Myelination and axonal regeneration in the central nervous system of mice deficient in the myelin-associated glycoprotein

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Summary
The myelin-associated glycoprotein, a member of the immunoglobulin superfamily, has been implicated in the formation and maintenance of myelin sheaths. In addition, recent studies have demonstrated that myelin-associated glycoprotein is inhibitory for neurite elongation in vitro and it has therefore been suggested that myelin-associated glycoprotein prevents axonal regeneration in lesioned nervous tissue. The generation of mice deficient in the expression of myelin-associated glycoprotein by targeted disruption of the mag gene via homologous recombination in embryonic stem cells has allowed the study of the functional role of this molecule in vivo. This review summarizes experiments aimed at answering the following questions: (i) is myelin-associated glycoprotein involved in the formation and maintenance of myelin in the CNS? and (ii) does myelin-associated glycoprotein restrict axonal regeneration in the adult mammalian CNS? Analysis of optic nerves from mutant mice revealed a delay in myelination when compared to optic nerves of wild-type animals, a lack of a periaxonal cytoplasmic collar from most myelin sheaths, and the presence of some doubly and multiply myelinated axons. Axonal regeneration in the CNS of adult myelin-associated glycoprotein deficient mice was not improved when compared to wild-type animals. These observations indicate that myelin-associated glycoprotein is functionally involved in the recognition of axons by oligodendrocytes and in the morphological maturation of myelin sheaths. However, results do not support a role of myelin-associated glycoprotein as a potent inhibitor of axonal regeneration in the adult mammalian CNS.

Myelin-associated glycoprotein has been implicated in the formation and maintenance of myelin

The presence of a multilayered myelin sheath electrically insulates axons. Generation of action potentials is thus confined to myelin-free regions of axons, i.e. the nodes of Ranvier, resulting in a saltatory and rapid propagation of nerve impulses. The formation of myelin around axons proceeds in various steps and requires complex interactions between the myelin-forming glial cell and the axon. Glial cells first recognize and adhere to myelination-competent axons and then enwrap these axons. Finally, the cytoplasm of the glial cells is extruded from the spiraling process to form compact myelin (for reviews, see Bray et al., 1981; Peters et al., 1991; Hildebrand et al., 1993).

Myelin-associated glycoprotein (MAG) is one of several molecules believed to mediate both neuron-glia interactions and the morphological maturation of myelin. Myelin-associated glycoprotein, a heavily glycosylated transmembrane protein, is a member of the immunoglobulin (Ig) superfamily with five Ig-like domains (Arquint et al., 1987; Lai et al., 1987; Salzer et al., 1987). The glycoprotein is a minor constituent of CNS and PNS myelin (Trapp, 1990), where it is exclusively expressed by oligodendrocytes and Schwann cells, respectively (Sternberger et al., 1979; Martini & Schachner, 1986; Bartsch et al., 1989, 1994; Trapp et al., 1989). Two isoforms of MAG with apparent molecular weights of 67 kDa and 72 kDa after deglycosylation are generated from a single gene on chromosome 7 in the mouse by alternative splicing (Barton et al., 1987; Lai et al., 1987; D'Eustachio et al., 1988; Tropak et al., 1988). Expression of both isoforms in the CNS is developmentally regulated; the 72 kDa isoform is the abundant form early in development whereas the 67 kDa form predominates in the adult. In the PNS, expression of the 67 kDa isoform predominates during all stages of development and in...
the adult (Frail et al., 1985; Tropak et al., 1988; Inuzuka et al., 1991).

The expression of MAG in oligodendrocytes and Schwann cells occurs early during myelination of axons (e.g. Sternberger et al., 1979; Bartsch et al., 1989; Owens & Bunge, 1989). For instance, myelinating oligodendrocyte processes are MAG immunoreactive when axons are being enwrapped (Fig. 1A; Bartsch et al., 1989; Trapp et al., 1989). It has therefore been hypothesized that the molecule might be of functional relevance during initial stages of myelin formation (Bartsch et al., 1989; Trapp et al., 1989). Then, after compact myelin has formed, MAG becomes restricted to the periaxonal region of myelinated axons (Fig. 1B) and is weakly expressed in paranodal regions (Bartsch et al., 1989; Trapp et al., 1989). Myelin-associated glycoprotein might thus play a role in stabilizing oligodendrocyte-axon contacts in mature myelin sheaths.

The identification of MAG as a member of the Ig superfamily, the expression of the protein during early stages of myelination, and the predominant location at the interface between myelin sheaths and axons (see above) suggests that MAG plays a crucial role in the interaction between glial cells and neurons. This view is strengthened by the observation that Fab fragments of a monoclonal anti-MAG antibody interfere with oligodendrocyte-oligodendrocyte and oligodendrocyte-neuron adhesion in vitro (Poltorak et al., 1987). Moreover, it was demonstrated that purified MAG incorporated into liposomes binds specifically to MAG-negative neurons (Poltorak et al., 1987; Sadoul et al., 1990), demonstrating that MAG is a heterophilic ligand for a yet to be identified neuronal receptor(s).

Experimental evidence for a functional involvement of MAG in myelination has been provided by in vitro experiments using co-cultures of rat Schwann cells and dorsal root ganglion (DRG) neurons. Schwann cells infected with a retrovirus expressing MAG antisense RNA failed to segregate large caliber axons. A 1:1 relationship between axons and Schwann cells did not form and Schwann cells thus failed to form myelin (Owens & Bunge, 1991). In contrast, when MAG cDNA was introduced into Schwann cells, initial investment of DRG axons by Schwann cells was accelerated (Owens et al., 1990). Based on these observations, it was postulated that MAG plays a critical role during initial stages of myelination. Evidence was also provided for an involvement of MAG in the establishment and maintenance of morphologically intact myelin sheaths. In the PNS of the quaking mutant, myelin sheaths show regions with a fused Schwann cell periaxonal cytoplasmic collar and dilated periaxonal space. Regions of myelin sheaths with such morphological defects are MAG negative whereas regions of the same sheaths with a morphologically intact periaxonal cytoplasmic collar and periaxonal space were found to be MAG positive (Trapp et al., 1984).

**Myelination in the optic nerve of MAG-deficient mice**

Defects in the formation of myelin, either in spontaneously occurring mutants or in genetically engineered animals provide a unique opportunity to gain insights into both interactions between myelinating glial cells and axons and the differentiation of myelin sheaths at the molecular level (reviewed by Hudson, 1990, Snipes et al., 1993; Lemke, 1993; Nave, 1994). Mice completely lacking expression of MAG were generated by inactivation of the mag gene and were used to investigate the functions of this molecule in the intact organism (Li et al., 1994; Montag et al., 1994).

In the developing optic nerve of MAG-deficient mice, formation of compact myelin around retinal ganglion cell axons was delayed. We found approximately 40% to 50% fewer myelinated axons in the optic nerve of 10- or 11-day old MAG mutants when compared to optic nerves of age-matched wild-type animals (Montag et al., 1994). This observation confirms the view of a critical role of MAG during initial stages of myelination in the CNS and highlights the functional relevance of the early expression of MAG by oligodendrocytes (Bartsch et al., 1989; Trapp et al., 1989). The time course of myelination in the PNS of MAG-deficient mice was, however, similar to that in wild-type animals (Montag et al., 1994). This latter observation is in marked contrast with the in vitro findings that myelination is retarded in co-cultures of Schwann cells and DRG neurons in which Schwann cells express low amounts of MAG (see above). Although the reasons for these apparently contradictory results are presently unknown, it is reasonable to assume that other molecules compensate for the lack of MAG in the mutant mouse but not in the co-cultures (see below).

The ultrastructure of compact myelin was unaffected in the mutants (Figs 2, 3 and 4; Li et al., 1994; Montag et al., 1994; Bartsch et al., 1995a). Similarly, we found a normal spacing between axons and myelin sheaths in both CNS (Figs 2 and 3) and PNS (Montag et al., 1994; Bartsch et al., 1995a) whereas others reported a disorganized periaxonal space for some myelinated CNS and PNS axons (Li et al., 1994). The oligodendrocyte periaxial cytoplasmic collar of the majority of myelin sheaths was, however, either reduced in length or completely lacking in the CNS (Figs 2 and 3; Li et al., 1994; Montag et al., 1994). Quantitative analysis revealed that the length of the periaxonal cytoplasmic collar was shorter than half of the axonal circumference in approximately 94% of all myelin sheaths in MAG mutants whereas such observations were made in only about 24% of all myelin sheaths in...