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Phorbol Diester 12-O-Tetradecanoylphorbol 13-Acetate (TPA) Up-Regulates the Expression of Estrogen Receptors in Human THP-1 Leukemia Cells

Maurizio Cutolo,[1] Giuseppe Carruba,[2] Barbara Villaggio,[1] Domenico A. Coviello,[3] Jean-Michel Dayer,[4] Ildegarda Campisi,[5] Monica Miele,[5] Rosalba Stefano,[2] and Luigi A.M. Castagnetta[2,5]*

[1]Division of Rheumatology, Department of Internal Medicine, University of Genoa, Italy
[2]Institute of Oncology, University Medical School, Palermo, Italy
[3]Institute of Biology and Genetics, University of Genoa, Italy
[4]Division of Immunology and Allergy, Department of Internal Medicine, Hôpitaux Universitaire de Geneve, Geneve, Switzerland
[5]Experimental Oncology, Palermo Branch of IST-Genoa, c/o 'M. Ascoli' Cancer Hospital Center, ARNAS, Palermo, Italy

Abstract In the present work, we have inspected expression of estrogen receptors (ER) and their regulation by the phorbol diester 12-O-tetradecanoylphorbol 13-acetate (TPA) in a leukemic cell line, the THP-1 cells, using multiple experimental approaches. Firstly, ligand binding assay (LBA) revealed that control (unstimulated) THP-1 cells express type I (high affinity, limited capacity) ER in the nuclear fraction only, whilst treatment of cells with TPA resulted in the appearance of type I ER in the soluble fraction as well, with the 50 ng/ml dose and the 48 h incubation time being the most effective experimental condition. A concomitant increase of type II ER was also seen in both soluble and nuclear cell fractions. Unstimulated THP-1 cells were found to be ER negative by immunocytochemistry; conversely, cells exposed to 50 ng/ml TPA for 48 h stained positively for ER, with the majority of cells having a strong nuclear staining. Scrutiny of ER mRNA expression using reverse transcriptase-polymerase chain reaction showed the presence of a wild type ER transcript in both control and TPA-treated THP-1 cells, though levels of ER mRNA were found to be comparatively higher in the latter. This combined evidence would imply that the TPA-induced differentiation of THP-1 cells is accompanied by the rise of high affinity (type I) ER, suggesting that estrogens may play a role in the regulation of macrophage activity during the inflammatory and/or the immune response. J. Cell. Biochem. 83: 390–400, 2001.

Key words: phorbol diester TPA; estrogen receptors; THP-1 leukemia cells; cellular activation; monocytes/macrophages

Differentiation of monocytic cells is accompanied by the expression of specific surface antigens, cytokine production and interaction with specific ligands. These activities can be regulated by both various cytokines and steroid hormones [Johnston, 1988].

The THP-1 is a leukemic cell line derived from a patient with acute monocytic leukemia that, unlike other leukemic cell lines, has non-prominent chromosomal abnormalities [Tsuchiya et al., 1980; Koeffler, 1986]. Previous studies have shown that several cytokines and/or hormones may induce differentiation of the THP-1 cell line along the monocyte/macrophage pathway. The triggering stimuli include interferon-gamma (IFN-γ) [Gaffney et al., 1988], 1α,25 dihydroxyvitamin D_3 (1,25$(OH)_2D_3$) [Vey et al., 1992], as well as pharmacological agents such as retinoic acid and 12-O-tetradecanoylphorbol 13-acetate (TPA) [Tsuchiya et al., 1982; Matikainen and Hurme, 1994]. In particular, IFN-γ induces the expression of HLA-DR, FC-receptors, and CD54 (intercellular adhesion molecule-1) on THP-1 cell surface and enhances their anti-microbial, tumoricidal, and antigen-presenting capacities [Basham and Merigan,

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*Correspondence to: Prof. Luigi A.M. Castagnetta, PhD, Institute of Oncology, University Medical School, Via Marchese Ugo, 56-90141, Palermo, Italy.
E-mail: lucashbl@unipa.it

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1983; Gaffney et al., 1988]. The active form of vitamin D_3, $1,25(OH)_2D_3$, has been reported to induce myeloid differentiation of human mononuclear cells [Munker et al., 1986], and to affect metalloproteinase expression in THP-1 cells [Lacraz et al., 1994]. Exposure of THP-1 cells to TPA induces the appearance of a macrophage-like phenotype, featured by several morphological and functional changes, including adhesion to plastic, growth arrest, increase of phagocytic activity, and release of mediators such as prostaglandin E_2 (PGE_2) and TNFα [Tsuchiya et al., 1982; Matikainen and Hurme, 1994; Schwende et al., 1996]. Therefore, this cell line has been adopted as useful model system for studies on mechanisms implicated in macrophage differentiation and induction or regulation of macrophage-specific genes.

Several reports have revealed that estrogens exert immunomodulatory activities both in vivo and in vitro by interacting with several cell lineages involved in the immune response [Maoz et al., 1985; Paavonen et al., 1985; Hu et al., 1988; Cutolo et al., 1995]. In particular, it has been reported that estrogens positively affect IL-1β and TNFα mRNA expression in TPA-stimulated THP-1 cells [Shanker et al., 1994a,b]. Estrogen receptors (ER) have been identified in immune cells, such as thymocytes [Danel et al., 1983], CD8+ T lymphocytes [Cohen et al., 1983], mononuclear cells [Carbone et al., 1986], and recently, synovial macrophages [Cutolo et al., 1993, 1996].

In the present study, we have investigated the expression of ER in THP-1 cells and their regulation by the phorbol diester TPA at both protein and transcript levels, as well as the TPA effects on the characteristics of estrogen binding.

MATERIALS AND METHODS

Cell Cultures

THP-1 cells were cultured in RPMI-1640 supplemented with 10% FCS (containing <0.5 EU/ml endotoxin), 2 mM L-glutamine, 100 mg/ml streptomycin, and 100 U/ml penicillin, in a 5% CO_2-humidified atmosphere at 37°C. Cells were maintained in logarithmic growth (between 2×10^5 and 1×10^6 cells/ml) by passage every 3 to 4 days. For TPA studies, cells were seeded into 6-wells flat-bottom plates (5×10^5 cells/well) and then either exposed to increasing TPA concentrations (0.5–500 ng/ml) for 24–48 h or incubated for various times (12–120 h) with 50 ng/ml TPA. This latter concentration was selected since it yielded the maximum effect in dose–response experiments. In all experiments, control cells received vehicle (DMSO 0.01%) alone. After incubation, cells were harvested and processed for immunocytochemical assay (ICA) or ligand binding assay (LBA) of ER as described below.

Expression of Surface Antigens

In order to determine the potential effect of TPA on the expression of the surface antigens CD14, CD54, CD68, and HLA-DR, THP-1 cells were exposed for 48 h to 50 ng/ml TPA; control cell cultures received vehicle alone. Cells were then incubated to primary mouse monoclonal antibodies directed against the human cell surface antigen CD54 (Cymbus Bioscience Ltd., Hants, UK), CD14 (Immunotech, Marseille, France), HLA-DR (Immunotech) and CD68 (Dako SpA, Milan, Italy) for 30 min at room temperature in a humidified chamber. The reaction was visualized through incubation with a secondary fluorescein-conjugate goat anti-mouse IgG (Immunotech) for 30 min as before. Both the proportion of stained cells and the intensity of staining were assessed using the Leica Q500 MC Image Analysis System (Leica, Cambridge, UK). Stain intensity was expressed as the ratio of number of pixels over micrometer square of positive area.

Immunocytochemical Studies

Both treated and untreated cells were centrifuged on glass slides, fixed in ice-cold 4% formaldehyde for 5 min, then incubated for 30 min at room temperature with a mouse monoclonal antibody (dilution 1:70) directed against the DNA-binding domain of ER (anti-ER mAb) (BioReagents, Neshomic Station, NJ). The second step was performed using the improved biotin–streptavidin-amplified detection system (BioGenex Laboratories, San Ramon, CA). Briefly, after two washes with PBS, cells were exposed for 30 min at room temperature to a secondary concentrated link antibody (biotinylated anti-mouse IgG; 1:100 dilution). After several washes with PBS, cells were incubated as before with the concentrated enzyme label (alkaline phosphatase). Control slides (negative control) were treated in an identical manner, except the first step that was replaced by nonspecific mouse IgG. The slides were

dehydrated in ethanol, fixed in xylol, and finally mounted using the Eukitt mounting medium (O.Kindler, GmbH, Freiburg, Germany). All samples were examined with a Leitz microscope (Leitz, Rockleigh, NJ).

LBA

ER content and status of THP-1 cells was determined by means of LBA, using a modification of a previously described method [Castagnetta et al., 1983, 1987b; Cutolo et al., 1993, 1996]. The cell pellets were washed twice in ice-cold PBS-A, resuspended in 4 ml of HED buffer (Hepes 10 mM, EDTA 1.5 mM, Dithiothreitol 0.5 mM, pH 7.4) and gently teflon-glass homogenized for 2×10 s bursts at a setting of 600–800 rpm on a Potter S cooling system homogenizer (B.Braun Biotech Int. GmbH, Melsungen, Germany). 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 2000*g* for 5 min at 4°C to remove cell debris; the nuclear pellet was subsequently washed three times in ice-cold buffered saline (Hepes 10 mM, NaCl 150 mM, 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 [2,4,6,7-^{3}H]17β-estradiol as radioligand. One hundred fold excess of unlabeled diethylstilbestrol (DES) was used for competition studies; in addition, a constant excess (10^{-7} M) of the synthetic androgen methyltrienolone (R1881) was also used to measure possible displacement of estradiol binding. 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, each tube was mixed for 15 min with 0.5 ml DCC suspension (0.15% w/v Charcoal, 0.0015% w/v Dextran) in HES buffer (Hepes 10 mM, EDTA 1.5 mM, Sucrose 250 mM, pH 7.4) to strip the unbound radioligand. Samples were centrifuged at 3000*g* for 5 min at 4°C. One mililiter aliquots of the resulting supernatant were counted on a β-counter (Beckman). For the nuclear fraction, 100 μl aliquots of the nuclear suspension were filtered through Whatman GF/C glass fiber filters (Whatman Ltd., Maidstone, UK) on a Millipore apparatus (Millipore), the unbound ligand being washed out using saline. Filters were then removed, dried overnight at room temperature, and counted up 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 [Leake et al., 1987], run on an IBM-PC, yielding both dissociation constant (K_d) and concentration values (fmol/ml homogenate); the latter were expressed either as fmol/mg protein or DNA or as number of sites per cell, for any cell compartment. Data were also analysed using a model for one of two binding sites, depending on the best fitting achieved.

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

Total RNA was extracted from both THP-1 cells and the MCF7 human mammary cancer cell line; the latter was used as positive control being ER-positive and estrogen-responsive. Pellets containing 10^7 cultured cells were resuspended with 1 ml Trizol Reagent (Life Technologies, Gaithersburg, MD) and 200 μl chloroform. After the extraction, the RNA was precipitated with absolute isopropanol 1:1 and washed with 500 μl of 75% ethanol; the resulting RNA pellet was then resuspended in 40 μl sterile double distilled water containing 0.1% diethylpyrocarbonate (DEPC).

The cDNA synthesis was performed in a 30 μl reaction volume containing 1 μg total RNA, 200 U Moloney Murine Leukemia Virus Reverse Transcriptase (Promega, Madison, WI), 150 ng Random Primers (Promega), 10 mM HCl pH 8.3, 3 mM $MgCl_2$, 10 mM DTT, 75 mM KCl, 30 U RNasin ribonuclease inhibitor (Promega), 500 μM each of four deoxyribonucleotide triphosphates (dNTPs) (Promega). The reaction was incubated at 37°C for 70 min followed by 5 min denaturation.

To verify the presence of specific mRNA for ER, we amplified a fragment of 229 base pairs (bp) corresponding to the entire DNA binding domain of ER (amino acid 185–261) as previously described [Cutolo et al., 1996]. The sequence of the sense primer was 5′-GTG CAG TGT GCA ATG ACT ATG C-3′, that of the reverse primer was 5′- CCT CTT CGGTCT TTT CGT ATC C-3′, corresponding to nucleotides 846–867 and nucleotides 1053–1074 of the ER mRNA sequence, respectively (GenBank, sequence accession number M12674).

The PCR amplification was carried out in a total volume of 50 μl, containing 5 μl cDNA, 1.25 U TAQ Polymerase (Promega), 1× TAQ Buffer, 200 μM each of the four dNTPs, 1.5 mM $MgCl_2$, 100 ng of each primer. The reaction was performed with a Perkin Elmer (9600) thermal cycler (Perkin-Elmer Italia, Monza, Italy) using 4 min at 95°C for initial denaturation, 35 cycles of 1 min at 59°C (annealing time), 1 min at 72°C (elongation time), and 30 s at 95°C (denaturation time), and a final step of 5 min at 72°C. Parallel amplifications were carried out in the absence of RNA (blank control) or without the RT reaction (negative control) in order to exclude possible DNA contamination.

Ten microliter aliquots of the amplified products were electrophoresed on a 6% polyacrylamide non-denaturing gel, stained by ethidium bromide, and photographed under UV illumination.

RESULTS

Expression of Cell Surface Antigens

Exposure of THP-1 cells to 50 ng/ml TPA for 48 h resulted in a significant increase in the expression of individual surface antigens with respect to control cells (Table I). TPA-treated cells stained in fact positively for CD68 (55–60%), CD54 (>90%) and HLA-DR (80–90%), whilst unstimulated THP-1 cells were found to be negative for CD68 and stained weakly, though uniformly, for CD54 (70%) and HLA-DR (80–90%). Both control and TPA-stimulated cells remained negative for CD14.

Immunocytochemical Assay of ER

Both expression and cellular localization of ER in THP-1 cells were explored using immunocytochemistry. Unstimulated, control cell cultures (receiving vehicle alone) were found to be ER negative (Fig. 1a). By contrast, cells exposed for 48 h to 50 ng/ml TPA displayed a positive staining for ER, with the majority of positive cells having a small size, a dense nucleus, and a strong stain intensity mostly located within the nucleus with some perinuclear cytoplasmic staining (Fig. 1b). The proportion of ER positive cells ranged from 60 up to 75% over different cell cultures. The appearance of ER in TPA-treated THP-1 cells was also evident after shorter (36 h) exposure times (not shown). The absence of a positive stain in control slides (negative control), receiving a nonspecific mouse IgG, confirmed that the staining was ER-specific.

RT-PCR of ER Trasncripts

Presence of mRNAs for ER was investigated using reverse transcription and PCR amplification of total RNA extracted from THP-1 cells. As illustrated in Figure 2, a single band of 229 bp was observed in both control (unstimulated) and TPA-treated THP-1 cells, as well as in the ER-positive estrogen-responsive MCF7 breast tumor cells, used as positive control. This band is quite consistent with to the expected length (nucleotides 846 to 1074 of the ER sequence) of a wild type ER transcript, as determined by comparison with the ϕx-HaeIII marker fragments. Although this RT-PCR method could not be used to estimate relative amounts of mRNA for ER, a comparison of ER mRNA amplification products was however possible, also taking into account slight sample to sample variations. As can be seen from Figure 2, control THP-1 cells expressed markedly lower amounts of ER mRNA with respect to MCF7 cells. Interestingly, treatment of cells with TPA resulted in an apparent, significant increase of this message. No amplification product could be detected in

TABLE I. Expression of Cell Surface Antigens in THP-1 Cells

	Control		TPA	
Antigen	Positive cells (%)	Intensity of staining	Positive cells (%)	Intensity of staining
HLA-DR	80–90	22.1	80–90	45.3
CD14	ND	—	ND	—
CD54	70	25.2	>90	89.3
CD68	ND	—	55–60	23.5

Data represent average percent positive cells and intensity of staining (calculated as the number of pixels:μm^2 of positive area) from triplicate experiments performed in the absence (control) or presence (TPA) of 50 ng/ml TPA for 48 h. ND, not detectable. For methodological details see text.

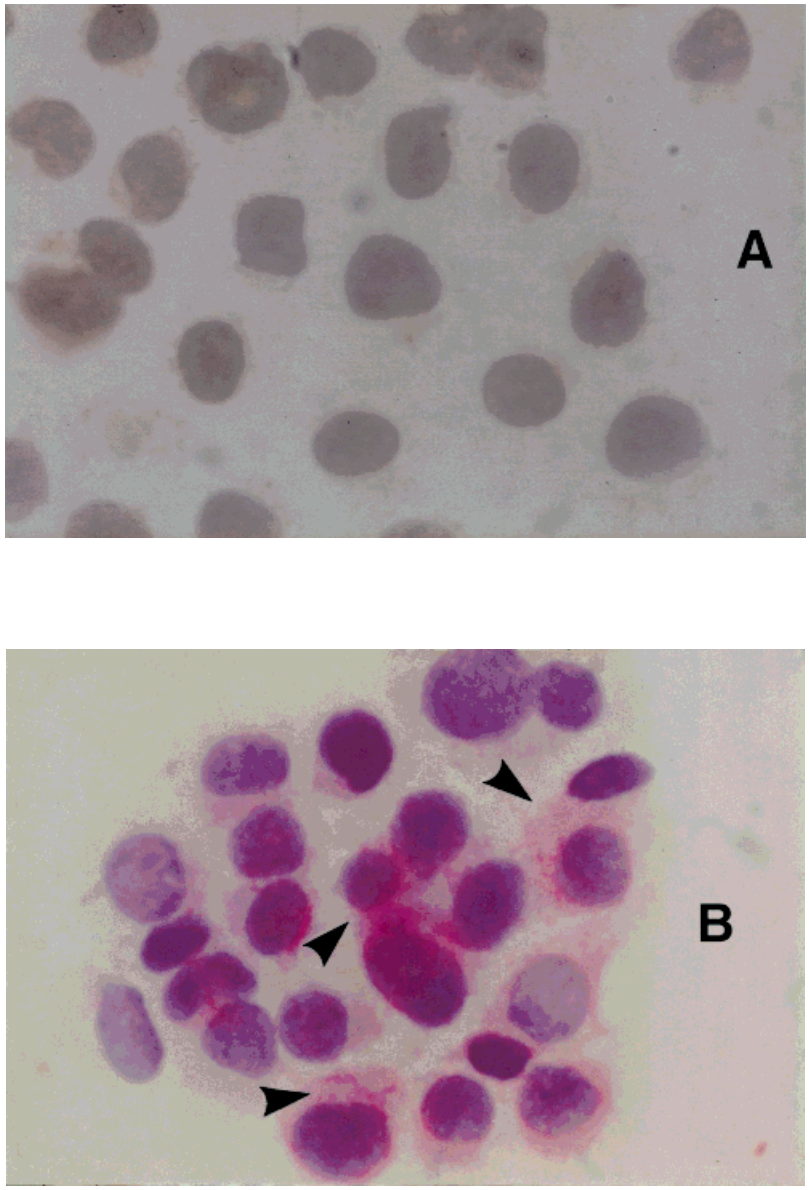

Fig. 1. Immunocytochemical assay of ER in THP-1 cells. **a**: Unstimulated, control cells; **b**: TPA-stimulated cells. Cells were exposed to a primary mouse anti-ER mAb and the specific binding revealed through a secondary step biotinylated anti-mouse IgG, using alkaline phosphatase as concentrated enzyme label. Control cell cultures received vehicle alone. Staining was mostly located within the nucleus, with some peri-nuclear cytoplasmic staining (arrowheads).

either the blank control or the negative control, obtained by amplifying RNA without the RT reaction, thus excluding any potential DNA contamination.

Type I and II ER Status and Content

LBA was used to define the presence of type I (high affinity, lower capacity) and II (low affinity, greater capacity) ER in both soluble and nuclear fractions of THP-1 cells.

Overall, the results obtained indicate that TPA is able to induce or increase type I ER in either cell fraction. In particular, control cell cultures, receiving vehicle alone, did not express type I ER, while showing type II ER only in the soluble fraction; by contrast, treatment of THP-1 cells with TPA resulted in the appearance of soluble type I ER, along with a substantial increase of type II ER in cytosol. On the other hand, TPA treatment increased both

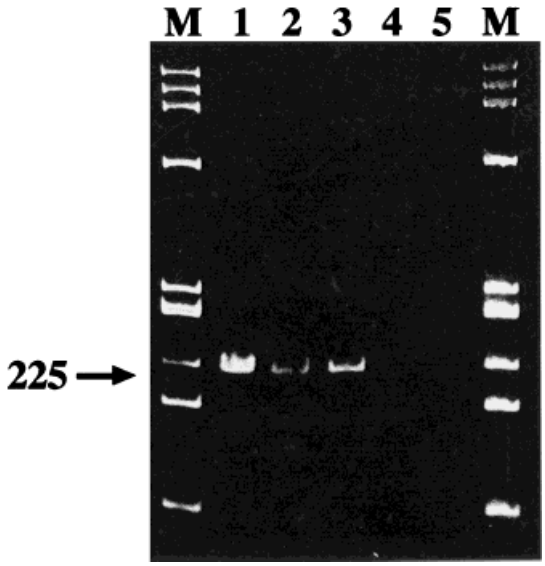

Fig. 2. RT-PCR analysis of ER. The figure shows the amplification products of an ethidium bromide-stained 6% polyacrylamide gel. **Lane 1**: MCF7 cells (positive control); **lane 2**, unstimulated (control) THP-1 cells; **lane 3**, TPA-stimulated THP-1 cells; **lane 4**, RT-PCR of an RNA-free sample (blank control); **lane 5**, RT-PCR of a RT-free sample (negative control). Marker lane, ϕx-HaeIII digest fragments (length given in base pairs).

type I and type II ER concentrations in the nuclear cell fraction with respect to controls.

As reported in Table II, the induction of type I ER in cytosols of THP-1 cells was observed after either 24, 36, or 48 h incubation with 50 ng/ml TPA, the maximal effect being found after 48 h, whilst it was no more evident at longer incubation times, e.g., 72 and 120 h, when only type II ER could be detected. This TPA effect was also dose-related, in that 50 ng/ml TPA and, to a lesser extent, 5 ng/ml appeared to be the most effective doses; this was true at both 24 and 48 h (see Table III). Figure 3 illustrates typical Scatchard plots and ligand saturation curves for soluble ER in control and TPA-treated THP-1 cells.

As far as the nuclear cell fraction is concerned, type I ER were expressed in both controls and TPA-treated cells. TPA treatment significantly increased nuclear type I ER concentrations with respect to controls; again, 50 ng/ml represented the most effective dose, especially after 24 or 48 h incubation. However, only type II ER could be detected in the nuclear fraction of THP-1 cells treated for 48 h with 500 ng/ml TPA (Table II).

The overall outcome of TPA treatment on type I and II ER levels in both soluble and nuclear fractions of THP-1 cells is portrayed in Figure 4. As can be seen, the appearance of type I ER in the cytosol of TPA-stimulated THP-1 cells was accompanied by the increase of type II ER concentrations (peak of 2.2-fold at 24 h), whilst soluble type II ER levels dropped to 65 and 37% with respect to time 0, respectively at 72 and 120 h, namely when the TPA-induced rise of type I ER could not be measured. On the other hand, both type I and II ER concentrations concurrently raised in the nuclear fraction of THP-1 cells, a peak of 2.3- (type I) and 2.6-fold (type II) increase being seen after 120 h TPA treatment.

TABLE II. ER Concentration and Dissociation Constant (Kd) Values of THP1 Cells in the Presence of TPA (50 ng/ml) for Various Incubation Times

	Soluble		Nuclear		
Time (h)	Kd (Nm)	Sites/cell	Kd (nM)	Sites/cell	Type I ER Status S/N
0	2.97* ±0.31	12,046* ±897	0.24 ±0.06	1,445 ±121	0/+
24	0.49 ±0.04	2,745 ±289	0.31 ±0.02	1,556 ±188	+/+
36	0.33 ±0.05	3,252 ±137	0.29 ±0.03	1,087 ±209	+/+
48	0.52 ±0.03	3,457 ±549	0.41 ±0.05	2,033 ±311	+/+
72	1.37* ±0.11	7,830* ±934	0.11 ±0.07	1,355 ±102	0/+
120	1.04* ±0.15	4,457* ±597	0.56 ±0.04	3,373 ±372	0/+

Data represent mean±SD values from duplicate experiments each performed in triplicate.
*Type II ER only. N.B. Type I ER are commonly defined by a Kd $\leq$ 0.55 nM.
S, soluble fraction: N, nuclear fraction.

TABLE III. ER Concentration and Dissociation Constant (Kd) Values in THP-1 Cells in the Absence or Presence of Various TPA Doses After 48 h Treatment

	Soluble		Nuclear		
TPA (ng/ml)	Kd (nM)	Sites/cell	Kd (nM)	Sites/cell	Type I ER Status S/N
None	2.70* ±0.20	11,154* ±1,502	0.24 ±0.07	1,449 ±178	0/+
0.5	0.31 ±0.05	1,788 ±225	0.31 ±0.08	947 ±103	+/+
5	0.48 ±0.05	2,071 ±287	0.29 ±0.06	1,308 ±183	+/+
50	0.53 ±0.05	5,129 ±433	0.41 ±0.08	1,710 ±256	+/+
500	1.32* ±0.29	3,627* ±686	2.11* ±0.33	5,699* ±829	0/0

Data represent mean±SD values from duplicate experiments each performed in triplicate.
*Type II ER only. N.B. Type I ER are commonly defined by a Kd $\leq$ 0.55 nM. S, soluble fraction; N, nuclear fraction.

DISCUSSION

Differentiation of human myeloid stem cells consists of two major pathways, the granulocyte and the monocyte/macrophage pathway, which dichotomize since the promyelocytic stage of differentiation. Several compounds have been shown to induce irreversible commitment of precursor cells throughout either pathway. They include retinoic acid and vitamin D_3, but

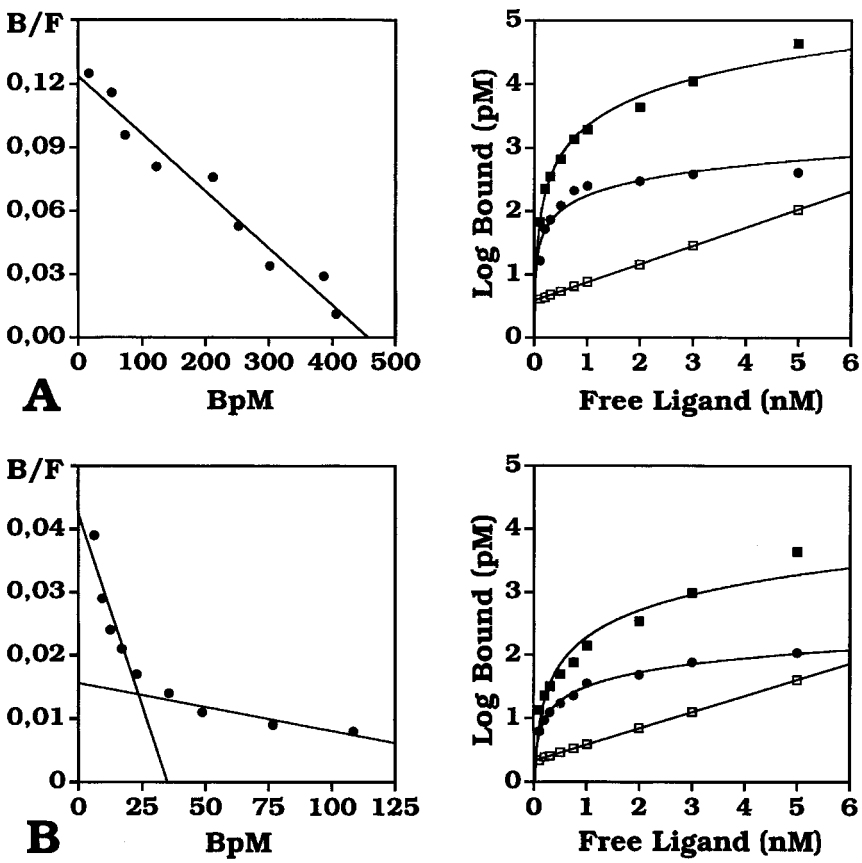

Fig. 3. LBA of estrogen receptor in THP-1 Cells. Typical Scatchard plots (left) and tracer ligand saturation curves (right) of either type I or II sites of estrogen binding in soluble fraction of (**A**) control and (**B**) TPA-stimulated THP-1 cells are illustrated. Each data point was performed in triplicate. BpM = bound picomolar; B/F = bound over free. ■ = total, ● = specific, □ = non-specific binding of tritiated estradiol, as calculated from competition studies using a 100-fold excess of unlabeled DES. For methodological details see text.

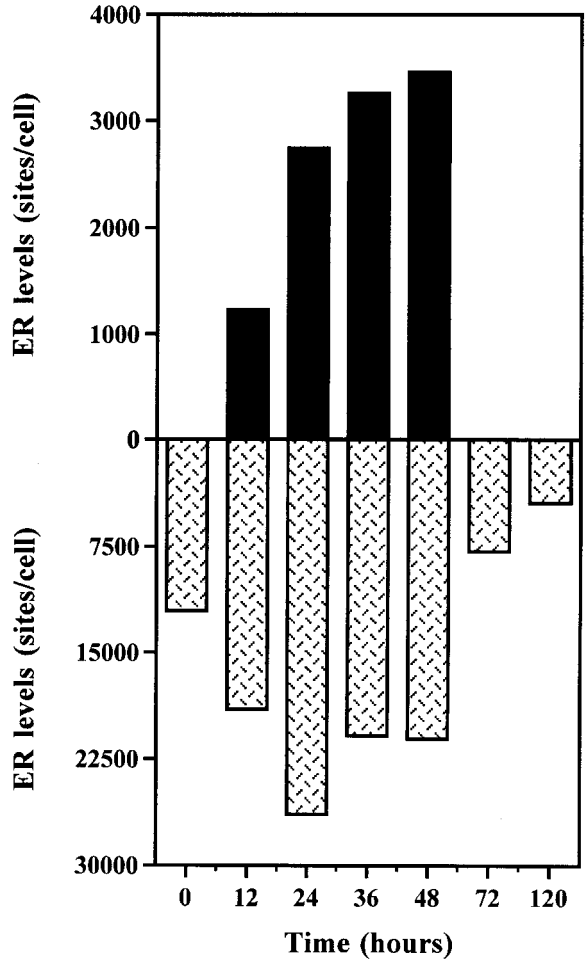

Fig. 4. Time-course patterns of type I (top) and II (bottom) ER in TPA-stimulated THP-1 cells. Cells were exposed for various times (12–120 h) to 50 ng/ml TPA and ER binding measured through LBA, as described in the Materials and Methods section.

also activators of the protein kinase C (PKC) enzyme, such as phorbol esters [Gallagher and DeLuca, 1991; Ways et al., 1994; Bhatia et al., 1996]. The monocyte/phagocyte system has been reported to be exquisitely sensitive to estrogens, supporting the view that gonadal steroids are primary regulators of various sides of the immune response [Grossman, 1989]. The presence of ER in different immune cell types has been repeatedly reported by others and our own group [Cohen et al., 1983; Danel et al., 1983; Carbone et al., 1986; Cutolo et al., 1993, 1996]. In addition, estradiol has been involved in the regulation of macrophage activity, especially in the bone [Oursler et al., 1991]. Based on the above evidence, monocytes can be considered target cells of estradiol.

Here we describe ER expression in human monocytic leukemic THP-1 cells. LBA of ER revealed that high-affinity type I receptors are present solely in the nuclear fraction of unstimulated (control) THP-1 cells, whilst the soluble fraction contains type II ER only. Accordingly, unstimulated THP-1 cells were found to be ER negative by ICA. 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., 1987a, 1992]. We report that the phorbol diester TPA induces the appearance of type I ER in the soluble fraction of THP-1 cells and increases nuclear type I ER content. This evidence was reinforced by both ICA and RT-PCR approaches, where exposure of cells to TPA, under exactly the same experimental conditions used for LBA studies, resulted in the rise of ER expression and the marked increase of ER transcript levels, respectively. The presence of wild type ER mRNA in unstimulated THP-1 cells is seemingly inconsistent with data obtained using ICA, where control cells were found to be ER negative. Notably, however, the low levels of ER mRNA seen in THP-1 cells may well give rise to insufficient amounts of the receptor protein that may turn out to be undetectable by cytochemical assay.

Our data apparently conflict previous reports by which TPA drastically decreases ER expression, at both transcriptional and protein level, in MCF7 human mammary cancer cells [Martin et al., 1995; Gierthy et al., 1996; Lee et al., 1996]. However, Migliaccio et al. [1998] have observed that phorbol ester reduces PKC activity, but significantly increases cytosol ER binding in the uterus, suggesting that TPA-induced changes of PKC may play an important role in the modulation of uterine ER levels. In addition, short-term treatment of MDBK normal, nontransformed bovine renal cells with TPA causes a transient but significant increase of immunoreactive ER and its binding capacity for estradiol [Miller et al., 1994]. The above evidence agrees with our observation that TPA treatment of THP-1 cells results in the appearance of type I ER in the soluble fraction and that

the TPA-induced rise of ER occurs at 24–48 h incubation, but disappears after longer exposure times (72–120 h). It ought to be emphasized that TPA-treated THP-1 cells may be considered as differentiated monocytic cells having phagocytic function, as also supported by the present evidence for the appearance or the increased expression of individual cell surface markers (CD68, CD54, and HLA-DR) specific for macrophages and/or mature monocytes. This may also account for the divergent effects of TPA on ER expression seen in different normal or cancer cells. Recently, the expression of the two different estrogen receptor forms, ERα and ERβ, has been investigated in the U937 human monoblastoid cell line [Vegeto et al., 1999]. The authors report that both estradiol and progesterone increase survival and prevent apoptosis of PMA-differentiated, macrophage-like U937 cells through the activation of specific receptors, suggesting that female steroid receptors may play a role in the regulation of immune response by preventing apoptosis of mononucleated cells.

Other authors have indicated that cell- and promoter-specific pathways exist for transcriptional regulation of the expression of a set of cell cycle genes in mammalian cells; this includes TPA-mediated repression of cdc2 promoter in THP-1 cells. On this basis, TPA effects on ER expression may well be dependent on the cell type, other than being dose- and time-related [Sugarman et al., 1995].

The crosslink between estrogens and TPA in the regulation of THP-1 cell growth and differentiation stems from various experimental results. In the first place, physiological concentrations of estrogens increase the inducible expression of IL-1β mRNA in THP-1 cells activated by TPA; similar effects were reported on TNFα mRNA expression [Shanker et al., 1994a,b]. Furthermore, estrogen-modulated synthesis/secretion of IL-1β and TNFα has been observed in cultured human peripheral monocytes [Polan et al., 1989; Loy et al., 1992]. On the other hand, TPA, likewise estrogen and antiestrogen, produces a marked increase of ER phosphorylation in SV40-transformed COS-1 monkey kidney cells stably transfected with wild type ER; this effect is mediated through activation of the PKC signal transduction pathway [LeGoff et al., 1994].

Interestingly, it has recently been reported that TPA stimulates estrogen response element (ERE)-mediated transcription and that the combination of TPA and estradiol results in a transcriptional synergism in MCF7 cells [Cho and Katzenellenbogen, 1993] or in the ER positive BG-1 human ovarian adenocarcinoma cells [Ignar-Trowbridge et al., 1996]. The same authors failed however to reveal such a synergistic effect in the ER-transfected Chinese hamster ovary (CHO) cells, suggesting that the ERE-dependent transcriptional activation may differ between cell types [Cho and Katzenellenbogen, 1993].

Jakob et al. [1995] have documented that treatment of vitamin D-differentiated THP-1 cells with TPA induces both a significant reduction of the 17βhydroxysteroid dehydrogenase type 4 (17βHSD4) and a concurrent increase of the aromatase enzyme activities. Since 17βHSD4 governs the oxidation of estradiol into estrone, and aromatase directs transformation of testosterone into estradiol, the net TPA effect would create a dominating estrogenic milieu which may represent an essential requirement for ER-mediated functions of myeloid cells.

Overall, combined evidence suggests that the TPA-activated THP-1 cells may provide a helpful in vitro model system to investigate the effects of estrogens on macrophages, possibly leading to significant insights into estrogen implication in the inflammatory and immune responses.

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