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Ligand binding to the cell surface receptor for reovirus type 3 stimulates galactocerebroside expression by developing oligodendrocytes

(glial differentiation/myelination/optic nerve/anti-idiotypes)

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ABSTRACT **Viruses utilize normal cell surface structures as attachment sites. Interaction of viral components with these structures may alter target cell growth. In the present study, the expression and function of the cell surface receptor for reovirus type 3 (Reo3R) was studied in neonatal rat optic nerve glial cultures. The Reo3R is expressed by mature oligodendrocytes and astrocytes but not by O-2A progenitor cells. It appears at an early stage of oligodendrocyte development, coincident with the O4 marker but prior to galactocerebroside or myelin basic protein. Anti-Reo3R antibodies stimulate the expression of galactocerebroside by developing oligodendrocytes. Divalent Reo3R-binding peptides are similarly active. Maximal stimulation of galactocerebroside expression occurs with treatment as short as 4 hr, consistent with a receptor-mediated process. Cell surface structures used as an attachment site by reovirus type 3 may also play a role in the regulation of oligodendrocyte differentiation.**

Myelination in the central nervous system involves the migration and proliferation of oligodendrocyte precursors, the coordinated synthesis of myelin components, and morphological adaptation of the oligodendrocyte plasma membrane to form the myelin sheath. The regulation of this complex process probably involves autonomous mechanisms intrinsic to the oligodendrocyte and its precursors; interactions of oligodendrocytes with the substratum, axons, and other glia; and the actions of soluble regulatory factors. Neonatal rat optic nerve glial cultures provide a useful model system with which to dissect these phenomena (1). In this system, O-2A glial progenitor cells have been identified, which differentiate into oligodendrocytes and type 2 (fibrous) astrocytes. Type 1 (protoplasmic) astrocytes, also present in these cultures, probably arise from a separate lineage. Each of these cell types can be identified by morphological criteria and by the expression of characteristic antigenic markers (2–4).

The nervous system is an important target tissue of reovirus infection. Serotype-specific differences in the viral $\sigma 1$ cell-attachment protein and the differential expression of serotype-specific receptor structures on target cells determine the cellular tropism pattern of the virus. In primary central nervous system cultures, mature neurons, oligodendrocytes, and both type 1 and type 2 astrocytes express immunoreactive cell surface receptors for reovirus type 3 (Reo3R), but ependymal cells do not (5–8). Ependymal cells express receptors for reovirus type 1 but not type 3 (6, 7). O-2A glial progenitors do not express receptors for either reovirus type 3 (8) or type 1 (present study).

In the present study, the timing of the appearance of the Reo3R relative to other oligodendrocyte differentiation markers is delineated. The Reo3R appears at an early stage of oligodendrocyte differentiation prior to galactocerebroside (GalC) or myelin basic protein (MBP). Because of the biochemical and antigenic similarity of the Reo3R to the β_2-adrenergic receptor (β_2AR) and other members of the rhodopsin-like family of receptors (8–10), we postulated that Reo3R perturbation would alter oligodendrocyte differentiation. The present studies demonstrate that anti-Reo3R antibodies and Reo3R-binding peptides stimulate GalC expression by developing oligodendrocytes in culture, suggesting that the appearance of the Reo3R may be an important feature of oligodendrocyte differentiation.

MATERIALS AND METHODS

Cell Culture. Neonatal rat optic nerve glia were cultured according to the methods described by Raff and co-workers (3). Briefly, single-cell suspensions were prepared from optic nerves on the postnatal day indicated. Cells (3×10^3) 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 (3, 11) was added, bringing the final serum concentration to 0.5% (N2/0.5% FCS).

Antibodies. The isolation and characterization of the anti-Reo3R antibodies anti-ID3 (6) and 87.92.6 (7) have been described. A2B5, a mouse monoclonal antibody specific for GQ ganglioside (12), was obtained from the American Type Culture Collection. The O4 mouse monoclonal antibody (13) was provided by M. Schachner (Swiss Federal Institute of Technology, Zurich). Mouse monoclonal anti-GalC antibody (14) 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, Saint Louis) 2.2B10.6, a rat monoclonal antibody specific for glial fibrillary acidic protein (GFAP) (15) was provided by V. Lee (University of Pennsylvania, Philadelphia). HO13.4 and HO22.1, mouse IgM (κ light chain) monoclonal antibodies specific for mouse Thy 1.2 and Thy 1.1, respectively (16), were obtained from the American Type Culture Collection. Fluorochrome-conjugated secondary antibodies were purchased from Tago.

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Abbreviations: Reo3R, cell surface receptor for reovirus type 3; GalC, galactocerebroside; MBP, myelin basic protein; β_2AR, β_2-adrenergic receptor; FCS, fetal calf serum; GFAP, glial fibrillary acidic protein; PND-x, postnatal day x.

For immunocytochemical studies, antibodies were used at a saturating dilution of concentrated culture supernatant or clarified ascites. Antibodies used to treat developing glial cultures were further purified. The mouse IgM (κ light chain) monoclonal antibodies 87.92.6, HO13.4, and HO22.1 were purified from clarified mouse ascites by adsorption to anti-mouse IgM-agarose (Sigma) columns and elution with 3.5 M $MgCl_2$. Anti-ID3 IgG was prepared from rabbit antiserum by ammonium sulfate precipitation and passage over a protein A-Sepharose CL-4B (Sigma) column. The purified antibodies were dialyzed extensively against phosphate-buffered saline, concentrated by ultrafiltration, filter sterilized, and stored at −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 Reo3R and other glial markers, the cells were fixed with 2% paraformaldehyde in Hanks' balanced salt solution (HBSS) for 5 min at 4°C. Antibody dilutions and subsequent washes were performed in HBSS supplemented with 10 mM Hepes, 4% FCS, and 0.2% NaN_3 (IFM buffer). The cells were incubated successively in anti-ID3 (50 μg/ml) followed by goat anti-rabbit IgG conjugated to rhodamine for 30 min each at room temperature. They were then incubated for 30 min at room temperature with saturating concentrations of A2B5, O4, anti-GalC, M1D3, or 2.2B10.6 followed by fluorescein isothiocyanate-conjugated goat secondary antibody of the appropriate specificity. Prior to staining for MBP with M1D3, the cells were permeabilized by incubation in acetone at room temperature for 10 min. Prior to staining for GFAP with 2.2B10.6, the cells were permeabilized with 5% glacial acetic acid in ethanol for 5 min at −20°C. After immunostaining, the coverslips were postfixed in 5% glacial acetic acid in ethanol for 5 min at −20°C and mounted in glycerol/PBS containing 0.2 M diazabicyclo[2.2.2]octane (17). The coverslips were examined with a Leitz Dialux 20 microscope equipped for phase contrast and epifluorescence microscopy.

To double label for A2B5 or O4 in addition to GalC, the cells were fixed with 2% paraformaldehyde in HBSS for 5 min at 4°C. They were then incubated successively in saturating concentrations of anti-GalC antibody, goat anti-mouse IgG conjugated to fluorescein isothiocyanate, A2B5 or O4 antibody, and goat anti-mouse IgM conjugated to rhodamine (μ-chain specific) for 30 min each at room temperature.

Virus Binding Studies. Reovirus types 1 and 3 (kindly provided by D. H. Rubin, University of Pennsylvania) were grown on murine L cells and purified by CsCl gradient centrifugation as described (18, 19). Coverslips were incubated in 100 μl of IFM buffer containing 10^8 viral particles at 4°C for 1 hr. After washing, the coverslips were incubated for 30 min each in rabbit reovirus-specific antiserum (20) followed by goat anti-rabbit IgG conjugated to fluorescein isothiocyanate. The cells were then double labeled for the glial markers (A2B5, O4, GalC, MBP, or GFAP) as described above.

Peptides. Reo3R-binding peptides (Table 1) were synthesized as described (21, 22). Peptides were further purified by gel filtration over a Sephadex G-25 column. Composition and purity were confirmed by amino acid analysis and reverse-phase high-performance liquid chromatography.

Statistical Analyses. Sample means were compared using a paired *t* test for populations with unknown, unequal variances or analysis of variance (24).

RESULTS

Normal Developmental Sequence in Optic Nerve Cultures. To allow detection of potentially subtle changes induced by Reo3R-binding ligands, it was necessary to carefully characterize the time course of oligodendrocyte differentiation in this culture system. Single-cell suspensions were prepared on postnatal day 1 (PND-1) or PND-4. Cells (3×10^3) were plated on poly(L-lysine)-coated coverslips in N2/0.5% FCS. After 1, 2, 4, or 7–10 days of culture, the cells were double immunostained for A2B5 and GalC, O4 and GalC, GalC and MBP, or A2B5 and GFAP.

Table 1. Reo3R-binding peptides

Peptide	Sequence
Reovirus type 3	QSM-WIGIVSYSGSGLN
σ1 protein	317 332
V_L peptide	KPGKTNKLLIYSGSTLQ
V_LSH peptide	CKPGKTNKLLIYSGSTLQ
Peptide F	CNGSHVPDHDVTEERDE

The amino acid sequence of the Reo3R-binding region of the reovirus type 3 σ1 protein (21–23) is listed. V_L peptide corresponds to a region of nearly identical sequence present in the light-chain complementarity determining region II of the 87.92.6 anti-Reo3R antibody. V_LSH peptide is the V_L sequence plus an amino-terminal cysteine to allow dimerization. Peptide F is a control peptide with pI, net charge, and hydrophobicity approximately equal to that of V_L.

The predominant cell in these cultures was the type 1 astrocyte, which is characterized by a flat triangular shape and expression of GFAP but neither A2B5 nor GalC. The absolute and relative numbers of these cells increased over time. Cells of the O-2A lineage were present in smaller numbers and comprised the majority of the remaining cells.

The phenotypic profile of cells in the O-2A lineage rapidly evolved in cultures prepared on PND-1 (Fig. 1 and Table 2). Initially, $A2B5^+$ $GalC^-$ progenitor cells with a simple bipolar morphology represented the most common cells of the O-2A lineage. The number of these cells was markedly decreased by day 2. By day 4 these cells were virtually absent. The O4 marker appears at an early stage of oligodendrocyte development prior to the appearance of GalC (13). Substantial numbers of $O4^+$ $GalC^-$ cells with short, simple processes were present on days 1 and 2 of culture but not later.

Small numbers of $GalC^+$ oligodendrocytes were present on day 1 of culture. These cells uniformly expressed A2B5 and O4 and exhibited long processes with multiple branches. The absolute number of $A2B5^+$ $GalC^+$ cells increased until day 2 or 3 of culture and then decreased as the cells further differentiated and ceased to express A2B5. Mature $A2B5^-$ $GalC^+$ oligodendrocytes with extensive, complex branching processes were rare before day 2 or 3 of culture. The number of such cells steadily increased up to day 4. No further increase was observed on day 7. The number of $O4^+$ $GalC^+$

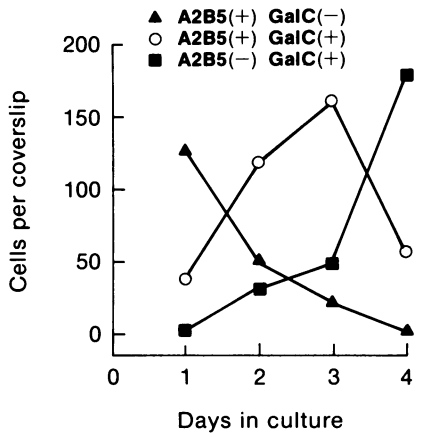

FIG. 1. *In vitro* differentiation of O-2A progenitor cells. Cells (3×10^3) isolated on PND-1 were cultured in N2/0.5% FCS. After 1, 2, 3, or 4 days in culture the cells were double immunostained for A2B5 and GalC. The values represent the mean number of cells of three coverslips. For every data point the SEM, omitted for clarity of presentation, represented 10–15% of the mean.

Table 2. Expression of the Reo3R during oligodendrocyte differentiation

Days in culture	Cells expressing Reo3R among cells expressing other glial markers									
	GFAP		A2B5		O4		GalC		MBP	
	%	Fraction	%	Fraction	%	Fraction	%	Fraction	%	Fraction
1	71.7	607/847	5.5	13/236	51.7	61/118	93.1	27/29		0/0
2	92.7	1124/1213	31.2	39/125	29.9	47/157	88.8	174/196		0/0
4	96.9	1648/1701	25.0	2/8	46.6	125/268	92.9	223/240	100	12/12

Cells (3×10^3) isolated on PND-4 were cultured in N2/0.5% FCS. After 1, 2, or 4 days of culture, the cells were double immunostained for Reo3R and one of the following glial markers: GFAP, A2B5, O4, GalC, or MBP. The percentages of Reo3R$^+$ cells among cells expressing each of the glial markers are indicated. The fractions represent total cell counts from individual coverslips. The denominators are the total number of cells expressing a given glial marker. The numerators are the numbers of such cells that also expressed Reo3R.

cells also progressively increased over the first 4 days of culture. After day 4, the numbers of O4$^+$ GalC$^+$ and A2B5$^-$ GalC$^+$ cells were virtually identical, suggesting that these two cell populations were the same. Similar results were obtained in studies of cultures set up on PND-4, aside from the earlier appearance of mature oligodendrocytes.

In four separate experiments, cells expressing immunoreactive MBP were first detected after 7 or 8 days of culture in cultures initiated on PND-1. In cultures set up on PND-4 (three separate experiments), MBP$^+$ cells appeared after 3 or 4 days. Thus, MBP appeared on the equivalent of PND-7 to -8, regardless of the day of culture. MBP$^+$ cells were uniformly GalC$^+$. Prior to PND-7, no GalC$^+$ cells expressed MBP. Subsequently, the proportion of GalC$^+$ cells that expressed MBP progressively increased. These results agree with previous studies (3, 13, 25–28) suggesting that cells of the O-2A lineage sequentially express the following antigenic phenotypes: A2B5$^+$ O4$^-$ GalC$^-$ MBP$^-$ → A2B5$^+$ O4$^+$ GalC$^-$ MBP$^-$ → A2B5$^+$ O4$^+$ GalC$^+$ MBP$^-$ → A2B5$^-$ O4$^+$ GalC$^+$ MBP$^-$ → A2B5$^-$ O4$^+$ GalC$^+$ MBP$^+$.

Expression Pattern of Reo3R During Oligodendrocyte Differentiation. Previous studies demonstrated that immunoreactive Reo3R is expressed by mature oligodendrocytes and astrocytes in culture but not by O-2A progenitor cells (8). To determine the timing of Reo3R appearance more precisely, optic nerve cultures were set up on PND-1 (two experiments) or PND-4 (two experiments). Coverslips were removed over the next 4 days, and the cultures were double immunostained for Reo3R and for A2B5, O4, GalC, MBP, or GFAP. The results of a representative experiment are presented in Table 2.

As noted above, type 1 astrocytes were the most numerous cells in these cultures. The relative numbers of these cells progressively increased as did the proportion expressing immunoreactive Reo3R. After 4 days in culture, 96–98% expressed Reo3R.

A2B5$^+$ cells were numerous on day 1 of culture; the majority were bipolar or exhibited short, simple processes typical of O-2A progenitor cells. Such cells were uniformly negative for Reo3R. The rare A2B5$^+$ Reo3R$^+$ cells observed probably represented early oligodendrocytes, as evidenced by their weak A2B5$^+$ reactivity and relatively complex processes. At early time points, few cells expressed the O4 marker. The number of O4$^+$ cells increased progressively over the 4-day culture period. At all time points, one-third to one-half of the O4$^+$ cells expressed Reo3R. Mature oligodendrocytes, expressing GalC followed by MBP, were rare at the beginning of the culture period but progressively increased in number. Virtually all expressed Reo3R.

Reovirus type 3 binding studies demonstrated a developmental expression pattern identical to that obtained with anti-Reo3R antibody. Reovirus type 1 did not bind to glial progenitors or to mature astrocytes or oligodendrocytes. When cultures were double labeled by using reovirus type 3 and anti-Reo3R antibody binding (nonsaturating concentrations of both), identical cell populations were stained. These results confirm earlier studies showing that the anti-Reo3R antibodies identify a binding site for reovirus type 3 expressed by central nervous system glia (6–8, 29).

Stimulation of GalC Expression by Anti-Reo3R Antibodies. Because of the developmentally regulated expression pattern of the Reo3R during oligodendrocyte differentiation and because of the biochemical and antigenic similarity of the Reo3R to the β_2AR (8–10), we hypothesized that Reo3R perturbation would alter oligodendrocyte development. To test this hypothesis, 3×10^3 cells isolated on PND-1 were cultured in N2 medium/0.5% FCS alone or with various concentrations of purified anti-Reo3R antibody 87.92.6. In seven separate experiments, addition of 87.92.6 to the cultures at concentrations of 1–50 μg/ml produced increased numbers of GalC$^+$ cells within 24 hr. The results of a representative experiment are presented in Fig. 2. Polyclonal anti-Reo3R antiserum, anti-ID3, also stimulated GalC expression. The weaker effect of this antiserum relative to 87.92.6 may reflect its polyclonal nature and a smaller proportion of antibody molecules that bind the Reo3R.

The number of GalC$^+$ cells in cultures containing HO13.4 or HO22.1, isotype-matched (IgM κ light chain) control antibodies purified in parallel in a manner identical to that of 87.92.6, at concentrations up to 50 μg/ml, was no different from cultures containing N2/0.5% FCS alone. To further demonstrate the specificity of the effect of anti-Reo3R antibody on GalC expression, the antigen-binding domain of 87.92.6 was blocked prior to addition to the cultures. Preincubation with purified 9B.G5, the anti-type 3 σ1 antibody used to generate 87.92.6 (7), abrogated the ability of anti-Reo3R antibody to stimulate GalC expression (data not shown). Finally, purified normal rabbit immunoglobulin at 50

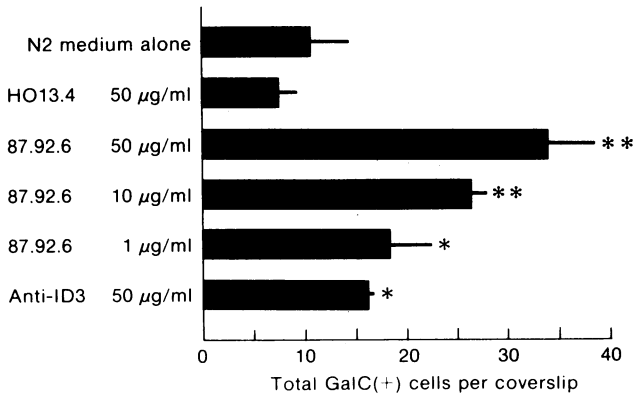

FIG. 2. Stimulation of GalC expression by anti-Reo3R antibodies. Cells (3×10^3) isolated on PND-1 were cultured in N2/0.5% FCS alone or in N2/0.5% FCS plus anti-Reo3R antibody (87.92.6 or anti-ID3) or control antibody (HO13.4) at the concentrations listed. After 24 hr in culture, the cells were immunostained for GalC. The values represent the mean number of GalC$^+$ cells per coverslip + SEM (*, $P < 0.05$; **, $P < 0.01$; $n = 3$).

μg/ml produced no change in the expression of GalC (data not shown).

Kinetics of Antibody-Mediated Induction of GalC Expression. Previous studies of clonal cell lines have shown that maximal Reo3R-mediated inhibition of proliferation requires incubation with anti-Reo3R antibody for only 1 hr (30). Therefore, studies were undertaken to determine the length of treatment necessary for maximal stimulation of GalC expression in neonatal rat optic nerve cultures. The 87.92.6 antibody at 50 μg/ml was added to PND-1 optic nerve cultures immediately after the cells attached. After incubation at 37°C for various lengths of time, bound antibody was removed by a 1-min wash with Dulbecco's modified Eagle's medium containing 25 mM sodium acetate (pH = 4.0). The cultures were washed several times with fresh N2/0.5% FCS and cultured for a total of 48 hr. As illustrated in Fig. 3 *Upper*, induction of the maximal number of cells expressing GalC at 48 hr required treatment of the cultures for 6–12 hr. No additional effect was observed by continued treatment for 24 hr or for the entire 48-hr culture period. We hypothesized that requirement for a 6- to 12-hr incubation with anti-Reo3R to induce maximal GalC expression in cultures treated immediately after plating may have resulted, in part, from the time required for Reo3R appearance. When antibody was added after 24 hr of culture, incubation for 4 hr produced a maximal effect (Fig. 3 *Lower*).

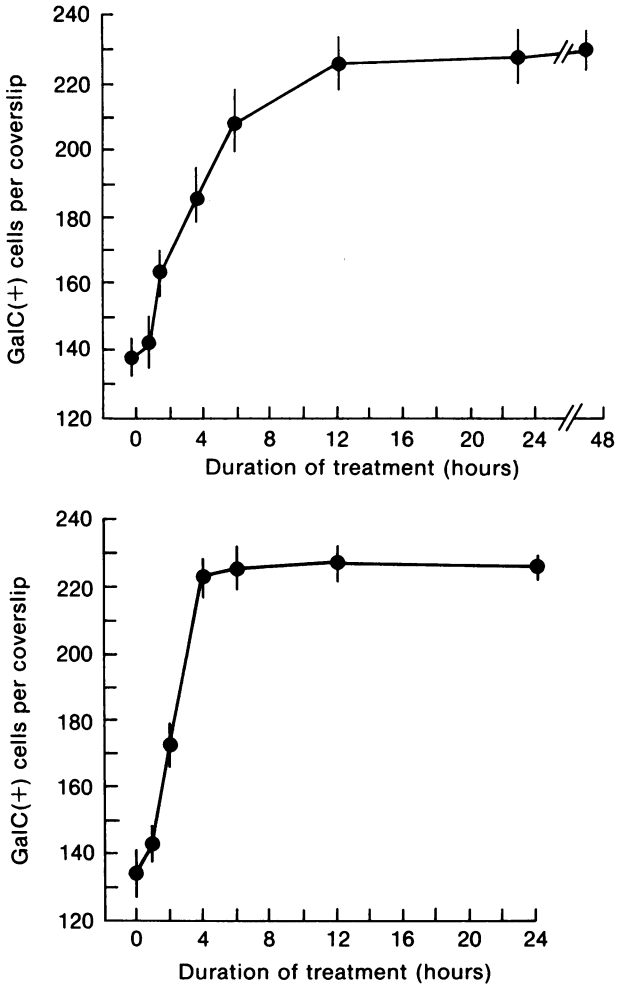

FIG. 3. Kinetics of GalC induction by anti-Reo3R antibodies. Cells (3×10^3) isolated on PND-1 were plated in N2/0.5% FCS. (*Upper*) The cells were treated at the beginning of the culture period with anti-Reo3R antibody 87.92.6 at 50 μg/ml for various lengths of time, washed, and cultured for a total of 48 hr. (*Lower*) The cells were cultured for 24 hr, incubated with anti-Reo3R antibody 87.92.6 at 50 μg/ml for various lengths of time, washed, and then incubated for an additional 24 hr. After a total of 48 hr in culture in both experiments, the cells were immunostained for GalC. The values represent the mean number of $GalC^+$ cells per coverslip ± SEM ($n = 3$).

Stimulation of GalC Expression by Reo3R-Binding Peptides. To molecularly define the structural features of Reo3R ligands necessary for modulation of oligodendrocyte differentiation, synthetic peptides corresponding to the receptor-binding regions of the reovirus type 3 σ1 protein and the anti-Reo3R antibody 87.92.6 were used (21–23). The sequences of these peptides are listed in Table 1. To produce a divalent Reo3R-binding peptide capable of crosslinking Reo3R molecules on the cell surface, an amino-terminal cysteine residue was added to the V_L sequence. The resultant peptide, designated V_LSH, was dimerized as described (21).

Like intact anti-Reo3R antibody, the divalent Reo3R-binding peptide V_LSH induced premature appearance of GalC when added to PND-1 optic nerve cultures at concentrations as low as 50 μg/ml (Fig. 4). Peptide F, a control peptide with similar pI, net charge, and solubility characteristics, was ineffective. Monomeric V_L peptide, which binds but does not crosslink Reo3R molecules, did not induce GalC expression.

DISCUSSION

These studies demonstrate that a cell surface structure utilized as an attachment site by reovirus type 3, although not expressed by glial progenitor cells, appears at an early stage of oligodendrocyte development and is expressed on virtually all mature oligodendrocytes and astrocytes in culture. Antibodies and peptides that bind the Reo3R stimulate GalC expression by developing oligodendrocytes. The differences between the control cultures and cultures treated with Reo3R ligands are significant. The stimulation of GalC expression represents a 200–400% increase in the number of $GalC^+$ cells. In this culture system, differences in the timing of the appearance of this marker of 1 to 2 days represent a substantial effect. Finally, these ligands are unique in their effect on oligodendrocyte differentiation.

The mechanisms by which Reo3R ligands alter oligodendrocyte development are unclear. Previous studies have demonstrated a region of sequence shared by the putative binding domain of the reovirus type 3 σ1 protein and antigen-binding region of the anti-Reo3R antibody 87.92.6 (23).

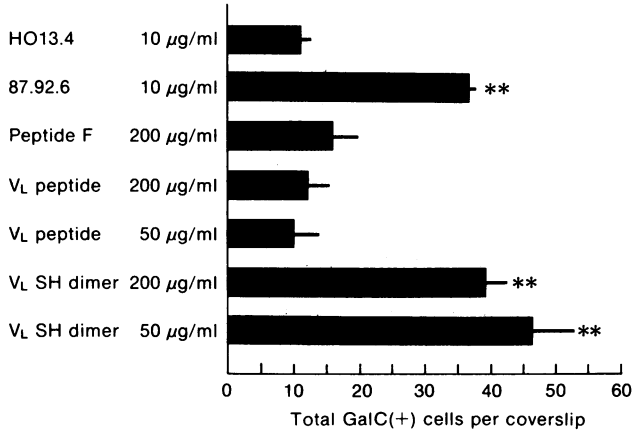

FIG. 4. Stimulation of GalC expression by Reo3R-binding peptides. Cells (3×10^3) isolated on PND-1 were cultured in N2/0.5% FCS plus Reo3R-binding antibody or peptides at the concentrations listed. HO13.4 is an isotype-matched control antibody for 87.92.6. Peptide F is a control peptide. After 24 hr in culture the cells were immunostained for GalC. The values represent the mean number of $GalC^+$ cells per coverslip + SEM (**, $P < 0.01$; $n = 4$).

Studies employing synthetic peptides demonstrated 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 to the Reo3R (21). These regions also mediate functional consequences of Reo3R–ligand interaction including down-modulation of Reo3R and inhibition of target cell growth (22, 31). The present studies demonstrate that these domains also mediate the observed effects on oligodendrocyte differentiation. Previous studies have emphasized the requirement for Reo3R crosslinking for Reo3R-mediated effects on receptor-bearing cells (22, 30). The present studies further confirm the requirement for Reo3R crosslinking for receptor-mediated alteration of target cell function.

Viruses have been postulated to interact with a variety of normal cell surface structures. This topic has been recently reviewed (32). The Reo3R and β_2AR are structurally and antigenically related and have similar distribution patterns (8–10). The expression and role of adrenergic receptors by developing and mature oligodendrocytes has been the subject of only a small number of studies. Adrenergic agonists stimulate cyclic AMP accumulation in oligodendrocyte cultures (33). Cyclic AMP and its analogs induce both GalC expression (34) and cyclic nucleotide phosphohydrolase activity (35), suggesting a role in the regulatory mechanisms of these cells. However, the receptor systems to which adenylate cyclase is linked in oligodendrocytes have not been established with certainty.

The precise relationship between the Reo3R and β_2AR requires further definition. Reo3R ligands and catecholamines do not compete for binding to either the Reo3R or the β_2AR (10, 36). Thus, if the β_2AR also serves as a serotype-specific reovirus receptor, virus and catecholamines probably utilize distinct binding domains. Reovirus type 3 and anti-Reo3R antibodies do not stimulate cyclic AMP accumulation in Reo3R-bearing cell lines (30, 37). Reo3R ligands do not affect isoproterenol-induced cyclic AMP accumulation (36). β-Adrenergic agonists neither reproduce nor alter Reo3R-mediated inhibition of cell growth (29). Thus, the functional consequences of reovirus type 3 and β-adrenergic ligand binding appear to be mediated by different second messenger systems. Classical adrenergic signaling pathways probably are not involved in Reo3R-mediated inhibition of target cell growth demonstrated in previous studies or in the stimulation of GalC expression by oligodendrocytes demonstrated in the present study.

The interaction of viruses, including reovirus, with normal cell surface structures may alter target cell growth, differentiation, or function by means of normal or aberrant activation of existent second messenger mechanisms. These effects could occur independent of subsequent cytopathic infection. Anti-receptor antibodies arising as anti-idiotypes during the normal immune response to systemic virus infection might similarly interact with normal cell surface structures with analogous functional consequences.

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