IGF-I Promotes Peripheral Nervous System Myelination

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ABSTRACT: Insulin-like growth factor-I (IGF-I) promotes the proliferation and differentiation of Schwann cells (SC). We use SC/dorsal root ganglion neuron (DRG) cocultures to examine the effects of IGF-I on the interaction between axons and SC. As SC extend processes toward the axon in the presence of IGF-I, these processes attach to and ensheath axons. Continued IGF-I exposure leads to enhanced P_0 expression and long-term myelination. No myelination occurs in the absence of IGF-I. These data imply that IGF-I is critical not only for SC attachment and ensheathment of axons but also for long-term myelination.

INTRODUCTION

Schwann cells (SC) are responsible for peripheral nervous system myelination. During development, SC precursors align and proliferate along extending axons, eventually ensheathing and myelinating axons.[1-3] As SC myelinate axons, they extend their cell processes and wrap around axons as part of a differentiation program. Differentiating SC express myelin proteins including P_0, myelin basic protein (MBP), myelin-associated glycoprotein (MAG), and PMP22. These proteins stabilize SC membranes and promote myelin sheath compaction.[3]

We are interested in the role of insulin-like growth factor-I (IGF-I) in the peripheral nervous system and find that IGF-I has prominent effects on SC. These biological actions of IGF-I are mediated by the type I IGF receptor (IGF-IR), a tyrosine kinase receptor. IGF-IR is important for the growth of several neural tissues,[4] and, like IGF-I, IGF-IR is expressed at the RNA and protein level during fetal development of the nervous system. Immunoreactive IGF-I and IGF-IR are present in SC and axons in developing nerves, reaching peak levels during periods of myelination.[5-7] In animal studies, there is a significant increase in IGF-I expression in injured peripheral nerves,[8,9] although SC may not be the only source of IGF-I during injury.[6] In the central nervous system, IGF-I is the only growth factor that promotes central nervous system myelinating cells (oligodendrocytes) to proliferate, differentiate, and myelinate.[10-12] In mice that are genetically altered to overexpress IGF-I, brain mass is increased because of enhanced myelin production by oligodendrocytes.[13] Disruption of IGF-I expression results in central nervous system hypomyelination and smaller overall brain size.[14]

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In this study, we report that IGF-I promotes the alignment of SC along axons, enhances the expression of myelinating genes, and facilitates long-term myelination. These results along with reports by other laboratories demonstrating IGF-I enhances nerve regeneration[5,6,15] suggest the therapeutic use of IGF-I in the treatment of demyelinating peripheral nervous system disorders.

IGF-I ENHANCES SC–AXONAL CONTACTS

In this study, IGF-I treatment of SC increases the likelihood that SC will contact axons. For these experiments, we use an *in vitro* model of developing peripheral nerve rat SC/dorsal root ganglion neuron (DRG) cocultures. Dissociated DRG neurons are plated at a density of approximately 20,000 neurons per dish for 5 days in a serum- and insulin-free defined medium (SIFDM) with 30 μM FUDR. Secondary SC are isolated from sciatic nerves of 3-day-old Sprague-Dawley rats and cultured in (Dulbecco's modified essential medium a.k.a DMEM) containing 10% fetal bovine serum (FBS), 2 μM forskolin, and 10 μg/ml bovine pituitary extract.[16] Cells are passaged when confluent and used for four passages. SC are prelabeled with a fluorescent dye (DiI). After washing with SIFDM, approximately 100,000 secondary SC are added to dissociated DRG axons. SC/DRG cocultures are maintained in SIFDM ± 10 nM IGF-I.

SC adhere to the culture dish by 6 hours (FIG. 1**A,B**) and are evenly distributed along the DRG axons (FIG. 1**C,D**, *arrows*). SC extend processes in the presence of IGF-I (FIG. 1**B**, *arrows*). By 12 hours, SC contact axons and align along axons (FIG. 2**B,D**, *arrows*). SC have fewer processes and are less likely to align along axons in the absence of IGF-I

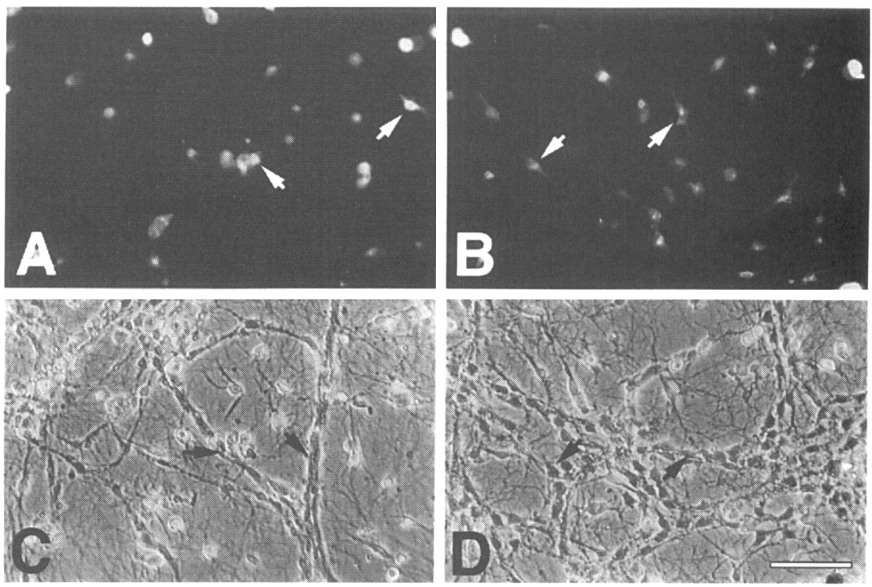

FIGURE 1. IGF-I enhances SC process extension in 6 hours. DiI-labeled SC were cocultured with DRG neurons in either SIFDM (**A,C**) or SIFDM with 10 nM IGF-I (**B,D**). (**A**) and (**B**) demonstrate SC morphology (*arrows*) using fluorescence microscopy. (**C**) and (**D**) are the corresponding phase-contrast photomicrographs of (**A**) and (**B**) respectively, showing localization of axons (*arrows*). Bar = 20 μM. Photomicrographs are representative of three independent experiments.

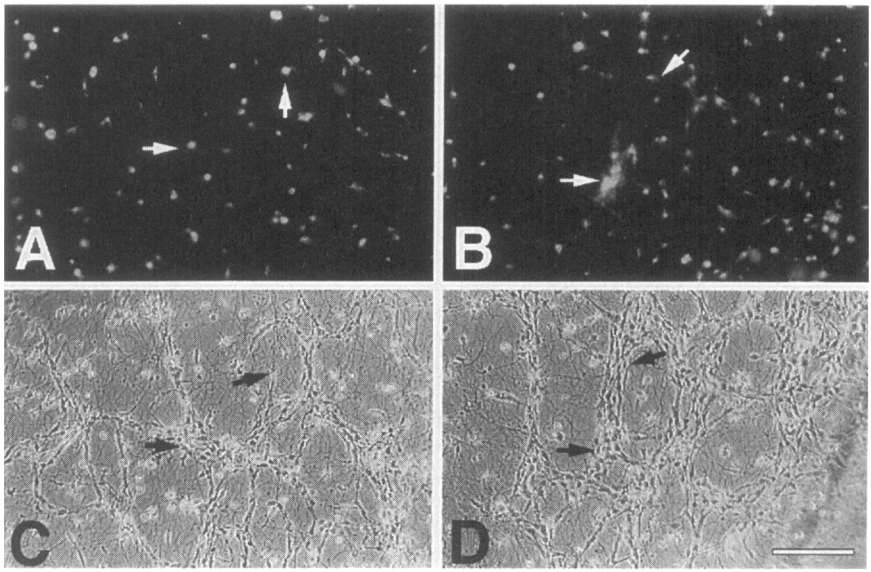

FIGURE 2. IGF-I enhances SC motility and the likelihood of SC contact witht DRG axons in 12 hours. DiI-labeled SC were cocultured with DRG neurons in either SIFDM (**A,C**) or SIFDM with 10 nM IGF-I (**B,D**). (**A**) and (**B**) demonstrate SC morphology (*arrows*) using fluorescence microscopy. (**C**) and (**D**) are the corresponding phase-contrast photomicrographs of (**A**) and (**B**), respectively, showing localization of axons (*arrows*). Photomicrographs are representative of three independent experiments. Bar = 40 μM.

(FIG. 2**A,C**, *arrows*). There is a twofold increase in the number of SC that have processes and align along axons with IGF-I treatment, and by 48 hours, most IGF-I-treated SC are aligned along axons.

IGF-I ENHANCES MYELIN PROTEIN EXPRESSION IN FORSKOLIN-TREATED SCHWANN CELLS

Differentiating SC express several myelin proteins including P_0, MAG, PMP-22, and MBP, adhesion molecules that facilitate myelin sheath compaction.[17] *In vitro*, forskolin mediates SC differentiation and induces myelin protein expression.[18-21] We examined the effects of IGF-I on the expression of myelin proteins in serum-deprived SC treated ± 1 μM forskolin (to mimic differentiating/nondifferentiating SC) and/or 10 nM IGF-I for 24 hours. Western blots for myelin proteins demonstrate P_0, MAG, and PMP22 levels were upregulated by forskolin treatment alone. IGF-I, by itself, has no effect on myelin protein expression, but enhances P_0, MAG, and PMP22 expression in the presence of forskolin (FIG. 3). These results implicate IGF-I as one of the factors important in allowing SC to attain a more differentiated phenotype. We see a similar effect of IGF-I treatment on P_0 expression in DRG/SC cocultures (data not shown). Our data are supported by developmental studies of Jessen and colleagues where IGF-I treatment permits pre-myelinating SC to convert to myelinating SC.[22-24]

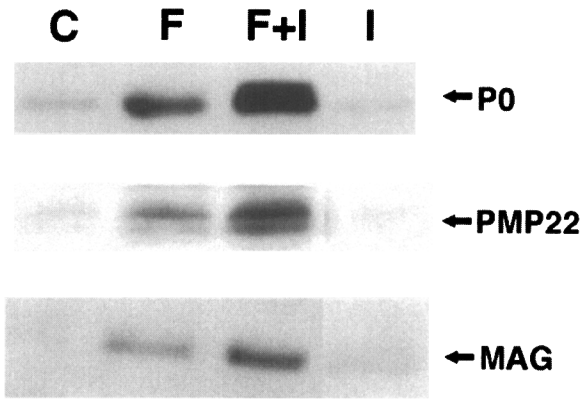

FIGURE 3. IGF-I enhances myelin protein in forskolin-treated SC. SC were treated with DM or DM ± 1 μM forskolin ± 10 nM IGF-I for 24 hours. Cell lysates were collected and processed for SDS-PAGE, followed by immunoblotting procedures for myelin proteins, including, P_0, MAG, and PMP22. IGF-I administration enhances myelin protein expression in the presence of forskolin. Results are representative of three independent experiments.

IGF-I ENHANCES MYELINATION

There are convincing reports that IGF-I enhances myelination in the central nervous system (CNS). IGF-I promotes oligodendrocyte myelination of CNS axons in organotypic cultures.[25] Transgenic mice that overexpress IGF-I have large, hypermyelinated brains[13] whereas mice that overexpress a binding protein that blocks IGF-I action have few CNS myelinated axons with thin myelin sheaths.[26] Targeted disruption of the IGF-I gene results in a similar poorly myelinated CNS phenotype, with hypomyelination and a decrease in white matter tracts.[14] We find that IGF-I has similar effects on PNS myelination.

In these experiments, SC/DRG cocultures are grown in SIFDM ± IGF-I for 21 days and prepared for transmision electron microscopy (TEM) to fully evaluate myelination. Under SIFDM conditions, we find no myelinated axons (FIG. 4**A**). When cocultures are maintained in SIFDM alone, most SCs retain an undifferentiated form, and there is little evidence of attachment of SC on axons or SC ensheathment of axons. On gross appearance there is little difference in the unmyelinated axons in SIFDM alone (control) when compared to unmyelinated axons in the presence of IGF-I. IGF-I promotes the formation of normal myelin (FIG. 4**B**). There is normal periodicity of the myelin and major dense lines between the loops of myelin (FIG. 4**B**).

SUMMARY

In summary, we find that IGF-I downstream signaling increases the likelihood that SC will contact axons. Upon contact, SC align and ensheath axons in the presence of IGF-I. Ensheathment is followed by enhanced P_0 expression and, after 21 days of continuous

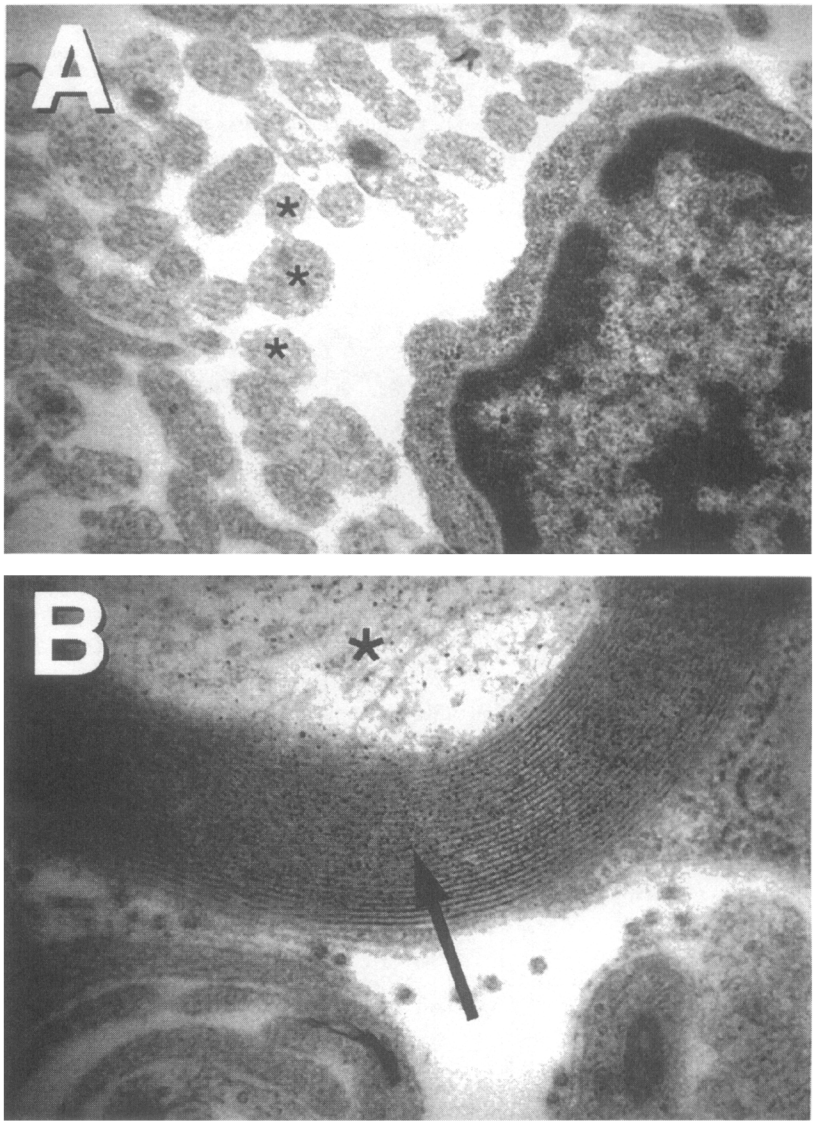

FIGURE 4. IGF-I promotes Schwann cell myelination of dorsal root ganglion axons. Dissociated E15 rat dorsal root ganglion neurons were treated to remove endogenous SCs and then were allowed to myelinate for 21 days in SIFMM with 10 ng/ml of NGF in the presence of added secondary rat SCs. Shown are transmission electron micrographs of cocultures containing (**A**) no addition or (**B**) 10 nM IGF-I. Axons (*) in the absence of IGF-I remain unmyelinated, whereas addition of IGF-I causes abundant myelination of axons with distinct myelin lamellae and clear major dense lines (*arrow*). Original magnifications 57,000× in A and 100,000× in B; reduced here by 10%. (From Leventhal *et al.*[27]; used with permission from Humana Press, Inc., Totowa, NJ.)

IGF-I exposure, long-term myelination. Collectively, these results suggest IGF-I may prove useful in the treatment of peripheral nervous system demyelinating disorders.

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