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Editorial

Growth factors: potential therapeutic applications in neurology

Growth factors are defined as signalling proteins required for normal cellular growth and division and, consequently, they are identified as important regulators of developmental processes. In addition, growth factors have a number of potentially important clinical applications, some of which may benefit patients with neurological diseases. This potential was appreciated soon after the purification of nerve growth factor (NGF),¹ the first member of this group of molecules to be identified. It exerts its predominant effects as a trophic agent for developing sympathetic and sensory neurons,^{2,3} and, in consequence, it was suggested that NGF deficiency might be relevant to the aetiology of at least one neuronopathy—namely, familial dysautonomia.⁴ A causal relation was not, however, established⁵ and, in the absence of a clear clinical application for treatment with NGF, its functional analysis became the exclusive province of developmental neurobiology for many years. During this period, three NGF homologues were also identified—namely, brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4)^{6–9}; collectively they became known as the neurotrophins and each was shown to exert distinct but overlapping activities within the developing peripheral and central nervous systems. Other growth factors, often initially identified as having systemic activities, were also found to exert significant effects within the developing nervous system.¹⁰ Molecules with these activities include ciliary neurotrophic factor (CNTF),¹¹ leukaemia inhibitory factor/cholinergic differentiation factor (LIF/CDF),^{12,13} the acidic and basic fibroblast growth factors (FGF-1 and FGF-2),^{14,15} epidermal growth factor (EGF),¹⁶ and platelet derived growth factor (PDGF).¹⁷

In recent years, there has been renewed interest in determining whether some of these growth factors could be useful therapeutic agents for the treatment of neurological diseases. Clinical trials are currently in progress to determine if they can prevent or protect against neural cell degeneration. This article explores these potential uses for growth factors, with emphasis given to the treatment of peripheral neuropathy, motor neuron disease, Parkinson's disease, Alzheimer's disease, and multiple sclerosis.

Peripheral neuropathy

Peripheral neuropathy encompasses a large array of diseases subclassified as either neuronopathies,

axonopathies, or demyelinating neuropathies according to whether the pathology primarily involves the neuronal cell body, axon, or Schwann cell, although in many neuropathies a combination of pathologies is manifest.¹⁸ If growth factors are to prove effective treatments for peripheral neuropathy, due consideration must be given to this heterogeneity; for example, growth factors that act predominantly as mitogens for Schwann cells, such as the glial growth factors,^{19,20} are unlikely to be beneficial in the treatment of primary axonopathies. Furthermore, motor, sensory, and autonomic neurons each require different combinations of neurotrophic factors for maintenance of their survival and for axonogenesis.²¹ It is thus likely that, ultimately, a number of growth factors will be used in the treatment of peripheral neuropathy, with the exact therapeutic regimen tailored to the specific clinical presentation. In the meantime, most interest is focusing on the therapeutic role of growth factors in three discrete clinical presentations and most of the preliminary studies have been undertaken with NGF.

Diabetic neuropathy has several manifestations, the most common being a sensory polyneuropathy of insidious onset, due to axonal degeneration.²² Neurotrophin deficiency has been hypothesised to be implicated in the pathogenesis of diabetic neuropathy,²³ and a streptozotocin induced rodent model of diabetic neuropathy exhibits slowed retrograde axonal transport of NGF in sciatic and mesenteric nerves.^{24,25} Although these findings do not prove a causal association, one rodent study showed that NGF given three times a week, and immediately after diabetes was induced with streptozotocin, prevented an increase in the tail flick threshold temperature over a three month period, suggesting that NGF may protect against diabetic sensory neuropathy.²⁶ As a result, a clinical trial to test the efficacy of NGF as a therapeutic agent for diabetic sensory neuropathy has been started (J A Kessler, personal communication). Clearly, however, there are other important metabolic and vascular pathogenetic mechanisms involved in this neuropathy, suggesting that a combination of treatment strategies will be required to alter its clinical course appreciably.

Neuropathies induced by cytotoxic drugs are potentially well suited to therapeutic intervention with growth factors because treatment can be coadministered with the causative agent. This strategy has the potential to prevent the dose limiting neurotoxicity of some important cytotoxic agents. One example is taxol, used in the treatment of solid tumours such as malignant melanoma and ovar-

ian carcinoma, which induces a sensory neuropathy²⁷ by promoting the abnormal assembly of microtubules within the cytoplasm.²⁸ It has been shown that NGF can induce coordinate microtubule assembly²⁹ and, consequently, it might be of use in ameliorating such microtubule associated pathology.³⁰ Indeed, animal studies have shown either prevention or delay of neuropathic changes when NGF is coadministered with taxol,³¹ vinblastine,^{32,33} and cisplatin.³⁴ It is postulated that NGF protects against the microtubule associated pathology in taxol and vinblastine toxicity but, in the case of cisplatin, the mechanism of action is unknown. It is now opportune to assess whether growth factors are also useful in preventing neuropathy induced by cytotoxic drugs in patients.

Peripheral nerve injuries may also be amenable to treatment with growth factors. The mRNAs of NGF, BDNF, CNTF, and LIF/CDF are all increased after peripheral nerve lesion in animal models, although they display differing and complex patterns of expression within Schwann cells around the injury site³⁵⁻³⁹; NGF, BDNF, CNTF, and LIF/CDF have all potentiated neuronal survival in neonatal rodent studies after peripheral nerve lesions.⁴⁰⁻⁴⁴ Detailed studies in the adult, assessing the degree of peripheral axonal regeneration after treatment with growth factor, need to be conducted before the potential applicability of this strategy to peripheral nerve injury can be adequately assessed.

Motor neuron disease

The diseases of the motor system comprise a number of discrete and often fatal disorders, grouped together because they share the common pathological process of progressive motor neuronopathy.^{45,46} Major insights into motor neuron cell biology have occurred in recent years and, in particular, an understanding of the potential role that specific growth factors play in supporting motor neuron development has been gained. It has been postulated that these same growth factors might ameliorate the devastating effects of motor neuron disease.

The relevant background studies show that CNTF, although not required for normal motor neuron development,⁴⁷ supports the *in vitro* and *in vivo* survival of superfluous motor neurons that would normally die in embryogenesis, during the period of neuronal modelling⁴⁸⁻⁵⁰; CNTF also prevents the degeneration of axotomised motor neurons in the perinatal rodent,^{51,52} and attenuates the decline in motor performance and neuropathology of the *pnn/pnn* mouse.^{51,52} It remains to be established, however, if the results seen in the *pnn* mouse are relevant to motor neuron disease, as the mouse exhibits a dying-back axonopathy, rather than primary neuronal degeneration.⁵³ Nevertheless, it has been reported that disruption of the CNTF gene results in a limited but significant degeneration of facial nerve motor neurons in adult mice, suggesting that CNTF, produced by Schwann cells,⁵⁴ plays a part in the maintenance of rodent motor neurons during postnatal life. By contrast, CNTF deficiency does not seem to be causally related to neurological disease in humans, as a recently identified null mutation of the CNTF gene is equally distributed in a normal cohort and in patients with neurological disease.⁵⁵ This does not preclude a therapeutic role for exogenous CNTF in pathological states such as motor neuron disease, and in concordance with this, two independent clinical trials, each giving systemic CNTF, have been established. It is important to note, however, that analysis of the pharmacokinetics of CNTF emphasises that its short half life, together with its induction of acute phase responses in liver, may limit the clinical usefulness of CNTF given systemically.⁵⁶

The growth factor LIF has structural similarities to CNTF and its cognate receptor shares subunits with the CNTF receptor.⁵⁷ It is thus not surprising to find that LIF also promotes the survival of embryonic spinal cord motor neurons *in vitro*.^{58,59} It is also retrogradely transported by motor neurons, especially after injury,³⁹ and its mRNA is expressed in muscle during development and in the distal segments of axotomised adult sciatic nerves.^{39,60} *In vivo*, LIF has been shown to rescue neonatal rat motor neurons from death in two independent axotomy studies.^{41,61} The systemic administration of LIF to rodents has, however, led to undesirable side effects,⁶² suggesting that careful consideration will need to be given to the dosage and route of administration, if it is to be clinically useful.

Nerve growth factor does not exert any effect on motor neurons but other members of the neurotrophin family do have significant effects and, as such, represent candidate therapeutic agents for motor neuron disease. For example, BDNF, NT-3, and NT-4 all promote the survival of cultured embryonic rat motor neurons and the high affinity receptors for these factors, *trkB* and *trkC*, are present on motor neurons, indicating that the neurotrophins act directly on the motor neuron pool.^{63,64} Furthermore, the mRNAs of BDNF and NT-3 are expressed by skeletal muscle, both at the time of synaptogenesis and in the adult, suggesting that they may act as target derived growth factors for motor neurons *in vivo*.^{64,65} Also, BDNF is retrogradely transported by perinatal and adult motor neurons and the expression of BDNF mRNA in adult animals is upregulated in denervated muscle,^{43,66} further supporting the hypothesis that it is important in maintaining motor neuron integrity. Most importantly, BDNF reduces motor neuron death in neonatal rats after either facial nerve or sciatic nerve axotomy^{38,43,63} and, in a murine deletion mutant of *trkB*, there is loss of both cranial and spinal motor neurons.⁶⁷ It is of particular note, however, that BDNF null mutant mice do not exhibit a reduction in motor neuron number,⁶⁸ raising the possibility that NT-4, which also acts through the *trkB* receptor,⁶⁹ could be responsible for promoting motor neuron survival *in vivo*.

Factors FGF-2 and FGF-5 have also been shown to promote the survival and neuritic extension of chick spinal cord motor neurons.⁷⁰ In recent studies, however, neither FGF-1 nor FGF-2 prevented lesion induced facial motor neuron death in newborn rodents.⁶¹ Finally, insulin like growth factor-1 (IGF-1) has been shown to be an effective survival factor for rodent motor neurons, both *in vitro* and *in vivo*.⁶¹

Clinical trials are proceeding to test the safety and potential efficacy of BDNF and IGF-1 for the treatment of patients with motor neuron disease. Preliminary reports from one of the CNTF trials indicate that there were serious side effects from treatment, and that a significant number of these patients had fared worse, as assessed by loss of muscle strength, than the control group.⁷¹

Parkinson's disease

The characteristic loss of nigral dopaminergic neurons in Parkinson's disease has stimulated the search for neurotrophic factors that support the survival of dopaminergic neurons *in vitro*. One such molecule is BDNF, in that it supports the survival of cultured foetal dopaminergic neurons.⁷² The detection of mRNA for the BDNF receptor in the substantia nigra,⁷³ the isolation of a BDNF like protein from the striatum,⁷⁴ and the demonstration of retrograde axonal transport of radiolabelled BDNF from the

striatum to the substantia nigra⁷⁵ are all consistent with a putative role of BDNF as a growth factor for nigral dopaminergic neurons. The survival of midbrain dopaminergic neurons is not affected, however, in mutant mice lacking BDNF.⁶⁸ Furthermore, a detailed study by Knusel and colleagues failed to show that BDNF alone could rescue lesioned nigral dopaminergic neurons in an *in vivo* model in the adult rat.⁷⁶ Despite this result, there is independent evidence that exogenous BDNF modulates dopaminergic activity in the adult rat brain,⁷⁷ suggesting that BDNF might yet prove to be clinically useful in the context of parkinsonism.

The isolation of neurotrophic factor derived from a glial cell line (GDNF) has provided renewed hope that dopaminergic neuron specific neurotrophic factors will be identified.⁷⁸ Firstly, GDNF mRNA is present in striatal dopaminergic target areas of the developing rat brain.⁷⁹ Secondly, GDNF promotes the survival, differentiation, and high affinity dopamine uptake of cultured foetal dopaminergic neurons⁷⁸ but unlike BDNF, NT-3, and NT-4/5, it has no effect on cultured nigral derived GABAergic neurons. It also potentiates the survival of dopaminergic neurons in grafts of foetal mesencephalon transplanted to the anterior eye chamber.⁷⁹ This finding suggests that GDNF might be useful in potentiating the survival of mesencephalic grafts transplanted for therapeutic purposes into the forebrain of patients with Parkinson's disease.

Although the evidence suggests that GDNF is a trophic agent for developing dopaminergic neurons, GDNF has not been detected by the method of *in situ* hybridisation in adult rat brain, suggesting that GDNF may not be a physiologically relevant molecule in the adult. Despite this lack of expression, *in vivo* studies giving exogenous GDNF are encouraging, suggesting that either GDNF or a related molecule may be a useful therapeutic agent for Parkinson's disease. Firstly, injection into the substantia nigra results in enhanced tyrosine hydroxylase activity locally at the injection site, as well as distally in the ipsilateral striatum.⁸⁰ Secondly, electron microscopic examination at the injection site showed short lived axonal sprouting and synapse formation.⁸⁰ Thirdly, GDNF injection into the substantia nigra of adult rats results in increased motor activity, although this effect is seen with high doses only.⁸¹ Preliminary studies are also underway to evaluate the effects of GDNF on the recovery of adult rats from 6-OH dopamine lesioning of the nigrostriatal pathway.

Many mitogenic factors that primarily stimulate glial cells have been reported to promote the survival and differentiation of nigral dopaminergic neurons. This list includes FGF-2,^{82,83} IGF-1,⁸⁴ transforming growth factor- α ,⁸⁵ and EGF.⁸⁶ The mitogenic subgroup of dopaminergic neurotrophic factors all share the clinically undesirable side effect, however, of promoting glial cell proliferation. By contrast, GDNF and BDNF do not stimulate gliosis and thus are more likely to represent useful therapeutic agents for Parkinson's disease.

Alzheimer's disease

The neuropathology of Alzheimer's disease includes pathognomonic features (neurofibrillary tangles and amyloid plaques) as well as widespread cortical atrophy and diminished levels of multiple neurotransmitters within the neocortex.^{87,88} Despite these extensive abnormalities, some investigators have espoused the view that the demise of cholinergic forebrain neurons is central to the clinical presentation of Alzheimer's disease, raising the possibility that treatment with growth factors could be

useful. This view is supported by an extensive literature, which suggests that NGF is a neurotrophic factor for these neurons. Firstly, NGF protein and mRNA have been detected in hippocampal and cortical neurons,⁸⁹ the projection fields of forebrain cholinergic neurons. Secondly, the low affinity NGF receptor is colocalised with choline acetyl transferase (ChAT),⁸⁴ the neurotransmitter phenotype specific enzyme for basal cholinergic neurons, and *trk* mRNA encoding the high affinity NGF receptor also has been identified in the basal forebrain.⁹⁰ Thirdly, there is selective retrograde transport of labelled NGF from either the neocortex or hippocampus to the basal forebrain nuclei.⁶⁶ It is important to note, however, that a recent report characterising a null mutation of the NGF gene failed to show a reduction in basal forebrain neurons, despite a reduction in ChAT levels; this suggests that the physiological function of NGF in the CNS may be to support neurotransmitter phenotype rather than to act primarily as a trophic agent.⁹¹

The efficacy of NGF in mediating basal forebrain neuronal rescue has been studied extensively in CNS axotomy models, performed in mammals ranging from rodents to non-human primates.^{92,93} Transection of the fimbria-fornix, the axonal projection of the cholinergic forebrain nuclei to the hippocampus, results in the death of a substantial number of cholinergic neurons, potentially mimicking the cholinergic neuronal loss seen in Alzheimer's disease. Treatment with NGF⁹⁴⁻⁹⁸ or transplantation of cell lines genetically engineered to secrete NGF⁹⁹ have been reported to result in cholinergic cell rescue and axonal arborisation. BDNF used in the same fimbria-fornix axotomy model is reported to rescue a smaller number of cholinergic neurons, as well as non-cholinergic neurons.⁷⁶ A third molecule reported to be effective in cell rescue in this axotomy model is FGF-2,¹⁰⁰ but, unlike the effects mediated by either NGF or BDNF, its effect is thought to be indirect.

To date, there is only a single case history reporting the testing of NGF as a putative therapeutic agent for Alzheimer's disease¹⁰¹; unfortunately, the patient developed an intercurrent illness, necessitating cessation of treatment and making it impossible to assess the long term efficacy of NGF. Even if NGF proves to be an effective therapeutic agent in Alzheimer's disease, it almost certainly will not provide definitive treatment, as pathogenetic mechanisms other than neurotrophin deficiency are clearly involved, and the pathology is clearly not restricted to NGF responsive neurons.

Multiple sclerosis

Multiple sclerosis is traditionally classified as an autoimmune disease that causes periaxial demyelination by specifically targeting oligodendrocytes¹⁰² and recent interest has centred on the part that the immunomodulatory cytokine, β -interferon, can play in diminishing the frequency of recurrent attacks.^{103,104} Also, growth factors could potentially subserve a restorative function in multiple sclerosis, by enhancing oligodendrocyte replenishment and, therefore, remyelination. It has been reported that there is a small steady state turnover of non-neuronal cells, including oligodendrocytes, within the normal adult rodent brain.¹⁰⁵ It is possible that in multiple sclerosis the demands on this physiological process are increased and ultimately perturbed, consistent with the finding that the capacity to replenish lost oligodendrocytes is less in patients with long term multiple sclerosis than in patients with recent onset demyelination.¹⁰⁶ Functional oligodendrocyte replenishment could be achieved by potentiating either the survival or, theoretic-

cally, the division of pre-existing oligodendrocytes, as reported to occur in vitro with NGF stimulation.¹⁰⁷ It also may be possible to upregulate the division and subsequent differentiation of oligodendrocytic progenitor cells,¹⁰⁸ which persist into adult life.¹⁰⁹ In vitro data with rodent cells suggest that the proliferative potential of neonatal oligodendrocytic progenitors can be enhanced by PDGF,¹¹⁰⁻¹¹¹ FGF-2,¹¹²⁻¹¹⁴ and NT-3.¹¹⁵

The challenge, if growth factors are to be useful for patients with multiple sclerosis, is to discover how to regulate the proliferative and differentiative potential of progenitor cells without depleting progenitor cell numbers. Recently, enhanced generation of oligodendrocytes has been reported after culture with either CNTF or LIF,¹¹⁶ and continued proliferation of their progenitors can be induced in culture with FGF-2¹¹³⁻¹¹⁴; together, these findings suggest that, ultimately, either the combined or sequential administration of cytokines could represent useful treatment. Expanded populations of progenitors have also been transplanted into animals with demyelinating lesions and the engrafted cells migrate significant distances, differentiate and myelinate in vivo.¹¹⁷ It is uncertain if this strategy will be applicable to the long term treatment of a multifocal disease, such as multiple sclerosis.

Conclusion

In this article, we have presented an optimistic view of the potential therapeutic uses of growth factors in selected neurological diseases, where definitive treatments are currently lacking. Although there is an extensive body of literature on the basic neurobiology of growth factors, much more study is required to bring these potential therapeutic agents into the clinic as established treatments. Disappointingly, the hope that growth factors will alter the clinical course of fatal neurodegenerative disease has been tempered with the recent report of lack of efficacy and adverse side effects when CNTF was given to patients with motor neuron disease. This failure highlights important issues, which were also considerations when the neurobiology of CNTF was being assessed. Firstly, many of the animal models currently available are, at best, poor approximations to the corresponding human neurodegenerative diseases; yet animal studies are a crucial and necessary step before clinical trials. Secondly, pharmacokinetics need to be thoroughly investigated, to ensure both effective delivery of the growth factor to the site of disease and to minimise unwanted systemic or neurological side effects.

The potential clinical application of growth factors to neurological disease parallels the use of other factors, such as colony stimulating factors, in haemato-oncology, where they are now used to induce bone marrow precursor cells to replenish peripheral blood cells in patients with marrow aplasia.¹¹⁸ As a corollary, it is tempting to speculate that recently described precursor cells, found to exist in the adult mammalian brain,¹¹⁹⁻¹²⁰ could be eventually induced, by the use of exogenous growth factors, to replace dead neurons in a variety of neurological diseases. The more immediate aim is to use either single or combinations of growth factors to protect neural cells or to enhance axonal regeneration. If this can be achieved, growth factors will have assumed an important place in clinical neurology.

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- Cohen S. Purification of a nerve-growth promoting protein from the mouse salivary gland and its neuro-cytotoxic antiserum. *Proc Natl Acad Sci USA* 1960;46:302-11.
- Levi-Montalcini R, Booker B. Destruction of the sympathetic ganglia in mammals by an antiserum to a nerve-growth protein. *Proc Natl Acad Sci USA* 1960;46:384-91.
- Johnson Jr. EM, Gorin PD, Brandeis LD, Pearson J. Dorsal root ganglion neurons are destroyed by exposure in utero to maternal antibody to nerve growth factor. *Science* 1980;210:916-8.
- Pearson J, Axelrod F, Dancis J. Trophic functions of the neuron. V. Familial dysautonomias. Current concepts of dysautonomia. *Ann NY Acad Sci* 1974;228:288-300.
- Breakfield XO, Orloff G, Castiglione C, Coussens L, Axelrod FB, Ullrich A. Structural gene for B-nerve growth factor not defective in familial dysautonomia. *Proc Natl Acad Sci USA* 1984;81:4213-6.
- Barde Y-A, Edgar D, Thoenen H. Purification of a new neurotrophic factor from mammalian brain. *EMBO J* 1982;1:549-53.
- Leibrock J, Lottspeich F, Hohn A, et al. Molecular cloning and expression of brain-derived neurotrophic factor. *Nature* 1989;341:149-52.
- Hohn A, Leibrock J, Bailey K, Barde Y-A. Identification and characterization of a novel member of the nerve growth factor/brain-derived neurotrophic factor family. *Nature* 1990;344:339-41.
- Hallbook F, Ibanez CF, Persson H. Evolutionary studies of the nerve growth factor family reveal a novel member abundantly expressed in *Xenopus* ovary. *Neuron* 1991;6:845-58.
- Bartlett PF, Drago J, Kilpatrick TJ, Richards LJ, Wookey P, Murphy M. Regulation of the early development of the nervous system by growth factors. In: Sharma C, Goffinet A, eds. *Development of the vertebrate nervous system*. London: Academic Press, 1992:1-21.
- Stockli K, Lottspeich F, Sendtner M, et al. Molecular cloning, expression and regional distribution of rat ciliary neurotrophic factor. *Nature* 1989;342:920-3.
- Hilton DJ, Nicola NA, Metcalf D. Purification of a murine leukaemia inhibitory factor from Krebs ascites cells. *Anal Biochem* 1988;173:359-67.
- Hilton DJ, Nicola NA, Gough NM, Metcalf D. Resolution and purification of three distinct factors produced by Krebs ascites cells which have differentiation-inducing activity on murine myeloid leukemic cell lines. *J Biol Chem* 1988;263:9238-43.
- Esch F, Baird A, Ling N, et al. Primary structure of bovine pituitary basic fibroblast growth factor (FGF) and comparison with the amino-terminal sequence of bovine brain acidic FGF. *Proc Natl Acad Sci USA* 1985;82:6507-11.
- Gimenez-Gallego G, Rodkey J, Bennett C, Rios-Candelore M, DiSalvo J, Thomas K. Brain-derived acidic fibroblast growth factor: complete amino acid sequence and homologies. *Science* 1985;230:1385-8.
- Cohen S. The stimulation of epidermal proliferation by a specific protein (EGF). *Dev Biol* 1965;12:394-407.
- Hammacher A, Hellman U, Johnsson A, et al. A major part of platelet-derived growth factor purified from human platelets is a heterodimer of one A and one B chain. *J Biol Chem* 1988;263:16493-8.
- Thomas PK, Ochoa J. Clinical features and differential diagnosis. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, eds. *Peripheral neuropathy*. Vol 2. Philadelphia: WB Saunders, 1993:749-74.
- Lemke GE, Brookes JP. Identification and purification of glial growth factor. *J Neurosci*, 1984;4:75-83.
- Goodearl ADJ, Davis JB, Mistry K, et al. Purification of multiple forms of glial growth factor. *J Biol Chem* 1993;268:18095-102.
- Korsching S. The neurotrophic factor concept: a reexamination. *J Neurosci* 1993;13:2739-48.
- Thomas PK, Tomlinson DR. Diabetic and hypoglycemic neuropathy. In: Dyck PJ, Thomas PK, Griffin JW, Low PA, Poduslo JF, eds. *Peripheral neuropathy*. Vol 2. Philadelphia: WB Saunders, 1993:1219-50.
- Editorial. Understanding diabetic neuropathy. *Lancet* 1991;338:1496-7.
- Jakobsen J, Brimijoin S, Skau K, Sidenius P, Wells D. Retrograde axonal transport of transmitter enzymes, fucose-labeled protein, and nerve growth factor in streptozotocin-diabetic rats. *Diabetes* 1981;30:797-803.
- Schmidt RE, Plurad SB, Saffitz JE, Grabau GG, Yip HK. Retrograde axonal transport of ¹²⁵I-nerve growth factor in rat ileal mesenteric nerves—effect of streptozotocin diabetes. *Diabetes* 1985;34:1230-40.
- Apfel SC, Arezzo JC, Brownlee M, Federhoff H, Kessler JA. Nerve growth factor treatment ameliorates diabetic sensory neuropathy [abstract]. *Diabetes* 1992;41(suppl):140A.
- Lipton R, Apfel S, Dutcher J, et al. Taxol produces a predominantly sensory neuropathy. *Neurology* 1989;39:368-73.
- Schiff PB, Horowitz SB. Taxol stabilizes microtubules in mouse fibroblast cells. *Proc Natl Acad Sci USA* 1980;70:1561-6.
- Drubin DG, Feinstein SC, Shooter EM, Kirschner MW. Nerve growth factor-induced neurite outgrowth in PC12 cells involves the coordinate

- induction of microtubule assembly and assembly-promoting factors. *J Cell Biol* 1985;101:1799-807.
- 30 Peterson ER, Crain SM. Nerve growth factor attenuates neurotoxic effects of taxol on spinal cord-ganglion explants from fetal mice. *Science* 1982;217:377-9.
 - 31 Apfel SC, Lipton RB, Arezzo JC, Kessler JA. Nerve growth factor prevents toxic neuropathy in mice. *Ann Neurol* 1991;29:87-90.
 - 32 Menesini-Chen MG, Chen JS, Calissano P, Levi-Montalcini R. Nerve growth factor prevents vinblastine destructive effects on sympathetic ganglia in newborn mice. *Proc Natl Acad Sci USA* 1977;74:5559-63.
 - 33 Johnson Jr EM. Destruction of the sympathetic nervous system in neonatal rats and hamsters by vinblastine: prevention by concomitant administration of nerve growth factor. *Brain Res* 1978;141:105-18.
 - 34 Apfel SC, Arezzo JC, Lipson L, Kessler JA. Nerve growth factor prevents experimental cisplatin neuropathy. *Ann Neurol* 1992;31:76-80.
 - 35 Heumann R, Lindholm D, Bandtlow C, et al. Differential regulation of mRNA encoding nerve growth factor and its receptor in rat sciatic nerve during development, degeneration, and regeneration: role of macrophages. *Proc Natl Acad Sci USA* 1987;84:8735-9.
 - 36 Meyer M, Matsuoka I, Wetmore C, Olson L, Thoenen H. Enhanced synthesis of brain-derived neurotrophic factor in the lesioned peripheral nerve: different mechanisms are responsible for the regulation of BDNF and NGF mRNA. *J Cell Biol* 1992;119:45-54.
 - 37 Friedman B, Scherer SS, Rudge JS, et al. Regulation of ciliary neurotrophic factor expression in myelin-related Schwann cells in vivo. *Neuron* 1992;9:295-305.
 - 38 Sendtner M, Stockli KA, Thoenen H. Synthesis and localization of ciliary neurotrophic factor in the sciatic nerve of the adult rat after lesion and during regeneration. *J Cell Biol* 1992;118:139-48.
 - 39 Curtis R, Scherer SS, Somogyi R, et al. Retrograde axonal transport of LIF is increased by peripheral nerve injury: correlation with increased LIF expression in distal nerve. *Neuron* 1994;12:191-204.
 - 40 Cheema SS, Richards LJ, Murphy M, Bartlett PF. Leukaemia inhibitory factor prevents the death of axotomised sensory neurons in the dorsal root ganglia of the neonatal rat. *J Neurosci Res* 1994;37:213-8.
 - 41 Cheema SS, Richards LJ, Murphy M, Bartlett PF. Leukaemia inhibitory factor rescues motoneurons from axotomy-induced cell death. *Neuroreport* 1994;5:989-92.
 - 42 Yip HK, Rich KM, Lampe PA, Johnson Jr. EM. The effects of nerve growth factor and its antiserum on the postnatal development and survival after injury of sensory neurons in rat dorsal root ganglia. *J Neurosci* 1984;4:2986-92.
 - 43 Yan Q, Elliott J, Snider WD. Brain-derived neurotrophic factor rescues spinal motor neurons from axotomy-induced cell death. *Nature* 1992;360:753-5.
 - 44 Sendtner M, Kreutzberg GW, Thoenen H. Ciliary neurotrophic factor prevents the degeneration of motor neurons after axotomy. *Nature* 1990;345:440-1.
 - 45 Chancellor AM, Warlow CP. Adult onset motor neuron disease: worldwide mortality, incidence, and distribution since 1950. *J Neurol Neurosurg Psychiatry* 1992;55:997-1001.
 - 46 Chancellor AM. The Scottish motor neuron disease register: a prospective study of adult motor neuron disease in Scotland. Methodology, demography and clinical features of incident cases in 1989. *J Neurol Neurosurg Psychiatry* 1992;55:536-41.
 - 47 Stockli KA, Lillien LE, Naher NM, et al. Regional distribution, developmental changes, and cellular localization of CNTF-mRNA and protein in the rat brain. *J Cell Biol* 1991;115:447-59.
 - 48 Arakawa Y, Sendtner M, Thoenen H. Survival effect of ciliary neurotrophic factor (CNTF) on chick embryonic motoneurons in culture: comparison with other neurotrophic factors and cytokines. *J Neurosci* 1990;10:3507-15.
 - 49 Oppenheim RW, Prevette D, Yin QW, Collins F, MacDonald J. Control of embryonic motoneuron survival in vivo by ciliary neurotrophic factor. *Science* 1991;251:1616-8.
 - 50 Oppenheim RW. Cell death during development of the nervous system. *Annu Rev Neurosci* 1991;14:453-501.
 - 51 Sendtner M, Arakawa Y, Stockli KA, Kreutzberg GW, Thoenen H. Effect of ciliary neurotrophic factor (CNTF) on motoneuron survival. *J Cell Sci Suppl* 1991;15:103-9.
 - 52 Sendtner M, Schmalbruch H, Stockli KA, Carroll P, Kreutzberg GW, Thoenen H. Ciliary neurotrophic factor prevents degeneration of motor neurons in mouse mutant progressive motor neuropathy [see comments]. *Nature* 1992;358:502-4.
 - 53 Schmalbruch H, Jensen HJS, Bjaerg M, Kamieniecka Z, Kurland L. A new mouse mutant with progressive motor neuropathy. *J Neuropathol Exp Neurol* 1991;50:192-204.
 - 54 Masu Y, Wolf E, Holtmann B, Sendtner M, Brem G, Thoenen H. Disruption of the CNTF gene results in motor neuron degeneration. *Nature* 1993;365:27-32.
 - 55 Takahashi R, Yokojii H, Misawa H, Hayashi M, Hu J, Deguchi T. A null mutation in the human CNTF gene is not causally related to neurological disease. *Nature Genetics* 1994;7:79-84.
 - 56 Dittrich F, Thoenen H, Sendtner M. Ciliary neurotrophic factor: pharmacokinetics and acute-phase response in rat. *Ann Neurol* 1994;35:151-63.
 - 57 Hall AK, Rao MS. Cytokines and neurokinins: related ligands and related receptors. *TINS* 1992;15:35-37.
 - 58 Martinou J, Martinou I, Kato A. Cholinergic differentiation factor [CDF/LIF] promotes survival of isolated rat embryonic motoneurons in vitro. *Neuron* 1992;8:737-44.
 - 59 Richards L, Kilpatrick T, Bartlett P. Leukemia inhibitory factor promotes the neuronal development of the spinal cord precursors from the neural tube. *J Neurosci Res* 1992;33:476-84.
 - 60 Murphy M, Reid K, Brown MA, Bartlett PF. Involvement of leukemia inhibitory factor and nerve growth factor in the development of dorsal root ganglion neurons. *Development* 1993;117:1173-82.
 - 61 Hughes RA, Sendtner M, Thoenen H. Members of several gene families influence survival of rat motoneurons in vitro and in vivo. *J Neurosci Res* 1993;36:663-71.
 - 62 Metcalf D, Nicola NA, Gearing DP. Effects of injected leukemia inhibitory factor on hematopoietic and other tissues in mice. *Blood* 1990;76:50-6.
 - 63 Koliatsos VE, Clatterbuck RE, Winslow JW, Cayouette MH, Price DL. Evidence that brain-derived neurotrophic factor is a trophic factor for motor neurons in vivo. *Neuron* 1993;10:359-67.
 - 64 Henderson CE, Camu W, Mettling C, et al. Neurotrophins promote motor neuron survival and are present in embryonic limb bud. *Nature* 1993;363:266-70.
 - 65 Schecterson LC, Bothwell M. Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. *Neuron* 1992;9:449-63.
 - 66 DiStefano PS, Friedman B, Radziejewski C, Schnick C, Lindsay RM, Wiegand SJ. The neurotrophins BDNF, NT-3, and NGF display distinct patterns of retrograde axonal transport in peripheral and central neurons. *Neuron* 1992;8:983-93.
 - 67 Klein R, Smeyne RJ, Wurst W, et al. Targeted disruption of the trkB neurotrophin receptor gene results in nervous system lesions. *Cell* 1993;75:113-22.
 - 68 Enfers P, Lee KF, Jaenisch R. Mice lacking brain-derived neurotrophic factor develop with sensory deficits. *Nature* 1994;368:147-50.
 - 69 Davies AM. Switching neurotrophin dependence. *Current Biology* 1994;4:273-6.
 - 70 Hughes RA, Sendtner M, Goldfarb M, Lindholm D, Thoenen H. Evidence that fibroblast growth factor 5 is a major muscle-derived survival factor for cultured spinal motoneurons. *Neuron* 1993;10:369-77.
 - 71 Barinaga M. Neurotrophic factors enter the clinic. *Science* 1994;264:772-4.
 - 72 Hyman C, Hofer M, Barde YA, et al. BDNF is a neurotrophic factor for the dopaminergic neurons of the substantia nigra. *Nature* 1991;350:230-2.
 - 73 Hyman C, Juhasz M, Jackson C, Wright P, Ip NY, Lindsay RM. Overlapping and distinct actions of the neurotrophins BDNF, NT-3 and NT4/5 on cultured dopaminergic and GABAergic neurons of the ventral mesencephalon. *J Neurosci* 1994;14:335-47.
 - 74 Dal Toso R, Giorgio O, Soranzo C, Kirschner G. Development and survival of neurons in the dissociated fetal mesencephalic serum-free cell cultures. Effects of cell density and of adult mammalian striatal derived neurotrophic factor. *J Neurosci* 1988;8:733-45.
 - 75 Weigard SJ, Anderson K, Alexander C, et al. Receptor binding and axonal transport of I125-labelled neurotrophins in the basal ganglia and related brain regions. *Mov Disord* 1992;7(suppl 1):147B.
 - 76 Knusel B, Beck KD, Winslow JW, et al. Brain derived neurotrophic factor administration protects basal forebrain cholinergic but not nigral dopaminergic neurons from degenerative changes after axotomy. *The Adult Rat Brain* 1992;12:4391-402.
 - 77 Altar CA, Boylan CB, Jackson C, et al. Brain derived neurotrophic factor augments rotational behaviour and nigrostriatal dopamine turnover in vivo. *Proc Natl Acad Sci USA* 1992;89:11347-51.
 - 78 Lin LFH, Doherty DH, Lile JD, Bektess S, Collins F. GDNF: A glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 1993;260:1130-2.
 - 79 Stromberg I, Bjorklund L, Johansson M, et al. Glial cell line-derived neurotrophic factor is expressed in the developing but not adult striatum and stimulates developing dopamine neurons in vivo. *Exp Neurol* 1993;124:401-12.
 - 80 Henry MA, Granholm AC, MacKerlovian L, Hudson J, Hoffer BJ, Collins F. Electron microscopic study following GDNF injection into the substantia nigra reveals sprouting and synaptogenesis within the striatum. *Society for Neuroscience Abstracts* 1993;275.4:652.
 - 81 Hudson J, Granholm AC, Gerhardt G, et al. In-vivo activity of GDNF, a glial-cell-line derived neurotrophic factor, on the rat nigrostriatal dopamine system. *Society for Neuroscience Abstracts* 1993;275.5:652.
 - 82 Ferrari G, Minozzi MC, Toffano G, Leon A, Skaper SD. Basic fibroblast factor affects the survival and development of mesencephalic neurons in culture. *Dev Biol* 1989;133:140-7.
 - 83 Ferrari G, Minozzi MC, Toffano G, Leon A, Skaper SD. Basic fibroblast factor affects the survival and development of mesencephalic neurons in culture. *Adv Exp Med Biol* 1990;265:93-9.
 - 84 Beck K, Knusel B, Hefti F. The nature of the trophic actions of brain-derived neurotrophic factor, des(1-3)-insulin-like growth factor-I, and basic fibroblast growth factor on mesencephalic dopaminergic neurons developing in culture. *Neuroscience* 1993;52:855-66.
 - 85 Alexi T, Hefti F. Trophic actions of transforming growth factor on mesencephalic dopaminergic neurons developing in culture. *Neuroscience* 1993;55:903-18.
 - 86 Casper D, Mytilineou C, Blum M. EGF enhances the survival of dopamine neurons in rat embryonic mesencephalon primary cell culture. *J Neurosci* 1991;30:372-81.
 - 87 Beal MF, Martin JB. Somatostatin: normal and abnormal observations in the CNS. In: Wurtman RJ, Corkin SH, Growdon JH, eds. *Alzheimer disease: advances in basic research and therapies*. Cambridge MA: Center for Brain Sciences and Metabolism Charitable Trust, 1984:229-58.
 - 88 Sasaki H, Muramoto O, Kanazawa I. Regional distribution of amino acid transmitters in postmortem brains of presenile dementia of the Alzheimer type. *Ann Neurol* 1986;19:263-9.
 - 89 Korsching S, Auburger G, Heumann R, Scott J, Thoenen H. Levels of nerve growth factor and its mRNA in the central nervous system of the rat correlate with cholinergic innervation. *EMBO J* 1985;4:1389-3.
 - 90 Vazquez ME, Ebendal T. Messenger RNAs for trk and the low-affinity NGF receptor in the rat basal forebrain. *Neuroreport* 1991;2:593-6.
 - 91 Crowley C, Spencer S, Nishimura M, et al. Mice lacking nerve growth factor display perinatal loss of sensory and sympathetic neurons yet develop basal forebrain cholinergic neurons. *Cell* 1994;76:1001-11.
 - 92 Tuszyński MH, U HS, Amaral DG, Gage FH. Nerve growth factor infusion in primate brain reduces lesion-induced cholinergic neuronal degeneration. *J Neurosci* 1990;10:3604-14.
 - 93 Koliatsos VE, Nauta HJ, Clatterbuck RE, Holtzman DM, et al. Mouse nerve growth factor prevents degeneration of axotomised basal forebrain cholinergic neurons in the monkey. *J Neurosci* 1990;10:3801-13.
 - 94 Hefti F. Nerve growth factor promotes survival of septal cholinergic neurons after fimbrial transections. *J Neurosci* 1986;6:2155-62.
 - 95 Williams LR, Varon S, Peterson GM, et al. Continuous infusion of nerve growth factor prevents basal forebrain neuronal death after fimbria fornix transection. *Proc Natl Acad Sci USA* 1986;83:9231-5.
 - 96 Gage FH, Armstrong DM, Williams LR, Varon S. Morphological response of axotomised septal neurons to nerve growth factor. *J Comp Neurol* 1988;269:147-55.
 - 97 Montero CN, Hefti F. Rescue of lesioned septal cholinergic neurons by nerve growth factor: specificity and requirement for chronic treatment. *J Neurosci* 1988;8:2986-99.

- 98 Koliatsos VE, Applegate MD, Knusel B, *et al.* Recombinant human nerve growth factor prevents retrograde degeneration of axotomized basal forebrain cholinergic neurons in the rat. *Exp Neurol* 1991;112:161-73.
- 99 Kawaja MD, Gage FH. Reactive astrocytes are substrates for growth for adult CNS axons in the presence of elevated levels of nerve growth factor. *Neuron* 1991;7:1019-30.
- 100 Otto D, Frotscher M, Unisicker K. Basic fibroblast growth factor and nerve growth factor administered in gel foam rescue medial septal neurons after fimbria fornix transection. *J Neurosci Res* 1989;22:83-91.
- 101 Olson L, Norberg A, VonHolst H, *et al.* Nerve growth factor affects 11C-nicotine binding, blood flow, EEG, and verbal episodic memory in Alzheimer patients. *J Neural Transm* 1992;4:79-95.
- 102 Mathews WB, Compston A, Allen IV, Martyn CN. Immunological aspects of multiple sclerosis. In: *McAlpin's multiple sclerosis*. Edinburgh: Churchill Livingstone, 1991:332-5.
- 103 The IFN β Multiple Sclerosis Study Group. Interferon beta-1b is effective in relapsing-remitting multiple sclerosis. I. Clinical results of a multicenter, randomised, double-blind, placebo-controlled trial. *Neurology* 1993;43:655-61.
- 104 Arnason BGW. Interferon beta in multiple sclerosis. *Neurology* 1993;43:641-3.
- 105 Elias SB. Oligodendrocyte development and the natural history of multiple sclerosis. *Arch Neurol* 1987;44:1294-9.
- 106 Lassmann H. *Comparative neuropathology of chronic experimental allergic encephalomyelitis and multiple sclerosis*. In: Lassmann H, ed. *Schriftenreihe Neurologie*. New York: Springer Verlag, 1983:60-2.
- 107 Althaus HH, Kloppner S, Schmidt-Shultz T, Schwartz P. Nerve growth factor induces proliferation and enhances fiber regeneration in oligodendrocytes isolated from adult pig brain. *Neurosci Lett* 1992;135:219-23.
- 108 Raff MC, Miller RH, Noble M. A glial progenitor cell that develops in vitro into an astrocyte or an oligodendrocyte depending on the culture medium. *Nature* 1983;303:390-6.
- 109 Wolswijk G, Noble M. Identification of an adult-specific glial progenitor cell. *Development* 1989;105:387-400.
- 110 Noble M, Murray K, Stroobant P, Waterfield MD, Riddle P. Platelet-derived growth factor promotes division and motility and inhibits premature differentiation of the oligodendrocyte/type-2 astrocyte progenitor cell. *Nature* 1988;333:560-2.
- 111 Raff MC, Lillien LE, Richardson WD, Burne JF, Noble MD. Platelet-derived growth factor from astrocytes drives the clock that times oligodendrocyte development in culture. *Nature* 1988;333:562-5.
- 112 Bogler O, Wren D, Barnett SC, Land H, Noble M. Cooperation between two growth factors promotes extended self-renewal and inhibits differentiation of O-2A progenitor cells. *Proc Natl Acad Sci USA* 1990;87:6368-72.
- 113 McKinnon RD, Matsui T, Dubois-Dalcq M, Aaronson SA. FGF modulates the PDGF-driven pathway of oligodendrocyte development. *Neuron* 1990;5:603-14.
- 114 Mayer M, Bogler O, Noble M. The inhibition of oligodendrocytic differentiation of O-2A progenitors caused by basic fibroblast growth factor is overridden by astrocytes. *Glia* 1993;8:12-9.
- 115 Barres BA, Schmid R, Sendtner M, Raff MC. Multiple extracellular signals are required for long-term oligodendrocyte survival. *Development* 1993;118:283-95.
- 116 Mayer M, Bhakoo K, Noble M. Ciliary neurotrophic factor and leukemia inhibitory factor promote the generation, maturation and survival of oligodendrocytes in vitro. *Development* 1994;120:143-53.
- 117 Groves AK, Barnett SC, Franklin RJ, *et al.* Repair of demyelinating lesions by transplantation of purified O-2A progenitor cells. *Nature* 1993;362:641-3.
- 118 Metcalf D. Control of granulocytes and macrophages: molecular, cellular, and clinical aspects. *Science* 1991;254:529-33.
- 119 Reynolds B, Weiss S. Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science* 1992;255:1707-10.
- 120 Richards L, Kilpatrick T, Bartlett P. De novo generation of neuronal cells from the adult mouse brain. *Proc Natl Acad Sci USA* 1992;89:8591-5.