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Anti-reovirus receptor antibody accelerates expression of the optic nerve oligodendrocyte developmental program

(glial differentiation/myelination/anti-idiotypes/virus receptor)

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ABSTRACT **Previous studies showed that the cell-surface receptor for reovirus serotype 3 (Reo3R) appears at an early stage of oligodendrocyte differentiation and that anti-Reo3R antibodies and Reo3R-binding peptides induce galactocerebroside expression by developing oligodendrocytes. In the present studies, anti-Reo3R antibodies are shown to stimulate additional features of the program of oligodendrocyte development, including the loss of the A2B5 marker and expression of myelin basic protein. In anti-Reo3R antibody-treated cultures, galactocerebroside was expressed by cells having the morphology of immature oligodendrocyte precursors. Reo3R binding did not appear directly to inhibit or stimulate proliferation of glial progenitor cells or to affect their lineage commitment. Cell-surface structures utilized as a receptor by reovirus type 3 appear to play a role in the regulation of the initiation or rate of execution of the oligodendrocyte developmental program.**

Oligodendrocytes and type-2 astrocytes in the optic nerve develop from bipotential O-2A glial progenitor cells (1), which themselves likely are derived from immature neuroectodermal cells located in the germinal matrix of the cerebral subventricular zone (2, 3). In culture, cells in the O-2A lineage can be identified by the sequential expression of characteristic morphological features and antigenic markers (1, 4, 5). O-2A progenitors are motile cells with a simple bipolar morphology that react with the A2B5 (6) and anti-GD3 (3) antibodies. Immature oligodendrocyte precursors extend simple branching processes and react with both the A2B5 and O4 antibodies (7–9). As oligodendrocytes assume a more mature morphology with extensive branching processes, they lose A2B5 reactivity and express a variety of myelin-associated molecules including galactocerebroside (GalC), 2′,3′-cyclic nucleotide 3′-phosphodiesterase (CNPase), myelin basic protein (MBP), myelin-associated glycoprotein, and proteolipid protein. Astrocytes can be identified by the expression of glial fibrillary acidic protein (GFAP) and lack of expression of GalC. Type 2 (fibrillary) astrocytes have long processes and the $A2B5^+$ $GFAP^+$ phenotype. Type 1 (protoplasmic) astrocytes, also present in these cultures, probably arise from a separate lineage. They have a flat triangular shape and $A2B5^-$ $GFAP^+$ phenotype.

The neonatal rat optic nerve culture system provides a useful model with which to dissect the mechanisms that regulate cellular differentiation. We have utilized this culture system to investigate the interaction between a well-characterized neurotropic virus, reovirus type 3, and developing central nervous system glia. Previous studies demonstrated that mature oligodendrocytes and types 1 and 2 astrocytes express the immunoreactive receptor for reovirus serotype 3 (Reo3R), but O-2A progenitor cells do not (10–15). The Reo3R appears in a developmentally regulated manner at an early stage of oligodendrocyte differentiation prior to the appearance of GalC or MBP (15). Because of the early appearance of the Reo3R during oligodendrocyte development and because of the biochemical and antigenic similarity of the Reo3R to the β_2-adrenergic receptor and other members of the rhodopsin-like family of receptors (13, 16, 17), we postulated that perturbation of the Reo3R would alter glial differentiation.

Previous studies have demonstrated that the putative binding domain of the reovirus type 3 σ1 protein and the antigen-binding region of the anti-idiotypic anti-Reo3R antibody, 87.92.6 (18), exhibit striking sequence similarity. Studies using synthetic peptides have confirmed that these domains mediate the binding of the σ1 protein and 87.92.6 antibody to both the neutralizing anti-σ1 antibody, 9B.G5, and the Reo3R (19). These regions also mediate the functional consequences of Reo3R–ligand interaction, including down-modulation of Reo3R, inhibition of target cell growth, and stimulation of GalC expression by developing oligodendrocytes (15, 20, 21). In the present studies, these observations were extended by the demonstration that Reo3R–ligand binding induces several additional features of oligodendrocyte maturation.

MATERIALS AND METHODS

Cell Culture. Neonatal rat optic nerve glia were cultured by the method of Raff and coworkers (1) as described (15). Cells (3 or 6 × 10^3) were plated on poly(L-lysine)-coated 12-mm glass coverslips in 24-well culture plates (Falcon/Becton Dickinson Labware) in 25 μl of Dulbecco's modified Eagle's medium (GIBCO) supplemented with 10% heat-inactivated fetal calf serum (FCS; HyClone). After 1 hr to allow cell attachment 475 μl of N2 medium (1, 22) was added, bringing the final serum concentration to 0.5% (N2/0.5% FCS).

Antibodies. The isolation and characterization of the anti-Reo3R antibody, 87.92.6, has been described (12). The mouse A2B5 monoclonal antibody specific for GQ ganglioside (6) was obtained from the American Type Culture Collection. Mouse monoclonal anti-GalC antibody (23) was provided by B. Ranscht (La Jolla Cancer Research Center, La Jolla, CA). The rat monoclonal antibody specific for MBP, M1D3, was provided by W. Hickey (Washington University, St. Louis). A rat monoclonal antibody specific for GFAP, 2.2B10.6, (24) was provided by V. Lee (University of Pennsylvania, Philadelphia). HO13.4, a mouse IgM (κ light chain) monoclonal antibody specific for mouse Thy-1.2 (25), was obtained from the American Type Culture Collection. Fluorochrome-conjugated secondary antibodies were purchased from Tago.

For immunocytochemical studies, antibodies were used at a saturating dilution of concentrated culture supernatant or

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Abbreviations: Reo3R, cell surface receptor for reovirus type 3; GalC, galactocerebroside; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; PDGF, platelet-derived growth factor.

clarified ascites. Antibodies used to treat developing glial cultures were further purified. The mouse IgM (κ light chain) monoclonal antibodies 87.92.6 and HO13.4 were purified from clarified mouse ascites by adsorption to anti-mouse IgM–agarose (Sigma) columns and elution with 3.5 M $MgCl_2$. The purified antibodies were dialyzed extensively against phosphate-buffered saline, concentrated by ultrafiltration, filter-sterilized, and stored −70°C until use. Protein concentration was determined by absorbance at 280 nm. Purity was confirmed by SDS/PAGE.

Immunocytochemical Studies. To double label for A2B5 and GalC, cells were fixed with 2% paraformaldehyde in Hanks' balanced salt solution for 5 min at 4°C. Antibody dilutions and subsequent washes were performed in Hanks' balanced salt solution supplemented with 10 mM Hepes, 4% FCS, and 0.2% NaN_3. The cells were incubated successively in saturating concentrations of anti-GalC antibody, fluorescein isothiocyanate-conjugated goat anti-mouse IgG, A2B5 antibody, and rhodamine-conjugated goat anti-mouse IgM (μ chain specific) for 30 min each at room temperature. After immunostaining, the coverslips were postfixed in 5% glacial acetic acid in ethanol for 5 min at −20°C and mounted in glycerol/phosphate-buffered saline containing 0.2 M diazabicyclo(2.2.2)octane (26). The coverslips were examined by using a Leitz Dialux 20 microscope equipped for phase contrast and epifluorescence microscopy.

Prior to incubation with M1D3, the cells were permeabilized by incubation in acetone at room temperature for 10 min. Prior to incubation with 2.2B10.6, the cells were permeabilized with 5% glacial acetic acid in ethanol for 5 min at −20°C.

Determination of Proliferation by Immunostaining for BrdUrd Incorporation. The effect of anti-Reo3R antibody on cellular proliferation was determined by an adaptation of the BrdUrd incorporation method (27). Glial cultures were cultured for 24 hr in N2/0.5% FCS containing 10 μM BrdUrd (Sigma) and anti-Reo3R or control antibody. At the end of the culture period, the medium was replaced with fresh medium without BrdUrd, and the cells were incubated for an additional 30 min. The cells were mixed in 2% paraformaldehyde in Hanks' balanced salt solution for 5 min at 4°C and then stained with A2B5 followed by rhodamine-conjugated goat anti-mouse IgM or with anti-GalC followed by rhodamine-conjugated goat anti-mouse IgG. The cells were permeabilized with 70% ethanol for 30 min at −20°C and then incubated in 2 M HCl at room temperature for 10 min followed by 0.1 M sodium borate at room temperature for 10 min. The coverslips were incubated sequentially in anti-BrdUrd antibody (Becton Dickinson) diluted 1:10 followed by fluorescein isothiocyanate-conjugated goat anti-mouse IgG. The coverslips were mounted and examined as described above. The additional surface-staining resulting from the reactivity of fluorescein isothiocyanate-conjugated goat anti-mouse IgG with bound A2B5 or anti-GalC was easily distinguished from the staining of BrdUrd in the nucleus.

Mitomycin C Treatment. Cultures were treated with mitomycin C (Sigma) at 25 μg/ml diluted in N2/0.5% FCS for 30 min at 37°C in the dark. The cultures were washed twice with N2/0.5% FCS and cultured for an additional 24 or 48 hr.

RESULTS

Effects of Anti-Reo3R Antibody on A2B5 and GalC Expression. Previous studies demonstrated that addition of anti-Reo3R antibodies and certain Reo3R-binding peptides to neonatal rat optic nerve cultures increased the number of cells expressing GalC after 24 hr (15). Further studies were undertaken to determine the origin and fate of these cells. Optic nerve cells (3×10^6) were isolated on postnatal day 1 and cultured in N2/0.5% FCS containing 87.92.6 or an isotype-matched control antibody at 10 μg/ml. After 1–5 days in culture, the cells were double-immunostained for A2B5 and GalC. The number of $A2B5^+$ $GalC^-$ precursors rapidly decreased in both cultures (Fig. 1 *Top*). However, the rate of disappearance was more rapid in cultures containing anti-Reo3R antibody.

A substantial increase in the number of $GalC^+$ cells was observed after 24 hr of culture in the presence of 87.92.6 at 10 μg/ml. At this early time point the predominant effect represented an increase in the number of $A2B5^+$ $GalC^+$ cells (Fig. 1 *Middle*). After 2 days of culture, the number of $A2B5^+$ $GalC^+$ cells remained higher in cultures treated with anti-Reo3R antibody relative to control cultures. By day 3 of culture, the number of $A2B5^+$ $GalC^+$ cells in cultures treated with anti-Reo3R antibody began to decrease, reflecting further differentiation of these cells and loss of A2B5 reactivity. In contrast, under these culture conditions the number of $A2B5^+GalC^+$ cells in control cultures typically peaked on day 3.

After 1 day of culture, $A2B5^-$ $GalC^+$ cells were more numerous in cultures treated with 87.92.6 relative to cultures containing control antibody (Fig. 1 *Bottom*). On days 2 and 3 of culture, there were substantially greater numbers of these cells. By day 5 of culture, the numbers of $A2B5^-$ $GalC^+$ cells in treated and control cultures were equal. Thus, anti-Reo3R antibody induced GalC expression in immature cells, manifested as an increased number of $A2B5^+$ $GalC^+$ cells after 1 day of culture. Anti-Reo3R antibody also stimulated loss of A2B5 reactivity leading to the early appearance of $A2B5^-$ $GalC^+$ cells.

Dissociation of Phenotypic from Morphological Maturation. In control cultures, the first cells observed to express surface GalC were weakly $A2B5^+$ and exhibited long processes with multiple branches. In contrast, after 24 hr of culture in the

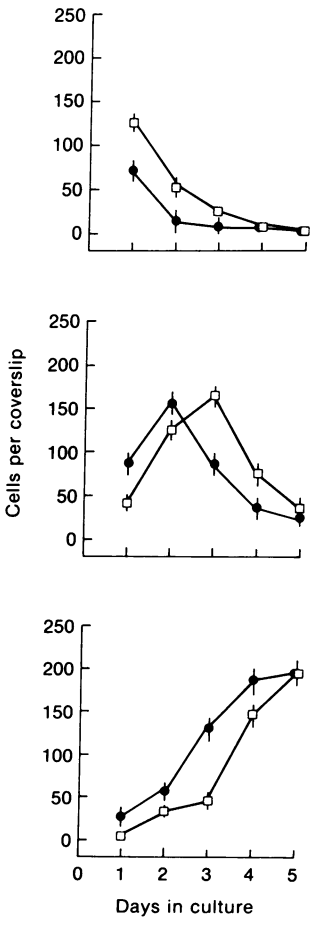

FIG. 1. Anti-Reo3R antibody accelerates the appearance of mature oligodendrocytes. Cells (3×10^3) isolated from neonatal rat optic nerves on postnatal day 1 were cultured in N2/0.5% FCS containing the anti-Reo3R antibody, 87.92.6 (●), or an isotype-matched control antibody, HO13.4 (□), at 10 μg/ml. After 1–5 days in culture, the cells were double-immunostained for A2B5 and GalC and examined by epifluorescence microscopy. The total numbers of cells per coverslip expressing the $A2B5^+$ $GalC^-$ (*Top*), $A2B5^+$ $GalC^+$ (*Middle*), and $A2B5^-$ $GalC^+$ (*Bottom*) phenotypes were determined. The values represent the mean ± SEM (n = 3).

presence of anti-Reo3R antibody, a large proportion of GalC$^+$ cells reacted strongly with the A2B5 antibody and had the morphological appearance of immature oligodendrocyte precursors (9) with short, simple processes (Fig. 2 *A* and *B*). Lack of staining with other isotype-matched control antibodies confirmed that the reactivity with anti-GalC antibody was specific. When left in culture for several additional days, these cells extended processes indistinguishable from those of normal oligodendrocytes in culture (Fig. 2*C*), suggesting that the simple processes exhibited by the early appearing GalC$^+$ cells did not merely result from a toxic effect of anti-Reo3R antibody. Rather, it appears that these cells prematurely expressed GalC relative to their state of morphological maturation.

Induction of MBP. A later appearing myelin component, MBP, also appeared in an accelerated manner in cultures containing anti-Reo3R antibody. Cells (6×10^3) from postnatal day 4 rat pups were cultured in N2/0.5% FCS containing 87.92.6 anti-Reo3R antibody or isotype-matched control antibody at 10μg/ml. Previous studies by our group and by others have shown that developing oligodendrocytes expressing MBP first can be detected in small numbers on the equivalent of postnatal day 7 or 8 regardless of the day of culture (9, 15, 28). The number of MBP$^+$ cells typically increases gradually over the next several days in culture. In two experiments, anti-Reo3R antibody stimulated the accelerated appearance of MBP (Fig. 3). In both experiments MBP$^+$ cells were first detected in anti-Reo3R antibody-treated cultures on day 3 (equivalent to postnatal day 7). Substantial numbers of MBP$^+$ cells were observed after day 4 of culture. In contrast, only rare MBP$^+$ cells were detected in control cultures on day 4. By day 7 of culture, the numbers of MBP$^+$ cells in the treated and control cultures were equal. Under both culture conditions, immunoreactive MBP initially was confined to the cytoplasm. When cultures were maintained for several additional days, MBP was detected in the membranous outer processes. Thus, developing oligodendrocytes stimulated to express MBP early appeared to transport the protein normally.

Lack of Effect on Lineage Commitment. Anti-Reo3R antibody did not appear to alter the lineage commitment of glial precursors. Cultures were set up on postnatal day 4 and grown in N2 medium containing either 0.5% or 10% (vol/vol) FCS and either 87.92.6 or control antibody at 10 μg/ml. After either 48 or 96 hr of culture, the coverslips were double-immunostained for A2B5 and GFAP or GalC and GFAP. No difference in the number of process-bearing type 2 astrocytes was observed at either time point (data not shown). In addition, no increase in the number of GalC$^+$ GFAP$^+$ cells occurred (data not shown).

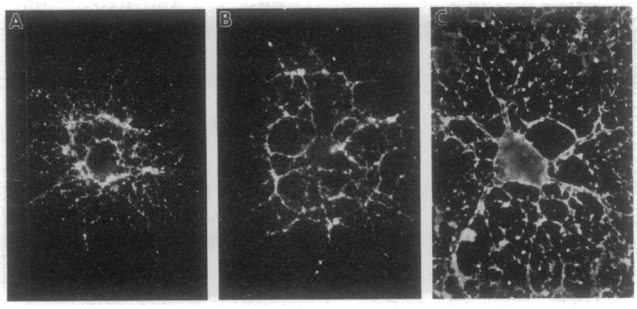

FIG. 2. Anti-Reo3R antibody induces GalC expression by cells having the morphology of immature oligodendrocytes. Cells isolated from neonatal rat optic nerves on postnatal day 1 were cultured in N2/0.5% FCS containing 87.92.6 anti-Reo3R antibody. After 1 or 3 days of culture, the cells were immunostained for anti-GalC. (*A* and *B*) Morphologically immature GalC$^+$ cells after 1 day of culture. (*C*) A morphologically mature GalC$^+$ cell after 3 days in culture with anti-Reo3R antibody. (×25.)

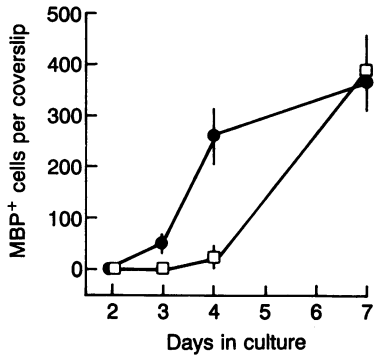

FIG. 3. Stimulation of MBP expression by anti-Reo3R antibody. Cells (6×10^6) isolated from neonatal rat optic nerves on postnatal day 4 were cultured in N2/0.5% FCS containing 87.92.6 anti-Reo3R antibody (●) or an isotype-matched control antibody, HO13.4 (□), at 10 μg/ml. After 2, 3, 4, or 7 days in culture, the cells were immunostained for MBP and examined by epifluorescence microscopy. The total numbers of cells per coverslip expressing MBP were determined. The values represent the mean ± SEM (n = 3).

Lack of Effect on O-2A Proliferation. The onset of differentiation and expression of myelin markers is associated with the cessation of O-2A cell proliferation (1, 29, 30). High concentrations of anti-Reo3R antibodies and Reo3R-binding peptides inhibit the proliferation of a number of receptor-bearing cells in culture (20, 21, 31). To test whether inhibition of proliferation *per se* affected the timing of GalC$^+$ appearance, optic nerve cultures prepared on postnatal day 1 were incubated with mitomycin C at 25 μg/ml for 30 min. The cultures were washed and then carried for an additional 24 or 48 hr. Decreased numbers of A2B5$^+$ cells were observed at both time points without a concomitant increase in the proportion of GalC$^+$ cells (data not shown).

Lower concentrations of anti-Reo3R antibodies and Reo3R peptides comparable to those that stimulate GalC expression by developing oligodendrocytes (15) weakly stimulate the proliferation of selected cells (J.A.C., unpublished data). Anti-Reo3R antibodies could have produced the apparent early appearance of oligodendrocyte markers by stimulating proliferation and expansion of the O-2A cell pool. Several lines of evidence suggest that this was not the case. First, no increase in the number of A2B5$^+$ GalC$^-$ cells was observed after 1 day of culture in the presence of anti-Reo3R antibody (Fig. 1 *Top*). Thus, there was no significant expansion of the O-2A pool. Second, the maximum numbers of A2B5$^+$ GalC$^+$ and A2B5$^-$ GalC$^+$ cells were equal in anti-Reo3R antibody-treated and control cultures. In the anti-Reo3R antibody-treated cultures, these cells appeared earlier rather than in increased numbers.

Third, no effect on oligodendrocyte precursor proliferation was observed upon direct testing. Cells were labeled over the first 24 hr of culture with BrdUrd in the presence of 10–50 μg of anti-Reo3R or control antibody per ml. After 24 hr of culture, the coverslips were double-immunostained for BrdUrd incorporation and for A2B5 or GalC to determine the proportion of cells expressing these markers that had undergone cell division over the preceding day. In three separate experiments, 2–5% of both the A2B5$^+$ and the GalC$^+$ cells exhibited nuclear staining for BrdUrd in both the 87.92.6- and HO13.4-treated cultures. This proportion agrees with previous estimates of the mitotic index of O-2A cells cultured in N2/0.5% FCS (1, 29, 30, 32) and confirms the rapidity with which cells of the O-2A lineage drop out of the mitotic pool when placed in culture in N2 medium with low serum. No difference between the proliferative rates of control and anti-Reo3R antibody-treated cultures could be discerned. Given

the low level of BrdUrd incorporation, inhibition of proliferation would be difficult to detect. More importantly, no increase in the number of cells incorporating BrdUrd was observed. Thus, the increased number of $GalC^+$ oligodendrocytes observed in cultures treated with anti-Reo3R antibody did not merely result from a mitogenic effect leading to expansion of the precursor pool. These data suggest that Reo3R binding stimulates oligodendrocyte differentiation independent of direct effects on cellular proliferation.

DISCUSSION

Previous studies demonstrated that the Reo3R appears at an early stage of oligodendrocyte development. Addition of Reo3R ligands to neonatal rat optic nerve cultures leads to increased numbers of $GalC^+$ cells after 24 hr (13–15). The present studies show that anti-Reo3R antibody stimulates several additional features of oligodendrocyte differentiation. Anti-Reo3R antibody stimulates accelerated conversion of O-2A progenitor cells into immature oligodendrocytes with the $A2B5^+$ $GalC^+$ phenotype. These cells subsequently differentiate into $A2B5^-$ $GalC^+$ MBP^+ oligodendrocytes at an increased rate. GalC is first detected on cells having the appearance of immature oligodendrocyte precursors, which normally do not express GalC (9), suggesting that the rate at which developing oligodendrocytes can extend processes may be relatively fixed. Several lines of evidence argue that the effect on differentiation is independent of potential effects on precursor proliferation. Treatment with anti-Reo3R antibody also did not appear to alter the lineage commitment of bipotential glial progenitors grown in either N2/0.5% medium or medium containing 10% serum. Rather, Reo3R binding appeared to accelerate the differentiation into oligodendrocytes of precursors already committed to that path.

Myelination in the central nervous system occurs during the first 3 weeks after birth in rodents. This process depends upon the coordinated execution of a complex developmental sequence that includes migration of glial precursors, expansion of the precursor pool via proliferation, exit of precursors from the cell cycle, synthesis of myelin-associated proteins and lipids, myelin assembly, and myelin maintenance. O-2A cells migrate into the rat optic nerve beginning on approximately embryonic day 15 (33). *In vivo* they continue to proliferate for several weeks (34, 35), gradually dropping out of the mitotic pool and differentiating into oligodendrocytes or type-2 astrocytes. When cultured in defined medium, O-2A cells cease dividing and initiate the sequence of oligodendrocyte differentiation regardless of the age of the animal from which they are isolated (30). A variety of soluble factors and hormones have been proposed to play a role in the regulation of oligodendrocyte development and function. Several receptor systems represent regulatory mechanisms with which the Reo3R system may interact.

Oligodendrocytes express several receptors linked to adenylate cyclase, whose activity is modulated by cell-substratum interactions (36). Which of these receptor systems represent the principal regulators of intracellular cAMP concentration in oligodendrocytes *in vivo* is unknown. Nevertheless, it appears that cAMP has potent effects on developing oligodendrocytes. Treatment of glial cultures derived from postnatal day 1 rat cerebral hemisphere with 1 mM dibutyryl cAMP leads to increased numbers of cells expressing immunoreactive CNPase, GalC, and MBP (37). The dibutyryl cAMP analog inhibits O-2A proliferation and accelerates the rate of differentiation into oligodendrocytes without altering the lineage choice of the precursor cells. These observations are of particular interest in view of the biochemical and antigenic similarity of the Reo3R to the β_2-adrenergic receptor and other members of the rhodopsin-like family of receptors (13, 16, 17). However, there are several lines of evidence showing that Reo3R-mediated effects in other cells do not occur through classic adrenergic receptor transduction mechanisms. Competition studies show that catecholamine and reovirus type 3 binding sites are distinct (16, 17, 38). β-Adrenergic agonists neither reproduce nor alter Reo3R-mediated inhibition of L-cell proliferation (31). Reo3R ligands do not affect isoproterenol-induced cAMP accumulation (38). Finally, Reo3R-mediated effects on cell growth are not mediated by changes in intracellular cAMP concentration (17, 38). Thus, the precise relationship between the Reo3R and members of the rhodopsin-like family of receptors, including their relationship in oligodendrocytes, requires better definition.

Several lines of evidence argue that platelet-derived growth factor (PDGF) plays an important role in oligodendrocyte differentiation. The normal timing of oligodendrocyte differentiation can be restored by culture in the presence of type-1 astrocyte-conditioned medium (30). Recent studies demonstrate that PDGF, particularly the AA homodimeric form, is a potent mitogen for O-2A cells (32, 39, 40), prevents the premature differentiation of O-2A cells in culture (9, 29, 30, 41), and probably is the factor elaborated by astrocytes that is responsible for these activities (32, 39, 41).

These findings suggest that Reo3R ligands could stimulate oligodendrocyte differentiation by decreasing PDGF elaboration or effects in the cultures. Type-1 astrocytes, which comprise the most abundant cells in the cultures used in the present studies, uniformly express Reo3R (15). The binding of Reo3R ligands might inhibit PDGF production by these cells, affecting oligodendrocyte differentiation indirectly. However, preliminary experiments using purified cultures of rat type-1 astrocytes treated with Reo3R ligands indicate no effect on the level of PDGF A chain mRNA (J.A.C., unpublished data). It is possible that Reo3R ligands compete with PDGF for its receptor. However, comparison of the sequences of the binding domains of the reovirus type 1 σ1 protein and 87.92.6 anti-Reo3R antibody (18, 19) with the PDGF A and B chains demonstrates no homology. Also, the Reo3R (16, 42) and PDGF receptor (43) are biochemically distinct. BALB/c 3T3 fibroblasts persistently infected with reovirus type 3 exhibit a decreased DNA synthetic response to epidermal growth factor, a key mitogen for these cells (44), associated with a 70–90% decrease in the surface expression of epidermal growth factor receptor. By analogy, Reo3R perturbation could accelerate oligodendrocyte differentiation via decreased expression of PDGF receptors. This possibility is unlikely, given the absence of an effect of Reo3R ligands on O-2A proliferation.

Insulin-like growth factor I appears to have several effects on precursor cells in the oligodendrocyte lineage. It stimulates the proliferation of intermediate oligodendrocyte precursor cells and induces commitment to differentiation to oligodendrocytes (9, 45, 46). In addition, it increases expression of immunoreactive MBP (47). There is no apparent homology between the Reo3R ligands (18, 19) and insulin-like growth factor I (48). The Reo3R (16, 42) is structurally distinct from the insulin-like growth factor I receptor (49–51). Thus, it is unlikely that the effects of Reo3R ligands on oligodendrocyte development are mediated by the receptor for insulin-like growth factor I.

Antibodies against other surface markers have been shown to alter oligodendrocyte differentiation in culture. The O4 antibody reacts with sulfatide and seminolipid (8, 52). Reactivity with O4 appears at approximately the time that developing oligodendrocytes begin to express Reo3R (15). Interestingly, treatment of glial cultures with O4 stimulates oligodendrocyte differentiation and is manifest as increased expression of MBP mRNA, immunoreactive MBP, and CNPase activity (53). However, in contrast with Reo3R ligands,

the initial manifestation of O4 treatment is pronounced cell aggregation.

It does not appear that accelerated differentiation is a universal consequence of antibody binding to developing oligodendrocytes. Antibodies against GalC inhibit oligodendrocyte differentiation (54, 55). Oligodendrocytes is anti-GalC-treated cultures exhibited long processes with sparse branching. In such cultures there was decreased synthesis of GalC and sulfatide, MBP mRNA and protein, and CNPase activity. These effects are reversible after the removal of anti-GalC antibody. Treatment with antibodies reactive with a variety of other oligodendrocyte surface markers, including N-CAM, HNK-1, O1, 1A9, and cholesterol, produce no effect on oligodendrocyte development (54).

Associated with loss of the A2B5 and GD3 markers, developing oligodendrocytes express myelin-associated proteins and lipids in a characteristic temporal sequence. The appearance of surface lipids recognized by the O4 antibody is followed by expression of GalC and CNPase, MBP and myelin-associated glycoprotein, and finally proteolipid protein. Like O4 antibody, insulin-like growth factor-I, cAMP analogs, and anti-GalC antibody, Reo3R ligands appear to modulate several features of oligodendrocyte development, not merely the expression of a single myelin component. The intracellular signaling mechanisms by which the Reo3R and its cognate ligand regulate the initiation or rate of execution of this developmental program have not been elucidated. Clarification of these mechanisms will be of interest.

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