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ESTRIOL: A POTENT REGULATOR OF TNF AND IL-6 EXPRESSION IN A MURINE MODEL OF ENDOTOXEMIA

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Abstract—The increased incidence of autoimmune disease in premenopausal women suggests the involvement of sex steroids in the pathogenesis of these disease processes. The effects of estrogen on autoimmunity and inflammation may involve changes in the secretion of inflammatory mediators by mononuclear phagocytes. Estradiol, for example, has been reported to regulate TNF, IL-6, IL-1 and JE expression. In the present study the effects of the estrogen agonist, estriol, on cytokine expression have been investigated in mice administered a sublethal lipopolysaccharide, LPS, challenge. Pretreatment of mice with pharmacologic doses of estriol, 0.4–2 mg/kg, resulted in a significant increase in serum TNF levels in both control and autoimmune MRL/lpr mice, following LPS challenge. This increase in TNF over the placebo group was blocked by the estrogen antagonist tamoxifen. Estriol treated mice also exhibited a rapid elevation in serum IL-6 levels following LPS challenge with the peak increase occurring 1 hr post LPS. This contrasted with the placebo group in which maximal serum IL-6 levels were detected at 3 hrs post challenge. This shift in the kinetics of IL-6 increase by estriol was inhibited by tamoxifen. The estriol mediated effects on TNF and IL-6 serum levels were consistent with the changes in TNF and IL-6 mRNA observed ex vivo in elicited peritoneal macrophages. Macrophage cultures from estriol treated animals however, did not demonstrate significant differences from the placebo group for TNF or NO secretion following in vitro LPS challenge. These results suggest that the estrogen agonist estriol can have significant quantitative, TNF, and kinetic, IL-6, effects on inflammatory monokines produced in response to an endotoxin challenge.

INTRODUCTION

The beneficial role of estrogen replacement therapy for reductions in cardiovascular disease in post menopausal women has long been recognized (1, 2). Estrogen therapy induces a shift in the lipoprotein profile resulting in a lowering of

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LDL cholesterol and an elevation of HDL cholesterol (3, 4). While part of the reduction in cardiovascular disease is through the shift to a more beneficial lipoprotein profile, estrogen effects within the arterial wall and more specifically within a developing atheroma have been proposed as also playing an important role in disease reduction. As the macrophage plays a critical role in atheroma development through the elaboration of cytokines, metalloproteinases, and by LDL oxidation (5–7), the effects of estrogen agonists on macrophage effector functions serves as an important link between cardiovascular disease, autoimmunity and inflammation. To this extent, estrogen receptors on macrophages have been described (8) and in vitro effects of estrogens on LDL oxidation (9, 10) and cytokine elaboration (11–16) have been reported. The in vitro data on cytokine expression however, has been contradictory with estrogen reported to both reduce and increase IL-1 production (11–14). Estrogen has also been reported to decrease IL-6 expression in stromal cells (17, 18) to decrease JE mRNA expression in mouse macrophages (19), to increase PDGF-A chain mRNA (20) and to stimulate TNF production (16).

Estrogens also have both beneficial and detrimental roles in autoimmune disease. Estrogen therapy for example, has been reported to ameliorate rheumatoid arthritic disease (21, 22). Studies in murine models with type II collagen induced arthritis suggest direct effects of estrogen on T cell function (23). In contrast, oral estrogens are contraindicated in Lupus (21, 24). Estrogens in murine models of Lupus have resulted in increases in both anti DNA and anti phospholipid titers (25, 26). While the effects of 17β or oral conjugated estrogens have been studied in murine models of autoimmunity and inflammation, the effects of other estrogen agonists on inflammation have not been considered in vivo.

Estriol has been classified as a short acting estrogen agonist-antagonist based on minimal uterotropic responses following injections into rodents (27). However, chronic exposure of animals to estriol does result in uterotropic effects (28). While estriol measurements in plasma and urine have been used to monitor fetal health, little is known regarding the effects of chronic estriol treatment on inflammatory processes. To this extent, the present study has focused on the effects of pharmacologic doses of estriol on the regulation of TNF and IL-6 expression in a murine model of endotoxemia. Parenteral estriol treatment for 1 week at concentrations between 0.4 to 2.2 mg/kg resulted in significant increases in TNF following endotoxin challenge when compared with placebo treated mice. These effects were apparent in both normal, BALB/c, and autoimmune, MRL/lpr, mice and were antagonized by tamoxifen. Estriol treatment also resulted in a shift in the kinetics of IL-6 expression with peak levels of serum IL-6 detected 1 hr post endotoxin challenge in the estriol treated animals while at 3 hrs in the placebo group. These results provide further evidence to the effects of other estrogen agonists on exacerbating inflammatory responses.

MATERIALS AND METHODS

Animal Model. Female BALB/c, MRL/lpr, or MRL/++ mice were implanted with formulated pellets containing estriol, 17β estradiol, estrone, or placebo (Innovative Research of America, Toledo, Ohio). These 1.5 mg pellets deliver approximately 2.2 mg/kg of estriol per day over a 21 day period. In dose response experiments, lower doses of estriol from 0.5 to 0.01 mg pellets were also used. Mice were anesthetized with metophane and implanted subcutaneously behind the neck with pellets using a 10 gauge trochar. A surgical wound clip was used to insure skin closure. In most experiments, animals were used 5-7 days post surgery. In experiments with tamoxifen (Sigma Chemical, St. Louis, Missouri), animals were treated orally with tamoxifen at 10 mg/kg which was formulated in β cyclodextran. Mice were injected i.v. with 200 μg of lipopolysaccharide (E. coli O55:B5, Difco, Detroit, Michigan) and bled at 1, 3, and 5 hrs for cytokine determinations. In certain experiments, mice were injected i.v. with 200 μg of a TNF specific monoclonal antibody, TN3-19.12 (29) or an irrelevant hamster immunoglobulin control (Cappel, West Chester, Pennsylvania).

Cytokine and NO Quantitation. Serum TNF levels were quantitated with a murine TNF specific ELISA (Genzyme, Cambridge, Massachusetts) using the conditions specified by the supplier. IL-6 serum levels were quantitated by a sandwich ELISA using two distinct monoclonal antibodies as previously described (30). TNF was also quantitated in macrophage culture supernatants by ELISA. In both serum and macrophage culture supernatants, the appropriate sample dilution was employed to insure that all determinations were within the linear portion of the standard curve. NO was measured in macrophage culture supernatants as the combined nitrite and nitrate products using the Griess reaction (31).

Macrophage Cultures. Pellet implanted mice approximately 5 days post surgery were injected with 1.5 ml of thioglycollate broth and elicited peritoneal macrophages were harvested by lavage 3 days later. Macrophages were maintained in 24 well plates (Costar, Cambridge, Massachusetts) at 2.5×10^5 cells per well for supernatant TNF and NO determinations or in 6 well plates at 1×10^6 cells per well for mRNA quantitation by PCR. LPS at 100 ng/ml was used as the in vitro challenge and supernatants were harvested at 24 and 48 hrs for TNF and NO respectively. In select ex vivo experiments, macrophage cultures were primed with 1000 units/ml of recombinant murine γ interferon (Genzyme).

Semiquantitative Reverse Transcriptase Polymerase Chain Reaction. Mice that had been implanted with estriol or placebo pellets were thioglycollate injected four days post implant and used in all ex vivo PCR studies three days later. Thioglycollate primed estriol or placebo implanted mice were injected i.v. with 200 μg of LPS and macrophages were harvested from three mice per group at 30 minute intervals until approximately 2 hrs and 45 minutes post challenge. Macrophages were allowed to adhere in 6 well plates, 1×10^6 cells per well for 90 minutes, non adherent cells were removed by washing and the adherent monolayer was lysed and total RNA prepared using a micro-scale total RNA isolation protocol (Clontech Laboratories, Palo Alto, California). PCR amplimer and mimetic sets for mouse TNFα, IL-6, and actin were from Clontech Laboratories. Preparation of cDNA, PCR mimetic amplifications, and electrophoresis of PCR products on agarose gels were performed as previously described (30). PCR products were stained with Sybr green I (Molecular Probes, Eugene, Oregon) which was incorporated into the loading buffer. Images from the photographic negative of the UV transilluminated gels were captured by a Sony CCD video camera model AVC-D7 with a Nikon AF Nikkor 28 mm F2.8 lens connected to a Mac II equipped with a Frame Grabber Board (Data Translation Inc., Marlboro, Massachusetts). Band intensities were analyzed on a Macintosh II VX computer using the public domain NIH Image program (developed at the U.S. National Institutes of Health and available on floppy disk from the National Technical Information Service, Springfield, Virginia, part number PB95-500195GFI). Attomoles of mRNA

per μg of total RNA was calculated for the PCR products and was based on a linear regression anaysis using 5–6 dilutions of mimetic for each sample. Ratios of product to mimetic which approached unity were used for all calculations.

RESULTS

The profile and kinetics of increase of circulating cytokines following a bolus inflammatory stimulus has been well characterized in many mammalian species. The murine response to a bolus LPS challenge for example is associated with a rapid rise in TNF peaking at 1 hr and a subsequent increase in other cytokines. In the present study, BALB/c mice exposed to estriol at 2.2 mg/kg for 4 days when injected with 200 μg of LPS also demonstrated peak serum TNF levels approximately 1 hr post challenge (Figure 1). While the kinetics of

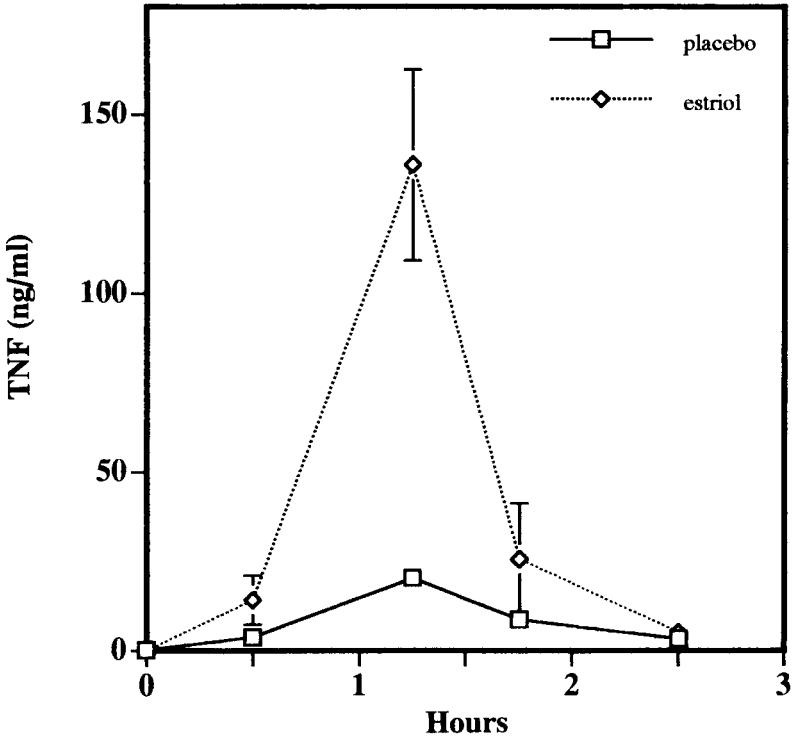

Fig. 1. Effect of estriol on serum TNF following endotoxin challenge. Mice were implanted with 1.5 mg estriol or placebo pellets and challenged with 200 μg i.v. of LPS one week later. At the designated intervals animals were bled and serum TNF quantitated by ELISA. Representative experiment of 4. Brackets indicated standard deviation, $n = 3$ animals at each time point.

TNF increase were similar to that observed with the placebo group, the magnitude of the TNF elevation was significantly greater. The placebo treated mice had a peak value of 20.37 ng/ml of circulating TNF while the estriol treated animals had 135.87 ng/ml. This increase in TNF releative to the placebo group was observed in all experiments performed during a 9 month time period although the magnitude of the increase varied from 3–7 fold over the parallel placebo group.

The effects of estriol on the peak serum TNF levels were not limited to BALB/c mice. Autoimmune MRL/lpr male and female mice as well as animals with a milder form of disease, MRL/++ also demonstrated a parallel increase in TNF when the estriol group was compared with the placebo group (Table 1). Therefore, the increases in circulating TNF observed in mice exposed to pharmacologic doses of estriol were apparent in normal and autoimmune mice and was independent of sex.

The increase in serum TNF in endotoxin challenged estriol treated mice was mediated through an estrogen receptor as mice treated with 2.2 mg/kg estriol and orally dosed with 10 mg/kg of tamoxifen exhibited an increase in TNF which while greater than the placebo was clearly reduced relative to estriol alone (Figure 2). Furthermore, although estradiol has been previously reported to increase the magnitude of the serum TNF response (30), estriol treatment consistently resulted in greater increases following endotoxin challenge. Therefore, while the estriol doses are pharmacologic, the effect appears to be mediated through an estrogen receptor dependent mechanism and that the magnitude of the TNF increase is dependent on the nature of the estrogen agonist.

In addition to the effects on TNF, estriol treated animals also displayed significant changes in the circulating IL-6 response following endotoxin challenge. Whereas placebo treated mice demonstrated peak IL-6 levels 3 hrs post LPS, the kinetics of IL-6 increase were significantly shifted by estriol (Figure

Table 1. Estriol Mediated Increases in Serum TNF Following Endotoxin Challenge in Autoimmune Mice.

Mouse strain	Treatment	TNF (ng/ml)
MRL/lpr female	Placebo	77.9 (18.9)
MRL/lpr female	Estriol	186.3 (24.2)
MRL/l++ female	Placebo	38.1 (4.4)
MRL/++ female	Estriol	152.3 (47.2)
MRL/lpr male	Placebo	60.7 (33.4)
MRL/lpr male	Estriol	147 (27.9)

MRL mice were between 10–20 weeks of age prior to implantation of 1.5 mg estriol or comparable placebo pellets. Following 1 week of estriol exposure mice were challenged with 200 ug of LPS i.v. and bled 1 hr later. Sera were diluted and TNF quantified by ELISA. Parentheses indicate *SD*, $n = 4$.

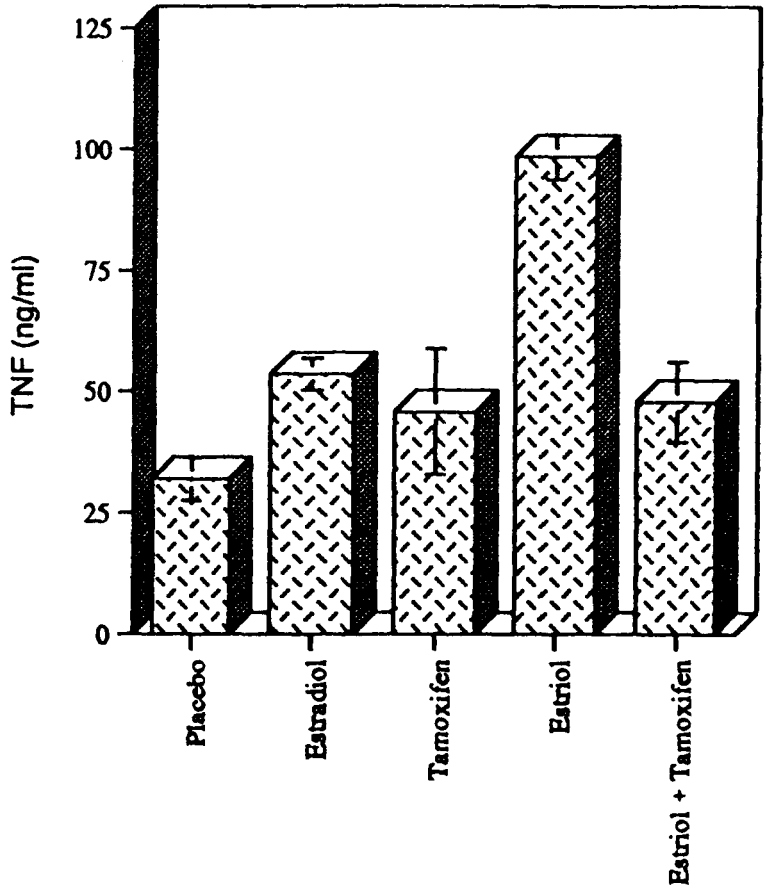

Fig. 2. Inhibition of estriol mediated increases in serum TNF by the estrogen antagonist tamoxifen. Mice were implanted with 1.5 mg pellets of estriol or estradiol and orally dosed with 10 mg/kg of tamoxifen or vehicle for one week prior to LPS challenge. Mice were challenged with 200 μg i.v. of LPS, 2 hrs after the final dose of tamoxifen and bled 1 hr later for TNF quantitation. Brackets indicate standard deviation, $n = 5$ animals per group.

3). Maximal increases in circulating IL-6 were apparent 1 hr after LPS injection and these levels were significantly reduced by 3 hrs. Therefore, while the peak IL-6 levels were comparable between the estriol and placebo animals, the kinetics of IL-6 increase were significantly different.

The estriol mediated increase in IL-6 levels was then compared with a different estrogen agonist, estrone, which had also been implanted subcutaneously 1 week prior. These results demonstrated that estriol treated animals had

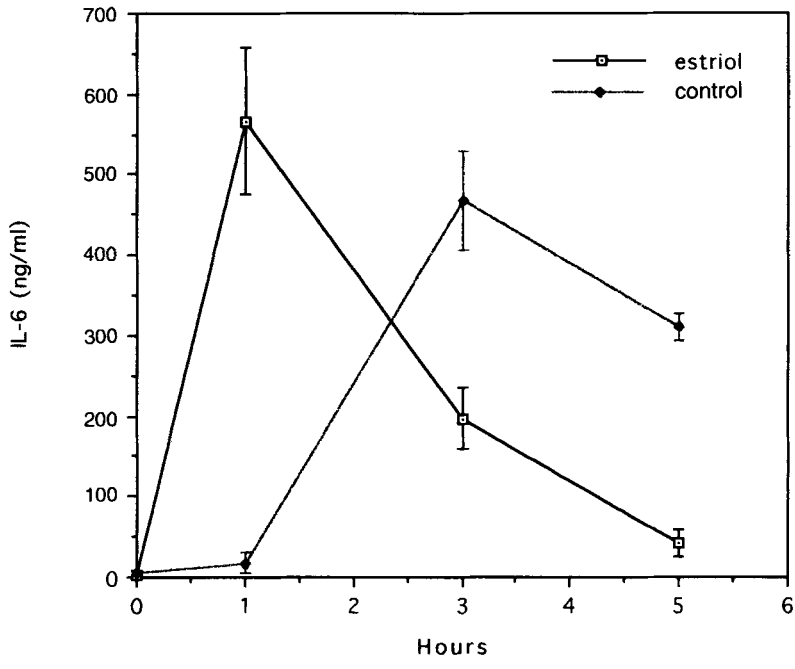

Fig. 3. Estriol treated mice demonstrate an earlier onset of circulating IL-6 following LPS challenge. Mice exposed to 1.5 mg estriol or placebo pellets for 7 days were challenged with 200 μg LPS and bled at the designated intervals. Sera were quantitated for IL-6 by ELISA. Brackets indicate standard deviation, $n = 5$ animals per group. Representative experiment of 4.

a greater increase in serum IL-6 one hr after LPS challenge than the estrone group (Figure 4). The IL-6 increase in the placebo group was modest compared with non LPS challenged mice. Therefore, among the estrogen agonists evaluated, estriol resulted in the greatest increase in serum IL-6 one hr post LPS challenge.

The effects of estriol on both elevating circulating TNF and shifting the increase in IL-6 to 1 hr post LPS were dose related. While most studies were performed with the 1.5 mg pellets, comparable effects on both TNF and IL-6 were observed with pellets containing 0.5 and 0.25 mg of estriol representing doses of 800 and 400 μg/kg per day (Figure 5A, B). The response to increasing concentrations of estriol was non linear. Doses below 400 μg/kg per day were without any effect on either IL-6 or TNF while above this concentration resulted in maximal increases. The somewhat reduced IL-6 increase seen at the highest dose of estriol was not reproducible in other experiments.

While the estriol mediated increase in IL-6 observed 1 hr post LPS chal-

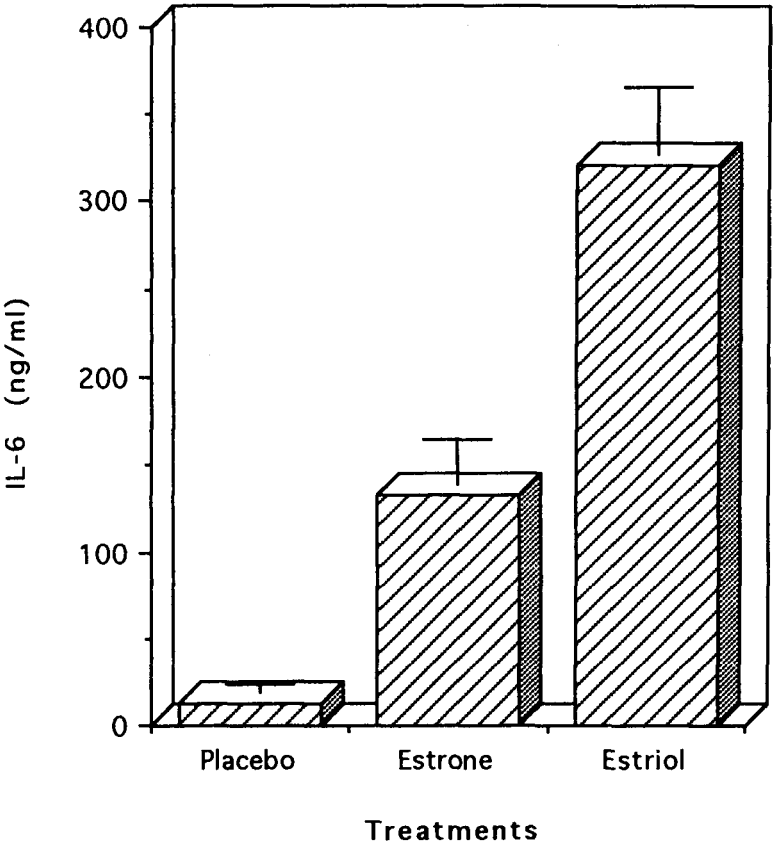

Fig. 4. Effect of the estrogen agonist estrone on IL-6 levels. Mice were implanted with 1.5 mg pellets of estrone or estriol and 1 week later challenged with 200 μg LPS i.v. Animals, 5 per group, were bled 1 hr later and serum IL-6 levels determined. Brackets indicate *SD*.

lenge required pharmacologic doses of agonist, it was estrogen receptor dependent, as the IL-6 shift was inhibited in mice orally dosed with 10 mg/kg of tamoxifen (Figure 6). In the estriol plus vehicle mice, the maximal increase in IL-6 following LPS challenge was at 1 hr which progressively decreased at the 3 and 5 hr time points. The IL-6 time course following tamoxifen resembled the kinetics observed in normal mice with maximal IL-6 increase observed by 3 hrs. Therefore, the earlier increase in serum IL-6 following LPS challenge in estriol treated mice was sensitive to the estrogen receptor antagonist tamoxifen. In additional experiments animals were pretreated with a monoclonal antibody against murine TNF (TN3–19.12) to determine the relationship between the

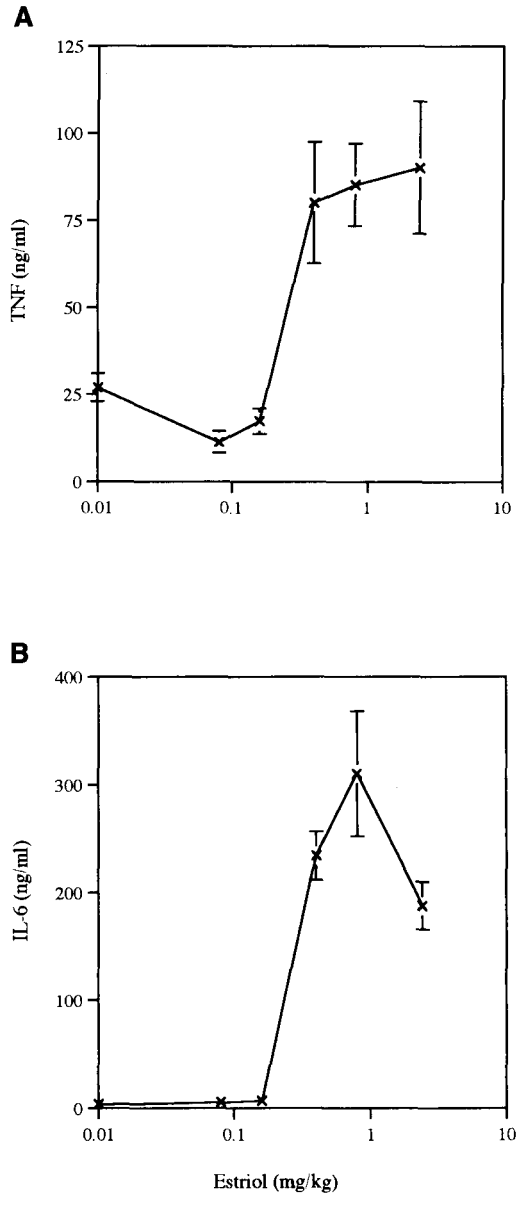

Fig. 5. Estriol effects on TNF and IL-6 were dose related. Mice, 5 animals per group, were implanted with estriol pellets containing varying amounts of estriol from 0.01 to 1.5 mg or placebo designated as 0. After 2 weeks mice were challenged with LPS, 200 μg, and bled 1 hr later. Appropriate sera dilutions were quantified for TNF (A) and IL-6 (B) levels. Brackets indicate *SEM*.

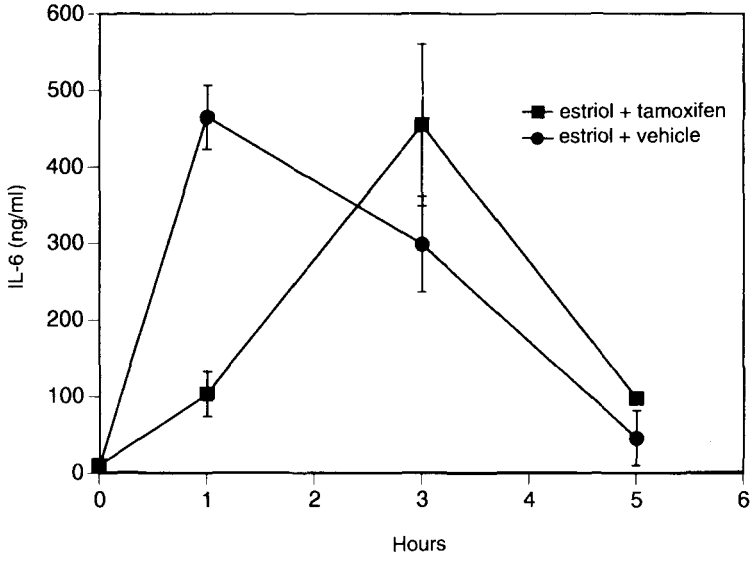

Fig. 6. Tamoxifen reverses the estriol mediated shift in IL-6 expression following LPS challenge. Mice were implanted with estriol and half the animals were dosed orally with 10 mg/kg of tamoxifen while the remaining animals received vehicle alone. After 1 week, all animals were challenged with 200 μg of LPS i.v. and at designated intervals, 5 animals per group were sacrificed and sera quantified for IL-6 by ELISA. This experiment was repeated twice, brackets indicate *SD*.

increase in circulating TNF with the earlier peak in serum IL-6. While the anti TNF, when compared to a hamster immunoglobulin control, did result in reduced IL-6 levels in placebo treated mice (126.4 vs 428.85 ng/ml, $p < 0.02$), the TNF specific monoclonal antibody had no effect on reversing the shift in serum IL-6 kinetics (data not shown). This would suggest that the earlier onset of the IL-6 peak in estriol mice was not likely to be mediated by the exacerbated increase in circulating TNF.

The increase in TNF and IL-6 observed 1 hr following LPS challenge necessitated additional studies designed to evaluate the effects of estriol on both TNF and IL-6 mRNA levels in peritoneal macrophages from pellet treated animals. Thioglycollate elicited peritoneal macrophages from placebo or estriol pellet implanted mice were harvested at approximately 30–45 minute intervals following LPS challenge. As demonstrated (Figure 7), macrophages from estriol treated mice showed a progressive increase in TNF mRNA which peaked approximately 75 minutes post challenge. The placebo group in contrast achieved peak levels at 30 minutes post challenge and was decreased by 75 minutes. The difference in TNF mRNA levels however, between the estriol and placebo groups

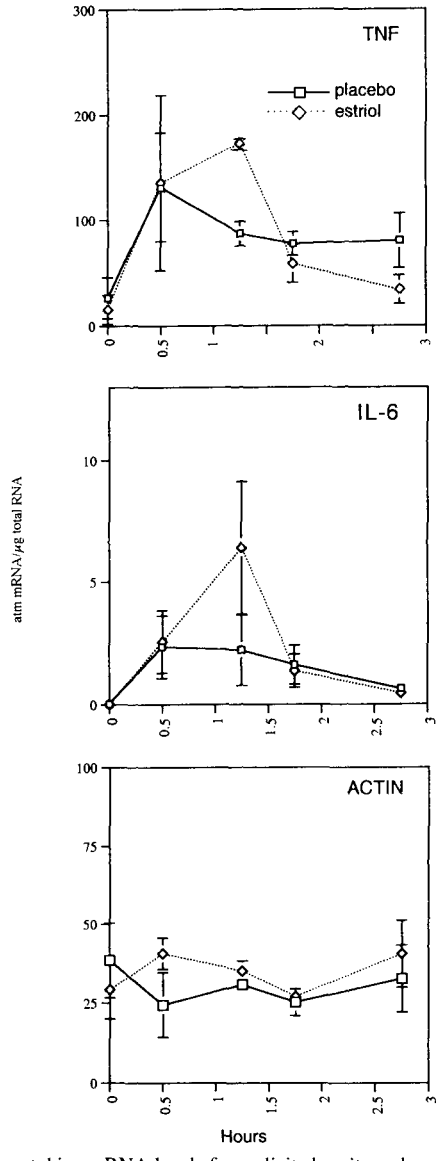

Fig. 7. Estriol effects on cytokine mRNA levels from elicited peritoneal macrophages. Mice exposed to 1.5 mg estriol or placebo pellets were thioglycollate primed, 4 days after implant and challenged with 200 μg LPS 3 days later. At designated intervals mice, 3 per group, were sacrificed, bled for circulating cytokine measurements and peritoneal cells harvested by lavage. Peritoneal cells were counted and 1×10^6 macrophages were adhered to 6 well plates. Following removal of non adherent cells at 90 min, monolayers were lysed, RNA was prepared and RT-PCR performed as described. Messenger RNA levels for TNF, IL-6, and actin were quantitated by mimetic analysis. All values represent the mean from a pool of 3 separate experiments. Brackets indicate the *SEM*.

did not exceed two fold and was only significant at the 75 minute time point, suggesting that post transcriptional regulatory events were involved in the more significant increase in circulating TNF. Changes in IL-6 mRNA were also apparent and as with TNF, while the peak in IL-6 mRNA in the macrophages from the placebo mice was apparent 30 minutes after in vivo challenge, the IL-6 levels increased to a peak value at approximately 75 minutes after LPS injection. This increase was approximately 2–3 fold that observed in the placebo group at the same time point but did not achieve statistical significance. In contrast to the changes observed in both TNF and IL-6 mRNA levels in peritoneal macrophages following endotoxin challenge, the actin mRNA levels were relatively constant at all time points evaluated and did not differ between the estriol and placebo groups.

Finally, while estriol treatment resulted in significant increases in circulating cytokine levels following endotoxin challenge and changes in cytokine mRNA levels, ex vivo macrophage cultures from estriol treated mice failed to demonstrate any significant increase over the placebo group in TNF or NO secretion in response to an in vitro LPS challenge (Table 2). Whereas LPS resulted in a significant increase in both TNF and NO accumulation in the supernatants, the values obtained were comparable between the ex vivo macrophage cultures from estriol and placebo treated animals. The interferon γ primed macrophages from both groups demonstrated a further increase in NO secretion following LPS challenge but again, the values were comparable between the ex vivo estriol and placebo macrophage cultures. Therefore, the significant effects in vivo on circulating cytokine levels did not result in increased secretion of inflammatory mediators in vitro by LPS stimulated peritoneal macrophage cultures from estriol pretreated animals.

Table 2. Estriol Effects on NO and TNF in Ex Vivo LPS Stimulated Macrophage Cultures.[a]

Treatments	NO (Moles)	TNF (ng/ml)
Placebo-Control	8.15 (0.34)	<0.1
Placebo-IFNγ	19.4 (1.94)	<0.1
Placebo-LPS	71.85 (1.78)	11.8 (1.02)
Placebo-LPS + IFNγ	109.78 (0.4)	14.47 (0.85)
Estriol-Control	8.59 (0.34)	<0.1
Estriol-IFNγ	17.7 (1.7)	0.4 (0.36)
Estriol-LPS	60.1 (2.07)	9.99 (0.60)
Estriol-LPS + IFNγ	95.26 (2.94)	11.23 (0.41)

[a]Thioglycollate elicited peritoneal macrophages were obtained from BALB/c mice 1 week after implant of estriol (1.5 mg) or placebo pellets. Cells were plated at 2.5×10^5 per well in 24 well plates and stimulated with LPS at 100 ng/ml in the presence or absence of IFN γ at 1000 units/ml. Supernatants were harvested at 24 hrs for TNF and 48 hrs for NO measurements. Parentheses indicate standard deviations from triplicate cultures. Representative experiment of 3.

DISCUSSION

The increased incidence of autoimmune disease in premenopausal women and in animal models have provided evidence for the involvement of sex steroids in inflammation and autoimmunity. Estrogens have been demonstrated to impact on immune cell function by suppressing T cell and yet to increase B cell and macrophage functions (21, 24–26, 30). Estrogenic effects in vitro on macrophage cytokine expression have included both positive and negative affects on IL-1 secretion (11–14), decreased JE and increased PDGF-A chain mRNA levels (19, 20), modest effects on TNF (16) and a reduction in IL-6 secretion (30). Estrogen has also been demonstrated to inhibit macrophage mediated oxidation of LDL (9, 10) and in vivo, to increase splenic cell macrophage Fc receptor activity (32). The mechanism(s) by which estrogens effect macrophage effector function however remain unknown. Estrogen receptors have been reported on macrophages (8, 33) and macrophage like synoviocytes (34) and yet as reported by Ray et al. (35) repression of the IL-6 promoter in estrogen receptor transfectants by the receptor-ligand complex was not dependent upon high affinity binding of the complex to the promoter. Clearly, to what extent estrogenic modulation of macrophage effector functions in vivo are dependent upon estrogen-macrophage interactions or are mediated by an accessory cell type requires further study. Previous studies from this laboratory have demonstrated that pharmacologic oral doses of 17 α ethinyl estradiol or parenteral administration of 17 β estradiol resulted in an increase in circulating levels of TNF and yet reductions in IL-6 following endotoxin challenge (30). Estrogens including estradiol and estriol in female MRL/lpr mice were also observed to induce a shift in the serum lipoprotein profile resulting in a significant increase in LDL cholesterol (36). While the lipid composition and oxidation state of these LDL particles have not yet been defined, these studies as well as earlier investigations on estrogenic effects in murine lupus models support a role for estrogen in the exacerbation of the inflammatory response.

In the present study, the relationship between estrogens and inflammation have been further explored by considering the effects of a distinct estrogen agonist, estriol, on the nature of the cytokine response to an in vivo endotoxin challenge. As with the previous study related to estradiol (30), estriol treatment also resulted in a 3–7 fold increase in serum TNF levels 1 hr post LPS challenge. While the doses of estriol 400 μg to 2.2 mg/kg were pharmacologic, the effects appear to be dependent upon an estrogen receptor as tamoxifen was able to reverse the estriol effects on both TNF and IL-6 expression. The possible relationships between estrogen, IL-6 expression, and osteoporosis (18) as well as the earlier observation that estradiol reduced circulating levels of IL-6 following endotoxin challenge (30) necessitated an understanding of the effects of estriol on serum IL-6. In contrast to the estradiol data, estriol treatment resulted in an

earlier increase in circulating IL-6. Whereas peak values of IL-6 in the placebo group occurred 3 hrs post LPS challenge, in agreement with other bolus endotoxin models (37), maximal levels within the estriol group occurred at 1 hr. Therefore, measurements derived only from the peak time point would have provided the erroneous conclusion that estriol had significantly reduced circulating IL-6.

The mechanism by which the IL-6 peak is shifted to exhibit kinetics similar to TNF is unclear. The tamoxifen data would suggest that this effect is dependent upon estrogen receptors and yet was not observed with estradiol at comparable doses (30). This effect did not appear to be related to the significant increase in circulating TNF following endotoxin challenge in the estriol treated animals as administration of the TNF neutralizing monoclonal antibody TN3-19.12, did not shift the peak level of IL-6 back to the placebo. While the mRNA data from peritoneal macrophages does not support transcriptional regulation as explaining the accelerated kinetics for the serum IL-6 increase, parallel studies with Kupffer cells and splenic macrophages may provide some insight. Whether estriol treatment results in a priming of an IL-6 secreting cell type resulting in an increase in steady state IL-6 mRNA levels which is then more rapidly translated following an inflammatory stimulus remains to be determined. To the best of our knowledge, this represents the first report of a pharmacologic treatment which alters the time course rather than magnitude of a systemic cytokine response to an inflammatory stimulus.

Additional studies have focused on the effects of the altered cytokine responses in estriol treated mice to the sequella associated with endotoxemia. While TNF levels were significantly higher in the estriol group, there was no increase in lethality observed when the estriol and placebo groups were challenged with an LD_{15} concentration of LPS (data not shown). Furthermore, the estriol treated animals were competent in becoming endotoxin tolerant as these animals did not mount a significant increase in circulating TNF in response to a secondary LPS challenge (SZ and NP unpublished observations). This distinguishes the estriol treated mice from adrenalectomized mice which previously had been demonstrated to have increased circulating TNF levels following LPS challenge and yet did not become endotoxin tolerant (38).

While the in vivo effects of estriol on cytokine expression are highly reproducible, ex vivo studies on peritoneal macrophages from these animals have not revealed significant differences in TNF or NO secretion (Table 2), and only modest changes in cytokine mRNA levels (Figure 7). Furthermore, in flow cytometry experiments evaluating MAC-1, class I and class II antigens, and Fcγ II/III receptor expression, detected by 2.4G2 staining, peritoneal macrophages from estriol treated BALB/c mice did not exhibit any significant changes in surface antigen expression when compared to macrophages from the placebo treated animals (SEA and SHZ unpublished observations).

The pleiotropic responses of various target cells to IL-6 raises the question as to the significance of the shift in IL-6 appearance in the estriol treated animals. As IL-6 is a potent regulator of the acute phase response (39), the earlier increase in circulating IL-6 may influence the kinetics and nature of the acute phase response to endotoxin. This may represent a compensatory mechanism in the estriol treated animals which based on the relative increases in systemic TNF exhibit an exaggerated cytokine response to an inflammatory stimulus. The use of IL-6 knockout mice (40) will permit a greater understanding of the possible protective role of IL-6 in the estriol treated animals. Finally, as estrogen treatment has been reported to increase disease severity in lupus prone strains of mice (25, 26) and with the well described role of IL-6 in B cell-plasma cell differentiation (39), the relevance of the earlier increase in circulating IL-6 following an inflammatory stimulus in estriol treated animals may provide a model to understand the role of estrogens in disease progression through IL-6 regulation.

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