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In vivo modulation of murine serum tumour necrosis factor and interleukin-6 levels during endotoxemia by oestrogen agonists and antagonists

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SUMMARY

Oestrogen has been reported to modulate tumour necrosis factor (TNF), interleukin (IL)-1 and IL-6 cytokine levels in human mononuclear cell cultures. In the present study, the effects of exogenous oestrogen administration on the cytokine response to an endotoxin challenge was investigated in a murine model of endotoxemia. Animals pretreated for 4 days with 17α ethinyl oestradiol exhibited divergent regulation of TNF and IL-6 levels in sera from endotoxin-stimulated mice. Oestrogen treatment resulted in a significant increase in serum TNF while serum IL-6 levels, relative to the placebo group, decreased in response to an endotoxin challenge. These oestrogenic effects were dose dependent with maximal elevations observed in TNF at 1 mg/kg and maximal reduction in IL-6 at 0·1 mg/kg of 17α ethinyl oestradiol. The increase in TNF levels by ethinyl oestradiol was blocked by co-administration of the oestrogen receptor antagonist tamoxifen. Oestrogen-mediated modulation of the TNF and IL-6 response to endotoxin was also apparent in animals implanted with 17β oestradiol pellets. The oestrogen-mediated effects on serum IL-6 were consistent with a reduction in IL-6 mRNA in peritoneal macrophages from oestrogen-treated mice. The effects of oestrogen on TNF and IL-6 production were also investigated *in vitro*. Oestradiol-treated macrophage cultures produced three- to fourfold lower amounts of IL-6 without any significant modulatory effects on TNF secretion. The combined *in vivo* and *in vitro* results demonstrate the modulation of IL-6 and TNF during endotoxemia by oestrogen analogues through an oestrogen receptor-dependent mechanism.

INTRODUCTION

The central role of oestrogens in the regulation of bone resorption and in autoimmune disease, as well as its protective effects on the cardiovascular system, have been well documented both through animal models as well as by epidemiological studies comparing disease incidence between pre-menopausal and post-menopausal women.[1–3] While the beneficial effects of oestrogen-replacement therapy on bone resorption[4–6] and the ensuing favourable shift in lipoprotein profiles to a less atherogenic one suggests a protective role, the increased incidence of autoimmune disease in pre-menopausal women and the exacerbating effects of oestrogen in animal models of systemic lupus[7,8] would indicate a contributory role of oestrogen for both chronic and acute inflammation. However, the effects of oestrogen on autoimmune disease and inflammation are complex and depend on the cellular response involved.[9] Women with rheumatoid arthritis for example, appear to be less symptomatic during pregnancy and show some benefit from oestrogen-containing oral contraceptives.[10–12] Using the type II collagen-induced arthritis model in mice to simulate certain aspects of rheumatoid arthritis, oestrogen treatment has been reported to reduce the severity and incidence of arthritis.[13]

While the mechanism by which oestrogen impacts on the acute or chronic inflammatory processes associated with autoimmune disease remain speculative, oestrogen effects on cytokine expression as well as on endothelial cell-adhesion molecules have been reported. These effects, as with the role of oestrogen in autoimmune disease progression, also appear somewhat contradictory. Oestradiol has been reported to increase leucocyte binding to tumour necrosis factor-(TNF) stimulated endothelial cells by upregulating the induction of vascular cell adhesion molecule-1 (VCAM-1), E selectin, and intercellular adhesion molecule-1 (ICAM-1);[14] and yet, in a separate study has been found to inhibit the interleukin-1 (IL-1) induction of VCAM-1.[15] Oestrogen has also been reported to inhibit IL-6 production by endometrial stromal cells,[16] and osteoblasts[17] and yet synergize with IL-1 to stimulate IL-6 production by human articular chondrocytes.[18] Oestrogen has also been reported to both increase and decrease IL-1 production by mononuclear cells *in vitro*.[19–22]

In view of the contradictory data regarding oestrogen effects on cytokine regulation *in vitro* the present study was undertaken to investigate the effects of oestrogen agonists and antagonists on cytokine expression in a murine model of

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endotoxemia. This model characterized by a bolus endotoxin challenge represents a system in which cytokine regulation can be evaluated during an acute inflammatory process.[23–25] Quantitation of serum TNF and IL-6 in both placebo and oestrogen-treated mice following endotoxin challenge was performed and the effects of the oestrogen-receptor antagonist/agonist tamoxifen have been evaluated. These studies demonstrate that following endotoxin challenge oestrogen-pretreated mice had an increase in serum TNF, antagonized by tamoxifen, and yet lower IL-6 levels than the parallel endotoxin-stimulated vehicle-treated controls. These results suggest that oestrogen or oestrogen analogues can shift the cytokine balance during endotoxin-induced inflammation.

MATERIALS AND METHODS

Animal model

Female BALB/c mice (Charles River Labs, Portage, MI) were treated by oral administration of 17α ethinyl oestradiol in β cyclodextrin vehicle at concentrations of 1 μg to 1 mg per kg for periods between 1–7 days. In experiments involving tamoxifen, animals were dosed orally concomittant with oestradiol. Unless otherwise indicated, all reagents were obtained from Sigma (St Louis, MO). Two hours after the final oral dose, mice were injected with the specified concentrations of lipopolysaccharide (LPS) (*E. coli* O55:B5, Difco, Detroit, MI) and animals were bled at 1 hr for TNF and at 3 hr for IL-6 quantitation. These intervals for cytokine quantitation were based on preliminary experiments which detected that 1- and 3-hr post-LPS challenge resulted in maximal elevation in TNF and IL-6 respectively. In most experiments mice were stimulated with 20 or 200 μg of LPS for evaluating changes in TNF and IL-6 respectively. These concentrations were chosen as preliminary experiments demonstrated that maximal elevations in the non-oestradiol-treated animals were obtained with these endotoxin concentrations. The effects of chronic 17β oestradiol were determined by implant of formulated pellets (Innovative Research of America, Toledo, OH) which result in sustained release of compound over a 3-week period.

Cytokine quantitation

Serum TNF levels were determined with a murine TNF-specific enzyme-linked immunosorbent assay (ELISA) (Genzyme, Cambridge, MA) using the conditions specified by the supplier. Mouse IL-6 was quantitated by a sandwich ELISA utilizing two monoclonal antibodies (mAb) directed against different epitopes.[26] Briefly, microwells (Dynatech Immulon IV; Dynatech, Chantilly, VA) were coated with clone MP5–20F3 anti-IL-6 capture mAb at 0·1 μg/well in bicarbonate coating buffer overnight at 4°. Wells were washed with phosphate-buffered saline (PBS) + 0·05% Tween-20 (PBS-T) and blocked with 5% milk/PBS-T. After 2-hr incubation at room temperature, wells were washed and incubated with serial dilutions of mouse sera prepared in PBS + 3% bovine serum albumin (BSA) for 3 hr. Wells were then washed followed by the addition of clone MP5–32C11 biotinylated anti-IL-6 detection mAb at 0·05 μg/well. After a 3-hr incubation, wells were washed and received streptavidin-peroxidase conjugate (Sigma, St Louis, MO) for 45 min at room temperature. After washing, wells received 2.2′-asino-di[3-ethyl-benzthiazoline sulfonate(6)] (ABTS) substrate according to manufacturers instructions (Kirkegaard & Perry, Gathersburg, MD). Optical density was measured using a Molecular Devices Vmax plate reader. Recombinant mouse IL-6 (PharMingen, San Diego, CA) was used as an ELISA standard. Serum oestradiol levels were quantitated by ELISA using a comercial kit (Medix Biotech, Foster City, CA). This kit detected 17β oestradiol but not 17α ethinyl oestradiol.

Primary macrophage cultures

Mice were injected intraperitoneally with 1·5 ml of thioglycollate broth and 96-hr later elicited peritoneal macrophages were harvested by lavage, allowed to adhere for 1 hr in 6- or 24-well plates (Costar, Cambridge, MA) in RPMI-1640 (Gibco, Grand Island, NY) supplemented with 2% fetal calf sera (FCS) (HyClone, Logan, UT) and non-adherent cells removed. Cultures were immediately lysed for polymerase chain reaction (PCR) analysis or incubated for 48 hr with varying concentrations of 17β oestradiol. LPS was added for the final 6 hr at 100 ng/ml and supernatants were quantitated for both IL-6 and TNF.

Semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)

Total RNA was prepared from 1×10^6 macrophages using a micro-scale total RNA separator protocol with reagents and procedure specified by Clontech Laboratories (Palo Alto, CA). RNA, 2 μg, was converted to first-strand cDNA using a Gibco/BRL Superscript first-strand cDNA synthesis kit (Gibco/BRL, Gathersburg, MD). PCR amplimer sets for mouse TNF-α and IL-6 were obtained from Clontech Laboratories as were the respective PCR mimics. Semi-quantitative PCR was performed in thin-walled tubes (Perkin-Elmer, Norwalk, CT) containing the respective 5′ and 3′ primers and varying amounts of mimic with amplification occurring over 34 cycles (Perkin-Elmer Cetus Thermal Cycler) using a 45-s denaturation at 94°, re-annealing at 59° for 45 s and 2-min extension at 72°. Aliquots of the PCR reactions were electrophoresed on 1·8% agarose gels containing 0·5 μg/ml of ethidium bromide. Characterization of the PCR products were based on predicted band size relative to a φx174 HAE III ladder and in initial experiments by hybridization with the relevant oligonucleotide probes. Bands were photographed from the UV transilluminated gel and negatives obtained from Polaroid type 665 film (Kodak, Rochester, NY) were scanned by densitometry using a Bio Image scanner and Visage software (Bio Image Products, Ann Arbor, MI). Quantitation of attomoles of specific message per μg of total RNA was based on a linear-regression analysis using five to six dilutions of mimetic for each RNA sample analysed. Ratios of product to mimetic that approached unity were used for all calculations.

RESULTS

Oral dosing of mice with 17α ethinyl oestradiol over a 4-day period resulted in a dose-dependent increase in serum TNF levels in response to an intravenous challenge with 20 μg of LPS (Fig. 1). Maximal increases in serum TNF were observed at oestradiol doses of 1 mg/kg and higher levels of oestradiol at 3 mg/kg did not result in any further augmentation (data not shown). The increase in TNF was only apparent following the LPS stimulus and sera measurements from oestrogen-treated animals prior to LPS challenge were comparable to normal

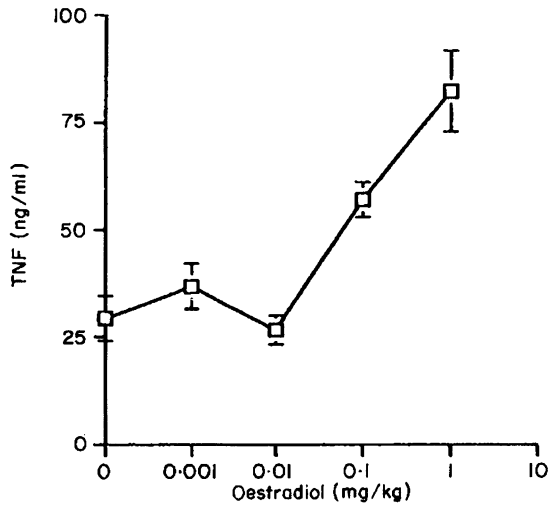

Figure 1. Oestradiol increases serum TNF levels in LPS-stimulated mice. Mice were dosed orally with 17α ethinyl oestradiol for 4 days at the indicated concentrations in 20% cyclodextrin. Two hours after the final dose, mice were injected i.v. with 20 μg of LPS and bled 1 hr later. Appropriate sera dilutions were quantitated for TNF by ELISA. The 0 mg/kg oestradiol group represented mice dosed for 4 days with the cyclodextrin vehicle alone. Representative experiment of three subjects, $n = 6$: brackets indicate SEM. The differences in TNF levels between the 0 and the 0·1 and 1 mg/kg oestradiol doses were significant ($P < 0·01$ and $P < 0·001$) respectively by a two-tailed Student's t-test.

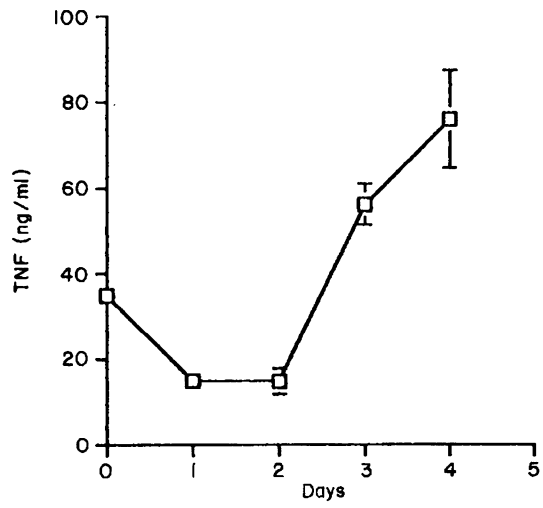

Figure 2. Oestradiol effects on TNF require multiple days of administration. Mice were dosed orally for the indicated number of days prior to the LPS challenge with 1 mg/kg of 17α ethinyl oestradiol. Group 0 represents control animals dosed for 4 days with vehicle alone. Two hours after the final dose mice were injected i.v. with 20 μg of LPS and bled 1 hr later for TNF quantitation. Representative experiment of three subjects; $n = 7$; brackets indicate SEM. The differences in TNF between days 1–4 with day 0 were significant ($P < 0·01$) by a two-tailed Student's t-test.

mouse sera and the placebo controls. While 0·1 mg/kg of oestradiol also resulted in a significant increase in serum TNF following a LPS challenge, all further studies involving oestradiol effects on TNF levels were performed at the 1 mg/kg dose.

The time course for the 17α ethinyl oestradiol effect on LPS-induced TNF release was investigated following 1–4 days of daily dosing. As demonstrated in Fig. 2, oestradiol effects were most apparent following 4 days of daily dosing and resulted in a significant increase in serum TNF levels. While similar effects were observed after 3 days, the increase in serum TNF was in some experiments more variable at 3 days. Therefore, all further experiments were based on 4 days of oral dosing at 1 mg/kg. The reduction seen in serum TNF after 1 or 2 days of dosing appears to be related to a stress response.

The increase in serum TNF was observed at both optimal and suboptimal LPS concentrations. 10-fold dilutions of the LPS challenge dose from 20 μg to 20 ng per animal resulted in increased serum TNF and at each LPS concentration the oestradiol-treated group had a significant increase in TNF relative to the vehicle-treated animals (Fig. 3). Therefore, while not shifting the dose response to endotoxin, oestradiol-treated animals demonstrated increased serum TNF at all LPS concentrations evaluated.

While oral dosing of mice with 17α ethinyl oestradiol resulted in significant increases in serum TNF, similar effects were also apparent following subcutaneous implant of 17β oestradiol tablets at a calculated dose of 2·1 mg/kg/day. The increase in serum TNF in the 17β oestradiol-treated animals was consistent with a significant elevation in serum oestradiol levels (Table 1).

In an attempt to determine whether the effects of relatively high doses of oral 17α ethinyl oestradiol on increased serum TNF following an LPS challenge were through a classic oestrogen receptor, animals were dosed for 4 days with 1 mg/kg oestradiol concomittant with the oestrogen-receptor agonist/antagonist tamoxifen. While tamoxifen at 3 mg/kg

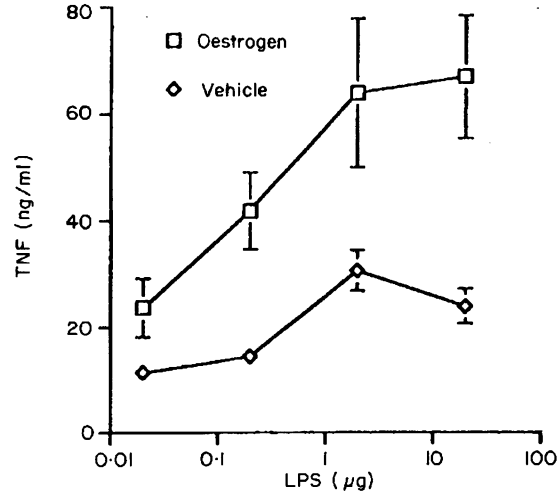

Figure 3. Oestradiol-mediated increases in serum TNF occur at both optimal and suboptimal LPS dilutions. Mice were dosed for 4 days with 1 mg/kg of 17α ethinyl oestradiol or vehicle alone prior to i.v. challenge with logarithmic dilutions of LPS from 20 μg to 20 ng. Mice were bled and sera quantitated for TNF as described. Representative experiment of three subjects, $n = 5$; brackets indicate SEM. The differences between the vehicle and oestradiol groups were significant at $P < 0·1$ (LPS 0·02 μg), $P < 0·05$ (LPS 2 μg), and $P < 0·01$ (LPS 0·2 and 20 μg) by Student's two-tailed t-test.

Table 1. Serum oestradiol and TNF levels following pellet implant. Animals were implanted subcutaneously with 1·5 mg of 17β oestradiol or placebo as formulated pellets, resulting in sustained release of compound at a calculated rate of 2·1 mg/kg/day. At 1-week post-implant, three to six animals per group were bled and sera quantitated for 17β oestradiol. Parallel animals were stimulated with 20 μg of LPS and bled 1 hr later for TNF determination. (Representative experiment of four subjects; parenthesis indicate standard deviation.)

Pellet	Oestradiol (nmol/l)	TNF (ng/ml)
Oestradiol	4·5 (0·2)	63·91 (0·15)
Placebo	< 0·25	30·61 (3·8)

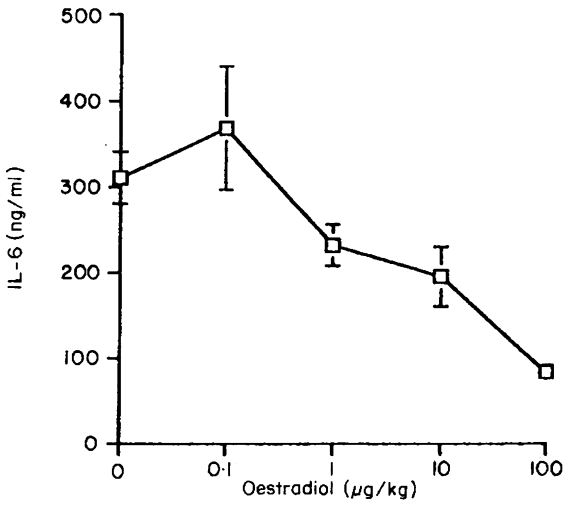

Figure 5. Dose-dependent reduction in serum IL-6 by 17α ethinyl oestradiol in LPS-stimulated mice. Mice were dosed for 4 days with the indicated concentrations of 17α ethinyl oestradiol prior to injection of 200 μg of LPS. The 0 group of mice were dosed for 4 days with vehicle alone. Animals were bled 3 hr later and sera were quantitated for IL-6. Representative experiment of three subjects, $n = 4$; brackets indicate SEM. The differences between the IL-6 in the 0 group when compared with the 1, 10, and 100 μg/kg oestradiol doses was significant at the $P < 0·1$, 0·05, 0·001 levels respectively by a two-tailed Student's t-test.

alone had no apparent effect on serum TNF levels in LPS-stimulated mice (Fig. 4), tamoxifen was capable of inhibiting the augmentation of serum TNF levels following a LPS challenge in oestradiol treated animals. These results suggest that the priming effect of oestradiol on increasing serum TNF levels following an endotoxin challenge was mediated through a classic oestrogen receptor.

The effects of oestradiol pretreatment on LPS-induced increases in serum IL-6 were also evaluated by ELISA. While increases in both TNF and IL-6 were detected in the serum following endotoxin challenge, the IL-6 levels in oestradiol-treated animals demonstrated a dose-dependent decrease following a 200 μg LPS challenge (Fig. 5). Pretreatment of mice for 4 days with 17α ethinyl oestradiol at 1–100 μg per kg significantly reduced the amount of serum IL-6 following LPS challenge. Increasing the oestradiol concentration to 1 mg/kg did not result in any further decrease in the IL-6 response (data not shown). In contrast to the oestradiol effects on TNF increase in which oestradiol effects were no longer detected at 10 μg/kg, reductions in serum IL-6 following endotoxin challenge were still observed at 1 μg/kg. At 100 ng/kg, oestradiol pretreatment no longer had any effect on reducing IL-6 levels. As was observed for oestrogenic effects on TNF increase, maximal reductions in IL-6 were apparent following 4 days of oral dosing (data not shown).

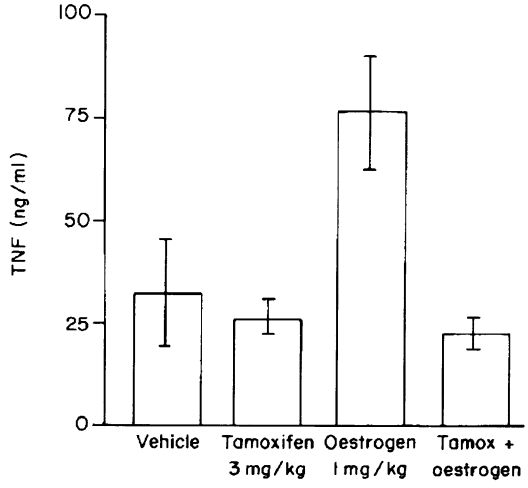

Figure 4. Tamoxifen inhibits the oestrogen-mediated increase in serum TNF in LPS-stimulated mice. Mice were dosed orally with vehicle, 1 mg/kg of 17α ethinyl oestradiol, 3 mg/kg of tamoxifen, or both for 4 days prior to LPS challenge. Sera obtained 1 hr after LPS injection were assayed for TNF. Representative experiment of three subjects, $n = 5$; brackets indicate SEM. The difference between the vehicle and oestradiol groups was significant ($P < 0·05$) by a two-tailed Student's t-test.

PCR analysis of *ex vivo* macrophages was then performed to determine whether the reduction in serum IL-6 also correlated with decreased IL-6 mRNA expression. Macrophages from thioglycollate-primed mice that had been dosed orally for 4 days with 0·1 mg/kg of 17α ethinyl oestradiol were harvested just prior to the LPS stimulus ($T = 0$) or at 1 and 3 hr post-challenge. PCR analysis was then performed following adherence and removal of non-adherent cells. As evident in Fig. 6a, the decrease in IL-6 serum levels correlated with a similar reduction in IL-6 mRNA (Fig. 6b) from the peritoneal macrophages of the LPS-stimulated oestrogen-treated animals. At intervals between 0 and 5 hr post LPS challenge, serum IL-6 peaked at approximately 3 hr in both vehicle and oestradiol groups. As with the respective serum peaks of IL-6, a comparable reduction in IL-6 mRNA levels were detected. In contrast to the oestradiol-mediated reduction in IL-6 mRNA, quantitation of TNF mRNA from these same samples revealed no significant difference in message levels (Fig. 6c). Therefore, the oestradiol-mediated reduction in IL-6 mRNA from the *ex vivo* macrophages was specific for IL-6 and not apparent for TNF. The reduction in IL-6 mRNA may be mediated by a direct effect of oestrogen on the macrophage as similar oestrogen effects on IL-6 synthesis were apparent *in vitro*

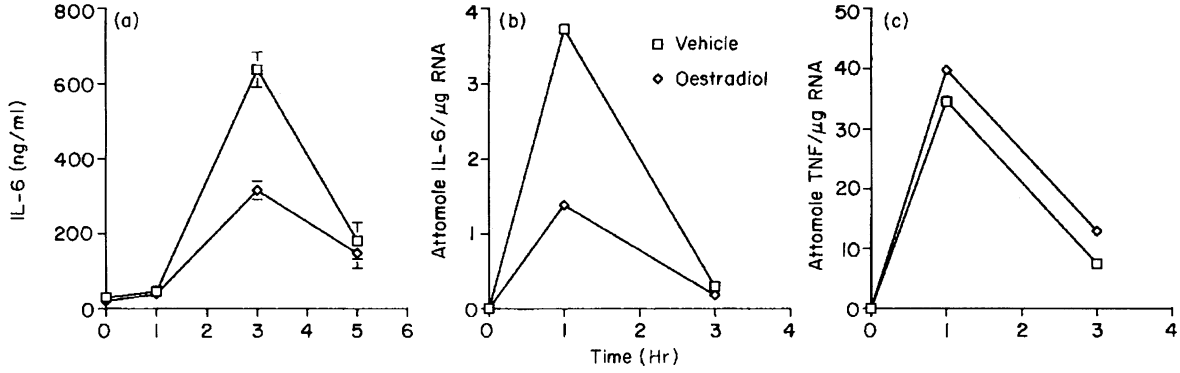

Figure 6. Reduced IL-6 mRNA in elicited peritoneal macrophages from LPS-stimulated oestradiol-treated mice. Mice were administered 0·1 mg/kg of 17α ethinyl oestradiol or vehicle alone for 4 days. One day after the initial dose mice were injected i.p. with thioglycollate broth to elicit macrophages. Two hours after the final oestradiol or vehicle dose, mice were injected i.v. with 200 μg of LPS and at 0, 1, 3 and 5 hr post-LPS, mice were bled and peritoneal macrophages were harvested at the 0, 1 and 3 hr time points. The macrophages within each experimental group were pooled and adhered to flasks for 1 hr, washed to remove non-adherent cells and then lysed for PCR analysis. Serum from each mouse was quantitated for IL-6 by ELISA (a). PCR quantitation of IL-6 (b) and TNF (c) mRNA was by mimetic analysis. This experiment was repeated twice, $n = 3$ for IL-6 protein determinations: brackets indicate SEM. The difference between the vehicle and oestradiol groups for serum IL-6 (a) at the 3-hr time point was significant ($P < 0{\cdot}01$) by a two-tailed Student's *t*-test.

under conditions in which macrophage cultures were pretreated with oestrogen for 2 days prior to the addition of LPS. Oestrogen-mediated reduction in IL-6 secretion (Fig. 7a) was apparent at the lowest oestrogen concentration evaluated, 5 μM, and further reductions in IL-6 secretion were apparent at higher concentrations. Effects on TNF secretion, in contrast, were minimal (Fig. 7b) with no increase detected at any concentration and some inhibition of secretion observed at 20 μM oestradiol. Therefore, the combined *in vitro* and *in vivo* results demonstrate the independent regulation of TNF and IL-6 by oestrogen in response to an endotoxin challenge.

DISCUSSION

While the association between oestrogen metabolites with inflammation and autoimmunity has been well documented, the mechanisms by which exogenous oestrogen can impact on the disease process remain unclear. Oestrogen has been demonstrated to suppress T-cell function and yet exacerbate humoral immunity.[9] In murine models of autoimmunity, oestrogen treatment has been demonstrated to suppress type II collagen-induced arthritis[13] and yet to increase disease severity in the NZB Lupus model.[7,8] Clearly, the effect of oestrogen and metabolites on autoimmunity are complex, involving multiple cell types, signal pathways and the involvement of soluble mediators. The demonstration of oestrogen receptors on macrophages[27] suggests that oestrogen effects on pathophysiology may in part be mediated by oestrogen–macrophage interactions and the modulation of subsequent cellular effector functions. As diverse macrophage functions are mediated through cytokine secretion, the role of oestrogen agonists and antagonists on *in vivo* TNF and IL-6 secretion has been investigated in the present study.

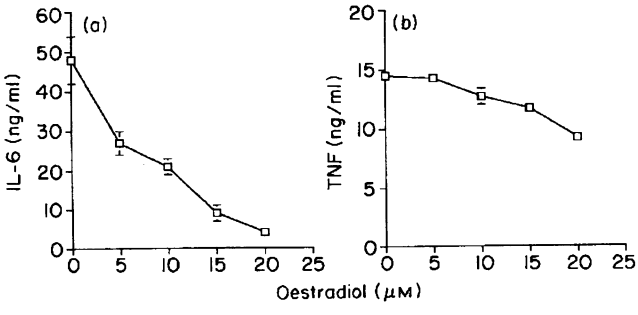

Figure 7. *In vitro* effects of 17β oestradiol on LPS-stimulated macrophage secretion of IL-6 and TNF. Elicited peritoneal macrophages were maintained *in vitro* in escalating concentrations of 17β oestradiol, 5–20 μM for 48 hr. Cells were then stimulated with 100 ng/ml of LPS and supernatants were quantitated for both IL-6 (a) and TNF (b) 6 hr after LPS addition. This experiment was repeated twice, $n = 3$, brackets indicate SEM. The reductions in IL-6 (a) by oestradiol when compared with the parallel control cultures were significant ($P < 0{\cdot}05$) by a two-tailed Student's *t*-test.

Treatment of mice with oestradiol resulted in the increase in serum TNF levels and a reduction in IL-6 following an endotoxin challenge when compared with vehicle-treated littermates. The effects of oestrogen metabolites on increased TNF were apparent only *in vivo* and were antagonized by tamoxifen. Furthermore, the oestrogenic effects on increasing systemic TNF levels was not unique to BALB/c mice. Preliminary experiments using the oestrogen agonist oestriol resulted in a two- to three-fold increase in TNF levels in MRL/*lpr* mice following 1-week exposure to this agonist (data not shown). However, in contrast to the *in vivo* data, attempts to demonstrate a similar increase in TNF secretion *in vitro* were negative with oestrogen pretreated endotoxin-stimulated cells. These results would suggest that the dose-dependent effect of oestrogen on augmented TNF secretion following endotoxin challenge may occur indirectly through a non-macrophage cell population. Oestrogen effects on lymphocytes or endothelial cells, for example, may contribute to secretion of other cytokines that could prime the macrophage to secrete

increased amounts of TNF following endotoxin challenge. However, the potential priming effects on macrophage TNF secretion are distinct from those reported for other macrophage-activating factors. Interferon-γ (IFN-γ) priming for example, results in a shift in the dose response to LPS[28] and this was not apparent in the present study (Fig. 3).

The stimulatory effects of oestrogen on TNF were in contrast to the oestrogen-mediated reduction in IL-6. While elevated following endotoxin challenge, serum IL-6 levels were significantly lower than the parallel vehicle littermates. In addition, while tamoxifen directly antagonized the oestradiol-mediated effects on TNF, experiments to demonstrate a tamoxifen-antagonist effect on IL-6 reduction were unsuccessful (data not shown). The inability to block oestradiol-mediated reductions in IL-6 *in vivo* with tamoxifen represents an area under further consideration. However, the *in vivo* oestrogen-mediated reduction in serum IL-6 was consistent with reductions in macrophage IL-6 secretion *in vitro* as well as with earlier *in vitro* studies concerning oestrogen effects on IL-6 gene expression in endometrial and osteoblast-like cells.[16,17] While the effects of oestrogen on these *in vitro* cultures involves an interaction between the oestrogen receptor and oestradiol, recent studies suggest that repression of IL-6 expression by oestrogen may occur in the absence of high-affinity DNA binding by the oestrogen receptor.[29]

While the mechanism by which oestrogen metabolites regulate cytokine expression *in vivo* remains unclear, several *in vitro* and *in vivo* observations have relevance. Oestrogen has been demonstrated to augment certain macrophage-related functions including Fc-receptor-mediated clearance of antibody-coated erythrocytes in guinea pigs[30] and to increase IL-1 secretion in primary peritoneal macrophages[21] and in myeloid cell lines.[22] Cyclic expression of IL-1, IL-6 and TNF was reported in mouse uterus and induction of these cytokines in ovariectomized mice was reported following oestrogen and progesterone treatment.[31] However, oestrogen treatment has also been reported to inhibit IL-1 production by mononuclear cells[19,20,32] and to inhibit monocyte- and macrophage-mediated oxidation of low-density lipoproteins.[33,34] Accordingly, while the female pre-menopausal predominance in several autoimmune diseases is established, the role of oestrogen and cytokine modulation during autoimmunity and inflammation requires further analysis in well-characterized animal models.

The bolus endotoxin mouse model has been extensively investigated and the profile, as well as kinetics of cytokine expression in the serum and cytokine gene transcription in target organs, has been elucidated.[23–25,35,36] While serum TNF increase represents one of the earliest cytokines to peak within the serum, subsequent increases in IL-1, colony-stimulating factor (CSF) and IL-6 have been reported. The observation by Aderka *et al.* that IL-6 injection into mice resulted in a subsequent decrease in LPS-stimulated serum TNF levels suggested to these investigators the possible role of IL-6 in TNF regulation.[37] Further evidence of a role for IL-6 in the regulation of TNF was suggested in the evaluation of the cytokine response in mice in which the IL-6 gene has been deleted through gene targeting.[38] LPS treatment of these mice resulted in a threefold increase in TNF compared with the controls. This threefold increase in the IL-6-deficient mice is similar to the change observed in TNF in the present study in the oestradiol-treated mice in which serum IL-6 levels were depressed. While oestrogen modulation of TNF required higher concentrations than for IL-6 regulation, the tamoxifen results would suggest that although the oestrogen concentrations are non-physiologic, these effects are through an oestrogen receptor.

While activation of both IL-6 and TNF gene transcription involves regulatory elements including NF-KB and is mediated by similar inflammatory stimuli including endotoxin and IL-1, concurrent inhibition of both TNF and IL-6 synthesis and secretion appears to be a property limited to glucocorticoids.[39,40] Endocrine-immune cell interactions play a central role in the regulation of the inflammatory response. While endogenous and exogenous steroids have been demonstrated to modulate immune cell function, the present study demonstrates that a clear distinction can be made concerning the effects of oestrogen on the regulation of TNF and IL-6.

The present investigations have concentrated on the characterization of the effects of oestrogen agonists and antagonists on cytokine levels in control non-ovarectomized mice following endotoxin challenge. The focus in the present study on non-ovariectomized mice was based on evaluating the role of exogenous oestrogens on acute inflammation in an animal model with relevance to pre-menopausal women. Clearly, the effects of exogenous oestrogen therapy on cytokine expression in ovariectomized mice may serve as a model for understanding the regulation of inflammatory cytokine levels in post-menopausal women. This latter system, as well as the effects of exogenous oestrogen treatment on cytokine secretion and transcription in autoimmune murine models subjected to endotoxin challenge, represents studies that are currently underway.

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