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IFNγ and TNFα synergistically induce neurite outgrowth on PC12 cells

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Abstract

PC12 cells are commonly used in the study of neuronal cells. It was reported that IFNγ enhances neurite outgrowth of PC12 cells by NGF-stimuli. Accordingly, IFNγ was examined to determine if it could solely produce neurite outgrowth. In addition, because the synergism between TNFα and IFNγ is well-known, this study investigated whether or not a mixture of IFNγ and TNFα might augment neurite outgrowth on PC12 cells. Finally, this study examined how an AG490 treatment, which was used to inhibit the IFNγ signal in this study, affected the cytokine-mediated phenomenon. The results showed that the cytokines did not cause an increase in apoptosis in the PC12 cells and the serum-starved condition blocked the cytokine-mediated neurite outgrowth. Interestingly, AG490 enhanced this effect. In conclusion, it was shown that IFNγ has the potential to form neurites, and TNFα can enhance this ability.

Keywords: PC12 cells; Neurite outgrowth; IFNγ; TNFα; AG490; Cell death

Neurite outgrowth is essential for the communication of the nervous system. PC12 cells are commonly used to investigate the nervous system, as a characteristics of these cells are similar that of sympathetic neuronal cells [25]. It is well-known that exogenous stimuli such as NGF induce neurite outgrowth from PC12 cells [3,25]. It has also been reported that an IFNγ treatment facilitates neurite outgrowth on PC12 cells when it is treated in combination with NGF [15]. However, the molecular mechanism of this process is unclear. Differentiation induced by IFNγ has been well-studied in other cell types [7,13,14,22,26,27,31,32,35,36]. Hence, the aims of this study were: (1) to determine if IFNγ solely affected neurite outgrowth; and (2) to examine whether or not TNFα synergistically affected the IFNγ-mediated cellular events because recent reports have been shown some synergism between IFNγ and TNFα in various cell systems [2,4–6,11,16,18,19,33,34].

IFNγ was initially examined to determine if caused neurite outgrowth (Fig. 1a), because Improta et al. [15] reported that IFNγ treatment enhanced NGF-mediated neurite outgrowth. As shown in Fig. 1a, the mixture induced neurite outgrowth (Fig. 1a and c) although IFNγ (R&D systems) alone also caused this effect. As TNFα (R&D systems) did not cause neurite outgrowth, it was assumed that IFNγ had the potential to induce neurite outgrowth, and TNFα augmented the IFNγ-mediated effect. In order to confirm this assumption, the IFNγ signal was blocked with AG490 treatment (Fig. 1b). The PC12 cells were pretreated with 10 uM AG490 at 30 min before the cytokine treatment. Fig. 1a shows how the cells forming the neurites were counted. In contrast to Fig. 1 and our assumption, the AG490 treatment induced neurite outgrowth more potently, and a treatment of this inhibitor combined with the mixture was effective for even 3 days compared with a treatment with the mixture alone. A recent study showed synergism between IFNγ and TNFα for cell survival, which resulted from the cytoprotective function of NFkB signaling [5,6]. However, others reported that these cytokines worked for cell death [2,11,16,19,34]. Overall, these cytokines might trigger cell death in NGF-stimulated PC12 cells, but not in naïve PC12

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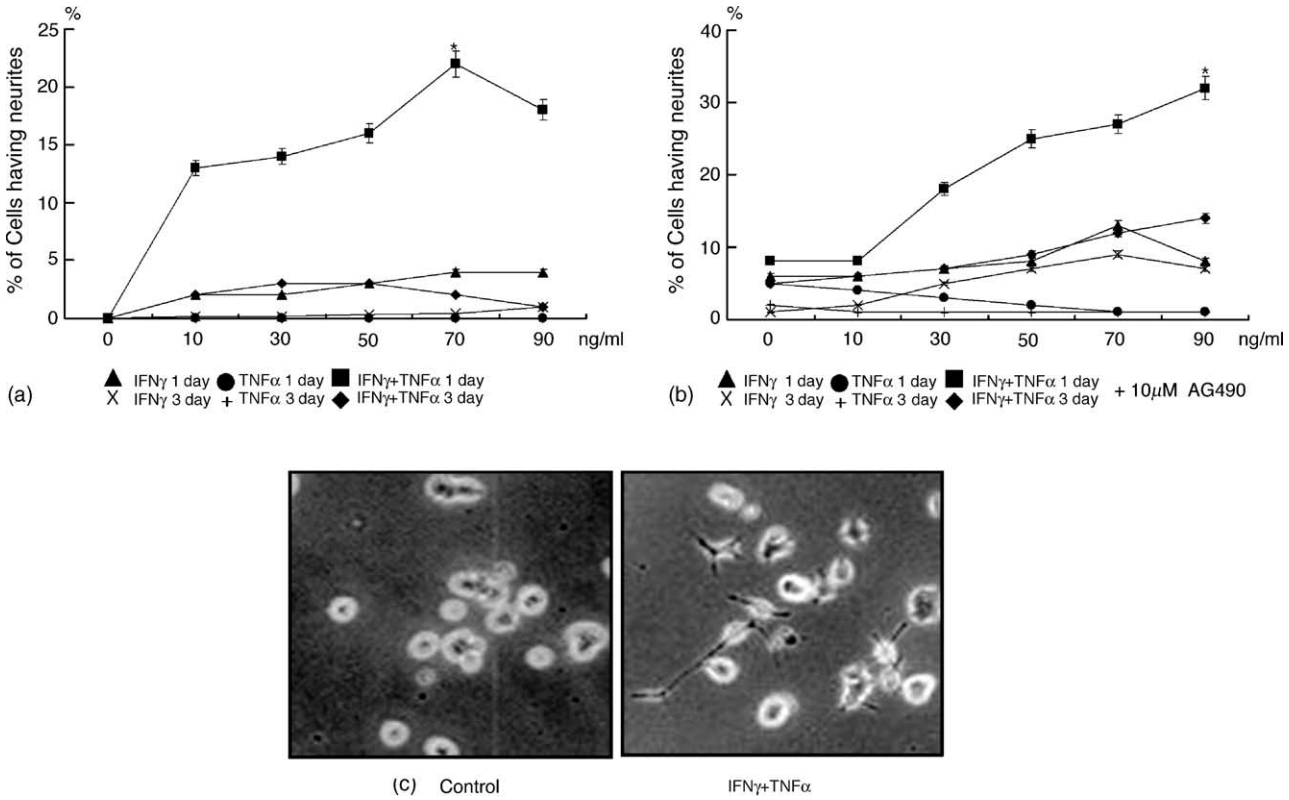

Fig. 1. IFNγ plus TNFα synergistically induce neurite outgrowth. (a and b) PC12 cells grown on poly-L-lysine (Sigma-Aldrich) coated culture dish were treated with IFNγ, TNFα, and the mixture on indicated concentration (0, 10, 30, 50, 70, and 90 ng/ml) for indicated days (Day 1 and 3). Neurite outgrowth was determined by counting at least 10,000 cells having neurites longer than one cell body. The values are the mean ± S.E.M. of three separate experiments. (c) Pictures were taken 1 day after addition of the cytokines. Magnification: 400×; * $P < 0.05$ vs. control.

cells. Although studies of cytokine-mediated cell death on the two types of PC12 cells showed that this different sensitivity might have a similar cause [9,12,21], the precise mechanism has not been reported. Therefore, this study investigated whether or not the cytokines affected cell death on cytokine-treated PC12 cells, and how neurite outgrowth was related to this (Fig. 2). The Fig. 1 showed that a combined cytokine treatment could generate neurite outgrowth. However, the cells were no longer differentiated because 3 day after the cytokine treatment, there was a lower number of cells forming neurites. Presumably, the population of decreased cells might progress to cell death. Therefore, this study examined cell death on the cytokine-treated PC12 cells (Fig. 2) in order to determine how the PC12 cells respond to the cytokine treatment. The cells treated with the cytokines, under similar treatment condition to that shown in Fig. 1 for one day, were subjected to a MTT assay (Fig. 2a). Compared with TNFα, IFNγ and the mixture caused a decrease in the cell viability. A treatment with TNFα did not alter the cell viability, which agrees with recent reports regarding the TNFα-responsiveness on the PC12 cells [9,23,24]. The decreased pattern on both IFNγ and the mixture suggest that it is unlikely that these treatments caused apoptosis on PC12 cells. This is because the MTT assay did not reflect apoptotic cell death, and a decreased pattern was also shown in the cell condition, which is similar to the cessation of proliferation [17]. Moreover, only one method could not prove whether this decreased pattern reflected apoptotic cell death because the caspase-3 activity did not cause PC12 cell death [28]. Hence, the cytokine-treated PC12 cells were sampled for FACS analysis. As shown in Fig. 2b, IFNγ did not bring about apoptotic cell death. Therefore, it was concluded that the decreased pattern of the IFNγ-treated PC12 cells in the MTT assay was not an apoptosis but a necrotic cell death population or a cessation of proliferation. In addition, it was also shown in the mixture-treated PC12 cells that the G1 phase percentage of TNFα-treated PC12 cells was lower. Subsequently, the PC12 cells were placed under serum-starvation conditions in order to determine if the death condition could result in a change of cell viability inclination from the MTT assay (Fig. 2c). It is well-known that serum-starvation supports NGF-mediated neurite outgrowth on PC12 cells [29]. These results did not show any differences between the cytokines alone and the cytokines plus serum-starvation. Hence, the decreased pattern in the MTT assay results might not reflect cell death, and cytokines used in this study did not cause cell death under serum-starvation. The neurite-forming cells were counted in order to confirm if the decreased pattern by IFNγ and the mixture under serum-starvation was also matched to