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Tumor Necrosis Factor Stimulates Ornithine Decarboxylase Activity in Human Fibroblasts and Tumor Target Cells

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Abstract The activity of the polyamine biosynthetic enzyme, ornithine decarboxylase (ODC), has been shown to be rapidly modulated by a variety of growth regulatory molecules. In this report the effect of the growth modulatory peptide, tumor necrosis factor, on ODC activity was examined on two cell lines which express equivalent TNF binding properties, but differ in their growth response when exposed to this factor. TNF treatment of WI-38 fibroblasts stimulated both their growth and induced ODC activity 5–10-fold when measured 6–24 h after TNF incubation. TNF induced cytotoxicity in ME-180 cervical carcinoma cells and, interestingly, stimulated both ODC activity (3–6-fold) and putrescine accumulation when measured prior to the onset of cytotoxicity. Induction of ODC was TNF concentration-dependent and paralleled the concentration-dependency for cytotoxicity. Based upon studies with cycloheximide, de novo protein biosynthesis was required for TNF-mediated ODC induction in ME-180 cells.

The effects of other growth inhibitory peptides and growth factors were analyzed for their combined effect on ODC activity in TNF-treated or untreated ME-180 cells. Interferon gamma treatment had no significant effect on basal ODC activity but inhibited TNF-mediated ODC induction by ~50%. EGF treatment resulted in a potent stimulation of ODC activity which was not effected by TNF pre-treatment or coadministration on ME-180 cells. These results suggest that TNF has properties which are similar to those of a growth factor and distinct from those of other growth inhibitory peptides. The early growth factor-like actions of TNF occur on both normal fibroblasts and some tumor cells and evidence suggests that these effects are antagonistic to the antiproliferative effects of TNF.

Key words: cytokines, cell proliferation, polyamines, cytotoxicity, growth factors

Tumor necrosis factor (TNF), a peptide hormone secreted by cells of monocyte lineage, has been shown to be a potent modulator of mammalian cell growth and differentiation [1–4]. Incubation of TNF with tumor cells in culture has been shown to result in cytotoxicity or cytostasis, suggesting that this endogenous peptide may play a role in host defense mechanisms involving the control of neoplastic cell growth [5,6]. In contrast, TNF treatment of normal fibroblasts results in the stimulation of thymidine incorporation, increased expression of proto-oncogenes involved in the initiation of cell growth, and an overall increase in fibroblastic cell growth [1,7,8]. Thus, TNF, like other growth factors or cytokines can mediate both positive or negative influences on the growth of target cells [9–11]. The cellular mechanisms responsible for the generation of these stimulatory or inhibitory effects on cell growth have not been elucidated, but do not appear to be dependent on the differential expression of a cellular receptor for TNF which exists on the surface of both fibroblasts and tumor cells. Studies examining the characteristics of TNF binding have demonstrated the presence of equivalent quantities of TNF receptors with similar or identical affinity for TNF on cells sensitive to the cytotoxic action of TNF and on cells in which TNF behaves as a mitogen [1,2]. Based upon these studies, it has been proposed that TNF induces growth stimulatory events in both normal fibroblasts and tumor cells and it is the inability of tumor cells to process or regulate this growth-stimulatory signal which leads to eventual cytotoxicity or growth inhibition [7]. However, few comparative studies have ana-

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lyzed the effect of TNF on cellular proteins or enzymatic activities known to be involved in the process of cell growth or cell-cycle progression in tumor or normal cells.

Several biochemical elements have been shown to play a role in the complex process of cellular proliferation. The increased biosynthesis of polyamines has previously been shown to correlate with both the intracellular actions of growth factors, phorbol esters, and anabolic hormones, as well as with the G_1 to S phase transition in cycling cells [12–14]. Biosynthesis of polyamines is finely controlled by the activity of the rate-limiting enzyme in the polyamine biosynthetic pathway, ornithine decarboxylase (ODC) [15]. The activity of this enzyme has been shown to be increased rapidly and manyfold by a variety of biological and chemical agents, including growth factors, phorbol esters, hormones, and agents which increase the intracellular concentration of cyclic AMP [16–20].

Several studies have shown that accumulation of polyamines precedes increased cellular synthesis of RNA, DNA, and protein, and these activities correlate with overall alterations in cell growth, differentiation, and cell maintenance [for reviews, see 12,13,21]. Thus, the induction of ODC activity represents an early biochemical response in the signal transduction pathway for agents which are known to alter cell growth, differentiation, and homeostasis. In the present report, we examine the effects of TNF treatment on ODC activity in a cell line in which TNF displays mitogenic properties (WI-38) and compare this response with the actions of TNF in cervical carcinoma cells (ME-180), in which TNF behaves as a cytotoxic peptide. These two cell lines have previously been shown to express equivalent quantities of TNF receptor at their surface with similar affinities for this cytokine [1]. We report that TNF appears to stimulate ODC activity in both cell lines independent of whether or not TNF has an overall mitogenic or cytotoxic effect.

MATERIALS AND METHODS

Cell Lines

Human ME-180 cervical carcinoma and WI-38 fibroblast cells were obtained from ATCC (American Type Culture Collection, Rockville, MD) and were grown in minimal essential media containing 50 μg/ml gentamicin and 10% or 20% fetal bovine serum (GIBCO, Grand Island, NY), respectively. Cells were grown to confluence and maintained by trypsinization of cell monolayers and reseeding at a lower density in culture flasks. Both cell lines were found to be free of infection with mycoplasma.

Measurement of TNF Effect on Cell Growth

ME-180 or WI-38 cells were plated into individual wells of a 96-well culture plate in cell growth media and incubated at 37°C for 24 h prior to the addition of increasing concentrations of TNF in a total volume of 0.2 ml. TNF was diluted into cell growth media and filtered (0.22 μm membrane filter) prior to incubation with plated cells. ME-180 and WI-38 cells were plated at a density which would result in linear cell growth through the 24–72 h TNF incubation period (4×10^3 cells/well and 8×10^3 cells/well, respectively).

After 72 h in the presence of TNF, cell monolayers were rinsed three times in phosphate-buffered saline (PBS), tapped dry on absorbent paper, fixed, and stained by the addition of 0.5% crystal violet in 20% methanol (0.1 ml/well) as described by Sugarman et al. [22]. Following a 10-min incubation, plates were rinsed three times in deionized water and crystal violet was extracted from adherent cells with the addition of 0.2 ml of Sorenson's buffer/well (0.1 M sodium citrate, pH 4.2, in 50% ethanol). Cell plates were vortexed for 30 min at room temperature and the absorbance was read at 540 nm (Bio-Tek Instruments, Winooski, VT) and compared with control wells (medium alone).

ODC Activity Assay

Measurement of ODC activity was performed as previously described [23]. Briefly, cells were grown to confluence in 100 × 20 mm culture dishes. Cell monolayers were rinsed and cultured an additional 12 h in serum-free media prior to treatment with cytokines or phorbol ester. After incubation (as indicated) cell monolayers were rinsed three times with ice-cold phosphate-buffered saline, collected by scraping with a rubber policeman, and pelleted by centrifugation (300*g* for 5 min) at 4°C. The cell pellet was sonicated (Kontes Glass Co, Vineland, NJ) on ice in 0.6 ml of ODC assay buffer (50 mM Na-K phosphate, pH 7.2, 0.1 mM EDTA, 1.0 mM dithiothreitol, 40 μM pyridoxal phosphate) and following centrifugation (12,000*g* for 15 min), the supernatant (150 μl) was assayed in triplicate for ODC activity. Samples were assayed in a total volume of 0.2 ml containing 0.25 μCi L-[^{14}C-1] ornithine in a final concentration of 0.25 mM

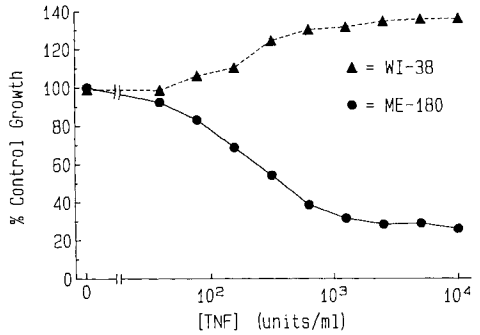

Fig. 1. Effect of TNF on the growth of WI-38 fibroblasts and ME-180 cervical carcinoma cells. WI-38 (8×10^3 cells/well) or ME-180 (4×10^3 cells/well) were plated into individual wells of a 96-well plate and incubated 24 h prior to the addition of TNF to the growth media. After 72 h of TNF exposure, cell plates were rinsed and analyzed for cell growth as described in Materials and Methods. The results represent the average of 8 determinations ± S.E.M. (error bars are included in the size of the symbol).

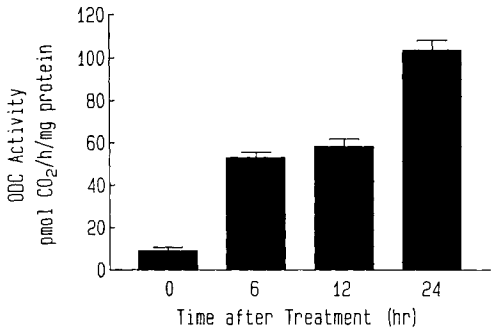

Fig. 2. Effect of TNF on ODC activity in WI-38 fibroblasts. WI-38 cells equilibrated in serum-free media were incubated with 10,000 units of TNF/ml for 0, 6, 12, or 24 h and cells were assayed for ODC activity. Each bar represents the average ± S.E.M. of triplicate assays on triplicate plates.

total ornithine. Assays were incubated 60 min at 37°C and reactions were terminated by the addition of 0.5 ml of 2 M citric acid injected directly into the reaction mixture. Activity was measured by trapping the evolved $^{14}CO_2$ in 0.2 ml hyamine hydroxide, which was suspended in a well above the assay mixture. Trapped $^{14}CO_2$ was counted by liquid scintillation and activity is defined in units of ODC activity (pmoles CO_2 evolved/h/mg protein). Protein was determined by Bio-Rad protein assay using bovine serum albumin as a standard.

Polyamine Analysis

ME-180 cells were grown to confluence in 100 × 20 mm culture dishes in complete growth media and equilibrated in serum free media as described above. Cells were treated for 6 h with cytokine or phorbol ester and cell monolayers were rinsed, scraped, and pelleted (as described above). Cell pellets were extracted with 1 N perchloric acid and soluble material was neutralized with 1 N NaOH and analyzed by automated polyamine analyzer as described by Sunkara and Nishioka [24]. Polyamines were quantitated based upon absolute levels of polyamine standards. Polyamine values are reported in picomoles per 1×10^6 cells.

Materials

Recombinant human TNF (6×10^7 units/mg protein) and human interferon gamma (1×10^7 units/mg protein) were kindly supplied by Dr. H. Michael Shephard of Genentech Corporation (South San Francisco, CA). Both lymphokines migrate as single homogeneous peptides when analyzed by Na-dodecyl sulfate polyacrylamide gel electrophoresis. EGF was purchased from Collaborative Research, Inc. (Bedford, MA). Phorbol ester (TPA) was obtained from Chemicals for Cancer Research, Inc. (Chanhassen, MN). L-[1-^{14}C] ornithine (specific activity = 59 mCi/mmol) was purchased from Amersham Corp (Arlington Heights, IL). Difluoromethylornithine was kindly supplied by Merrell-Dow Pharmaceuticals (Cincinnati, OH).

RESULTS

The effect of TNF on the growth of WI-38 fibroblasts and ME-180 cervical carcinoma cells is shown in Figure 1. Increasing doses of TNF had a mitogenic effect on WI-38 cells with maximal mitogenic stimulation observed at doses > 1,000 units/ml. Incubation of WI-38 cells with maximal stimulatory doses of TNF (10,000 units/ml) enhanced WI-38 cell growth by 35% when compared with control cell proliferation. Inhibition of WI-38 cell growth was not achieved even at doses of 100,000 units of TNF/ml. In contrast, TNF reduced the viability of ME-180 cells in a dose-dependent fashion. Concentrations of TNF as low as 100 units/ml significantly reduced ME-180 cell growth when compared with untreated cells. Maximal cytotoxic effects of TNF on ME-180 cells were observed at concentrations of TNF > 1,000 units/ml.

The time dependent effect of TNF on ODC activity in WI-38 fibroblasts is shown in Figure 2. Incubation with TNF at 10,000 units/ml (~10 nM) resulted in a 5–6-fold induction of ODC

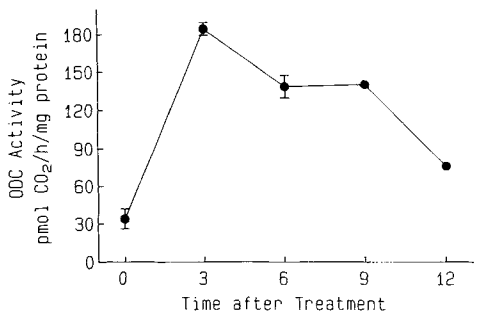

Fig. 3. Time course of TNF effects on ODC activity in ME-180 cells. ME-180 cells were treated with 1,000 units/ml of TNF for various times at 37°C. Cells from triplicates dishes for each time point were prepared and assayed for ODC activity as described above. Results are reported as the average of triplicate assays of triplicate dishes ± S.E.M.

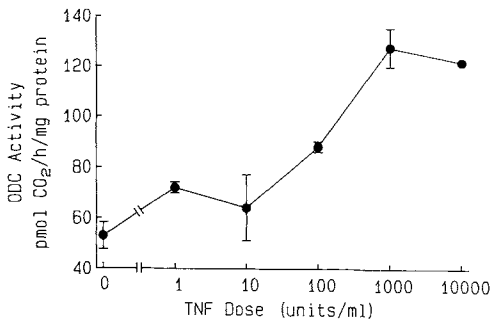

Fig. 4. Effect of TNF concentration on ME-180 cell ODC activity. Increasing concentrations of TNF were incubated with ME-180 cells (equilibrated in serum-free media) for 3 h and cell supernatants were prepared and assayed for ODC activity. ODC activity is reported in units of enzyme activity per mg protein and each point represents the average ± S.E.M. of triplicate assays on triplicate dishes.

activity when analyzed 6 or 12 h after TNF addition. Twenty-four hours after TNF addition to quiescent fibroblasts, an 11-fold induction of ODC activity was measurable when compared with untreated cells. Studies of the regulation of ODC activity in WI-38 cells by growth factors and other mitogens have shown similar patterns of ODC induction or gene expression in these cells [25,26].

To determine the effect of TNF on ODC activity in a tumor cell line in which TNF induces cytotoxicity, the time-dependent effects of TNF on ODC activity were measured in ME-180 cervical carcinoma cells. Treatment of ME-180 cells with 1,000 units/ml of TNF resulted in a 5–6-fold ODC induction when measured 3 h after TNF addition (Fig. 3). ODC activity remained elevated 6–9 h after TNF addition and declined toward control activity 12 h after treatment. Peak ODC activity was consistently measurable 3–4 h after TNF addition to quiescent ME-180 cells (results of four separate experiments).

The concentration-dependent effect of TNF on ME-180 cell ODC activity was measured 3 h after addition of TNF to cell cultures (Fig. 4). Doses as low as 1–10 units/ml of TNF resulted in changes of basal ODC activity. A dose-dependent induction of ODC activity was achieved at TNF concentrations > 100 units/ml. Maximal activity was measurable at a dose of 1000 units/ml (1 nM TNF), which was not significantly affected by 10-fold higher doses of TNF.

Accumulation of polyamines in ME-180 cells following incubation with TNF was also measured and compared with the effects of EGF or phorbol ester. As illustrated in Table I, TNF (1,000 units/ml, 20 ng/ml) stimulated > 6-fold increased putrescine content 6 h after incubation with quiescent serum-free ME-180 cells. Stimulation with either EGF (20 ng/ml) or TPA (0.5 μg/ml) resulted in 18–23-fold increased putrescine content when analyzed following treatment for 6 h with these agents. Of the three agents tested, TNF treatment resulted in the most significant change in polyamines of higher molecular weight: 1.6-fold increase in spermidine vs. 1.2 to 1.5 for EGF or TPA, respectively. Additionally, TNF treatment of ME-180 cells

TABLE I. Polyamine Levels in ME-180 Cells Following Treatment*

Treatment	Putrescine	Spermidine	Spermine
Control	2.9 (1.0)	86.1 (1.0)	694.3 (1.0)
TNF (20 ng/ml)	19.1 (6.6)	138.9 (1.6)	564.6 (0.8)
TPA (0.5 μg/ml)	66.5 (22.9)	127.1 (1.5)	680.8 (1.0)
EGF (20 ng/ml)	52.8 (18.2)	101.7 (1.2)	681.2 (1.0)

*ME-180 cells, grown to confluence in 100 mm dishes (1.5×10^7 cells), were equilibrated in serum-free media (18 h) and were treated for 6 h prior to the extraction of polyamines from cell pellets. Polyamines were analyzed with a Beckmann automated analyzer and are reported as pmoles per 10^6 cells, based upon quantitation with absolute levels of polyamine standards. The fold-increase in individual polyamines for each treatment over that of control is shown in parentheses. Similar results were obtained in additional experiments.

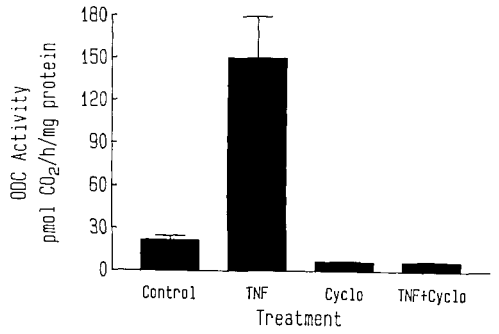

Fig. 5. Effect of cycloheximide on TNF-mediated ODC induction. Quiescent ME-180 cells in 100 mm plates were treated with 10 μg/ml cycloheximide (cyclo) 60 min prior to the addition of 1,000 units/ml TNF. Three hours after TNF addition, cells were harvested and supernatants were assayed in triplicate for ODC activity.

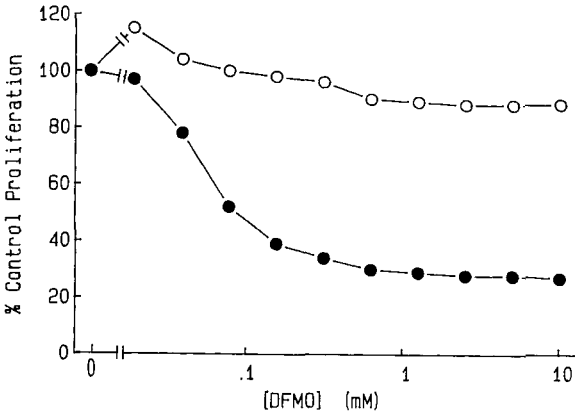

Fig. 6. Effect of DFMO on ME-180 cell proliferation. Four × 10^3 ME-180 cells were dispensed into individual wells of a 96-well culture plate and incubated at 37°C for 24 h prior to the addition of DFMO at various concentrations as noted. Following an additional 72 h incubation in the presence of DFMO, cells were rinsed three times with PBS, fixed, and stained with 0.5% crystal violet in 20% methanol. After extraction of dye with Sorenson's buffer, the absorbance was read and the effect of treatment was compared to untreated cells and quantitated as described above. The result of treatment with DFMO in the presence (○) or absence (●) of 1 mM putrescine is shown. The results represent the mean ± S.E.M. of four determinations.

resulted in an apparent reduction in the cellular spermine content when compared with untreated cells.

Cycloheximide was utilized to determine the role of protein synthesis in TNF-induced ODC activity. TNF treatment of ME-180 cells (3 h) resulted in a 5.5-fold induction of ODC activity (Fig. 5). Cycloheximide (10 μg/ml) pretreatment of ME-180 cells 1 h prior to TNF addition abolished TNF-induced ODC activity, suggesting that protein synthesis is required for TNF-mediated ODC induction.

The effects of the specific irreversible inhibitor of ODC, difluoromethylornithine (DFMO), on the proliferation of ME-180 cells was examined. As shown in Figure 6, incubation of ME-180 cells with increasing doses of DFMO for 72 h resulted in a dose-dependent inhibition of ME-180 cell growth when compared with untreated cells. In the presence of 1 mM putrescine, the inhibitory effects of DFMO on the proliferation of ME-180 cells could be reversed, suggesting that depletion of putrescine in ME-180 cells can inhibit their proliferation. In addition, cell viability was measured in TNF-treated ME-180 cells in the presence or absence of DFMO. As shown in Figure 7, treatment of quiescent ME-180 cells with DFMO (1 mM) did not significantly affect ME-180 cell viability after 24 h of treatment. Incubation with TNF alone (1,000 units/ml) resulted in a 25% reduction in ME-180 cell number. Pretreatment of ME-180 cells with 1 mM DFMO prior to the addition of TNF, significantly reduced ME-180 cell viability (>55%) when compared with TNF alone.

Interferon gamma has been shown to synergistically improve the antiproliferative activity of TNF in ME-180 cells [1]. To determine the influence of interferon gamma alone and in combination with TNF on ODC activity in ME-180

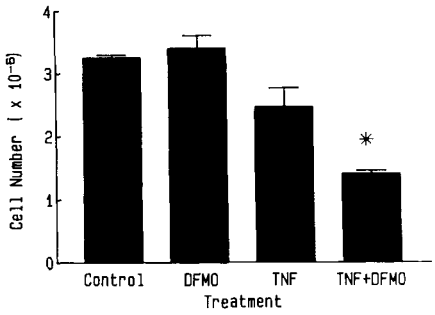

Fig. 7. Effect of TNF and DFMO on ME-180 cell viability. ME-180 cells were grown to confluence in 6-well culture plates and shifted to serum-free media overnight. Quiescent cells were treated with DFMO (1 mM), TNF (1,000 units/ml) or a combination of these two molecules. When combined treatments were performed, cells were treated with DFMO 4 h prior to the addition of TNF. After incubation for 24 h, cell monolayers were rinsed three times in PBS and released by trypsinization. Cell washes and released cells were pooled and counted in the presence of trypan blue to determine cell number and viability. The effects of individual or combined treatment on ME-180 cell viability is shown. Each bar represents the mean ± S.E.M. of 3 determinations. * = $P < 0.05$ when compared with TNF alone.

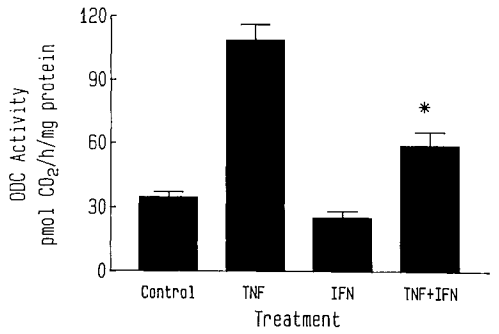

Fig. 8. Effect of cytokine treatment on ME-180 cell ODC activity. ME-180 cells in serum-free media were treated with TNF (1,000 u/ml), or IFN-γ (100 u/ml) alone or a combination of the two cytokines. ODC activity was assayed after 3 h of incubation at 37°C and results are reported in units of enzyme activity per mg protein. Each bar represents the average of triplicate assays on triplicate samples ± S.E.M. * = $P < 0.04$ when compared with TNF alone.

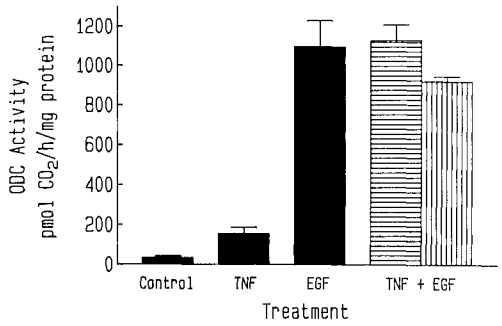

Fig. 9. Effect of TNF on EGF-stimulated ODC induction in ME-180 cells. Quiescent ME-180 cells, equilibrated in serum-free media, were treated with TNF (20 ng/ml) or EGF (20 ng/ml) alone and assayed for ODC activity after 3 h of incubation at 37°C. The effects of simultaneous addition of TNF with EGF on ODC activity (horizontal hatched bar) or 90 min pretreatment of cells with TNF prior to EGF incubation for 3 h (vertical hatched bar) are also reported. Results represent the average of triplicate assays on triplicate dishes of treated cells ± S.E.M.

cells, ODC activity assays were performed on cytokine treated cells. TNF treatment (3 h) stimulated ODC activity ~3-fold when compared to untreated cells (Fig. 8). Interferon treatment (3 h) of ME-180 cells reduced basal ODC activity only marginally when compared with control cells. Combination of both cytokines reduced TNF-stimulated ODC activity by ~50%, suggesting that interferon treatment may oppose the stimulatory effect of TNF on ODC activity.

The cytotoxic activity of TNF has recently been reported to be antagonized by EGF pretreatment of ME-180 cells [22]. To determine the influence of EGF, ODC activity measurements were performed on TNF-treated and untreated ME-180 cells in the presence or absence of EGF. Preliminary studies of the kinetics of ODC induction in ME-180 following EGF treatment revealed an activity profile similar to that obtained following TNF treatment of ME-180 cells. Maximal ODC induction occurred 3–4 h after the addition of EGF (20 ng/ml) to confluent ME-180 cells and activity returned to basal levels 9–12 h after the addition of growth factor (data not shown). The effect of combined growth factor/TNF treatment is shown in Figure 9. TNF (20 ng/ml) alone stimulated ODC activity ~3.5-fold when compared with untreated cells. EGF treatment resulted in ~23-fold increased ODC activity, which was not significantly affected when co-administered with TNF (horizontal-hatched bar).

DISCUSSION

Previous studies have demonstrated the presence of TNF receptors expressed in equivalent quantities and with similar affinities for TNF on the surface of both ME-180 (K_a = 0.31 nM, ~2250 TNF binding sites/cell) and WI-38 cells (K_a = 0.22 nM, ~2172 TNF binding sites/cell) [1], and evidence suggests that TNF receptor is required and essential for the induction of TNF biological responses. Due to the dose-dependent but opposite effects of TNF on the growth of WI-38 and ME-180 cells and the similar reported TNF binding properties on these two cell types, these cell lines appear to be ideal to examine the process of TNF-induced growth regulation.

We analyzed the effect of TNF on the regulation of the growth-related, highly inducible polyamine biosynthetic enzyme, ODC. To determine whether or not differential regulation of cell growth by TNF in WI-38 and ME-180 cells was reflected in divergent regulation of ODC activity in these cells, ODC activity measurements were made on both cell types following exposure to TNF. Treatment of WI-38 fibroblasts with TNF resulted in an enhancement of their growth and induction of ODC activity. The growth factor-like actions of TNF on fibroblastic cell growth appear to correlate with the time-dependent stimulation of ODC activity in these cells. The mechanism by which TNF induces ODC activity in these cells is unknown. However, the cascade

of events resulting in increased ODC activity and stimulation of cell growth is presumably initiated through TNF interaction with its specific cell surface receptor. Of particular interest was the observation that the early cellular effects of TNF on ODC activity appear to be similar regardless of whether or not the ultimate cellular response is growth inhibition or growth stimulation. Treatment of ME-180 cells with TNF resulted in the concentration- and time-dependent induction of ODC through a mechanism requiring protein synthesis. Induction of ODC activity and maximal activation in ME-180 cells occur prior to the onset of TNF-mediated cytotoxicity (~ 18–24 h in confluent, serum-free ME-180 cells), suggesting that induction of ODC activity was mediated in both tumor target cells and normal fibroblasts and may play a role in the onset of TNF-mediated alterations in cell growth. Although there appears to be a dose-dependent correlation between TNF effects on both cell growth and ODC induction, the precise role and relationship between these two processes is unclear.

ODC has been shown to control the biosynthesis and accumulation of putrescine in mammalian cells [15]. Conversely, recent studies also demonstrate that the accumulation of intracellular putrescine can regulate the expression of ODC activity by a reduction in the efficiency of translation of ODC mRNA [27,28]. To determine their involvement in TNF-induced ODC activity, the intracellular polyamine concentration was measured in ME-180 cells following exposure to TNF. The effects of TNF were compared with those of epidermal growth factor (EGF) and the tumor promoting phorbol ester, TPA, both of which have previously been shown to alter both cellular polyamine content and expression of ODC mRNA [26,29,30]. Treatment with TNF significantly increased putrescine content in ME-180 cells, although not to the extent measurable in EGF or phorbol ester treated cells (Table I). TNF treatment also influenced cellular content of high molecular weight polyamines, inducing increases in the content of spermidine, while spermine content was reduced. Whether or not the reduction in spermine concentration is due to the conversion of spermine to spermidine and putrescine is not known. However, of the three agents tested, TNF was most active in mediating changes in spermidine and spermine, and the rapid induction of these alterations (6 h) may be of some significance in TNF-mediated cellular toxicity. Overall, these results suggest that increased ODC activity in ME-180 cells following TNF treatment correlates with intracellular putrescine and spermidine accumulation and do not appear to be due to cellular reduction in putrescine content and reciprocally increased translation efficiency of ODC mRNA. However, alterations in the subcellular distribution of polyamines have not been examined in ME-180 cells following incubation with TNF.

Depletion of intracellular polyamines by specific inhibitors of ODC enzymatic activity has been shown to alter mammalian cell proliferation [31] and ME-180 cells appear to be no exception. Incubation of ME-180 cells with DFMO inhibits their growth dose-dependently and was reversible in the presence of putrescine. These results demonstrate that modulation of ODC activity and intracellular polyamine content appear to be important elements in the regulation of ME-180 cell growth and provide a potential mechanism to determine the biological significance of ODC induction and modulation of polyamine biosynthesis in TNF-treated tumor cells. Under conditions of cellular confluence, incubation with DFMO had no measurable effect on ME-180 cell viability. TNF, at a concentration of 1,000 units/ml, reduced ME-180 cell viability by ~25% after 24 h, and cytotoxicity was significantly improved when TNF treated cells were preincubated with DFMO (~55%). These results demonstrate that the inhibitory effects of DFMO on polyamine biosynthesis can markedly alter the induction of TNF-mediated cytotoxicity. Due to the specific irreversible interaction of DFMO with ODC, the results also suggest that inhibition of ODC activity in TNF-treated ME-180 cells can enhance the cytotoxic actions of this cytokine. These results suggest that induction of ODC activity may represent one of a class of biological responses to TNF which antagonizes the ability of TNF to function as an antiproliferative agent [32].

Combined treatment of some tumor cells with growth modulatory cytokines has been reported to synergistically improve their cytotoxic potential [1]. Interferon gamma has been shown to enhance the antiproliferative effects of TNF on ME-180 cells and the effect of individual and combination TNF/IFN on ODC activity was examined. Interferon consistently reduced the in-

duction of ODC activity in TNF-treated cells, demonstrating antagonism between the actions of these cytokines on ODC activity. Interferon has been shown to reduce the expression of ODC mRNA in growth factor stimulated cells [33] and may represent one mechanism which accounts for the opposing effects of these molecules. Although the molecular mechanism is unknown, these data suggest that the inhibition of TNF-stimulated ODC induction by interferon may contribute to the synergistic growth inhibitory properties of these cytokines.

In opposition to the synergistic antiproliferative actions of TNF and IFN on ME-180 cell growth, growth factors such as EGF have been shown to inhibit the growth suppressive actions of TNF. Our results demonstrate that like TNF, EGF stimulated ODC activity in ME-180 cells, although the magnitude of the induction was markedly different. These results demonstrate that the relative potential of TNF to induce ODC activity is limited with respect to the actions of a well-characterized growth factor. In addition, the presence of TNF did not alter the ability of EGF to stimulate ODC enzymatic activity, suggesting that early effects of TNF on tumor cells are not expressed through an inhibition of EGF-mediated biological activity. EGF-treatment of tumor cells in the presence of TNF did not result in additive induction of ODC activity, which may be expected if TNF exposure resulted in increased expression and secretion of additional growth factors, as has been previously shown to occur in TNF-treated fibroblasts [34,35]. We have recently shown that short-term incubation (10–30 min) of ME-180 cells with TNF alone results in an increased phosphorylation of EGF receptor which correlated with a stimulation of EGF receptor tyrosine protein kinase activity [36]. Whether or not ODC induction is related to TNF stimulation of EGF receptor phosphorylation is currently under investigation.

Recent studies by Endo et al. [37] have suggested a relationship between the suppression of basal ODC activity and the growth inhibitory activity of TNF on A375 melanoma cells. The results obtained in ME-180 tumor cells suggest that the early actions of TNF are similar to those of a growth factor: inducing both ODC activity and putrescine accumulation. When compared with fibroblasts (Fig. 2), TNF stimulated rapid ODC induction (6 h), which was sustained or elevated in fibroblasts incubated with TNF for extended periods of time (12–24 h). These results contrast with those obtained in TNF (or EGF) treated tumor cells in which maximal ODC induction was achieved after relatively short exposure to these factors (3–6 h) and returned to control levels after 9–12 h of incubation. The earliest ODC activity measurements examined in TNF-treated melanoma cells by Endo et al. were conducted 24 h after TNF exposure and utilized both attached and detached cells. Our studies were confined to the early events initiated upon TNF exposure to ME-180 cells to determine the molecular events which occur prior to the onset of cytotoxicity. Under the conditions of treatment of ME-180 cells (serum-free, confluent), cytotoxicity following TNF incubation was evident as early as 18 h after exposure. ODC activity and putrescine content were concomitantly reduced in ME-180 cells when measured after the onset of cytotoxicity (24 h, data not shown). However, since several biochemical activities may be altered indirectly through the complex process of TNF-induced cytotoxicity [38], it is difficult to assign a direct or causal role for ODC regulation in the antiproliferative actions of TNF.

Bladder carcinoma cells (T24) also express specific cell surface TNF receptors, but are resistant to TNF-mediated cytotoxicity [1]. We have recently demonstrated that TNF treatment of T24 cells results in the stimulation of ODC activity to an extent similar to that obtained with EGF [39]. These results suggest that ODC activity may be regulated by TNF, but that alterations in enzymatic activity appear to be uncoupled from and antagonistic to the cytotoxic properties of this molecule. These results also suggest that TNF can, at least in some tumor cell types, mimic the early actions of growth factors, as suggested by the observations of Vilcek et al. [7]. These results also increase our understanding of the pleiotropic actions of TNF and may aid in defining the complex growth regulatory properties of this cytokine.

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