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Myelin-associated glycoprotein in myelinogenesis and demyelinating processes

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The data concerning localization of myelin-associated glycoprotein (MAG) in myelin, functional significance of MAG and changes of MAG in demylinating processes are presented. In our studies we have not seen any appreciable changes in the content of MAG both in the early and later stages of central Wallerian degeneration in the optic nerve. We have also found that the decrease of MAG content in triethyl tin poisoning is proportional to the drop of total myelin proteins and is not indicative of a specific sensitivity of that glycoprotein. Changes in the content of MAG in brains of children who died from SSPE were also only negligible and myelin fraction was even relatively enriched in this glycoprotein.

Key words: myelin-associated glycoprotein, myelinogenesis, demyelinating processes.

Introduction

Glycoproteins, the less known constituents of myelin sheaths, are likęly to be of importance for the formation, structure and perhaps for the pathological breakdown of myelin. The basic glycoprotein of the central myelin is a glycoprotein of high molecular weight, unique to myelin and referred to as the myelin-associated glycoprotein (MAG). Although MAG is quantitatively a minor constituent of the whole myelin fraction, it probably accounts for a considerably higher proportion of the protein molecules exposed on the surface of the myelin membrane, and plays the role of a receptor. Hence, it is possible to speculate about their significance in molecular events leading to myelinogenesis and demyelination.

The myelin-associated glycoprotein (MAG) is a 100 K-Dalton, integral membrane glycoprotein containing 30% carbohydrate that is in central and

peripheral myelin sheaths. MAG can be purified from isolated myelin by extraction with lithium diiodosalicylate, partitioning against phenol, and gel filtration on sepharose-C16B.

MAG in developing nervous system

In the early stages of development of the optic nerve the increase of MAG content runs parallel to the degreee of myelination of its axons, which indicates the function of this component in the formation of myelin sheaths. It can be, therefore, suggested that the external surface membrane components of myelin sheaths to which the glycoproteins belong may be involved in specific recognitation roles during the process of myelination.

The interpretation of the last findings is rather complicated for two reasons: firstly, each brain structure is myelinated at different times during ontogeny and, secondly, there are regional differences in myelin composition of the CNS.

Localization of MAG in the myelin

Based on immunocytochemical studies and radioimmunoassays using polyclonal antisera raised in rabbits against MAG, it appears that the myelin-associated glycoprotein is a nervous system specific protein. Furthermore, the immunocytochemical studies indicate that it is restricted to myelin-forming oligodendrocytes in the CNS and Schwann cells in the PNS. Recently, however, there have been a number of studies with monoclonal antibodies that bind to MAG, which have suggested that MAG or a MAG-like molecule is present in other cell types both in the nervous system and in the immune system. However, there is a conflict in the literature about whether or not MAG is a constituent of compact myelin, or whether it is restricted to non-compact membranes associated with myelin sheaths, including the periaxonal membrane of oligodendrocytes and Schwann cells. A variety of biochemical experiments on CNS tissue involving the subfractionation of myelin and myelin-related membranes had suggested that MAG was most concentrated in oligodendroglia membranes that were different from compact myelin, but biochemical experiments cannot precisely define the localization of this protein. Therefore, the immunocytochemical studies of S t e r n b e r g e r et al. [3], which first showed immune staining for MAG only in the periaxonal part of the central and peripheral myelin sheaths and not in compact myelin, were a very important step in defining the localization of this protein. Light microscopic immunochemical studies have shown that MAG is present in the periaxonal region of the developing myelin sheaths. On the contrary, some other experiments have proved on the basis of electron microscopic and immunochemical studies that MAG is localized within the compact CNS myelin. It was also shown that MAG increases in the whole brain during development. In the peripheral nervous system, where unlike in the CNS the major PO-glycoprotein constitutes a considerably greater part of myelin proteins, it is localized both in the intraperiod and major dense lines. In iminodipropionitrile neuropathy the presence of MAG in Schwann cell periaxonal membranes has also been demonstrated unequivocally by staining the axonal ingrowths of axolemma and Schwann cell periaxonal membrane. Quantitative biochemical studies on the mutants provide supporting data for the presence of MAG in membranes that are different from compact myelin in the PNS.

Functional significance of MAG

The presence of MAG in periaxonal Schwann cell and oligodendroglia membranes strongly suggests that this glycoprotein is involved in maintaining the junction between the myelin sheaths and the axon. Correlation of the presence or absence of MAG determined by immunocytochemistry with ultrastructural changes in the tissue continue to support a role for MAG in the formation and maintenance of glia-axon junctions. Since glycoproteins are known to be cell-surface antigens and receptors for some viruses, there are theoretical reasons to suspect that MAG could be involved in autoimmune or viral aspects of multiple sclerosis or other demyelinating diseases. The finding that a carbohydrate epitope on the surface of a subset of lymphocytes is shared with MAG and other glycoconjugates in the nervous system may be of significance with regard to autoimmune demyelination diseases. In any case, the existence of a shared epitope between lymphocytes and carbohydrates determinant on MAG and other glycoconjugates of the nervous system is a factor that needs to be considered with regard to autoimmune demyelinating diseases. The nature of the molecule in lymphocytes containing this epitope has not yet been established conclusively. An abstract has appeared indicating that the antigen consists of a protein or proteins with M similart to MAG. The involvement of glycoproteins in pathological processes connected with myelin breakdown is assumed from some experimental data, particularly from the great loss of MAG in the degenerating myelin isolated from hexachlorophene intoxicated rats [2].

MAG in demyelinating processes

In our own studies central Wallerian degeneration was evoked in rabbits' optic nerves by ophthalmectomy. The incorporation of radioactive fucose into MAG of the optic nerve undergoing Wallerian degeneration was studied [7]. In other groups of experimental animals, changes in the content of MAG were evaluated [6]. The results were compared with myelin lipid and protein studies.

Chemical evaluation of MAG. The soluble proteins of the delipidated myelin fraction were separated by means of polyacrylamide gel electrophoresis and stained with Schiff's reagent. The electrophoregrams were evaluated densitometrically. Results are expressed in μg of MAG per mg of total myelin protein.

Incorporation of radioactive fucose into MAG. Bilaterally ophthalmectomized rabbits were injected intracisternally with ^{14}C of ^{3}H-labelled fucose (40 and 100 μCi respectively) Twenty-four hrs or 7 days after surgery both experimental groups were killed 20 hrs after injection of the radioisotope, and the isolated myelin fraction of the optic nerve was lyophilized. The soluble proteins of the delipidized fraction were electrophoresed on polyacrylamide gel and radioactivity of the electrophoregrams cut into 2 mm sections committed. The results obtained in central Wallerian degeneration showing an essentially unchanged metabolism of MAG, i.e. an only negligibly reduced incorporation of radioactive fucose (Table 1), as well as lack of changes in the content of this glycoprotein fraction (Table 2), thus do not support the assumption that MAG is seriously involved in biochemical events occurring during the early stages of all central demyelinating processes. In our studies we have not seen any appreciable changes in the content of MAG both in the early and later stages of central Wallerian degeneration of the optic nerve. Furthermore, tracing the rate of radioactive fucose incorporation into MAG, we were unable to demonstrate eminent changes in MAG metabolism in this experimental model.

Table 1. Incorporation of ^{3}H-fucose into myelin-associated glycoprotein in central Wallerian degeneration

	Control	24 hrs after enucleation	7 days after enucleation
MAG	39,0	40,8	34,3

Incorporation of ^{14}C-fucose into myelin-associated glycoprotein in central Wallerian degeneration

		Control	24 hrs after enucleation
MAG		54,3	43,6

Note: Expressed in percentages of total protein radioactivity.

Table 2. Total protein and MAG content in the myelin of the optic nerve undergoing Wallerian degeneration

	Dry weight of myelin in μg/mg of optic nerve	Total myelin protein in mg per one optic nerve	MAG in arbitrary units per mg of total myelin proteins
Control	117,8± 7,9	1,54±0,06	4,95±1,03
1 day after enucleation	113,5± 8,9	1,67±0,15	3,96±0,79
7 days after enucleation	139,3±16,0	1,07±0,10	5,45±1,06
14 days after enucleation	83,2± 3,6	1,06±3,6	5,15±1,09
28 days after enucleation	94,5± 3,5	1,01±0,05	7,11±0,64

Number of animals in each group : 6 ; mean ± S. E. ; significant differences underlined ; one unit of MAG is equivalent to one μg of fetuin.

In central Wallerian degeneration of optic nerves there are other chemical events which are more pronounced. Following perikaryon-axon disconnection a marked reduction of the most sensitive protein fraction (the basic myelin protein) is observed. This reduction, however, is preceded by an early appearance of esterified cholesterol. From these results we are inclined to assume that undue esterification of cholesterol and not the alterations of MAG or basic protein could function at least as one of the primary factors injuring the molecular architecture of myelin membranes in the course of central Wallerian degeneration.

In the peripheral nervous system, where unlike in the CNS the major Po-glycoprotein constitutes a considerably greater part of the myelin proteins and is licalized both in the intraperiod and major dense lines, it was shown to disappear more rapidly than other proteins during Wallerian degeneration of the rat sciatic nerve [8]. The discrepant behaviour of the glycoprotein components in the central and peripheral nervous system are not surpising when the above mentioned differences in the structural localization of glycoproteins between the central and peripheral myelin are borne in mind. We have also found [5] that the decrease of MAG content in triethyl tin (TET) poisoning is proportional to the drop of total myelin proteins and thus is not indicative of a specific sensitivity of that glycoprotein to TET poisoning (Table 3). The slight decrease of the MAG component of total myelin proteins occurring on the top of the pathological process seems to result from the localization of MAG within the intraperiodic line, where the splitting of myelin lamellae takes place during TET poisoning.

Table 3. Total protein and MAG content in the myelin of the optic nerve in rats intoxicated with triethyl tin sulfate (TET)

	Total myelin proteins in μg per g of dry myelin	MAG in arbitrary units* per mg of total myelin proteins	MAG in arbitrary units* per mg of dry myelin
Control animals	204±8,90	20,7±1,14	4,22±0,41
5 hrs after intoxication	128±2,14	18,4±0,54	2,35±0,19
24 hrs after intoxication	137±5,50	17,1±1,04	2,34±0,24
7 days after intoxication	84±2,81	16,6±0,92	1,39±0,17
28 days after intoxication	135±3,93	24,8±0,88	3,35±0,23

Number of animals in each group: mean ± S.E.; significant differences underlined;
* one unit of MAG is equivalent to one μg of glucose oxidase (E.C. — 1.1.3.4.).

Table 4. MAG in SSPE cases (in arbitrary units per mg of total myelin proteins)

SSPE (4 cases)	Control (6 cases)
4,81±0,53	2,50±0,15

Myelin proteins in SSPE cases (in % of total myelin proteins)

	SSPE (4 cases)	Control (6 cases)
Wolfgram protein	27,0±1,7	22,6±1,4
Proteolipid	50,2±0,7*	38,7±0,7
Basic protein	22,8±2,1*	38,7±1,2

Note: Mean value for 5 brain pieces.

Table 5. CSF anti-MBP and anti-MAG antibody level (in cpm per 0,5 μg of CSF IgG)

	SSPE 10 cases	MS 18 cases	Neurotics 6 cases
Anti-MBP	2721±764*	832±452*	384±221
Anti-MAG	1337±480*	42±225*	183± 90

Changes in the content of MAG in brains of children who died from SSPE were also only negligible (Table 4), and the myelin fraction was even relatively enriched in this glycoprotein. By contrast, a very high level of antibodies against MAG was found by solid-phase radioimmunoassay in the CSF of SSPE cases (Table 5). That is why, we conclude that the alterations of MAG should not be considered as a trigger mechanism for demyelination in SSPE.

Itoyama et al. [1] reported a decreased MAG immunostaining in early acute multiple sclerosis lesions that extended far beyond the margin of acute demyelination. The loss of immunoreactivity could represent either a progressing loss of MAG molecules at the periphery of the developing plaque, or a great change in their antigenic properties, so that they no longer reacted

adequately with the appropriate antiserum. Contrary to that, immunocytochemical studies on leukodystrophies conducted by Ulrich and Heitz [4] on sections of brains from various forms of leukodystrophy showed that the distribution of MAG was remarkably little altered. In view of the results pertaining to the behaviour of MAG in various demyelinating processes, it should be stated that, although MAG shows considerable sensitivity towards definite pathological factors, it does not constitute a specifically weak chain in the molecular structure of the myelin sheaths that is affected independently by the agent that brings about demyelination.

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