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Alpha/Beta Interferon Is a Neuronal Growth Factor

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Abstract
We investigated the effect of alpha/beta interferon on neuronal survivability. E16 murine telencephalic vesicles were dissected aseptically and grown on collagen-coated coverslips. After 2 weeks of culturing, the media was supplemented with mouse alpha/beta interferon (1,500 U/ml). After an additional 2 weeks of culturing, all of the control cultures demonstrated significant neuronal death. In the 16 interferon-treated cultures, neuronal death took place only in 1 culture, baseline survivability occurred in 2 and proliferation in 13 cultures ($p < 0.005$). Thus, alpha/beta interferon sustains neuronal growth.

Introduction

The effects of alpha/beta interferon on cultured fibroblasts and leukocytes have been well investigated. These include decreased rates of mitosis, locomotion, membrane ruffling and staltatory movements of intracellular granules [1, 2]. Interferon treatment also changes the amount and cellular distribution of actin and fibronectin [2, 3] and produces defective lymphocyte capping following concanavalin A administration [4].

Alpha/beta interferon may play a physiologic role in the mature central nervous system (CNS). Interferon is present in the cerebrospinal fluid [5]. In monkeys the CNS can produce interferon-dependent RNA following intrathecal administration of interferon [6]. Neurons in vivo and in culture are sensitive to interferon, thus suggesting the possibility of functional interferon receptors in the CNS [7].

There have been few investigations of the effect of alpha/beta interferon on the developing nervous system. Fetal and newborn human fibroblasts and mononuclear cells can produce adult levels of virus-induced interferon [8, 9]. If the effect of alpha/beta interferon on the developing CNS is similar to that on fibroblasts and leukocytes, one would expect the interferons to be neuronal growth inhibitors. Forty-eight hours of alpha/beta interferon treatment of cultured fetal murine CNS increases the immunohistochemical expression of the 210-kD neurofilament subunit [10]. This observation does not answer the question of what effects interferon exposure may have on neuronal growth and survival.

We decided to investigate the effect of prolonged (2 weeks) exposure of interferon on neuronal growth and survival using cultured fetal murine CNS neurons.

Materials and Methods

Pregnant mice were sacrificed by cervical dislocation. Using sterile techniques, E16 normal fetuses were removed from the uterine sacks. Their telencephalic vesicles were dissected and homogenized through a coarse stainless steel sieve into a trypsin-EDTA solution for 2 min. The homogenates were washed several times in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum and plated onto 32 separate collagen-coated coverslips at plating density of 10^6 cells/ml. The cultures were incubated at 37°C in a 94% air/6% CO_2 atmosphere. The media was replaced every 4 days. After 2 weeks, the media of 16 cultures was supplemented with mouse

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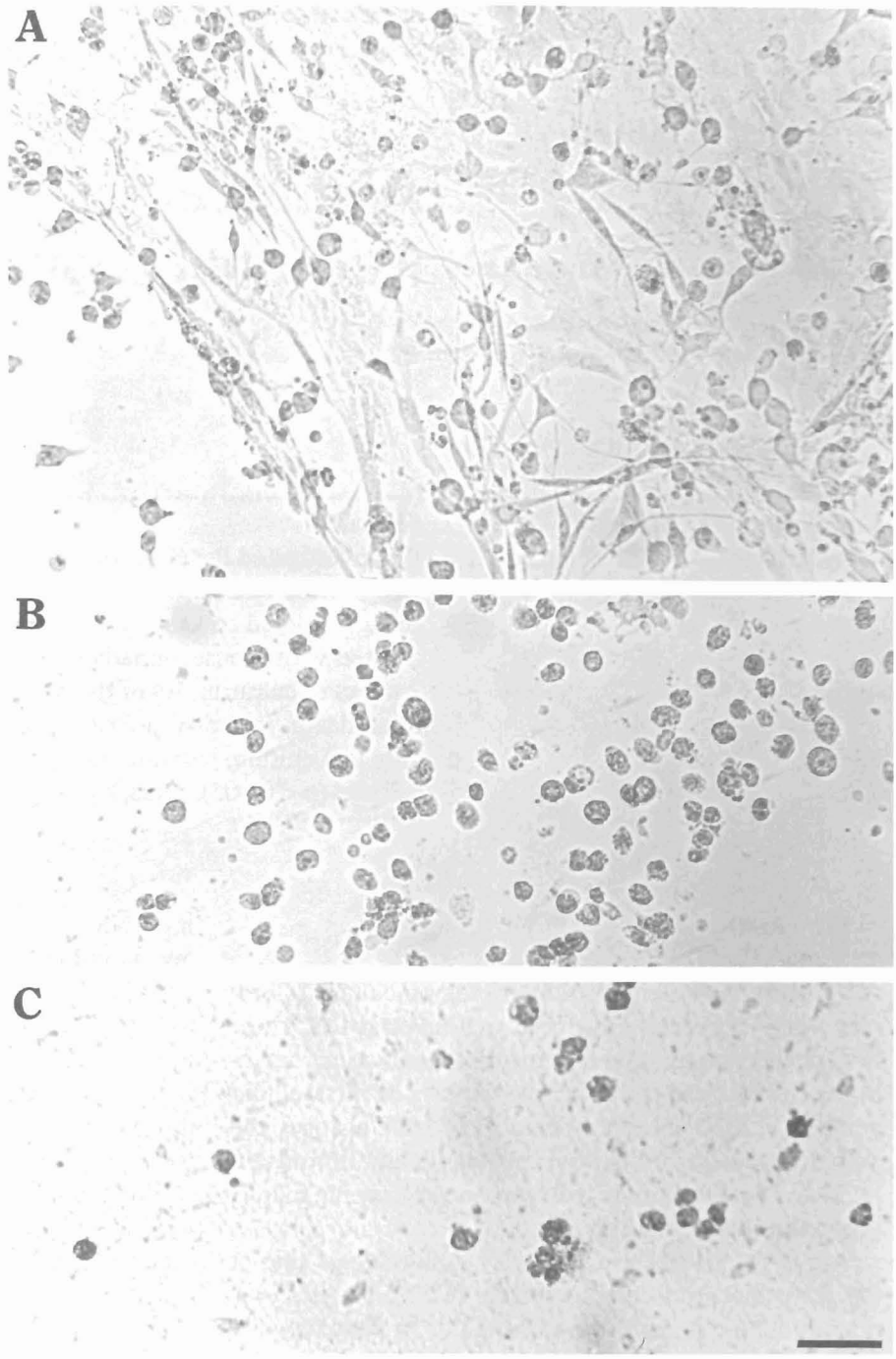

Fig. 1. Phase-contrast photomicrographs of control murine E16 telencephalic vesicle neurons grown in tissue culture. Each photomicrograph is of the same microscopic field. **A** = After 2 weeks in culture; **B** = after 3 weeks; **C** = after 4 weeks. In **A** there is exuberant neuronal growth with multiple pyramidal cells evident. In **B** all cellular profiles have contracted indicative of cellular death. In **C**, with subsequent replacement of culture media, there is less cellular debris than in **B**. Scale bar = 50 μm.

alpha/beta interferon (Sigma, Inc.) in a concentration of 1,500 U/ml. This dosage of interferon is comparable to that used in other studies of peripheral tissues [1, 2, 11, 12] and CNS tissue [10]. The media of the control and interferon-treated cultures was changed every 4 days for an additional 2 weeks. The media for the interferon-treated cultures continued to be supplemented with alpha/beta interferon in a concentration of 1,500 U/ml.

Phase-contrast photomicrographs were taken before and after 1 and 2 weeks of treatment. Identical fields in each culture were photographed and used for analysis. The photographs were compared to determine whether a decrease or increase in neuronal numbers occurred. For statistical analysis χ^2 testing was used.

Six additional cultures were established and grown using the above-described techniques. After 2 weeks the cultures were fixed in

−20 °C acetone for 5 min. After several rinses, two of the coverslips were incubated for 2 h in monoclonal antibody N210 (mabN210). The production and characterization of mabN210 has been described previously [13]. MabN210 recognizes specifically the highly phosphorylated 210-kD subunit of neurofilaments and has been used for a number of immunohistochemical studies [13–15]. After several rinses HRP-conjugated rabbit antimouse immunoglobulin (Dako, Inc.) was applied for 2 h. The reaction product was visualized with 4-chloro-1-naphthol as colorant [16]. Photomicrographs revealed patterns of neurofilament staining similar to previous reports [17]. Preparations in which the primary antibody was omitted gave no staining. Similar techniques were followed to visualize the immunohistochemical expression of the 68-kD neurofilament subunit and neuron-specific enolase using monoclonal antibodies (Boehringer Mannheim). Two coverslips were stained with the anti-68-kD neurofilament monoclonal antibody and two with the anti-neuron-specific enolase monoclonal antibody.

Results

After 2 weeks in culture, all of the coverslips had exuberant neuronal growth. Figure 1A shows a dense growth of multipolar and pyramidal cells with extensive cellular processes. Without interferon treatment, in 7 days most of the cells had retracted their cellular processes and were dying (fig. 1B). Seven days later only scattered cellular debris could be identified (fig. 1C).

An entirely different sequence of events took place with interferon treatment. After 2 weeks in culture, before interferon was added, multipolar cells and axonal networks are evident (fig. 2A). After 7 days of interferon treatment (fig. 2B) there is extension of neuronal processes, cellular migration is taking place and neurons are maturing. These proliferative events continued over the subsequent 7 days of interferon treatment (fig. 2C).

A summary of results after 1 week of interferon treatment on the neuronal cultures is presented in table 1. With interferon there was proliferation in 6 of the 16 cultures, no change in 9 of them and a decrease in numbers in only one. In the untreated control cultures, there was no change in one, a decrease in 4 and neuronal death in 11 ($p < 0.005$).

After 2 weeks of interferon treatment the results were even more dramatic (table 2). With interferon treatment there was proliferation in 13 cultures, no change in 2 and cellular death in one. All of the untreated cultures died ($p < 0.005$).

The additional neuronal cultures, after 2 weeks in culture, revealed extensive immunohistochemical reactivity for the 68-kD and the 210-kD neurofilament subunits and for neuron-specific enolase (not illustrated).

Table 1. Effects of interferon on neuronal numbers after 1 week of treatment

	Interferon	Control
Proliferation	6	0
No change	9	1
Decrease	1	4
Died	0	11

Table 2. Effects of interferon on neuronal numbers after 2 weeks of treatment

	Interferon	Control
Proliferation	13	0
No change	2	0
Decrease	0	0
Died	1	16

Discussion

Our results show that mouse alpha/beta interferon has neuronal growth-sustaining effects in murine CNS cultures. It is unlikely that the photomicrographs revealed a population of cells other than neurons. Using identical tissue preparation and culturing techniques, immunocytochemicals specific for neurons (68- and 210-kD neurofilament subunits and neuron-specific enolase) revealed that the majority of cells were neurons. With the concentration of interferon used we would not expect to see fibroblast growth because this should be inhibited [1–3, 12]. Interferon in the concentration used is a strong supressor of human glial cell lines in culture [Olapade, pers. commun.] suggesting that it is unlikely that glial cell proliferation took place in our cultures. Of more significance is the fact that all of the untreated cultures died. In our laboratory we have been able to routinely maintain fibroblast cultures for up to 6 months, but we have had consistent difficulty in maintaining neuronal cultures. If our cultures were primarily glial or fibroblast in nature, the control cultures should have survived.

It is unlikely that the observed effects are due to other factors that may have been present in the alpha/beta interferon preparation. Using identical CNS culturing techniques and the same commercial source of interferon, alpha/beta interferon enhances the immunohistochemical

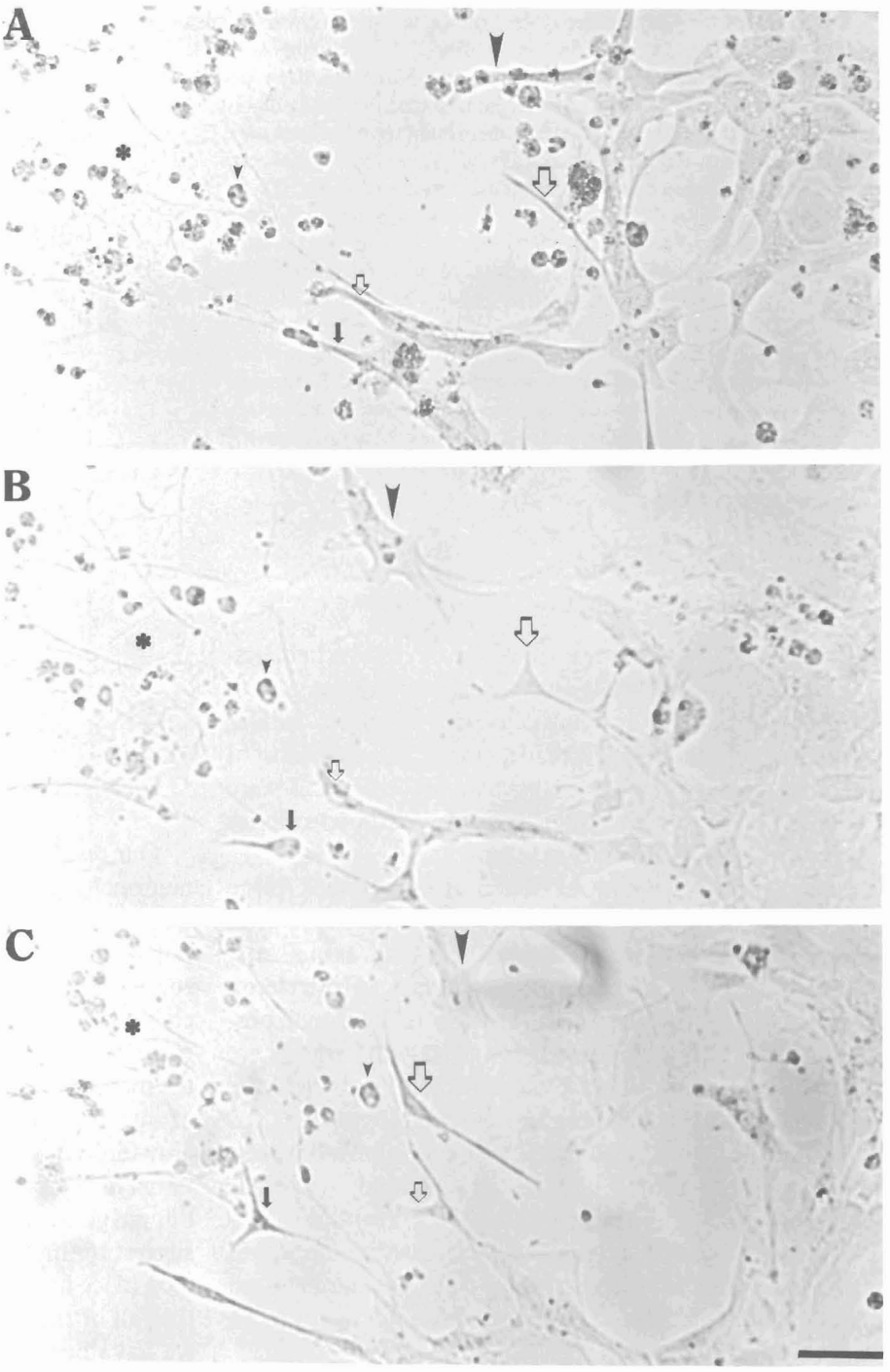

Fig. 2. Phase-contrast photomicrographs of interferon-treated murine E16 telencephalic vesicle neurons grown in tissue culture. Each photomicrograph is of the same microscopic field. **A** = After 2 weeks in culture, just before alpha/beta interferon was added; **B** = after 1 week of interferon treatment and **C** = after 2 weeks of interferon treatment. The large open arrow indicates an outgrowing cellular process in **A**, the formation of a pyramidal cell in **B**, and further migration in **C**. The small open arrow, the solid arrow and large arrowhead all indicate progressive cellular process extension, migration and maturation from **A** through **C**. The small arrowhead indicates a granule of cellular debris that is constant in all photomicrographs. The asterisk indicates a field of axons which progressively becomes more elaborate from **A** through **C**. Scale bar = 50 μm.

expression of the 210-kD neurofilament subunit [10]. This effect is totally blocked by the concomitant administration of oxyphenbytazone [10] which is a specific intercellular inhibitor of the interferon-induced antiviral state [18].

For many years the only recognized neurotrophic factor was nerve growth factor. Recently, an entire family of neurotrophins has been described including brain-derived neurotrophic factor [19], NT-3 [20], NT-4 [21], NT-5 [22] and ciliary neurotrophic factor [23]. What rela-

tion, if any, alpha/beta interferon has to this family of neurotrophins remains to be elucidated.

Since alpha/beta interferon has neuronal growth sustaining properties, it may play a role in human neurogenesis. Also, alpha/beta interferon may play a role in maintaining function of the mature CNS and interference in interferon-mediated metabolic pathways may be a contributing cause of CNS degenerative conditions [10, 24].

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