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17β-Estradiol Antiinflammatory Activity in Carrageenan-Induced Pleurisy

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ABSTRACT

We have recently demonstrated that 17β-estradiol (E_2) opposes cytokine-dependent increase of inducible nitric oxide synthase (iNOS) activity in rat smooth muscle cells and proposed that this effect might be associated to an antiinflammatory activity of this hormone. In the present study, we examine the E_2 effects on a well-known *in vivo* model of inflammation. We show that, in carrageenan treatment of ovariectomized rats, prior exposure to E_2 significantly attenuated inflammatory response as measured by histological examination and exudate production. The effect was visible with a single injection of a physiological dose of E_2 1 h before the carrageenan treatment and was blocked by coadministration of the estrogen receptor antagonists ICI 182,780 or tamoxifen. This latter observation suggests that the effect is receptor mediated. The mechanisms by which estradiol has beneficial effects in this model of inflammation are unclear: we show that in hormonally treated rats there is a decrease in polymorphonuclear cells migration as shown by cell counting and myeloperoxidase measurement. In addition, E_2 pretreatment opposes carrrageneen-induced high lipid peroxidation maintaining malondialdehyde activity at control levels. E_2 treatment decreases NO production and the activity of iNOS with consequent diminished nitrite synthesis and nitrosine accumulation. Finally, immunohistochemical analysis for poly (ADP-ribose) synthase revealed a positive staining in lungs from carrageenan-treated rats that was blocked by estradiol treatment. We conclude that E_2 attenuates the degree of inflammation and tissue damage associated with carrageenan-induced pleurisy in the rat. (*Endocrinology* **141:** 1455–1463, 2000)

ESTROGENS ARE FEMALE sex steroid hormones that have a plethora of effects on a wide range of tissues. These effects are mediated through well characterized intracellular receptors (ERα and ERβ) (1). Traditionally, E_2 has been associated to a proinflammatory activity because in the reproductive tract, which represents E_2 most studied target organ, the hormone stimulates epithelial cell proliferation, increases vascular permeability, edema and influx of macrophages, and eosinophils in the uterine stroma. The recent observation of an E_2-dependent blockage of cytokine-dependent induction of inducible nitric oxide synthase (iNOS) (2) led us to speculate that estrogens may also exert antiinflammatory activity. An evaluation of the data present in the literature partly supports this view. It is well known that gender affects the susceptibility to immune-mediated inflammatory diseases such as rheumatoid arthritis and multiple sclerosis, and women have increased risk of developing these diseases as compared with men. Clinical remissions of both diseases occur during pregnancy, and estrogens are of benefit in rheumatoid arthritic disease (3, 4), leading to hypothesis that sex steroids are a factor in its pathogenesis (4, 6). In addition, estrogens were shown to have an antiinflammatory activity also in several animal models (7–15). On the other hand, females have an impressively higher incidence of collagen diseases like systemic lupus erythematosus (SLE), and the severity of SLE and other inflammatory pathologies (*e.g.* gingivitis) are worsened by alterations in hormonal balance at puberty and during menses (16). This suggests that estrogens may also play a proinflammatory role in specific diseases or districts. The mechanisms underlying estrogen effects in these complex pathologies are not known; their comprehension is therefore of relevance in view of the increasing number of women chronically treated with estrogens for oral contraception or replacement therapy.

Estrogens exhibit immunomodulatory effects, *in vivo* and *in vitro*. Murine models with type II collagen-induced arthritis suggest direct effects of estrogens on T cell function (17); in other models of SLE, estrogens are shown to increase anti-DNA and antiphospholipid titers (7, 18). The administration of estradiol suppresses the delayed-type of hypersensitivity, which is an antigen-specific T cell-mediated inflammation, whereas it enhances the antigen-specific antibody response. E_2 has also an antiinflammatory property that is T cell independent, not mediated by modulation of corticosteroid production, but rather, by suppression of the production of leukocytes by the bone marrow (10). These effects may be linked to the gender difference in the incidence of collagen diseases such as SLE. Finally, few studies report on an effect of estrogen on the polymorphonuclear leuko-

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cytes (PMNs), which play an important role in inflammation (20–22).

The aim of the present study was to investigate the activity of estrogens in a well-known murine model of inflammation (carrageenan-induced pleurisy). The cellular and molecular mechanism of the carrageenan-induced pleurisy is well characterized. The early phase of the carrageenan-induced inflammation is related to the production of histamine, leukotrienes, platelet-activating factor, and possibly cyclooxygenase products, while the delayed phase of the carrageenan- induced inflammatory response has been linked to neutrophil infiltration and the production of neutrophil-derived free radicals and oxidants, such as hydrogen peroxide, superoxide, and hydroxyl radical, as well as to the release of other neutrophil-derived mediators (22–26) that causes tissue necrosis. To characterize the effect of E_2 in this model of acute inflammation, we have determined the following endpoints of the inflammatory response in ovariectomized rats: 1) exudate formation; 2) PMN infiltration; 3) peroxynitrite formation (immunohistochemistry); 4) activation of the nuclear enzyme poly (ADP-ribose) polymerase (PARP); and 5) expression of the iNOS protein (immunohistochemistry) and activity, (7, 8), lipid peroxidation, and lung injury. In addition, we have investigated the effects of the systemic administration (pretreatment) of estrogen receptor antagonists (tamoxifen and ICI) on the above parameters of inflammation.

Materials and Methods

Reagents

Primary antinitrotyrosine antiserum was from Upstate Biotech (DBA, Milan, Italy). All other reagents and compounds used were obtained from Sigma (Milan, Italy).

Animals

Female Sprague Dawley rats (300–350 g; Charles River Laboratories, Inc.; Milan; Italy) were housed in a controlled environment and provided with standard rodent chow and water. Animal care was in compliance with Italian regulations on protection of animals used for experimental and other scientific purposes (D.M. 116192) as well as with the EEC regulations (O.J. of E.C. L 358/1 12/18/1986).

Ovariectomy

All surgical procedures were performed under halothane (2%) anesthesia followed by nitrous oxygen/O_2 anesthesia for about 18 min. Ovariectomy (OVX) was performed through a single dorsal midline cutaneous incision followed by bilateral muscle incisions.

Experimental groups

OVX animals were treated with carrageenan (carrageenan group) or vehicle (saline) (sham group). Both of this group were subdivided into four subgroups each treated: 1 h before carrageenan with E_2 (50 μg/kg; ip) (carrageenan + E_2 group), 1 h before E_2 with tamoxifen (50 μg/kg ip) (carrageenan + tamoxifen group), with ICI 182 780 (500 μg/kg ip) (carrageenan + ICI group) or with vehicle.

Carrageenan-induced pleurisy

Rats were anesthetized with isoflurane and submitted to a skin incision at the level of the left sixth intercostal space. The underlying muscle was dissected and saline (0.2 ml) or saline containing 1% λ-carrageenan (0.2 ml) were injected into the pleural cavity. The skin incision was closed with a suture and the animals were allowed to recover. At 4 h after the injection of carrageenan, the animals were killed by inhalation of CO_2. The chest was carefully opened and the pleural cavity rinsed with 2 ml of saline solution containing heparin (5 $U \cdot ml^{-1}$) and indomethacin (10 $\mu g \cdot ml^{-1}$). The exudate and washing solution were removed by aspiration and the total volume measured. Any exudate, which was contaminated with blood, was discarded. The amount of exudate was calculated by subtracting the volume injected (2 ml) from the total volume recovered. The leukocytes in the exudate were suspended in PBS and counted with an optical microscope in a Burker's chamber after vital Trypan blue staining.

Measurement of nitrite/nitrate

Nitrite + nitrate production, an indicator of NO synthesis, was measured in the exudate as previously described (26). Briefly, the nitrate in the exudate was first reduced to nitrite by incubation with nitrate reductase (670 $mU \cdot ml^{-1}$) and NADPH (160 μM) at room temperature for 3 h. The nitrite concentration in the samples was then measured by the Griess reaction, by adding 100 μl of Griess reagent (0.1% naphthylethylenediamide dihydrochloride in H_2O and 1% sulfanilamide in 5% concentrated H_2PO_4; vol. 1:1) to 100 μl samples. The optical density at 550 nm (OD_{550}) was measured using ELISA microplate reader (SLT Labinstruments, Salzburg, Austria). Nitrate concentrations were calculated by comparison with OD_{550} of standard solutions of DMEM.

Histological examination

Lung biopsies were taken at 4 h after injection of carrageenan. The biopsies were fixed for 1 week in buffered formaldehyde solution (10% in PBS) at room temperature, dehydrated by graded ethanol and embedded in Paraplast (Sherwood Medical, Mahwah, NJ). Tissue sections (thickness 7 μm) were deparaffinized with xylene, stained with trichromic Van Gieson and studied using light microscopy (Dialux 22 Leitz, Milan, Italy).

Immunohistochemical localization of nitrotyrosine and PARS

Tyrosine nitration, an index of the nitrosylation of proteins by peroxynitrite and/or oxygen-derived free radicals, was determined by immunohistochemistry as previously described (27). At the end of the experiment, the relevant organs were fixed in 10% buffered formaldehyde, and 8 μm sections were prepared from paraffin-embedded tissues. After deparaffinization, endogenous peroxidase was quenched with 0.3% H_2O_2 in 60% methanol for 30 min. The sections were permeabilized with 0.1% Triton X-100 in PBS for 20 min. Nonspecific adsorption was minimized by incubating the section in 2% normal goat serum in PBS for 20 min. Endogenous biotin or avidin binding sites were blocked by sequential incubation for 15 min with avidin and biotin. The sections were then incubated overnight with primary antinitrotyrosine antiserum (1:1000) or with primary antipoly (ADP-ribose) antiserum (1:500) or with control solutions. Controls included buffer alone or nonspecific purified rabbit IgG. Some sections were also incubated with the primary antibody (antinitrotyrosine) in the presence of excess nitrotyrosine (10 mM) to verify the binding specificity. Specific labeling was detected with a biotin-conjugated goat antirabbit IgG and avidin-biotin peroxidase complex. Diaminobenzidine was used as a cromogen.

Myeloperoxidase (MPO) activity

MPO activity, an indicator of polymorphonuclear leukocyte (PMN) accumulation, was determined as previously described (28). Four hours after the intrapleural injection of carrageenan, lung tissues were obtained and weighed. Each pieces of tissue was homogenized in a solution containing 0.5% hexa-decyl-trimethyl-ammonium bromide dissolved in 10 mM potassium phosphate buffer (pH 7) and centrifuged for 30 min at 20,000 × g at 4 C. An aliquot of the supernatant was then allowed to react with a solution of tetra-methyl-benzidine (1.6 mM) and 0.1 mM H_2O_2. The rate of change in absorbance was measured spectrophotometrically at 650 nm. MPO activity was defined as the quantity of enzyme degrading 1 μmol of peroxide min^{-1} at 37 C and was expressed in milliunits per gram weight of wet tissue.

Malondialdehyde (MDA) measurement

MDA levels in the lung tissue were determined as an indicator of lipid peroxidation (29). Lung tissues, collected at the specified time, were homogenized in 1.15% KCl solution. An aliquot (100 μl) of the homogenate was added to a reaction mixture containing 200 μl of 8.1% SDS, 1500 μl of 20% acetic acid (pH 3.5), 1500 μl of 0.8% thiobarbituric acid, and 700 μl distilled water. Samples were then boiled for 1 h at 95 C and centrifuged at 3,000 × *g* for 10 min. The absorbance of the supernatant was measured by spectrophotometry at 650 nm.

Determination of nitric oxide synthase activity

The calcium-independent conversion of L-arginine to L-citrulline in the homogenates of lungs (obtained 4 h after carrageenan treatment in the presence or the absence of E_2) served as an indicator of iNOS activity (30). Cells were scraped into a homogenization buffer composed of 50 mM Tris·HCl, 0.1 mM EDTA, and 1 mM phenylmethylsulphonyl fluoride (pH 7.4) and homogenized in the buffer on ice using a tissue homogenizer. Conversion of [^{3}H]-L-arginine to [^{3}H]-L-citrulline was measured in the homogenates as described (31). Briefly, homogenates (30 μl) were incubated in the presence of [^{3}H]-L-arginine (10 μM, 5 kBq per tube), NADPH (1 mM), calmodulin (30 nM), tetrahydrobiopterin (5 μM), and EGTA (2 mM) for 20 min at 22 C. Reactions were stopped by dilution with 0.5 ml of ice-cold HEPES buffer (pH 5.5) containing EGTA (2 mM) and EDTA (2 mM). Reaction mixtures were applied to Dowex 50W (Na+ form) columns and the eluted [^{3}H]-L-citrulline activity was measured by a Beckman Coulter, Inc. (Milan, Italy) scintillation counter.

Statistical analysis

All values in the figures and text are expressed as mean ± SE (SEM) of the mean of *n* observations. For the *in vivo* studies, *n* represents the number of animals studied. In the experiments involving histology or immunohistochemistry, the figures shown are representative of at least three experiments performed on different experimental days. The results were analyzed by one-way ANOVA followed by a Bonferroni *posthoc* test for multiple comparisons. A *P* value less than 0.05 was considered significant.

Results

Effects of E_2 in carrageenan-induced pleurisy

All carrageenan-injected rats developed an acute pleurisy, with edema of the lung, production of a peritoneal turbid exudate, and neutrophil infiltration (Table 1; Fig. 1A). The volume of the exudate was visibly smaller in E_2-pretreated rats (Table 1). Trypan blue exclusion counting in the exudate revealed $87.3 \pm 4.97 \times 10^6$/rat polymorphonuclear leukocyte in comparison to sham rat ($2 \pm 0.8 \times 10^6$/rat) (Fig. 1A). Sham animals demonstrated no abnormalities in the pleural cavity or fluid. The polymorphonuclear migration was significantly reduced in rats pretreated with E_2 (Fig. 1A). E_2 pretreatment did not cause significant changes in these parameters in sham rats (Table 1; Fig. 1A). Coadministration of E_2 and tamoxifen or ICI significantly blocked the effect of E_2 (Table 1; Fig. 1A).

TABLE 1. Exudate volume in pleural cavity at 4 h after carrageenan injection

	Volume (ml)	
	Vehicle	Carrageenan
Control	0.12 ± 0.06	2.3 ± 0.17[a]
E_2	0.11 ± 0.08	0.66 ± 0.14[b]
E_2 + ICI	0.1 ± 0.08	2.14 ± 0.18[a,c]
E_2 + Tamoxifen	0.16 ± 0.07	1.76 ± 0.21[a,c]

[a] $P < 0.01$ *vs.* vehicle.
[b] $P < 0.01$ *vs.* carrageenan.
[c] $P < 0.01$ *vs.* E_2.

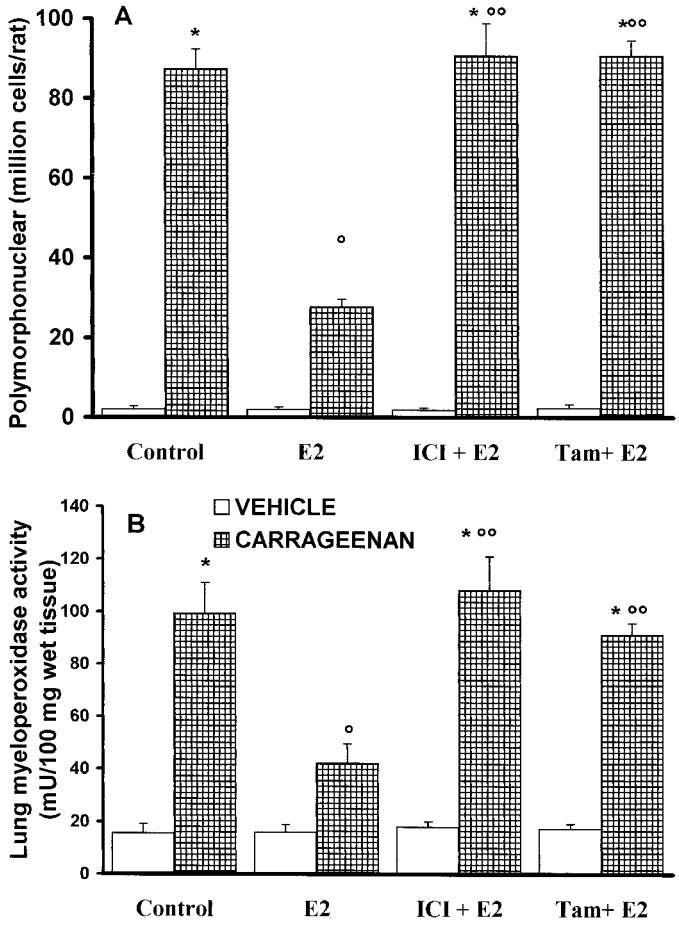

FIG. 1. Effect of E_2 on accumulation of polymorphonuclear cells in pleural cavity (A) and myeloperoxidase activity (B) in the lung at 4 h after carrageenan injection: E_2 significantly reduced polymorphonuclear cells infiltration in the pleural cavity as well as the myeloperoxidase activity in the lung. Tamoxifen and ICI treatments significantly reverted the effect of E_2. Data are means ± SE means of 10 rats for each group. *, $P < 0.01$ *vs.* sham. °, $P < 0.01$ *vs.* carrageenan. °°, $P < 0.01$ *vs.* E_2.

A decreased neutrophil infiltration was also indicated by the measurement of myeloperoxidase activity in lungs after carrageenan administration. As shown in Fig. 1B, myeloperoxidase activity (99.26 ± 11.7 mU/100 mg wet tissue) was significantly ($P < 0.01$) increased at 4 h after carrageenan injection compared with sham rats (15.6 ± 3.5 mU/100 mg wet tissue). E_2 pretreatment prevented the carrageenan effect only in the absence of the two antagonists (Fig. 2B).

Histological examination of lung sections of rats treated with carrageenan showed tissue injury (Fig. 2A) as well as infiltration of the bronchial (Fig. 2B) and perivascular space (Fig. 2C) with PMNs. E_2 pretreatment reduced both tissue injury (Fig. 2D) and infiltration of the bronchial (Fig. 2E) and perivascular space (Fig. 2F). Tamoxifen and ICI treatments significantly blocked the effect of E_2 (data not shown).

NOx levels were also significantly ($P < 0.01$) increased in the exudate after carrageenan challenge (76.3 ± 4.3 nmol/rat *vs.* 9.98 ± 0.77 nmol/sham rat) (Fig. 3A). In the lungs obtained from animals subjected to carrageenan-induced pleurisy, a significant increase of inducible NO synthase activity

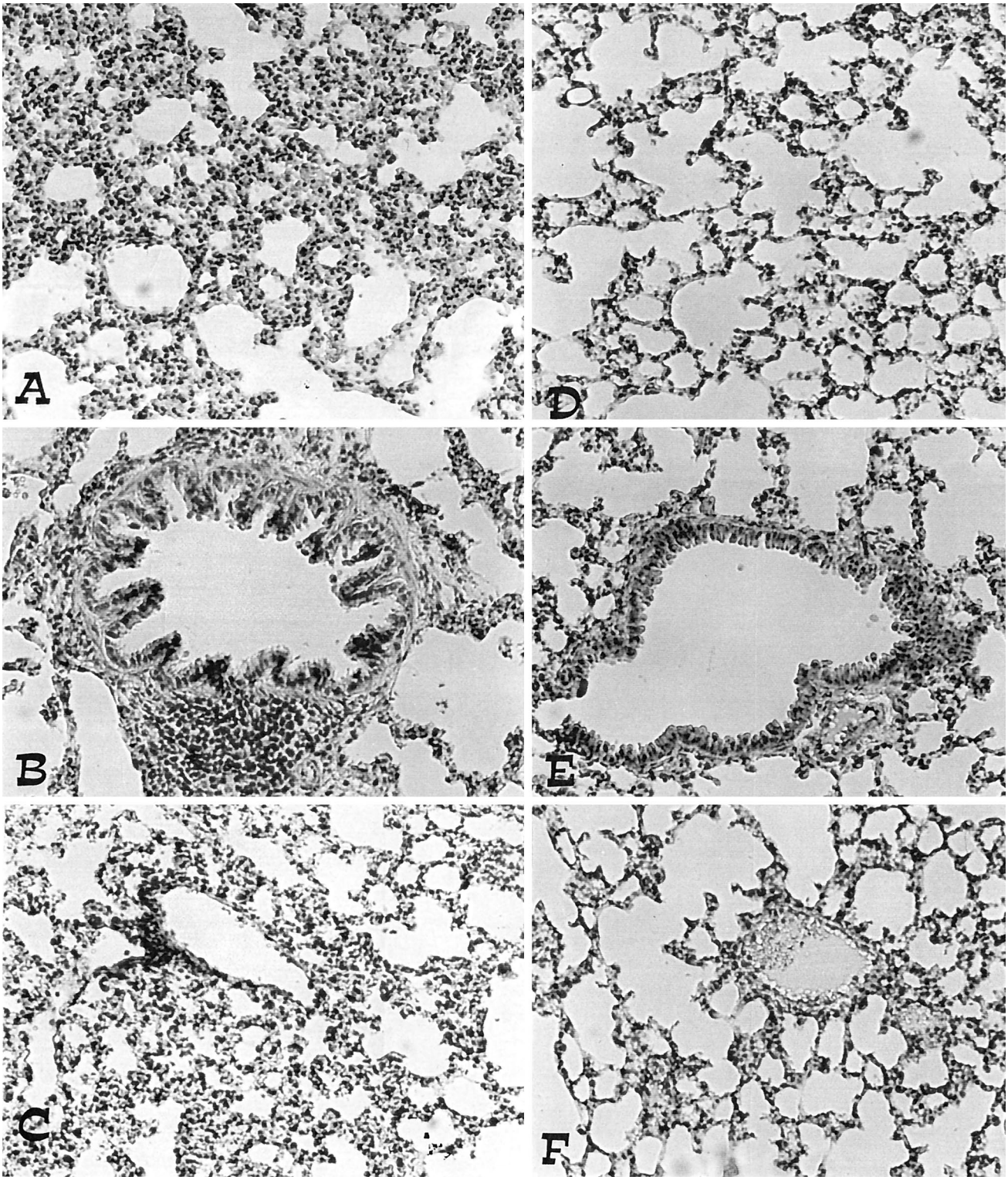

FIG. 2. Effect of E_2 on lung injury: The lung section from a carrageenan-treated rat demonstrates interstitial hemorrhage (A), infiltration of the bronchial (B), and perivascular space (C) with polymorphonuclear leukocyte. The lung section from a carrageenan-treated rat that had received E_2 demonstrates reduced interstitial hemorrhage (D) and a lesser cellular infiltration in the bronchial (E) and perivascular space (F). Original magnification: 125×. Figure is representative of at least three experiments performed on different experimental days.

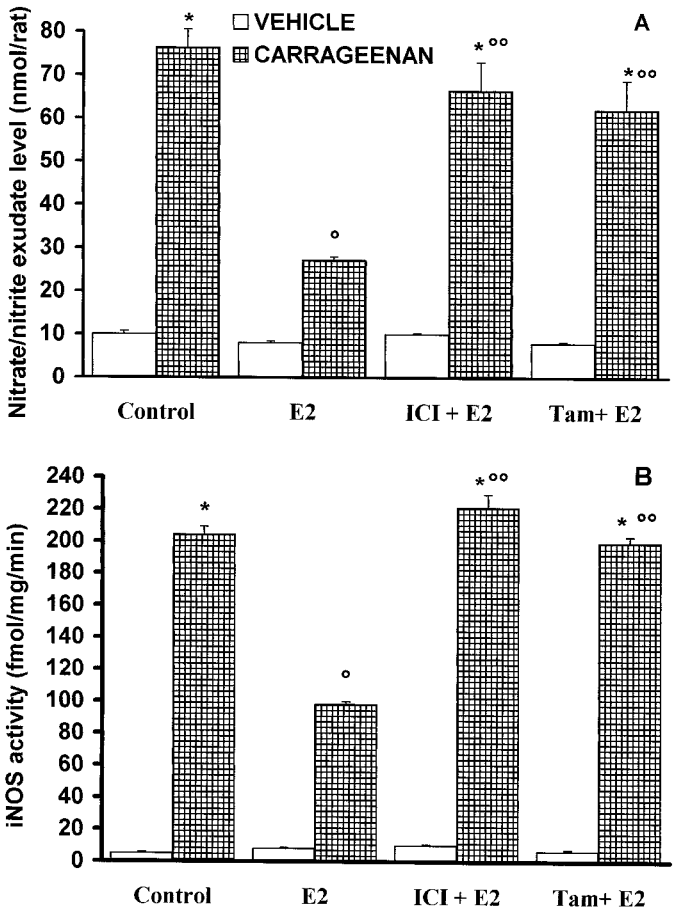

FIG. 3. Effect of E_2 on nitrite and nitrate concentrations in pleural exudate (A) and iNOS activity in lungs (B) at 4 h after carrageenan administration. Nitrite and nitrate levels and iNOS activity in carrageenan-treated rats were significantly increased *vs.* sham group. E_2 treatment significantly ameliorated the carrageenan-induced elevation of nitrite and nitrate levels and the expression of iNOS activity. Tamoxifen and ICI treatments significantly reverted the effect of E_2. Data are means ± SE means of 10 rats for each group. *, $P < 0.01$ *vs.* sham. °, $P < 0.01$ *vs.* carrageenan. °°, $P < 0.01$ *vs.* E_2.

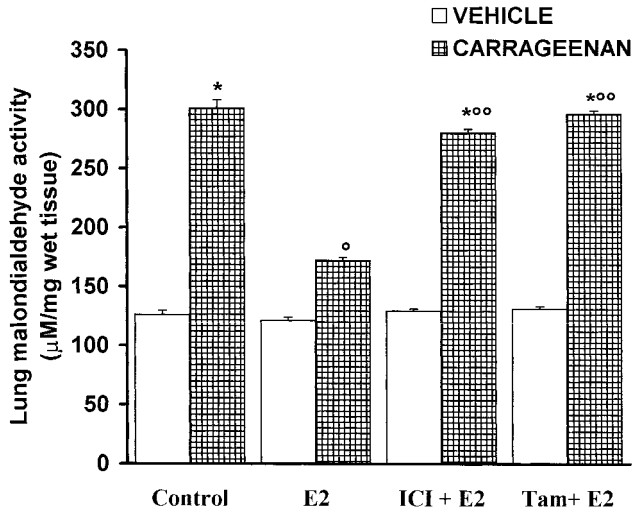

FIG. 4. Effect of E_2 on MDA levels in the lungs of carrageenan-treated rats killed at 4 h. MDA levels were significantly increased in the lungs of the carrageenan-treated rats in comparison to sham rats (*, $P < 0.01$). E_2 reduced the carrageenan-induced increase in MPO activity and MDA levels. Tamoxifen and ICI treatments significantly reverted the effect of E_2. Data are means ± SE means of 10 rats for each group. *, $P < 0.01$ *vs.* sham. °, $P < 0.01$ *vs.* carrageenan. °°, $P < 0.01$ *vs.* E_2.

was detected at 4 h (204 ± 2.97 fmol/mg/min) (Fig. 3B). NOx levels and iNOS activity were significantly reduced in rats pretreated with E_2 (Fig. 3, A and B). Tamoxifen and ICI treatments significantly blocked the effect of E_2 (Fig. 3, A and B).

A significant ($P < 0.01$) increase of MDA was found in the lung of carrageenan-treated rat (301 ± 6.5 μM/mg wet tissue) compared with sham rats (126 ± 3.5 μM/mg wet tissue) (Fig. 4). E_2 pretreatment prevented the carrageenan effect only if administered in the absence of the two antagonists (Fig. 4).

At 4 h following the intrapleural injection of carrageenan, lung sections were analyzed for the presence of nitrotyrosine. Immunohistochemical analysis in lungs from carrageenan-treated rats, using a specific antinitrotyrosine antiserum, revealed a positive brown staining (see *arrows)* in perivascular (Fig. 5A) and in the apical portion of the epithelial cell (Fig. 5B) as well as in inflammatory cells (Fig. 5C). No positive staining was found in perivascular (Fig. 5D) and in the apical portion of the epithelial cell (Fig. 5E) as well as in inflammatory cells (Fig. 5F) in the lungs of the carrageenan-treated rats when they were pretreated with E_2. Immunohistochemical analysis for PARS from lung sections obtained from rats treated with carrageenan also revealed a positive brown staining (see *arrows*) in perivascular (Fig. 6A) and in the apical portion of the epithelial cell (Fig. 6B) as well as in inflammatory cells (Fig. 6C) (Fig. 6A). In contrast, no positive staining for PARS was found in perivascular (Fig. 6D) and in the apical portion of the epithelial cell (Fig. 6E) as well as in inflammatory cells (Fig. 6F) in the lungs of carrageenan-treated rats, which had been pretreated with E_2. Tamoxifen and ICI treatments significantly blocked the effect of E_2 (data not shown). Please note that there was no staining for either nitrotyrosine or PARS in lungs obtained from sham-operated rats (data not shown).

Discussion

Here we demonstrate that in rat E_2 reduces the development of carrageenan-induced pleurisy, the infiltration of the lung with PMNs (histology and MPO activity), the degree of lipid peroxidation in the lung, NO production, and the degree of lung injury (histology). All of these findings support the view that E_2 attenuates the degree of inflammation and lung injury caused by carrageenan in the rat. The mechanisms underlying this antiinflammatory-like activity are still to be clarified. E_2 might act at several steps of the inflammatory process. In the present model, system general effects like suppression of leukocyte production and bone marrow distribution of peripheral blood neutrophils (10) or direct stimulation of CRH (32) are unlikely to occur. E_2 was in fact administered at short time before the inflammatory stimulus: a period of time insufficient to significantly alter white cell activities. In the present model, however, the decreased PMN infiltration in carrageenan-treated lungs might be due to a hormone-dependent modification of PMN chemotaxis. Izumi and colleagues in fact reported that E_2 significantly

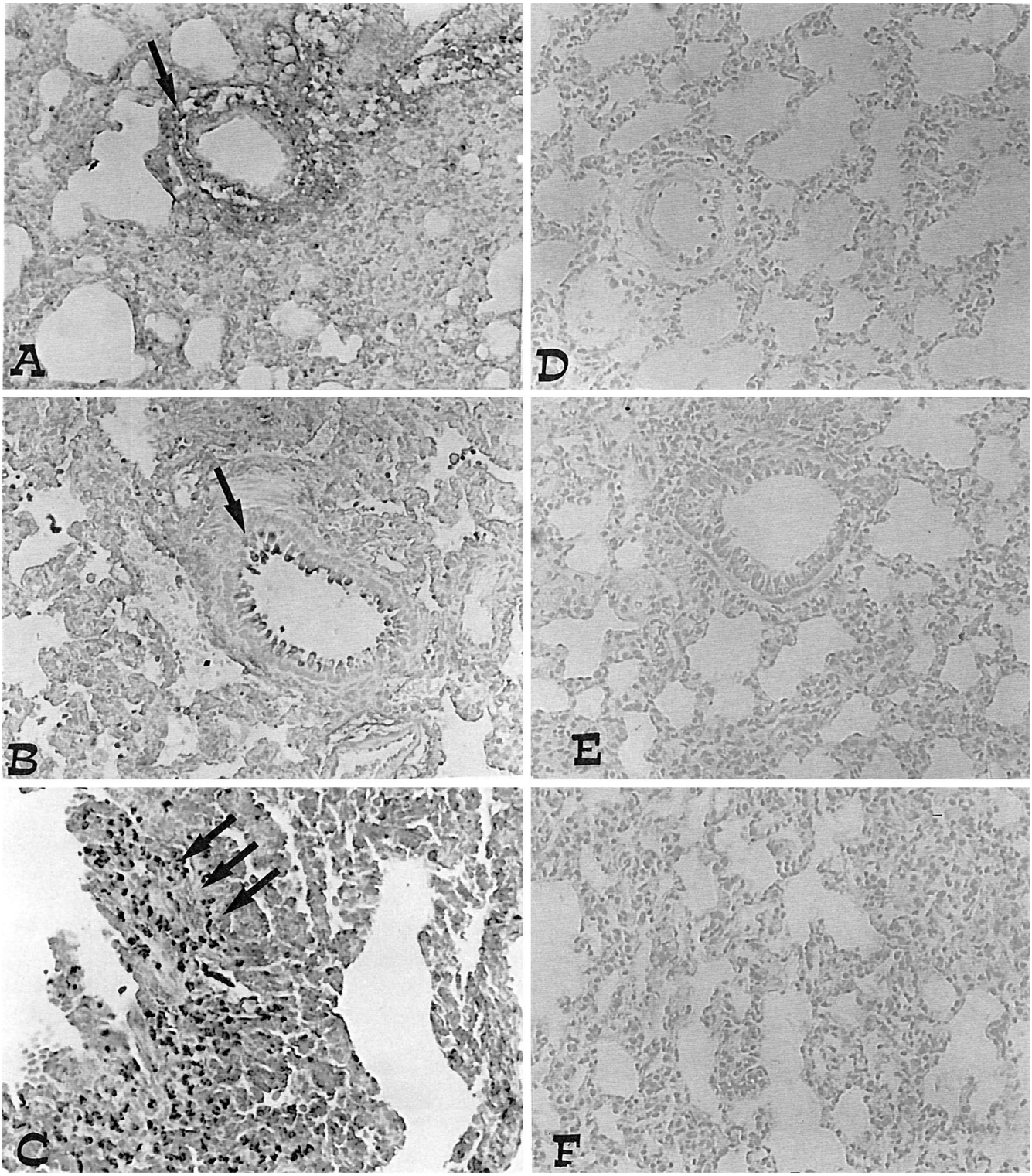

FIG. 5. Effect of E_2 on nitrotyrosine formation: Immunohistochemical localization of nitrotyrosine in the rat lung. Four hours following carrageenan injection, positive staining for nitrotyrosine (typical areas are indicated by *arrows*) was observed in perivascular (A) and epithelial cell (B) as well as in inflammatory cells (C). There was a marked reduction in the immunostaining in perivascular (D) and epithelial cell (E) as well as in inflammatory cells (F) from the lungs of carrageenan-treated rats pretreated with E_2 (B). Original magnification: 125×. Figure is representative of at least three experiments performed on different experimental days.

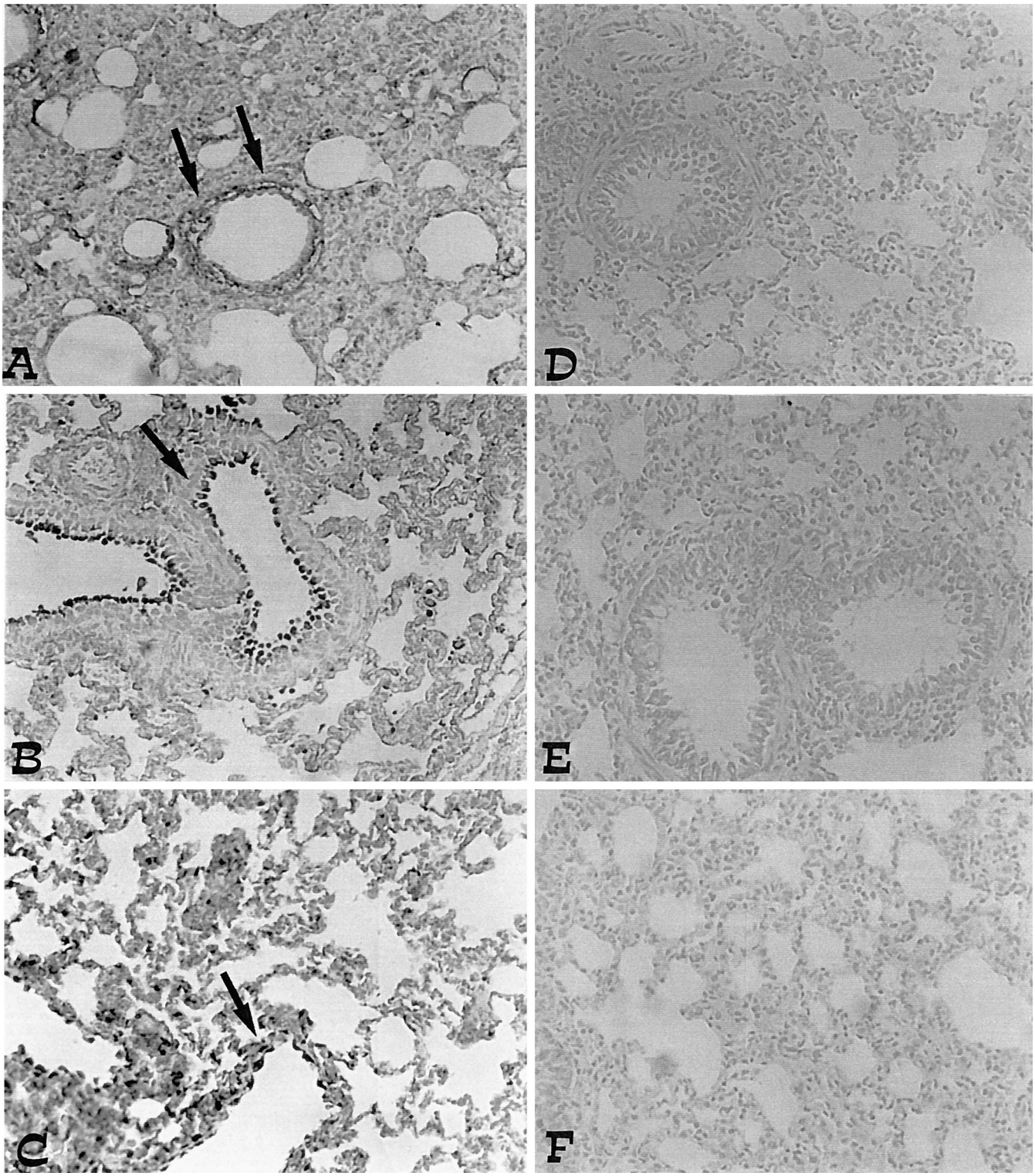

FIG. 6. Effect of E_2 on PARS activity: Four hours following carrageenan injection, positive staining for PARS (typical areas are indicated by *arrows*) was observed in perivascular (A) and epithelial cell (B) as well as in inflammatory cells (C). No positive staining was found in perivascular (D) and epithelial cell (E) as well as in inflammatory cells (F) from the lungs of carrageenan-treated rats pretreated with E_2 (B). Original magnification: 125×. Figure is representative of at least three experiments performed on different experimental days.

inhibits the response of PMNs to FMLP or LTB4 (33). PMNs chemotaxis to FNILP was reduced dose dependently, whereas that to LTB4 was reduced in smaller concentration of E_2 than that to FNILP. Alternatively, E_2 might have altered the expression of membrane adhesion molecules as reported by Genco and colleagues (34).

We here show that E_2 decreases iNOS activity and the consequent formation of free radical species. Previous studies have shown that NOS inhibitors reduce the development of carrageenan-induced inflammation and support a role for NO in the pathophysiology associated with this model of inflammation (27, 35–38).

In addition, we demonstrate that E_2 attenuates the nitrosilation of proteins in the lung of rats treated with carrageenan. Nitrotyrosine formation, along with its detection by immunostaining, was initially proposed as a relatively specific marker for the detection of the endogenous formation "footprint" of peroxynitrite (39). There is, however, recent evidence that certain other reactions can also induce tyrosine nitration; *e.g.*: the reaction of nitrite with hypochlorous acid and the reaction of myeloperoxidase with hydrogen peroxide can lead to the formation of nitrotyrosine (40). Increased nitrotyrosine staining is considered, therefore, as an indication of "increased nitrosative stress" rather than a specific marker of the generation of peroxynitrite.

Reactive oxygen species (ROS) and peroxynitrite produce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage. ROS produce strand breaks in DNA that trigger energy-consuming DNA repair mechanisms and activates the nuclear enzyme PARS resulting in the depletion of its substrate NAD *in vitro* and a reduction in the rate of glycolysis. As NAD functions as a cofactor in glycolysis and the tricarboxylic acid cycle, NAD depletion leads to a rapid fall in intracellular ATP. This process has been called the PARS Suicide Hypothesis. There is recent evidence that the activation of PARS may also play an important role in inflammation (27, 42, 43). We demonstrate here that E_2 attenuates the increase in PARS activity caused by carrageenan in the lung. Thus, we propose that the antiinflammatory effects of E_2 reported here are, at least in part, due to the prevention of the activation of PARS.

Estrogens have been reported to have antioxidative activities that might in part explain some of the findings reported in the present study. However, the antioxidant activity of E_2 is observed at pharmacological concentrations of the hormone and is not blocked by antagonists of the estrogen receptors. We thus believe that the lack of lung tissue injury in E_2 -pretreated rats here reported is not due to estrogens antioxidant action because observed in rats treated with low doses of the hormone and prevented by the coadministration of two antagonists of estrogen receptor: tamoxifen and ICI 182,780. The effects here reported therefore are most likely receptor mediated.

In conclusion, our study shows that E_2 may have an antiinflammatory activity in a well known model for the study of antiinflammatory drugs. This model will hopefully be useful for the dissection of the mechanisms by which estrogen might influence a number of human pathologies.

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References

1. **Kuiper GGJM, Gustafsson JA** 1997 The novel oestrogen receptor-β subtype: potential role in the cell and promoter specific actions of estrogens and antiestrogens. FEBS Lett 410:87–90
2. **Zacan V, Santagati S, Bolego C, Vegeto E, Maggi A, Puglisi L** 1999 17 β-Estradiol decreases nitric oxide synthase II synthesis in vascular smooth muscle cells. Endocrinology 140:2204–2209
3. **Holmdahl R** 1989 Estrogen exaggerates Lupus but suppresses T-cell dependent autoimmune disease. J Autoimmun 2:651–662
4. **Vandenbroucke JP, Valkenburg HA, Boersma JW, Cats A, M. Festen JJ, Huber-Bruning O, Raske JJ** 1982 Oral contraceptives and rheumatoid arthritis: further evidence for a preventive effect. Lancet 2:839–853
5. Deleted in proof
6. **Da Silva JAP, Hall GM** 1992 The effects of gender and sex hormones on outcome in rheumatoid arthritis. Baillieres Clin Rheumatol 6:193–219
7. **Carlsten H, Nilsson N, Jonsson R, Backman K, Holmdahl R, Tarkowski A** 1992 Estrogen accelerates immune complex glomerulonephritis but ameliorates T cell mediated vasculitis and sialadenitis in autoimmune MRL Ipr/mice. Cell Immunol 144:190–198
8. **Josefsson E, Tarkowski A** 1997 Suppression of type II collagen-induced arthritis by the endogenous oestrogen metabolite 2-methoxyestradiol. Arthritis Rheum 40:154–63
9. **Shirai M, Sato A, Chida K** 1995 The influence of ovarian hormones on the granulomatous inflammatory process in the rat lung. Eur Respir J 8:272–277
10. **Josefsson E, Tarkowski A, Carlstein H** 1992 Anti-inflammatory properties of oestrogen. I. In vivo suppression of leukocyte production in bone marrow and redistribution of peripheral blood neutrophils. Cell Immunol 142:67–78
11. **Da Silva JAP, Colville-Nash P, Spector TD, Scott DL, Willoughby DA** 1993 Inflammation-induced cartilage degradation in female rodents. Protective role of sex hormones. Arthritis Rheum 36:1007–1013
12. **Schroder J, Kahlke V, Staubach KH, Zabel P, Stuber F** 1998 Gender differences in human sepsis. Arch Surg 133:1200–1205
13. **Iwamura K** 1979 Clinical significance of estrogens in chronic aggressive hepatitis. Acta Hepatogastroenterol 26:267–278
14. **Jansson L, Holmdahl R** 1998 Estrogen-mediated immunosuppression in autoimmune diseases. Inflamm Res 47:290–301
15. **Hayashi T, Yamada K, Esaki T, Muto E, Chaudhuri G, Iguchi A** 1998 Physiological concentrations of 17β-estradiol inhibit the synthesis of nitric oxide synthase in macrophages via a receptor-mediated system. J Cardiovasc Pharmacol 31:292–298
16. **Persellin RH** 1997 The effect of pregnancy on rheumatoid arthritis. Bull Rheum Disease 27:922–927
17. **Holmdahl R, Jansson L, Meyerson B, Klareskog L** 1987 Oestrogen induced suppression of collagen arthritis: I. Long term oestradiol treatment of DBA/1 mice reduces severity and incidence of arthritis and decreases the anti type II collagen immune response. Clin Exp Immunol 70:372–378
18. **Roubinian JR, Talal N, Greenspan JS, Goodman JR, Siiteri PK** 1978 Effect of castration and sex hormone treatment on survival, anti-nucleic acid antibodies, and glomerulonephritis in NZB/NZW F1 mice. J Exp Med 147:1568–1575
19. Deleted in proof
20. **Calrsten H, Holmdahl R, Tarkowski A, Nilsson LA** 1989 Oestradiol and testosterone-mediated effect on the immune system in normal and autoimmune mice are genetically linked and inherited as dominant traits. Immunology 68:209–214
21. **Hammerschimidt DE, Knabe AC, Silberstein PT, Lamche HR, Coppo PA** 1988 Inhibition of granulocyte function by steroids is not limited to corticoids: studies with sex steroids. Inflammation 12:277–284
22. **Buyon JP, Korchak HM, Rutherford LE, Ganguly M, Weissmann G** 1984 Female hormones reduce neutrophil responsiveness *in vitro.* Arthritis Rheum 27:623–630
23. **Miyagi M, Kaoyama H, Morishita M, Iwamoto Y** 1992 Effects of sex hormones on chemotaxis of human peripheral polymorphonuclear leukocytes and monocytes. J Periodontolology 63:28–32
24. **Salvemini D, Wang ZQ, Bourdon DM, Stern MK, Currie MG, Manning PT** 1996 Evidence of peroxynitrite involvement in the carrageenan-induced rat paw edema. Eur J Pharmacol 303:217–20
25. **Peskar BM, Trautmann M, Nowak P, Peskar BA** 1991 Release of 15-hydroxy-5,8,11,13-eicosatetraenoic acid and cysteinyl-leukotrienes in carrageenin-induced inflammation: effect of non-steroidal anti-inflammatory drugs. Agents Actions 33:240–246
26. **Da Motta JI, Cunha FQ, Vargaftig BB, Ferreira SH** 1994 Drug modulation of antigen-induced paw oedema in guinea-pigs: effects of lipopolysaccharide, tumour necrosis factor and leucocyte depletion. Br J Pharmacol 112:111–116
27. **Cuzzocrea S, Caputi AP, Zingarelli B** 1998 Peroxynitrite-mediated DNA strand breakage activates poly (ADP-ribose) synthetase and causes cellular energy depletion in carrageenan-induced pleurisy. Immunology 93:96–101
28. **Cuzzocrea S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabó C** 1998 Anti-inflammatory effects of mercaptoethylguanidine, a combined inhibitor of nitric oxide synthase and peroxynitrite scavenger, in carrageenan-induced models of inflammation. Free Rad Biol Med 24:450–459
29. **Mullane KM, Westlin W, Kraemer R** 1988 Activated neutrophils release

mediators that may contribute to myocardial injury and dysfunction associated with ischemia and reperfusion. Ann NY Acad Sci 524:103–121
30. **Ohkawa H, Ohishi N, Yagi K** 1979 Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358
31. **Szabó C, Mitchell JA, Thiemermann C, Vane JR** 1993 Nitric oxide-mediated hyporectivity to norepinephrine precedes the induction of nitric oxide synthase in endotoxin shock. Br J Pharmacol 108:786–794
32. **Heller B, Wang ZQ, Wagner EF, Radons J, Burkle A, Fehsel K, Burkart V, Kolb H** 1995 Inactivation of the poly (ADP-ribose) polymerase gene affects oxygen radical and nitric oxide toxicity in islet cells. J Biol Chem 270:11176–11180
33. **Chrousos GP, Torpy DJ, Gold PW** 1998 Interactions between the hypothalamic-pituitary-adrenal axis and the female reproductive system: clinical implications. Ann Intern Med 129:229–240
34. **Zumi I, Hayashj T, Yamada K, Kuzuya M, Naito M, lguchi A** 1995 Physiological concentration of estradiol inhibits polymorphonuclear leukocyte chemotaxis via a receptor mediated system. Life Sci 56:2247–2253
35. **Genco RJ, Vandyke TE, Levine MJ, Nelson RD, Wilson ME** 1985 J Dent Res 65:1379–1391
36. **Tracey WR, Nakane M, Kuk J, Budzik G, Klinghofer V, Harris R, Carter G** 1995 The nitric oxide synthase inhibitor, L-NG-monomethylarginine, reduces carrageenan-induced pleurisy in the rat. J Pharmacol Exp Ther 273:1295–1299
37. **Wei XQ, Charles IG, Smith A, Ure J, Feng GJ, Huang FP, Xu D, Muller W, Moncada S, Liew FY** 1995 Altered immune responses in mice lacking inducible nitric oxide synthase. Nature 375:408–411
38. **Salvemini D, Wang ZQ, Wyatt P, Bourdon DM, Marino MH, Manning PT, Currie MG** 1996 Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br J Pharmacol 118:829–838
39. **Cuzzocrea S, Zingarelli B, Gilard E, Hake P, Salzman AL, Szabó C** 1997 Protective effect of melatonin in carrageenan-induced models of local inflammation. J Pineal Res 23:106–116
40. **Beckman JS** 1996 Oxidative damage and tyrosine nitration from peroxynitrite. Chem Res Toxicol 9:836–844
41. Deleted in proof
42. **Szabó C, Lim LHK, Cuzzocrea S, Getting SJ, Zingarelli B, Flower RJ, Salzman AL, Perretti M** 1997 Inhibition of poly (ADP-ribose) synthetase exerts anti-inflammatory effects and inhibits neutrophil recruitment. J Exp Med 186:1041–1049
43. **Szabó C, Viràg L, Cuzzocrea S, Scott GS, Hake P, O'Connor M, Zingarelli B, Ma Y, Hirsch R, Boiovin GP, Salzman AL, Kun E** 1998 Protection against peroxynitrite-induced fibroblast injury and arthritis development by inhibition of poly (ADP-ribose) synthetase. Proc Natl Acad Sci USA 95:3867–3872