Influence of the Sympathetic Nervous System on the Growth of Neuroblastoma in Vivo and in Vitro

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ABSTRACT

The sympathetic nervous system exerts a trophic-mitogenic influence on C-1300 mouse neuroblastoma. We now report that sympathetic axotomy suppresses growth of the S-20 clonal line of neuroblastoma but does not influence the growth in vivo of two other clonal lines, NIE-115 and C-46. Sympathetic ganglia-conditioned medium significantly increases proliferation of S-20 cells in vitro. Growth of NIE-115 and C-46 clonal neuroblastoma lines is not influenced by sympathetic ganglia-conditioned medium. We postulate that the sympathetic nervous system secretes a mitogenic-trophic factor that favors growth of C-1300 neuroblastoma in vivo. Sensitivity to this factor varies between neuroblastoma clonal lines.

INTRODUCTION

NB is the most common tumor of infancy and early childhood. There is no effective treatment for this tumor at present. NB and the SNS share a common embryological origin, the neural crest, and not surprisingly, given this fact, NB also shares several neural properties with sympathetic neurons.

C-1300 NB arose spontaneously in A/J mice and has been carried s.c. in this strain since. C-1300 NB resembles human neuroblastoma in many respects and is commonly used as an experimental model for the latter. C-1300 NB and clones derived from C-1300 NB are able to synthesize in culture several known or putative neurotransmitters, acetylcholine, dopamine, norepinephrine, tyramine, octopamine, histamine, serotonin, and γ-aminobutyric acid. These cells contain enzymes involved in neurotransmitter synthesis and degradation, show high affinity uptake systems for specific neurotransmitters and their precursors, and possess receptors which are sensitive to acetylcholine, dopamine, norepinephrine, prostaglandin-E1, and morphine. A limited number of clones derived from C-1300 have been identified which produce a single neurotransmitter (1-4).

6-OHDA is an adrenergic neurotoxin. Administration of 6-OHDA to adult animals results in transient atrophy of peripheral sympathetic endings. Treatment of a newborn animal results in permanent destruction of the peripheral sympathetic nervous system, sympathectomy (5, 6). We have reported previously that 6-OHDA-induced chemical sympathectomy and chemical axotomy of sympathetic nerve endings suppress growth of mouse C-1300 NB in the living animal (7, 8). We have also shown that treatment of newborn mice with chlorisondamine, an agent that blocks afferent cholinergic input into sympathetic ganglia and arrests maturation of the SNS, slows C-1300 NB growth (9). In an opposite situation NB growth is augmented significantly in newborn mice pretreated with NGF (a treatment that causes hypertrophy of the SNS) (8). These findings argue for a control exerted by the SNS on NB growth.

We have reported recently that coculturing of C-1300 NB explants with sympathetic ganglia in vitro augments growth of NB and that sympathetic ganglia-conditioned medium exerts a mitogenic effect on S-20 cells (a cholinergic clonal line derived from C-1300 NB) (10). We now present data comparing the influences of chemical axotomy on growth of different clonal lines of C-1300 NB in vivo with the response of these same clonal lines to sympathetic ganglia-conditioned medium in vitro.

MATERIALS AND METHODS

C-1300 NB clonal lines, S-20 cholinergic, NIE-115 adrenergic, and C-46, a nonproducer of neurotransmitters were obtained as a gift from Dr. R. Rosenberg. The NIE-115 clonal line lacked adrenergic properties at the time of our studies. Cells were maintained in culture in Dulbecco's modified Eagle's medium (catalogue No. 420-2100; Grand Island Biological Co., Grand Island, NY) containing 10% FBS. Cell passages 18-24 were used in these experiments.

Experiments Done in Vivo. To induce a chemical axotomy, adult 6- to 8-wk-old A/J mice were given i.p. injections of 6-OHDA (Sigma Chemical Co., St. Louis, MO) daily for 10 days in doses of 50 μg/g of body weight. The solution used contained 6-OHDA, 10 mg/ml, and ascorbic acid (as an antioxidant) 0.1 mg/ml. Control mice received 0.9% NaCl ascorbic acid solution. This treatment schedule has been shown in prior work to deplete tissue norepinephrine markedly.

Two days after the last injection of 6-OHDA, a time chosen so as to avoid any possible direct influence of 6-OHDA on NB tumor, mice were given s.c. injections in the flank of 10⁶ dispersed viable NB S-20, NB NIE-115, or NB C-46 cells. Tumors were removed 10 days later and weighed. Statistical analyses of tumor weight were done with Student's t test.

Experiments Done in Vitro. SCG were obtained from 1-day-old Wistar-Furth rats.

Each sympathetic ganglion was cut into eight pieces and 3-4 explants were seeded onto collagen-coated Petri dishes (surface area, 9 cm²). SCG explants were cultured in Dulbecco's modified Eagle's medium enriched with 10% FBS and NGF, 84 ng/ml, at 37°C in humidified 5% CO₂ balanced with air. Outgrowth of fibers from SCG explants was visible after 24 h. SCG-conditioned medium was obtained from cultured SCG that had extended multiple processes. Culture medium containing 1% FBS and NGF was substituted for regular medium for the 24-h period prior to supernatant removal. Control medium contained 1% FBS and NGF and was incubated for 24 h in collagen-coated Petri dishes. S-20, NIE-115, or C-46 cells were seeded onto Petri dishes at 5 x 10⁴ cells/
In each experiment, three experimental dishes were incubated with SCG-conditioned medium and three control dishes were incubated with collagen-conditioned medium. Medium was changed once. After 4 days in culture, cells were removed and counted. Statistical analyses were done using the paired t test.

RESULTS

In Vivo Experiments. Growth of the S-20 tumors was significantly suppressed in axotomized mice (Chart 1). Mean S-20 tumor weight in axotomized animals was 270 ± 40 (SE) mg (n = 32) and 460 ± 40 mg (n = 36) in controls. The difference was highly significant (P < 0.001).

Growth of NIE-115 tumor was not influenced by axotomy. Mean tumor weight for axotomized animals was 275 ± 38 mg (n = 59) and 287 ± 45 mg (n = 50) for controls. Growth of C-46 tumor was not suppressed by axotomy; to the contrary, tumors in axotomized mice were marginally larger than in controls. Mean tumor size in axotomized animals was 790 ± 90 mg (n = 15) (4 experiments) and in controls 550 ± 80 mg (n = 22).

In Vitro Experiments. Growth of S-20 cells was significantly augmented in the presence of conditioned medium (Chart 2). The number of S-20 NB cells per dish grown in the presence of conditioned medium was 2.2 x 10^5 ± 0.29 x 10^5 (8 experiments). The number of S-20 cells grown in control medium was 1.2 x 10^5 ± 0.31 x 10^5 (8 experiments). The difference was statistically significant (P < 0.01). Growth of NIE-115 cells and C-46 cells was not influenced by the presence of SCG-conditioned medium in the culture medium. The number of NIE-115 cells in conditioned medium was 2.7 x 10^5/dish ± 0.82 x 10^5 (8 experiments) versus 2.07 x 10^5/dish ± 0.51 x 10^5 (8 experiments) for control medium. The number of C-46 cells in conditioned medium was 1.22 x 10^6 ± 0.22 x 10^6 and in control medium 1.29 x 10^5 ± 0.32 x 10^5 (4 experiments).

DISCUSSION

In earlier work we have shown that the SNS modulates growth of mouse NB both in vivo and in vitro. We have now extended this work to clonal lines of NB. Growth of the S-20 clone was significantly suppressed in mice with a destroyed SNS and significantly augmented in vitro in the presence of SCG-conditioned medium. Growth of C-46 and NIE-115 NB tumors was neither influenced by axotomy in vivo nor by presence of SCG-conditioned medium in vitro.

In our previous experiments we showed that coculturing C-1300 NB explants or S-20 cells in the presence of SCG augments growth of these tumors significantly (11). We demonstrated that this effect is relatively specific for neuroblastoma since coculturing of SCG with A-10 mouse breast adenocarcinoma did not influence growth of this tumor. We also presented evidence that the putative trophic factor that we postulate is responsible for this effect is probably of neural origin because fibroblast-conditioned medium (fibroblasts are present in SCG cultures) did not influence neuroblastoma growth.

That the nervous system contains trophic factors is well documented. In studies on regeneration of amputated limbs of newts and salamanders denervation immediately after leg amputation prevents limb regeneration and decreases DNA, RNA, and protein synthesis in the stump (11-15). The latter can be totally or partially restored by injecting brain or nerve homogenate into the regenerating stump. The nature of the trophic factor responsible for this effect remains unknown. When denervation was performed after differentiation of the regenerating limb had already started, it did not prevent limb formation but the regenerated limb was smaller than normal (16).

Several growth factors of nervous system origin have been identified. Fibroblast growth factor was isolated from mammalian brain and pituitary and was shown to be mitogenic for cells of mesodermal origin (17-19). Glial growth factor was isolated from bovine brain and human acoustic neuroma and was shown to be mitogenic to Schwann cells, astrocytes, and fibroblasts (20-22). It has been found more recently that glial growth factor is present in the early stage of a regenerating blastema, undifferentiated cells which arise at the site of amputation in amphibians, and is lost on denervation (22). Plasma membrane fragments from cultured neurites are mitogenic to Schwann cells (23-26). Embryonic sympathetic neurons have been demonstrated to cause ganglionic nonneural cell proliferation (27, 28). A trophic effect of sympathetic ganglia on normal and dystrophic skeletal muscle has also been shown (29).

While neurotrophic effects on normal tissues are well documented, little is known concerning neurotrophic influence on tumor growth. However, altered tumor growth has been noticed by several investigators following manipulation of the nervous
growth of C-1300 NB and a clonal cholinergic line derived from the investigations of investigators other than ourselves. This group showed that the influence of acetylcholine on neuroblastoma growth has been studied by only one group (11). Singer, M. Neurotrophic activity of brain extracts in forelimb regeneration of the newt Triturus. J. Exp. Zool., 196: 131–150, 1976.

Based on our previous and the present studies, we postulate that the SNS secretes a trophic-mitogenic factor that favors growth of C-1300 NB and a clonal cholnergic line derived from it. Two other clonal lines derived from C-1300 NB, neither of which were producing neurotransmitters at the time of study, were insensitive to the SNS factor. The results obtained in vivo found their counterpart in vitro. We do not know at present why sensitivity to the SNS factor varies between different clonal lines of neuroblastoma. It may relate to specific neural properties of neuroblastoma cells or to the presence of receptors for the SNS trophic factor on selected neuroblastoma cells.

We hope that this finding will aid in an understanding of the biology of neuroblastoma and will have future implications for the treatment of human neuroblastoma.

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