ORIGINAL

Opposing effects of estradiol and progesterone on the oxidative stress-induced production of chemokine and proinflammatory cytokines in murine peritoneal macrophages

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Abstract : In inflammatory and oxidative liver injury, virus proteins and reactive oxygen species are involved in the regulation of proinflammatory cytokine production by macrophages. This study investigated the effects of estradiol (E2) and progesterone on the unstimulated and oxidative stress-stimulated production of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, macrophage inflammatory protein (MIP)-2, and macrophage chemotactic protein (MCP)-1 by peritoneal macrophages isolated from male and female mice. E2 inhibited the cytokine production of TNF-α, IL-1β, MIP-2, and MCP-1 by the unstimulated macrophages from males and females, which was then further stimulated by progesterone. The exposure to hydrogen peroxide in the macrophages from both sexes induced the production of cytokine. The hydrogen peroxide-stimulated cytokine production was suppressed by E2 and enhanced by progesterone. The sex hormone effects on the unstimulated and stimulated macrophages were blocked by their receptor antagonists and showed no significant difference between male and female subjects. These findings suggest that E2 may play a favorable role in the course of persistent liver injury, by inhibiting proinflammatory cytokine production, which, in addition, progesterone may counteract the favorable E2 effects through their receptors. J. Med. Invest. 55 : 133-141, February, 2008

Keywords **:** *estradiol, progesterone, oxidative stress, macrophage, ER, progesterone receptor, proinflammatory cytokine*

INTRODUCTION

It has been reported that chronic hepatitis C and B appear to progress more rapidly in men than in women (1, 2), and that the decline in estradiol (E2) production with menopause is associated with spontaneous increase in proinflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β (3). In inflammatory and oxidative liver injury, macrophages and monocytes are recruited both outside and inside the liver, and they as well as Kupffer cells, the resident hepatic macrophages,

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accumulate at the sites of injury and inflammation in the liver. This accumulation is thought as being mediated by such cytokines as chemokines, including IL-8 and macrophage chemotactic protein (MCP)-1. These inflammatory cells play essential roles in the innate immune system, thereby functioning to produce reactive oxygen species (ROS) as well as chemokines and proinflammatory cytokines, thus leading to the occurrence of persistent liver injury. In addition, excess amounts of ROS are able to lead to cell damage and also involved in several intracellular pathways that ultimately lead to the activation of the macrophages and monocytes (4). E2, at physiological concentrations (10^{-11} - 10^{-8} mol/L), has been shown to inhibit the spontaneous secretion of these proinflammatory cytokines in whole blood cultures (5) or peripheral blood mononuclear cells (6). Our previous studies have shown that hepatocytes mainly possess functional estrogen receptor (ER) subtypes ER β, but not ER α, while in cultured hepatocytes in a state of oxidative stress, E2 inhibited the activation of nuclear factor κB (NF-κB), a key transcription factor that induces multiple genes in response to inflammation and oxidative stress, through ER β (7, 8). The NF-κB pathway plays an important role in cellular response to a variety of extracellular stimuli, including TNF-α and ROS. In comparison to other types of ROS, hydrogen peroxide is more stable and membrane permeable, thus leading to the hypothesis that it acts as a second messenger in regulating the signaling events leading to activate NF-κB. These findings suggest that E2 could enhance antiinflammatory activity in the injured liver by decreasing the proinflammatory cytokine production, and might play a cytoprotective role through ER β in the liver (9). However, regarding E2 and another female sex steroid, progesterone, there is still little information about the direct effects of these sex hormones on the production of chemokines and proinflammatory cytokines from macrophages and monocytes.

Therefore, the present study investigated the effects of E2 and progesterone on the production of TNF-α, IL-1β, macrophage inflammatory protein (MIP)-2, and MCP-1 by peritoneal macrophages from male and female mice after stimulation with hydrogen peroxide. MIP-2 (also known as CXC chemokine ligand 8) is the mouse homology of IL-8. The functions of the ER subtypes and progesterone receptor (PR) in the macrophages were also examined.

MATERIALS AND METHODS

Animals

The adherent macrophages were harvested from the peritoneal cells of male and female C3H/He mice (7 weeks old ; Japan SLC, Shizuoka, Japan) 3 days after the intraperitoneal injection of thioglycolate (50 mL/kg body weight). The peritoneal macrophages were then seeded at a concentration of 1×10^6/mL in buffered RPMI 1640 supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 1% L-glutamine (RPMI culture medium) with 10% heat-inactivated fetal bovine serum. The viability of cell preparations was determined by trypan blue exclusion. Routinely, peritoneal cell morphology was evaluated by microscopic analysis at least 200 cells in Wright's stained cytocentrifuge preparations.

After settling for 2-3 h, the non-adherent cells were washed with phosphate-buffered saline. The adherent macrophages were cultured at 37°C in a 5% CO_2 atmosphere and 100% humidity in serum-free RPMI culture medium for 24 h, and oxidative stress was then induced in the macrophages by incubation with hydrogen peroxide (10^{-7} - 10^{-5} mol/L) for up to 24 h in the presence or absence of 17 β-E2 (Sigma, St Louis, MO, USA) or progesterone (Wako, Osaka, Japan), with or without the estrogen receptor antagonist, ICI-182,780 (ICI : Tocris Cookson, Ballwin, MO, USA), or the PR antagonist RU486 (RU : Wako). The steroid sex hormones and receptor antagonists were initially prepared as an ethanolic stock solution (10^{-2} mol/L) and then were diluted with the culture medium in order to obtain an appropriate working solution concentration, with a final concentration of 0.1% ethanol. Culture media with 0.1% ethanol were used as a blank control.

Cytokine and female sex hormone assays

The cytokines of TNF-α, IL-1β, and MIP-2, and the female sex hormones of E2 and progesterone secreted into the culture supernatant were measured by an enzyme-linked immunosorbent assay (ELISA) using commercial kits (R&D Systems, Minneapolis, MN, USA) and by a radioimmunoassay using commercial kits (CIS Diagnostic, Tokyo, Japan), respectively, according to the manufacturer's instructions.

Western Blot Analysis

The ER subtype and PR proteins in the macrophages were immunologically detected using antibodies against ER α (1D5 : DAKO, Glostrup, Denmark ; 1 : 1000 dilution), ER β (H-150 : Santa Cruz

Biotechnology, Santa Cruz, CA, USA ; 1 : 1000 dilution), and PR (C-20 : Santa Cruz Biotechnology ; 1 : 1000 dilution). The immunoreactive bands were visualized with an ECL Western blotting detection system (chemiluminescence) kit (Amersham, Arlington Heights, IL, USA) as described elsewhere (10, 11), and then were evaluated by a densitometric analysis. A rabbit antibody against β actin (Biomedical Technologies, Stoughton, MA, USA) was used as the control for protein loading.

Real-time Polymerase Chain Reaction

For an analysis of the mRNA expressions of ER subtypes and MCP-1 in the macrophages, the cells were collected using ISOGEN (Nippon Gene, Tokyo, Japan) and the total RNA was prepared according to the manufacturer's instructions. A real-time polymerase chain reaction (PCR) was performed using a SYBR Green PCR Core Reagents kit (Biosystems, Warrington, UK) according to the manufacturer's protocol. For PCR amplification, the following primer pairs were employed : 1) ER α, 5'-GCCGAGGAGGGAGAATGTTG-3' (sense) and 5'-CGCCAGACGAGACCAATCAT-3' (antisense) ; 2) ER β, 5'-CATCAGTAACAAGGGCATGG-3' (sense) and 5'- CACTGAGACTGTAGGTTCTG-3' (antisense) ; 3) MCP-1, 5'-GCTCTAGACACCATGCAGGTCCCTGTC-3' (sense) and 5'-CACTGTCACTTGATCACATTCGAACC-3' (antisense). The primers for 18S rRNA were purchased from a commercial vendor, and were included in the reactions as an internal standard. A quantitative PCR was performed on the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's protocol as follows : 2 min at 50°C, 10 min at 95°C, and then 40 cycles with denaturation at 95°C for 15 sec, and annealing at 60°C for 1 min. The cDNA content for a specific gene in each sample was semiquantitatively assessed by comparing the experimentally determined crossing point with the crossing points and respective concentrations of a linearized standard plasmid DNA (qPCR Plasmid Standards : Invitrogen, Carlsbad, CA, USA). All results were normalized by the 18S rRNA content to ensure comparability.

Statistical Analysis

The data were presented as the mean ± SD, unless otherwise indicated. The means were compared between the two groups using the Wilcoxon's signed-rank test and the Mann-Whitney U test. All p values were two-tailed. A p value of less than 0.05 was considered to be statistically significant.

RESULTS

Expressions of ER subtypes and PR in the mouse peritoneal macrophages from male and female mice

The purity of the macrophages was usually 90% or better as judged by microscopic differential. Viability of recovered cells was always greater than 95%. We first performed a Western blot analysis of the extracts from the mouse uterus using the antibodies against ER α (1D5), ER β (H-150) and PR (C-20) (data not shown). When the lysates of mouse peritoneal macrophages were analyzed by Western blotting in the same manner, we also found 66- and 55-kD bands for ER α and ER β (12) (Fig. 1A), respectively, and 94- and 114-kD bands for PR (12) (Fig. 1C). A densitometric analysis revealed the expression intensity of ER β to be significantly greater

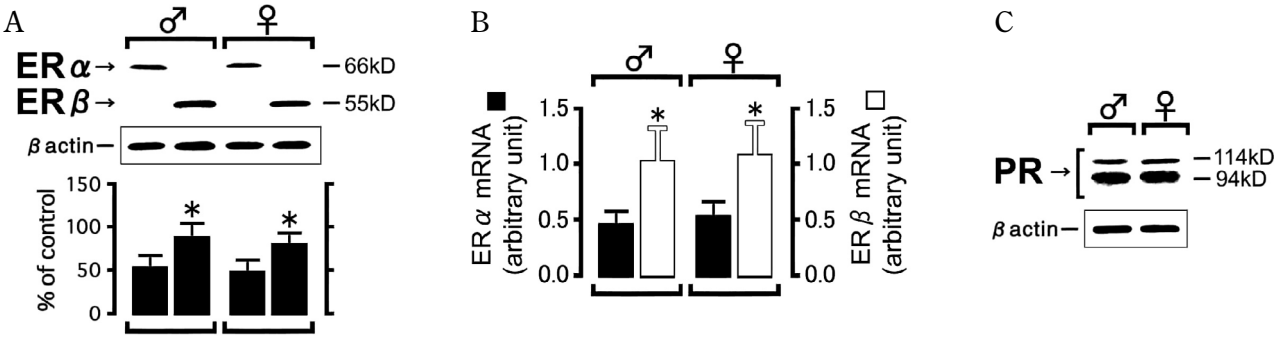

Fig. 1. Western blot and real-time PCR analyses of ER α and ER β, and PR in macrophages from male and female mice
The cell lysates were subjected to 12% SDS-polyacrylamide gel electrophoresis and transferred onto nylon membranes, and ER α and ER β (A), and PR (C) were detected immunologically. The results of a densitometric analysis are presented as the mean percentages of the signal intensity of β-actin for ER subtype expression. ER α and ER β mRNAs (B) in the macrophages were quantified by real-time PCR, and their expression levels are given arbitrary units normalized by 18S rRNA expression. The graphs represent the typical results of four independent experiments. *P < 0.05 in comparison to the intensity of ER α mRNA or protein expression.

than that of ER α in the macrophages both from the males and females (Fig. 1A). No significant difference between males and females was found in the expressions of the ER subtypes and PR.

The divergent occurrence of ER α and ER β in the peritoneal macrophages from both genders was also investigated by real-time PCR (Fig. 1B). The expression level of ER β mRNA was significantly higher than that of ER α mRNA. The messages of the ER subtypes showed no significant difference between males and females.

Effects of E2 and progesterone on spontaneous expression levels of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA by macrophages from male and female mice

After culturing for 24 h in serum-free RPMI, the macrophages from male and female mice expressed MCP-1 mRNA and released TNF-α, IL-1β, and MIP-2 into the culture medium (Table 1). The levels of E2 and progesterone in the culture supernatant were found to be under 10^{-12} mol/L. There was no significant difference between the cytokine expression levels in the hydrogen peroxide-unstimulated macrophages from males and females.

We next investigated the effects of E2 and progesterone on the spontaneous expression of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA by the hydrogen peroxide-unstimulated macrophages. When the macrophages were incubated for an additional 24 h without exposure to hydrogen peroxide, the mean percentages of each initial value for the cytokine expression reached up to 121-131% (Tables 1 and 2).

Table 1. Expression levels of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA by macrophages from male and female mice before exposure to hydrogen peroxide

	TNF-α	IL-1β (pg/ml supernatant)	MIP-2	MCP-1 mRNA (arbitrary unit)
Male mice (n=10)	22.5 ± 3.1	15.4 ± 2.5	84.4 ± 13.1	0.98 ± 0.13
Female mice (n=10)	21.9 ± 3.0	15.3 ± 2.6	82.8 ± 12.9	0.97 ± 0.13

The peritoneal macrophages from the male (n=10) and female (n=10) mice were cultured for 24 h in serum-free RPMI before exposure to hydrogen peroxide. The levels of TNF-α, IL-1β, and MIP-2 in the 24 h culture supernatant were then detected by means of an ELISA. MCP-1 mRNA in the macrophages was quantified by real-time PCR, and its expression levels are given arbitrary units normalized by 18S rRNA expression. The values are the means ± SD (n=10).

Table 2. Effects of E2 and progesterone on spontaneous expression levels of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA by macrophages from male and female mice without exposure to hydrogen peroxide

	Addition (mol/L)	TNF-α	IL-1β (pg/ml supernatant)	MIP-2	MCP-1 mRNA (arbitrary unit)
Male mice (n=10)	None	27.4 ± 3.9	19.6 ± 3.2	108.1 ± 16.3	1.28 ± 0.22
	+ E2 (10^{-10})	26.1 ± 3.7	19.4 ± 3.1	94.2 ± 14.5	1.20 ± 0.19
	+ E2 (10^{-9})	21.8 ± 3.0*	14.5 ± 2.4*	77.3 ± 12.7*	0.98 ± 0.16*
	+ E2 (10^{-8})	18.4 ± 2.6*	13.7 ± 2.2*	72.4 ± 10.9*	0.87 ± 0.15*
	+ E2 (10^{-8}) + ICI	26.3 ± 3.7	18.6 ± 3.0	98.4 ± 14.8	1.15 ± 0.20
	+ Progesterone (10^{-9})	29.6 ± 4.0	20.5 ± 3.4	112.0 ± 17.4	1.38 ± 0.21
	+ Progesterone (10^{-8})	32.8 ± 4.7*	24.5 ± 4.1*	131.5 ± 19.7*	1.62 ± 0.25*
	+ Progesterone (10^{-7})	37.3 ± 5.3*	27.6 ± 4.5*	145.7 ± 21.8*	1.89 ± 0.33*
	+ Progesterone (10^{-7}) + RU	28.8 ± 4.1	20.2 ± 3.3	119.9 ± 17.9	1.37 ± 0.24
Female mice (n=10)	None	26.8 ± 3.7	18.5 ± 3.0	103.0 ± 15.7	1.22 ± 0.16
	+ E2 (10^{-10})	23.7 ± 2.9	15.9 ± 2.7	97.7 ± 13.6	1.19 ± 0.15
	+ E2 (10^{-9})	20.9 ± 3.0*	13.8 ± 2.3*	78.6 ± 11.0*	0.99 ± 0.12*
	+ E2 (10^{-8})	18.8 ± 2.6*	12.2 ± 2.0*	68.0 ± 10.4*	0.82 ± 0.11*
	+ E2 (10^{-8}) + ICI	24.7 ± 3.4	19.1 ± 3.1	98.9 ± 15.1	1.16 ± 0.15
	+ Progesterone (10^{-9})	28.8 ± 4.3	20.7 ± 3.8	118.4 ± 13.8	1.35 ± 0.17
	+ Progesterone (10^{-8})	33.1 ± 4.7*	24.0 ± 4.0*	128.8 ± 15.7*	1.70 ± 0.20*
	+ Progesterone (10^{-7})	38.0 ± 5.3*	26.5 ± 4.3*	134.9 ± 17.3*	1.94 ± 0.25*
	+ Progesterone (10^{-7}) + RU	29.5 ± 4.1	19.4 ± 3.2	111.2 ± 17.0	1.35 ± 0.18

After culturing for 24 h in serum-free RPMI, the macrophages from male (n=10) and female (n=10) mice were then incubated for an additional 24 h with and without E2 (10^{-10} - 10^{-7} mol/L) (E2) or progesterone (10^{-10} - 10^{-7} mol/L) (Progesterone) in the presence and absence of 10^{-6} mol/L ICI (ICI) or 10^{-6} mol/L RU (RU). The spontaneous production levels of TNF-α, IL-1β, and MIP-2 in the culture supernatant were detected by means of an ELISA. MCP-1 mRNA in the unstimulated macrophages was quantified by real-time PCR, and its expression levels are given arbitrary units normalized by 18S rRNA expression. The values are the means ± SD (n=10). *$P < 0.05$ in comparison to the 24-h-cultures in the absence of the female sex hormones and receptor antagonists (None).

Whereas the treatment with E2 and progesterone in the unstimulated macrophages for 24 h significantly affected the cytokine expression in a dose-dependent manner. The cytokine expression levels decreased significantly in the macrophages treated with E2 at 10^{-9} and 10^{-8} mol/L, and they increased significantly in the cells treated with 10^{-8} and 10^{-7} mol/L progesterone (Table 2). There was no significant difference between the change rates of the cytokine expression in the males and females.

The inhibitory effects of 10^{-8} mol/L E2 on the unstimulated expression of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA in the males and females were blocked by the specific ER antagonist ICI at a dose of 10^{-6} mol/L, while the further enhancement effects of 10^{-7} mol/L progesterone on the unstimulated cytokine expression in both sexes were blocked by the PR antagonist RU at 10^{-6} mol/L (Table 2). The treatment with ICI or RU alone had no effect on any of the parameters examined herein (data not shown).

Effects of E2 and progesterone on hydrogen peroxide-stimulated expression of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA by macrophages from male and female mice

After culturing for 24 h in serum-free RPMI, the exposure to low doses of hydrogen peroxide (10^{-6} - 10^{-5} mol/L) in the macrophages from the male and female mice, incubated in serum-free RPMI for an additional 24 h, was observed to stimulate the expression of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA in a dose-dependent manner (data not shown). The subsequent studies used a dose of 10^{-5} mol/L of hydrogen peroxide for further stimulation of the macrophages. The exposure to hydrogen peroxide induced a time-dependent and transient expression of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA, peaking at 6-24 h, over a 24-h period (Fig. 2). There was no significant difference between the cytokine levels in the males and females (data not shown). The levels of TNF-α, IL-1β, and MIP-2 in the culture supernatant and the MCP-1 mRNA levels in the cells peaked after 24 h and 6 h, respectively. Subsequent studies used an incubation time of 6 h to measure the MCP-1 mRNA levels and 24 h to measure the levels of TNF-α, IL-1β, and MIP-2 after the hydrogen peroxide exposure.

The hydrogen peroxide-stimulated cytokine expression was inhibited by E2 (10^{-10} - 10^{-8} mol/L) in a dose-dependent manner (Fig. 3). The inhibitory effect of E2 at a dose of 10^{-8} mol/L was blocked by 10^{-6} mol/L ICI. In contrast to E2, progesterone treatment for up to 6 h resulted in the further expression of TNF-α, IL-1β, MIP-2, and MCP-1 mRNA in the hydrogen peroxide-stimulated macrophages (10^{-9} - 10^{-7} mol/L) in a dose-dependent manner (Fig. 3). The stimulatory effect of progesterone at 10^{-7} mol/L was blocked by 10^{-6} mol/L RU. No parameters examined in the macrophages were found to be significantly different between the males and females (data not shown).

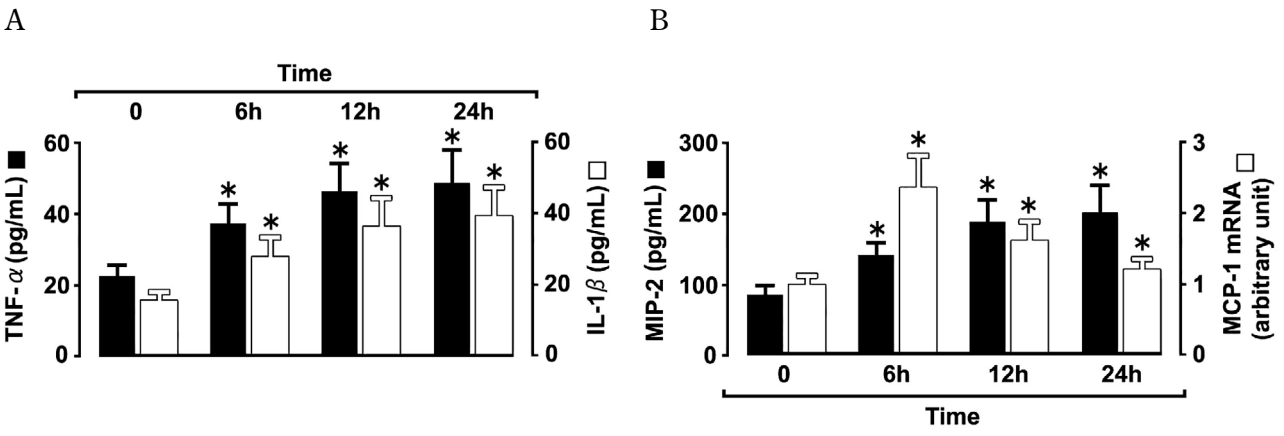

Fig. 2. Stimulation of TNF-α, IL-1β (A), MIP-2, and MCP-1 mRNA (B) expression after exposure to hydrogen peroxide by macrophages After culturing for 24 h in serum-free RPMI, the macrophages from female mice were then exposed to 10^{-5} mol/L hydrogen peroxide for up to 24 h. The levels of TNF-α, IL-1β, and MIP-2 in the culture supernatant were then detected by means of an ELISA. MCP-1 mRNA in the macrophages was quantified by real-time PCR, and its expression levels are given arbitrary units normalized by 18S rRNA expression. The values are the means ± SD (n=10). *$P < 0.05$ in comparison to the cultures before hydrogen peroxide exposure.

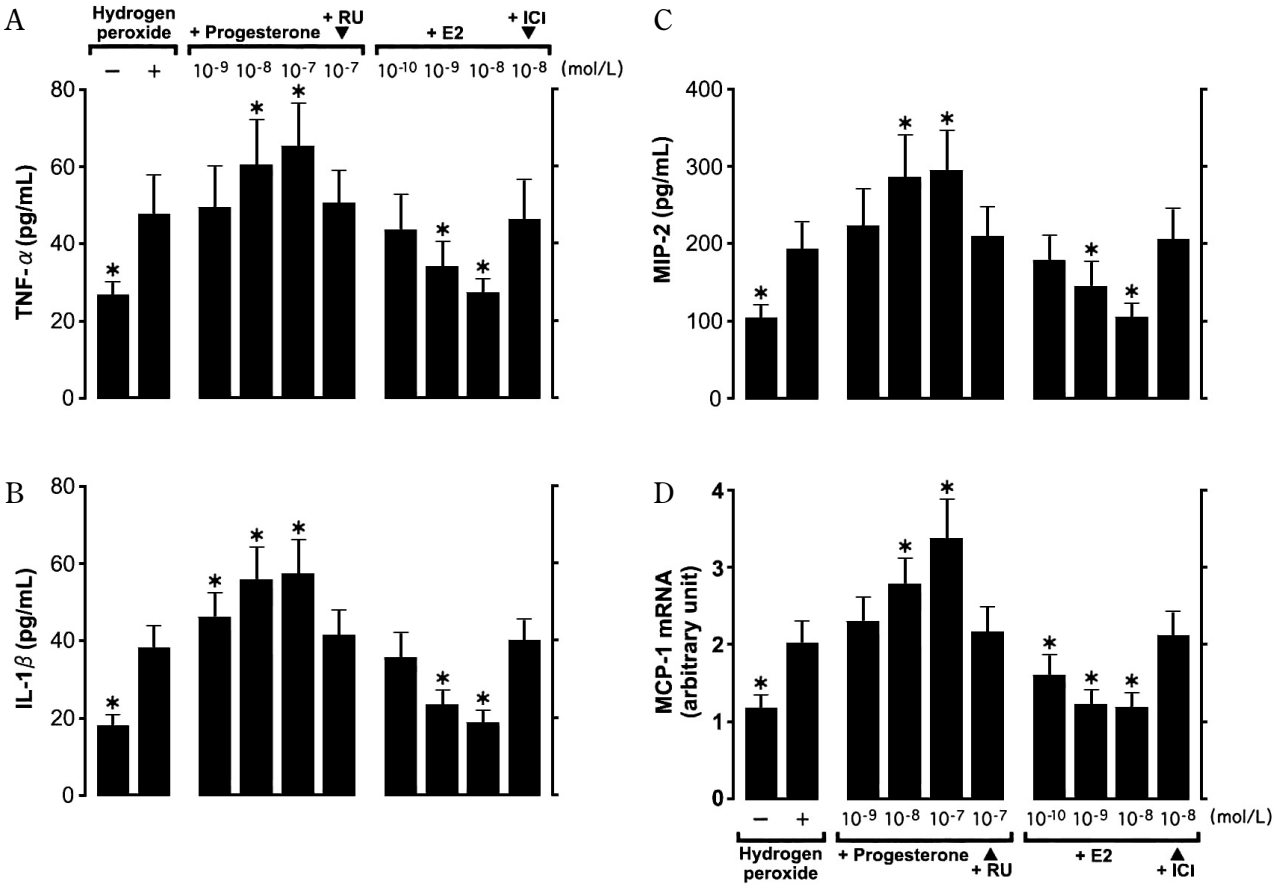

Fig. 3. Effects of E2 and progesterone on hydrogen peroxide-stimulated expression of TNF-α (A), IL-1β (B), MIP-2 (C), and MCP-1 mRNA (D) by macrophages
After culturing for 24 h in serum-free RPMI, the macrophages from female mice were then exposed to 10^{-5} mol/L hydrogen peroxide (Hydrogen peroxide) for up to 24 h in the presence or absence of E2 (10^{-10} - 10^{-8} mol/L) (E2) or progesterone (10^{-9} - 10^{-7} mol/L) (Progesterone) with and without 10^{-6} mol/L ICI (ICI) or 10^{-6} mol/L RU (RU). The hydrogen peroxide-stimulated production levels of TNF-α, IL-1β, and MIP-2 in the culture supernatant were then detected by means of an ELISA. MCP-1 mRNA in the stimulated macrophages was quantified by real-time PCR, and its expression levels are given arbitrary units normalized by 18S rRNA expression. The values are the means ± SD (n=10). *$P < 0.05$ in comparison to the cultures with hydrogen peroxide exposure (Hydrogen peroxide).

DISCUSSION

The present study indicates that the hydrogen peroxide-stimulated production of TNF-α, IL-1β, MIP-2, and MCP-1 by the cells were suppressed by E2, and were enhanced by progesterone in a dose-dependent manner through their receptors. The specificity of the E2-mediated antiinflammatory induction through the ER subtypes and the progesterone-mediated proinflammatory induction through the PR was shown by ICI and RU, respectively, in both the unstimulated and oxidative stress-stimulated macrophages. ICI belongs to a class of steroidal antiestrogens that bind to ER with a high affinity similar to that of E2 and with an ER β > ER α binding affinity (13). As a result, ICI acts as a pure antiestrogen on both receptor subtypes, especially on ER β. The inhibitory effect of E2 at a dose of 10^{-8} mol/L on the unstimulated and stimulated cytokine production was blocked by ICI in both males and females. The treatment with the progesterone receptor antagonist RU led to a blockage of further cytokine production induced with 10^{-7} mol/L progesterone by the unstimulated and stimulated macrophages from both sexes. No parameters examined in the cells were found to be significantly different between the male and female mice.

Some studies using mice lacking ER α and ER β genes, however, showed that the antiinflammatory activity of E2 in brain macrophages and protective effect of E2 on experimental autoimmune encephalomyelitis were mediated through ER α (14, 15). In addition to the action of ER as a classical estrogen response element, ER α and ER β also mediate gene transcription from an AP-1 enhancer element. Paech, *et al.* reported that ER α and ER β from an AP-1 site signaled in opposite ways when combined with E2 : with ER α, E2 activated transcription,

whereas with ER β, E2 inhibited transcription (16). We also demonstrated a high level of expression of ER β and a low level of ER α expression in human and rat hepatocytes (8, 12, 17). Although it is becoming evident that ER α and ER β genes may be responsible for regulating different biological functions based on their expression patterns, localization profiles, and protein structures (18), the precise roles of these ER subtypes in regulating cell- and tissue-specific responses remain to be elucidated.

The inflammatory cells of macrophages and monocytes are particularly sensitive to oxidative stress including such intracellular and extracellular stimuli as ROS, chemokines, and proinflammatory cytokines. These inflammatory cells, in turn, function to produce also ROS and cytokines in response to oxidative stress and further to activate themselves and immune system (4, 19). In addition, there is large body of evidence indicating that E2 regulates the innate immune response of macrophages and monocytes and serves as an antiinflammatory agent (3, 20). For example, treatment of brain macrophages and peripheral macrophages with E2 reduces the TNF-α production (21, 22), which were consistent with the present data. The decline in the ovarian function with menopause is associated with spontaneous increases in TNF-α, IL-1β, and IL-6 (3). The *in vivo* treatment with E2 transdermally in postmenopausal women has been reported to decrease the spontaneous IL-6 production by peripheral blood mononuclear cells (PBMCs) (6). E2 is able to ameliorate the burn-induced increase in the serum TNF-α levels in rats (23), and to attenuate IL-1β in ER expressing HepG2 cells (24). Furthermore, higher blood levels of TNF-α have been observed during the luteal phase of the menstrual cycle in comparison to the follicular phase (25). During the luteal phase, the serum concentration of endogenous progesterone rises up to a maxima of about 10^{-7} mol/L, which can be ten to a hundred times higher than E2. The male sex hormone testosterone has some structural and functional similarities to progesterone (12). Judging from these findings and the present data showing that treatment with E2 (10^{-9} and 10^{-8} mol/L) and progesterone (10^{-8} and 10^{-7} mol/L) significantly affected the cytokine production by the oxidative stress-stimulated macrophages, E2 may therefore exert an antiinflammatory action against both inflammation and oxidative stress, at least in part, by inhibiting the production of proinflammatory cytokines by inflammatory cells, whereas progesterone may counteract the favorable effects of E2.

Toll-like receptors (TLRs), an important arm of the innate immune system, are commonly expressed in macrophages and monocytes, and activate pathways that produce proinflammatory and antiinflammatory cytokines when stimulated by pathogen-derived ligands. Some TLRs are involved in detection of viral proteins including hepatitis C (HCV) and B (HBV) viruses (26, 27). Infection with all of these viruses results in a strong activation of inflammatory cytokine responses likely triggered by TLRs. HCV and HBV proteins have been reported to enhance the TLR signaling pathway in macrophages, leading to the activation of mitogen-activated protein kinase (MAPK) and NF-κB (26, 27). Furthermore, damage to the parenchymal cell membranes and liver mitochondria could produce ROS derived from lipid peroxidative processes, which constitute a general feature of a sustained inflammatory response and liver injury. Hydrogen peroxide acts as a second messenger in regulating the signaling events, including the MAPK activation. We have already reported that E2 inhibited the prooxidant-induced lipid peroxidation in rat liver mitochondria (7), attenuated ROS generation and NF-κB activation in cultured rat hepatocytes in a state of prooxidant-induced oxidative stress (8), while also suppressing the hydrogen peroxide-induced activation of MAPKs and transcription factors NF-κB and AP-1 in cultured rat hepatic stellate cells (28). In the present study, hydrogen peroxide exposure resulted in an increase in the TNF-α, IL-1β, MIP-2, and MCP-1 mRNA levels in the macrophages from the males and females. The oxidative stress-stimulated cytokine expressions mediated likely through the activation of MAPKs and NF-κB were attenuated by E2 and augmented by progesterone in a dose-dependent manner without any significant difference between the males and females. These effects of E2 and progesterone were blocked by their receptor antagonists ICI and RU, thus indicating that ER and PR could mediate female sex hormone action in the oxidative stress-stimulated macrophages.

Finally, the current data suggest that E2 may play a favorable role in the course of persistent liver injury, at least in part, by inhibiting the proinflammatory cytokine production through ER subtypes, especially ER β, whereas progesterone may counteract these positive E2 effects by enhancing the proinflammatory cytokine production through PR.

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