Lysenin-Sphingomyelin Binding at the Surface of Oligodendrocyte Lineage Cells Increases During Differentiation In Vitro

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We have investigated the relationship between the developmental expression of sphingomyelin, a major component of myelin, and oligodendrocyte lineage. Using lysenin as a cytochemical probe for membrane sphingomyelin, we have now determined the distribution pattern of sphingomyelin on the plasma membrane of rat cultured oligodendrocytes. Although lysenin does not bind to A2B5/NG2 bipolar oligodendrocyte progenitors, lysenin recognizes sphingomyelin on the cell bodies of multipolar A2B5+ cells, but not on their processes. O4+ and O1+ immature and MBP+ mature oligodendrocytes are strongly labeled by lysenin from cell bodies to the tips of processes. The content of sphingomyelin in immature and mature oligodendrocytes is approximately 2-fold higher than that in oligodendrocyte progenitors. These findings show that sphingomyelin increases during differentiation of cells in the oligodendrocyte lineage. In multipolar oligodendrocyte progenitors exposed to Triton X-100 at 4°C, lysenin labels cell processes in addition to cell bodies. In contrast, Triton X-100 extraction does not alter the distribution of lysenin binding on O4+, O1+ and MBP+ cells, although the immunocytochemical intensities of the lysenin bindings increase. Our data suggest that the alteration in sphingomyelin content and distribution in the oligodendrocyte lineage cells could have important consequences for cell recognition and downstream signaling events through sphingomyelin-rich domains. J. Neurosci. Res. 62:521–529, 2000.

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Key words: oligodendrocyte lineage; sphingomyelin; cholesterol; cell culture
sequences for cell recognition and downstream signaling molecules. Our data suggest that the alteration in sphingomyelin increases during development of OL, although the amount and distribution of cholesterol remain unchanged. Treatment of cells with Triton X-100 at 4°C, that does not normally solubilize lipid rafts, revealed cryptic lysenin binding sites, suggesting complex regulation of sphingomyelin expression in cells of the oligodendrocyte lineage. Sphingomyelin is a critical component of lipid rafts that contain specific classes of cell adhesion molecules and also signaling molecules. Our data suggest that the alteration in sphingomyelin content and distribution in cells of the oligodendrocyte lineage could have important consequences for cell recognition and downstream signaling events.

MATERIALS AND METHODS

Animals

Pregnant rats were obtained from the Japan SLC Inc. (Sizuoka, Japan). They were maintained on laboratory chow and water ad lib. “The Tokyo Metropolitan Institute of Medical Science Institutional Animal Care and Use Committee according to National Institutes of Health Animal Care and Use protocol” approved all experimental protocols.

Cell Cultures

Oligodendrocyte progenitor (OLP) and oligodendrocyte (OL) cultures were prepared from primary mixed cell cultures of embryonic rat cerebral cortex as described by Itoh et al. (2000) with slight modifications. Briefly, the cerebral cortex from an embryonic 16–18-day-old rat was mechanically dissociated through 140 μm pore-sized stainless mesh in 10% fetal calf serum (FCS) (JRH Biosci, Lenexa, KS) in Eagle’s minimum essential medium (EMEM) (Nissui, Tokyo, Japan). The dissociated cells were finally sieved through 70 μm pore-sized nylon mesh (Falcon, NJ) and then were centrifuged for 10 min, 100 × g at 4°C. Cells were resuspended in FCS/EMEM and were seeded on poly-L-lysine (PLL, 100 μg/ml, Sigma, St. Louis, MO)-coated 90 mm diameter culture dishes (Griner, Germany) at a density of 10 × 10^5 cells/dish. After 7 days in culture, the cells were passaged with 0.05% trypsin in Dulbecco’s phosphate buffered saline (D-PBS) (1st passage). Cells were resuspended in FCS/EMEM and were cultured for 7 days at a density of 8 × 10^6 cells on culture dish. After 7 days in culture, cells were passaged with 0.05% trypsin in D-PBS and were cultured for 2 days at a density of 3 × 10^6 cells in FCS/EMEM on 100 mm diameter Petri dish for OLP and for OL (second passage). On the second day of culture, the medium was exchanged to serum-free chemical defined medium in high glucose Dulbecco’s modified MEM (DMEM) (Gibco BRL, Grand Island, NY) supplemented with 5 μg/ml insulin, 0.5 μg/ml transferrin, 100 μg/ml BSA, 0.06 ng/ml progesterone, 16 μg/ml putrescine, 40 ng/ml sodium selenite, 40 ng/ml l-thyroxine, 30 ng/ml l-triiodothyronine) with 2 ng/ml basic fibroblast growth factor (bFGF) (all ingredients from Sigma). After Day 1, these cultures were used for OLP. To get OL, these cells were allowed to culture for 5 more days. Cells were then passaged with 0.05% trypsin in D-PBS and were seeded at a density of 2 × 10^6 cells on the same Petri dish as above in the serum-free chemical defined medium without bFGF (third passage). Six-day-old cultures from the third passage were used for these experiments. The procedures were necessary to eliminate neurons and astrocytes.

Immunocytochemistry

Monoclonal antibodies A2B5, O4, O1 were purchased from ATCC (Twinbrook, MD), anti-GalC polyclonal antibody was from BioMakor (Rehovot, Israel), anti-MBP monoclonal antibody was from Boehringer Mannheim (Germany), anti-Gst monoclonal antibody, anti-Gst polyclonal antibody were from Sigma. NG2 antibody was kindly provided by Dr. B. Stallcup. Monoclonal antibody TuJ1 was from Promega (Madison, WI), polyclonal GFAP antibody was from Dako (Denmark). Biotinylated anti-mouse IgG, biotinylated anti-rabbit IgG, fluorescein isothiocyanate (FITC)-conjugated avidin were from Amersham Pharmacia Biotech (Tokyo, Japan), tetramethyl rhodamine isothiocyanate (TRITC)-conjugated anti-rabbit IgG was from ICN (Costa Mesa, CA), TRITC-conjugated anti-mouse IgG (γ-chain specific) was from Kirkegaard & Perry Lab. (Gaithersburg, MD), TRITC conjugated anti-mouse IgM (μ-chain specific) was from Sigma.

Lysenin purified from earthworm Eisenia fetida and fusion protein of lysenin with glutathione s-transferase (Gst) constructed by Yamaji et al. (1998) were used for immunocytochemical analyses. Lysenin-staining was done as follows; cells cultured on Petri dishes were fixed by various fixations (3.7% formalin, 2–4% paraformaldehyde, 75 mM lysine-1% paraformaldehyde, 10 mM sodium periodate, 3.75 mM sodium phosphate [LPP], and on live). Depending on the purpose of experiments, cells were incubated with 0.05–0.5% Triton X-100 (Sigma) in D-PBS, or just in D-PBS as control, for 20 min at 4°C. Cells were washed with lysenin-Gst (1 μg/ml) for 2 hr, washed with D-PBS, incubated with anti-Gst antibody for 1 hr, washed with D-PBS, incubated with biotinylated anti-rabbit IgG antibody or with biotinylated anti-mouse IgG antibody for 30 min, washed with D-PBS, incubated with FITC-conjugated avidin (5 μg/ml) for 30 min before immunocytochemistry using marker antibodies. Filipin (Polysci, Warrington, PA) to stain unesterified cholesterol in the cells was done as follow; cells were incubated with filipin (50 μg/ml) for 1 hr at room temperature (RT) after double-immunocytochemistry using lysenin and marker antibodies. The cells were observed under a fluorescence microscope with UV-filter to assess changes in cellular staining pattern. Lineage-specific antigens were stained as fol-
low; cells were incubated with A2B5, NG2, O4, O1 or anti-galactosyleramidase (GalC) antibodies after 2% paraformaldehyde for 20 min. For anti-MBP antibody, cells were fixed for 30 min of 2% paraformaldehyde incubation followed by 10 min of 0.1% Triton X-100 incubation at RT. Cells were incubated with the 1st antibodies for 1 hr at RT or overnight at 4°C and then, incubated with TRITC-conjugated anti-rabbit IgG, TRITC-conjugated anti-mouse IgG or TRITC-conjugated anti-mouse IgM for 1 hr at RT. Stained-cells were mounted using Vectashield mounting medium (Vector Lab., Burlingame, CA). Fluorescence microscopy was performed with Bio–Rad confocal laser scanning MRC1000 system (Hercules, CA) with an inverted microscope (Axiophot; Carl Zeiss Inc., Germany) at magnification levels (×600–2100). Confocal images have single and reconstructions of 20 0.54-μm-thick optical sections of either lysenin+ and A2B5+ or O4+, O1+ and MBP+ OL, respectively.

**Enzyme Treatments on Cultured Oligodendrocytes**

Cells were treated with various enzymes, endo-β-N-acetyl-glucosaminidase H (Endoglycosidase H) (0.2 and 1 U/ml), N-glycosidase F (PNGase F) (0.4 and 0.8 U/ml), endo-β-galactosidase (0.1 and 0.5 U/ml), β-galactosidase (4 and 20 U/ml), phosphatidylinositol-phospholipase C (PI-PLC) (0.05, 0.5 and 5 U/ml), PLC (0.05, 0.5 and 5 U/ml) at 37°C, 5% CO2 (all enzymes from Boehringer Mannheim). Enzyme treatments were performed under wide range of incubation time (from 1 hr to overnight).

**Phospholipid and Cholesterol Determination**

Total lipids were extracted according to the method of Bligh and Dyer (1959). OL and OLP were incubated with 0.05% trypsin for 2 min at RT to remove the lipoproteins from the cell surface and harvested using rubber policeman. Aliquots were taken to determine cellular protein. The cell protein mass of each lysate was determined by BCA Protein Assay Reagent (Pierce, IL). The lower phase from the Bligh-Dyer extract was dried under a stream of nitrogen and the lipids were resuspended in CHCl3. The phospholipids were separated according to the method of Rouser et al. (1966) on TLC plate (Merck, Darmstadt, Germany) using the solvent system; chloroform/methanol/acetate/formic acid (50:30:4.5:6.5, v/v). The separated spots were visualized by primulin, and the silica gels of each spot were scraped. Lipids were eluted from the silica gel by chloroform/methanol/water (1:1:1 v/v), and the contents of phospholipids were quantified by the absorbance (660 nm) using a spectrophotometer (Beckman DU-530, Fullerton, CA). Aliquots of the Bligh-Dyer extract were taken to determine cellular cholesterol that was measured with an enzyme test kit (Boehringer Mannheim).

**RESULTS**

**The Phenotype of Cultured Oligodendrocytes**

The cell types isolated from embryonic day 16–18 rat cerebral cortex were identified using lineage-specific antibodies. The cell population quantified using oligodendrocyte lineage-specific antibodies at 3 days after the second passage (OLP) and 6 days after the third passage (OL) is shown in Table I. The OL, neuronal and astrocyte marker antibodies did not cross react with between OL, neurons and astrocytes in our culture system. When cells were labeled with any combination of OL markers, we found that the majority of OLP and OL cultures were labeled with any combination of OL markers, we found that the majority of OLP and OL cultures were labeled by A2B5 and NG2, O4, O1 and anti-MBP antibodies with anti-GalC antibody. Figures as the ratio of positive cells of each lineage-specific marker against total number of cells represent means ± SE of 3 independent cultures. ND, not detectable.

**TABLE I. Developmental Profile of Oligodendrocytes Expressing Lineage-Specific Markers**

<table>
<thead>
<tr>
<th>Markers</th>
<th>Oligodendrocyte progenitors</th>
<th>Oligodendrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>A2B5</td>
<td>89.8 ± 5.2</td>
<td>22.4 ± 9.5</td>
</tr>
<tr>
<td>NG2</td>
<td>85.9 ± 6.7</td>
<td>15.5 ± 9.4</td>
</tr>
<tr>
<td>O4</td>
<td>8.5 ± 6.4</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td>O1/GalC</td>
<td>6.2 ± 8.8</td>
<td>87.2 ± 8.5</td>
</tr>
<tr>
<td>MBP</td>
<td>ND</td>
<td>49.5 ± 19.6</td>
</tr>
<tr>
<td>NG2+/A2B5+</td>
<td>85.9 ± 6.7</td>
<td>15.5 ± 9.4</td>
</tr>
<tr>
<td>A2B5+/GalC+</td>
<td>80.1 ± 9.2</td>
<td>10.2 ± 8.5</td>
</tr>
<tr>
<td>A2B5+/GalC+</td>
<td>3.5 ± 3.4</td>
<td>12.2 ± 5.3</td>
</tr>
<tr>
<td>O4+/GalC+</td>
<td>6.2 ± 8.8</td>
<td>6.1 ± 1.2</td>
</tr>
<tr>
<td>MBP+/GalC+</td>
<td>ND</td>
<td>46.9 ± 16.9</td>
</tr>
</tbody>
</table>

*OLP and OL were double-labeled by A2B5, NG2, O4, O1 and anti-MBP antibodies with anti-GalC antibody. Figures as the ratio of positive cells of each lineage-specific marker against total number of cells represent means ± SE of 3 independent cultures. ND, not detectable.

**Distribution of Sphingomyelin Labeled by Lysenin in OL During Development**

To investigate the expression of sphingomyelin on the plasma membrane of rat cultured OLP and OL, cells were incubated with lysenin–Gst fusion protein and visualized with FITC-conjugated anti–Gst antibody and OLP and OL marker antibodies to identify developmental stages. To test influence of fixation on sphingomyelin-labeled and MBP+ cells represented approximately 50% of the OL. On the other hand, no labeling of cells with neuronal markers (Tuj1, NF) was revealed and GFAP+ astrocytes represented less than 2.5% of the cells.
Influence in Binding Activity of Lysenin to Sphingomyelin After Treatment With Various Glycosidases and Lipases

To determine whether other cell surface molecules and residues interfere with lysenin-sphingomyelin interaction, the cells were treated with various glycosidases and lipases before lysenin staining. Different concentrations of the enzymes and incubation times from 1 hr to overnight were tested. No damage to cell membranes was detected under phase contrast microscopy after treatment. Despite several kinds of enzyme used, no difference on lysenin-binding was observed (Table III). These findings indicate that existence of sugars and proteins of cell surface molecules do not influence interaction with lysenin and sphingomyelin.

Distribution of Cholesterol Labeled by Filipin in OL During Development

Recently, it was reported that protein-protein and protein-lipid interactions occur within specific regions in the plasma membrane, enriched in sphingomyelin and cholesterol (Sheets et al., 1999). Cholesterol may also influence in interactions with sphingomyelin and lysenin. To study the spatial relationship between sphingomyelin and cholesterol, OL and OLP were labeled by lysenin and filipin, a marker for cholesterol in the plasma membrane. Filipin labeling was detected ubiquitously on the cell surface of both OLP and OL (Fig. 2C). When cells were exposed to methyl-β-cyclodextrin, depleting approximately 60% cholesterol from the plasma membrane, lysenin immunoreactivity did not change markedly, despite the disappearance of filipin staining (data not shown). These results indicate that sphingomyelin-lysenin binding is not influenced by cholesterol in the plasma membrane.
Sphingomyelin Content Increases During OL Development

Because there was a difference in the distribution of sphingomyelin labeled by lysenin during OL development, we quantified the total phospholipid content as well as the relative amounts of individual phospholipids of OLP and OL. As shown in Table IV, the content of sphingomyelin in OL (13.5 nmol/mg cell protein) was significantly higher than that in the OLP (7.6 nmol/mg cell protein). Total phospholipid content did not change significantly, however, during development (180.4 and 183.5 nmol/mg cell protein in OLP and OL, respectively). Besides the obvious increase of sphingomyelin during development, there was no significant difference in the content of other phospholipids. Also, the content of cholesterol did not show a significant difference between OLP and OL (Table IV).

Effect of Triton X-100 on the Detection of Lysenin-Sphingomyelin Bindings

Membrane structures resistant to solubilization by nonionic detergents such as Triton X-100 are enriched in sphingomyelin and cholesterol. To test the influence of nonionic detergent in sphingomyelin-lysine binding, the cells were exposed to 0.05–0.5% Triton X-100 at 4°C or RT for 20 min after fixation. Although sphingomyelin-lysine binding was not found in bipolar OLP under normal conditions, lysenin positive signals clearly appeared on their cell bodies after exposure to Triton X-100 at 4°C (Fig. 3A and Table II). In multipolar OLP, sphingomyelin was detected on both cell bodies and processes after Triton X-100 extraction at 4°C (Fig. 3C and Table II). When cells were exposed to Triton X-100 at 4°C, lysenin immunoreactivity markedly increased, despite the disappearance of filipin staining (Fig. 2D). The immunoreactivities of A2B5, O4, or O1/GalC antibodies were not influenced by cold exposure to Triton X-100 (Fig. 3B,D,F). On the other hand, the immunoreactivities of A2B5, O4, and O1/GalC antibodies were reduced after exposure to Triton X-100 at RT as compared to cells not treated with Triton X-100 (data not shown). In the cells treated with Triton X-100 at RT, the distribution of glycolipids appeared like a “sprinkling of confection sugar” (data not shown). These results indicate that lysenin binding to sphingomyelin persists after Triton X-100 extraction at 4°C, but filipin binding to cholesterol binding does not.

DISCUSSION

In this study, we have analyzed the expression of sphingomyelin in the OL lineage cells by immunocytochemical and biochemical methods. We first demonstrated that the expression of sphingomyelin at the cell surface of cultured OL increases during their differentiation. To investigate the molecular events of OL lineage in vitro, we were able to get immunocytochemically enriched population of OLP and OL in our cultures. OL cultures contained several types of cells at different stages as shown in Table I, although the majority of cells was O1/GalC+OL. Interestingly, A2B5+/O1/GalC+ cells were morphologically indistinguishable from A2B5-/O1/GalC+OL. These findings indicate that the differentiation of OL occurs during the culturing period in our system. This provides evidence that a great majority (O1/GalC+) of the cells developed from A2B5+/ and NG2+ OL and O1/GalC+ OL are newly formed during 7 days in cultures. Therefore, this in vitro culture system can be used for understanding molecular events on the cell surface during OL development.

Sphingomyelin is well known as an important structural component of the myelin membrane. Little is known, however, about when and where sphingomyelin appears in the membrane of oligodendrocytes and how it participates in cellular functions, mainly due to a lack of appropriate methodologies for detecting sphingomyelin. We have developed a method using sphingomyelin-specific binding protein, lysenin, that allows direct detection of sphingomyelin in the cell surface (Mayaji et al., 1998). As shown in Figure 1 and Table II, the expression of sphingomyelin at the cell surface increased during the differentiation of OL and precedes not only the appear-

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Dose (U/mL)</th>
<th>Progenitors</th>
<th>Bipolar</th>
<th>Multipolar</th>
<th>Oligodendrocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>–</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endoglycosidase H</td>
<td>0.2–1</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>N-glycosidase F</td>
<td>0.4–0.8</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endo-β-galactosidase</td>
<td>0.1–0.5</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-galactosidase</td>
<td>4–20</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phosphatidylinositol-phospholipase C</td>
<td>0.05–5</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phospholipase C</td>
<td>0.05–5</td>
<td></td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Cells were treated with enzymes in various dilutions under wide range of incubation time (from 1 hr to overnight). No effect on the distribution of lysenin-sphingomyelin binding was shown. –, lysenin negative; +, lysenin positive.
Fig. 2. Influence in cholesterol on cell surface detected by filipin on Triton X-100 extraction at 4°C in OL. Cells without (A,C,E) or with (B,D,F) exposure to 0.5% Triton X-100 for 20 min at 4°C were triple-labeled with lysenin (A,B), filipin (C,D) and A2B5 (E,F). (A,C,E) and (B,D,F) were micrographs of the same field. Filipin-labeling was detected ubiquitously both on OLP (arrows) and OL (arrowheads) without exposure to cold Triton X-100 (B). When cells were exposed to cold Triton X-100, filipin-labeling was washed off (D), although lysenin-labeling was enhanced both on OLP and OL (B). Scale bars = 10 μm.
form a dense lateral packing in the plasma membrane through the amide-linkage of fatty acids of sphingomyelin (Slotte, 1997). Our previous studies have shown that sphingomyelin/lysenin interactions are enhanced when more than 30-mol% of cholesterol co-existed with sphingomyelin using liposomes (Yamaji et al., 1998). The content of cholesterol was lower than 30-mol% in OL that showed lysenin negative or weak signals (Table IV). Thus, cholesterol content and distribution at the cell surface must also be considered when evaluating lysenin binding. Although the lipid composition of myelin and OL has been already studied intensively, the lipid composition of OL lineage cells is still not determined (Pleasure et al., 1984; Szuchet et al., 1983). As shown in Table IV, phospholipids and cholesterol analyses in OL revealed little difference in the composition of OL lipids, except for an approximately 2-fold increase in sphingomyelin in OL as compared to OLP. This study, in which the cellular distribution of cholesterol was determined using filipin staining, showed that the levels of lysenin bound to sphingomyelin increased when the cells were treated with cold Triton X-100, despite depletion of cholesterol in OL lineage cells (Fig. 2). Recently, we have shown that the filipin-staining pattern was not significantly different between control and sphingomyelin-depleted cells (Porn and Slotte, 1995). Although there was a dramatic increase in cholesterol content during myelination (Kinney et al., 1994), the content of cellular cholesterol did not significantly change during development of the OL lineage cells (Table IV). Composition of cholesterol in OL, however, (38.2 ± 2.0%) significantly ($P < 0.025$) increased in comparison with OLP (27.3 ± 2.6%). This suggests a different role of cholesterol in content and composition during maturation of the OL and during myelination.

Recent attention has been drawn to lateral lipids assemblies of sphingomyelin and cholesterol (termed rafts) (Simons and Ikonen, 1997). In previous investigations, it has been shown that sphingomyelin and cholesterol-rich domains are insoluble in Triton X-100 at 4°C (Schroeder et al., 1994; Ledesma et al., 1999; Sheets et al., 1999). The binding of lysenin could be strongly detected on somas and processes after exposure to Triton X-100 at 4°C, despite depletion of phospholipids and cholesterol (Fig. 2B and Table II), suggesting that sphingomyelin clusters within insoluble domains at the cell surface. Besides sphingomyelin; sulfatide, galactosylceramide, gangliosides and GPI-anchored molecules such as F3 were also insoluble in cold Triton X-100, but PLP that is known to be detergent soluble was not (Gillespie et al., 1989; Pfeiffer et al., 1993; Koch et al., 1997). Indeed, the neural cell adhesion molecules F3 and L1 co-localized with lysenin bound to sphingomyelin and were not influenced by cold Triton X-100 exposure (data not shown). Taken together, these results strongly indicate that lysenin provides a useful cytochemical tool to detect sphingomyelin within rafts.

In conclusion, we have shown that sphingomyelin content increases as cells mature from OLP to OL, that the sphingomyelin is not extracted by Triton X-100 at 4°C, suggesting it is in detergent insoluble rafts and that it remains there even when much of cholesterol is depleted from the cells. It is well known that signaling molecules, such as src family kinases, are associated with lipid rafts in cells as are cell recognition molecules and growth factor receptors. Recently sphingomyelin have been implicated in the control of proliferation, differentiation and apoptosis through organizing these specialized membrane rafts (Jayadev et al., 1995; Brown and London, 1998; Sheets et al., 1999). Our data suggest that sphingomyelin may be involved in morphological and physiological differentiation and maturation of OL including myelination by altering the function of lipid membrane rafts. Clarifying the role of sphingomyelin in OL differentiation should open

### Table IV. Phospholipids and Cholesterol Composition and Content of OLP and OL

<table>
<thead>
<tr>
<th>Lipid</th>
<th>OLP (n = 5)</th>
<th>OL (n = 4)</th>
<th>Content (nmol/mg cell protein) b</th>
</tr>
</thead>
<tbody>
<tr>
<td>SM</td>
<td>4.8 ± 0.5</td>
<td>7.4 ± 0.2</td>
<td>7.6 ± 0.6</td>
</tr>
<tr>
<td>PC</td>
<td>53.1 ± 1.1</td>
<td>50.0 ± 0.6</td>
<td>78.0 ± 5.9</td>
</tr>
<tr>
<td>PI</td>
<td>7.3 ± 0.5</td>
<td>6.3 ± 0.3</td>
<td>11.3 ± 1.7</td>
</tr>
<tr>
<td>PS</td>
<td>8.9 ± 0.2</td>
<td>9.0 ± 0.3</td>
<td>14.0 ± 0.9</td>
</tr>
<tr>
<td>PE</td>
<td>27.5 ± 0.7</td>
<td>28.4 ± 0.4</td>
<td>43.6 ± 4.3</td>
</tr>
<tr>
<td>Total</td>
<td>100.0</td>
<td>100.0</td>
<td>180.4 ± 27.6</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>27.3 ± 2.6</td>
<td>38.2 ± 2.0</td>
<td>48.9 ± 7.8</td>
</tr>
</tbody>
</table>

*Progenitors and immature oligodendrocytes in vitro were used for phospholipid and cholesterol determinations. OLP, A2B5+/NG2+ oligodendrocyte progenitors; OL, O1 immature oligodendrocytes; SM, sphingomyelin; PC, phosphatidylcholine; PI, phosphatidylinositol; PS, phosphatidylserine; PE, phosphatidylethanolamine.

1. The number (mean ± SEM) of phospholipid composition (mol %) are represented as the percentage of total phospholipids.

2. The number (mean ± SEM) of phospholipid content are represented as the content of each phospholipid per cell protein. Statistical differences were analyzed as compared with OLP.

3. $P < 0.005$.

4. $P < 0.025$ (Student’s t-test). n = number of independent experiments.
new avenues for understanding demyelination and remyelination in the brain.

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