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Estrogen Regulates Cytokine Production and Apoptosis in PMA-Differentiated, Macrophage-Like U937 Cells

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Abstract We have investigated the effects of sex steroids, estradiol (E2), and testosterone (T) on the synthesis of tumor necrosis factor alpha (TNF-α) and interleukin-10 (IL-10) in phorbol-myristate-acetate (PMA)-differentiated human monoblastic U937 cells. The ability of both hormones to modulate the viability and programmed cell death of macrophage-like PMA-differentiated U937 cells was also inspected. E2 increased TNF-α synthesis, whereas T had no effect on the production of this cytokine. The combination of E2 and its antagonist tamoxifen or ICI-182,789 completely abolished the induction of TNF-α, while combination of T and its antagonist Casodex (CSDX) did not significantly affect TNF-α production by U937 cells. Exposure of cells to E2 resulted in a dose-dependent decrease of IL-10 synthesis, while again T did not show any detectable effect. In addition, E2 induced a significant increase of apoptosis in macrophage-like U937 cells and this increase was inhibited by the simultaneous addition of either tamoxifen or ICI-182. In contrast, T alone or in combination with CSDX did not modify apoptotic rates of U937 cells. This evidence, taken together, suggests that estrogens, but not androgens, exert a pro-inflammatory action through the modulation of TNF-α and IL-10, and regulate the immune effector cells by the induction of programmed cell death. J. Cell. Biochem. 90: 187–196, 2003.
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Key words: estrogen; TNF-α; IL-10; apoptosis; U937 cells

Experimental and clinical evidence has suggested the involvement of gonadal steroids in inflammation and autoimmunity [Lahita, 1993]. Generally, it is accepted that estrogens have an immune-stimulating role, whereas androgens could have immune-suppressive effects [Lahita, 1993]. Sex steroids have been reported to affect the immune system by modifying T-cell, B-cell, and macrophage functions [Carlsten et al., 1992; Van Vollenhoven and McGuire, 1994]. In addition, they regulate synthesis of cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-6, IL-1, IL-10, and nitric oxide production [Mori et al., 1992; Chao et al., 1995; Zuckerman et al., 1996; Squadrito et al., 1997; D'Agostino et al., 1999]. Various studies have revealed the expression of sex steroid hormone receptors in immune cells [Cutolo et al., 1995b]. The evidence for a sexual dimorphism in the immune-related diseases could provide a basis to better understand the frequent disorders affecting women in post-menopausal age, such as osteoporosis, cardiovascular, or neuro-degenerative diseases [Horowitz, 1993; Nathan and Chaudhuri, 1997]. These can be ascribed to the loss of sex hormone-dependent regulation of some physiological functions, or to the steroid-induced modification of activities in immune cells [Ben-Hur et al., 1995] or both.

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Recent studies have also demonstrated the involvement of estradiol (E2) and testosterone (T) in either induction or suppression of the apoptotic process in several cell systems [Hughes et al., 1996; Spyridopoulos et al., 1997; Mossuz et al., 1998; Olsen et al., 1998; Huber et al., 1999; Vegeto et al., 1999]. In particular, macrophage cells are important targets of sex steroid hormones, being endowed with both estrogen receptor (ER) and androgen receptor (AR), and involved in the pathogenesis of different sex hormone-related diseases (like rheumatoid arthritis or systemic lupus erythematosus) [Van Vollenhoven and McGuire, 1994].

In this study, we have investigated the effects of E2 and T on the synthesis of some cytokines (TNF-α, IL-10) in a human monoblastic leukemia U937 cell line, as well as their effects on U937 cell proliferative activity and programmed cell death.

MATERIALS AND METHODS

Reagents

RPMI 1640 tissue culture medium was obtained from Flow Laboratories (Irvine, UK). Medium supplements, including L-glutamine, penicillin–streptomycin, non-essential amino acids, and heat inactivated fetal calf serum (HI-FCS) were all from Hyclone Laboratories (Logan, UK). The phorbol-myristate-acetate (PMA), and the 3-[4,5-dimethyl-thiazol-2yl] 2,5-diphenyl-tetrazolium bromide (MTT) were obtained from Sigma (Poole, Dorset, UK). Testosterone (T), estrogen (E2), pure synthetic anti-estrogen, ICI-182,780 tamoxifen and non-steroidal anti-androgen Casodex (CSDX, ICI-176) (Sigma) was dissolved in ethanol. Tissue culture plasticware was purchased from NUNC (Roskilde, Denmark). Other reagents were obtained from Sigma.

Cell Cultures

The human monoblastic U937 cells were obtained from the American Tissue Culture Collection (ATCC, Rockville, MD). For routine maintenance, cells were grown in RPMI culture medium, supplemented with 10% (v/v) HI-FCS, 2 mM L-glutamine, penicillin–streptomycin, and 0.1 mM non-essential amino acids, at 37°C under a humidified 5% CO_2/95% air atmosphere. Endotoxin levels in FCS and RPMI medium were less than 0.03 EU ($<$10 ng/ml) as assessed by the limulus amebocyte lysate assay. Cells were sub-cultured in 25 cm^2 flasks (Corning Glassworks, Corning, NY) and periodically tested for both bacterial and mycoplasma contamination. Three days before experiments, medium was replaced with RPMI 1640 supplemented with 10% (v/v) steroid-deprived charcoal-treated (DCC) fetal calf serum (DCC-RPMI). In all cases, U937 cells were cultured in 10% (v/v) DCC-RPMI for 48 h in the presence of PMA (10 ng/ml/10^6) to induce their differentiation in macrophage-like cells.

Immunocytochemistry of AR and ER

The presence of both AR and ER in PMA-differentiated U937 cells was investigated using specific monoclonal antibodies as described elsewhere [Castagnetta et al., 1999]. Briefly, cells were grown in 10% (v/v) DCC-RPMI onto 2-well Lab-Tek Tissue Culture Chamber Slides (NUNC) for 24 h to 6 days. Medium was discarded and cells washed twice in ice-cold PBS-A and fixed for 10 min at 4°C using 3.7% (v/v) formaldehyde (ER) or picric acid (AR) solution. After two washing in PBS, cells were incubated for 2 h in the presence of primary mouse monoclonal antibodies raised against ER (0.1 μg/ml; DAKO, Glostrup, Denmark) and AR (0.05 μg/ml; PharMingen, San Diego, CA). A revelation system with secondary biotinylated antibodies and streptavidin–peroxidase complex (Supersensitive Multilink, HRP, Biogenex, Menarini, Italy) was used. Cells were counterstained with methyl green. Control slides were treated in an identical manner, except non-specific mouse IgG replaced the first step. A mean of 400 randomly selected fields from at least three different slides were analyzed using a quantitative image analysis application for the CAS200 Image Analyzer (Becton-Dickinson, Milan, Italy), which automatically yields the percentage of positively stained nuclei.

Ligand Binding Assay of AR and ER

The AR and ER content and status of both undifferentiated and PMA-treated U937 cells were determined by a radioligand binding assay, as previously described [Castagnetta et al., 1995]. Cell homogenates were spun at 800*g* for 5 min at 4°C to separate the soluble (supernatant) from the nuclear (pellet) cell fraction. The supernatant was further centrifuged at 2,000*g* for 5 min at 4°C to remove cell debris; the nuclear pellet was subsequently

washed three times in ice-cold buffered saline (10 mM HEPES and 150 mM NaCl, pH 7.4) and resuspended to the original volume in the same saline. Aliquots (150 μl) of each cell fraction were then incubated overnight at 4°C against increasing concentration (from 0.1 up to 5 nM) of either [2,4,6,7-^{3}H]17β-estradiol or [17α-methyl-^{3}H]mibolerone, as estrogen or androgen radioligand, respectively. After incubation, DCC absorption and filtration methods were used to separate bound from unbound ligand in soluble and nuclear fraction, respectively. For the soluble fraction, 1 ml aliquots of the resulting supernatant were counted in a β-counter. For the nuclear fraction, 100 μl aliquots of the nuclear suspension were filtered through Whatman GF/C glass fiber filters (Whatman, Maidstone, UK) using a Millipore apparatus (Millipore, SpA, Milan, Italy), and the unbound ligand was washed out using saline. Filters were then removed, dried overnight at room temperature, and counted for radioactivity.

Receptor data from both saturation and competition studies were analyzed and processed using Scatchard analysis and a modification (©OncoLog 2.2) of a least square fit routine, run on an IBM personal computer, yielding both dissociation constant (K_d) and concentration values (femtomoles per ml homogenate); the latter were expressed either as femtomoles per mg protein or DNA or the number of sites per cell for any cell compartment. Data were also analyzed using a model for one or two binding sites, depending on the best fitting achieved. An arbitrary K_d cut-off value of 1 nM was adopted for both receptors to separate type I (high affinity, low capacity) and type II (reduced affinity, greater capacity) binding sites. Protein and DNA cell contents were determined using the Bradford [Bradford, 1976] and modified Burton [Katzenellenbogen and RE, 1974] methods, respectively.

Induction and Measurement of Cytokine Production in the Supernatants

PMA-differentiated U937 cells (10^6/ml) were cultured in 10% (v/v) DCC-RPMI in 24-well plates for 24–48 h in the presence of different concentrations of sex hormones (10^{-9}–10^{-6} M) alone or in combination with relevant antagonists (antiestrogens: tamoxifen at 10^{-5} M, ICI-182,780 at 10^{-7} M; antiandrogen: CSDX 10^{-7} M). The culture supernatants were then collected for TNF-α and IL-10 measurement. The levels of cytokines were estimated using ELISA commercial kits (PharMingen), which employ a multiple antibody sandwich principle, according to the manufacturer's instructions.

Measurement of Cell Death

To establish the effects of gonadal steroids and their antagonists on apoptosis of U937 cells, both a TUNEL and an ELISA methods were used.

TUNEL assay. Cells were grown directly onto 2-well Lab-Tek Tissue Culture Chamber Slides (Nunc, Naperville, IL) and cell death rates measured using the In Situ Cell Death Detection Kit (Boehringer, Mannheim, Germany), according to the recommendations of the supplier. Briefly, cells were fixed in paraformaldehyde solution (4% v/v in PBS) and incubated with a 0.3% (v/v) H_2O_2 solution in methanol to block endogenous peroxidase. Slides were rinsed twice in PBS, and permeabilized using a 0.1% (v/v) Triton X-100 solution. After two washes in PBS, slides were incubated with appropriate amounts of a TUNEL (TdT-mediated dUTP nick end labeling) reaction mixture for 1 h at 37°C; in this step, terminal deoxynucleotidyl transferase (TdT) is used to achieve fluorescent labeling of DNA strand breaks. Slides were then rinsed three times in PBS and analyzed under a fluorescence microscope. In order to detect incorporated fluorescein, slides were incubated for 30 min at room temperature with an anti-fluorescein antibody conjugated with horseradish peroxidase. After three washes in PBS, slides were incubated for 10 min at room temperature with a DAB-substrate solution, rinsed in PBS, and finally mounted under glass coverslips. The proportion of stained (apoptotic) cell nuclei was assessed on at least 25 randomly selected fields.

Cell death detection by ELISA^{PLUS} assay. Apoptosis was also evaluated by an ELISA kit (Boehringer) which is based on a quantitative sandwich enzyme immunoassay principle using mouse monoclonal antibodies directed against histone-associated-DNA fragments [Tolomeo et al., 2001]. Macrophage-like U937 cells (1×10^4/well) were treated with lysis buffer. Twenty microliters of lysate (cytoplasmic fraction) were transferred into the streptavidin-coated microtiter plate. Subsequently, a mixture of anti-histone-biotin and anti-DNA-peroxidase conjugated were added. After the removal of unbound antibodies by a washing

step, the amount of nucleosomes was quantified by the peroxidase retained in the immunocomplex. Samples were read in a spectrophotometer at 405 nm. Fold increase in the level of mono- and oligo-nucleosomes in the cytoplasmic fraction was determined by comparing these results with those observed U937 control cells receiving vehicle (ethanol) alone.

Statistical Analysis

The standard deviation (SD) and standard error (SE) were calculated and the statistical significance was analyzed by using the Student's *t*-test (paired and unpaired) and by analysis of variance (Student–Newmann–Keuls test). The Mann–Whitney *U*-test was also used to compare data between groups. $P < 0.05$ was considered statistically significant.

RESULTS

Androgen and Estrogen Receptor Content and Status in U937 Cells

Results of cytochemical assay for both AR and ER in U937 cell line are reported in Table I. The PMA-differentiated U937 cells express both receptors under standard conditions, with a slight prevalence of AR. A highly significant decrease of ER or AR expression was observed when cells were exposed, respectively, to E2 or T at the concentration of 10^{-7} M, while T also induced a marked decrease of AR at 10^{-9} M (Table I). Immunocytochemical staining for ER and AR in U937 cells is illustrated in Figure 1.

Ligand binding assay was also used to define the presence of type I (high affinity, lower capacity) and type II (low affinity, greater capacity) ER and AR in both soluble and nuclear fractions of U937 cells. Undifferentiated U937 cells exhibited soluble and nuclear high affinity (type I) binding sites of both estrogen and androgen (Table II). PMA-treated cells showed the appearance of type II receptors in the soluble fraction, whilst nuclear type I receptor content was markedly increased. Concerning AR, treatment of cells with PMA resulted in the induction of type II receptors in the soluble fraction, while, as for ER, this agent produced a significant rise of type I nuclear AR.

TABLE I. Estrogen Receptor (ER) and Androgen Receptor (AR) Expression and Regulation in U937 Cells

Control	10^{-9} M	10^{-7} M
ER	Estradiol	
68 ± 7	63 ± 8	38 ± 5**
AR	Testosterone	
86 ± 10	56 ± 6*	22 ± 4**

Data are expressed as the percent of cells (nuclei) stained positively for ER or AR. Values represent mean ± SE of three separate experiments, each measuring a total of at least 400 cells.
*$P < 0.05$, **$P < 0.01$ with respect to control cell cultures.

Effects of E2 and T on TNF-α and IL-10 Production in U937 Cells

The effects of E2 and T on TNF-α production in the macrophage-like PMA-differentiated U937 cells are shown in Figure 2. After 24 h incubation, E2 was able to induce TNF-α synthesis at all doses used (range of 10^{-9}–10^{-6} M), with the most effective concentration (2.5-fold increase, $P < 0.01$) being 10^{-7} M (Fig. 2A). The simultaneous addition of the pure antiestrogen ICI-182 completely abolished the E2-induced synthesis of TNF-α at all the E2 concentrations used. Separate experiments using tamoxifen as antiestrogen, revealed that this mixed agonist-antagonist synthetic estrogen, at the concentration of 10^{-5} M, decreased the E2-induced TNF-α synthesis, even though it was less effective than the ICI-182 (data not shown). Conversely, T had no significant effect on the synthesis of TNF-α in macrophage-like U937 cell cultures, although a limited (30%) decrease was observed at 10^{-6} M (Fig. 2B). The combination of T and the pure antiandrogen CSDX did not affect TNF-α synthesis, apart from a not significant increase (42% above control) of this cytokine when T was used at 10^{-9} M (Fig. 2B).

Figure 3 illustrates the effects of E2 and T on IL-10 production in PMA-differentiated U937 cells. A 24 h incubation of U937 cell cultures with E2 resulted in a dose-dependent decrease of IL-10 synthesis (Fig. 3A), with a lowest point of nearly 40% ($P < 0.01$) at 10^{-6} M E2. By contrast, T did not induce any significant change in IL-10 production by U937 cells (Fig. 3B).

Effects of Exogenous E2 and T on U937 Cell Survival

To investigate the effects of sex steroids on cell viability and apoptosis of PMA-differentiated U937 cells, we carried out experiments where cells were incubated for 24 h with increasing concentrations of E2 or T, in the absence or presence of specific antihormones. Using the TUNEL technique, we observed that E2 significantly stimulates (nearly twofold,

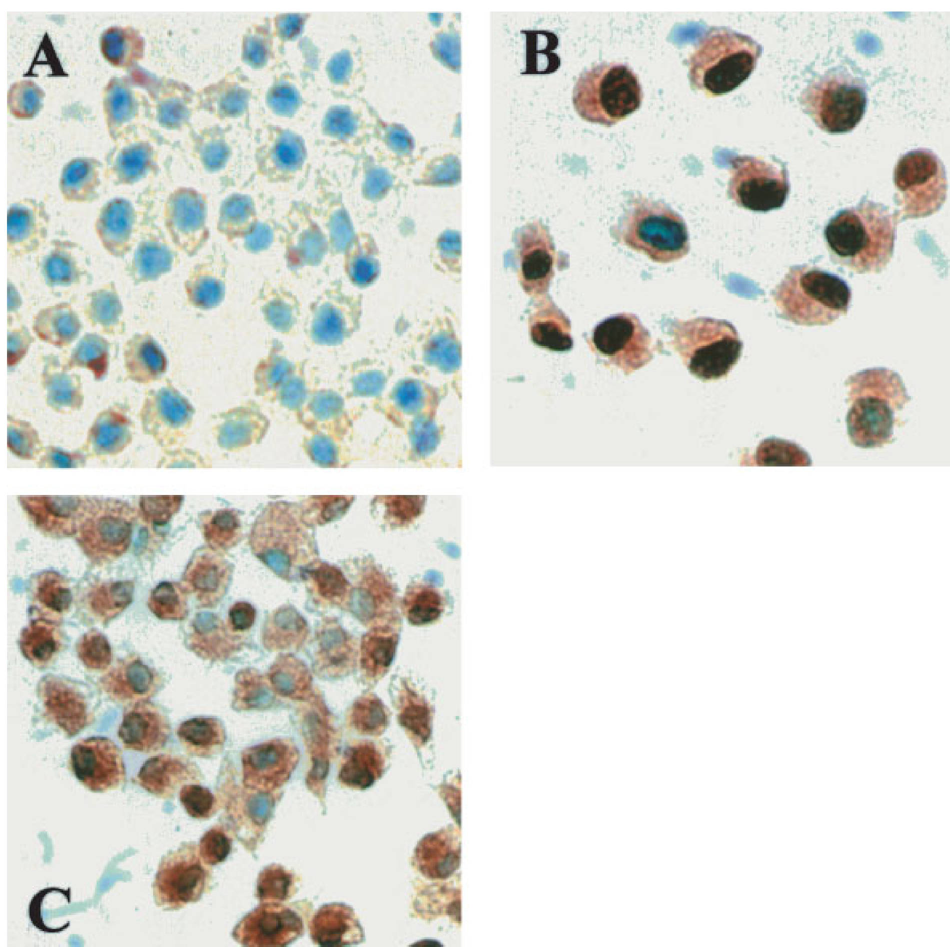

Fig. 1. Immunocytochemical staining for androgen receptor (AR) and estrogen receptor (ER) in U937 cells. **A**: Control; (**B**) androgen receptors; (**C**) estrogen receptors. Cells were exposed to anti-ER or anti-AR monoclonal antibody and the specific binding revealed through a secondary step biotinylated anti-mouse IgG and streptavidin–peroxidase complex. Control cell cultures received non-specific mouse IgG instead of primary antibody.

$P < 0.01$) apoptotic rates of the PMA-differentiated U937 cells at the concentration of 10^{-7} M, whilst it is ineffective at the 10^{-9} M dose (see Fig. 4A). In these cells, the simultaneous addition of the antiestrogen ICI-182 (10^{-7} M) or tamoxifen (10^{-5} M), almost completely reverted the E2-induced programmed cell death. On the contrary, treatment of U937 cell cultures with

TABLE II. Status of Soluble and Nuclear ER and AR Receptors in U937 Cells

	Soluble		Nuclear	
	K_d (nM)	Sites/cell	K_d (nM)	Sites/cell
ER				
Untreated	0.91 ± 0.08	$100{,}789 \pm 13{,}756$	0.10 ± 0.02	$17{,}749 \pm 1{,}939$
PMA	2.24 ± 0.29[a]	$445{,}628 \pm 56{,}321$[a]	0.35 ± 0.02	$64{,}736 \pm 5{,}789$
AR				
Untreated	0.69 ± 0.05	$75{,}433 \pm 8{,}211$	0.08 ± 0.01	$9{,}508 \pm 927$
PMA	1.27 ± 0.14[a]	$222{,}814 \pm 32{,}576$[a]	0.22 ± 0.03	$34{,}626 \pm 4{,}238$

ER and AR status of both untreated and PMA-differentiated U937 cells was assessed in both soluble and nuclear cell fractions using ligand binding assay (for methodological details see text). Values represent mean ± SD of quadruplicate experiments. K_d, dissociation constant.
[a]Type II receptors only. Type I ER and AR are defined by a K_d value ≤ 1 nM.

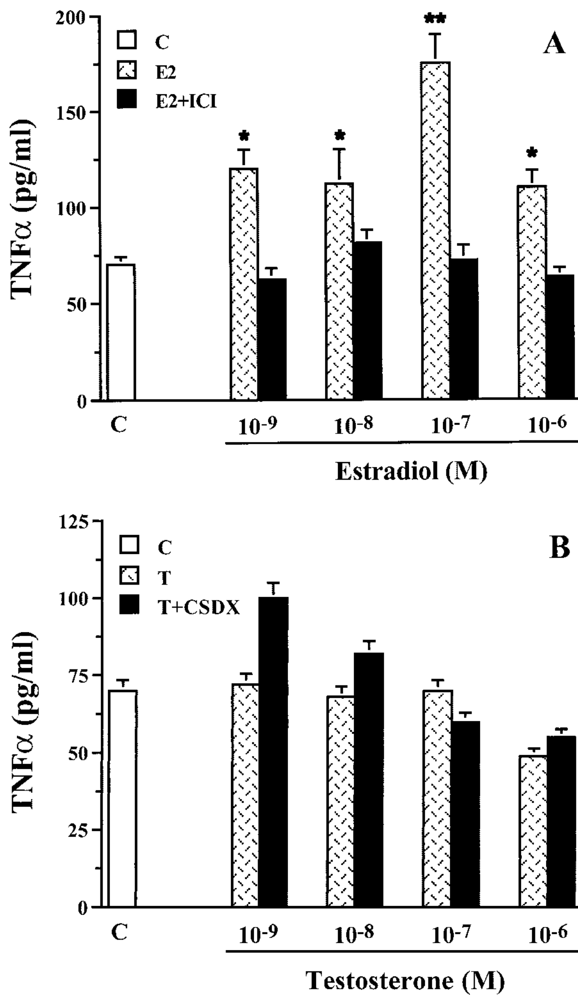

Fig. 2. Effects of exogenous E2 and T on TNF-α production by U937 cells. PMA-differentiated U937 cells (10^6/ml) were cultured in DCC-RPMI for 24 h in the presence of different doses (10^{-6}–10^{-9} M) of exogenous sex steroids alone or in combination with specific anti-hormones (10^{-7} M), precisely: (**A**) E2 alone (dotted columns) and E2 plus ICI-182,780 (black columns); (**B**) T alone (dotted columns) and T plus Casodex (CSDX, black columns). Data are representative of three independent experiments and expressed as mean ± SE. White columns represent control cell cultures receiving vehicle (ethanol 0.01%) alone. E2, estradiol; T, testosterone. *$P < 0.05$ and **$P < 0.01$ with respect to control.

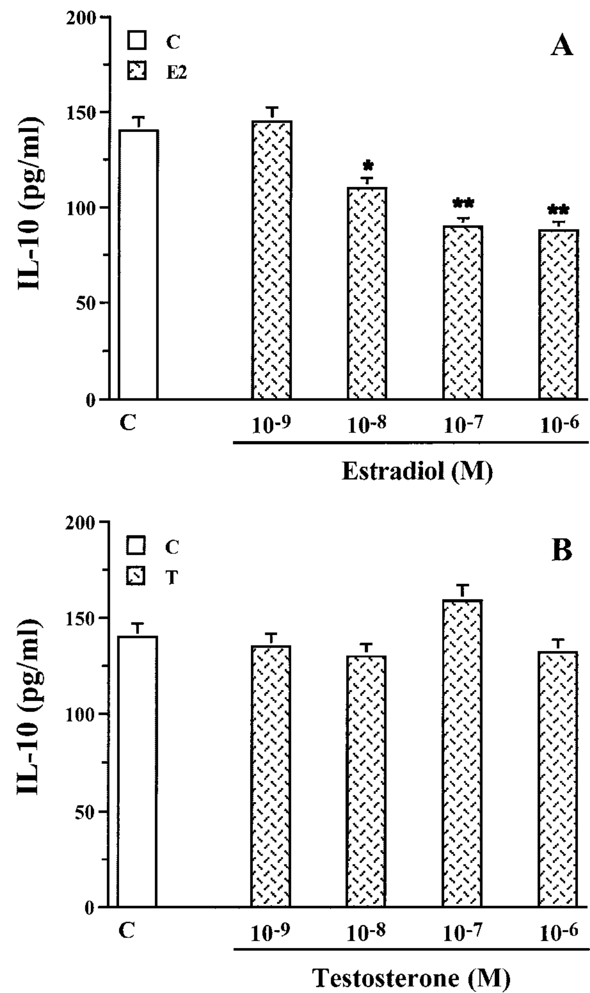

Fig. 3. Effects of exogenous E2 and T on IL-10 production by U937 cells. PMA-differentiated U937 cells (10^6/ml) were cultured in DCC-RPMI for 24 h in the presence of different doses (10^{-6}–10^{-9} M) of either estradiol (**A**) or testosterone (**B**). Data are representative of three independent experiments and expressed as mean ± SE. White columns represent control cell cultures receiving vehicle (ethanol 0.01%) alone. C, Control; E2, estradiol; T, testosterone. *$P < 0.05$ and **$P < 0.01$ with respect to control.

T, in the absence or presence of the antiandrogen CSDX, did not significantly influence survival of the U937 cells, with only a slight decrease (37%) of apoptotic rates being observed when T was used at 10^{-9} M (Fig. 4B). Typical cytochemical or fluorescent patterns of E2-induced apoptosis of U937 cells are shown in Figure 5.

Similar data were obtained using an alternative apoptosis assay (ELISA^{PLUS}) (data not shown).

DISCUSSION

In the present study, we report clear evidence that estrogen (E2), but not androgen (T), significantly affects cytokine production and apoptotic rates of the macrophage-like PMA-differentiated U937 cells. These results would imply that E2 can favor the induction of an inflammatory response in macrophage cells, as previously proposed [Zuckerman et al., 1996; D'Agostino et al., 1999]. The estrogen effects on

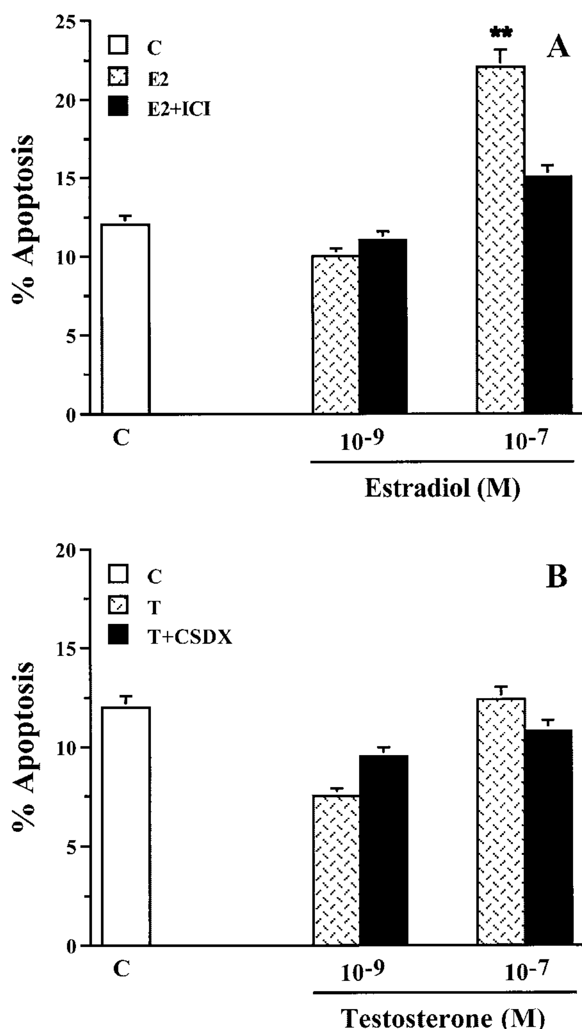

Fig. 4. Effects of exogenous E2 and T on apoptotic rates of U937 cells. PMA-differentiated U937 cells (10^6/ml) were cultured in DCC-RPMI for 24 h in the presence of different doses (10^{-7} and 10^{-9} M) of exogenous sex steroids alone or in combination with specific anti-hormones (10^{-7} M), precisely: (**A**) E2 alone (dotted columns) and E2 plus ICI-182,780 (black columns); (**B**) T alone (dotted columns) and T plus CSDX (black columns). Data are representative of three independent experiments and expressed as mean ± SE. White columns represent control cell cultures receiving vehicle (ethanol 0.01%) alone. C, control; E2, estradiol; ICI, ICI-182,780; T, testosterone; CSDX. $^*P < 0.05$ and $^{**}P < 0.01$ with respect to control.

TNF-α secretion appear to be receptor-mediated, being reversed by the antiestrogens tamoxifen and ICI-182,780, similar to what occurs for estriol in a murine model of endotoxemia [Zuckerman et al., 1996]. On the other hand, exogenous T did not significantly change both TNF-α and IL-10 production at all concentrations used.

Previous studies from our own and other groups have indicated that gonadal steroids, notably E2 and T, are important regulators of inflammatory cytokines in cultured macrophage model systems, even though male and female sex steroids appear to act in an opposite manner [Goemaere et al., 1992; Cutolo et al., 1995a; D'Agostino et al., 1999]. However, results from these studies are often conflicting and hardly comparable. Different groups have used peripheral blood mononuclear cells (PMBCs) or whole blood cells aiming to inspect hormone-induced changes in cytokine production [Asai et al., 2001; Deguchi et al., 2001]. The authors found that either E2 stimulates cytokine secretion, including IL-6 and TNFα, by male PMBCs or that IL-10 production increases in post-menopausal women. Furthermore, contradictory evidence also emerges from other reports whereby murine splenic or rat peritoneal macrophages have been used to study the effects of E2 on TNFα synthesis. It has been observed in fact that E2, respectively, reduces [Desphande et al., 1997] or increases [Chao et al., 1995] the production of TNFα in these in vitro model systems. Overall, this discrepancy among results of various studies would imply that estrogen-induced changes in cytokine production may be remarkably different depending on the target cell type and the physiological or pathophysiological condition.

Using ligand binding assay of both ER and AR, we have observed that PMA treatment of U937 cells results in the appearance of type II receptors in the soluble fraction and in a significant increase of nuclear type I receptors. This is worth noting since, in our own and other's experience, type I receptors only can be considered functional. In particular, the significance of type I ER stems from the original definition of biochemical and functional features relevant to distinct sites of estrogen binding [Clark and Peck, 1979] and, more, is illustrated by its discriminant value in the prognosis and treatment of both breast and endometrial cancer patients [Castagnetta et al., 1987, 1992]. Therefore, this PMA-induced change in both ER and AR status may suggest that the effects of sex steroids, notably estrogens, on cytokine production in PMA-differentiated U937 cells could be related to the increase of type I nuclear receptor and/or be combined to additional, non-receptorial mechanisms.

In our work, the E2-induced increase of TNF-α synthesis and decrease of IL-10 secretion, may eventually lead E2 to promote an inflammatory

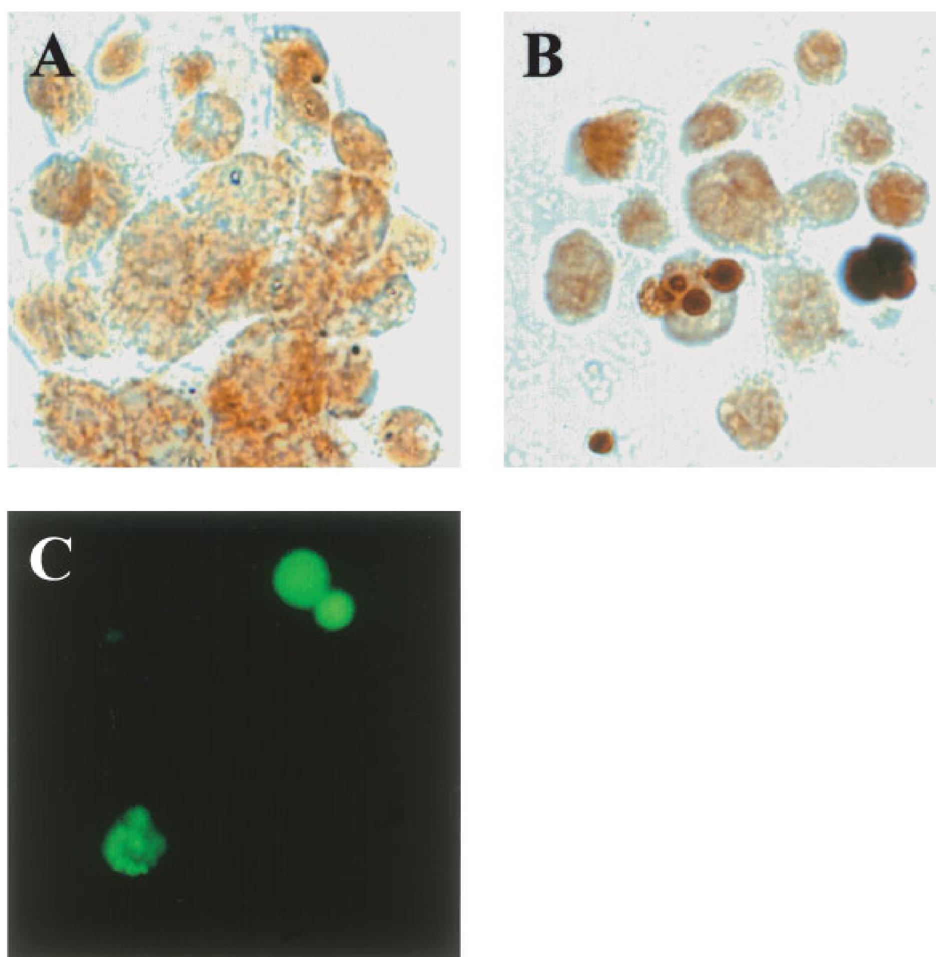

Fig. 5. Effects of E2 on apoptosis in U937 cells. PMA-differentiated U937 cells were incubated for 24 h in the presence of 10^{-9} or 10^{-7} M E2, while control cell cultures received vehicle (ethanol 0.01%) alone. Cell death rates were measured by a TUNEL assay that uses fluorescent labeling of DNA strand breaks. The incorporated fluorescein was revealed through an anti-fluorescein antibody conjugated with horseradish peroxidase, using DAB as substrate. The proportion of stained (apoptotic) cell nuclei was assessed on at least 25 randomly selected fields. **A**: Control; (**B**) E2 10^{-7} M DAB staining; (**C**) E2 10^{-7} M fluorescent staining.

status. In particular, the rising of endogenous TNF-α production in PMA-differentiated U937 cells could, at least in part, explain the ability of E2 to induce a significant increase of apoptotic rates in these cells. Conflicting reports exist on estrogen-induced apoptosis in immune effector cells, including macrophages. Recent studies have indicated that both E2 and T have cytostatic effects and may regulate apoptosis in U937 cells [Mossuz et al., 1998]. Some reports have also shown that androgens may favor apoptosis in different experimental models [Olsen et al., 1998; Huber et al., 1999]. Takada et al. [1999] have revealed that 12-*O*-tetradecanoylphorbol-13-acetate (TPA) induces apoptosis in U937 cell through the increase of TNF-α production. In another paper by Vegeto et al. [1999], the TNF-α-induced apoptosis of U937 cells was found to be prevented by E2 via a receptor-mediated mechanism. This evidence apparently conflicts with our results that E2 significantly increases apoptotic rates of PMA-differentiated U937 cells, presumably through the rise of TNF-α synthesis. However, it ought to be emphasized that Vegeto et al. revealed that E2 might prevent apoptosis induced by the exogenous administration of TNF-α to U937 cells, with an effective TNF-α concentration of 20 ng/ml. By contrast, in our work, we observed an E2-induced increase of endogenously produced TNF-α, up to nearly 200 pg/ml, that is to say TNF-α concentrations 100 times lower than those used in the paper by Vegeto et al.

Current evidence suggests that a dysfunctional cross-talk between endocrine and immune systems contributes to the pathogenesis, and/or activity, of several autoimmune diseases [Da Silva, 1995; Besedovsky and del Rey, 1996; Olsen et al., 1998]. Estrogens and androgens may operate, even in an opposite way, to suppress or activate immune effector cells on the basis of different stimuli in various target cells. For instance, the balance of gonadal steroid secretion, i.e., the androgen to estrogen ratio, could alternatively favor a shift in T helper 2-phenotype in systemic lupus erythematosus disease [Wilder, 1995], whereas a prolonged stimulation of TNF-α synthesis could be responsible for proliferation of synovial cells and activation of matrix metalloproteinases in rheumatoid arthritis [Feldmann et al., 1996; Wakisara et al., 1998].

Overall, our studies suggest that estrogens, but not androgens, may play a pro-inflammatory role in macrophage-like U937 cell system, through the concurrent increase of TNF-α synthesis and decrease of IL-10 production, and may affect cell viability by the induction of programmed cell death. In this respect, the local balance of gonadal steroids, namely, the androgen to estrogen ratio, may be important to regulate the activity of immune effector cells and to alternatively favor or prevent an inflammatory condition.

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