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# Stimulation of nerve myelinating cell differentiation and potential for treatment of neurodegenerative diseases

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### Multiple Sclerosis and other demyelinating neurodegenerative diseases

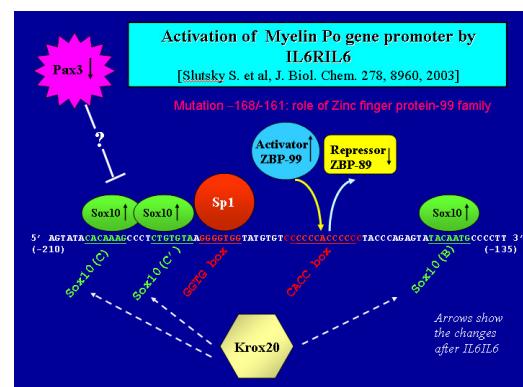
Myelin sheaths around axons are not only necessary for neural conduction but protect axonal integrity. In demyelinating diseases, such as Relapsing Remitting Multiple Sclerosis (MS), there is at first active remyelination (accounting for remissions) but when myelin loss exceeds repair there is progressive neuron death and brain atrophy. Current MS therapies use biological agents that slow down the autoimmune process destroying myelin. Our research in the 80s led to development of recombinant Interferon-beta (Rebif) produced in Israel by InterPharm within the Serono Group. Rebif and other IFN- $\beta$  are most widely used today to reduce progression of neurological disability and frequency of relapses in MS, another immunoregulatory drug being Copaxone (Teva). In the future, agents stimulating remyelination may offer new ways to prevent progression of the neurodegenerative disease. Such agents may be active also in Peripheral Neuropathies, due to diabetes or chemotherapy, which show early loss of myelin.

Myelin is made by membranes of oligodendrocytes [oligs] in the brain and spinal cord, and of Schwann cells [SC] in peripheral nerves. During mammalian embryonic development, the expression of myelin protein genes marks the last step of oligs and SC differentiation, occurring around birth and shortly after.

### Myelinating Schwann cell differentiation: effect of Interleukin-6 type cytokine

Dorsal root ganglia (DRG) from rat or mouse E14 embryo contain immature SC precursors that do not yet express myelin genes. We found that a recombinant molecule IL6RIL6, fusing Interleukin-6 (IL-6) to its soluble receptor (sIL-6R), promotes in

3-6 days the differentiation of myelinating SC, as denoted by induction of mRNAs for myelin protein zero (Po/MZP), myelin basic protein (MBP) and peripheral myelin protein (PMP22). Transcription factors which are high in embryonic SC and adult non-myelinating SC, such as Pax3, Oct6/SCIP and Egr1/Krox24, were down-regulated by IL6RIL6. Conversely, Egr2/Krox20 which characterizes the myelinating SC was increased. IL6RIL6 also enhanced neurogenesis in these DRG cultures.



**Fig. 1** Myelin Po gene promoter sequence involved in transcriptional induction by IL6RIL6. The arrows indicate the changes in transcription factors produced by the gp130 activator.

Transition from non-myelinating to myelinating SC is essential for myelin repair after nerve injury. After sciatic nerve section in rats, we found that IL6RIL6 i.p. administration increases 7-fold the number of myelinated nerve fibers present after 12 days at 5mm of the sutured nerve. Both IL6RIL6 and IL-6 alone were found active in rat models of diabetic and chemotherapy-induced neuropathies (work done in the Serono group). IL-6 injections had preventive and curative effects, with preservation of myelin sheaths

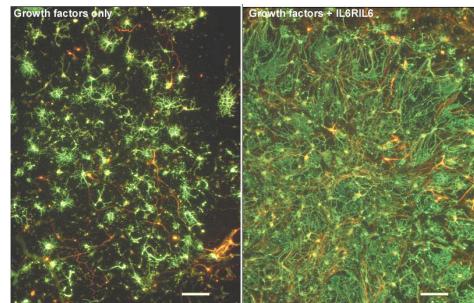
as well as motor and sensory nerve conduction. IL-6 (Atexakin-alpha, Serono) is presently in Phase II clinical trials for human neuropathies.

### Molecular mechanisms involved in the activation of a myelin gene

Po/MZP represents about 50% of the PNS myelin proteins. Activation of the Po gene promoter in response to IL6RIL6 could be demonstrated in SC-like cell lines, including murine melanoma B16/F10.9 cells that IL6RIL6 causes to transdifferentiate from a melanocytic to a SC phenotype. We identified the promoter sequence responding to IL6RIL6 and found that transcription factor ZBP99 and Sox10 act as positive regulators required for the induction by IL6RIL6 (Fig. 1). Silencing of the ZBP99 gene blocked Po induction by IL6RIL6, and interaction between ZBP99 and Sox10 was found by two-hybrid system. Krox20 also synergizes with Sox10, whereas Pax3 represses, in line with increase of Krox20 and decrease of Pax3 during myelinating SC maturation. Moreover, we found that transcription factor C\EBP- $\delta$  is consistently induced by IL6RIL6 and required for induction of myelin Po and MBP RNA accumulation in the cells. Overexpression of C\EBP- $\delta$  led by itself to Po and MBP mRNA accumulation.

### Enhancement of oligodendrocyte formation from Embryo Stem (ES) cells

Pluripotent ES cell lines derived from blastocysts inner cell mass are a source of neural stem cells (NSCs), precursors of neurons, astrocytes and oligs. With FGF-2, NSCs propagate as floating neurospheres and differentiate upon adherence to polycationic substrates. Addition of IL6RIL6 at this stage markedly promotes the development of oligs, which become much larger, with more arborization and longer branches contacting the axons. The effect of IL6RIL6 was seen in addition to that of growth factors EGF or PDGF (Fig. 2). Increased expression of CNS myelin genes MBP and CNPase, as well as lineage specific transcription factor Olig-1, was observed in response to IL6RIL6. The ES cell culture technology holds promises for transplantation into the brain and spinal cord to promote *in situ* remyelination, and we investigate the use of IL6RIL6 or IL-6 to promote the maturation of myelinating cells from transplantable NSCs.



**Fig. 2** Enhanced differentiation of oligodendrocytes from murine ES cells by IL6RIL6. Neurosphere cells were dissociated, plated on polyornithine and immunostained for oligodendrocytes (O4, green) and axons ( $\beta$ III-tubulin brown). Left: FGF-2, EGF, PDGF. Right: same + IL6RIL6.

### Selected Publications

Haggiag, S., Chebath, J. and Revel, M. (1999) Induction of myelin gene expression in Schwann cell cultures by an Interleukin-6 receptor – Interleukin-6 chimera. *FEBS Letters*, 457, 200-204.

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