Expression of a $\beta_1$-Related Integrin by Oligodendroglia in Primary Culture: Evidence for a Functional Role in Myelination

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Abstract. We have investigated the expression of integrins by rat oligodendroglia grown in primary culture and the functional role of these proteins in myelination. Immunochemical analysis, using antibodies to a number of $\alpha$ and $\beta$ integrin subunits, revealed that oligodendrocytes express only one detectable integrin receptor complex ($\alpha_{OL} \beta_{OL}$). This complex is immunoprecipitated by a polyclonal anti-human $\beta$ integrin subunit antibody. In contrast, astrocytes, the other major glial cell type in brain, express multiple integrins including $\alpha_1 \beta_1$, $\alpha_3 \beta_1$, and $\alpha_5 \beta_1$ complexes that are immunologically and electrophoretically indistinguishable from integrins expressed by rat fibroblasts. The $\beta_1$ subunit of the oligodendrocyte integrin ($\beta_{OL}$) and rat fibroblast $\beta_1$ have different electrophoretic mobilities in SDS-PAGE. However, the two $\beta$ subunits appear to be highly related based on immunological cross-reactivity and one-dimensional peptide mapping. After removal of N-linked carbohydrate chains, $\beta_{OL}$ and $\beta_1$ comigrated in SDS-PAGE and peptide maps of the two deglycosylated subunits were identical, suggesting differential glycosylation of $\beta_1$ and $\beta_{OL}$ accounts entirely for their size differences. The oligodendrocyte $\alpha$ subunit, $\alpha_{OL}$, was not immunoprecipitated by antibodies against well characterized $\alpha$ chains which are known to associate with $\beta_1$ ($\alpha_5$, $\alpha_4$, and $\alpha_6$). However, an antibody to $\alpha_5$, a more recently identified integrin subunit, did precipitate two integrin subunits with electrophoretic mobilities in SDS-PAGE identical to $\alpha_{OL}$ and $\beta_{OL}$. Functional studies indicated that disruption of oligodendrocyte adhesion to a glial-derived matrix by an RGD-containing synthetic peptide resulted in a substantial decrease in the level of mRNAs for several myelin components including myelin basic protein (MBP), proteolipid protein (PLP), and cyclic nucleotide phosphodiesterase (CNP). These results suggest that integrin-mediated adhesion of oligodendrocytes may trigger signal(s) that induce the expression of myelin genes and thus influence oligodendrocyte differentiation.

Myelination is a major developmental process of the nervous system, carried out by oligodendroglia in the central nervous system (CNS), and Schwann cells in the peripheral nervous system (PNS). Myelin is a membranous sheath that is an extension of the plasma membrane of the myelin-producing cell. It consists of numerous alternating lipid and protein-containing lamellae wrapped tightly around a segment of neuronal axon, functioning as an insulator to accelerate the velocity of electrical impulses transmitted between a neuronal cell body and its target cell (Raine, 1984). The brain also contains numerous process-bearing cells including neurons that are not myelinated. Little is known about the biochemical processes underlying specific recognition between oligodendroglia and their neuronal targets and/or their surrounding extracellular matrix (ECM). Adhesion events are likely to be critical in determining the ability of oligodendroglia to form myelin. A number of adhesion molecules have been suggested to take part in myelination. In Schwann cells myelin-associated glycoprotein (MAG) and L1 have been implicated (for review see Quarles, 1989). In neurons, L1 and N-CAM are likely to be involved (Nieke and Schachner, 1985; Martini and Schachner, 1986).

In addition to cell–cell interactions it is now evident that cell–matrix interactions play a significant role in development. Integrins are a family of cell surface receptors which translate signals outside the cell to alterations in cell behavior. A role for integrins and ECM in leukocyte development (for review see Hemler, 1990) and in neural development and migration (for review see Reichardt and Tomaselli, 1991) has been clearly demonstrated. Integrins bind certain components of the extracellular matrix, mainly glycoprotein...
teins such as fibronectin, laminin, and vitronectin (for review see Hynes, 1987; Ruoslahti and Pierschbacher, 1986). The integrins were originally classified into three major subfamilies (β1, β2, and β3) each having a common β subunit noncovalently associated with a distinct set of α subunits (Hynes, 1987). In addition to these three well characterized β subunits, there have been five other β subunits reported including β6 (Kajji et al., 1989; Suzuki and Naito, 1990; Hoggervost et al., 1990), β8 (Ramaswamy and Hemler, 1990; McLean et al., 1990), β7 (Sheppard et al., 1990), β3 (Yuan et al., 1992; Erle et al., 1991), and β5 (Moyle et al., 1991). It has also been shown that some α subunits can associate with more than one β subunit and therefore there is no longer a clear demarcation between subfamilies (Cheresh et al., 1989; Kajji et al., 1989; Vogel et al., 1990; Krissansen et al., 1990; Dedhar and Gray, 1990).

Some integrins (e.g., the fibronectin receptor) recognize the tripeptide sequence RGD (Arginine-Glycine-Aspartic Acid) which appears to play a key role in cell adhesion (for review see Ruoslahti and Pierschbacher, 1987). Our laboratory previously reported that isolated oligodendrocytes are able to bind to components of a matrix derived from glial cells in culture via a protein which appeared to have integrin-like binding properties (Cardwell and Rome, 1988a). In this report we have investigated the biochemical nature of this protein and present direct evidence that it is a member of the integrin superfamily. In addition, this receptor appears to play a regulatory role in CNS myelination.

Materials and Methods

Cell Culture and Astroglial Matrix Preparation

Purified oligodendrocytes were prepared from neonatal rat cerebral cortex after the method of McCarthy and de Vellis (1980) with modifications (Rome et al., 1986; Cardwell and Rome, 1988a). Two days after isolation, greater than 80% of the cells stain positively for the oligodendrocyte marker, galactocerebroside, and most cells that score as negative for galactocerebroside possess oligodendrocyte morphology, and, in the presence of 5% calf serum-containing medium, go on to express galactocerebroside with time in culture (Cardwell and Rome, 1988a). Astroglial matrix (AGM) was prepared as described earlier (Rome et al., 1986; Cardwell and Rome, 1988a). Briefly, mixed glial cells were cultured in 100-mm tissue culture plates to confluence. Media was then removed and 10 ml of distilled water was added to each plate. After 2 h or longer incubation at room temperature, the lysed cell material was removed. The plates were washed two times with PBS, once with serum-free medium, and stored in medium at 37°C until use. The material remaining on the culture surface after water was added to each plate. After 2 h or longer incubation at room temperature, the lysed cell material was removed. The plates were washed two times with PBS, once with serum-free medium, and stored in medium at 37°C until use. The material remaining on the culture surface after water lysis is referred to as AGM (astroglial matrix). Typical AGM contained 0.5-1 µg protein per cm² surface.

We use the term equivalent "brain age ~ to mean the days in culture plus the age of the rat plus the time at the time of dissection (2 d in these studies). Isolated oligodendrocytes (10-12 d equivalent brain age) were either used for surface labeling or plated onto AGM-coated tissue culture plates and maintained in DMEM/F12 (1:1) containing Hepes (15 mM, pH 7.1), NaHCO₃ (1.2 g/l), and 5% calf serum (HyClone, Logan, UT) for RNA preparation. Astrocytes, prepared by the method of McCarthy and de Vellis (1980), were maintained in DMEM/F12 as above. Rat and human skin fibroblasts were maintained in DMEM/F12 as above. Rat and human skin fibroblasts were maintained in DMEM/F12 as above.

Cell Surface Labeling and Immunoprecipitation

For surface-labeling, oligodendrocytes from 80 cortices (from 40 neonatal rat pups) were removed from mixed glial cells by overnight shaking (McCarthy and de Vellis, 1980) and pelleted by brief centrifugation in a table-top centrifuge at 450 x g. The pellet (5 x 10⁶ cells) was washed twice with PBS by resuspension and centrifugation and the final pellet was suspended in 1 ml PBS containing 1 mM Ca²⁺ and 1 mM Mg²⁺. Astrocytes (10⁶ cells) and fibroblasts (5 x 10⁶ cells) were removed from flasks by incubating in 20 mM EDTA in PBS (2 ml per flask) for 10-20 min. Detached cells were collected in PBS containing 1 mM each Ca²⁺ and Mg²⁺ and pelleted by centrifugation at 1400 x g for 15 min followed by incubation with non-immune rabbit or mouse serum and protein A-Sepharose beads (anti-mouse IgG-agarose for mouse serum). Integrin heterodimers were then immunoprecipitated using one or more of the following polyclonal antibodies raised against either an intact integrin subunit (anti-β), or a synthetic peptide of the cytoplasmic domain (anti-β, anti-β, anti-α, anti-α, anti-α, anti-α, anti-α, anti-α subunits) or a monoclonal antibody (anti-α). The antibodies were generous gifts of Dr. Martin Hemler (anti-β, anti-β, anti-β, anti-α), Dr. Richard Hynes (anti-β, anti-α, anti-α, and anti-α), Dr. Stephen Kaufman (anti-α), Drs. Lynn Schnapp and Robert Pytelia (anti-α), Dr. Dean Sheppard (anti-α), and Dr. Louis Reichardt (anti-α). The immune complexes were recovered with protein A-Sepharose or anti-mouse IgG-Agarose. After extensive washing, immune complexes were dissociated from the beads by boiling in sample buffer (2% SDS, 100 mM Tris-HCl, pH 6.8, 10% glycerol, 10 mM EDTA). Samples were analyzed by SDS-PAGE using 4% and 6% acrylamide in the stacking and running gels, respectively (Laemmli, 1970).

Treatment of Integrins with N-Glycosanase F

Oligodendrocyte and fibroblasts integrins were immunoprecipitated using anti-β antibody as described above. Immunoprecipitated protein was denatured by boiling in 1% SDS for 3 min. Sodium phosphate buffer (20 mM, pH 7.2) containing 10 mM sodium azide, 50 mM EDTA, and 0.5% n-octylglucoside was then added to bring the SDS concentration to 0.1% and the samples boiled again for 3 min. After cooling, N-glycosidase F (0.5 unit; Boehringer Mannheim Corp., Indianapolis, IN) was added to each sample followed by incubation for 16 h at 37°C before analysis on SDS-PAGE.

Northern Blot Analysis

Oligodendrocytes (10 d equivalent brain age) were plated onto 150-mm tissue culture plates coated with AGM (see above). At 17 d equivalent brain age, select cultures were treated for a period of 48 h with 0.1 mg/ml GRGDSP peptides, or 0.1 mg/ml GRGESP peptides, or 0.1 µg/ml cycloheximide, or 0.1 mg/ml GRGESP + 0.1 µg/ml cycloheximide. Total RNA was prepared by the method of Chomczynski and Sacchi (1987) using acid guanidinium thiocyanate-phenol-chloroform extraction. For developmental studies, isolated oligodendrocytes were plated in 150-mm tissue culture plates and RNA was prepared from each culture at various stages of development. The earliest time point was at day 13 and the latest was at day 23 (equivalent brain age). RNA separation was carried out on 1.0% agarose/formaldehyde gels before transfer to nylon membranes (ICN Biobios). Blots were prehybridized in 50% formamide, 0.2% SDS, 5 x Denhardt's, 5 X Pipes, and 10 µg/ml salmon sperm DNA overnight at 42°C. Hybridization probes (myelin basic protein [MBP], proteolipid protein [PLP], and cyclic nucleotide phosphodiesterase [CNP] and human β integrin, generous gifts of Drs. Anthony Campagnoni, Robert Milner, Sally Lewis, and Michael Schiati, respectively) were labeled with [α-35S]dCTP to a specific activity of 4 x 10⁶ cpm/µg by the random priming method (BRL). Hybridization was carried out overnight at 42°C in the same solution as prehybridization buffer except Denhardt's was used at 1 x and salmon sperm DNA was added to 100 µg/ml. After hybridization, blots were washed two times (1 h each) in 2 x SSC containing 0.2% SDS for 1 h at 42°C followed by a final wash in 2 x SSC, 0.5% SDS for 1 h and exposed to Kodak XAR-5 film. To normalize for the amount of RNA loaded in each well, blots were stripped and reprobed for chicken f-actin (Cleveland et al., 1980). Autoradiographs were scanned with an Ultrascan XL laser densitometer (Pharmacia LKB Biotechnology, Piscataway, NJ) and analyzed with the GelScan XL 2.1 software package (Pharmacia LKB).

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Results

Immunohistochemical Analysis

To demonstrate directly the existence of integrins on the surface of rat oligodendrocytes, surface iodinated cells were solubilized and immunoprecipitated using antibodies to various integrin subunits. Antibodies specific for two different α chains (α5 and α3) and three different β chains (β1, β3, and β5) were initially tested. Only one antibody, anti-β1, gave a positive reaction with the labeled oligodendrocytes. In contrast, anti-α5, α3, and β5 were all able to precipitate integrin subunits from detergent-solubilized surface labeled rat fibroblast samples run in parallel as a control. As indicated in Fig. 1 A, under non-reducing conditions, antiserum to a peptide representing the cytoplasmic domain of the human α5 integrin subunit coprecipitated an α5 and an associated β1 chain from surface-iodinated fibroblasts (Fig. 1 A, lane 1) while the same antiserum failed to detect any immunoreactive material in the detergent solubilized extract of surface-iodinated oligodendrocytes (Fig. 1 A, lane 2). Similarly, antiserum to a peptide representing the cytoplasmic domain of chicken α5 integrin subunit immunoprecipitated an α5 and associated β1 from fibroblasts (Fig. 1 A, lane 5). However this antiserum also failed to detect any immunologically cross-reactive protein in the oligodendrocyte lysate (Fig. 1 A, lane 6). In contrast, antiserum against human β1 integrin coprecipitated two polypeptides, a putative β and an associated α5 chain from fibroblasts (Fig. 1 A, lane 4); we will refer to these polypeptides as αOL and βOL (OL, for oligodendrocytes). The βOL subunit had lower electrophoretic mobility in SDS-PAGE under non-reduced conditions (Fig. 1 A, lane 4) than β1 from fibroblasts (Fig. 1 A, lanes 3 and 5). In addition, oligodendrocytes maintained for two weeks in culture expressed this same arrangement of integrin chains (αOL and βOL), indicating that the pattern of integrin expression was unchanged between days 12 and 21 equivalent brain age.

As mentioned above, we also tested the possibility of expression by oligodendrocytes of other β subfamily integrins such as β3 and β5. Both antibodies failed to detect any immunoreactive material in the oligodendrocytes lysate (data not shown). However, we examined whether the αOL chain could be α5, or α3, two other subunits that have been reported to associate with β1 (Hemler et al., 1987; Vogel et al., 1990). Both anti-α5 and α3 antibodies were found to be unreactive with the surface-labeled oligodendrocyte extracts (data not shown). While this work was in process, we obtained antibodies raised against integrin subunits α5 (Song et al., 1992), α3 (L. Schnapp and R. Pytela, personal communication), and α3 (Palmer et al., 1993), the more recently identified α subunits that appear to associate with a β1 chain. Immunoprecipitation of an 125I-labeled extract of oligodendrocytes using the above antibodies showed that only the α5 antibody reacted with the labeled extract (Fig. 1 B). Two polypeptides were precipitated, a putative α that has a smaller size from that reported for chick α5 (Bossy et al., 1991) and an associated β subunit (Fig. 1 B, lane 1). The protein doublet immunoprecipitated with anti-α5 had a mobility in SDS-PAGE identical to the doublet immunoprecipitated with anti-β1 (αOL, βOL) (Fig. 1 B, lanes 1 and 2, respectively) suggesting that αOL could be α5 or an α5-related subunit.

Expression of integrins by rat astrocytes, the other major glial cell type in the CNS, was also examined using some of the same antibodies as above to immunoprecipitate extracts of surface-iodinated cells. An antibody to the α5 integrin subunit coprecipitated and an α and an associated β subunit (Fig. 1 C, lane 4) maintained for two weeks in culture expressed this same arrangement of integrin chains (αOL and βOL), indicating that the pattern of the experiment was unchanged between days 12 and 21 equivalent brain age.

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Figure 2. Comparison of rat oligodendrocyte $\beta_{ol}$ and rat fibroblast $\beta_i$ by one-dimensional peptide mapping with V8 protease. $^{125}$I-labeled integrins from detergent-solubilized extracts of oligodendrocytes and fibroblasts were immunoprecipitated with antibodies raised to human $\beta_i$ integrin; individual subunits were separated by SDS-PAGE. (A) Gel slices containing $\beta_{ol}$ (lane 1) and $\beta_i$ (lane 2) were each treated with 1 $\mu$g V8 protease during a second electrophoresis on a 15% SDS-polyacrylamide gel (Cleveland et al., 1977). Arrowheads indicate the differences in peptide maps of $\beta_i$ and $\beta_{ol}$. Numbers at the left indicate the position and size in kD of molecular weight markers. (B) Integrin subunits were immunoprecipitated from rat fibroblasts and oligodendrocytes by antisera raised against human $\beta_i$ and either remained as control (lanes 1 and 2) or treated with N-glycanase F (lanes 3 and 4, see Materials and Methods). Arrowhead indicates position of migration of $\beta_i$ (lane 3) and $\beta_{ol}$ (lane 4) after deglycosylation. Samples were analyzed under nonreducing conditions by SDS-PAGE. (C) Comparative peptide maps of fibroblast $\beta_i$ (lane 1) and oligodendrocyte $\beta_{ol}$ (lane 2) integrin subunits after deglycosylation by N-glycanase F.

Chemical Characterization of the Oligodendrocyte Integrin

The $\beta_{ol}$ polypeptide was further compared to the putative fibroblast $\beta_i$ chain by one dimensional peptide mapping (Cleveland et al., 1977). Both integrin $\beta_{ol}$ and $\beta_i$ subunits yielded several identical peptide fragments (Fig. 2 A, compare lanes 1 and 2), as well as distinct fragments (Fig. 2 A, arrows). In the 18-30-Kd region, there are 2-3 fragments generated from the fibroblasts $\beta_i$ while in the same region $\beta_{ol}$ produced only one peptide fragment that did not comigrate with any of those from fibroblasts (see arrows). In the region below 14 Kd, the $\beta_i$ digest has an additional peptide that is missing in the $\beta_{ol}$ digest (arrow). There was too little material from the $\alpha_{ol}$ sample to generate a distinct map.

To test whether differential glycosylation accounts for the differences in molecular weight and peptide maps of the fibroblast $\beta_i$ and oligodendrocyte $\beta_{ol}$ chains, the polypeptides were treated with N-glycanase F to remove N-linked carbohydrate chains. After digestion, the two $\beta$ subunits ran as smaller proteins which comigrated in a non-reduced SDS gel (Fig. 2 B, lanes 3 and 4, $\beta_i$ and $\beta_{ol}$, respectively, arrow). The $\alpha$ chains were also reduced in size but did not appear to run at identical mobilities. The deglycosylated $\alpha_{ol}$ chain ran as a closely spaced doublet in this experiment, likely due to incomplete deglycosylation, since in other experiments only a single band at the lower size was seen (not shown). Peptide maps of the deglycosylated $\beta$ chains were generated and found to be identical (Fig. 2 C).

Developmental Expression of $\beta_{ol}$ mRNA

The $\beta_{ol}$ and $\beta_i$ subunits were also highly related at the mRNA level, Northern blots of total oligodendrocyte mRNA probed with a full-length cDNA specific for the human $\beta_i$ integrin subunit, revealed a single 3.2-Kb message (Fig. 3,
Figure 3. Northern blot analysis of integrin β subunit message expressed by oligodendrocytes. Rat and human fibroblasts were used as control cells. Total mRNA from human fibroblasts (lane 1, 10 μg), rat fibroblasts (lane 2, 10 μg) and rat oligodendrocytes (lane 3, 30 μg) were prepared, transferred to a nylon membrane, and probed with a human β1 integrin cDNA as described in Materials and Methods.

We used the human probe since to our knowledge, the rat β1 has not been cloned. This message was approximately the same size as that seen in both human and rat fibroblast mRNA (Fig. 3, lanes 1 and 2, respectively). We recently isolated a putative βOL cDNA from an oligodendrocyte cDNA library (Malek-Hadayat, S., and L. H. Rome, manuscript in preparation). This clone shows >90% identity to mouse β1 cDNA and detects the same size oligodendrocyte mRNA in a Northern blot (not shown).

To examine whether expression of βOL mRNA is developmentally regulated, total mRNA was prepared from isolated oligodendrocyte cultures at various developmental stages between day 13 (the earliest age at which we can obtain pure cells) and day 23 (a time beyond the peak period of myelin synthesis). The mRNAs were analyzed by Northern blots using the βOL cDNA as a hybridization probe. Results shown in Fig. 4 A indicated no significant differences between the levels of mRNA expressed. This was confirmed by densitometric quantitation relative to β-actin expression which was probed in the same gel (Fig. 4 B).

The results were compared to mRNA expression in the presence of a control non-specific peptide, GRGESP, and normalized to expression of β-actin. Cells grown in the presence of 0.1 mg/ml GRGDSP peptides showed a 74% reduction in expression of βOL message at day 23 (not shown).

Effect of GRGDSP Peptides and Cycloheximide on Expression of Integrin and Myelin-Specific Messages by Oligodendrocytes

We have previously reported that GRGDSP synthetic peptides can block the initial attachment of oligodendrocytes to their substratum, AGM (Cardwell and Rome, 1988a). However, the addition of GRGDSP peptides to established oligodendrocyte cultures does not cause cell detachment, yet these peptides significantly reduced the synthesis of MBP (Cardwell and Rome, 1988b). To further analyze the mechanism of action of the GRGDSP peptides, we examined the effect of these peptides on the level of expression of mRNAs for several myelin genes, including MBP, CNP, and PLP.

Figure 4. Expression of oligodendrocyte integrin β subunit (βOL) during maturation of oligodendrocytes. (A) Total mRNA was isolated from purified oligodendrocytes at different developmental stages and analyzed by Northern blots using rat βOL integrin cDNA (see results). (B) Densitometric quantitation of mRNAs in A relative to β-actin probed in the same gel (not shown). This experiment was repeated twice with essentially the same result.

Figure 5. (A) Effect of GRGDSP and GRGESP synthetic peptides on expression of mRNAs for MBP, CNP, PLP, and integrin (βOL) by oligodendrocytes. Purified oligodendrocytes cultured on AGM (control, first lane each probe), or treated for 48 h with 0.1 mg/ml GRGDSP (center lane each probe), or 0.1 mg/ml GRGESP (third lane each probe). Total mRNAs were extracted, separated on a 1% agarose gel (12 μg per well) and transferred to a nylon membrane. Blots were probed with 32P-labeled MBP, PLP, CNP, oligodendrocyte integrin βOL, and β-actin cDNAs as described in Materials and Methods. (B) mRNAs from each blot in 5A were quantitated by densitometry and normalized to the amount of β-actin in each lane. This experiment was repeated several times with the following results (mean ± SD): the inhibition of mRNA expression by GRGDSP was 74 ± 7% for MBP (n = 3), 64 ± 7% for PLP (n = 3), and 44 ± 8% for CNP (n = 2).
the amount of MBP mRNA relative to untreated control cells (Fig. 5, A and B, MBP, lanes 2 and 1, respectively). In rat, PLP message is expressed as two different species of 1.6 and 3.2 Kb, both messages were reduced by ~64% compared to control (Fig. 5, A and B, PLP, lanes 2 and 1, respectively). Similarly, the CNP mRNA was decreased by ~40% relative to control (Fig. 5, A and B, CNP, lanes 2 and 1, respectively). In contrast, the control peptide, GRGESP, showed no significant inhibitory effect on expression of any of the myelin genes (Fig. 5, third lane for each probe). In all experiments the level of peptide added (0.1 mg/ml) did not result in a significant detachment of cells from the culture substrate (less than 3% of the cells detached). The effect of RGD-containing peptides on expression of βₒα₅ mRNA was also examined. In contrast to the myelin genes, Northern blot analysis showed that RGD-containing peptides had no effect on the level of this putative βₒα₅ mRNA (Fig. 5, A and B as indicated), which supports the selective regulation of myelin genes by RGD-containing peptides.

Inhibition by RGD-containing peptides could be a direct effect on transcription of myelin genes or the peptides could be acting indirectly, perhaps by affecting genes encoding intermediary acting factors. To differentiate these two mechanisms, we treated cells with GRGDSP in the presence of cycloheximide (Table I). Cycloheximide blocked the GRGDSP inhibition to a significant extent, allowing maintenance of MBP, and PLP messages near the control levels. Cycloheximide alone did not super-induce message for either of the myelin proteins (Table I).

Discussion

In this study we have described the expression of a single integrin receptor complex by rat cerebral cortex oligodendroglia, a cell type restricted to the central nervous system and responsible for the synthesis of CNS myelin. Evidence presented here indicate that this integrin is an alternately glycosylated member of the β subfamily. Preliminary results suggest that the associated α subunit is α₆, however, definitive proof will require additional chemical and/or molecular analysis.

In addition to the chemical characterization, we have also presented evidence for a potential regulatory role for this receptor in synthesis of myelin components. We previously reported that isolated oligodendroglia in primary culture interact specifically with matrix components derived from mixed glial cells. Moreover, a synthetic hexapeptide containing the RGD sequence disrupts this interaction and inhibits the synthesis of myelin components such as MBP and sulfatides by oligodendrocytes (Cardwell and Rome, 1988a,b). These results prompted us to study the nature of this interaction and its effect on myelination. Based on the RGD and divalent cation dependence of oligodendrocyte adhesion, we speculated that a likely candidate for the adhesion receptor could be a member of the integrin family of receptors for ECM proteins. In the present study we used antibodies raised against several α and β integrin subunits to probe for the presence of an oligodendrocyte integrin. Rat fibroblasts were used as control cells since these cells are known to express a number of integrin chains including α₅β₁, α₅β₁, and α₅β₁ (Malek-Hedayat and Rome, 1992). Of nine antibodies tested, only anti-β₁ and anti-α₅ were able to immunoprecipitate an integrin complex from oligodendrocytes. Furthermore, the complexes immunoprecipitated with both antibodies were strikingly similar to each other with respect to electrophoretic mobility on non-reduced SDS-PAGE. Using a similar battery of antibodies we found that astrocytes, the other major glial cell type in brain, express multiple integrin receptors including α₅β₁, α₅β₁, and α₅β₁. This combination of integrin chains is also expressed in C6 glioma cells, a chemically induced tumor cell line from rat brain (Malek-Hedayat and Rome, 1992). Using a monoclonal antibody (3A3), which recognizes an α₅β₁ heterodimer, Tawil et al. (1990) previously demonstrated the presence of this integrin on the surface of rat astrocytes. Astrocytes from mouse can be stained with anti-fibronectin receptor antibodies (Pesheva et al., 1988), which is consistent with our finding of α₅β₁ in rat astrocytes.

The oligodendrocyte integrin β subunit (βₒα₅) and the β₁ subunit expressed by rat fibroblasts displayed different mobilities on non-reducing SDS-PAGE. However, peptide maps of the two subunits indicated that they were highly related. After removal of N-linked carbohydrate chains by N-glycanase F both subunits were found to comigrate on SDS gels. In addition, peptide maps of the two deglycosylated subunits were indistinguishable, suggesting that βₒα₅ and β₁ are identical at the amino acid level and that differential glycosylation occurs in oligodendrocytes and fibroblasts. Due to the unique role of the oligodendrocyte integrin in regulation of myelin synthesis, it is possible that this cell-specific glycosylation may play a role in receptor function. A number of studies have recently attempted to examine the role of integrin carbohydrate chains on the adhesive properties and biological function of these receptors. During development, mouse T cells have been shown to express two different β₁ subunits that differ in the extent of N-linked glycosylation and sialylation. The differential glycosylation of the β₁ subunit appears to effect binding of the receptor to fibronectin (via VLA-4 and VLA-5) and laminin (via VLA-6) (Wadsworth et al., 1993). The effect of an altered glycosylation
of the $\beta$ subunit on binding of the cells to fibronectin and laminin has been shown as well by other investigators (Ak-06576 and a grant from the Multiple Sclerosis Society (RG 2200-A-1).

We have not characterized the $\alpha_{OL}$ subunit to the same extent as $\beta_{OL}$. It appears to be either expressed in lower abundance or less efficiently iodinated than $\beta_{OL}$. Our preliminary results using antibodies to a number of recently described $\alpha$ subunits suggest that $\alpha_{OL}$ might be $\alpha_1$ since antibody raised against the cytoplasmic domain of human integrin $\alpha_1$ subunit immunoprecipitated two polypeptides from oligodendrocytes with the identical mobilities on SDS-

Despite the recent studies of oligodendrocyte integrins, the identity of ligand(s) for us-containing integrins is still unknown. Antibodies to several known ECM proteins including fibronectin, laminin, tenascin, and vitronectin failed to inhibit oligodendrocytes adhesion to AGM (Cardwell and Rome, 1988a; Malek-Hedayat, S., and L. H. Rome, unpublished observations). However, the AGM ligand appears to be highly insoluble since the adhesive activity can not be removed from AGM by strong detergents, chaotropic agents, high salt, or low pH (Hamilton, S. P., and L. H. Rome, unpublished observations). It is possible that the AGM ligand recognized by the oligodendrocyte integrin is still unknown. Antibodies to several known ECM proteins including fibronectin, laminin, tenascin, and vitronectin failed to inhibit oligodendrocytes adhesion to AGM (Cardwell and Rome, 1988a; Malek-Hedayat, S., and L. H. Rome, unpublished observations). However, the AGM ligand appears to be highly insoluble since the adhesive activity can not be removed from AGM by strong detergents, chaotropic agents, high salt, or low pH (Hamilton, S. P., and L. H. Rome, unpublished observations). It is possible that the AGM ligand recognized by the oligodendrocyte integrin described here is a novel ECM component. In light of our preliminary results with anti-$\alpha_1$ antibody, it is intriguing that the identity of ligand(s) for $\alpha_1$-containing integrins is still unknown (Bossy et al., 1991).

If the oligodendrocyte integrin we describe here does in fact regulate myelin expression in vivo, we might expect its level of expression to change during glial cell development. Thus far we have examined $\beta_1$ expression in oligodendrocytes only over a limited period (13–21 d) without detecting a significant change in mRNA level. However, changes in $\beta_1$ mRNA expression could occur earlier than day 13 or alternatively, the expression of the $\alpha$ chain may be regulated.

We are now in the process of determining the integrin-mediated signaling events that regulate myelin synthesis. The matrix target of the oligodendrocyte integrin also remains an important issue. Despite continued efforts, the biochemical nature of the AGM ligand recognized by the oligodendrocyte integrin is still unknown. Antibodies to several known ECM proteins including fibronectin, laminin, tenascin, and vitronectin failed to inhibit oligodendrocytes adhesion to AGM (Cardwell and Rome, 1988a; Malek-Hedayat, S., and L. H. Rome, unpublished observations). However, the AGM ligand appears to be highly insoluble since the adhesive activity can not be removed from AGM by strong detergents, chaotropic agents, high salt, or low pH (Hamilton, S. P., and L. H. Rome, unpublished observations). It is possible that the AGM ligand recognized by the oligodendrocyte integrin described here is a novel ECM component. In light of our preliminary results with anti-$\alpha_1$ antibody, it is intriguing that the identity of ligand(s) for $\alpha_1$-containing integrins is still unknown (Bossy et al., 1991).

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