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Demyelinating diseases and remyelination: Actions of interferon-β and of an interleukin-6 chimera

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Interferon-beta in the therapy of Multiple Sclerosis

Multiple Sclerosis (MS) is one of the more common human diseases resulting from nerve demyelination. In the brain white matter and spinal cord, an autoimmune process attacks the cells responsible for myelin synthesis (oligodendrocytes) and digests away the myelin sheath around the nerves. The ensuing impairment of nerve conductivity as well as atrophy of the nerves are at the basis of the neurological disabilities experienced by the patients. The current therapy of MS aims primarily at preventing progression of disability and achieving prolonged relapse-free remission states. In extensive clinical trials, the sustained administration of Interferon-beta (IFN-β) has proven to be an efficient mean to achieve these therapeutic goals. In particular, the human IFN-β produced by genetically engineered hamster CHO cells – a process originally developed in our laboratory and now industrially applied, among other in the Israeli biotechnology company InterPharm for the Serono pharmaceutical group – has allowed high dose and three times weekly injections, giving higher efficacy than other regimen. The identical structure of the recombinant protein and of the natural IFN-β in the human body, minimizes side-effects. There are today over 200,000 patients worldwide under treatment with IFN-β for relapsing-remitting MS. Moreover, clinical trials in which treatment is started as soon as suspected MS is diagnosed (based on Magnetic Resonance Imaging at the time of first symptoms) show promise to prevent the development of irreversible neurological disability, and therefore of clinically definite MS.

The mechanism of action of IFN-β in MS is through a decrease in the activation of immune cells (macrophages and lymphocytes) as a result of the down regulation of pro-inflammatory cytokines such as IFN-gamma and Tumor necrosis factor, and increase in suppressory cytokines such as Interleukins-4 and –10. The major drugs used today in MS, IFN-β and Copaxone, provide means to decrease the auto-immune process and thereby reduce the destruction of myelin and axons. However, a future goal remains to be able to repair the nerve damage by stimulating the regeneration and remyelination of nerves, which is being lost during the progression of the disease.

Interleukin-6 cytokine family in the differentiation of neurons and myelinating cells

Cytokines of the Interleukin-6 (IL-6) family, which share a common receptor chain gp130, have neurotrophic activity. CNTF and LIF increase for example the survival of neurons in culture. Moreover, inactivation of the gp130 gene in newborn mice leads to loss of myelin. We have created a recombinant protein designated IL6RIL6, which binds with a high affinity to gp130 and acts as a potent activator of this receptor intracellular signaling. We study the effects of IL6RIL6 on neuroglial differentiation in cultures of embryonic cells obtained from the dorsal root ganglia (DRG). At day 14 of the embryonic development of the rat, the DRG are still rich in early precursor cells that will give rise to peripheral neurons and glial cells among which the myelinating Schwann cells (SC). Precursor cells can be enriched by FACS for expression of the early cell marker p75NGF-receptor, in order to examine their differentiation during in vitro cultures. Addition of IL6RIL6 to these cultures produces a striking stimulation of neurogenesis, with proliferation of neural cell bodies and formation of dense axonal fascicular bundles. Moreover, there are much more elongated glial cells that align and bind along the axons, acquiring markers of myelinating cells (such as transcription factor Krox-20). IL6RIL6 induces the transcription products of myelin genes, such as myelin basic protein and

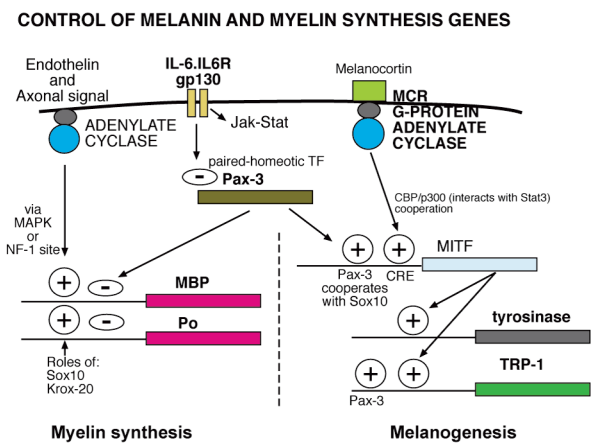

Fig. 1 *Signaling through the IL-6 family gp130 receptor causes down-regulation of Pax-3 and of the melanogenesis pathway, while activating the expression of myelin genes.*

Po (representing 50% of the total proteins of peripheral nerve myelin). In brain slices or cortical cell cultures treated with IL6RIL6, neuroprotective effects and differentiation of myelinating oligodendrocyte with increase in MBP gene expression have also been observed.

Among the many genes that regulate the differentiation of neural crest cells into myelinating SC, the paired-homeodomain Pax-3 gene plays a particularly crucial role. Pax3 is expressed in the differentiating SC but has to disappear before the SC mature into nerve myelinating cells. In the adult, Pax-3 continues to be expressed only in melanocytes, the pigmented epidermal cells originating also from neural crest stem cells, and in non-myelinating SC. Interestingly, following nerve traumatism, myelin is degraded and myelinating SC disappear to be replaced by Pax-3 expressing non-myelinating SC. These new SC proliferate and must undergo again terminal maturation to myelin-synthesizing cells characterized by repression of Pax-3. Hence, Pax-3 is one of the gene markers which must be repressed in order for myelin synthesis to be switched on.

Our studies have identified Pax-3 as a major target of IL6RIL6 action through the gp130 receptor. In embryonic DRG cell cultures, addition of IL6RIL6 causes a quasi-complete disappearance of Pax-3 correlating with induction of the myelin genes Po and MBP. From DRG, we isolated clones of glial cells whose growth and differentiation is stimulated by IL6RIL6 (therefore, called CH cells). In co-cultures of CH cells with neurons, IL6RIL6 induces the synthesis of myelin Po and MBP, and causes the disappearance of Pax-3. The CH cells are still multipotent embryonic precursors, which can be differentiated into myelinating SC by IL6RIL6 or else into smooth muscle cells by Activin-β A, a member of the TGF-β family.

Transdifferentiation of melanoma into Schwann cells

We have discovered that a murine metastatic melanoma, B16/F10.9, responds to IL6RIL6 by growth arrest and transdifferentiation from a melanocytic to a myelinating Schwann cell phenotype. Melanoma tumor cells have, like normal melanocytes, an active synthesis of melanin pigments and as a rule express high levels of Pax-3. The Pax-3 protein is a survival factor for melanoma and also serves as a required transcription factor for the expression of the microphtalmia associated transcription factor (MITF), a key gene in melanogenesis. MITF, in turn, controls the expression of the tyrosinase gene, which encodes the rate-limiting enzyme for conversion of tyrosine into the polymeric melanin pigments (Fig. 1). Treatment by IL6RIL6 causes a down-regulation of Pax-3 mRNA and protein thereby inactivating the MITF gene promoter and inhibiting tyrosinase and melanogenesis. At the same time, there is an activation of the promoters of the myelin Po and MBP genes, as well as synthesis of other myelin components such as CNPase. If Pax-3 is expressed in these cells by an ectopic expression plasmid, IL6RIL6 does not decrease the MITF gene activity and the induction of Po and MBP genes is impaired, showing that down-regulation of Pax3 by IL6RIL6 is crucial for the transdifferentiation and the switch-on of myelin genes (Fig.1).

Pax-3 synergizes with Sox-10 to activate the MITF gene promoter, an effect which is lost after treatment by IL6RIL6. However, unlike Pax-3, Sox-10 is actually increased after IL6RIL6. The switch-on of the myelin genes by IL6RIL6 appears to correlated with this increase in the ratio of Sox-10 over Pax-3. Our analyses of the promoter of the myelin Po gene shows that it is stimulated synergistically by Sox-10 and Krox-20 (myelinating SC specific transcription factor), but inhibited by Pax-3. By further studying this cellular transdifferentiation system, we hope to elucidate how myelin gene expression is controlled and could be triggered when needed, such as in demyelinating diseases.

In vivo efficacy of IL6RIL6 to stimulate myelination during nerve regeneration

Experiments were done in rats that had been subjected to surgical transection and resuture of the sciatic nerve. This causes a Wallerian degeneration of the axons and loss of their myelin sheaths. After 12 days, the number of re-myelinated fibers was quantified by electron microscopy of the sciatic nerve at 5 mm below the suture. A group of rats that had received injections of IL6RIL6 showed up to 7-fold increase in the number of myelinated axons and increase in the thickness of the myelin sheath. Applications in neuropathies are being studied.

Selected Publications

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