextran-treated fetal bovine serum (Perbio- 
sciences, Cheshire, United Kingdom); water-soluble estradiol, progester- 
one, RU486, and all remaining chemicals unless otherwise stated were from 
Sigma-Aldrich (Dorset, United Kingdom).

Neutrophil isolation

Neutrophils were isolated from healthy male (n = 26) and female 
(n = 26) volunteers with an age range of 26 to 33 years; fully informed 
consent was given by all volunteers. Institutional ethical committee 
approval from the Mater Misericordiae Hospital was received for the 
study period. The technique used was dextran (3%) sedimentation and 
centrifugation through a discontinuous Ficoll gradient; red blood cells 
were lysed using E-Lyse.20 The neutrophil pellet was resuspended in 
DMEM culture medium supplemented with 10% FCS, 1% glutamate, 1% 
penicillin/streptomycin solution, and amphotericin B at a concentra- 

tion of 1000 units/mL. Cells were pelleted using E-Lyse.20 The neutrophil 
pellet was resuspended in 400 μL hypotonic fluorochrome solution (200 μL phosphate-buffered saline [PBS], 10 mg propidium iodide, 3.4 mM sodium chloride, 1 mM Tris [tris(hydroxymethyl)aminomethane], 0.1 mM EDTA [ethylenediami- 
netetraacetic acid], 0.1% Triton X-100). They were placed on ice 
for 10 minutes before they were analyzed using the Coulter Epics 
XL-MCL cytofluorometer (Miami, FL). A minimum of 5000 events were 
collected and analyzed. Apoptotic nuclei were distinguished from normal nuclei 
by their hypodiploid DNA and debris was excluded from analysis by 
raising the forward threshold. All measurements were performed under 
the same instrument settings.

Quantification of apoptosis

Spontaneous apoptosis of neutrophils was quantified by flow cytometry 
as the percent of cells with hypodiploid DNA.20,21 Cells (1 × 106/mL) were 
centrifuged at 130 g for 5 minutes and then gently resuspended in 
400 μL hypotonic fluorochrome solution (200 μL phosphate-buffered 
saline [PBS], 10 mg propidium iodide, 3.4 mM sodium chloride, 1 mM Tris 
[tris(hydroxymethyl)aminomethane], 0.1 mM EDTA [ethylenediam- 
etetraacetic acid], 0.1% Triton X-100). They were placed on ice 
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by their hypodiploid DNA and debris was excluded from analysis by 
raising the forward threshold. All measurements were performed under 
the same instrument settings.

Quantification of cell surface antigen expression

The expression of CD11b and Fas antigens on the surface of neutrophils 
was measured by flow cytometry; 500 μL cells (1 × 106/mL) were treated 
with 10 μL PE-CD11b or control antibody and incubated at 4°C for 20 minutes. 
The cells were washed 3 times with 400 μL cold PBS at 130 g for 
10 minutes and finally resuspended in 400 μL Isoton II solution and stored 
on ice before they were analyzed by flow cytometry. Similarly, 500 μL 
neutrophils (1 × 106/cells/mL) were stained with 5 μL Fas antibody 
(50 μg/mL) and centrifuged at 150 g for 5 minutes. The supernatant was 
aspirated, the pellet resuspended in 400 μL PBS, and the mean channel 
fluorescence assessed by flow cytometry.

Western blot analysis

Total protein was isolated from 9 × 106 human neutrophils using NP-40 isolation 
solution (0.5% NP-40, Tris 10 mM, pH 8.0, 60 mM KCl, 1 mM EDTA, pH 8.0, 
1 mM DTT [dithiothreitol], 10 mM phenylmethylsulfonyl fluoride [PMSF], 1 
μM leupeptin, 1 μM aprotinin, and 2 μM pepstatin). Total protein was measured 
by the Bradford assay protein detection kit (Bio-Rad, Hercules, CA) and loaded 
at 50 μg/well. Samples were then run on 12% sodium dodecyl sulfate 
(SDS)–polyacrylamide gradient gel (140 V for 60 minutes), and electrophoreti- 
cally transferred to Immobilon P (Millipore, Bedford, MA; 100 V, 80 minutes). 
The remaining gel was stained after transfer with Coomassie blue solution to 
confirm equal loading. Blots were incubated with rabbit antihuman caspase 3 
antibody (1:1000) or rabbit antihuman caspase 9 antibody (1:1000) in 1% bovine 
serum albumin (BSA), triethanolamine-buffered saline (TBS), and 0.1% Tween 
20 for 1 hour at room temperature. The blots were then incubated with 
horseradish peroxidase–conjugated antirabbit IgG at 1:5000 dilution for 1 hour.

Isolated neutrophils were prepared for cytochrome c evaluation by 
initially pelleting the cells followed by resuspension in 100 μL CLAMI 
buffer (250 mM sucrose, 70 mM KCl in PBS) containing 200 μg/mL 
digitation. Following a 5-minute incubation on ice, the cells were pelleted at 
1000 g for 5 minutes at 4°C. The supernatant or cytosol fraction was stored 
at −70°C until 30 μL was run on a Western blot. The pellet containing 
the mitochondrial fraction was resuspended in 100 μL universal immunoprecipita- 
tion buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 2 mM 
ethylene glycol tetraacetic acid [EGTA], 0.2% Triton X-100, 0.3% NP-40, 
1 mM PMSF, leupeptin, aprotinin) for 10 minutes on ice. The solution was 
then pelleted at 10 000 g for 10 minutes at 4°C and the supernatant or 
mitochondrial fraction stored at −70°C until 30 μL was run on a Western 
blot. The anti–cytochrome c antibody (1:1000) was a generous gift from Dr 
Seamus Martin (Trinity College, Dublin, Ireland). The secondary antibody 
used was horseradish peroxidase–conjugated antirabbit IgG at 1:5000 
dilution for 1 hour.

MnSOD is used as a specific marker of the mitochondrial fraction of 
the cell lysate22 and confirmed the purity of the cytosolic and mitochondrial 
fractions. Blots were incubated with the antibody at 1:1000 and the 
secondary antibody used was horseradish peroxidase–conjugated antirabbit 
IgG at 1:5000 dilution for 1 hour.

All blots were developed using the enhanced chemiluminescence 
system. The density of each band was measured using the UN-SCAN-ITgel 
Version 5.1 program (Silk Scientific, Orem, UT).

Respiratory burst activity

Generation of ROIs was evaluated by flow cytometry using the technique of 
Smith and Wiedemann.23 Neutrophils (500 μL [1 × 106/mL]) were incu- 
atated with DHR 123 (100 μM) at 37°C for 10 minutes. Cells were 
stimulated with 1 μL (16 μM) phorbol 12-myristate 13-acetate (PMA) for 
20 minutes at 37°C. The reaction was then halted by placing samples on ice. 
Neutrophil fluorescence intensity was assessed by flow cytometry and 
expressed as Ln mean channel fluorescence (LnMCF). DHR 123 has been 
shown to detect mainly intracellular H2O2 and OH radical production.23

Caspase extraction and activity assay

Cell lysates were prepared from 10 × 106 cells using caspase isolation 
buffer (25 mM HEPES [N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic 
acid], pH 7.8, 5 mM MgCl2, 1 mM EDTA, 10 mM leupeptin, 5 mM 
peptatin, 100 mM PMSF, and 10 mM DTT) and caspase incubation buffer 
(100 mM HEPES, pH 7.5, 10% sucrose, 0.1% CHAPS [3-(3-cholamidopro- 
pyl)dimethylammonio]-1-propane-sulfonic acid], 10 mM Leupeptin, 5 mM 
peptatin, 100 mM PMSF, and 10 mM DTT). Aliquots of the lysates 
(40 μL) were diluted in caspase incubation buffer (40 μL) and 20 mM 
(5 μL) Ac-LEHD-AMC (caspase 9) or 20 mM Ac-DEVD-AMC (caspase 3)

Figure 1. Sex differences in spontaneous neutrophil apoptosis. Neutrophils 
(1 × 106 cells/mL) were isolated from healthy male (n = 26) and female 
(n = 26) volunteers and incubated in vitro for 24 hours. Percentage neutrophil apoptosis 
was evaluated by propidium iodide DNA staining and flow cytometry. *P < .05 versus 
men. Error bars indicate mean ± SD.
were also expressed as the Ln mean channel fluorescence using flow cytometry assessed neutrophil apoptosis at physiologic doses. In addition, coincubation of DHT with estradiol or progesterone did not alter the female sex steroid hormone-mediated delay in neutrophil apoptosis (results not shown).

The effects of estradiol and progesterone on neutrophil function

The ability of these hormones to stimulate increases in neutrophil function was also assessed. There was no significant difference in control CD11b expression or ROI activity between male and female neutrophils. CD11b expression was not significantly altered in response to estradiol and progesterone in male or female volunteers (Figure 3A). There was no significant alteration in baseline ROIs in men and women but PMA-induced respiratory burst was increased in women in response to estradiol and progesterone (Figure 3B-C). Female neutrophils had an increased baseline ROI production on coincubation with estradiol and progesterone in combination and therefore the Δ difference between baseline and PMA-stimulated was reduced. (Figure 3B). The ability of these hormones to prime for an additive response to lipopolysaccharide (LPS) was also investigated. Estradiol and progesterone did not increase the ability of LPS to delay neutrophil apoptosis or to increase CD11b expression (Figure 4). CD33 and CD34 were also evaluated as markers of altered neutrophil maturity and were unchanged by these female sex hormones (data not shown). Therefore, physiologic levels of estradiol and progesterone alone or in combination do not appear to alter neutrophil CD11b expression despite causing an increase in female ROI production and delaying apoptosis in both sexes.

**Figure 3. Effects of estradiol and progesterone on markers of inflammatory cell activation.** Normal neutrophils (1 x 10^6 cells/mL) were incubated for 6 hours with estradiol, progesterone, or both in combination (10^-8 g/mL). Cells were then assessed for CD11b (A) using a PE-labeled mAb and the mean channel fluorescence analyzed using flow cytometry. □ indicates male (n = 8); ■ indicates female (n = 8). (B) ROI activity was assessed in neutrophils preincubated with sex steroids for 6 hours and then stimulated with DHR (baseline [BL]) alone or in addition to PMA (respiratory burst [RB]). Results were expressed as the Ln mean channel fluorescence ± SEM. (C) Results were also expressed as the Δ difference of the Ln mean channel fluorescence between DHR-treated cells with and without PMA stimulation. * P < .05 versus control (male, n = 8; female, n = 8). Error bars indicate mean ± SD.

**Results**

**Sex differences in neutrophil apoptosis and the role of estradiol and progesterone**

Human neutrophils die spontaneously by the process of apoptosis both in vivo and in vitro. This study demonstrated a significant delay in the spontaneous rates of apoptosis after 24 hours of in vitro incubation in healthy women of reproductive age compared with age-matched men (Figure 1). The addition of physiologic levels of estradiol (E2) and progesterone (P) further delayed female neutrophil apoptosis and reduced the rates of apoptosis in male neutrophils in a dose-dependent manner. There was no significant additive effect when these steroid hormones were added in combination over the same physiologic range (Figure 2).

Testosterone is converted to dihydrotestosterone (DHT) in the cell by 5α-reductase. DHT is the active metabolite mediating androgenic effects in the cell. Testosterone can also be converted to estradiol by aromatase. Testosterone-induced responses would therefore not give a true indication of androgenic effects. We studied DHT (10^-8 M), which had no significant effect on male (control = 24.2% ± 0.5%; DHT = 23.0% ± 2.5%; n = 4) and female (control = 16.0% ± 4.1%; DHT = 12.2% ± 3.0%; n = 4) neutrophil apoptosis at physiologic doses. In addition, coincubation of DHT with estradiol or progesterone did not alter the female sex steroid hormone-mediated delay in neutrophil apoptosis (results not shown).

**Figure 2. Dose-dependent effects of estradiol, progesterone, or both in combination on spontaneous neutrophil apoptosis.** Neutrophils (1 x 10^6 cells/mL) isolated from male (●, n = 10) and female (◇, n = 10) controls were incubated in vitro with increasing physiologic doses (10^-14 to 10^-6 g/mL) of estradiol (A), progesterone (B), or both in combination (C). Propidium iodide DNA-binding staining using flow cytometry assessed neutrophil apoptosis at 24 hours. *P < .05 versus control (Con). Error bars indicate mean ± SD.

**Statistics**

Statistical analysis was carried out using ANOVA one-way analysis of variance with Student-Newman correction and Student t test using Minitab statistical software 13.32 (http://www.minitab.com). Significance was assumed for P values less than .05. Results are expressed as mean ± SD unless stated otherwise.
reversed the estradiol-mediated (10^{-7} g/mL) delay in apoptosis after 24 hours (Figure 5). This demonstrates that the delay in apoptosis associated with female sex steroids is mediated through their respective steroid hormone receptors.

**Mechanism of female sex hormone-mediated delay in neutrophil apoptosis**

Previous studies in our laboratory have demonstrated that delays in neutrophil apoptosis are associated with resistance to Fas antibody-induced apoptosis and inhibition of caspase activity.\(^\text{20}\) To determine how estradiol and progesterone delay apoptosis, we investigated their effects on Fas antibody–induced apoptosis. Preincubation for 1 hour with estradiol or progesterone had no effect on Fas antibody–induced apoptosis (Figure 6A). This response was not mediated by Fas expression on the surface of the neutrophil, because this was unchanged by these steroid hormones (Figure 6B). Etoposide-induced apoptosis was also not reversed by estradiol and progesterone (data not shown).

The ability of these hormones to block spontaneous caspase activity was then assessed. The expression of the (active) proteolytic cleavage product of caspase 3 protein was increased following 6 and 24 hours of neutrophil incubation in vitro. Neutrophils incubated with progesterone or estradiol demonstrated a significant decrease in active caspase 3 protein expression at 6 hours that was confirmed by densitometry (Figure 7A). Progesterone but not estradiol treatment did result in a decrease in active caspase 3 protein expression at 24 hours (Figure 7B). Neutrophils incubated with progesterone demonstrated a decrease in active caspase 9 (Figure 8). There was a smaller decrease in active caspase 9 following incubation with estradiol at 24 hours (Figures 7A and 8).

To further validate altered active caspase protein expression we assessed caspase activity using fluorescent substrates. Both caspase 3 and 9 activities were reduced at 24 hours by estradiol and progesterone (Figure 9).

**Cytochrome c release and female sex steroid hormones**

To understand how these caspases are activated we examined the effects of neutrophil aging on spontaneous release of cytochrome c from the mitochondria. Cytochrome c is an activator of the caspase cascade and triggers neutrophil apoptosis.\(^\text{25}\) This is confirmed in Figure 10, which demonstrates that progressive cytosolic cytochrome c release in aging neutrophils corresponds to increasing neutrophil apoptosis. LPS (1 μg/mL) inhibited the release of cytochrome c and previous studies have also confirmed LPS-induced inhibition of apoptosis, as well as caspase 3 and 9 activity.\(^\text{20}\) In addition, estradiol and progesterone diminished mitochondrial cytochrome c release into the cytosol (Figure 11).

MnSOD is used as a specific marker of the mitochondrial fraction of the cell lysate.\(^\text{22}\) Purity of the cytosolic and mitochondrial fractions was confirmed by MnSOD staining (Figure 11).

**Discussion**

In this study, we demonstrated that neutrophil survival was significantly increased in women. Estradiol and progesterone delayed neutrophil apoptosis, although functional activity remained intact. This represents a possible mechanism for the elevated systemic neutrophil count seen in women compared with men.\(^\text{26}\) The link between the timing of maximum female neutrophil counts during normal menstruation and elevated estradiol and progesterone levels has been established.\(^\text{27,28}\) Similarly, neutrophils isolated from pregnant women show a more exaggerated delay in neutrophil apoptosis.\(^\text{29}\) This is reflected in the marked neutrophilia associated with term pregnancy\(^\text{30}\) and coincides with significantly elevated levels of female steroid hormones.\(^\text{19}\)

Estrogen has been shown to have pro- and anti-inflammatory properties depending on the cell type involved. Both cell proliferation and cell death have been described in the setting of breast cancer in response to sex steroids.\(^\text{31}\) Female sex hormone have been shown to delay apoptosis in the peripheral blood mononuclear cells of women with normal menstrual cycles,\(^\text{2}\) B cells,\(^\text{3}\) and endothelial cells.\(^\text{33}\) Tumor necrosis factor (TNF)–induced apoptosis in monoblastoid cells is also delayed by these hormones.\(^\text{34}\) Estrogen also has proinflammatory effects on the uterus and promotes neutrophil influx. In addition, progesterone can antagonize proinflammatory estrogenic activities via the progesterone receptor.\(^\text{35}\)

The specific receptor antagonists, ICI 182 780 and RU486, reversed the delays in apoptosis following co-cultivation with exogenous estradiol and progesterone, respectively. Recently, both estradiol receptor (ER) α and β have been described on the surface of neutrophils from men and women. However, although both receptor subtypes are up-regulated by 17β-estradiol (10^{-8} M) in premenopausal women, only ERα expression was enhanced in men.\(^\text{37,38}\) These studies with the respective specific receptor antagonists, ICI 182 780 and RU486, demonstrate that the delay in
apoptosis is a receptor-mediated effect. Estradiol- and progesterone-mediated interactions may therefore be involved in the transcriptional or translational regulation of antiapoptotic genes.

To further understand the role of male sex steroids and sex in neutrophil apoptosis we incubated neutrophils with DHT. Testosterone is converted to DHT in the cell by 5α-reductase and by aromatase to estradiol. These enzymes have been shown to have a sex-dependent distribution in immune cells. T cells from women have displayed aromatase activity and male-derived T cells have increased 5α-reductase activity.49 This, in turn, may mediate the cellular immunosuppression seen in men following trauma-hemorrhage. Neither aromatase nor 5α-reductase activities have been described in the mature neutrophil, although HL60 cells and progenitor hematopoietic CD34 cells have aromatase activity.49 Because testosterone can be converted to estradiol by aromatase and would therefore not give a true indication of androgenic effects, we studied DHT rather than testosterone. DHT had no significant effect on male and female neutrophil apoptosis in physiologic doses.

PMA-induced ROI release was enhanced by estradiol and progesterone in women. The combination of these hormones elevated the baseline ROI production. Both increased41,42 and decreased ROI43-46 production have been reported in response to female sex hormones. Increased ROI production was found when an intracellular dye was used, whereas decreased ROIs were found in studies measuring extracellular ROI release. Our study used the intracellular dye DHR 123. This is consistent with the published data that describe an enhanced intracellular ROI production in neutrophils from pregnant women, when estradiol and progesterone are at maximum physiologic levels.47 Differences between the PMA-induced ROI production with these sex hormones in men and women may be due to a sex-specific alteration in the neutrophil phenotype. ER subtypes are differentially expressed on the neutrophil phenotype. ER subtypes are differentially expressed on male and female neutrophils exposed to estradiol.38 Sex differences have also been described in the expression of protein kinase C, which is a primary target for PMA. This may explain the differences between the PMA-induced responses.48 Neutrophil CD11b expression in men and women was unaffected by physiologic doses of female sex hormones. These hormones had no priming effect on CD11b expression or delays in apoptosis following activation with LPS.

Despite the effects of these hormones on spontaneous apoptosis they had no effect on Fas antibody–induced apoptosis. This is in contrast to LPS and granulocyte-macrophage colony-stimulating factor (GM-CSF), which delay apoptosis and prevent the induction of Fas antibody–induced apoptosis.20 The delay in neutrophil apoptosis with estradiol and progesterone is relatively subtle and not as exaggerated as that seen with LPS and GM-CSF. An inhibitory effect may be seen at a lower dose of Fas antibody. This may partially explain the failure of these hormones at physiologic doses to reverse Fas-induced apoptosis or to completely inhibit caspase activity.

The mechanisms by which these hormones delay spontaneous neutrophil apoptosis have yet to be fully elucidated. The current study demonstrated that after 6 hours of estradiol and progesterone treatment there was an inhibition of the proteolytic activation of caspase 3, an important activator of the apoptotic cascade. At 24 hours the decreased activity of caspase 3 was not associated with a significant decrease in the corresponding active protein expression. This may indicate the ability of these hormones to delay but not completely inhibit apoptosis. Estradiol has previously been shown to induce an undefined inhibitor of active caspases through a receptor-mediated nongenomic pathway in neurons.49 Estrogen withdrawal stimulates the processing of caspase 3 to its active form.
in chick oviduct, culminating in the induction of apoptosis. Decreased apoptosis in MCF-7 breast cancer cell line and in B cells is believed to be due to increased Bcl-2 expression. The fact that the active protein is expressed but the activity is inhibited and apoptosis delayed would indicate that these hormones inhibit the functional activity of the caspase. However, because similar effects are seen with caspase 9 activity, we investigated cytochrome c release and the mitochondria as a site for apoptotic inhibition.

Cytochrome c is an important activator of the caspase cascade in the neutrophil and is released from the mitochondria to initiate spontaneous apoptosis. Caspase activation in the neutrophil requires 100-fold less cytochrome c release than other cells such as Jurkats. We believe that even though we did not detect cytochrome c at the earlier time points, undetectable quantities are released from the mitochondria enabling activation of the caspases. Both estradiol and progesterone had an inhibitory effect on cytochrome c release indicating the ability of these hormones to regulate apoptosis at the level of the mitochondria and thus preventing caspase activation and apoptosis. Therefore, the apparent time delay in the up-regulation of cytochrome c at 18 hours compared with the caspase 3 activity at 6 hours may be due to late detection by the relatively insensitive method of Western blotting.

These studies demonstrate a novel and interesting paradox whereby estradiol increases neutrophil survival without an increase in neutrophil activation. This would be immunologically advantageous by enhancing the number of circulating neutrophils available to combat infection. The neutrophil phenotype in this case is also different from the extreme immune activation seen in SIRS. Female sex hormones have been shown to induce a significant female survival advantage in a number of disease states including neurodegeneration, cardiac disease, and sepsis. These beneficial effects may be partially mediated by the effects of these hormones on the immune system.

The mechanism and use of these hormones as an adjunctive treatment in sepsis or shock warrant further investigation. This study also highlights the importance of equal male-to-female ratios in control samples used in future neutrophil research. The effect of therapeutic sex steroid hormone agonists and antagonists on immune function is often overlooked and we suggest that further study is required to assess the impact of immune modulation on disease progression. In conclusion, estradiol and progesterone increase neutrophil survival while preserving normal function. We postulate that by altering the phenotype of the neutrophil these hormones prepare the female host for potential inflammatory insults.

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