The Amino Acid Sequences of the Myelin-associated Glycoproteins: Homology to the Immunoglobulin Gene Superfamily

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Abstract. The myelin-associated glycoproteins (MAG) are integral plasma membrane proteins which are found in oligodendrocytes and Schwann cells and are believed to mediate the axonal-glial interactions of myelination. In this paper we demonstrate the existence in central nervous system myelin of two MAG polypeptides with Mr of 67,000 and 72,000 that we have designated small MAG (S-MAG) and large MAG (L-MAG), respectively. The complete amino acid sequence of L-MAG and a partial amino acid sequence of S-MAG have been deduced from the nucleotide sequences of corresponding cDNA clones isolated from a lambda gt11 rat brain expression library. Based on their amino acid sequences, we predict that both proteins have an identical membrane spanning segment and a large extracellular domain. The putative extracellular region contains an Arg-Gly-Asp sequence that may be involved in the interaction of these proteins with the axon. The extracellular portion of L-MAG also contains five segments of internal homology that resemble immunoglobulin domains, and are strikingly homologous to similar domains of the neural cell adhesion molecule and other members of the immunoglobulin gene superfamily. In addition, the two MAG proteins differ in the extent of their cytoplasmically disposed segments and appear to be the products of alternatively spliced mRNAs. Of considerable interest is the finding that the cytoplasmic domain of L-MAG, but not of S-MAG, contains an amino acid sequence that resembles the autophosphorylation site of the epidermal growth factor receptor.

The myelin-associated glycoproteins (MAG) are plasma membrane proteins of myelin-forming oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (reviewed in reference 40). Although the precise role of these proteins in the formation and maintenance of the myelin sheath is not known, it has been proposed that they are important in maintaining the apposition of the myelin sheath to the axon (40). Consistent with this idea is the localization of MAG to the periaxonal glial membrane, and its absence in compact myelin (50, 55, 56, 27). Furthermore, studies of the dysmyelinating mouse mutant, Quaking (the primary defect of which is not known, but which has only 15% of the normal levels of MAG [20]), have revealed an abnormally widened space between the axon and the innermost turn of the myelin sheath in discreet regions where MAG cannot be detected immunocytochemically (57). Lastly, MAG has an extensive extracellular exposure (35) and shares a carbohydrate determinant (HNK-1) with several other molecules that are proposed to mediate cell-cell interactions in the developing nervous system, including the neural cell adhesion molecule (N-CAM) and L1 (28, 21). Whether all of these presumptive nervous system adhesion molecules, including MAG, share general structural and amino acid sequence homologies has not yet been elucidated.

Two MAG polypeptides (M, 72,000 and 67,000) are detectable in in vitro translation systems programmed with total brain mRNA (10). Presumably, these polypeptides, when glycosylated in vivo, co-migrate on SDS PAGE as the single, characteristically broad band (Mr, 100,000) that corresponds to native MAG (40). The precise structural differences between the two MAG proteins are not known. Peptide maps of the two polypeptides are nearly identical (10) and suggest that the polypeptides differ by a single segment that is present only in the larger protein. Interestingly, the larger protein is expressed during the early rapid phase of myelination while the smaller protein is synthesized primarily in the adult, when myelination is nearly complete (10). This may reflect different functions for the individual MAG proteins in myelination.

In the present study we report the complete amino acid sequence of the large MAG polypeptide (L-MAG) and a partial amino acid sequence of the small MAG polypeptide (S-
MAC), deduced from the nucleotide sequences of corresponding cDNA clones isolated from a rat brain lambda gt11 expression library. Analysis of the primary amino acid sequences reveals several features of these proteins that may be related to their postulated function as glial–neuron recognition molecules. These include: (a) the tripeptide sequence Arg-Gly-Asp (RGD), which has been found to mediate binding in several receptor–ligand systems (42), (b) five tandem repeats of a highly conserved peptide domain within the extracellular portion of the protein, and (c) a cytoplasmically disposed region that is shorter in S-MAG than in L-MAG. The conserved extracellular domains, which are centered around cysteine residues, are significantly homologous to the variable regions of immunoglobulins and related membrane receptors, as well as to N-CAM. Finally, we report that the 3' end of L-MAG is identical to a brain-specific cDNA clone, pIB236 (52), which has been well studied and was believed to be neuron-specific.

Materials and Methods

Generation of Anti-MAG Antibodies

MAG was isolated by the method of Quarles and Pasnak (39). In brief, purified rat brain myelin (32) was extracted with chloroform/methanol (2:1 vol/vol). The insoluble residue was treated with 0.25 M lithium diiodosalicylate and partitioned with phenol. The aqueous phase, which is enriched in MAG, was dialyzed and lyophilized. MAG was electrophoretically separated by preparative SDS PAGE (10% acrylamide) and the broad 100-kD MAG band was excised and electroeluted (16). Antibodies were raised by injecting purified MAG (50 μg) into rabbit popliteal lymph nodes in complete Freund's adjuvant and boosting every other week with 100 μg of MAG in incomplete adjuvant. Antiserum was affinity purified (26) against a lithium diiodosalicylate extract of myelin that had been endoglycosidase F-treated (New England Nuclear, Boston, MA) before coupling to cyanogen bromide-activated Sepharose CL-4B beads. The affinity-purified antibody was eluted with 4 M sodium thiocyanate, and dialyzed against several changes of sodium PBS (pH 7.5). By immunoblot analysis (54), this antibody detected a single broad band of 100 kD that was present in extracts of whole rat brain and was markedly enriched in a purified rat central myelin preparation. Immunocytochemical analysis of tissue sections of 4% paraformaldehyde-fixed adult rat brain demonstrated that the antibody specifically recognized myelinated fiber tracts.

Construction and Screening of a Rat Brain cDNA Library

10 μg of Poly(A)+ RNA isolated from rat brain (postnatal day 20) was used as a template for cDNA synthesis. The first strand was synthesized using the protocol provided with M-MLV reverse transcriptase (Bethesda Research Laboratories, Gaithersburg, MD), and second strand synthesis was performed as described (13). Double-stranded cDNA (2 μg) was treated (20 min, 37°C) with 5 U of mung bean nuclease and the double-stranded cDNA was size-fractionated on a preparative SDS PAGE (10% acrylamide) and the broad 100-kD MAG band was excised and electroeluted (16). Double-stranded cDNA (2 μg) was treated (20 min, 37°C) with 5 U of mung bean nuclease (PL-Pharmacia, Piscataway, NJ), in 50 mM NaCl, 30 mM Na acetate, pH 5.5, 1 mM ZnCl2, and 3% glycerol (100 μl final volume). The double-stranded cDNA was then methylated in a standard ligation reaction. Redundant linker sequences were excised with Eco RI enzyme and the double-stranded cDNA was size-fractionated on a Sepharose CL-4B (PL-Pharmacia) column (10 ml). cDNAs larger than 1.5 kb were ligated to lambda phage gII1 arms (Strategene), that had been cleaved and dephosphorylated at the single Eco RI site, and packaged into bacteriophage with a commercial packaging extract (Strategene Cloning Systems, San Diego, CA). This library contained 3 × 108 independent recombinants.

Affinity-purified anti-MAG antibodies were used to screen a portion (106 recombinants) of this library (66). Positive colonies were identified with a goat anti-rabbit antibody (Cappel Laboratories, Malvern, PA) conjugated to horseradish peroxidase in a standard reaction.

Nucleic Acid Blotting and Hybridization

RNAs (5-10 μg of total, 3 μg of Poly(A)+) were subjected to electrophoresis on 1.7% agarose gels containing formaldehyde, and transferred by capillary blotting to filters (Genescreen, New England Nuclear). These filters were probed with cDNA inserts which were nick-translated with deoxycytidine 5'-triphosphate [α32P] to a specific activity of 5 × 106 cpm/μg. Hybridization and washing were carried out as recommended by the manufacturer (New England Nuclear). DNA blotting was performed by the method of Southern (47, 7) and plaque hybridization by methods described in Maniatis et al. (25).

DNA Sequencing

DNA sequencing was performed by the dye deoxyxy chain termination technique (45). Restriction endonuclease fragments were directionally cloned into the M13 bacteriophages mp18 and mp19 (Pharmacia Fine Chemicals) and M13 um20 (International Biotechnologies, Inc., New Haven, CT), which were digested to yield compatible cloning sites. In one case an oligonucleotide primer, synthesized with a DNA synthesizer (Applied Biosystems, Foster City, CA), was used to complete the sequence.

Iodination of Myelin and Immunoprecipitations

Aliquots (1 μg protein) of purified myelin (32) were washed three times by suspension in 1 ml of 50 mM Na borate buffer (pH 8), pelletted by centrifugation (100,000 g, 10 min), and resuspended. Iodination was carried out with the Bolton–Hunter reagent according to the manufacturer's instructions (ICN Biomedicals, Inc., Irvine, CA). The 125I-labeled myelin was washed extensively (5 × 1 ml) in 400 mM Tris-HCl (pH 7.5) and then solubilized in 100 μl 2% SDS (80°C) before immunoprecipitation by the procedure of Goldman and Blobel (12).

Protein Blotting and Epitope Selection

Escherichia coli strain Y1089 was lysogenized with a recombinant bacteriophage clone (66). Approximately 106 lysogenized bacteria were incubated at 42°C for 20 min and 10 mM isopropylthio-β-D-galactoside was added for 1.5 h to induce the β-galactosidase fusion protein. The bacteria were recovered by centrifugation, sonicated in 10% SDS, and the proteins were fractionated by preparative SDS PAGE. The fusion protein was identified by light staining with Coomassie Blue and was recovered from the gel by electroelution. 100 μg was injected into rabbits every other week to generate antibodies. These antibodies were affinity purified against the fusion protein before being used on Western blots of myelin. Alternatively, electrophoretically separated proteins extracted from the bacteria were transferred onto nitrocellulose paper and the fusion protein band was identified (by brief staining with 0.1% Fast Green), excised, and used as an adsorption matrix for the anti-MAG antiserum. This band was incubated overnight with a 1:10 dilution of the antiserum at 4°C. The nitrocellulose strip was then washed four times with PBS containing 0.1% Triton X-100 and 0.02% gelatin, and the bound antibody was eluted with 0.1 M glycine HCl (pH 3.0) for 1 min and rapidly neutralized with Tris HCl, pH 8.9. The eluted antibodies were used to probe myelin immunoblots and visualized with a goat anti-rabbit IgG conjugated to horseradish peroxidase in a standard reaction mixture.

In Vitro Transcription and Translation of L-MAG

Restriction mapping and sequence analysis of cDNA clone M10D (Fig. 5) revealed that two Apa 1 sites existed in the 5' and 3' untranslated regions, respectively. This allowed the subcloning of the entire coding region into the Bluescript vector (Strategene). mRNA was transcribed with T3 polymerase from 2 μg of purified plasmid in a 10-μl reaction mixture, using the riboprobe system II (Promega Biotec, Madison, WI), according to the manufacturer's instructions. The reaction mixture also contained mG(5'-PPP)5'G(Pharmacia Fine Chemicals) to cap the mRNA. 1 μl of the mixture was used to program a wheat germ translation system containing [35S]methionine and incubated for 2 h at 28°C.

Computer Analysis of the Amino Acid Sequence of MAG

The protein data base of the National Biomedical Research Foundation was searched for homologous protein sequences by using the FASTP computer program (24). This program was also used to obtain optimized similarities.
and the percent identity for each homology found. The significance of each homology was determined by the RDF program and expressed as a Z score (24). Internal segments of homology were visually aligned and also compared pairwise by the ALIGN program (49, 6). The ALIGN program was also used to optimize identities between N-CAM and MAG. The hydrophobicity analysis was performed by the ANALYSEP program (22).

Results

Anti-MAG Antibodies Recognize Two Polypeptides in Myelin

The polyclonal antibody was used to immunoprecipitate MAG from \(^{32P}\)-labeled (CNS) myelin. SDS PAGE analysis of the immunoprecipitate revealed that, as expected, MAG migrates as a broad band of \(M_r 100,000\) (Fig. 1, lane a). After enzymatic deglycosylation of the immunoprecipitate with Endo F, two polypeptides of \(M_r 72,000\) and 67,000 were detected (Fig. 1, lane b). We have designated these proteins as L-MAG and S-MAG, respectively. This result directly demonstrates that adult rat CNS myelin contains two MAG proteins, whose presence had been previously inferred by in vitro translation studies of total brain mRNA (10). It is of interest that although L-MAG is apparently synthesized early and S-MAG is synthesized later in development (10), we have detected both proteins in equal abundance in the mature myelin sheath (Fig. 1, lane b).

Isolation of cDNA Clones that Encode Two MAG Polypeptides

A rat brain cDNA library constructed in the lambda gt11 vector was screened with the affinity-purified antibody and six immunopositive clones were identified. Three of these six cDNAs were found to cross-hybridize, and one clone, designated M10, was selected for further study. The insert of this clone was verified as a MAG cDNA by immunologic criteria. The M10 clone contains a cDNA insert of 663 bp; this clone expresses a \(\beta\)-galactosidase fusion protein of \(M_r 140,000\). Since \(\beta\)-galactosidase has a molecular mass of \(\sim 117\) kD, we estimated that the MAG portion of the fusion protein was \(\sim 23\) kD, encompassing about one-third of the MAG polypeptide. This fusion protein was used in an epitope selection experiment (61), in which antibodies that specifically cross-reacted with the M10 fusion protein (immobilized on nitrocellulose paper) were isolated from the polyclonal MAG antiserum. The reactivity of the affinity-purified antibodies was then compared with GEN S-3, an anti-MAG monoclonal antibody (31), on immunoblots (Fig. 2). Both antibodies recognize the same 100-kD MAG band on Western blots of CNS myelin. In a control experiment, the fusion protein of a lambda gt11 myelin basic protein clone (isolated from this library), failed to select any antibodies that reacted with MAG (Fig. 2, lane d). Finally, polyclonal antibodies were raised directly against the M10 fusion protein in several rabbits. These antibodies also specifically recognize MAG, as demonstrated on immunoblots of rat myelin (Fig. 2, lane c), and by immunoprecipitation of both native and the deglycosylated MAG polypeptides from iodinated myelin (data not shown).

We rescreened the lambda gt11 library by plaque hybridization with the \(^{32P}\)-labeled, M10 cDNA insert and identified two larger homologous clones. One of these clones, M10D, contains a cDNA insert of 2348 bp that is long enough to encode either MAG polypeptide. This cDNA was subcloned into the Bluescript plasmid vector and was further characterized by in vitro transcription and translation (Fig. 3). The synthetic mRNA transcribed with T3 polymerase was used to program a wheat germ cell-free translation system. The primary translation product had an \(M_r 73,000\) as measured on SDS PAGE (similar in size to the large MAG polypeptide), and was immunoprecipitable with anti-MAG antiserum (Fig. 3, lane b). The immunoprecipitation was completely inhibited by the addition of unlabeled MAG purified from myelin (Fig. 3, lane c), confirming the identity of the translation product and of the clone. The other large clone, M10E, contains a cDNA insert of 1083 bp. Sequence data, discussed below, reveals that it corresponds to an S-MAG cDNA.

Tissue and Temporal Expression of MAG mRNA

MAG mRNA levels were assessed by RNA blot analysis in

Figure 1. Two MAG polypeptides can be detected in myelin. An aliquot (1 \(\mu\)g protein) of purified osmotically shocked myelin was iodinated with Bolton-Hunter reagent, solubilized with detergents, and immunoprecipitated (12) with anti-MAG antiserum. Immunoprecipitates were subjected to electrophoresis directly on 10% polyacrylamide gels (lane a) or first treated with 2 U of endoglycosidase F for 10 h at 37°C before electrophoresis (lane b). Native MAG (lane a) has an \(M_r\) of 100,000; the L-MAG and S-MAG polypeptides (lane b) have \(M_r\) values of 72,000 and 67,000, respectively.

Figure 2. Characterization of the M10 fusion protein by immunoblot analysis. Each lane corresponds to 10 \(\mu\)g of purified myelin that was fractionated by SDS PAGE (10% acrylamide), electrophoretically transferred to nitrocellulose paper, and processed for immunoblotting. Nitrocellulose strips were incubated with the following antibody preparations: (lane a) a mouse anti-MAG monoclonal (31); (lane b) polyclonal MAG antiserum adsorbed to and eluted from the M10 fusion protein; (lane c) antibody raised and affinity purified against the M10 fusion protein; and (lane d) polyclonal anti-MAG antiserum absorbed and eluted from a myelin basic protein fusion protein.
Figure 3. Characterization of the M10D translation product. The complete coding region of the M10D cDNA insert was subcloned into the Apa I site of the plasmid vector Bluescript and mRNA was transcribed with T7 polymerase using the riboprobe system II according to the manufacturer’s instructions. (Lane a) ~0.3 μg of RNA transcribed in vitro was translated in a wheat germ cell-free system with [35S]methionine. (Lanes b and c) Immunoprecipitations of the translated products with anti-MAG antiserum in the absence (b) or presence (c) of 10 μg of unlabeled MAG purified from myelin. Immunoprecipitates were separated by SDS PAGE (8% acrylamide), and treated for fluorography with EN3HANCE (New England Nuclear).

Figure 4. RNA blot analyses of MAG mRNA expression. (A) Tissue distribution of MAG mRNA. 3 μg of Poly (A)⁺ RNA was isolated from various tissues of 20-d-old rats and subjected to electrophoresis in a 1.7% agarose gel containing formaldehyde. The RNA was transferred to GeneScreen membranes and hybridized to 0.5 μg of the nick-translated, 32P-labeled M10 cDNA. (Lane a) Brain; (Lane b) sciatic nerve; (Lane c) thymus; (Lane d) liver. The mRNA sizes indicated were estimated from the position of the 18S and 28S RNA bands. (B) Expression of MAG mRNA during development. Total brain RNA was prepared from rats killed on the postnatal day indicated and 5 μg were electrophoretically separated on formaldehyde containing agarose gels, transferred to GeneScreen, and hybridized as above.

Nucleotide and Deduced Amino Acid Sequences of the MAG Proteins

The sequencing strategy used is illustrated in Fig. 5 and the complete nucleotide sequence and deduced amino acid sequence for both M10D and M10E are shown in Fig. 6. M10D is 2348 nt long and contains an open reading frame of 1878 nt that begins with an ATG 126 nt downstream from the 5' end of the clone and 24 nt downstream from an in-frame stop codon. This open reading frame is followed by a TGA (position 2004–2006) and 342 bp of 3' untranslated sequence. M10E is 1083 nt long. It is identical to the 3' half of M10D except for an additional internal sequence of 45 nt (that begins after nt 1841 of M10D). This segment introduces a stretch of 10 amino acids followed by an in-frame termination codon that shortens the open reading frame by 135 nt (Fig. 6). Both cDNA inserts contain the Poly A acceptor sequence (AATAAA) (36) and M10E ends in a Poly A tract.

M10D encodes a polypeptide of 626 amino acids with a calculated molecular mass of 69.3 kD. This value agrees well with the molecular masses estimated by SDS PAGE for the translation product of the M10D transcript (73 kD) and for the deglycosylated L-MAG protein (72 kD). M10E encodes a MAG polypeptide that is calculated to be smaller at the carboxy terminus by 5.1 kD. Because the native MAG polypeptides in myelin are known to differ by 5 kD, it is likely that M10D is a full-length L-MAG cDNA and M10E is an incomplete S-MAG cDNA.

A hydrophobicity analysis (22) of L-MAG revealed two extended hydrophobic segments. The first, which occurs at the amino terminus of L-MAG, is a stretch of ~20 nonpolar amino acids that may be a cleavable signal peptide similar to those typically present on virtually all secretory and many transmembrane proteins with an extracellularly disposed amino terminus (44). The NH₂-terminal amino acid of native MAG has not been identified. However, based on an analysis of the amino acids found near known signal sequence cleavage sites (58, 59), the predicted site of cleavage is between the glycines at positions 19 and 20. The glycine at position 20 is therefore a potential candidate for the NH₂-terminal amino acid of the mature protein.

The second hydrophobic region is a segment of 23 nonpolar amino acids (amino acids 514 to 536), which is long enough to traverse the bilayer and likely to be membrane embedded. This segment is followed immediately by an extremely basic sequence (amino acids 537 to 540), a feature of the cytoplasmic domain of many membrane proteins (44) that suggests that the ensuing portion of the polypeptide re-
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Figure 5. Restriction maps of MAG cDNA clones and sequencing strategy. Restriction enzyme sites in the three MAG cDNA clones were deduced by conventional procedures (25). All restriction sites were subsequently verified by nt sequence analysis. Restriction fragments were directionally cloned into compatible sites of M13 before sequencing. The direction and extent of the sequence determination is shown by the arrows. In one case, a synthetic primer was used and is indicated by a vertical line at the tail of an arrow. The scale is calibrated in kilobases and shows the 5' to 3' orientation of the cDNAs relative to MAG mRNA. Coding regions are represented by the open bars and 3' and 5' untranslated regions by heavy lines. The 5'→3' orientation of the M10D insert was deduced by sequence analysis and confirmed by the in vitro transcription/translation studies described in the text.

Discussion

The formation of the vertebrate myelin sheath requires an intimate and specific interaction of a myelinating glial cell with a closely apposed axonal process (48). The MAG proteins have been considered to be likely candidates for mediating

**Figure 6.** nt sequences and deduced amino acid sequences of the MAG polypeptide. nt and amino acid residue positions for clone M10D are numbered on the left. Clone M10E begins at residue No. 1379 of M10D and is completely identical at every position except for the 45-bp insert shown enclosed by the box. There are eight potential N-linked glycosylation sites in the predicted extracellular portion of MAG which are indicated by * over Asn. The Arg-Gly-Asp segment (position 118-120), described in the text, is bracketed from above and below. The predicted 32 residue membrane spanning segment is indicated above by the heavy bar. Both 5' and 3' untranslated segments are indicated as stretches of continuous *nt. A potential Poly A acceptor site in the 3' untranslated sequence is bracketed.
Figure 7. Alignment of internal homologies present in L-MAG. Portions of the primary sequence from Fig. 6 are shown in the single letter code for amino acids. The five segments of internal homology were visually aligned to maximize identities between segments. A break introduced into the sequence is indicated by a dashed line. The position of the initial amino acid of each of the five homology segments (I-V) is indicated to the left of the figure. Additional, but less striking, homologies were found to exist outside of the sequence shown and are described in the text.

The position of the initial amino acid of each of the five homology segments (I-V) is indicated to the left of the figure. Additional, but less striking, homologies were found to exist outside of the sequence shown and are described in the text.

Figure 8. Homology between MAG and N-CAM. Portions of the rat L-MAG and chicken N-CAM (14) primary sequence are shown in the single letter code for amino acids. The segments shown correspond to MAG homology units III and IV (lines 2 and 3) and the two internal repeats of N-CAM that they most closely resemble. Identities between segments were optimized by pairwise alignment with the ALIGN program (49). Only those amino acids that are identical on both a MAG and an N-CAM segment are boxed. Additional identities shared between the MAG homology units are indicated by dots between the second and third lines. Amino acid positions are indicated on the left and right margins.

Table I. Proteins Homologous to MAG*

| Identity region | MAG region | Z value |
|-----------------|------------|---------|
| N-CAM (169-367) | 27.8       | 224-408 | 14.3   |
| HLA class II, DR (53-167) | 19.7       | 181-294 | 8.91   |
| HLA class II, DC-1 (50-223) | 19.3       | 177-346 | 8.81   |
| H-2 class II, Eα (107-167) | 25.8       | 235-294 | 7.14   |
| Ig heavy chain V–III region (72-104) | 30.3       | 279-311 | 8.02   |
| Poly-Ig receptor (364-478) | 19.0       | 317-432 | 7.29   |
| Alpha 1β-glycoprotein (333-448) | 23.9       | 283-392 | 6.15   |

* A search of the National Biomedical Research Foundation protein data base with the amino acid sequence of L-MAG by the FASTP program (24) revealed significant homology to a number of immunoglobulin-related proteins. These proteins, which are listed above, include chicken N-CAM (14), human HLA class II antigens DR (5) and DC-1 (1), the murine H-2 antigen Eα (3) and immunoglobulin heavy chain V–III (60), the rabbit poly-Ig receptor (29), and human αβ-glycoprotein (19). Numbers in parentheses correspond to the sequences of each protein most homologous to the indicated sequences of MAG. The significance of the homology of each protein to MAG is given by its Z value (24), which corresponds to the number of standard deviations by which the score of an optimally aligned segment of MAG differs from the mean score of randomly permuted MAG sequences aligned with the same protein. It has been suggested (24), that Z scores >6 are probably significant, and those >10 are definitely significant.
The major structural features of the MAG polypeptides. This diagram summarizes the major structural features of the MAG polypeptides discussed in the text. Both S-MAG and L-MAG are thought to have an identical large extracellular domain and membrane spanning segment, but differ in their cytoplasmic domains. The extracellular domain contains five segments of immunoglobulin-like internal homology that are shown as boxes; regions of strong homology are shown by continuous lines, and less precise sequence homologies are dotted. Arrowheads indicate the position of cysteines. Note that each homology unit contains at least two cysteine residues spaced an average of 51 amino acids apart. The position of the RGD sequence that may participate in cell adhesion is also indicated.

cell–cell interactions in the nervous system, share such close homology. It may be that other cell adhesion molecules in the nervous system will be found to share sequence and structural homologies with N-CAM and MAG as well.

MAG may therefore be considered a member of the immunoglobulin gene superfamily (15, 17, 62, 63). This is a family of proteins that share a common extracellular subunit structure termed the immunoglobulin homology unit (17). This unit is ∼100 amino acids long and contains cysteine residues postulated to form an intrachain disulfide linkage that stabilizes a series of characteristically folded anti-parallel beta sheets (62, 63).

At present nothing is known about how the extracellular portion of MAG is folded. It is of interest to note however, that each of the five homology units contains at least two cysteine residues that are spaced, on average, 51 amino acids apart. Furthermore, the sequences surrounding these cysteines are highly conserved in each unit, suggesting that these cysteines and their flanking sequences are structurally important. In view of the homologous sequences and similarly spaced cysteine residues shared by the MAG and immunoglobulin domains, it is reasonable to suggest that these protein domains are structurally similar as well, and that two cysteines within each MAG homology unit are disulfide linked. Although MAG contains sequences resembling those of variable domains, the cysteine spacing (as well as a preliminary analysis of the secondary structure of the extracellular segments) is more closely related to that described for the constant domains of immunoglobulins (62, 63).

Our data therefore predicts that MAG contains five extracellularly disposed, disulfide-linked homologous domains (summarized in Fig. 9). In this proposed structure it would resemble the alpha 1 β-glycoprotein (19), and the poly-Ig receptor (29). As has been proposed for these proteins and other members of the immunoglobulin gene superfamily, MAG may have evolved by gene duplication from an ancestral immunoglobulin-like gene involved in cell recognition phenomena (19, 29, 17).

A most unexpected finding of the homology search was that a cDNA with an identical amino acid and nucleotide sequence to the 3′ end of L-MAG had been previously isolated. This cDNA, clone IB236 (52), corresponds to the 3′ half of M10D except that it contains an additional 90 nt in the 3′ untranslated region, including an alternate Poly A acceptor site, and ends in a Poly A tract. IB236 had been considered to be a neuron-specific cDNA. This conclusion was based on immunocytochemical studies in which antibodies, raised against chemically synthesized peptides (based on the deduced amino acid sequence of the cDNA), were reported to show staining of specific neuron groups in the CNS (4, 23). We cannot reconcile these observations with the results of the previously discussed immunocytochemical studies that localized MAG to myelinating cells (50, 55, 27). If a localization to neurons is confirmed, however, it would suggest that MAG may have a more general role in cell–cell interactions in the nervous system than previously appreciated.

We have demonstrated that the two MAG proteins encoded by M10D and M10E differ in the extent of their intracytoplasmic domains (Fig. 6). This is likely to be the only difference between these proteins, since the calculated molecular mass difference (5 kD) of their carboxy terminal regions is in close agreement with the directly determined molecular mass difference between the native polypeptides (Fig. 1). Furthermore, protease V8 peptide maps of the two MAG polypeptides also suggest that they differ by a single peptide fragment unique to L-MAG (10). Until a full-length cDNA for S-MAG has been characterized, other amino acid differences cannot be ruled out. It is of interest that N-CAM, whose similarities to MAG in structure and function have already been noted, also exists in multiple forms that differ in the extent of their cytoplasmic domains (14). These proteins, termed large domain and small domain N-CAM (14), contain cytoplasmic segments of 362 and 101 amino acids, respectively, and, like MAG, are expressed differentially during development (11).

The data presented in this paper suggest that the MAG proteins are products of a single gene whose primary transcript may be alternatively spliced to yield the two MAG mRNAs. This is consistent with the identity of M10D and M10E at all nt positions, with the exception of the internal 45-nt segment present only in M10E (Fig. 6). Furthermore, preliminary Southern blot studies of rat genomic DNA are also consistent with the presence of a single MAG gene (data not shown). Finally, we have also detected a much larger difference in the size of MAG mRNAs by RNA blot analysis (Fig. 4), e.g., mRNAs of 2,500 and 3,000 nt. This is almost certainly due to sequence differences in the untranslated regions, possibly an alternate polyadenylation site.

The functional significance of the two different intracytoplasmic domains is not known. One possibility is that the cytoplasmic segments may have important and perhaps distinct interactions with intracytoplasmic constituents, particularly with cytoskeletal elements. In this regard it is noteworthy that the putative cytoplasmic segment of MAG is homologous to a similarly disposed cytoplasmic segment of integrin, a plasma membrane receptor that binds actin intracellularly (53). Specifically, the carboxy-terminal 21 amino acid segment of integrin shares eight identical and eight conserved amino acids with amino acids 551-573 in both L-MAG and S-MAG when two gaps are allowed (data not shown). It is also of interest that actin is known to have a similar periaxonal localization to that of MAG (64). Additional studies will be necessary to establish whether either or both MAG proteins directly interact with actin or other cytoskeletal elements.

It is also possible that the two cytoplasmic segments of
MAG are phosphorylated differently, as has been demonstrated for the two alternate cytoplasmic domains of N-CAM (46). Based on the amino acid sequences surrounding known phosphorylation sites of other proteins (30), several serine and threonine of the predicted cytoplasmic segments of the MAG proteins may be phosphorylated in vivo. These include potential phosphorylation sites for calcium/calmodulin-dependent protein kinase at amino acids 537–543 (RRKKNVT) and for protein kinase C at 575–582 (KRLGSERR) and 604–608 (KRPTK). In addition, a tyrosine of L-MAG (amino acid 620) lies in a sequence (TEELAEY) that closely resembles the tyrosine autophosphorylation site of the EGF receptor (TAENAY) (8, 18). Phosphorylation of the EGF receptor at this tyrosine may be an important modulator of the activity of this receptor in vivo (18). Whether these sites on the MAG proteins are actually phosphorylated in vivo and their relevance for the roles of the two MAG proteins during development will require further investigation. It is intriguing to note that three of the potential sites described above are present only in L-MAG.

In summary, we have isolated cDNA clones that encode alternate forms of the MAG proteins. Sequence analysis of these clones revealed homologies to the immunoglobulin gene superfamily, particularly to N-CAM, and the presence of an RGD sequence that may be important for the postulated role of the MAG proteins in glial–axonal interactions. The availability of these clones will facilitate future studies directed at the precise role of the MAG proteins in cell–cell interactions and the significance of their distinct cytoplasmic domains.

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Note Added in Proof: After this paper was accepted for publication the nucleotide sequence of a partial MAG cDNA was reported (Arquint, M., Dunn, 1987, Proc. Natl. Acad. Sci. USA, 84:600–604).

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