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Tumor necrosis factor is markedly synergistic with interleukin 1 and interferon-γ in stimulating the production of nerve growth factor in fibroblasts

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Abstract

A possible interaction between tumor necrosis factor-α (TNF) and other cytokines/growth factors in stimulating the production of nerve growth factor (NGF) in Swiss 3T3 cells was studied. TNF's stimulatory activity on fibroblast NGF production was synergized by interleukin-1α (IL-1α), IL-1β and interferon-γ (IFN-γ), but was antagonized by transforming growth factor-β (TGF-β). The most remarkable synergistic effect was observed between TNF and IL-1α/β; as little as 0.003 ng/ml of IL-1β markedly enhanced TNF's stimulatory activity on NGF production in the cells. These findings reinforce the idea that TNF, in concert with IL-1α/β, plays an essential role in regulating the regeneration of peripheral nerves following injury through an indirect mechanism by which it stimulates NGF production in fibroblasts.

Key words: Tumor necrosis factor; Nerve growth factor; Interleukin 1; Interferon-γ; Fibroblast

1. Introduction

Tumor necrosis factor-α (TNF), a macrophage/monocyte-derived cytokine, was originally identified as a factor with anti-tumor activity in vitro and in vivo, but is now known to be implicated in diverse biological processes including inflammation, immunoregulation, antiviral defense, endotoxic shock, cachexia, angiogenesis, and mitogenesis (reviewed in [1]). Synergistic and/or antagonistic interactions of TNF with other cytokines in eliciting such a wide spectrum of biological activities have been reported. For example, the anti-proliferative activity of TNF on some tumor cells is synergized by IFN-γ [2] and IL-1 [3], or is antagonized by TGF-α and TGF-β [4]; antiviral activity of TNF is synergized with sub-effective concentrations of IFN-β [5]; growth-promoting action of TNF on fibroblasts is synergized by epidermal growth factor (EGF), platelet-derived growth factor (PDGF), basic fibroblast growth factor (bFGF) and TGF-β, or is inhibited by IFN-β and -γ [6].

We have recently shown that TNF is also involved in modulating neuronal cell function through an indirect mechanism by which it stimulates the synthesis and secretion of NGF in fibroblasts and glial cells [7]. Here we have asked whether or not this newly found action of TNF is influenced by the presence of other cytokines/growth factors.

2. Materials and methods

2.1. Cell culture

Swiss albino mouse fibroblasts (3T3 cells; American Type Culture Collection, CCL92) was obtained through the Japanese Cancer Research Resources Bank. Subconfluent cultures of Swiss 3T3 cells in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum were rendered quiescent by incubation in serum-free medium (DMEM containing 5 mg/ml of bovine serum albumin, 10 μg/ml soy bean lipid, 1 μg/ml insulin, 2 μg/ml transferin, 20 nM Na_2SeO_3, and 10 mM HEPES, pH 7.4) for 24 h; TNF and/or other cytokines/growth factors were then added [7].

2.2. Two-site enzyme immunoassay of NGF

NGF contents in the conditioned media of Swiss 3T3 cells, treated with TNF and/or other cytokines for 48 h, were assayed by the two-site enzyme immunoassay (EIA) specific for mouse submaxillary gland β-NGF [8]. Before the assay, each conditioned medium was passed through a 0.22-μm filter to remove cell debris.

2.3. Materials

Recombinant human TNF-α was produced in *Escherichia coli* and purified to homogeniety as described previously [9]. Recombinant human IL-1α, -1β, recombinant human PDGF (B–B homodimer), recombinant human bFGF and recombinant human EGF were purchased from Toyobo (Osaka), recombinant mouse IFN-γ was from Genzyme (Cambridge), and recombinant human TGF-β was from Wako Pure Chemical Industries Ltd. (Osaka). Other chemicals and reagents were of the purest grade available.

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3. Results

As combinations of cytokines have been reported to act synergistically to produce a variety of effects in many cell systems, we examined whether or not TNF's stimulatory activity on NGF production in fibroblasts [7] is influenced by the presence of other cytokines/growth factors. TNF, IL-1α, IL-1β, EGF, bFGF, IFN-γ, and TGF-β by themselves stimulated the production of NGF in Swiss 3T3 cells, although to varying degrees, TNF being the most potent (Table 1). Combinations of TNF and EGF or bFGF showed that paired factors had roughly additive effects on fibroblast NGF production, which was also the case with the combinations of IL-1β and bFGF, EGF (data not shown) or IFN-γ. On the contrary, simultaneous addition of TNF and IL-1α, IL-1β or IFN-γ stimulated the production of NGF in Swiss 3T3 cells to much greater degrees than did each agent alone; these effects were apparently synergistic since the amount of NGF produced by the cells incubated in TNF plus IL-1α, TNF plus IL-1β, or TNF plus IFN-γ exceeded the sum of the NGF produced by fibroblasts incubated with the cytokines separately (Table 1). Although TGF-β by itself stimulated the production of NGF in Swiss 3T3 cells, it rather antagonized the stimulatory effects of TNF and IL-1β on fibroblast NGF production when used in combination.

Next we examined the optimal concentrations of combinations of TNF, IL-1 and IFN-γ that stimulate the NGF production in Swiss 3T3 cells. As shown in Fig. 1, each cytokine stimulated the production of NGF in the cells in a dose-dependent manner with a maximum being noted with ~10 ng/ml of TNF, ~1 ng/ml of IL-β, and ~10 U/ml of IFN-γ. Stimulatory effects of IL-1β and IFN-γ on fibroblast NGF production were markedly enhanced by the presence of TNF at concentrations greater than 3 ng/ml (Fig. 1A), and that of TNF was synergistically increased by IFN-γ at concentrations greater than 3 U/ml (Fig. 1B). The most remarkable synergistic effect on the stimulation of NGF production in the cells was observed between IL-1 and TNF. As shown in Fig. 1C, as little as 0.003 ng/ml of IL-1β and IL-1α (data not shown) apparently synergized TNF's stimulatory activity on NGF production in Swiss 3T3 cells, which was maximal at a concentration of ~1 ng/ml.

The time–course of stimulation of the fibroblast NGF production by combinations of TNF, IL-1β, IFN-γ and bFGF was examined. We have previously shown that the stimulation of NGF production in TNF-treated Swiss 3T3 cells is a result of an increase in the NGF mRNA level [7]. It is, however, rather a late response of cells; the increase in the NGF mRNA level is detectable only after 6 h, and is maximal by 12–18 h of incubation with TNF. Accordingly, the increase in the NGF protein concentration in the culture medium of TNF-treated cells was observed with a lag time of ~12 h; this was also the case

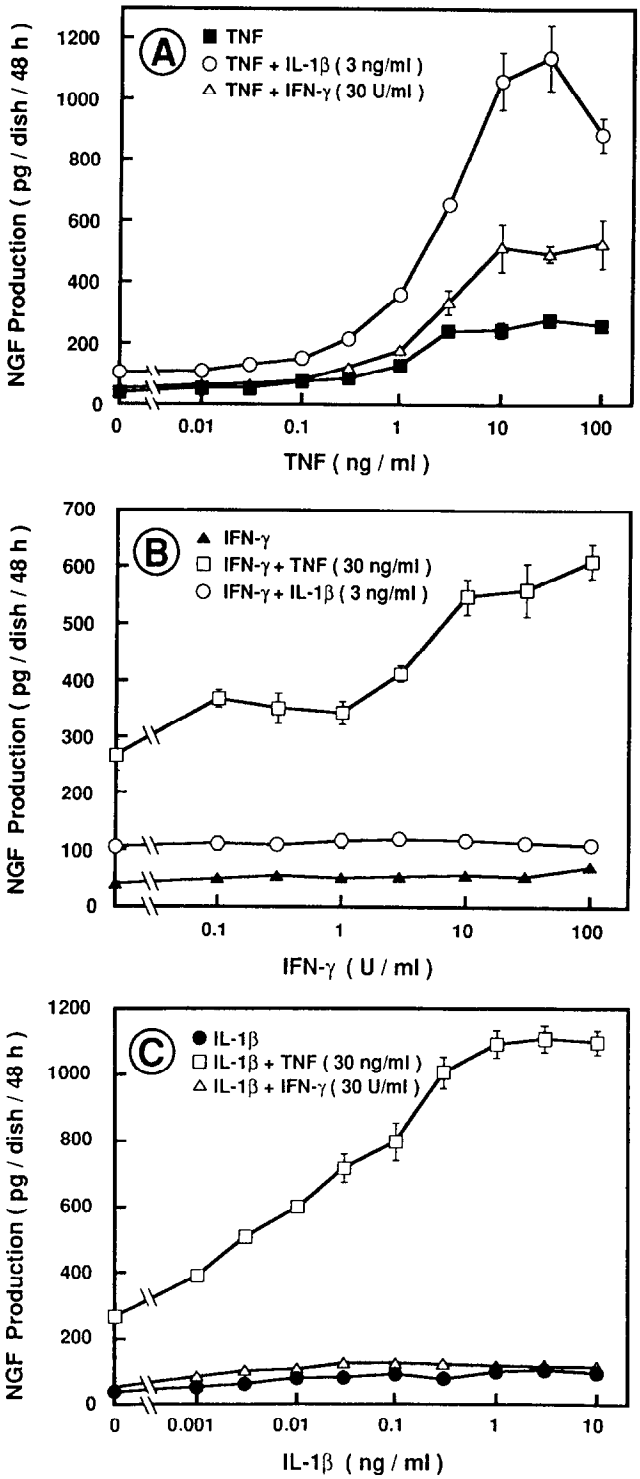

Fig. 1. Dose-dependence of combinations of TNF, IL-1β, and IFN-γ that stimulate the NGF production in Swiss 3T3 cells. Swiss 3T3 cells were cultured with varying doses of TNF (A), IFN-γ (B), or IL-1β (C) with or without TNF (30 ng/ml), IFN-γ (30 U/ml), or IL-1β (3 ng/ml) for 48 h, and then NGF content in the culture media were assayed by EIA. In this experiment, NGF concentration was not normalized to cell number at the end of culture: TNF, IL-1β and IFN-γ were weak mitogens for Swiss 3T3 cells, and the cell numbers after a 48-h treatment with these cytokines (used separately or in combination) were 100–121% of unstimulated control. Means ± S.E.M. of 8 determinations using two 3.5-cm dishes for each experimental condition are indicated. Data shown are representative of three experiments that gave essentially the same results.

in cells treated with IL-1β (Fig. 2A). In contrast, bFGF induced a more rapid stimulation of the fibroblast NGF production, which was maximal by 12 h. Such a rapid stimulation of NGF production in acidic FGF-treated astorocytes has been reported previously [10]. The markedly synergistic effect of the combination of TNF and IL-1β or IFN-γ on the NGF production in Swiss 3T3 cells was apparent after 24 h; even when used in combination, these cytokines did not elicit a more rapid production of NGF in the cells than did bFGF (Fig. 2B). Simultaneous addition of TNF and bFGF showed only an additive effect on the fibroblast NGF production at any op the time points examined.

4. Discussion

The present study has clearly shown that TNF's stimulatory activity on NGF production in fibroblasts we found recently [7] is synergized by IL-1α, -β and IFN-γ, and is antagonized by TGF-β. Similar synergistic and/or antagonistic interactions of TNF with various cytokines in eliciting its other wide spectrum of biological activities have been reported [2–6,11].

The most remarkable synergistic effect on NGF production in Swiss 3T3 cells is observed between TNF and IL-1α/β; as little as 0.003 ng/ml of IL-1β markedly enhances the TNF's stimulatory activity on NGF production in the cells (Fig. 1C). This observation sounds very interesting because (i) it has been suggested that the macrophage-dependent process of Wallerian degeneration is a necessary prologue for peripheral sensory nerve regeneration [12], (ii) macrophages invading the site of nerve lesion during Wallerian degeneration have been shown to be important in the regulation of NGF synthesis [13], and (iii) TNF, IL-1α and IL-1β are the major inflamma-

Table 1
Stimulation of NGF production in Swiss 3T3 cells by various cytokines/growth factors

Treatment	NGF production[a] (fg/cell/48 h)	Fold	
Control	0.570 ± 0.051	**1.00**	
TNF (30 ng/ml)	3.101 ± 0.138	5.44	
IL-1α (3 ng/ml)	1.374 ± 0.162	2.41	
IL-1β (3 ng/ml)	1.260 ± 0.073	2.21	
IFN-γ (30 U/ml)	0.770 ± 0.040	1.35	
bFGF (5 ng/ml)	1.436 ± 0.223	2.52	
EGF (10 ng/ml)	0.638 ± 0.114	1.12	
TGF-β (3 ng/ml)	1.357 ± 0.179	2.38	
TNF (30 ng/ml) + IL-1α (3 ng/ml)	13.395 ± 1.921	23.50	(6.85)[b]
+ IL-1β (3 ng/ml)	13.076 ± 0.711	22.94	(6.65)
+ IFN-γ (30 U/ml)	6.766 ± 0.255	11.87	(5.79)
+ bFGF (5 ng/ml)	4.680 ± 0.140	8.21	(6.96)
+ EGF (10 ng/ml)	2.662 ± 0.333	4.67	(5.56)
+ TGF-β (5 ng/ml)	2.177 ± 0.105	3.82	(6.82)
IL-1β (3 ng/ml) + IFN-γ (30 U/ml)	1.676 ± 0.295	2.94	(2.50)
+ bFGF (5 ng/ml)	2.234 ± 0.421	3.92	(3.73)
+ TGF-β (5 ng/ml)	1.043 ± 0.177	1.83	(3.59)

[a] NGF content in the culture medium of Swiss 3T3 cells treated with various cytokines/growth factors for 48 h was assayed by EIA and then normalized with respect to cell numbers at the end of culture [7]. Values are expressed as the means ± S.E.M. of 8 determinations using 2 dishes for each experimental condition. The amount of NGF produced by the cells varied to some extent from one experiment to another (0.45–0.87 fg of NGF/cell/48 h for unstimulated cells), but the stimulatory effect of these growth factors (used separately or in combination) on NGF production was essentially identical in six separate experiments.
[b] Values for the sum of NGF produced by Swiss 3T3 cells incubated with the cytokines separately are in parentheses.

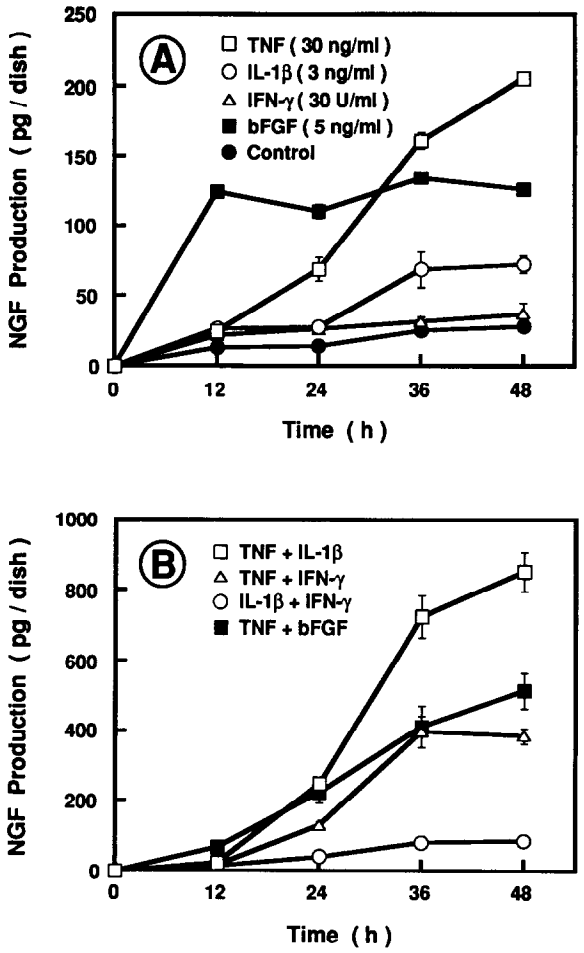

Fig. 2. Time-dependent increase of NGF production in Swiss 3T3 cells by various cytokines/growth factors. NGF content in the culture media of Swiss 3T3 cells treated for varying times with each cytokine/growth factor separately (A), or in combination (B), were assayed by EIA. In this experiment, NGF concentration was not normalized to cell number at the end of culture: unlike TNF, IL-1β and IFN-γ, bFGF strongly stimulated cell proliferation and cell numbers after a 48-h treatment with bFGF was 184% and that with bFGF/TNF was 174% of unstimulated control. Means±S.E.M. of 8 determinations using 2 dishes for each experimental condition are indicated. Data shown are representative of three experiments that gave essentially the same results.

tory cytokines characteristically produced at the site of inflammation by macrophages/monocytes [11]. Considering all this, it seems very likely that TNF, in concert with IL-1α/β, plays an essential role in regulating the regeneration of peripheral nerves following injury through an indirect mechanism by which it stimulates NGF production in fibroblasts.

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