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RAPID COMMUNICATION

Oligodendrocyte-myelin glycoprotein (OMgp) is an inhibitor of neurite outgrowth

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Abstract

A protein fraction purified from bovine brain myelin, previously called arretin because of its ability to inhibit neurite outgrowth, has been identified as consisting predominantly of oligodendrocyte-myelin glycoprotein (OMgp). We show that it is a potent inhibitor of neurite outgrowth from rat cerebellar granule and hippocampal cells; from dorsal root ganglion explants in which growth cone collapse was observed; from rat retinal ganglion neurons; and from NG108 and PC12 cells. OMgp purified by a different procedure from both mouse and human myelin behaves identically in all bioassays tested.

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The failure of injured axons to regenerate long distances in the adult mammalian CNS leads to permanent paralysis and other functional deficits such as those seen after spinal cord injuries. Although axons do not regenerate through adult CNS tissue, they retain the ability to regrow for long distances if provided with an appropriate cellular environment, for example a peripheral nerve graft (David and Aguayo 1981). Work by Schwab and colleagues led to the discovery that this failure of axons to regenerate was likely to be caused, in part, by the influence of axon growth inhibitory activity associated with myelin (Caroni and Schwab 1988; Schwab and Bartholdi 1996; Horner and Gage 2000). Nogo-A (Chen *et al*. 2000; Fournier *et al*. 2001; GrandPre *et al*. 2002), myelin-associated glycoprotein (MAG) (McKerracher *et al*. 1994; Mukhopadhyay *et al*. 1994), and several proteoglycans (Niederost *et al*. 1999) have been identified as myelin associated molecules that impede axonal regeneration in the adult mammalian CNS.

In our studies on MAG as a neurite growth inhibitor (McKerracher *et al*. 1994), we observed non-MAG containing chromatographic fractions eluting in 2–3 M NaCl solution from an anion exchange column that contained very low concentrations of protein but were highly active in bioassays. Further chromatographic purification on a peanut agglutinin lectin, as an affinity support, resulted in column fractions having very potent neurite growth inhibition. We called this potent activity 'arretin'. However, these biologically active fractions still contained several electrophoretically separable components detected on silver-stained gels. We now show that: (i) the dominant protein of 'arretin' is oligodendrocyte-myelin glycoprotein (OMgp); (ii) authentic OMgp isolated by a different procedure and recombinant OMgp both inhibit neurite outgrowth. Aspects of this work were presented at the Twenty Third International Symposium on Spinal Cord Trauma, Montreal, 2001b.

Materials and methods

Purification of arretin and OMgp

Myelin was prepared from bovine brain, extracted with octylglucoside salt, and chromatographed on diethylaminoethyl (DEAE)-Sepharose (Pharmacia) as previously described (McKerracher *et al*. 1994). Column fractions eluting in 1.5–2 M NaCl solution were pooled, diluted three-fold, and loaded on a peanut agglutinin (PNA)-agarose column. Following a wash with 2 M NaCl in 20 mM triethanolamine (pH 7.5) and a cocktail of protease inhibitors arretin was eluted with 0.5 M D-galactose in the wash buffer. The eluate was dialysed against H_2O and lyophilized. The protein was re-dissolved in a minimum volume of H_2O. OMgp from either mouse or human brain was purified following the method described by Mikol and Stefansson (1988). The anti-OMgp used for western blots and for immunodepletion experiments was raised in rabbits and affinity purified (Dr Mikol, University of Michigan, MI, USA). All chemicals were purchased from Sigma-Aldrich Canada Ltd.

Immunodepletion of OMgp

In order to establish that the bioactivity in our sample of OMgp was not caused by minor contaminants, we subjected the sample to four rounds of immunodepletion (McKerracher *et al*. 1994). Briefly, the mouse OMgp sample in 50 mM Tris buffer, pH 7, was incubated with the affinity purified rabbit anti-OMgp overnight at 4°C with rotation. Protein A beads were added for 3 h with rotation, centrifuged (50 000 *g*, 10 min) and the supernatant was subjected to three repeat treatments as described above. The final depleted samples, as well as the protein A beads containing the immunosorbed OMgp, were separated by sodium dodecyl sulfate − polyacrylamide gel electrophoresis (SDS–PAGE) for silver staining and western blotting, and bioassays were performed as described below.

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Abbreviations used: DEAE, diethylaminoethyl; MAG, myelin-associated glycoprotein; OMgp, oligodendrocyte-myelin glycoprotein; PNA, peanut agglutinin; SDS–PAGE; sodium dodecyl sulfate − polyacrylamide gel electrophoresis.

Neurite outgrowth assays and growth cone collapse

Assays for neurite outgrowth have been described (Xiao *et al*. 1996; Huang *et al*. 1999). Briefly, inhibitors were applied as a spot to nitrocellulose and poly-L-lysine coated wells and incubated overnight at 37°C in a humidified atmosphere. Wells were rinsed, and NG108-15, PC12 or primary cells were plated in chemically defined media. Cells were allowed to grow for 48 h, fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), stained with Coomassie blue, washed and dried. Cerebellar and hippocampal neurons, rat dorsal root ganglion cells, and rat retinal ganglion neurons were cultured, and neurites quantified as described (Huang *et al*. 1999). Growth cone collapse assays with image analysis and quantification, are being performed in the laboratory of Dr Lisa McKerracher (Université de Montréal, Montreal, QC, Canada).

Expression of recombinant OMgp

The full length cDNA encoding rat OMgp was cloned by RT-PCR and subcloned into the pCMS-EGFP vector for expression in eukaryotic cells. Transfected cells were collected and lysed in 50 mM Tris pH 8, 1% octylglucoside and protease inhibitors at 4°C for 1 h, then sonicated for 30 s and centrifuged at 15 000 *g* for 30 min at 4°C. Lysates were collected, diluted 3-fold, and PNA-agarose beads were added and incubated overnight at 4°C with mixing. The beads were washed, and the OMgp was eluted as in the arretin purification.

Results

We previously described preliminary attempts to characterize a low abundance but highly active growth inhibitor that eluted with 1.5–2.0 M NaCl from a DEAE ion exchange column (Fig. 1a; McKerracher *et al*. 1994; Xiao *et al*. 1997). Further chromatography on a lectin support (PNA) yielded an inhibitory fraction (~1 μg protein, starting from 100 mg of bovine brain myelin protein). The silver-stained electrophoretic profile is shown in Fig. 1(b). Mass spectrophotometric analysis of each silver-stained band revealed that either OMgp (~110 kDa) or its degradation fragments collectively account for the major protein, aside from the band of PNA that spuriously eluted from the column. Several other minor components are evident, including tenascin-R (J1-160/180), and versican 3. Western blots (not shown) with anti-OMgp confirmed the identity of OMgp. An authentic sample of mouse OMgp, purified independently by a different procedure (Mikol and Stefansson 1988) proved to have minor contaminants that we also identified by mass spectrometry (Fig. 1c); none of these are known to have growth inhibitory properties in our assays.

Figure 2 demonstrates the neurite growth inhibitory properties in a bioassay of 'arretin' at 1–2 nM, using NG108 cells (Fig. 2a), rat dorsal root ganglia (Fig. 2b), cerebellar granule cells and hippocampal neurons (Fig. 2c). Growth cone collapse in response to arretin was also observed in early experiments, but quantification is not yet complete. Figure 3(a) shows the inhibition of neurite outgrowth by mouse OMgp (1–2 nM) in

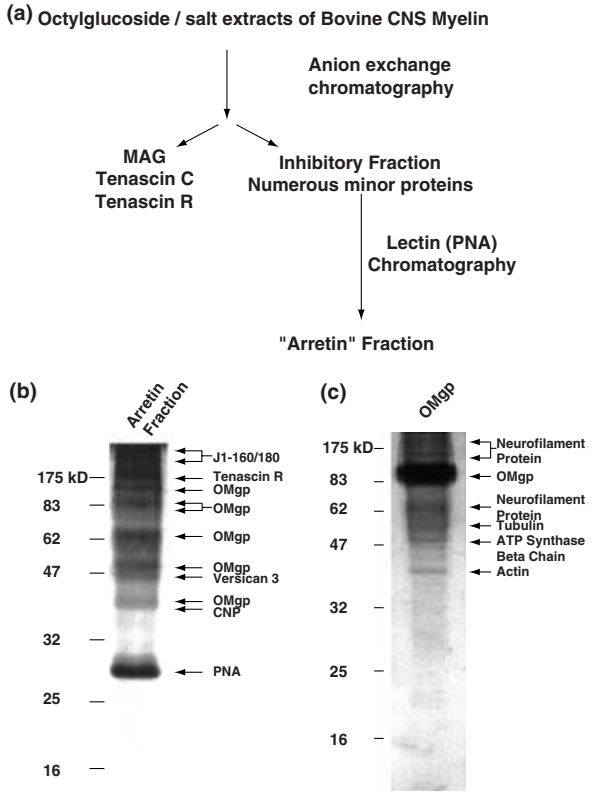

Fig. 1 (a) Scheme summarizing the purification of arretin. (b) SDS–PAGE profile of arretin. Proteins were separated on a 12% polyacrylamide gel, and stained with silver. All visible bands were subjected to mass spectrometric analysis to identify proteins. CNP, 2′,3′-cyclic nucleotide 3′ phosphodiesterase; PNA, peanut agglutinin. (c) SDS–PAGE profile of purified OMgp. OMgp was purified as previously described (Mikol and Stefansson 1988) and analyzed on a 12% gel as in (b).

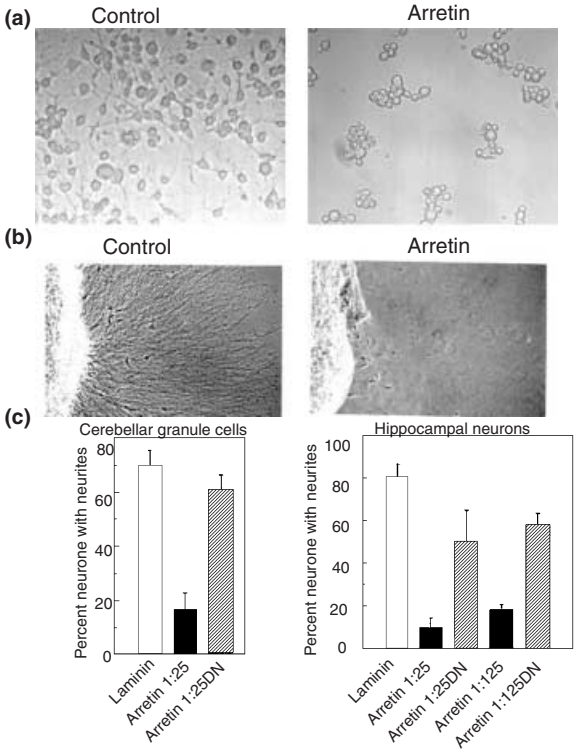

Fig. 2 Inhibition of neurite outgrowth by arretin. (a) Arretin (1–2 nM) is strongly inhibitory for neurite extension from NG108 cells. (b) Neurite outgrowth from mouse dorsal root ganglion explants (P6–7) is inhibited by arretin (1–2 nM). The control was the laminin substrate alone. (c) In both cerebellar granule cells and hippocampal neurons, neurite outgrowth is inhibited (in a dose responsive manner for hippocampal neurons). This inhibitory effect is greatly reduced when the arretin is partially denatured (DN) by heating to 80°C for 15 min.

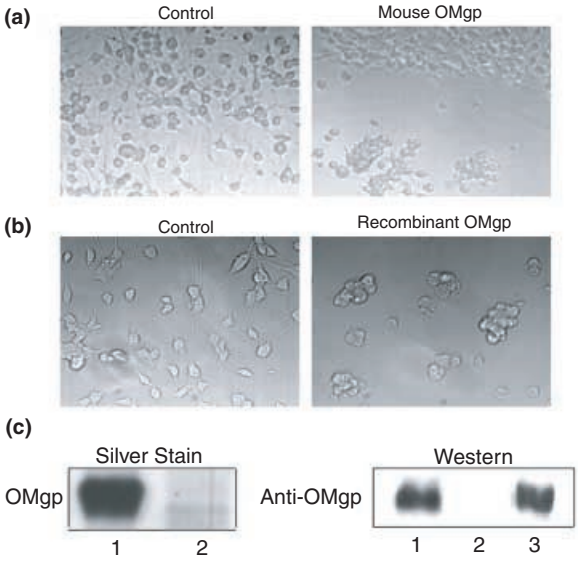

Fig. 3 Inhibition of neurite growth by mouse and recombinant OMgp. (a) NG108 cells grown in the presence of purified mouse OMgp (1–2 nM) manifest strong inhibition of neurite growth. (b) Neurite outgrowth from PC12 cells is inhibited by response to recombinant OMgp, in the form of an octylglucoside extract of transfected 293T cells that has been eluted from a PNA column. (c) Immunodepletion of purified mouse OMgp. Lane 1, the major OMgp band detected either by silver staining or by western blotting. Lane 2, absence of OMgp after four cycles of immunodepletion. Lane 3, OMgp electrophoretically recovered from the protein A beads following immunodepletion.

NG108 cells. When recombinant OMgp was expressed in 293T cells, a detergent extract of these cells demonstrated inhibition of neurite outgrowth from PC12 cells (Fig. 3b); a similar inhibitory response was observed with NG108 cells (not shown). Immunodepletion of the mouse OMgp sample with the affinity purified anti-OMgp (Fig. 3c) abolished the inhibitory response; the bioassay results looked exactly like the control NG108 cells seen in Fig. 3(a). Thus the minor contaminants found in purified samples do not contribute significantly to the inhibitory response. Human OMgp, prepared by the same procedure as that for mouse, elicited a vigorous inhibitory response in our neurite outgrowth assay with rat retinal ganglion cells (Fig. 4).

Discussion

Numerous observations suggest that the CNS myelin sheath possesses multiple inhibitors of axon regeneration. Although Nogo (Chen *et al*. 2000; GrandPre *et al*. 2000), MAG (McKerracher *et al*. 1994; Mukhopadhyay *et al*. 1994), and several chondroitin sulfate proteoglycans (Niederost *et al*. 1999) have been most prominently studied, we now conclude that a potent myelin-associated inhibitory activity, previously reported as 'arretin' (Xiao *et al*. 1997) is OMgp. Authentic samples of OMgp purified from mouse or human brain by a different procedure evinced the same inhibitory properties as arretin. This glycoprotein of 110–120 kDa (440 amino acids) is glycosylphosphatidylinositol-linked to the cell membrane in myelinating oligodendrocytes, and is localized to the glial–axonal interface of myelinated axons (Mikol *et al*. 1990a,b, 1993; Habib *et al*. 1998). The protein is a relatively minor component of CNS myelin, the temporospatial appearance of which during development parallels the pattern of myelination (Mikol and Stefansson 1988). Mouse and human OMgp are structurally very similar; the protein has a

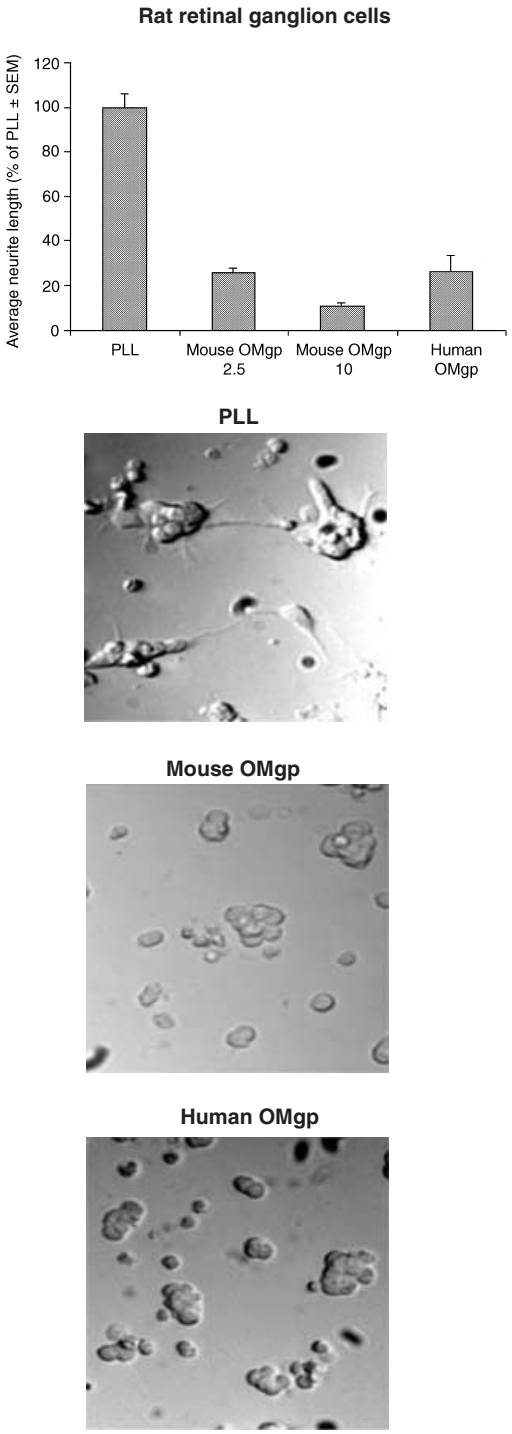

Fig. 4 Inhibition of neurite outgrowth from rat retinal ganglion neurons by mouse and human OMgp. Mouse OMgp was bioassayed at two concentrations (2.5 and 10 U), representing 1–2 nm, and 2–8 nm, respectively). Human OMgp was estimated to be between 2 and 10 nm. PLL, poly-L-lysine control.

series of tandem leucine-rich repeats, like those in a variety of adhesion molecules and receptors, including the Nogo receptor; this domain probably has a significant function role (Mikol *et al*. 1993). It is likely

that the observed ability to inhibit neurite outgrowth and, by extension, axonal regeneration is not the only function of OMgp, and a normal physiological role remains to be determined.

While this work was being prepared for publication, a *Nature* advance online publication reported that OMgp binds to the Nogo receptor with high affinity, and that this induces growth cone collapse and inhibition of neurite outgrowth (Wang *et al*. 2002). Our own observations on the potency of OMgp, and those reported by Wang *et al*. (2002) strongly support our view that this protein should be considered as a major obstacle to nerve regeneration. (Additionally, while our manuscript was in review, Liu *et al*. 2002 and Domeniconi *et al*. 2002 reported that MAG is also a functional ligand of the Nogo receptor).

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References

Caroni P. and Schwab M. E. (1988) Antibody against myelin-associated inhibitor of neurite growth neutralizes nonpermissive substrate property of CNS white matter. *Neuron* **1**, 85–96.

Chen M. S., Huber A. B., vander der Haar M. E., Frank M., Schnell L., Spillmann A. A., Christ F. and Schwab M. E. (2000) Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. *Nature* **403**, 434–439.

David S. and Aguayo A. J. (1981) Axonal elongation into peripheral nervous system 'bridges' after central nervous system injury in adult rats. *Science* **214**, 931–933.

Domeniconi M., Cao Z., Spencer T., Sivasankavan R., Wang K. C., Nikulina E., Kimura N. CaH., Deng K., Gao Y., He Z. and Filbin M. T. (2002) Myelin-associated glycoprotein interacts with the Nogo-66 receptor to inhibit neurite outgrowth. *Neuron* **35**, 283–290.

Fournier A. E., GrandPre T. and Strittmatter S. M. (2001) Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. *Nature* **409**, 341–346.

GrandPre T., Nakamura F., Vartanian T. and Strittmatter S. M. (2000) Identification of the Nogo inhibition of axon regeneration as a Reticulon protein. *Nature* **403**, 439–444.

GrandPre T., Li S. and Strittmatter S. M. (2002) Nogo-66 receptor antagonist peptide promotes axonal regeneration. *Nature* **417**, 547–551.

Habib A. A., Marton L. S., Allwardt B., Gulcher J. R., Mikol D. D., Hognason T., Chattopadhyay N. and Stefansson K. (1998) Expression of the oligodendrocyte-myelin glycoprotein by neurons in the mouse central nervous system. *J. Neurochem.* **70**, 1704–1711.

Horner P. J. and Gage F. H. (2000) Regenerating the damaged central nervous system. *Nature* **407**, 963–970.

Huang D. W., McKerracher L., Braun P. E. and David S. (1999) A therapeutic vaccine approach to stimulate axon regeneration in the adult mammalian spinal cord. *Neuron* **24**, 639–647.

Liu B. P., Fournier A., GrandPre T. and Strittmatter S. M. (2002) Myelin associated glycoprotein as a functional ligand for the Nogo-66 receptor. Science *on line*, June 27; 10.1126/Science.

McKerracher L., David S., Jackson D. L., Kottis V., Dunn R. J. and Braun P. E. (1994) Identification of myelin-associated glycoprotein as a major myelin-derived inhibitor of neurite growth. *Neuron* **13**, 805–811.

Mikol D. D. and Stefansson K. (1988) A phosphatidylinositol-linked peanut agglutinin-binding glycoprotein in central nervous system myelin and on oligodendrocytes. *J. Cell Biol.* **106**, 1273–1279.

Mikol D. D., Alexakos M. J., Bayley C. A., Lemons R. S., Le Beau M. M. and Stefansson K. (1990a) Structure and chromosomal localization of the gene for the oligodendrocyte-myelin glycoprotein. *J. Cell Biol.* **111**, 2673–2679.

Mikol D. D., Gulcher J. R. and Stefansson K. (1990b) The oligodendrocyte-myelin glycoprotein belongs to a distinct family of proteins and contains the HNK-1 carbohydrate. *J. Cell Biol.* **110**, 471–479.

Mikol D. D., Rongnoparut P., Allwardt B. A., Marton L. S. and Stefansson K. (1993) The oligodendrocyte-myelin glycoprotein of mouse: Primary structure and gene structure. *Genomics* **17**, 604–610.

Mukhopadhyay G., Doherty P., Walsh F. S., Crocker P. R. and Filbin M. T. (1994) A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. *Neuron* **13**, 757–767.

Niederost B. P., Zimmermann D. R., Schwab M. E. and Bandtlow C. E. (1999) Bovine CNS myelin contains neurite growth-inhibitory activity associated with chondroitin sulfate proteoglycans. *Neurosci.* **19**, 8979–8989.

Schwab M. E. and Bartholdi D. (1996) Degeneration and regeneration of axons in the lesioned spinal cord. *Physiol. Rev.* **76**, 319–370.

Wang K. C., Koprivica V., Kim J. A., Sivasankaran R., Guo Y., Neve R. L. and He Z. (2002) Oligodendrocyte-myelin glycoprotein is a Nogo receptor ligand that inhibits neurite outgrowth. *Nature* **417**, 941–944.

Xiao Z. C., Taylor J., Montag D., Rougon G. and Schachner M. (1996) Distinct effects of recombinant tenascin-R. domains in neuronal cell functions and identification of the domain interacting with the neuronal recognition molecule F3/11. *Eur. J. Neurosci.* **8**, 766–782.

Xiao Z.-C., David S., Braun P. and McKerracher L. (1997) Characterization of a new myelin-derived growth inhibitory activity. *Soc. Neurosci. Abstract* **23**, 777.2.