Molecular Neurobiology
Copyright ©1993 Humana Press, Inc.
All rights of any nature whatsoever reserved.
ISSN0893-7648/93/7(1): 1–22/$4.40

Novel Oligodendrocyte Transmembrane Signaling Systems

Investigations Utilizing Antibodies as Ligands

Charissa A. Dyer

Department of Biomedical Sciences, E. K. Shriver Center, Waltham, MA 02254

Contents

Abstract

Antibodies are increasingly being used as tools to study the function of cell surface markers. Several types of responses may occur upon the selective binding of an antibody to an epitope on a receptor. Antibody binding may trigger signals that are normally transduced by endogenous ligands. Moreover, antibody binding may activate normal signals in a manner that disrupts a sequence of events that coordinates either differentiation, mitogenesis, or morphogenesis. Alternately, it is possible that binding elicits either a modified signal or no signal. This article focuses on the cascade of events that occur following specific antibody binding to myelin markers expressed by cultured murine oligodendrocytes. Binding of specific antibodies to the oligodendrocyte

membrane surface markers myelin/oligodendrocyte glycoprotein (MOG), myelin/oligodendrocyte specific protein (MOSP), galactocerebroside (GalC), and sulfatide on cultured murine oligodendrocytes results in different effects with regard to phospholipid turnover, Ca^{2+} influxes, and antibody:marker distribution. The consequence of each antibody-elicited cascade of events appears to be the regulation of the cytoskeleton within the oligodendroglial membrane sheets. The antibody binding studies described in this article demonstrate that these myelin surface markers are capable of transducing signals. Since endogenous ligands for these myelin markers have yet to be identified, it is not known if these signals are normally transduced or are a modification of normally transduced signals.

Index Entries: Antibody; transmembrane signaling; oligodendrocytes; glycoproteins; transmembrane proteins; cytoskeleton; calcium fluxes; phospholipid turnover.

Introduction

Myelin is a specialized membrane that wraps around axons, insulating and facilitating conduction. It is produced by oligodendrocytes in the central nervous system (CNS) and Schwann cells in the peripheral nervous system (PNS). Although CNS and PNS myelin share a variety of unique lipids and proteins (Lees and Brostoff, 1984; Lemke, 1988), a few proteins are known to be exclusively expressed in CNS myelin, with other proteins selectively expressed in PNS myelin (Lees and Brostoff, 1984; Lemke, 1988; Mikol et al., 1990; Linington et al., 1984). One fundamental difference between these two myelin-forming cells is that an oligodendrocyte can myelinate as many as 40 different axons, whereas each Schwann cell myelinates a single axon (for reviews on myelination, *see* Raine, 1984; Bologa, 1985). Oligodendrocytes and Schwann cells express myelin components at specific times during their differentiation. It is possible that during differentiation interactions between these surface myelin components and molecules in the surrounding environment elicit signals that regulate the production of cytoskeleton for process and myelin sheath formation by oligodendrocytes, and axon engulfment and mesaxon extension by Schwann cells (for Schwann cell/axon interactions, *see* Owens and Bunge, 1990). Cytoskeleton may also play a role in the maintenance of myelin; here again, a major difference exists between CNS and PNS myelin. CNS myelin contains a cytoskeletal structure called radial component that is not present in PNS myelin (Kosaras and Kirschner, 1990; Karthigasan et al., 1991). Thus, understanding the signals mediated by surface components that regulate cytoskeleton may lead to an understanding of myelination and the maintenance of myelin in the CNS and PNS.

Oligodendrocytes grown in culture provide an excellent system in which to examine the signal transduction capabilities of myelin surface markers. This article reviews the cascade of events leading to cytoskeletal change that are mediated by surface markers after the binding of specific antibodies to cultured oligodendrocytes in the absence of complement.

Antibodies as Tools in Elucidating Signal Transduction Capabilities of Surface Membrane Components

Studies in many different systems demonstrate that antibody binding to a particular protein on a cell surface can mimic the action of a specific stimuli. Vandenberghe and Ceuppens (1991) have shown that monoclonal antibodies binding to T-cells elicit IL-2R expression, IL-2 production, and cell proliferation in T-cell cultures. Antibodies to markers on B-cells also have been shown to induce cellular responses (Deane et al., 1991).

Furthermore, antibody binding studies have elucidated the roles of lymphocyte markers that are involved in facilitating other cellular responses (Yamada et al., 1991; Geppert and Lipsky, 1987; Levine et al., 1991). Examples of antibody-induced cellular responses in nonleukocytes include

1. A monoclonal antibody reactive with the epidermal growth factor (EGF) receptor that induces both early and delayed biological effects normally mediated by EGF (Schreiber et al., 1981);
2. An antibody against the insulin receptor that induces glucose uptake in the absence of agonist (Jacobs et al., 1978); and
3. An antibody directed to the catecholamine receptor that stimulates adenyl cyclase activity in the absence of agonist (Courand et al., 1981).

Finally, several monoclonal antibodies have been produced that induce platelet activation (as discussed in Kornecki et al., 1990).

Antibody binding studies have also been used to study signal transduction via glycophosphatidylinosital-anchored proteins (GPI) in many cells types (for review, *see* Robinson, 1991). The responses include increases in intracellular Ca^{2+}, IL-1 and IL-2 production, superoxide production, respiratory bursts, and mitogenic responses. The mechanism of how GPI-linked molecules transduce signals remains to be elucidated; the ability to transduce signals is intriguing since GPI-anchored molecules do not span the lipid bilayer, but are present only in the outer face of the lipid bilayer.

Thus, there is precedence for antibody mimicking ligand binding to produce normal cellular responses. Some receptors are normally activated by ligand crosslinking, whereas others are not. There also is precedence for the binding of Fab fragments activating a cellular response. For example, $F(ab')_2$ fragments to Fc receptors on human granulocytes trigger cytoplasmic calcium fluxes (Lund-Johansen et al., 1991). In addition, the binding of Fab fragments to the mouse macrophage IgG Fc receptor indicates that these Fc receptors function as ligand-dependent ion channels (Young et al., 1983a,b).

The above studies demonstrate that antibody binding to cell surface components can elicit normal cellular responses. The following sections demonstrate that antibodies to oligodendrocyte surface markers also are capable of eliciting cellular responses, and although it is not known if these occur normally during myelination, they result in the modulation of the cytoskeleton in the membrane sheets elaborated by cultured oligodendrocytes.

GalC, Sulfatide, MOG, and MOSP—Receptors?

Evidence *suggests* that GalC, sulfatide, MOG, and MOSP are membrane receptors. Although no endogenous ligands for MOG and MOSP have as yet been identified, 34 and 68 kDa candidates for ligands to sulfatide have been identified in white matter (Law et al., 1988). In addition, three brain proteins have been identified that specifically bind to GalC (Dyer, unpublished data).

The specific locations of these markers in myelin in vivo suggest that they have distinct functions. GalC and sulfatide are sphingolipids present in the outer leaflet of the myelin membrane; their head groups extend away from the membrane surface. Both lipids are present throughout the myelin lamellae. MOG is a 24, 26 kDa glycoprotein; the different molecular weights are due to alternate carbohydrate chains attached to a single protein (Amiguet et al., 1991). MOG is present predominantly on the surface of the outer wrap of myelin (Brunner et al., 1989). MOSP is a recently identified oligodendrocyte specific protein (Dyer et al., 1991). It was identified by immunoprecipitation with anti-MOSP in (^{35}S)methionine labeled Triton X-100 solubilized proteins from cultured oligodendrocytes. The antibody used as a control is a well-characterized IgM antibody, A007, that has been shown to bind to sulfatide and other molecules on cells in the oligodendrocyte lineage (Bansal et al., 1992). Immunoprecipitations were performed using the following combination of reagents: 1. Goat anti-mouse IgM bound to protein A Sepharose; 2.

A007 IgM plus goat anti-mouse IgM bound to protein A Sepharose; or 3. Anti-MOSP IgM plus goat anti-mouse IgM bound to protein A Sepharose. The resultant immunoprecipitates essentially contained the same pattern of proteins except for a 48 kDa protein with a pI of 6.7, which was immunoprecipitated by anti-MOSP (Dyer et al., 1991); the similar sets of proteins brought down by both antibodies were likely nonspecifically bound to the protein A Sepharose/antibody complexes. A series of glycosphingolipids were tested for anti-MOSP reactivity and these were negative (Dyer et al., 1991). Although these data do not rule out the possibility that other components also react with anti-MOSP, these results indicate that the predominant molecule that reacts with anti-MOSP is a 48 kDa protein. By immunohistochemistry, MOSP appears to be present throughout the myelin sheaths in adult human spinal cords (Dyer et al., 1991).

The timed expression of oligodendrocyte/myelin components may be important for their interaction with molecules in the environment at various stages of differentiation. Indeed, GalC and sulfatide are detected on immature oligodendrocyte cell bodies and processes 2–3 d after birth (Bansal et al., 1989; Gard and Pfeiffer, 1989). Preliminary data indicate that MOSP is expressed on oligodendrocyte processes about the time that GalC and sulfatide are detected; MOSP later appears on cell bodies as well (Dyer, unpublished). Last, expression of MOG occurs about 1 wk after birth, shortly after MBP expression (Matthieu and Amiguet, 1990). At the time of expression of MBP, myelination begins (Barbarese et al., 1978).

Evidence suggests that GalC and/or sulfatide plays a role in regulating the differentiation of oligodendrocytes and Schwann cells. The Ranscht monoclonal antibody (R-mAb) (Ranscht et al., 1982; *see* Bansal et al., 1989 for specificities of the R-mAb) causes reversible inhibition of oligodendrocyte progenitor differentiation; data suggest that the target molecule in this study is sulfatide (Bansal and Pfeiffer, 1989). Other studies have shown that treatment of Schwann cells with the R-mAb results in the loss of GalC and sulfatide from the cell surface (Ranscht et al., 1987; Owens and Bunge, 1990). Under these conditions, Schwann cells depleted of GalC and sulfatide were capable of forming a single wrap-around axons, but elongation of mesaxons was inhibited. Thus, GalC and/or sulfatide appear to be important in transducing signals that continue the process of myelination. In both of the above studies, it is possible that inhibition of the production of the cytoskeleton was the mechanism blocking myelination.

Cytoarchitecture of Oligodendrocytes

To understand the changes that occur in the organization of the membrane sheets of cultured oligodendrocytes upon specific antibody binding to cultured oligodendrocytes, a brief description of the normal organization is necessary. Many investigators have described to varying degrees the flattened membrane sheets produced by oligodendrocytes in the absence of neurons from various in vitro preparations (Knapp et al., 1987; Rome et al., 1986; Pfeiffer, 1984). The shapes of the membrane sheets produced by cultured murine oligodendrocytes, as well as the number of sheets per cell, vary. Many of the membrane sheets are expansive, obtaining diameters of about 200 μm. Murine oligodendrocytes cultured from neonatal mice express a variety of myelin markers that are observed in vivo. These include the cytoplasmic myelin markers proteolipid protein (PLP), myelin basic protein (MBP), and 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNPase). The membrane surface markers that are the focus of this article, GalC, sulfatide, MOG, and MOSP, are normally expressed uniformly on the entire membrane sheet surface (Dyer and Benjamins, 1988a,b; Dyer and Matthieu, 1992; Dyer et al., 1991).

Within the membrane sheets, large colchicine-resistant microtubular structures extend from the cell body (Dyer and Benjamin, 1989a). Many

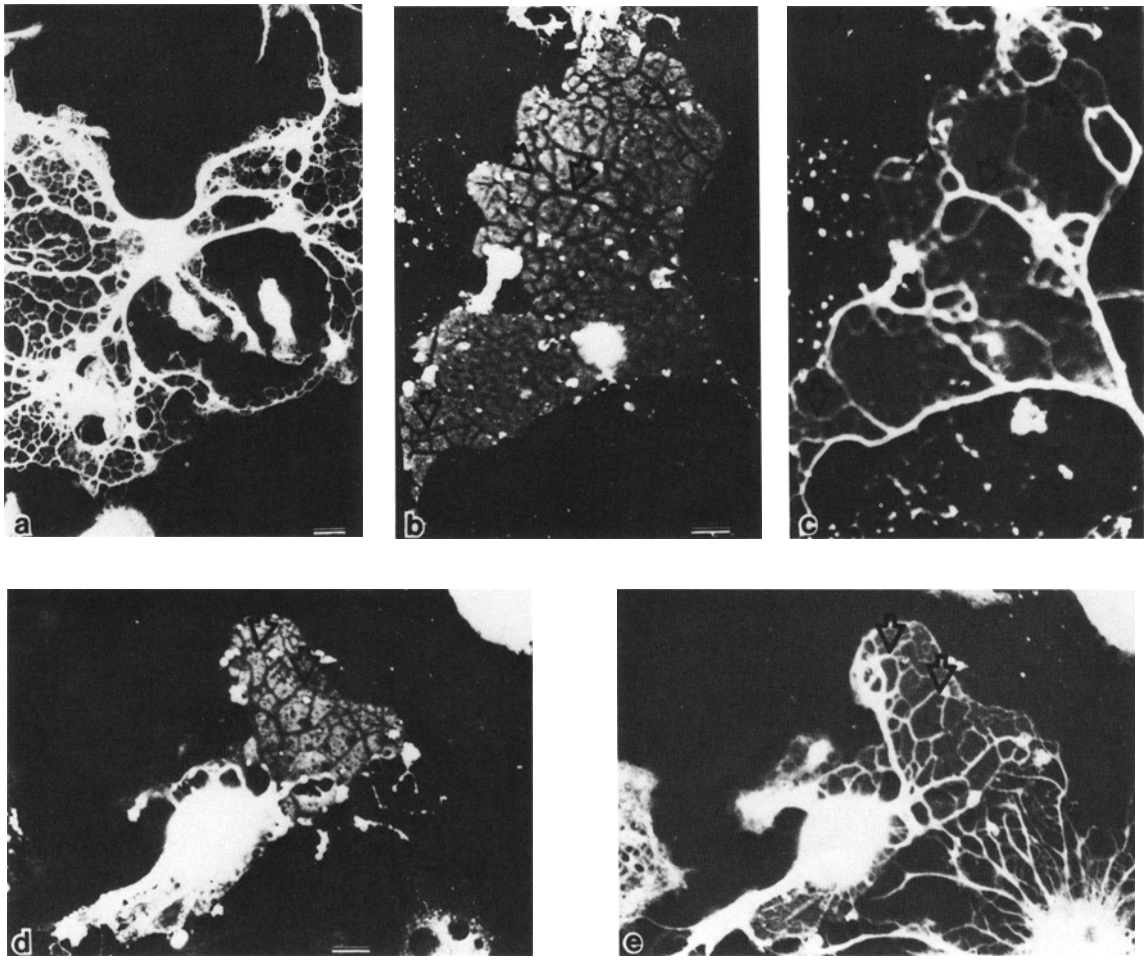

Fig. 1. Relationship of surface GalC patches to internal MBP domains and microtubular structures. **a:** Representative oligodendrocyte stained for tubulin after fixation and saponin treatment. **b:** Patched GalC surface staining on membrane sheet following exposure to anti-GalC for 15 min and GAR-FITC for 15 min. **c:** Internal tubulin staining of cell in **b** following fixation and saponin treatment. Note that many of the surface GalC patches (arrows) are surrounded by cytoplasmic tubulin-stained structures (arrows). **d:** MBP domains in oligodendroglia that were fixed, saponin-treated, and stained with anti-MBP with GAM-TRITC. **e:** Same cell in **d** double-labeled for tubulin. Note that MBP domains (arrows) are located inside microtubular structures (arrows). Bar = 15 μm. Reprinted from Dyer and Benjamins (1988) with permission.

small colchicine-sensitive microtubular structures branch off the large microtubular structures to form a lacy network throughout the membrane sheet (*see* Fig. 1 for microtubular structures in membrane sheet) (Dyer and Benjamins, 1989b). Antibodies specific for tubulin do not detect lacy networks of microtubular structures following colchicine treatment (Dyer and Benjamins, 1989a). Since colchicine is known to result in a loss of microtubules that are treadmilling, or turning over (Wilson and Farrell, 1986), these data indicate that the antibody is detecting microtubules within these membrane sheet. The cytoskeletal components microtubule associated protein 1B (MAP1B) (Fischer et al., 1990) and F-actin (Wilson and Brophy, 1989; Dyer and Benjamins, 1989a) have

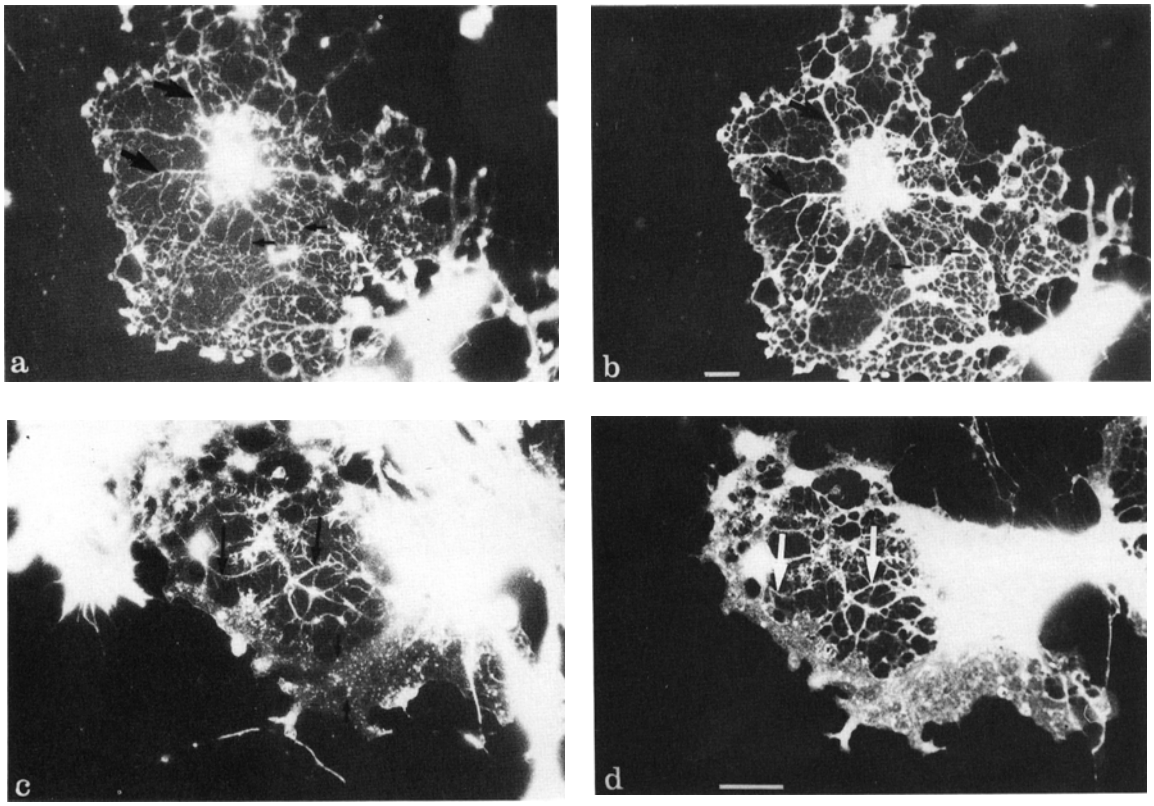

Fig. 2. Comparison of F-actin and CNPase staining in membrane sheets. **a:** Phalloidin-TRITC labeling demonstrates actin filaments colocalized along major veins (large arrows) and lacy networks (small arrow). **b:** CNPase staining of cell in **a** shows colocalization of CNPase with F-actin along major veins (large arrows) and lacy networks (small arrows). **c:** Phalloidin-TRITC staining of F-actin is both punctate (small arrows) and filamentous (large arrows) in this membrane sheet. **d:** CNPase staining of cell in **c** shows that the punctate F-actin staining is located in diffuse CNPase membrane regions and that filamentous actin is present along CNPase veins. Bars = 15 μm. Reprinted from Dyer and Benjamins (1989a) with permission.

been shown to be colocalized along microtubular structures in membrane sheets, as have the myelin markers, PLP (Konola et al., 1991) and CNPase (Wilson and Brophy, 1989; Dyer and Benjamins, 1989a). Furthermore, since cytochalasin B treatment of murine oligodendrocyte cultures results in the disruption of the lacy network of microtubules, F-actin and CNPase (these are colocalized) it appears that CNPase is associated with the cytoskeleton (Dyer and Benjamins, 1989a).

The extensive membrane sheets produced by murine oligodendrocytes in culture contain two types of cytoplasmic regions. The first type of region contains F-actin and CNPase that are *not* colocalized with microtubular structures (Dyer and Benjamins, 1989a) Although microtubular structures exist throughout these regions, F-actin is present in small aggregates and CNPase is distributed uniformly throughout (Fig. 2) (Dyer and Benjamins, 1989a). In addition, regions of uniformly stained CNPase lack MBP (Reynolds et al., 1989). It has been speculated that this type of membrane region represents a growth region for membrane extension (Reynolds et al., 1989; Dyer and Benjamins, 1989a). The second type of membrane region contains microtubular structures colocalized with F-actin and CNPase (Dyer and Benjamins, 1989a) (Fig. 2a,b, Fig. 3e,f). Areas, or

domains, solidly stained for MBP also exist in this type of membrane region (Dyer and Benjamins, 1989a) (Fig. 1d,e). Since domains of MBP are surrounded by vein-like structures of CNPase (Reynolds et al., 1989), and since CNPase in vein-like structures is colocalized with microtubules and F-actin (Dyer and Benjamins, 1989a), it appears that domains of MBP are surrounded by microtubules/F-actin/CNPase structures. It is likely that these are assembled membrane regions.

The mature membrane sheets are not identical to unfurled myelin sheaths, however. In vivo myelin sheaths are compact, i.e., the two membrane bilayers are apposed, with cytoplasm containing microtubules present predominantly in the perinodal loops, i.e., around the rim of the myelin sheaths (Kidd et al., 1991). Cultured oligodendrocytes loaded with the Ca^{2+}-binding fluorescent dye Indo-1 show an extensive network of fluorescent veins throughout the membrane sheets (Dyer and Benjamins, unpublished observations). These data suggest that in vitro membrane sheets have channels of cytoplasm that contains cytoskeletal structures. Therefore, it is possible that a signal(s) from axons is necessary for withdrawal of cytoplasm and depolymerization of the cytoskeletal structures that would leave the sheets resembling unfurled compact myelin sheaths.

Signaling Systems Observed Following Antibody Binding to Oligodendrocytes

Phospholipid Turnover Elicited by Antibody Binding

Myelin was once believed to be metabolically inert; however, it has been found to contain numerous intrinsic enzymes involved in ion transport, protein degradation, lipid metabolism, and phosphorylation/dephosphorylation (Suzuki, 1980; Lees and Sapirstein, 1983; Ledeen, 1984; Norton and Cammer, 1984; Barres et al., 1988; Soliven et al., 1988; Sontheimer et al., 1989). Moreover, polyphosphoinositides (Eichberg and Dawson, 1965; Eichberg and Hauser, 1973; Deshmukh et al., 1980; Kahn and Morell, 1988; Eichberg et al., 1989) and G-proteins (Braun et al., 1990; Golly et al., 1990) have been identified in purified myelin. Thus, myelin has a rich source of second messenger-generating element. This section will primarily focus on the phospholipid turnover elicited by anti-GalC in cultured oligodendrocytes.

Initial studies of oligodendrocyte cultures that have been prelabeled with ^{32}P phosphate showed that anti-GalC binding induces a dramatic loss of the ^{32}P-labeled phospholipids, phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidic acid (PA) compared to control cultures (Table 1). Upon further investigation, the changes in ^{32}P incorporation were found to be dependent on the length of time oligodendrocytes are exposed to antibody and the concentration of antibody added (Dyer, in preparation). A given concentration of antibody initially causes an increase in ^{32}P incorporation into phospholipids compared to controls and then incorporation drops to below control values with time (Dyer, in preparation). Experiments in which oligodendrocytes are labeled with (^3H)glycerol in the presence of anti-GalC or nonimmune IgG show no change in (^3H)glycerol incorporation, suggesting that the changes in ^{32}P labeling of these phospholipids during anti-GalC binding are *not* caused by changes in *de novo* synthesis, but by rapid cycling of the phospho-containing polar head groups (Dyer, in preparation).

Both Ca^{2+} and pertussis toxin appear to differentially affect the anti-GalC-induced breakdown of phospholipids. Preliminary results indicate that the anti-GalC-induced increased turnover of PI and PA are not affected by the absence of extracellular Ca^{2+}, whereas the increased turnover of PC is affected by the absence of extracellular Ca^{2+} (Table 2). The increased turnover of PI and PA owing to anti-GalC binding appears to be pertussis toxin-insensitive, but the anti-GalC-induced increased turnover of PC and PE appears to be pertussis toxin-sensitive (Table 3). Ribosyla-

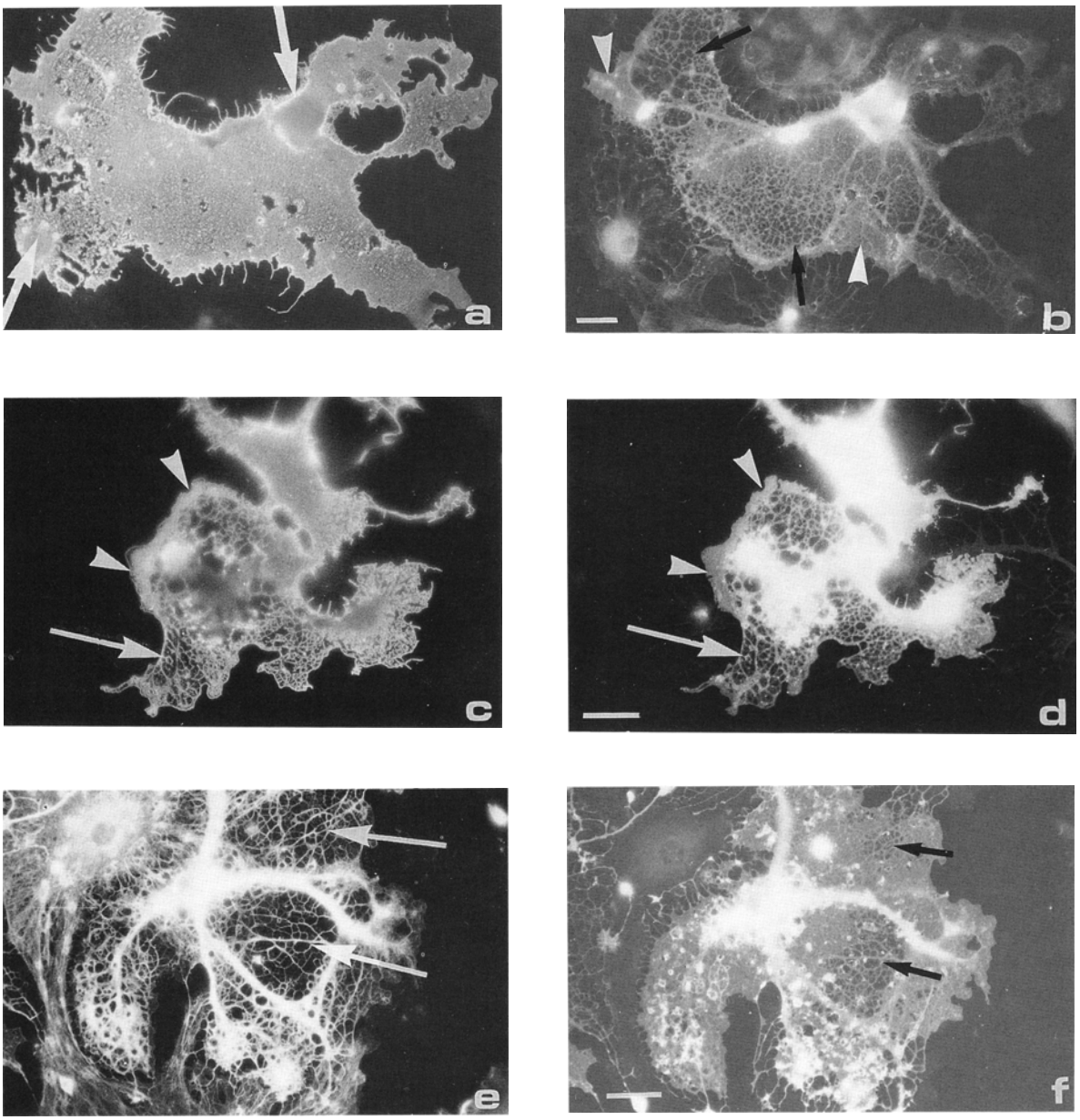

Fig. 3. MOSP either redistributes in a lacy network or remains uniformly distributed on membrane sheets. **a:** Representative oligodendrocytes fixed prior to immunostaining with anti-MOSP; note that the surface of the membrane sheets is solidly stained (arrow at cell bodies). **b:** Same cells in **a** permeabilized and stained for internal CNPase; note that CNPase is present in a lacy network in many membrane sheet regions (arrows), whereas other membrane sheet regions are solidly stained for CNPase (arrowheads). The cell at center top and the two cells at the bottom of the photograph are much less intensely stained than the large membrane sheet in the center of the photograph; this background is owing to second antibodies staining permeabilized cells other than membrane sheet bearing oligodendrocytes. **c:** Live oligodendrocytes were treated with anti-MOSP and second antibody; note that the surface anti-MOSP:MOSP complexes redistribute into a lacy network in some membrane sheet regions (arrows) but in other membrane sheet regions, surface anti-MOSP:MOSP remains solidly stained (arrowheads). **d:** Same cell in **c** fixed, permeabilized, and stained for internal CNPase shows that the internal CNPase is also present in a lacy network (arrows) or is solidly stained (arrowheads). Note that the surface anti-MOSP:MOSP complexes, which have redistributed (arrows in **c**), lie directly over internal veins of CNPase (arrows in **d**) and that the solidly stained regions of anti-MOSP:MOSP (arrowheads in **c**) directly overlie solidly stained regions of CNPase (arrowheads in **d**). **e:** Representative control membrane sheet stained for cytoplasmic microtubules shows lacy networks throughout the membrane sheet (arrows). **f:** Same cell in e stained for internal CNPase shows that the lacy network of CNPase is colocalized with microtubular veins (arrows). Note that microtubular veins run throughout solidly stained regions of CNPase. Bars = 20 μm.

Table 1

Turnover of ^{32}P in the Major Phopholipids Following Ab Exposure*

Ab	PI	PC	PA	PE
No Ab	11,106 ± 3036	329,400 ± 15,600	10,490 ± 505	79,875 ± 10,509
Anti-GalC	9471 ± 3380	133,660 ± 6220	4486 ± 2025	36,425 ± 7083

*Cultures were preincubated with (^{32}P)phosphate for 45 min followed by washing with cold phosphate. They were incubated with or without anti-GalC in buffer containing cold phosphate for 30 min. Anti-GalC was added in a concentration that caused patching over MBP domains. Culture contained approximately 50% OL and about 300 μg protein/sample. CPM plus and minus the range are presented.

Table 2

The Effect of Anti-GalC and Ca^{2+} on ^{32}P Incorporation into Phospholipids*

Ab	PI	PC	PA
Nonimmune IgG	41,461 ± 1558	23,409 ± 1163	22,965 ± 1022
Anti-GalC + Ca^{2+}	14,377 ± 4489	5631 ± 1200	2179 ± 1540
Anti-GalC, no Ca^{2+}	29,700 ± 2371	29,754 ± 2443	9435 ± 2431

*Data represent the means of duplicate samples plus and minus the ranges. Nonimmune rabbit IgG or anti-GalC was added at about 600 μg/mL. Nonimmune rabbit IgG was added in the presence of Ca^{2+}. Cultures were preincubated with or without Ca^{2+} for 1 h, then treated for 30 min with Ab and (^{32}P)phosphate under the same Ca^{2+} conditions, followed by washing in cold phosphate, lipid extraction, and HPTLC. Each sample contained about 50% oligodendrocytes and approx 450 μg protein.

Table 3

The Effect of Pertussis Toxin (PT)
in Anti-GalC-Induced Changes in ^{32}P Incorporation into Phospholipids*

Treatment	PI	PC	PA	PE
Anti-GalC	7786 ± 2807	11,368 ± 604	7,898 ± 1472	1499 ± 228
Anti-GalC + PT	13,758 ± 739	20,675 ± 110	11,875 ± 146	3108 ± 587
Nonimmune IgG	26,929 ± 4027	19,476 ± 1826	18,376 ± 3223	2868 ± 248

*Data represent the means of duplicate samples plus and minus the ranges. (^{32}P)Phosphate and either nonimmune IgG or anti-GalC was added during the last 30 min of a 4-h incubation with 100 μg/mL pertussis toxin. Each sample contained about 50% oligodendrocytes and approx 200 μg of protein.

tion by pertussis toxin inhibits a subpopulation of G-proteins that normally activate phospholipases (Kopf and Woolkalis, 1991). Therefore, these data suggest that anti-GalC IgG binding activates PC-specific phospholipases that are regulated by Ca^{2+} and pertussis toxin-sensitive G-proteins. Further, data suggest that anti-GalC binding activates PI-specific phospholipases that do not appear to be regulated by Ca^{2+} and may be regulated by pertussis toxin-insensitive G-proteins.

Evidence indicates that antibody binding itself does not nonspecifically activate phospholipases, but that *specific* signaling events are triggered by antibody binding. Unregulated entry of Ca^{2+} via

ionomycin does not mimic the ^{32}P incorporation data (Dyer, in preparation), thus demonstrating that the anti-GalC-induced changes in ^{32}P labeling of phospholipids are not nonspecifically activated by increases in Ca^{2+}. Furthermore, data show that anti-GalC IgG, anti-MOG IgG, and anti-MOSP IgM each causes a distinct pattern of phospholipid turnover when cultures are prelabeled with ^{32}P and then treated with antibody (Dyer, preliminary data).

The mechanism of GalC-mediated phospholipase activation is unknown. Two mechanisms that may account for the activation of phospholipases by anti-GalC include endocytosis of the antibody:glycolipid complex and subsequent clustering of the complexes (Dyer and Benjamin, 1988a). However, activation of phospholipases via receptor clustering is unlikely, since changes in ^{32}P incorporation into phospholipids are observed as soon as 10 min after antibody binding and patching of GalC occurs after 2 h of exposure to anti-GalC IgG alone. One possible model for activation involves MBP as a mediator; indeed, MBP has been implicated in signaling ovine oligodendrocytes to remyelinate in culture (Vartanian et al., 1986). In the murine oligodendrocyte culture system, surface membrane anti-GalC:GalC complexes redistribute directly over cytoplasmic MBP domains (Dyer and Benjamins, 1988b,1989b). The anti-GalC:GalC complexes appear to form an association with cytoplasmic MBP, since GalC and MBP become Triton X-100 insoluble after anti-GalC binding (Dyer, unpublished data). It is possible that this association is formed via a transmembrane protein; similarly, a transmembrane protein has been postulated to link cholera toxin:ganglioside GM1 complexes with the lymphocyte cytoskeleton and to mediate signals elicited by the binding of cholera toxin to GM1 (for review, *see* Fishman, 1982). Tompkins and Moscarello (1991) have recently demonstrated that the most positively charged forms of MBP stimulate PI-specific phospholipase C activity in vitro. Thus, it is possible that MBP may play a role in activation of phospholipases following anti-GalC binding.

Antibody-Induced Ca^{2+} Influxes

Ca^{2+} has been shown to play a role in modulation of the cytoskeleton in many cell types (Marcum et al., 1978; Yamamato et al., 1985; Keith et al., 1986). Indeed, extracellular Ca^{2+} was shown to be necessary for anti-GalC-induced microtubular depolymerization to occur in cultured oligodendrocytes (Dyer and Benjamins, 1990). Additional studies of Ca^{2+} fluxes in cultured oligodendrocytes revealed that the rat monoclonal antibody A007 reactive with sulfatide (IgM), and the mouse monoclonal anti-MOSP IgM (Dyer et al., 1991) also trigger an influx of Ca^{2+} into cultured oligodendrocytes (Table 4) (Dyer and Benjamins, 1990,1991; Dyer and Matthieu, 1992). Binding of the mouse monoclonal anti-MOG IgG (Linington et al., 1984) to cultured oligodendrocytes does not appear to elicit a Ca^{2+} response (Table 4). Interestingly, there are differences between the Ca^{2+} responses elicited by each of these antibodies.

Three types of responses are observed after antibody binding (Dyer and Benjamins, 1990,1991; Dyer and Matthieu, 1992) (Table 5). Data from studies due to EGTA indicate that all responses are owing to an influx of extracellular Ca^{2+} and not to release from intracellular Ca^{2+} stores. The first type of response is a large delayed sustained response. The kinetics for the time between addition of antibody and the initiation of the Ca^{2+} response generally range from 20–390 s (Table 4). The second type of response is a large delayed transient response. The kinetics for the response time are similar to those above. However, these responses are not sustained but are transient, returning to near baseline values within about 12 min. For both sustained and transient delayed responses, Ca^{2+} increases about 300–500 n*M* above the resting value of approx 5–20 n*M*. The third type of response is a small immediate transient response. These responses are mimicked by depolarizing membranes with 60 m*M* K^+ and are blocked by 500 m*M* Cd^{2+}. The magnitude of the small responses is less than one-tenth that of the large responses. Thus, these responses are referred to as voltage-operated Ca^{2+} channel-like, or VOC-like Ca^{2+} channels.

Table 4

Ca^{2+} Responses to Anti-GalC, A007, Anti-MOSP, and Anti-MOG*

Ab	*n*	Number responding	Range of times to response, s	Range of reversal times, s	Ratio change
Anti-MOG	9	0			
Anti-GalC IgG (large delayed sustained response)	28	21	20–360	Sustained	1.0 ± 0.5
Anti-GalC Fab	5	4	50–180	Sustained	1.4 ± 0.6
Anti-MOSP IgM (large delayed transient responses)	18	9	70–370[a]	220–610	1.8 ± 1.0
Anti-MOSP IgM (VOC-like)	18	3	0	40–100	0.1 ± 0.02
A007	53	10	40–390[b]	300–700	1.4 ± 0.5
60 m*M* K^+	3	3	0	30–60	0.1 ± 0.2

*Cell were loaded with 1 μm Indo-1 for 1 h, and the changes in intracellular Ca^{2+} examined using the ACAS laser cytometer. Cells were examined for changes in intracellular-free Ca^{2+} using the line scanning technique. Briefly, two points were identified on either side of the cell that defines the line through the cell that is scanned every 2 s for 5–15 min. Changes in bound and unbound Indo-1 were calculated for each scan and plotted against time. The time to response represents the time taken for the initial increase in Ca^{2+} to be observed after addition of Ab. The reversal time refers to the length of time it takes for peak Ca^{2+} values to return to near resting values. The ratio change represents the magnitude of the Ca^{2+} increase in units.

[a]These data represent values taken from nine responses; although the other 11 responses are documented sustained responses, gliches in the computer caused a loss of parts of the data for their responses and they were not included in the statistics.

[b]These data represent values taken from nine responses.

Table 5

Sustained vs Transient Calcium Responses Elicited by Antibodies to GalC, Sulfatide, MOG, and MOSP*

Types of responses	Cells treated with anti-GalC, %	Cells treated with A007, %	Cells treated with anti-MOSP, %	Cells treated with anti-MOG, %
No response	7 (25)	32 (61)	3 (18)	9 (100)
Delayed sustained response	21 (72)	6 (11)	2 (12)	0 (0)
Transient response:				
Large, delayed	1 (3)	10 (19)	9 (53)	0 (0)
Small, immediate		5 (9)	3 (18)	0 (0)

*These numbers were tabulated from the same data shown in Table 4.

As shown in Tables 4 and 5, the majority of anti-GalC-responding oligodendrocytes exhibit a large delayed sustained response (Dyer and Benjamins, 1990). Unlike anti-GalC treated cells, the majority of cells responding to A007 sulfatide-reactive antibody show a large, delayed, transient response; a few cells exhibit large, delayed, sustained responses, and fewer still show VOC-like Ca^{2+} channel responses. Anti-MOSP binding causes Ca^{2+} influxes in a pattern similar to A007 antibody.

Different numbers of oligodendrocytes appear to respond to anti-GalC, A007, and anti-MOSP

(Table 5). These differences exist despite the fact that virtually all oligodendrocytes stain intensely for both glycolipids (Dyer and Benjamins, 1991) and proteins (Dyer and Matthieu, 1993). Nonspecific crosslinking does not appear to play a role in triggering the anti-GalC-induced influx of Ca^{2+} since Fab fragments of the anti-GalC IgG trigger Ca^{2+} increases in oligodendrocytes similar to those elicited by intact IgG (Dyer and Benjamins, 1990,1991). In addition, if nonspecific crosslinking triggers the response, all cells should exhibit an influx of Ca^{2+} and this is not observed. The class of antibody also does not appear to play a role in triggering an influx of Ca^{2+} since 39% of the A007 IgM treated cells respond, whereas 83% of anti-MOSP IgM treated cells exhibit a Ca^{2+} response (Table 5). Furthermore, anti-GalC IgG elicits a Ca^{2+} response in the majority of oligodendrocytes treated, but anti-MOG IgG does not elicit an increase in intracellular Ca^{2+} in any of the cells examined. Thus, crosslinking of surface components on oligodendrocytes does not appear to be the mechanism of induction of the influxes of Ca^{2+} observed after the binding of these antibodies.

The mechanism underlying the Ca^{2+} influxes is unknown. The Ca^{2+} influxes triggered by anti-GalC IgG, A007 IgM, and anti-MOSP IgM do not appear to be via nonspecific pore formation since no nuclear staining was observed after treatment of cultures with propidium iodide and either anti-GalC, A007, or anti-MOSP (Dyer and Benjamins, 1991; Dyer, unpublished data). Delayed Ca^{2+} responses may reflect the time necessary to recruit Ca^{2+} channels from intracellular membrane compartments, as shown for transporters in other systems (Kono et al., 1982; Simpson and Cushman, 1985; Schwartz and Al-Awqati, 1986; Wade, 1986). It is also possible that antibody binding may inhibit efflux of Ca^{2+} while allowing influx to occur, so that Ca^{2+} transporters are induced to work in reverse as a result of antibody binding (Dixon et al., 1987). Anti-GalC and A007 are internalized in vesicles following antibody binding; thus some Ca^{2+} may enter oligodendrocytes via this route. However, the kinetics of endocytosis and Ca^{2+} entry are not similar (Dyer and Benjamins, 1991). It is important to note that all the increases in intracellular Ca^{2+} observed do not increase unchecked, but either reach a new steady state level or return to near resting levels. Therefore, the mechanism of Ca^{2+} influx induced by antibody binding appears to be a regulated process.

It is possible that antibody binding causes activation of second messenger-operated Ca^{2+} channels (SMOCs), since second messengers have been reported to mediate Ca^{2+} influxes in cell types that do not use VOCs as a means of regulating Ca^{2+} entry (Exton, 1988; Penner et al., 1988; Hockberger and Swandulla, 1987; Meldolesi and Pozzan, 1987; Merritt and Rink, 1987). The delayed Ca^{2+} responses may be triggered when a threshold level of a second messenger has accumulated. Indeed, this is likely in the oligodendrocyte system since data show that turnover of phospholipids occurs following antibody binding (*see* section above regarding turnover of phospholipids); thus, phospholipid-derived second messengers may be generated. Results by Martinson et al. (1990) show that muscarinic receptor stimulation in astrocytoma cells results in a delay of several minutes before accumulation of the second messenger 1,2-diglyceride. These results support the Ca^{2+} data from the oligodendrocyte system, which show that the large delayed Ca^{2+} influxes are delayed by 20–390 s, suggesting that SMOCs are specifically activated by antibody binding.

Antibody: Surface Marker Redistribution

Antibody-induced changes in cytoskeleton are only observed following redistribution of antibody:marker complexes (Dyer and Benjamins, 1988a,b; Dyer et al., 1991; Dyer and Matthieu, 1992). In each case, specific antibody complexed to GalC, sulfatide (Dyer and Benjamins, 1988b,1989b), or MOG (Dyer and Matthieu, 1993) results in the redistribution of these complexes

to regions on the surface of the membrane sheets that directly overlie cytoplasmic MBP domains (*see* Fig. 1b–e for anti-GalC:GalC redistribution). These patches of surface antibody:myelin marker complexes are excluded from tracts of membrane that directly overlie veins of cytoplasmic microtubular structures (Fig. 1b,c). In contrast, anti-MOSP:MOSP complexes redistribute in a lacy network on the membrane surface that directly overlies cytoplasmic CNPase colocalized with microtubular structures (Fig. 3) (Dyer and Matthieu, 1993). These complexes are excluded from regions on the membrane surface that directly overlie cytoplasmic MBP domains (Dyer and Matthieu, 1993).

There are several differences between the events that occur following antibody binding to oligodendrocytes and "capping and patching" events on lymphocytes. Patching and capping of many ligand:receptor complexes on lymphocytes is the earliest event, followed by endocytosis (Taylor et al., 1971; Sedlacek et al., 1976; Sela et al., 1978; Geiger and Singer, 1979). Generally, crosslinking of numerous receptors on lymphocyte results in rapid patching of the immune complexes over actin and associated proteins within 30 min (Ash and Singer, 1976; Ash et al., 1977; Bourguignon et al., 1985; Flanagan and Koch, 1978; Geiger and Singer, 1979). In contrast, endocytosis of small numbers of GalC and sulfatide molecules occurs within minutes of antibody binding, and patching of the majority of GalC and sulfatide molecules occurs after 2 h of continuous antibody exposure (Dyer and Benjamins, 1988a,b). Unlike lymphocytes, surface components on oligodendrocytes do not nonspecifically patch over actin and its associated proteins (Dyer and Benjamins, 1989a,b). Furthermore, surface anti-GalC:GalC, A007:sulfatide, and anti-MOG:MOG complexes patch over cytoplasmic MBP domains, whereas anti-MOSP:MOSP complexes redistribute over cytoplasmic CNPase colocalized with microtubular structures in membrane sheets. These events suggest that these redistribution events are specifically induced by antibody binding.

Cytoskeletal Changes Elicited by Antibody Binding

Short-term exposure (15 min of primary antibody followed by 15 min of secondary antibody) of cultures to anti-GalC IgG (Dyer and Benjamins, 1988b,1989b), A007 IgM (Dyer and Benjamins, 1988b,1991), and anti-MOG IgG (Dyer and Matthieu, 1993) results in loss of the lacy network of microtubules in membrane sheets. It is interesting that in all three cases, patching over cytoplasmic MBP occurs, followed by microtubule depolymerization. In contrast, short-term exposure to anti-MOSP does not result in depolymerization of microtubules (Dyer et al., 1991).

Additional events that occur following anti-GalC IgG:GalC complex patching and loss of the lacy network of microtubules are F-actin rearrangement, and MBP domain fusion with concomitant GalC surface patch fusion (Dyer and Benjamin, 1989b) (*see* Fig. 4 for MBP fusion). Exposure to anti-GalC for 6 d followed by 15 min of secondary antibody results in a reversible contraction of the majority of membrane sheets, whereas long-term exposure to A007 (sulfatide-reactive antibody) does not (Dyer and Benjamins, 1988b).

Long-term exposure to anti-MOSP (3–6 d) causes a dramatic increase in staining intensity of surface MOSP on oligodendrocytes with large membrane sheets; both the staining intensity and the amount of lacy network per membrane sheet are greatly increased compared to the nonspecific IgM treated controls (Fig. 5) (Dyer and Matthieu, 1993). In addition, there is a concomitant increase in both the thickness and the number of the underlying microtubules (Fig. 5). The membrane sheets do not appear contracted, unlike sheets exposed long-term to anti-GalC (Dyer and Benjamins, 1988b). Immature process-bearing oligodendrocytes exposed long-term to anti-MOSP have foci of processes that are intensely stained for MOSP that may represent accelerated growth regions (Dyer and Matthieu, 1992). Therefore, it is possible that the increases in amount of lacy networks reflect an upregula-

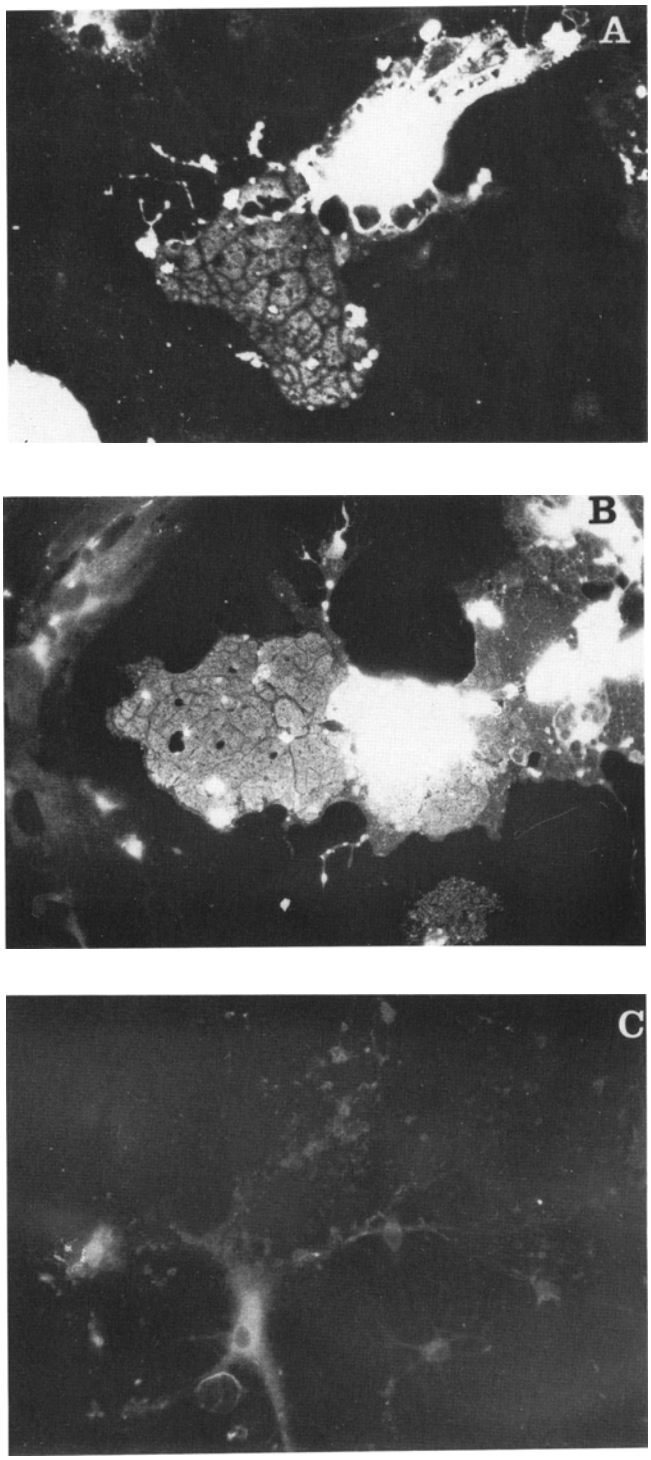

Fig. 4. MBP domains fuse following prolonged exposure to anti-GalC IgG. **A:** Normal distribution of MBP domains within membrane sheets. Note the unstained tracts surrounding the MBP staining. **B:** After 6 h of anti-GalC exposure at 37°C, the unstained tracts surrounding MBP are lost, leaving the membrane sheets virtually solidly stained. Cells were treated with rabbit anti-GalC IgG for 6 h, fixed, permeabilized, and staining for MBP with mouse monoclonal anti-MBP IgG and goat anti-mouse IgG conjugated to rhodamine. **C:** Staining performed as in **b** except that anti-MBP IgG was not added. The goat anti-mouse IgG conjugated to rhodamine did not react with the rabbit anti-GalC IgG; these results demonstrate the MBP staining in **B** is specific.

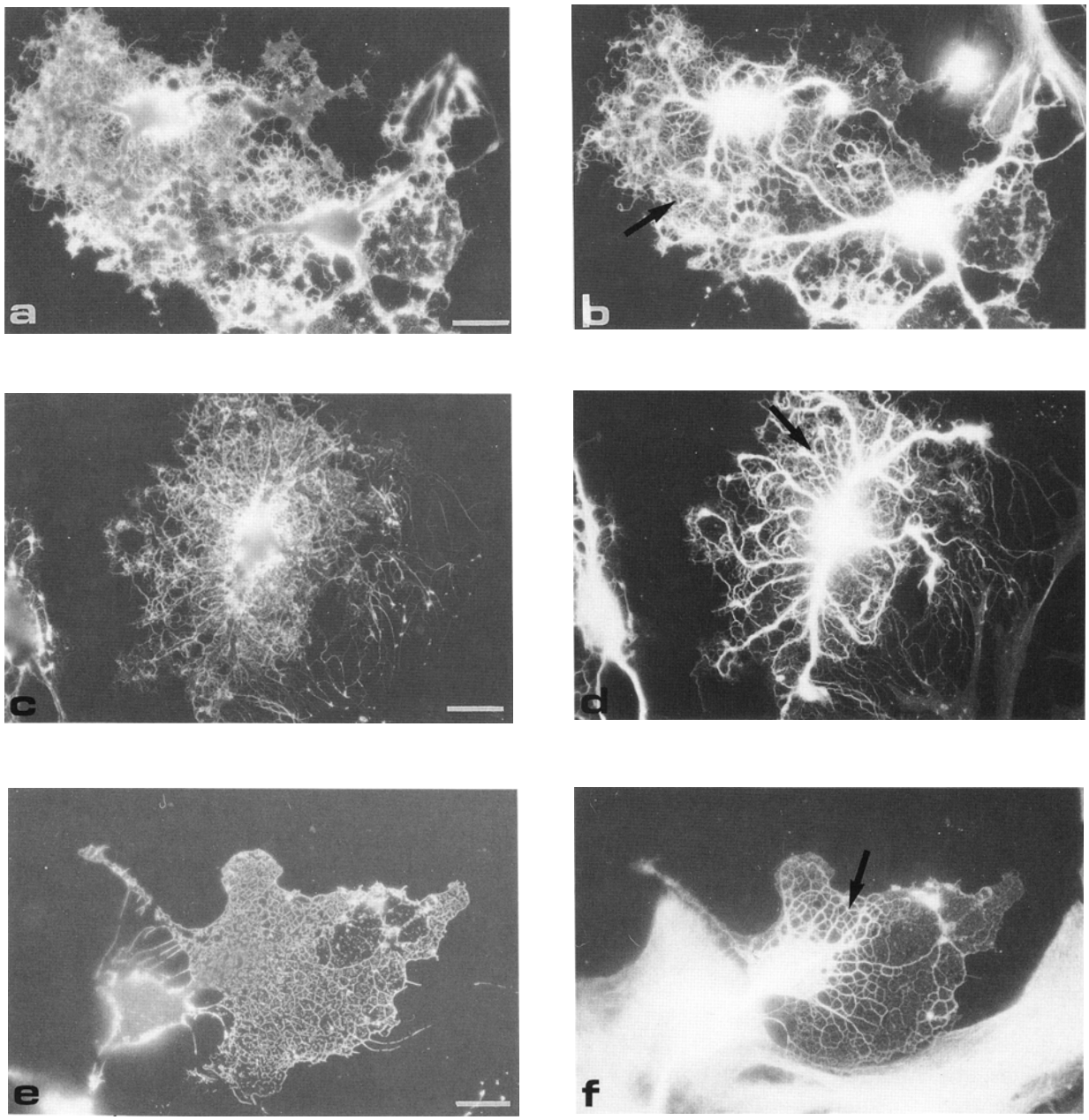

Fig. 5. Long-term exposure to anti-MOSP results in increased surface MOSP and cytoplasmic microtubular staining. Cultures were treated with anti-MOSP IgM for 6 d and then treated with goat anti-mouse IgM conjugated with fluorescein for 15 min. Cells were fixed, permeabilized, and stained for tubulin with mouse monoclonal anti-tubulin IgG and goat anti-mouse IgG conjugated to rhodamine. **a:** Increased lacy network of MOSP on two representative oligodendrocytes. **b:** Same cells in **a** labeled for tubulin show that there is a concomitant increase in microtubules; note that areas of diffuse MOSP staining show relatively little tubulin staining. **c:** Representative oligodendrocytes showing increased lacy network of MOSP on surface of membrane sheets. **d:** Same cell in **c** intensely stained for tubulin. **e:** Control live oligodendrocyte treated with anti-MOSP and second antibody for 15 min each; note that the amount of lacy network on the surface of the membrane sheet is much less than that on cells in **a** and **c**. **f:** Same cell in **e** stained for internal tubulin shows microtubule staining; note that the amount and thickness of the microtubular veins are much less than in cells in **b** and **d** (*see* arrows). Bar = 20 μm.

tion of both surface MOSP expression and internal microtubules that may be important in process outgrowth.

Long-term exposure to anti-MOG does not have a detectable effect on MOG surface expression (Dyer and Matthieu, 1993). However, long-term exposure to anti-MOG *does* have an effect on the redistribution of anti-MOSP:MOSP complexes; they are no longer redistributed in a lacy network on oligodendrocyte membrane sheets but are present as clumps and fragments of veins (Dyer and Matthieu, 1993) (Fig. 6). It is likely that the clumped and fragmented redistribution of anti-MOSP:MOSP complexes is owing to the fact that anti-MOG induces a loss of microtubular structures in membrane sheets and anti-MOSP:MOSP complexes redistribute over what remains of these structures (Dyer and Matthieu, 1993).

Although both anti-GalC and anti-MOG result in depolymerization of microtubules and both patch over MBP, the signals transduced by anti-MOG IgG compared to anti-GalC IgG are not identical since anti-MOG does not cause an increase in intracellular Ca^{2+} and anti-GalC does elicit an increase. It is possible that two known regulatory pathways that control assembly/disassembly of microtubules, a Ca^{2+}-dependent pathway; and a Ca^{2+}-independent pathway (Yamamoto et al., 1985) are activated by the binding of these antibodies.

It is interesting that anti-GalC IgG, anti-sulfatide IgM, and anti-MOSP IgM all trigger Ca^{2+} influxes, but the anti-glycolipid antibodies cause depolymerization of microtubules, whereas anti-MOSP antibody causes microtubule growth. It has been demonstrated in other systems that there is no simple relationship between changes in intracellular Ca^{2+} and cellular response (for review, *see* Rasmussen, 1990). Indeed, when ionomycin was added to cultured oligodendrocytes so that intracellular Ca^{2+} concentration was increased to levels equal to those induced by anti-GalC IgG or anti-MOSP IgM, no change in the appearance of microtubules was observed (Dyer, unpublished data). These results indicate that an influx of Ca^{2+} alone is not sufficient to induce either assembly or disassembly of microtubules. Thus, it is likely that Ca^{2+} fluxes take place within cellular compartments, and can differentially regulate microtubule assembly and disassembly. In this way, the role of Ca^{2+} may be dependent on which surface membrane component has been activated.

Relevance to In Vivo Myelination

The signals generated by the binding of specific antibodies to GalC, sulfatide, MOG, or MOSP may mimic those induced by hypothesized endogenous ligands in vivo during myelinogenesis and myelin maintenance. Cytoskeleton is likely to be important in immature oligodendrocytes for process extension and growth of membrane sheaths; it is possible that MOSP is involved in transducing signals that induce upregulation of cytoskeleton during process extension and the production of the membrane sheath. Once processes are extended in vivo during myelinogenesis and membrane sheaths are wrapped around axons, compaction of the myelin lamellae occurs. Results from antibody binding to murine oligodendrocytes suggest that GalC may be involved in membrane compaction, i.e., the disassembly of microtubules since anti-GalC binding causes depolymerization and fusion of MBP domains in vitro (Dyer and Benjamins, 1989b). A007 binding results suggest that ligands binding to sulfatide produce signals that block or terminate the GalC response (Dyer and Benjamins, 1991). MOG also may be important in generating signals that are involved in myelin compaction, since antibody binding results in loss of microtubules in cultured oligodendroglia membrane sheets. If new membrane is added to myelin via the outer wrap where MOG is predominantly located (Brunner et al., 1989), MOG may be important in regulating compaction at this location. Because GalC and MOSP appear to be present throughout myelin lamellae, they also

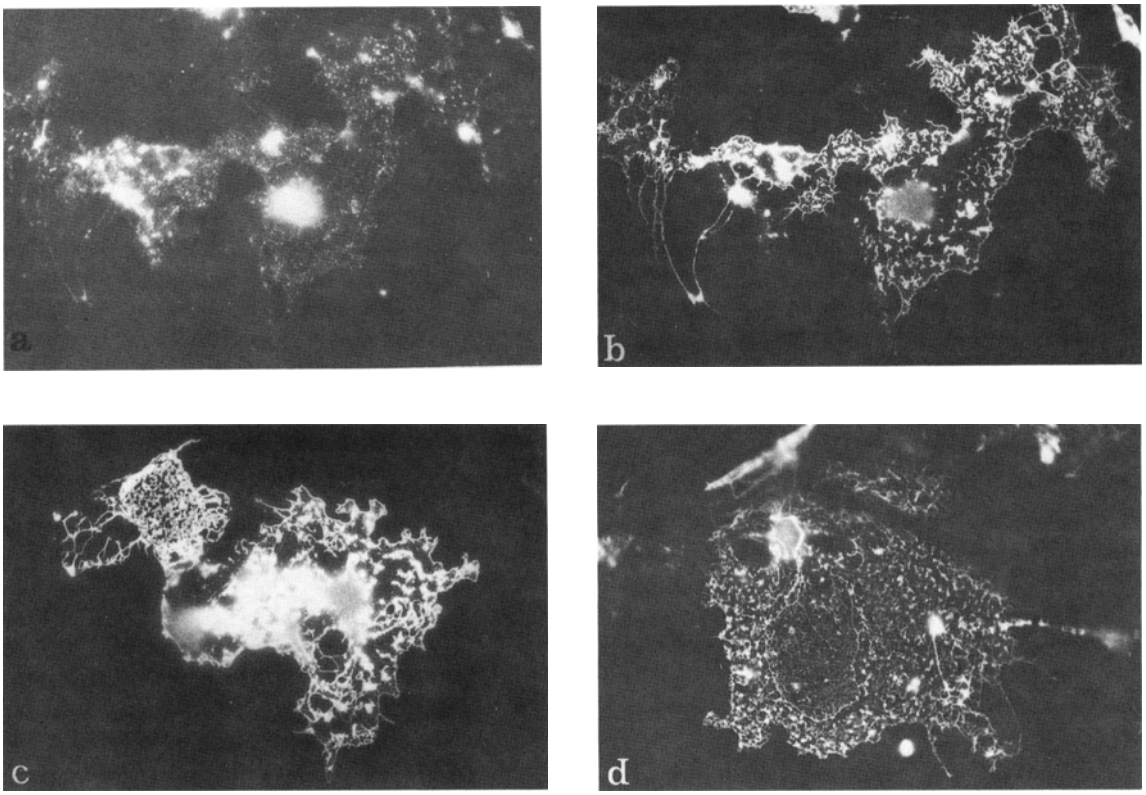

Fig. 6. Long-term exposure of oligodendrocytes to anti-MOG IgG results in fragmented and clumped redistributed MOSP. Live cultures were treated with anti-MOG IgG for 6 d and then treated with anti-MOSP and second antibody for 15 min each at 37°C. **a:** Patched anti-MOG:MOG complexes on oligodendrocyte membrane sheet. **b:** Same cell shown in **a** stained for MOSP; what normally would be continuous veins of anti-MOSP:MOSP complexes appear to be fragmented and clumped except for the single continuous vein on the edge of the sheet (arrow at cell body). **c:** Representative oligodendrocyte demonstrating staining of anti-MOSP:MOSP complexes after long-term anti-MOG IgG treatment. **d:** Representative example of anti-MOSP:MOSP clumped staining pattern on membrane sheets treated 6 d with anti-MOG. Bars = 20 μm.

may be involved in regulating cytoskeleton in mature CNS myelin. This may be possible since the radial component, which is composed of actin and tubulin as well as other molecules, is present in mature CNS myelin (Kosaras and Kirschner, 1990; Karthigasan et al., 1991).

Defining the signal transduction capabilities of these myelin surface markers is essential for understanding their roles in diseases that selectively affect CNS myelin, such as multiple sclerosis. Several lines of evidence indicate that MOG serves as an antigenic target in primary demyelinating diseases (Kerlero de Rosbo et al., 1990; Linington et al., 1988; Lassmann et al., 1988; Lassmann and Linington, 1987; Schluesener et al., 1987). Many investigators have previously reported that anti-GalC in the absence of complement has a pronounced effect on myelin in vivo and in cultured myelinating systems (Bornstein and Raine, 1970; Bornstein and Appel, 1961; Diaz et al., 1978; Fry et al., 1972,1974; Niedieck et al., 1965; Raine et al., 1981). In the presence of complement-depleted antiserum to white matter, the myelin lamellae show increased birefringence and doubling of the periodicity between the major dense lines (Raine et al., 1981; Diaz et al., 1978). At the same

time, the oligodendrocytes begin production of disorganized layers of membrane that accumulate in the extracellular space rather than wrapping around axons. The production of disorganized layers of membrane may result from inappropriate overstimulation by anti-GalC binding the GalC mediated signal transduction triggering pathway in oligodendrocytes. Since anti-GalC causes a loss of cytoskeleton in vitro, it is possible that anti-GalC is also causing a loss of cytoskeleton in myelin, which is manifested in the production of disorganized layers of myelin membrane. Therefore, it is conceivable that immune attack via binding to GalC, sulfatide, MOG or MOSP may mimic normal signals in a manner that disrupts the sequence of events that coordinates myelination or the maintenance of myelin in vivo.

Summary

Antibodies specifically reactive with GalC, sulfatide, MOSP, and MOG have different effects on cultured murine oligodendrocytes with regard to phospholipid turnover, Ca^{2+} influxes, and antigen redistribution that ultimately lead to modulation of cytoskeleton within membrane sheets. It is possible that these signaling systems operate in vivo to regulate the oligodendrocyte cytoskeleton during myelination and the maintenance of myelin. If so, it is conceivable that congenital defects resulting in the loss or altered expression of these markers could have an impact on the ability of oligodendrocytes to myelinate normally. Furthermore, antibody binding to GalC, sulfatide, MOG, or MOSP in pathologic diseases affecting CNS myelin could produce inappropriate signaling leading to production of abnormal myelin and/or loss of normal myelin

Acknowledgments

The author wishes to thank Joyce Benjamins and Jean-Marie Matthieu for their support and Ady Kendler for helpful discussion on the manuscript. This work was supported by the Multiple Sclerosis society grant PP0172, NIH 13413, the Swiss National Science Foundation (#31-30787.91), and the Multiple Sclerosis Society of Switzerland.

References

Amiguet P., Gardinier M. V., Zanetta J. P., and Matthieu, J. M. (1991) Purification and partial structural and functional characterization of mouse myelin/oligodendrocyte glycoprotein. *J. Neurochem.* **58,** 1676–1682.

Ash J. F. and Singer S. J. (1976) Concanavalin-A-induced transmembrane linkage of concanavalin-A receptors to intracellular myosin-containing filaments. *J. Cell Biol.* **73,** 4575–4579.

Ash J. F., Louvard D., and Singer S. J. (1977) Antibody-induced linkages of plasma membrane proteins to intracellular actomyosin-containing filaments in cultured fibroblasts. *J. Cell Biol.* **74,** 5584–5588.

Bansal R., Stefansson K., and Pfeiffer S. E. (1992) Proligodendroblast antigen (POA), a developmental antigen expressed by A007/04-positive oligodendrocyte progenitors prior to appearance of sulfatide and galactocerebroside. *J. Neurochem.* **58:** 2221–2229.

Bansal R. and Pfeiffer S. E. (1989) Reversible inhibition of oligodendrocyte progenitor differentiation by a monoclonal antibody against surface galactolipids. *Proc. Natl. Acad. Sci. USA* **86,** 6181–6185.

Bansal R., Warrington A. E., Gard A. L., Ranscht B., and Pfeiffer S. E. (1989) Multiple and novel specificities of monoclonal antibodies 01, 04, and mAb used in the analysis of oligodendrocyte development. *J. Neurosci. Res.* **58,** 2221–2229.

Barbarese E., Carson J. H., and Braun P. E. (1978) Accumulation of the four basic proteins in mouse brain during development. *J. Neurochem.* **31,** 779–782.

Barres B. A., Chun L. L. Y., and Corey D. P. (1988) Ion channel expression by white matter glia: type 2 saturates and oligodendrocytes. *Glia* **1,** 10–30.

Bologa L. (1985) Oligodendrocytes, key cells in myelination and target in desalinating diseases. *J. Neurosci. Res.* **14,** 1–20.

Bornstein M. B. and Raine C. S. (1970) Experimental allergic encephalomyelitis antiserum inhibition of myelination in vitro. *Lab. Invest.* **23,** 536–542.

Bornstein M. B. and Appel S. H. (1961) The application of tissue culture to the study of experimental allergic encephalomyelitis. I. Pattern of desalination. *J. Neuropath. Exp. Neurol.* **20,** 141–157.

Bourguignon L. Y. W., Suchard S. J., Nagpal A. L., and Glenney, J. R. (1985) A T-lymphoma transmembrane glycoprotein (gp180) is linked to the cytoskeletal protein, fodrin. *J. Cell Biol.* **101,** 477–487.

Braun P. E., Horvath E., Yong V. W., and Bernier L. (1990) Identification of GTP-binding proteins in myelin and oligodendrocyte membranes. *J. Neurosci. Res.* **26,** 16–23.

Brunner C., Lassmann H., Waehneldt T., Matthieu J. M., and Linington C. (1989) Differential ultrastructural localization of myelin basic protein, myelin/oligodendroglial glycoprotein, and 2',3'-cyclic nucleotide 3'-phosphodiesterase in the CNS of adult rat. *J. Neurochem.* **52,** 296–304.

Courand P.-O., Delavier-Klutchko C., Durieu-Trautmann O., and Strosberg A. D. (1981) Antibodies raised against β-adrenergic receptors stimulate adenyl cyclase. *Biochem. Biophys. Res. Comm.* **99,** 1295–1302.

Deane D. L., Harvey E., and Steel C. M. (1991) Differential effects of CD45, CD45R, and CD45R0 monoclonal antibodies in modulating human B cell activation. *Clin. Exp. Immunol.* **83,** 175–181.

Deshmukh D. S., Kuizon S., Bear W. D., and Brockeroff, H. (1980) Distribution of phosphoinositides among subfractions of rat brain myelin. *Lipids* **15,** 14–18.

Diaz M., Bornstein M. B., and Raine C. S. (1978) Disorganization of myelinogenesis in tissue culture by anti-CNS antiserum. *Brain Res.* **154,** 231–239.

Dixon S. J., Stewart D., Grinstein S., and Spiegel S. (1987) Transmembrane signalling by the B subunit of cholera toxin: Increased cytoplasmic free calcium in rat lymphocytes. *J. Cell Biol.* **105,** 1153–1161.

Dyer C. A. and Matthieu J. M. (1993) Antibodies to MOSP and MOG regulate cytoskeletal structure in cultured oligodendrocytes. Submitted.

Dyer C. A., Hickey W. F., and Geisert E. E., Jr. (1991) Myelin/oligodendrocyte-specific protein: a novel surface membrane protein that associates with microtubules. *J. Neurosci. Res.* **28,** 607–613.

Dyer C. A. and Benjamins J. A. (1991) Galactocerebroside and sulfatide independently mediate Ca^{2+} responses in oligodendrocytes. *J. Neurosci. Res.* **30,** 699–711.

Dyer C. A. and Benjamins J. A. (1990) Glycolipids and transmembrane signaling: antibodies to galactocerebroside cause an influx of calcium in oligodendrocytes. *J. Cell Biol.* **111,** 625–633.

Dyer C. A. and Benjamins J. A. (1989a) Organization of oligodendroglial membrane sheets. I: Association of myelin basic protein and 2',3'-cyclic nucleotide 3'-phosphohydrolase with cytoskeleton. *J. Neurosci. Res.* **24,** 201–211.

Dyer C. A. and Benjamins J. A. (1989b) Organization of oligodendroglial membrane sheets: II. Galactocerebroside:antibody interactions signal changes in cytoskeleton and myelin basic protein. *J. Neurosci. Res.* **24,** 212–221.

Dyer C. A. and Benjamins J. A. (1988a) Redistribution and internalization of antibodies to galactocerebroside by oligodendroglia. *J. Neurosci.* **8,** 883–891.

Dyer C. A. and Benjamins J. A. (1988b) Antibody to galactocerebroside alters organization of oligodendroglial membrane sheets in culture. *J. Neurosci.* **8,** 4307–4318.

Eichberg J., Berti-Mattera L. N., Day S-F., Lower, J., and Zhu X. (1989) Basal and receptor-stimulated metabolism of polyphosphoinositides in peripheral nerve myelin. *J. Neurochem.* **52 (Suppl.),** 524.

Eichberg J. and Dawson R. M. C. (1965) Polyphosphoinositides in myelin. *Biochem. J.* **96,** 644–650.

Eichberg J. and Hauser G. (1973) The subcellular distribution of polyphosphoinositides in myelinated and unmyelinated rat brain. *Biochem. Biophys. Acta* **326,** 210–223.

Exton J. H. (1988) Mechanisms of action of calcium-mobilizing agonists: some variations on a young theme. *Faseb J.* **2,** 2670–2676.

Fischer I., Konola J., and Cochary E. (1990) Microtubule associated protein (MAP1B) is present in cultured oligodendrocyte and co-localizes with tubulin. *J. Neurosci. Res.* **27,** 112–124.

Fishman P. H. (1982) Role of membrane ganglioside in the binding and action of bacterial toxins. *J. Membrane Biol.* **69,** 85–97.

Flanagan J., and Koch G. L. E. (1978) Cross-linked surface IgG attaches to action. *Nature* **273,** 278–281.

Fry J. M., Weissbarth S., Lehrer G. M., and Bornstein M. B. (1974) Cerebroside antibody inhibits sulfatide synthesis and myelination and desalination in cord tissue cultures. *Science* **183,** 540–542.

Fry J. M., Lehrer G. M., and Bornstein M. B. (1972) Sulfatide synthesis: inhibition by EAE serum. *Science* **175,** 192–194.

Gard A. L. and Pfeiffer S. E. (1989) Oligodendrocyte progenitors isolated directly from developing telencephalon at a specific phenotypic stage: myelinogenic potential in a defined environment. *Development* **106,** 119–132.

Geiger B. and Singer S. J. (1979) The participation of α-actinin in the capping of cell membrane components. *Cell* **16,** 213–222.

Geppert T. D. and Lipsky P. E. (1987) Accessory cell independent proliferation of human T4 cells stimulated by immobilized monoclonal antibody to CD3. *J. Immunol.* **138,** 1660–1665.

Golly F., Larocca J. N., and Ledeen R. W. (1990) Phosphoinositide breakdown in isolated myelin is stimulated by GTP analogues and calcium. *J. Neurosci. Res.* **27,** 342–348.

Hockberger P. E. and Swandulla D. (1987) Direct ion channel gating: A new function for intracellular messengers. *Cell. Mol. Neurobiol.* **7,** 229–236.

Jacobs S., Chang K. J., and Cuatrecasas P. (1978) Antibodies to purified insulin receptor have insulin-like activity. *Science* **200,** 1283–1284.

Kahn D. W. and Morell P. (1988) Phosphatic acid and phosphoinositide turnover in myelin and its stimulation by acetylcholine *J. Neurochem.* **50,** 1542–1550.

Karthigasan J., Kosaras B., and Kirschner D. A. (1991) Isolation and composition of radial component from CNS myelin. *Trans. Amer. Soc. Neurochem.* **22,** 161.

Kerlero de Rosbo N., Honegger P., Lassmann H., and Matthieu J.-M. (1990) Desalination induced in aggregating brain cell cultures by a monoclonal antibody against myelin oligodendrocyte glycoprotein (MOG). *J. Neurochem.* **55,** 583–587.

Keith C. H., Bajer A. S., Ratan R., Maxfield F. R., and Shelanske M. L. (1986) Calcium and calmodulin in the regulation of the microtubular cytoskeleton. *Ann. NY Acad. Sci.* **466,** 375–391.

Kidd G. J., Andrews S. B., and Trapp B. D. (1991) Microtubule organization in myelinating Schwann cells. *Trans. Amer. Soc. Neurochem.* **22,** 262.

Knapp P. E., Bartlett W. P., and Skoff R. P. (1987) Cultured oligodendrocytes mimic in vivo phenotypic characteristics: cell shape, expression of myelin specific antigens, and membrane production. *Dev. Biol.* **120,** 356–365.

Konola J. T., Tyler B. M., Yamamura T., and Lees M. B. (1991) Distribution of proteolipid protein and myelin basic protein in cultured mouse oligodendrocytes: primary vs. secondary cultures. *J. Neurosci. Res.* **28,** 49–64.

Kono T., Robinson F. W., Blevins T. L., and Ezaki O. (1982) Evidence that translocation of the glucose transport activity is the major mechanism of insulin action on glucose transport in fat cells. *J. Biol. Chem.* **257,** 10,942–10,946.

Kopf G. S. and Woolkalis M. J. (1991) ADP-ribosylation of G proteins with pertussis toxin. *Meth. Enzymol.* **195,** 257–266.

Kornecki E., Walkowiak B., Naik U. P., and Ehrlich Y. H. (1990) Activation of human platelets by a stimulatory monoclonal antibody. *J. Biol. Chem.* **265,** 10,042–10,048.

Kosaras B. and Kirschner D. A. (1990) Fine structure and supramolecular organization of the radial component of CNS myelin. *Ann. NY Acad. Sci.* **605,** 430–434.

Lassmann H., Brunner C., Bradl M., and Linington C. (1988) Experimental allergic encephalomyelitis: the balance between encephalitogenic T lymphocytes and desalinating antibodies determine size and structure of demyelinated lesions. *Acta Neuropathol. (Berl.)* **75,** 566–576.

Lassmann H. and Linington C. (1987) The role of antibodies against myelin surface antigens in desalination and in chronic EAE, in *A Multidisciplinary Approach to Myelin Diseases* (Crescenzi G. S., ed.), Plenum, London, pp. 219–225.

Law H., Itkonnen O., and Lingwood C. A. (1988) Sulfogalactolipid binding protein SLIP 1: A conserved function for a conserved protein. *J. Cell. Physiol.* **137,** 462–468.

Ledeen R. W. (1984) Lipid-metabolizing enzymes of myelin and their relation to the axon. *J. Lipid Res.* **25,** 1548–1544 .

Lees M. B. and Brostoff S. W. (1984) Proteins of myelin, in *Myelin* (Morell, P., ed.), Plenum, NY, pp. 197–217.

Lees M. B. and Sapirstein V. S. (1983) Myelin-associated enzymes, in *Handbook of Neurochemistry,* vol. 4, (Lajtha A., ed.), Plenum, NY, pp. 435–460.

Lemke G. (1988) Unwrapping the genes of myelin. *Neuron* **1,** 535–543.

Levine S., Xian C. Y., Agocha B., Allopenna J., Welte K., Armstrong, D., and Evans, R. L. (1991) Differential modulation of the CD-2 and CD-3 T cell activation pathways by a monoclonal antibody to Leu-13. *Cell. Immunol.* **132,** 366–376.

Linington C., Bradl M., Lassmann H., Brunner C., and Vass, K. (1988) Augmentation of desalination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am. J. Pathol.* **130,** 443–454.

Linington C., Webb M., and Woodhams P. L. (1984) A novel myelin-associated glycoprotein defined by a mouse monoclonal antibody. *J. Neuroimmunol.* **6,** 387–396.

Lund-Johansen F., Olweus J., Aarli A., and Bjerknes R. (1991) The IgG FcRII and the PI-linked IgG FcRIII trigger cytoplasmic calcium fluxes independently in human granulocytes. *Scand. J. Immunol.* **33,** 261-266.

Marcum J. M., Dedman J. R., Brinkley B. R., and Means A.R. (1978) Control of microtubule assembly-disassembly by calcium-dependent regulator protein. *Proc. Natl. Acad. Sci. USA* **75,** 3771–3775.

Martinson E. A., Trilivas I., and Brown J. H. (1990) Rapid protein kinase C-dependent activation of phospholipase D leads to delayed 1,2-diglyceride accumulation. *J. Biol. Chem.* **265,** 22,282–22,287.

Matthieu J. N. and Amiguet P. (1990) Myelin/oligodendrocyte glycoprotein (MOG) expression during development in normal and myelin-deficient (mld) mice. *Dev. Neurosci.* **12,** 293–302.

Meldolesi J. and Pozzan T. (1987) Pathways of Ca^{2+} influx at the plasma membrane: voltage, receptor, and second messenger-operated channels. *Exp. Cell Res.* **171,** 271–283.

Merritt J. E. and Rink T. J. (1987) Regulation of cytosolic free calcium in fura-2-loaded rat parotid acinar cells. *J. Biol. Chem.* **262,** 17,362–17,369.

Mikol D. D., Gulcher J. R., and Stefansson, K. (1990) The oligodendrocyte-myelin glycoprotein belongs to a distinct family of proteins and contains the HNK-1 carbohydrate. *J. Cell Biol.* **110,** 471–479.

Niedieck B., Kuwart E., Palacious O., and Drees O. (1965) Immunochemical and serological studies on the lipid hapten of myelin with relationship to EAE. *Ann. NY Acad. Sci.* **122,** 266–276.

Norton W. and Cammer W. (1984) Isolation and characterization of myelin, in *Myelin* (Morell P., ed.), Plenum, NY, pp. 147–195.

Owens G. C. and Bunge R. P. (1990) Schwann cells depleted of galactocerebroside express myelin-associated glycoprotein and initiate but do not continue the process of myelination. *Glia* **3,** 118–124.

Penner R., Matthews G., and Neher E. (1988) Regulation of calcium influx by second messengers in rat mast cells. *Nature* **334,** 499–504.

Pfeiffer S. (1984) Oligodendrocyte development in culture systems, in *Advances in Neurochemistry,* vol. 5, (Agranoff B. W., and Aprison M. H., eds.), Plenum, NY, pp. 233–298.

Raine C. (1984) Morphology of myelin and myelination, in *Myelin* (Morell, P., ed.), Plenum, NY, pp. 1–41.

Raine C. S., Johnson A. B., Marcus D. M., Suzuki A., and Bornstein M. B. (1981) Desalination in vitro. Absorption studies demonstrate that galactocerebroside is a major target. *J. Neurol. Sci.* **52,** 117–131.

Ranscht B., Wood P. M., and Bunge R. P. (1987) Inhibition of in vitro peripheral myelin formation by monoclonal anti-galactocerebroside. *J. Neurosci.* **7,** 2936–2947.

Ranscht B., Clapshaw P. A., Price J., Noble M., and Seifert W. (1982) Development of oligodendrocytes and Schwann cells studied with a monoclonal antibody against galactocerebroside. *Proc. Natl. Acad. Sci. USA* **79,** 2709–2713.

Rasmussen H. (1990) The complexities of intracellular Ca^{2+} signalling. *Physiol. Chem. Hoppe-Seyler* **371,** 191–206.

Reynolds R., Carey E. M., and Herschkowitz N. (1989) Immunohistochemical localization of myelin basic protein and 2',3'-cyclic nucleotide 3'-phosphohydrolase in flattened membrane expansions produced by cultured oligodendrocytes. *Neuroscience* **28,** 181–188.

Robinson P. J. (1991) Phosphatidylinositol membrane anchors and T-cell activation. *Immunology Today* **12,** 35–41.

Rome L. H., Bullock P. N., Chiappelli F., Cardwell M., Adinolfi, A. M., and Swanson, D. (1986) Synthesis of a myelin-like membrane by oligodendrocytes in culture. *J. Neurosci. Res.* **15,** 49–65.

Schluesener H. J., Sobel R. A., Linington C., and Weiner H. L. (1987) A monoclonal antibody against a myelin oligodendrocyte glycoprotein induces relapses and desalination in central nervous system autoimmune disease. *J. Immunol.* **139,** 4016–4021.

Schreiber A. B., Lax I., Yarden Y., Eshhar Z., and Schlessinger, J. (1981) Monoclonal antibodies against receptor for epidermal growth factor induce early and delayed effects of epidermal growth factor. *Proc. Natl. Acad. Sci. USA* **78,** 7535–7539.

Schwartz G. J. and Al-Awqati Q. (1986) Regulation of transepithelial H^+ transport by exocytosis and endocytosis. *Annu. Rev. Physiol.* **48,** 153–161.

Sedlacek H. H., Stourk J., Seiler F. R., Ziegler W., and Wiegandt H. (1976) Cholera toxin induced redistribution of sialoglycolipid receptor at the lymphocyte membrane. *FEBS Lett.* **61,** 272–276.

Sela B.-A., Raz A., and Geiger B. (1978) Antibodies to ganglioside GM1 induce mitogenic stimulation and cap formation in rat thymocytes. *Eur. J. Immunol.* **8,** 268–274.

Simpson I. A. and Cushman S. W. (1985) Regulation of glucose transporter and hormone receptor cycling by insulin in the rat adipose cell. *Curr. Top. Membr. Trans.* **24,** 459–503.

Soliven B., Szuchet B. G., Arnason W., and Nelson D. J. (1988) Forskolin and phorbol esters decrease the same K^+ conductance in cultured oligodendrocytes. *J. Membr. Biol.* **105,** 177–186.

Sontheimer H., Trotter J., Schachner M., and Dettenmann, H. (1989) Channel expression correlates with differentiation stage during the development of oligodendrocytes from their precursor cells in culture. *Neuron* **2,** 1135–1145.

Suzuki K. (1980) Myelin-associated enzymes, in *Neurologic Mutations Affecting Myelination* (Baumann N., ed.), Elsevier/North Holland Biomedical Press, Amsterdam, Netherlands, pp. 333–347.

Taylor R. B., Duffus W. P. H., Raff M. C., and dePetris S. (1971) Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules induced by a anti-immunoglobulin antibody. *Nature* **233,** 225–229.

Tompkins T. A. and Moscarello M. A. (1991) Myelin basic protein stimulates phospholipase C activity. *Trans. Am. Soc. Neurochem.* **22,** 163.

Vandenberghe P. and Ceuppens J. L. (1991) Immobilized anti-CD5 together with prolonged activation of protein kinase C induce interleukin 2-dependent T cell growth: evidence for signal transduction through CD5. *Eur. J. Immunol.* **21,** 251–259.

Vartanian T., Szuchet S., Dawson G., and Campagnoni A. T. (1986) Oligodendrocyte adhesion activates protein kinase C-mediated phosphorylation of myelin basic protein. *Science* **234,** 1395–1398.

Wade J. B. (1986) Role of membrane fusion in hormonal regulation of epithelial transport. *Annu. Rev. Physiol.* **48,** 213–223.

Wilson R. and Brophy P. J. (1989) Role for the oligodendrocyte cytoskeleton in myelination. *J. Neurosci. Res.* **22,** 439–448.

Wilson L. and Farrell K. W. (1986) Kinetics and steady state dynamics of tubulin addition and loss at opposite microtubules ends: the mechanism of action of soliciting. *Ann NY Acad. Sci.* **466,** 690–708.

Yamada A., Nojima Y., Sugita K., Dang N. H., Schlossman S. F., and Morimoto C. (1991) Cross-linking of VLA/CD29 molecule has a co-mitogenic effect with an anti-CD3 on CD4 cell activation in serum-free culture system. *Eur. J. Immunol.* **21,** 319–325.

Yamamoto H., Fukunaga K., Goto S., Tanaka E., and Miyamoto E. (1985) Ca^{2+}, calmodulin-dependent regulation of microtubule formation via phosphorylation of microtubule-associated protein 2, t factor, and tubulin, and comparison with cAMP-dependent phosphorylation. *J. Neurochem.* **44,** 759–768.

Young J. D.-E., Unkeless J. C., Kaback H. R., and Cohn Z. A. (1983a) Mouse macrophage Fc receptor for IgGg2b/g1 in artificial and plasma membrane vesicles functions as a ligand-dependent ionophore. *Proc. Natl. Acad. Sci. USA* **80,** 1636–1640.

Young J. D.-E., Unkeless J. C., Young T. M., Mauro A., and Cohn Z. A. (1983b) Role for mouse macrophage IgG Fc receptor as ligand-dependent ion channel. *Nature* **306,** 186–189.