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DEXAMETHASONE INHIBITS THE CYTOTOXIC ACTIVITY OF TUMOR NECROSIS FACTOR

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Effect of dexamethasone (DEX) on the cytotoxic activity of tumor necrosis factor (TNF) was examined using murine fibroblast cell line (L929 cells). DEX protected cells from the cytotoxic action of TNF. Protection of cytotoxic action was apparent when cells were pre-treated with DEX for 12h and no protection was observed in the presence of cycloheximide. These results suggested that de novo synthesis of new proteins was required for DEX-mediated protection. Moreover, prolonged simultaneous treatment with TNF and DEX resulted in the enhancement of cell growth, suggesting that TNF acted as a growth factor when cells were protected from the cytotoxic action of TNF. These results suggested that the signal transduction system for fibroblast growth enhancing and cytotoxic action of TNF were different from each other and that the interaction between TNF and glucocorticoids may play a modulating role in some inflammatory processes in vivo.

Tumor necrosis factor (TNF) is a monocyte derived protein originally defined on the basis of its ability to produce hemorrhagic necrosis of some animal tumors and direct cytotoxic effects on tumor cells in culture (1). However, recent evidence shows that TNF is a regulatory cytokine with multiple biological activities, which include a mitogenic effect in fibroblasts (2,3), activation of granulocyte functions (4,5), stimulation of cytokine production (6-8), stimulation of collagenase and prostaglandin E_2 production (9) and modulation of T-cell functions (10). Moreover, TNF is shown to be a major mediator of monocyte cytotoxicity (11,12). These results suggest that TNF might indeed act as a mediator of some inflammatory or immunological processes.

Availability of recombinant human TNF has made it possible to examine the biological action of TNF extensively (13,14).

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Consequently, the importance of TNF as inflammatory and/or immunoregulatory mediator is becoming more evident. However, for preclinical and clinical evaluation, it is still necessary to elucidate the interaction of TNF with other mediators.

In this paper, we examined the effect of dexamethasone (DEX) on the cytotoxic action of TNF. We found that DEX inhibited the cytotoxic action of TNF to murine L929 cells. Moreover, TNF acted as a growth factor to the cells which protected from the cytotoxic action of TNF. These results suggest that the interaction between TNF and glucocorticoids at the same microenvironment modulate the process of inflammation *in vivo*.

MATERIALS AND METHODS

Materials

Human recombinant TNF was produced in *Escherichia coli* by using a plasmid which has a T4 phage-derived promoter and pBR322 vector. TNF was purified to homogeneity (>99%) from *E. coli* lysate by serial chromatography on DEAE-Sepharose, phenyl-Sepharose, and Q-Sepharose columns (15). Specific activity of TNF was 3.2×10^6 u/mg protein based on L929 cytotoxic assay measured in the absence of actinomycin D. DEX were purchased from Sigma (St.Louis, MO).

Assay of Cell Growth

The L929 murine fibroblast cell line was used in all experiments. Cells were seeded in 96-well plates and cultured in a CO_2 incubator at 37 C in the presence of desired reagents (final 200µl/well). Four to six replicate wells were used for each experimental condition. At intervals, plates were removed from the incubator and medium (Eagle's minimal essential medium containing 5% fetal bovine serum) was removed by rapid decantation. The cells were fixed for 30min with 10% formalin in 9% acetic acid/0.1M sodium acetate buffer. The cells were then stained with solutions of 0.05% naphthol blue black (Aldrich Chemical Co. Milwarkee, WI) in 9% acetic acid with 0.1M sodium acetate. The plates were then washed with H_2O and the dye was eluted by adding 50mM NaOH (150µl per well). Absorbance of each well was read at 630nm on Dinatech Microelisa Autoreader (3).

RESULTS AND DISCUSSION

In the initial experiments, we examined the effect of DEX on growth of L929 cells. As shown in Fig.1, untreated control cells grew linearly for 3 days. Addition of DEX (10^{-8}-10^{-6}M) to the cultures resulted in a significant decrease of cell growth which was seen at day 2. Moderate suppression of cell growth was also seen at DEX dose of 10^{-9}M. Under the microscope, DEX-treated cells were intact at least up to day 9 of the incubation time.

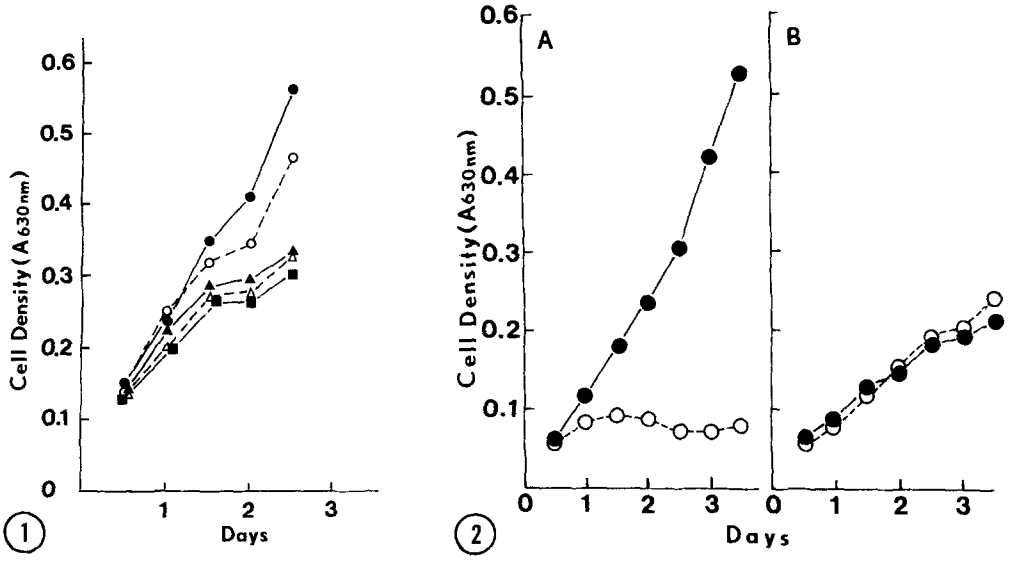

Fig.1 Effect of DEX on growth of L929 cells. Cells were seeded in 96-well plates (8,000 cells/sell). After attachment, cells were treated with 10^{-6}M (■), 10^{-7}M (▲), 10^{-8}M (△), or 10^{-9}M (○) of DEX and incubated for indicated times. Untreated cells (●) were also incubated as cell control.

Fig.2 Cytotoxic action of TNF to L929 cells (A) and its inhibition by DEX (10^{-6}M) (B). Cells were seeded (8,000 cells/sell) and incubated in 96-well plates in the presence (○) or absence (●) or TNF (20 ng/ml) for indicated times.

Since L929 cells are quite sensitive to the cytotoxic action of TNF, we examined the effect of DEX on TNF-mediated cytotoxicity on the cells. As shown in Fig.2, cells incubated with 20 ng/ml of TNF were killed significantly at day 3 after seeding. However, in the presence of DEX, although the growth was suppressed significantly, TNF showed little effect on cell growth.

Next, we examined whether DEX-mediated protection required protein synthesis or not. Cells in confluent cultures recieved TNF and incubated further with or without cycloheximide. Figure 3 shows the effect of DEX on TNF-mediated killing of L929 cells. In the absence of cycloheximide, TNF-mediated cytotoxicity was measured 60h after the addition of TNF. DEX inhibited the cell killing almost completely. On the other hand, it is well known that the inhibitor of protein synthesis enhances the killing activity of TNF (16). In the presence of cycloheximide, TNF-mediated cytotoxicity became apparent 8h after treatment. However, DEX had no effect on cell killing. These results suggested that DEX-mediated protection from cytotoxic action of TNF required de novo synthesis of new proteins.

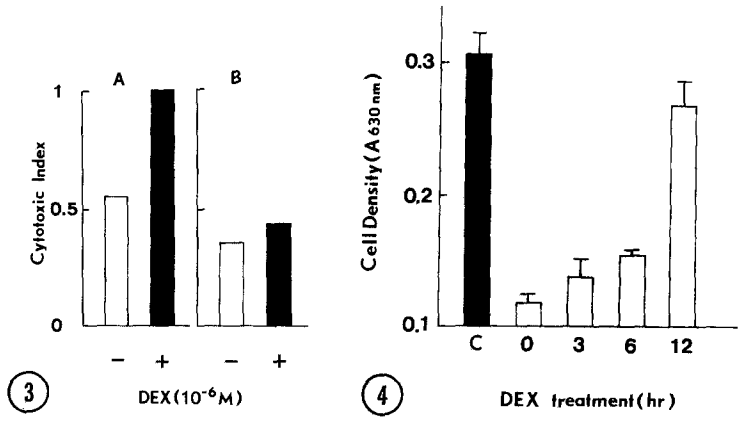

Fig.3 Cycloheximide inhibits DEX-mediated protection of L929 cells from cytotoxic action of TNF. Growth medium was removed from confluent cultures of L929 cells in 96-well plates and replaced with fresh medium with (A) or without (B) cycloheximide (100 μg/ml). After incubation in the presence or absence of TNF (20 ng/ml) for 60h (A) or 12h (B), cell density was determined as described in "Materials and Methods". Effect of TNF was expressed as cytotoxic index as follows:

$$\text{Cytotoxic index} = \frac{\text{A630 measured in the presence of TNF}}{\text{A630 measured in the absence of TNF}}$$

Fig.4 Effect of preincubation with DEX on cytotoxic action of TNF. L929 cells were seeded (8,000 cells/sell) and incubated with DEX (10^{-6}M) for indicated times. Thereafter, cells were washed to remove DEX and fresh medium containing TNF (20 ng/ml) was added. As cell control, cells were incubated with DEX for entire incubation time. Cell density was determined 4 days after seeding as described in "Materials and Methods".

Then, we examined the effect of preincubation of cells with DEX. Cells were treated with DEX, washed and resuspended in fresh medium containing TNF. Control cells were treated with DEX alone for entire incubation time. As shown in Fig.4, significant protection was observed only when DEX was removed 12h after initiation of the treatment. These results suggested that the critical event sensitive to DEX action occures between 6 and 12h.

In our previous paper, we reported that TNF enhanced growth of human fibroblast cell line, FS-4 (3). Because L929 cell is a fibroblast cell line, we next examined the effect of TNF on the cells incubated with DEX. As shown in Fig.5 TNF enhanced cell growth was observed when measured at day 7. These results indicated that when fibroblast cells were protected from cytotoxic action of TNF, even the growth of malignant fibroblast cells was enhanced by TNF. And the enhancement cell growth was apparent only in the late phase of the incubation time.

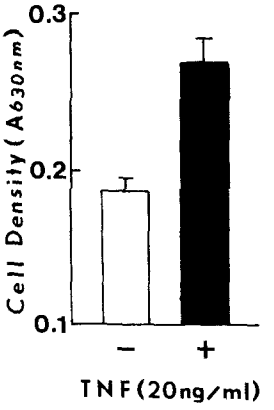

Fig.5 Effect of TNF on growth of DEX-treated L929 cells. Cells were seeded (8,000 cells/well) and incubated with DEX (10^{-6}M). After 7day incubation in the absence (open bar) or presence (solid bar) of TNF (20 ng/ml), cell density was determined as described in "Materials and Methods".

As for the mechanism of cytotoxic action of TNF, few data are available at present. We and others have shown that the number of TNF receptors in different cell lines shows no correlation to the cytotoxic action of TNF (17-19). On the other hand, several mechanisms which might be involved in TNF-mediated cell killing were proposed. These proposed mechanisms, which led to cell death, inclued protease action (20), DNA fragmentation (21), and membrane perturbation (22,23). Synergistic actions between TNF and interferon-γ (24) or interleukin-1 (25) were also demonstrated. On the other hand, transforming growth factors antagonized the antiproliferative activity of TNF (26). These results indicated that the cytotoxic action of TNF was positively or negatively regulated by other mediators. In this study, we presented the data concerning another example of regulators of cytotoxic action of TNF. Nonmalignant cells are generally resistant to the cytotoxic action of TNF. However, even normal fibroblasts undergo rapid lysis in the presence of inhibitors of protein and RNA synthesis (27). These results suggested that the insensitive cells synthesized proteins which protected them from the cytotoxic action of TNF and that failure to synthesize these proteins resulted in cell death. The protective effect of DEX was not observed in the presence of cycloheximide, suggesting that de novo synthesis of new proteins was required for the protection. DEX might induce proteins which was responsible for the protection of L929 cells from the cytotoxic activity of TNF.

Indeed, DEX was shown to regulate several gene expression such as interferon-β_2 (28), acute phase proteins (29), and lipocortin (30), which might affect the signal transduction pathway of TNF.

When the cytotoxic action was inhibited, TNF acted as growth factor even on a malignant fibroblast cell line. These results confirmed the notion that TNF is fibroblast growth factor (3). Modulation of fibroblast growth enhancing activity of TNF by interferon-γ (3), -β_2 (8) and glucocorticoids (28) suggested the significance of the interaction of these mediators at the same microenvironment in vivo.

Taking all the data together, the present data suggest that glucocorticoids participate in the modulation of some biological effects of TNF. Because glucocorticoids inhibit the mediator release in number of in vitro model of allergic reactions (31,32), interaction between TNF and glucocorticoids might be pathologically or physiologically significant in modulating the inflammatory processes.

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