Binding partners for the myelin-associated glycoprotein of N_2A neuroblastoma cells

Karen Strenge, Roland Schauer, Sørge Kelm*

Institute of Biochemistry, University of Kiel, Olshausenstrasse 40, 24098 Kiel, Germany

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Abstract The myelin-associated glycoprotein (MAG) has been proposed to be important for the integrity of myelinated axons. For a better understanding of the interactions involved in the binding of MAG to neuronal axons, we performed this study to identify the binding partners for MAG on neuronal cells. Experiments with glycosylation inhibitors revealed that sialylated *N*-glycans of glycoproteins represent the major binding sites for MAG on the neuroblastoma cell line N_2A. From extracts of [^{3}H]glucosamine-labelled N_2A cells several glycoproteins with molecular weights between 20 and 230 kDa were affinity-precipitated using immobilised MAG. The interactions of these proteins with MAG were sialic acid-dependent and specific for MAG.

Key words: Myelin-associated glycoprotein; Siglec; Sialic acid; Cell-cell interaction

1. Introduction

The myelin-associated glycoprotein (MAG) is a 100-kDa integral membrane glycoprotein expressed by myelinating glia cells in the central and peripheral nervous system [1]. It consists of five extracellular immunoglobulin-like (Ig-like) domains with 8–9 potential glycosylation sites, and a carboxy-terminal intracellular domain with several phosphorylation sites [2]. Structurally most closely related proteins are the Schwann cell myelin protein (SMP), sialoadhesin (Sn), CD22 and CD33, which all bind to sialic acid (Sia)-containing cell surface glycoconjugates [3,4]. Based on this specificity and some unique structural features, the name siglecs has been proposed for this group of Ig-like proteins [5].

For MAG, several functions in interactions between glia cells and neurones have been proposed [6–8]. Whereas in vitro experiments provided evidence that MAG is important for the initiation of myelination during development [9], myelination of axons occurs also in mice in which the MAG gene had been disrupted ($MAG^{-/-}$ mice) [10,11]. However, several abnormalities were observed in the morphology of myelinated axons in these animals. Animals older than 8 months show increased signs of a disturbed maintenance of their axon-myelin units indicating that MAG plays a role in long-term maintenance of the integrity of both myelin and axons [12,13].

An interesting aspect of MAG is its influence on neuronal growth. In vitro, MAG promotes neurite outgrowth of embryonal dorsal root ganglion neurones [14–16], whereas it inhibits the neurite outgrowth of cerebellar neurones, adult dorsal root ganglion neurones [15] and neuroblastoma cells [17].

The role of MAG as a neurone regeneration inhibiting molecule in vivo has remained controversial [6,7] since first experiments with $MAG^{-/-}$ mice gave no clear evidence for a significant contribution of MAG [18,19]. However, improved axonal regrowth has been demonstrated in $MAG^{-/-}$ mice cross-bred with animals of the strain C57BL/Wld^s that have delayed lesion-induced myelin degradation and axonal regrowth in the peripheral nervous system [20], indicating a significant inhibitory effect of MAG on neurone regeneration processes also in vivo.

Although the role of MAG as a cell adhesion molecule has been under investigation for many years, and binding activities to various extracellular components have been reported [21,22], no neuronal binding partners for MAG have been characterised so far. First evidence for Sia-containing glycoconjugates as binding partners for MAG came from the observation that MAG binds to sialylated glycans on erythrocytes [23]. The specificity of MAG for such glycans has been investigated in detail using gangliosides [24–26] or synthetic sialylated mono- and oligosaccharides [27,28]. Whereas experiments with resialylated red blood cells suggested that MAG binds preferentially to Neu5Aca2,3Galβ1,3GalNAc, a structure typically found on *O*-glycans of glycoproteins and on gangliosides [23], a direct comparison of free monovalent oligosaccharides in hapten inhibition assays revealed that structures found on *N*-glycans, i.e. Neu5Aca2,3Galβ1,3GlcNAc and Neu5Aca2,3Galβ1,4GlcNAc, were at least as well recognised as Neu5Aca2,3Galβ1,3GalNAc [28].

The functions of MAG mentioned above are likely to involve binding partners (counter-receptors) on neuronal cells. Although it is known that MAG binding is mediated by sialylated glycans, it has remained unclear by which structures, e.g. glycoproteins and/or glycolipids, these glycans are presented. Specific binding to gangliosides has been clearly demonstrated [23,24,26]. However, MAG binding to neuronal cells may be mainly to glycoproteins, since it is trypsin-sensitive [16]. The aim of this study has been the isolation of binding partners for MAG from the neuroblastoma cell line N_2A.

2. Materials and methods

2.1. Reagents

Affinity-purified anti-human IgG antibodies were from Biødesign, Kennebunk, ME, USA; carrier-free $Na^{125}I$ was from Amersham,

*Corresponding author. Fax: (49) (431) 880-2238.
E-mail: skelm@biochem.uni-kiel.de. URL: http://www.uni-kiel.de/biochemie/kelm/index.html

Abbreviations: a2,3SL, a2,3-sialyllactose; benzyl-GalNAc, benzyl-*N*-acetyl-a-D-galactosamine; Con A, concanavalin A haemagglutinin; FBS, foetal bovine serum; Ig-like, immunoglobulin-like; MAG, myelin-associated glycoprotein; $MAG^{-/-}$ mice, mice in which the MAG gene has been disrupted; PBS, phosphate-buffered saline; PNA, peanut haemagglutinin; Sia, sialic acid; SMP, Schwann cell myelin protein; Sn, sialoadhesin; VCS, sialidase from *Vibrio cholerae*

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Braunschweig, Germany; protein A beads were from Pharmacia Biotech, Uppsala, Sweden; sialidase from *Vibrio cholerae* was purchased from Behringwerke, Marburg, Germany.

2.2. Fc chimeras

Plasmids encoding the Fc chimeras containing the N-terminal three domains of MAG (Fc-MAG_{d1-3}) or the N-terminal five domains of MAG (Fc-MAG_{d1-5} [23], the N-terminal domain of sialoadhesin (Sn) (Fc-Sn_{d1}) [29] or the N-terminal three domains of murine CD22 (Fc-$CD22_{d1-3}$) [23] have been described. Fc chimeras were produced by transient expression of the plasmid in COS cells followed by purification from the tissue culture supernatants by immunoaffinity chromatography on protein A agarose [30]. Fc chimeras containing the N-terminal part of NCAM [31] were a kind gift from Dr Paul R. Crocker.

2.3. Binding assays

Purified Fc-MAG was labelled with ^{125}I and complexed with equimolar concentration of anti-human IgG to be used in binding assays as described [23,30,32].

2.3.1. Binding to adherent cells

Cells were grown on 10-mm coverslips, washed with phosphate-buffered saline (PBS) and fixed with 0.25% glutardialdehyde in PBS for 10 min at room temperature [33]. 200 μl radio-iodinated Fc-MAG/anti-human IgG complex was added to the cells, which were incubated overnight at 4°C. Unbound radioactivity was removed by dipping the coverslips three times in PBS containing 0.25% bovine serum albumin. Bound radioactivity was quantified by gamma counting. As a control for Sia specificity, binding to sialidase-treated cells was estimated.

2.3.2. Binding to cells in suspension

Adherent cells were detached from the cell culture plates with PBS containing 10 mM EDTA and 0.02% azide and fixed as described above. 25 μl radio-iodinated Fc-MAG/anti-human IgG complex was mixed with 25 μl cell suspension. After overnight incubation at 4°C bound radioactivity was quantified as described [30]. As a control for Sia specificity, binding to sialidase-treated cells was estimated and subtracted as background.

2.3.3. Elution of cell bound Fc-MAG/anti human IgG-complexes with a2,3-sialyllactose (a2,3SL)

Fc-MAG/anti-human IgG complexes were allowed to bind to cells in suspension and unbound radioactivity was removed by washing the cells. 20 μl a2,3SL at different concentrations was added to the cells and the mixture was incubated for 30 min at 4°C. The amounts of radioactivity remaining on the cells and in the supernatants were quantified by gamma counting. As a control for Sia specificity, binding to sialidase-treated cells was estimated and subtracted as background.

2.4. Glycosylation inhibition

N_2A cells were cultured for 3 days in the presence of 5 mM benzyl-*N*-acetyl-a-D-galactosamine (benzyl-GalNAc) for the inhibition of *O*-glycosylation or in the presence of 10 μM swainsonine to inhibit the processing of *N*-glycans.

2.5. [^{3}H]Glucosamine-labelled N_2A cell extracts

70% confluent N_2A cells were trypsinised in order to destroy MAG ligands on these cells, transferred to a new 100-mm cell culture dish at the same density and incubated in 6.6 ml DMEM containing 200 mM glutamine, 37.5 μl foetal bovine serum (FBS), 375 μl dialysed FBS and 20 MBq D-[6-^{3}H]glucosamine hydrochloride for at least 3 days. After labelling cells were washed with PBS and lysed with 1 ml 20 mM Tris pH 7.5 containing 150 mM NaCl, 5 mM EDTA, 1% NP-40 (lysis buffer) and the following protease inhibitors: 1.5 mM PMSF, 0.5 μg/ml leupeptin and 0.2 μg/ml trasylol. Insoluble material was removed by centrifugation for 15 min at 3500 min^{-1}.

2.6. Affinity precipitation of MAG binding partners

35 μg Fc chimeras were incubated for 1 h with 10 μl protein A beads at room temperature and unbound Fc chimeras were removed by three washes with 1 ml lysis buffer. 1 ml ^{3}H-GlcNAc-labelled N_2A cell extract was preincubated with 10 μl protein A beads for 1 h at 4°C, centrifuged and the supernatant was mixed with Fc-MAG-loaded protein A beads. After overnight incubation at 4°C under gentle shaking, the beads were centrifuged and washed four times with lysis buffer containing 0.1% NP-40. Bound proteins were eluted from the beads by boiling for 5 min in the presence of 20 μl of SDS-PAGE sample buffer.

2.7. SDS-PAGE and Western blotting

SDS-PAGE was performed according to Laemmli using 10% or 8% polyacrylamide gels. For Western blot analysis proteins were transferred to nitrocellulose and detected by autoradiography or by phosphor imaging.

3. Results

For the isolation of neuronal binding partners of MAG a suitable source had to be found. Since neurones from primary tissue culture are only available in limited amounts, several cell lines were compared for binding of MAG (Fig. 1). For all cell types, binding was Sia-dependent, since sialidase treatment of the cells reduced the amount of bound Fc-MAG to background levels. MAG showed high binding to the murine neuroblastoma cell line N_2A, whereas binding to PC12, C6, L929 and S-16 cells was significantly lower. Therefore, N_2A cells were chosen for the isolation of counter-receptors for MAG.

Up to now it has not been clear whether natural counter-receptors for MAG are glycoproteins and/or glycolipids. In order to investigate whether *N*- and/or *O*-glycans on glycoproteins are responsible for MAG binding to N_2A cells, these cells were cultivated in the presence of swainsonine, which prevents processing of *N*-glycans by inhibiting the Golgi mannosidase II [34], or the *O*-glycosylation inhibitor benzyl-GalNAc, which inhibits competitively the extension of GalNAcα-Ser/Thr on glycoproteins [35]. The effect of glycosylation inhibitors was checked by FACS-analysis with fluorescein-labelled lectins (data not shown). Binding of PNA to sialidase-treated cells revealed that *O*-glycosylation was reduced by benzyl-GalNAc but not by swainsonine. On the other hand, swainsonine-treated cells showed higher binding of concanavalin A (Con A) than untreated cells indicating that more accessible mannose residues were presented on these cells.

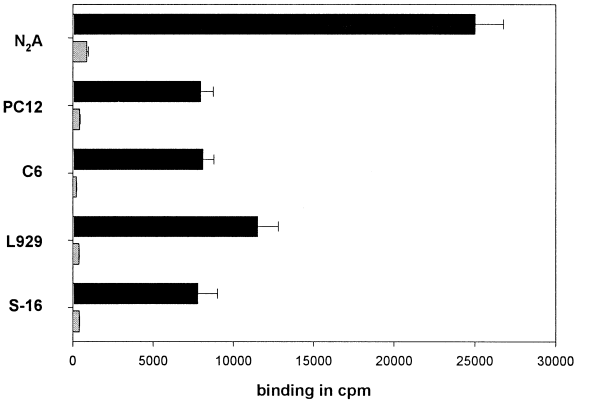

Fig. 1. Sialic acid-dependent binding of Fc-MAG_{d1-3} to different cell lines. Cells were grown on coverslips and fixed with glutardialdehyde. Binding of radio-iodinated antibody-complexed Fc-MAG to native and sialidase-treated cells was estimated as described in Section 2. N_2A = neuroblastoma cells, PC12 = cells from an rat adrenal phaeochromocytoma, C6 = rat glioma cells, L929 = mouse fibroblasts and S-16 = Schwann cell line.

Furthermore, the lectin staining demonstrated that the overall cell surface expression of *N*- or *O*-glycosylated proteins was not disturbed by swainsonine. If assayed for Sia-dependent MAG binding, swainsonine-treated N_2A cells did not bind Fc-MAG above background levels, whereas benzyl-GalNAc treatment apparently did not significantly influence MAG binding (Fig. 2). This is strong evidence that the majority if not all of the MAG binding sites are complex *N*-glycans on glycoproteins.

Therefore, MAG counter-receptors were purified from protein extracts of N_2A cells by affinity precipitation with Fc-MAG bound to protein A agarose. First, molecules which bind to protein A beads independent of MAG were removed by preincubation of the cell extract with protein A beads in the absence of Fc-MAG. In order to allow easy detection of neuronal glycoproteins after isolation N_2A cells were metabolically labelled with ^{3}H-GlcNAc. Since the affinity of MAG to a single glycan is relatively low, a high density of Fc-MAG bound to protein A beads was used for the precipitation in order to allow polyvalent interactions. Less than 5% of the radioactivity incorporated into the cells was precipitated with Fc-MAG-loaded protein A beads. The autoradiograph of proteins affinity-precipitated with MAG revealed several somewhat diffuse bands typical for glycoproteins (Fig. 3A, lane 2). The apparent molecular masses are between 20 and 130 kDa. The broad band above 116 kDa (Fig. 3A, lane 2) appears to be a double band of two proteins with molecular weights about 116 and 130 kDa, if proteins are separated on 8% polyacrylamide gels (Fig. 4A,C). In addition, glycoproteins with molecular masses of 180 and 230 kDa were precipitated. However, these were only detected by the more sensitive phosphor imaging technique (Fig. 4A,C), whereas they were hardly visible on autoradiographs (Fig. 3A, lane 2). Since the lysis of N_2A cells was performed in the presence of protease inhibitors and the pattern of glycoproteins precipitated was reproducible in several independent experiments, it is unlikely that the bands are the products of proteolytic cleavage.

The next question was whether these glycoproteins interact specifically with MAG or whether they also bind to other cell interaction molecules. Therefore, the same affinity purification was performed with Fc-NCAM (Fig. 3A, lane 3), Fc-CD22 (Fig. 3A, lane 4) or Fc-Sn (Fig. 3B, lane 2) instead of Fc-MAG. In these experiments several proteins were precipitated, but they were different from those obtained with Fc-MAG, even if Fc-Sn was used, a siglec which has a glycan specificity

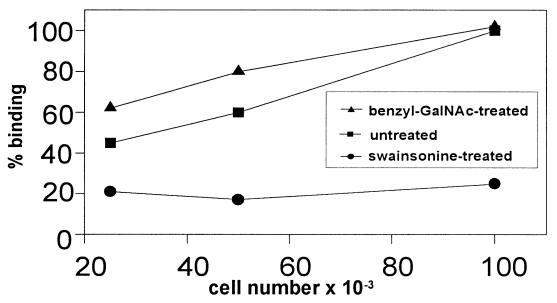

Fig. 2. Influence of glycosylation inhibitors on the Sia-dependent binding of Fc-MAG to N_2A cells. N_2A cells were grown for 3 days in the presence of the glycosylation inhibitors indicated and Sia-specific binding of radio-iodinated antibody-complexed Fc-MAG to 2.5×10^4, 5×10^4 and 10×10^4 of these cells or to control cells was estimated as described in Section 2.

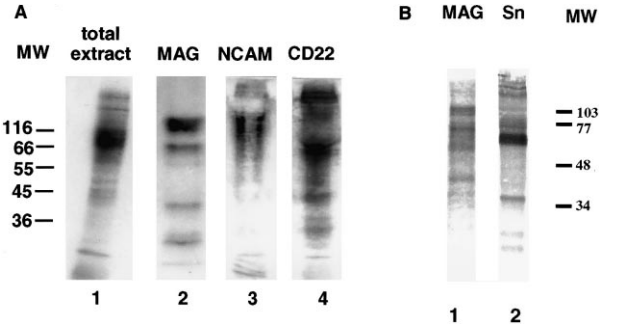

Fig. 3. SDS-PAGE of affinity-precipitated glycoproteins from N_2A cells with different adhesion molecules. Affinity precipitations of binding partners for different cell interaction molecules from extracts of [^{3}H]glucosamine-labelled N_2A cells were performed as described Section 2. A: Samples were analysed by SDS-PAGE on a 10% polyacrylamide gel. Lane 1: 10 μl of total cell extract; lane 2: glycoproteins from affinity precipitations with Fc-MAG_{d1-3}; lane 3: glycoproteins from affinity precipitation with Fc-$NCAM_{d1-5}$; lane 4: glycoproteins from affinity precipitation with Fc-$CD22_{d1-3}$. Molecular weight markers are shown on the left. B: Samples were analysed by SDS-PAGE on an 8% polyacrylamide gel. Lane 1: glycoproteins from affinity precipitations with Fc-MAG_{d1-5}; lane 2: glycoproteins from affinity precipitation with Fc-Sn_{d1}. Molecular weight markers are shown on the right.

similar to MAG. This indicates that the isolated glycoproteins bind MAG with high specificity. It is important to note that differences in the pattern of bands precipitated with Fc-MAG (Fig. 3A, lane 1 vs Fig. 3B, lane 1) are due to different percentages of polyacrylamide used for SDS-PAGE. Because of a better resolution at high molecular weights 8% polyacrylamide gels were used in all subsequent experiments.

The Sia dependence of the affinity precipitation has been shown by sialidase treatment of ^{3}H-GlcNAc-labelled N_2A cells before lysis. Only three bands were detectable in the extracts of sialidase-treated cells (Fig. 4A, lane 2). However, the intensities of these bands were much lower than those from parallel precipitations of extracts from untreated cells indicating that also these molecules were bound in a Sia-dependent manner. The same results were obtained if the Fc-MAG/protein A beads were preincubated with 10 mM a2,3SL as inhibitor for Sia-dependent binding (Fig. 4A, lane 3).

To investigate whether binding of MAG to its binding partners is reversible by the addition of competitive inhibitors, N_2A cells with bound radio-iodinated Fc-MAG/anti-human IgG complexes were incubated with different concentrations of a2,3SL as competitive inhibitor for the Sia binding site on MAG. Depending on the a2,3SL concentration the cell-bound radioactivity decreased, corresponding to an increase of radioactivity in the supernatant (Fig. 4B). Almost complete elution was obtained at an a2,3SL concentration of 50 mM, whereas no radioactivity was eluted by PBS (data not shown). These results indicate that counter-receptors can be eluted from MAG by suitable competitive inhibitors.

Taking advantage of this finding, a2,3SL was applied for the elution of MAG counter-receptors bound to the Fc-MAG/protein A beads as a further purification step. Some of the precipitated glycoproteins were eluted by incubation with 100 mM a2,3SL whereas no proteins could be eluted with PBS (Fig. 4C). The band pattern in this experiment seems to be somewhat different especially for bands in the 100–150-kDa range. This is due to aberrant running behaviour of samples

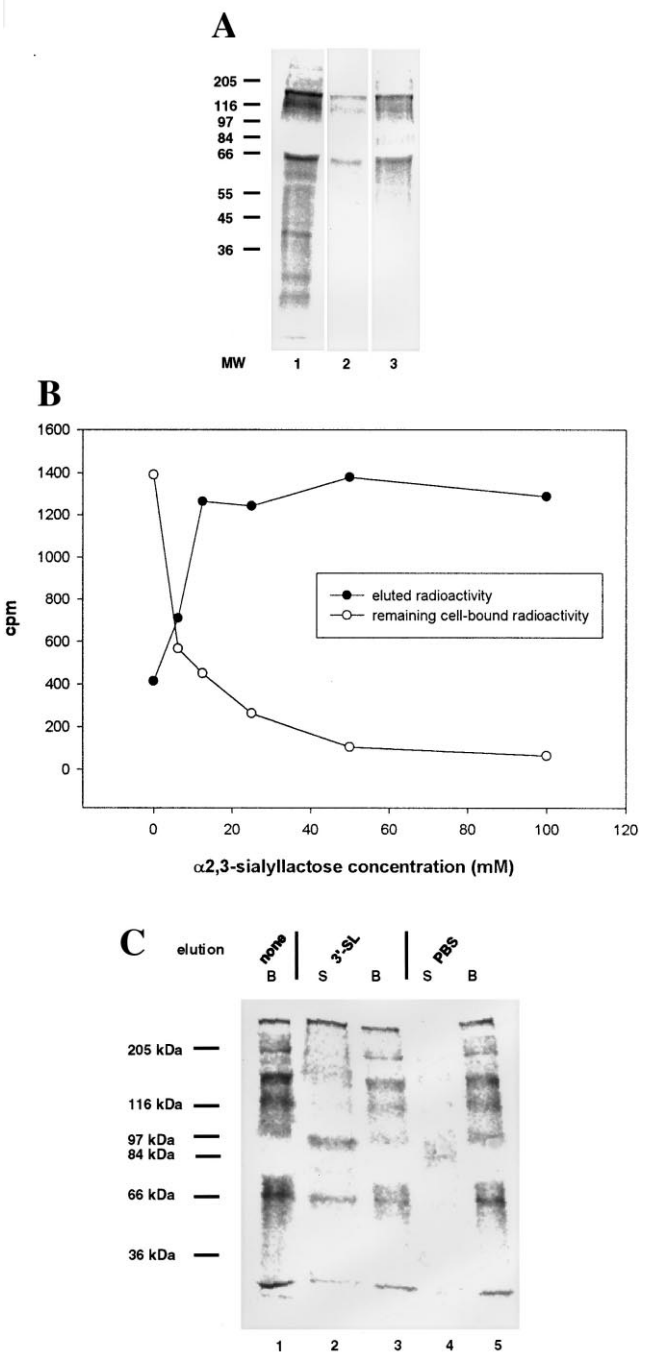

Fig. 4. Sialic acid dependence of affinity precipitations with Fc-MAG_{d1-3} from [^{3}H]glucosamine-labelled N_2A cell extract. A: SDS-PAGE of affinity precipitations with [^{3}H]glucosamine-labelled N_2A cell extract. Affinity precipitations of ligands for Fc-MAG_{d1-3} from [^{3}H]glucosamine-labelled N_2A cell extracts were performed as described in Section 2. Lane 1: glycoproteins from affinity precipitation with Fc-MAG_{d1-3}; lane 2: glycoproteins from affinity precipitation with Fc-MAG_{d1-3} of extract from VCS-treated [^{3}H]glucosamine-labelled N_2A cells; lane 3: glycoproteins from affinity precipitation with Fc-MAG_{d1-3} preincubated with 10 mM a2,3SL. Molecular weights are indicated on the left. B: Elution of prebound Fc-MAG from N_2A cells. Radio-iodinated antibody-complexed Fc-MAG was allowed to bind to N_2A cells and complexes were eluted by the addition of a2,3SL at different concentrations as described in Section 2. C: Elution of MAG binding partners with a2,3SL after affinity precipitations with Fc-MAG_{d1-3} from [^{3}H]glucosamine-labelled N_2A cell extract. SDS-PAGE of affinity precipitations of ligands for Fc-MAG_{d1-3} from [^{3}H]glucosamine-labelled N_2A cell extract were performed as described in Section 2. Lane 1: glycoproteins from affinity precipitation with Fc-MAG_{d1-3}; lane 2: glycoproteins eluted with 100 mM a2,3SL from Fc-MAG/protein A beads after affinity precipitation with Fc-MAG_{d1-3}; lane 3: glycoproteins which remained on the protein A beads after elution of ligands with a2,3SL; lane 4: glycoprotein eluted with PBS; lane 5: glycoproteins which remained on protein A beads after elution of ligands with PBS. Molecular weights are shown on the left. ←

from affinity precipitations as a result of large amounts of unlabelled Fc-MAG in these lanes.

4. Discussion

Various roles for MAG have been proposed in myelin/axon interactions. For a better understanding of these processes the neuronal binding partners of MAG have to be identified and characterised. In choosing a suitable source for MAG ligands several problems have to be considered. For a biochemical characterisation of the isolated molecules sufficient amounts are necessary, which may not be easy to obtain from freshly isolated neurones or primary cell cultures. Furthermore, it has to be kept in mind that in vivo neurones from different areas and stages of development do not necessarily carry the same counter-receptors for MAG. Therefore, we decided to use a cell line which can be obtained in large amounts. In addition, such cell lines allow easy metabolic labelling of glycoconjugates for a more sensitive detection. Since also a neuroblastoma cell line was used for studies showing MAG inhibition of neurite outgrowth [17], it is likely that molecules which are relevant for MAG-mediated signalling also occur on neuroblastoma cell lines. N_2A cells were used, since they showed high Sia-dependent binding of MAG (Fig. 1), indicating that possibly sufficient amounts of counter-receptors for MAG are expressed by these cells. Of course it remains uncertain whether these represent the same molecules expressed by neurones in vivo. First experiments with neurones from primary cell culture indicate that this is indeed the case (data not shown). Future studies will address this question.

Counter-receptors for MAG could be glycoproteins or glycolipids. In vitro MAG can bind to both [23–26]. The finding that MAG binding is trypsin-sensitive [16] has indicated that ligands for MAG are mainly glycoproteins. This is strongly supported by our finding that N_2A cells grown in the presence of the *N*-glycosylation inhibitor swainsonine loose their ability to bind MAG (Fig. 2). This finding implies that glycoproteins and not glycolipids are the main structures recognised by MAG on these cells. Swainsonine inhibits the processing of *N*-glycans of glycoproteins, whereas other types of glycans such as *O*-glycans on glycoproteins or glycolipids are not affected [34]. In spite of its effect on oligosaccharide biosynthesis, swainsonine seems not to impair the cell surface expression of incompletely processed glycoproteins [36] as demonstrated by the lectin staining experiments. In addition, inhibition of *O*-glycosylation by benzyl-GalNAc did not significantly influence binding of MAG to these cells (Fig. 2), strongly suggesting that *O*-glycans do not contribute to a large extent to MAG binding in this system. In hapten inhibition assays with synthetic oligosaccharides it has been demonstrated that the terminal sequences Neu5Aca2,3-

Galβ1,3GlcNAc and Neu5Aca2,3Galβ1,4GlcNAc occurring on *N*-linked glycans are recognised by MAG as well as Neu5Aca2,3Galβ1,3GalNAc found on *O*-linked glycans [28], although binding assays with derivatised erythrocytes had suggested that MAG binds only to Neu5Aca2,3Galβ1,3GalNAc [23]. However, this is likely to be due to the three-fold higher amount of Neu5Aca2,3Galβ1,3GalNAc than that of Neu5Aca2,3Galβ1,3/4GlcNAc on these cells [32]. Furthermore, if Neu5Ac is replaced by high-affinity Sia like Neu5ClAc or Neu5BrAc, Siaa2,3Galβ1,3(4)GlcNAc glycans efficiently support binding of MAG to resialylated erythrocytes, indicating that these glycans can serve as ligand determinants on cell surfaces [27].

The affinity precipitation experiments with Fc-NCAM, Fc-CD22 or Fc-Sn indicated that the isolation of potential counter-receptors is mediated by specific binding to the extracellular domains of MAG and not to other parts of the Fc chimeras. NCAM is a neuronal cell adhesion molecule with a substantial degree of sequence similarity to MAG [37]. The broad smear precipitated by Fc-NCAM could be NCAM which is expressed by N_2A cells and is known to exhibit homophilic interaction. CD22 is a member of the siglec family but recognises a2,6-linked Sia, whereas MAG binds to a2,3-linked Sia [3]. Therefore, it is unlikely that CD22 recognises the same glycan structure as MAG but it could bind the same glycoproteins if these contain a2,6-linked Sia. This seems not to be the case, since proteins precipitated with Fc-CD22 exhibit a distinct pattern different from that obtained with Fc-MAG.

Sn, another member of the siglec family, has a similar glycan specificity as MAG [27,28]. Therefore, it could be expected that the same glycoproteins isolated as ligands for MAG may also bind to Sn. However, the observation that different proteins were precipitated by the Fc chimeras of MAG and Sn indicates that additional structural features besides Sia recognition are involved. This is supported by the finding that modifications of the Sia residue [27] and the underlying glycan [28] contribute significantly to differences in the binding behaviour of MAG and Sn. Therefore, high specificity could also be caused by yet unknown glycans occurring on neuronal cells which are bound with high affinity by MAG but not by Sn. On the other hand, evidence has been reported that Sia-dependent binding may be a primary docking mechanism and that an additional Sia-independent binding site exists on MAG [38]. Such additional binding could also be a reason for the selective interaction of MAG observed in this study.

Most, if not all, of the interaction of the isolated glycoproteins with MAG is Sia-dependent. This has been demonstrated by sialidase treatment of N_2A cells before lysis, the inhibitory effect of preincubating MAG with a2,3SL and by the specific elution of bound glycoproteins with a2,3SL. Only small amounts of the precipitated proteins appear to bind to MAG independent of Sia. This could be due to insufficient sialidase treatment or incomplete inhibition by a2,3SL. However, it cannot be excluded completely that the precipitation of these proteins is mediated by Sia-independent interactions.

Since the MAG ligands are precipitated from a total cell extract, it is possible that besides cell surface molecules glycoproteins from intracellular compartments were also isolated. However, the fact that sialidase treatment of the intact cells before lysis prevents precipitation of most of the counter-receptors indicates that these proteins represent cell surface molecules.

Our results show that MAG binds to *N*-glycans on glycoproteins of the neuronal cell line N_2A in a Sia-dependent manner. A method for the isolation of counter-receptors for MAG from these cells has been developed and several glycoproteins have been isolated as potential ligands for MAG. One important question will be whether these glycoproteins also mediate the binding of MAG to neurones in vivo. To answer this question it will be necessary to determine the amino acid sequence of these proteins and to study their expression pattern during the development of the nervous system.

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