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Induction of myelin gene expression in Schwann cell cultures by an interleukin-6 receptor-interleukin-6 chimera

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Abstract Expression of myelin basic protein (MBP) and Po gene products is induced during the final postnatal maturation of Schwann cells and reinduced during nerve regeneration. We show that a chimeric protein containing interleukin-6 fused to its soluble receptor (IL6RIL6 chimera) induces MBP and Po RNAs and proteins in cultures of dorsal root ganglia (DRG) from 14 day old mouse embryos. Activation of gp130 signaling by IL6RIL6 appears comparable to cyclic AMP elevating agents to induce the myelin gene products in DRG and in pure Schwann cell cultures.

Key words: Myelination; Po protein; Myelin basic protein; Schwann cell; Interleukin-6; Interleukin-6 receptor

1. Introduction

Neuroprotective effects of interleukin-6 (IL-6) have been observed in cultures of cholinergic and catecholaminergic brain neurons (see [1,2] for review). Sympathetic neurons from the superior cervical ganglia [3] and sensory neurons of embryonic dorsal root ganglia (DRG) [4] respond by increased survival and neurite outgrowth when IL-6 is provided in combination with soluble IL-6 receptor (sIL-6R). This combination is needed, because in many cell types having or lacking membranal IL-6R, exogenous sIL-6R acts as an agonist of IL-6 by promoting the dimerization of gp130 which carries out the signal transduction [5–7]. For various neurons, the IL-6 and sIL-6R combination has an effect similar [3,4] to that of other neuropoietic cytokines such as CNTF and LIF signaling through gp130 in conjunction with the LIF receptor [8–10].

Forms of sIL-6R are present in body fluids [6,11] and act as IL-6 agonists also in vivo. In particular, transgenic mice overexpressing IL-6 and sIL-6R exhibit accelerated regeneration of axotomized hypoglossal nerve [4]. In wild-type mice, IL-6 is upregulated in the Schwann cells (SC) distal to the nerve lesion as well as in the neuronal bodies of the hypoglossal nuclei which also produce more IL-6R [4]. SC in contact with neurons produce IL-6 and there is a further increase of IL-6 within 12 h after sciatic nerve crush, highest distally to the site of injury [12]. IL-6 and IL-6R mRNA expressions increase in DRG during the postnatal period of myelination, mainly in neurons but also in SC for IL-6R mRNA [13]. Unlike IL-6, the CNTF levels in SC fall following axotomy, reincreasing only when the nerves regenerate [14,15]. While it is generally assumed that these cytokine changes contribute mainly to neuron survival [2–4,8], we were interested in their role in myelination. Following nerve lesions, myelinating SC (mSC) dedifferentiate to non-myelinating SC (nmSC) that express glial fibrillary acidic protein (GFAP) like embryonic SC (eSC), and proliferate as an essential part of nerve regeneration [16–19]. Remyelination requires differentiation of nmSC/eSC to mSC expressing myelin genes, this being a reversible axon-dependent process analogous to the postnatal maturation of eSC into mSC [17,18]. One agent able to induce peripheral nerve myelin gene products such as Po and myelin basic protein (MBP) in isolated SC is forskolin, which increases intracellular cAMP [16,18]. CNTF administered in vivo potentiates sciatic nerve regeneration and remyelination [20], but this may be secondary to neuroprotective effects since in vitro effects on myelin synthesis were not shown.

We examined the effect of IL-6 type signaling on myelin genes expression in embryo DRG cultures, in which Hirota et al. [4] showed increased neurite extensions by IL-6 and sIL-6R. The IL-6 type signal can be most efficiently delivered by recombinant proteins linking sIL-6R to IL-6 [3,21,22]. We used here a mammalian cell-produced IL6RIL6 chimera where human sIL-6R is directly fused to IL-6 [22] and which has a higher affinity for gp130 than IL-6 with sIL-6R [23]. This molecule enhanced axon sprouting from DRG as did nerve growth factor (NGF) but, in addition, IL6RIL6 induced expression of the MBP and Po gene products in SC.

2. Materials and methods

DRG explants were prepared [24] from C57Bl6/Sv129 F1 mice embryos at day E14 and cultured on glass coverslips coated with rat tail type VII collagen, or with poly-L-lysine, MW 30–70 000 (both from Sigma, St. Louis, MO, USA). The coverslips were placed in Nunc 12 well plates in 0.5 ml DMEM/F12 (1/1) with 10% fetal calf serum (FCS) and 2% horse serum, at 37°C in 5% CO_2. Additions were either 1.5 μg/ml rhIL6RIL6 chimera, produced in CHO cells as an 85 kDa glycoprotein [22,25], or 1 μg/ml rhIL-6 also from CHO cells [22], or mouse NGF 7S from submaxillary glands (Sigma) at 50–100 ng/ml, or forskolin 20 μM (Sigma). DRG were similarly cultured from Lewis rat embryos or newborns, but with a change on day 3 to MEM with 15% FCS and 50 μg/ml ascorbic acid to enhance myelination [26]. An SC line, cloned from primary cultures of newborn mouse sciatic nerve cells transfected by mutant p53 and neomycin resistance genes, and verified to align on demyelinated neurons in vitro, was kindly provided by Dr. Bernard Attali, Neurobiology Department, Weizmann Institute of Science.

DRG cultures were fixed in 2% paraformaldehyde, 0.1% glutaraldehyde, 0.1% tannic acid in PBS for 1 h at 20°C and treated with 0.1% Triton X-100, 10% goat serum for 2 h before immunostaining. Anti-MBP monoclonal antibody (MAB 386, Chemicon, Temecula, CA, USA) or anti-Po MAB (gift of Dr. Kitamura [27]) were added in 1% goat serum for 1 h at 37°C, followed by goat anti-mouse IgG

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(L+H)-Alexa 488 fluorescent dye conjugate (Molecular Probes, Eugene, OR, USA) for 1 h. Mowiol (Calbiochem) mounted slides were photographed (Olympus IX-70, Tokyo, Japan). Osmicated (0.1% OsO_4) Sudan black B stained preparations were observed as described [24,28].

Total cell RNA was extracted from pools of 5–10 DRG 8 day cultures by guanidinium thiocyanate-phenol-chloroform with the Tri-Reagent kit (Molecular Research Center, Cincinnati, OH, USA) and precipitated in isoamyl alcohol. Reverse transcriptase (SuperscriptII, Gibco-BRL) reactions were done in 20 μl with samples of 0.4 μg RNA, 0.5 μg oligo-dT primers (Promega) and 1–3 μl of the reaction used to amplify the cDNAs fragments with Dynazyme Taq polymerase (Finnzymes, Finland). Mixes with $MgCl_2$ contained 0.3 μM oligo nucleotide primers, 200 μM dNTPs and cycles were 30 s at 94°C, 45 s at 58°C, 1 min at 75°C. After 25–30 cycles (linear detection), the products were extended by 5 min incubation at 72°C. For murine MBP RNA, the primers were: forward, 5′-CACGGGCATCCTTGACTCCA and reverse, 5′-GAGATCCAGAGCGGCTGTCT. The major bands amplified were 345 bp and 240 bp long, corresponding to splicing forms of MBP and Golli-A transcripts [29]. For murine Po mRNA, the primers were: forward, 5′-GCTCTTCTCTTCTTTGGTGCTGTCT and reverse, 5′-GGCGTCTGCCGCCCTCGCTTCG, amplifying a 653 bp fragment [30]. Polymerase chain reaction (PCR) with G3PDH commercial primers (Clontech, Palo Alto, CA, USA) was used as control in each reaction. The intensities of the 345 bp MBP fragment and 653 bp Po fragments were quantified by the AlphaEase program (Alpha Innotech, San Leandro, CA, USA). The values were normalized for the G3PDH band using the same reverse transcription (RT) reaction.

3. Results

DRG of mouse E14 embryos were cultured on collagen coated coverslips and examined in phase contrast. Addition of IL6RIL6 induced the sprouting of axons from the DRG, which was visible already after 1 day (Fig. 1B). The number of axons was strikingly higher than without IL6RIL6 (Fig. 1A). Treatment of the DRG by NGF also increased the axonal growth markedly (Fig. 1C). However, when compared to the NGF treatment, the DRG cultures that had been treated by IL6RIL6 had a different morphology. There were more elongated cells adjacent to the axons giving a thicker appearance to the neural extensions (Fig. 1B). Treatment by IL6-RIL6 modified also the morphology of more mature rat E19 DRG that were cultured for 14 days, with addition of ascorbic acid, and then stained with Sudan black to examine myelin sheaths. With IL6RIL6 (Fig. 1D), the cultures contained stained smooth nerve fibers with the typical increased diameter and stiffness, as described by Bunge et al. [28] for myelinating nerve fibers in DRG. In contrast, NGF treated DRG (Fig. 1E) showed irregularly shaped thinner axon fibers, with poor Sudan black staining and more closely spaced SC, which are characteristics of the premyelinating phase [28].

These observations suggested that IL6RIL6 not only sus-

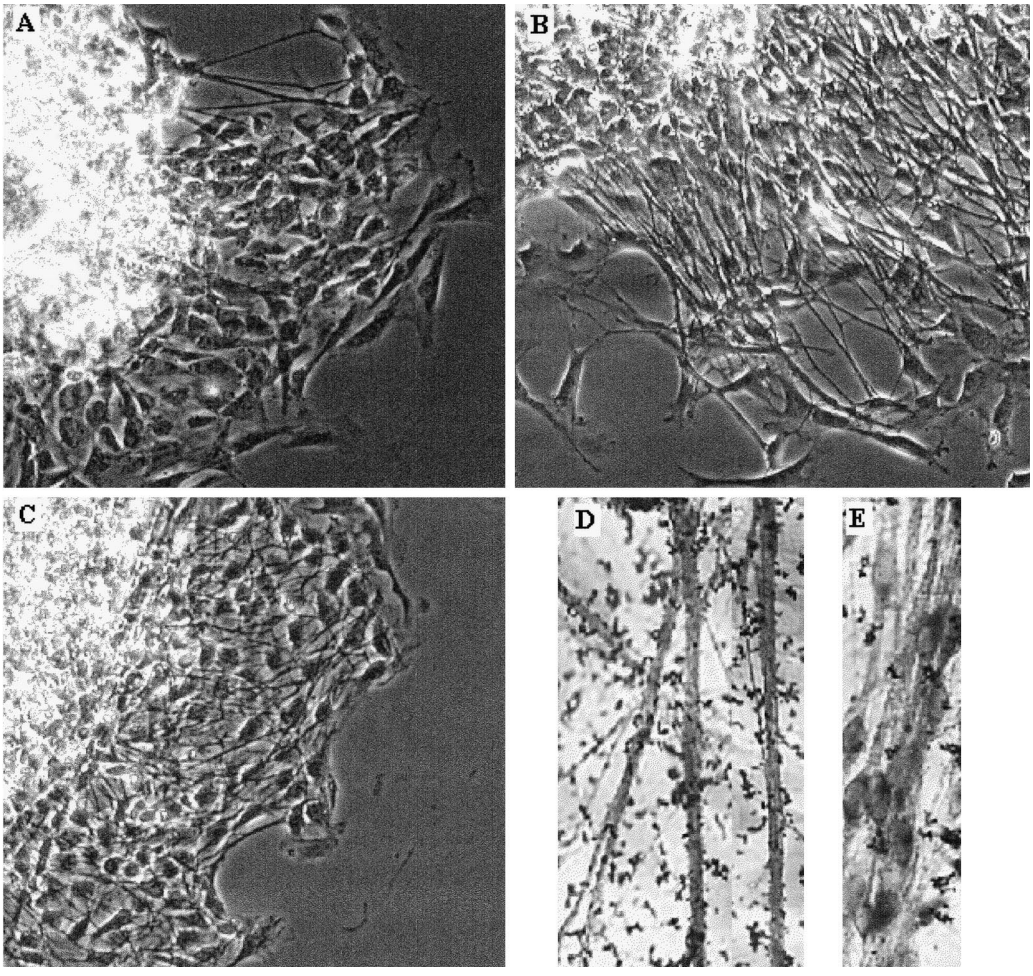

Fig. 1. Morphology of DRG cultures. A–C: Mouse E14 DRG on collagen after 1 day, phase contrast, magnification ×100. A: non-treated, B: with IL6RIL6, C: with NGF. D,E: Rat E19 DRG on poly-lysine for 14 days with ascorbic acid added at day 3, Sudan black stain, bright-field, magnification ×400. D: with IL6RIL6, E: with NGF.

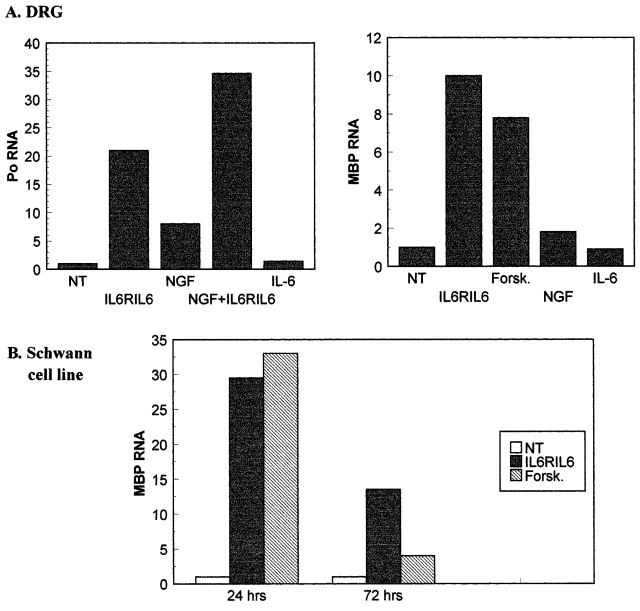

Fig. 2. Myelin Po and MBP gene transcripts. A: Mouse E14 DRG on poly-lysine after 8 days. B: Culture of isolated mouse SC line. The intensity of the Po and MBP RT-PCR products was normalized for the G3PDH bands. The fold increase over the non-treated culture value (taken as 1) is shown for each condition. Forsk: forskolin.

tains axon growth from the DRG explants but also affects development of the SC progenitors and their capacity to produce myelin components. Indeed, RNA extracted from mouse E14 DRG after 8 days of culture with IL6RIL6 showed an over 20-fold increase in transcripts from the major peripheral nerve myelin protein gene Po when analyzed by RT-PCR (Fig. 2A). In comparison, IL-6 alone had little effect. NGF-treated DRG also showed some increase in Po RNA over untreated cultures, but addition of IL6RIL6 together with NGF strongly increased the levels of Po RNAs as comparison to NGF alone (Fig. 2A). The IL6RIL6 treatment also increased the MBP RNA levels in the mouse E14 DRG cultures (Fig. 2A, right panel). The activity of IL6RIL6 was comparable to that of a cAMP elevating agent, such as forskolin, known to induce MBP gene transcripts in SC [16]. In contrast, treatment of the DRG cultures by NGF had little effect on MBP RNA and IL-6 alone had no effect (Fig. 2A).

Since myelination is a process that depends on contact of SC with axons [16], the induction of MBP RNA in the IL6-RIL6-treated DRG cultures may result from an effect on axon development. In order to examine the ability of IL6RIL6 to induce expression of the MBP gene in pure cultures of SC, we used a line of immortalized SC that had been isolated from sciatic nerve of newborn mice. Treatment of these cells by IL6RIL6 induced MBP gene transcripts within 24 h as measured by normalized RT-PCR (Fig. 2B). The activity of IL6-RIL6 was comparable to or higher than that of forskolin at 24 and 72 h (Fig. 2B).

Immunostaining with antibodies to myelin basic protein identified myelinating SC in the DRG cultures. IL6RIL6 treated mouse E14 DRG cultures (at 9 days) showed many cells positive for MBP, whereas such cells were not apparent in the NGF treated cultures (Fig. 3A–D), nor in the untreated cultures (not shown). With IL6RIL6, the MBP-positive cells were elongated and formed aligned patterns as typical of differentiated SC. This effect of IL6RIL6 on increasing the number of non-neuronal cells with elongated processes was consistently observed throughout the study. Immunostaining also confirmed the induction of the Po protein in IL6RIL6 treated DRG as comparison to non-treated or to NGF treated cultures. A marked increase in cells positive for the Po protein was produced by IL6RIL6 in both E14 DRG (not shown), and newborn rat DRG cultured 8 days with addition of ascorbic acid (Fig. 3E), as comparison to the same cultures with NGF (Fig. 3F). By phase contrast, the NGF cultures showed many SC attached to axons but negative for Po (not shown). Hence, the IL6RIL6 recombinant cytokine acts as an inducer of myelin gene products for SC, in addition to its effect on the neuronal cells.

4. Discussion

In the mouse embryo at days E12.5–E16, the neural crest derived precursors develop into committed embryonic eSC, positive for the S100 protein, but the MBP and Po gene products are late markers that are strongly induced only when the eSC mature into myelinating mSC after birth [17,18,31]. These late myelination markers are not formed by SC from Krox-20 deficient mice, which express S100 and early myelination markers [32]. In this study, we show that mouse E14 DRG cultures were induced to express Po and MBP RNA and proteins when treated with the IL6RIL6 chimera. Unlike the transition from precursors to eSC, the maturation of eSC to a myelinating phenotype is reversible as seen after nerve lesions, and is stimulated by interaction of SC with axons [16–18]. Whereas the IL-6 type signal from IL6RIL6 did promote neurite outgrowth from DRG explants, in line with Hirota et al. [4], the stimulation of axonal growth by NGF did not cause the marked induction of the Po and MBP gene products obtained with IL6RIL6. Furthermore, IL6RIL6 was also active to rapidly induce MBP gene transcripts in cultures of isolated SC. Although these SC were an immortalized line, this result shows some direct effect of the cytokine on SC cultured without neurons. The levels of MBP RNA reached were similar to those produced by using forskolin to increase cAMP levels, a treatment which palliates the absence of neurons [16]. Elevation of cAMP was reported to induce MBP gene expression in a SC line through a MAP kinase dependent pathway [33]. Signaling through gp130 activates in various cells the JAK/Stat pathway as well as the MAP kinase and PI 3-kinase pathways [34]. However, the mechanism by which IL6RIL6 induces MBP and Po in SC remains to be determined. We recently found that IL6RIL6 downregulates Pax-3 and induces MBP and other myelin proteins in transdifferentiating melanoma cells [25]. Pax-3 is a repressor of MBP gene expression [35], but there is no evidence that IL6RIL6 acts in this way on SC.

In the mouse E14 DRG cultures, IL6RIL6 increased the number of non-neuronal cells with elongated cell processes typical of the SC phenotype. With more mature newborn rat DRG, IL6RIL6 promoted the formation of Sudan black stained nerve fibers with the characteristics of myelinating fibers [28]. We have observed this in cultures with or without ascorbic acid, which helps lamina formation, Po synthesis and myelination [26]. These morphological changes support the notion that IL6RIL6 acts as a maturation factor for mSC. Other mechanisms cannot be excluded since constitutive ex-

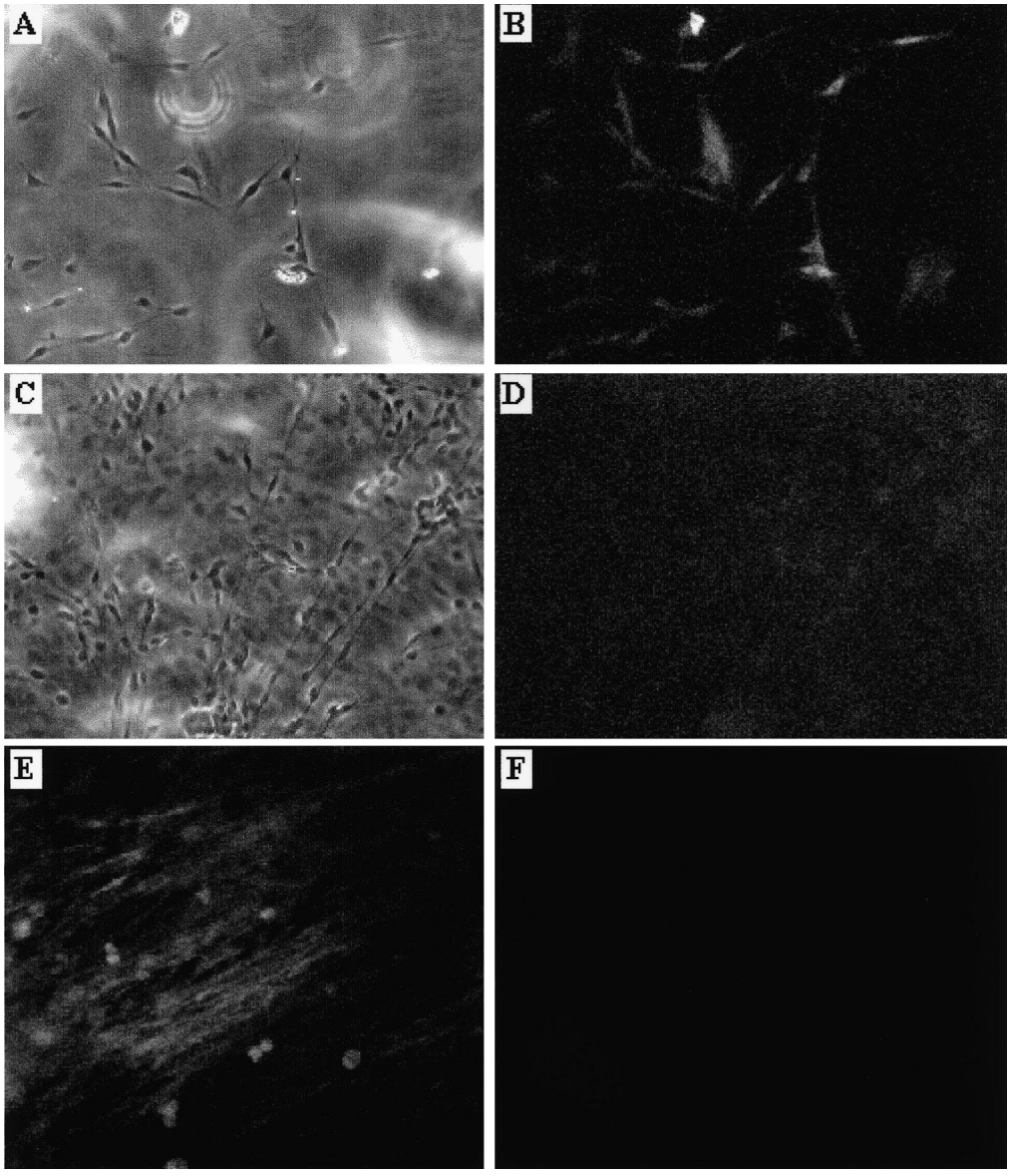

Fig. 3. Immunofluorescence for MBP and Po proteins. A–D: Mouse E14 DRG on collagen after 9 days stained with anti-MBP. E–F: Rat newborn DRG on poly-lysine for 7 days with ascorbic acid added at day 3, stained with anti-Po. A,B,E: with IL6RIL6, C,D,F: with NGF. Magnification ×200. A,C: Phase contrast; B,D,E,F: Ultraviolet light.

pression of Po and MBP was reported in rat postnatal SC cultures without serum [36] and it was suggested that inhibitors account for the absence of myelin gene products in early DRG. The effect of IL6RIL6 may then be to overcome this inhibition. However, this constitutive expression is a low basal level seen also in neural crest cells and eSC [37]. It is clear that the Po and MBP expression observed here is not a constitutive basal level since IL6RIL6 was an as efficient inducer as forskolin, which is a recognized mSC maturation stimulus [16,17,37,38]. Another possibility is that IL6RIL6 acts as a SC growth factor. The main growth and survival factors for precursors and eSC are the β-neuregulins/glial growth factors (NRGβ/NDFβ-2/GGF), working with fibroblast growth factor-2 (FGF-2) [39]. Neuregulins inhibit Po and MBP expression [36], and so does FGF-2 [40], in contrast to the IL6RIL6 stimulation demonstrated here. Other growth factors such as insulin-like growth factors IGF-I, and neurotrophins such as NT-3, BDNF and NGF, have also been reported not to induce late myelin markers unless forskolin is added [17]. IL6-RIL6 may stimulate SC maturation but also have a growth effect, since CNTF and LIF have indirect growth effects on SC through induction of the SC mitogen Reg-2 in neurons [19]. The mSC maturation functions of CNTF and LIF are still unclear. In DRG cultures, CNTF and LIF synergize with bFGF to induce Krox-20, but this transcription factor is still an eSC marker that is also induced by NDFβ-2 [41]. In cultures of oligodendrocyte-astrocyte progenitors, CNTF and LIF appear to increase survival of MBP^+ oligodendrocytes but they induce more strongly astrocytes that are $GFAP^+$ like nmSC [42–44]. Our results show for the first time that the IL-6 type signal produced by IL6RIL6 allows SC to undergo in vitro a late stage of differentiation to myelinating cells, characterized by the induction of MBP and Po gene products.

In vivo, IL-6 injections appeared to reduce demyelination in the murine encephalomyelitis produced by Theiler's virus infection [45]. However, this may be rather an immune mediated reduction of the virus pathogenic effect. In the hypoglossal nerve regeneration, by overexpression of IL-6 and sIL-6R [4], myelination was not examined. Based on our results, activation of gp130 by the IL6RIL6 chimera should be an interesting modality to study in vivo for stimulating remyelination in models of nerve regeneration [20,46] or of autoimmune demyelination of the central nervous system [47], in which GGF [46,47] and CNTF [20] have shown activity.

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References

[1] Hama, T., Kushima, Y., Miyamoto, M., Kubota, M., Takei, N. and Hatanaka, H. (1991) Neuroscience 40, 445–452.
[2] Gadient, R.A. and Otten, U.H. (1997) Prog. Neurobiol. 52, 379–390.
[3] Marz, P., Cheng, J.G., Gadient, R.A., Patterson, P.H., Stoyan, T., Otten, U. and Rose-John, S. (1998) Proc. Natl. Acad. Sci. USA 95, 3251–3256.
[4] Hirota, H., Kiyama, H., Kishimoto, T. and Taga, T. (1996) J. Exp. Med. 183, 2627–2634.
[5] Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. and Kishimoto, T. (1989) Cell 58, 573–581.
[6] Novick, D., Shulman, L.M., Chen, L. and Revel, M. (1992) Cytokine 4, 6–11.
[7] Paonessa, G., Graziani, R., DeSerio, A., Savino, R., Ciapponi, L., Lahmm, A., Salvati, A.L., Toniatti, C. and Ciliberto, G. (1995) EMBO J. 14, 1942–1951.
[8] Sendtner, M., Kreutzberg, G.W. and Thoenen, H. (1990) Nature 31, 440–441.
[9] Martinou, J.C., Martinou, I. and Kato, A.C. (1992) Neuron 8, 737–744.
[10] Ip, N.Y., Nye, S.H., Boulton, T.G., Davis, S., Taga, T., Li, Y., Birren, S.J., Yasukawa, K., Kishimoto, T., Anderson, D.J., Stahl, N. and Yancopoulos, G.D. (1992) Cell 69, 1121–1132.
[11] De Benedetti, F., Massa, M., Pignatti, P., Albani, S., Novick, D. and Martini, A. (1994) J. Clin. Invest. 93, 2114–2119.
[12] Bolin, L.M., Verity, A.N., Silver, J.E., Shooter, E.M. and Abrams, J.S. (1995) J. Neurochem. 64, 850–858.
[13] Gadient, R.A. and Otten, U. (1996) Brain Res. 724, 41–46.
[14] Friedman, B., Scherer, S.S., Rudge, J.S., Helgren, M., Morrisey, D., McClain, J., Wang, D.Y., Wiegand, S.J., Furth, M.E., Lindsay, R.M. and Ip, N.Y. (1992) Neuron 9, 295–305.
[15] Lee, D.A., Zurawel, R.H. and Windenbank, A.J. (1995) J. Neurochem. 65, 564–568.
[16] Lemke, G. and Chao, M. (1988) Development 102, 499–504.
[17] Zorick, T.S. and Lemke, G. (1996) Curr. Opin. Cell Biol. 8, 870–876.
[18] Kioussi, C. and Gruss, P. (1996) Trends Genet. 12, 84–86.
[19] Livesey, F.J., O'Brien, J.A., Li, M., Smith, A.G., Murphy, L.J. and Hunt, S.P. (1997) Nature 390, 614–618.
[20] Sahenk, Z., Seharaseyon, J. and Mendell, J.R. (1994) Brain Res. 655, 246–250.
[21] Fischer, M., Goldschmitt, J., Peschel, C., Brakenhoff, J.P.G., Kallen, K.-J., Wollmer, A., Grotzinger, J. and Rose-John, S. (1997) Nature Biotechnol. 15, 142–145.
[22] Chebath, J., Fischer, D., Kumar, A., Oh, J.-W., Kolett, O., Lapidot, T., Fischer, M., Rose-John, S., Nagler, A., Slavin, S. and Revel, M. (1997) Eur. Cytokine Netw. 8, 359–365.
[23] Kollet, O., Aviram, R., Chebath, J., Ben-Hur, H., Nagler, A., Shultz, L., Revel, M. and Lapidot, T. (1999) Blood 94 (in press).
[24] Kleitman, N., Wood, P.M. and Bunge, R.P. (1991) in: Culturing Nerve Cells (Banker, G. and Goslin, K., Eds.), pp. 337–377, MIT Press, Cambridge, MA.
[25] Kumar, A., Bertolotto, C., Haggiag, S., Revel, M. and Chebath, J. (1999) EMBO J. (in press).
[26] Fernandez-Valle, C., Fregien, N., Wood, P.M. and Bunge, M.B. (1993) Development 119, 867–880.
[27] Yoshimura, K., Negishi, T., Kaneko, A., Sakamoto, Y., Kitamura, K., Hosokawa, T., Hamaguchi, K. and Nomura, M. (1996) Neurosci. Res. 25, 41–49.
[28] Bunge, R.P., Bunge, M.B. and Bates, M. (1989) J. Cell Biol. 109, 273–284.
[29] Campagnoni, A.T., Pribyl, T.M., Campagnoni, C.W., Kampf, K., Amur-Umarjee, S., Landry, C., Handley, V., Newman, S., Garbay, B. and Kitamura, K. (1993) J. Biol. Chem. 268, 4930–4938.
[30] You, K.H., Hsieh, C.L., Hayes, C., Stahl, N., Francke, U. and Popko, B. (1991) Genomics 9, 751–757.
[31] Dong, Z., Sinanan, A., Parkinson, D., Parmantier, E., Mirsky, R. and Jessen, K.R. (1999) J. Neurosci. Res. 56, 334–348.
[32] Topilko, P., Schneider-Maunoury, S., Levi, G., Baron-Van Evercooren, A., Chennoufi, A.B., Seitanidou, T., Babinet, C. and Charnay, P. (1994) Nature 371, 796–799.
[33] Clark, R., Stewart, M., Miskimins, W.K. and Miskimins, R. (1998) Int. J. Dev. Neurosci. 16, 323–331.
[34] Takahashi-Tezuka, M., Yoshida, Y., Fukada, T., Ohtani, T., Yamanaka, Y., Nishida, K., Nakajima, K., Hibi, M. and Hirano, T. (1998) Mol. Cell. Biol. 18, 4109–4117.
[35] Kioussi, C., Gross, M.K. and Gruss, P. (1995) Neuron 15, 553–562.
[36] Cheng, L. and Mudge, A.W. (1996) Neuron 16, 309–319.
[37] Lee, M., Brennan, A., Blanchard, A., Zoidl, G., Dong, Z., Tabernero, A., Zoidl, C., Dent, M.A., Jessen, K.R. and Mirsky, R. (1997) Mol. Cell. Neurosci. 8, 336–350.
[38] Morgan, L., Jessen, K.R. and Mirsky, R. (1991) J. Cell Biol. 112, 457–467.
[39] Dong, Z., Brennan, A., Liu, N., Yarden, Y., Lefkowitz, G., Mirsky, R. and Jessen, K.R. (1995) Neuron 15, 585–596.
[40] Morgan, L., Jessen, K.R. and Mirsky, R. (1994) Development 120, 1399–1409.
[41] Murphy, P., Topilko, P., Schneider-Maunoury, S., Seitanidou, T., Baron-Van Evercooren, A. and Charnay, P. (1996) Development 122, 2847–2857.
[42] Mayer, M., Bhakoo, K. and Noble, M. (1994) Development 120, 143–153.
[43] Kahn, M.A. and De Vellis, J. (1994) Glia 12, 87–98.
[44] Bonni, A., Sun, Y., Nadal-Vicens, M., Bhatt, A., Frank, D.A., Rozovsky, I., Stahl, N., Yancopoulos, G.D. and Greenberg, M.E. (1997) Science 278, 477–483.
[45] Rodriguez, M., Pavelko, K.D., McKinney, C.W. and Leibowitz, J.L. (1994) J. Immunol. 153, 3811–3821.
[46] Chen, L.E., Liu, K., Seaber, A.V., Katragadda, S., Kirk, C. and Urbaniak, J.R. (1998) Neurochem. Int. 33, 341–351.
[47] Cannella, B., Hoban, C.J., Gao, Y.L., Garcia-Arenas, R., Lawson, D., Marchionni, M., Gwynne, D. and Raine, C.S. (1998) Proc. Natl. Acad. Sci. USA 95, 10100–10105.