**Prostacyclin Prevents Pericyte Loss and Demyelination Induced by Lysophosphatidylcholine in the Central Nervous System**

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**Background:** Pericyte damage is closely associated with the progression of neurodegenerative and neuronal dysfunction in the central nervous system (CNS).

**Results:** Prostacyclin attenuates pericyte damage following vascular barrier dysfunction in the adult CNS.

**Conclusion:** Prostacyclin therapy diminishes demyelination and neuronal deficits in pathophysiological conditions.

**Significance:** This study provides the first evidence that pericyte protection contributes to attenuate disease progression in adult CNS.

Vascular barrier dysfunction is a major anatomical feature in central nervous system (CNS) diseases, including multiple sclerosis, stroke, epilepsy, familial amyotrophic lateral sclerosis (1), and brain tumors (2). Because of an increase in vascular permeability, the CNS receives an influx of neurotoxic hemoglobin-derived products (3). Maintaining the integrity of the vascular barrier is therefore considered necessary for protection against expanding and exacerbating damage in CNS diseases (4).

Prostacyclin (prostaglandin I2) is a metabolite of arachidonic acid or prostaglandin endoperoxides and is released from vascular endothelial cells (9). The I-type prostaglandin receptor (IP3 receptor) is a G protein-coupled receptor that is primarily coupled to the activation of adenylate cyclase, which catalyzes the formation of 3',5' cyclic adenosine monophosphate (cAMP) (9), an enhancer of vascular barrier integrity (10). Expression of the IP receptor is reported on vascular smooth muscle and platelets and is involved in both vasorelaxants on smooth muscle and inhibition of platelet aggregation. Some drugs that target prostacyclin signaling have been approved for treatment of peripheral vascular diseases, including pulmonary arterial hypertension and arterial thrombosis. Concerning the role of prostacyclin in vascular barrier integrity, there have been

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**Notes:**

3 The abbreviations used are: IP, 1 type prostacyclin; BBB, blood-brain barrier; LPC, lysophosphatidylcholine; PDGFR, platelet-derived growth factor receptor; PFA, paraformaldehyde; Tuj1, class III β-tubulin; MBP, myelin basic protein; TEER, trans-endothelial electric resistance; LFB, luxol fast blue; GPR, G protein-coupled receptor; NS, not significant.
no reports that disrupting either prostacyclin or the IP receptor gene in mice leads to an abnormal vascular barrier. However, studies under pathological conditions reveal that after occlusion of the middle cerebral artery, a stable analog of prostacyclin inhibits brain edema that increases vascular permeability (11). One possible hypothesis for reducing edema formation with a prostacyclin analog is that prostacyclin signaling directly acts on endothelial cells and enhances endothelial barrier function (12). Moreover, it is known that vasodilatation is the result of relaxation of perivascular cells (e.g. vascular smooth muscle cells) wrapped around the endothelium. Therefore, perivascular cell activation by prostacyclin may also be involved in reducing edema formation. These observations prompted us to hypothesize that prostacyclin signaling in perivascular cells, such as pericytes, contributes to reducing capillary hydraulic permeability under pathological conditions in the adult CNS.

Lysoosphatidylcholine (LPC) is a bioactive proinflammatory lipid generated by hydrolysis of phosphatidylcholine via the action of phospholipase A₂. The majority of LPC formed in tissue is normally metabolized or reacylated; however, over-stimulation of phospholipase A₂ results in phosphatidylcholine breakdown and subsequent accumulation of LPC in the damaged tissue (13). Thus, accumulation of LPC is a recognized hallmark of pathological conditions and exacerbates pathological features. In the pathologically affected CNS, LPC production has been revealed by imaging mass spectrometry of experimental ischemic brain injury (14). Experimental accumulation of LPC into the CNS is known to cause neurodegeneration, which is characterized by demyelination (15, 16). Demyelination by LPC is understood to occur by phagocytosis (17) and direct changes of myelin-associated protein expression (18); however, other possible mechanisms have not been fully elucidated.

By studying the mouse spinal cord, we show that prostacyclin protects against LPC-mediated pericyte damage following vascular barrier dysfunction in the adult CNS. Intrathecal administration of a prostacyclin analog attenuates demyelination and motor deficits induced by LPC.

**EXPERIMENTAL PROCEDURES**

**Animals**—C57BL/6J mice and Wistar rats were obtained from Charles River Japan or Japan SLC. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Osaka University.

**Primary Cultures of Pericytes and Vascular Endothelial Cells**—Brain pericytes were prepared from cerebral cortices obtained from female rats 3 weeks postnatally as described previously with modifications (19). The cerebral cortices were digested in a mixture of 0.25% trypsin (Gibco) and DNase in phosphate-buffered saline (PBS) for 15 min at 37 °C followed by resuspension in DMEM containing 10% FBS. The isolated cells were plated on poly-l-lysine (Sigma)- precoated Lab-Tek 4-well chamber slides (Nalge Nunc International) at a density of 1 × 10⁶ cells/well.

**Primary Cultures of Microglia and Oligodendrocytes**—Microglia and oligodendrocytes were obtained from the brain of the postnatal day 1 rat or mouse. The tissues were digested in a mixture of 0.25% trypsin in PBS for 15 min at 37 °C followed by resuspension in DMEM containing 10% FBS. The isolated cells were plated on poly-l-lysine-precoated culture dishes in DMEM containing 10% FBS. One week after culturing, the dishes were rotated 20 times, and microglia were detached from the cell layer following centrifugation (300 × g, 5 min). Cell suspension was diluted with DMEM containing 10% FBS and plated on poly-l-lysine-precoated Lab-Tek 4-well chamber slides at a density of 5 × 10⁴ cells/well.

**Primary Culture of Cortical Neurons**—Cortical neurons were prepared from cerebral cortices of postnatal day 1 Wistar rats. The cerebral cortices were digested in a mixture of 0.25% trypsin (Gibco) and DNase in phosphate-buffered saline (PBS) for 15 min at 37 °C followed by resuspension in DMEM containing 10% FBS. The isolated cells were plated on poly-l-lysine-precoated culture dishes in DMEM containing 10% FBS. One week after culturing, the dishes were rotated 20 times, and microglia were detached from the cell layer following centrifugation (300 × g, 5 min). Cell suspension was diluted with DMEM containing 10% FBS and plated on poly-l-lysine-precoated Lab-Tek 4-well chamber slides at a density of 1 × 10⁵ cells/well.

**Primary Culture of Macrophages**—Macrophages were prepared from bone marrow of adult mice (female, 8 weeks)....
lected bone marrow cells were cultured in Roswell Park Memorial Institute 1640 medium containing 10% FBS and 50 ng/ml macrophage colony-stimulating factor (Sigma). After 1 day of cultivation, we used the cells for phagocytosis assay.

**Immunocytochemical Analysis**—After 24 h of culturing, cells were fixed with 4% paraformaldehyde (PFA) in PBS for 30 min at room temperature. After washing in PBS, cells were incubated with PBS containing 1% Triton X-100 and 5% BSA for 1 h at room temperature. Cells were incubated with primary antibodies for 2 h at room temperature and then incubated with fluorescently labeled secondary antibody for 1 h at room temperature. The primary antibodies used were as follows: rabbit anti-mouse IP receptor (Cayman Chemical, Ann Arbor, MI, catalog no. 160070, RRID, AB_10079417); goat anti-mouse CD13 (pericyte marker, 1:100; R&D Systems, catalog no. AF2335); biotinylated anti-mouse PDGFRβ (pericyte marker, 1:100; R&D Systems catalog no. BAF1042); mouse anti-class III β-tubulin (TuJ1, neuronal marker, 1:2000, Covance, catalog no. MMS-435P); goat anti-myelin basic protein (MBP, oligodendrocyte marker, 1:1000, Santa Cruz Biotechnology, catalog no. sc-13914); rabbit anti-β1a (microglia marker, 1:1000, Wako, catalog no. 019-19741); and rabbit anti-cleaved caspase-3 (1:250, Cell Signaling catalog no. 9661). Cells were incubated in the following secondary antibodies: Alexa Fluor 488 and Alexa Fluor 568 conjugated to isotype-specific antibodies (1:500, Invitrogen); streptavidin-conjugated Alexa Fluor 488 (1:500, Invitrogen). Nuclei were stained with a solution of DAPI at a final concentration of 1 μg/ml in PBS for 30 min at room temperature.

**Measurement of Electrical Resistance**—We used an in vitro BBB kit (PharmaCo-cell, catalog no. RBT-12), which is composed of rat brain vascular endothelial cells, pericytes, and astrocytes (20). To assess the vascular integrity, we measured the transendothelial electric resistance (TEER) across the upper and lower transwell chambers using ERS-2 (Millipore). Values were calculated relative to the TEER of the control at 0 h. To assess the effect of endothelial cells on the TEER value, we incubated with PBS containing 0.1% Triton X-100 and 10% swine serum (Vector Laboratories) for 1 h at room temperature. The sections were incubated with primary antibodies overnight at 4 °C and then incubated with fluorescent secondary antibodies for 2 h at room temperature. Th following antibodies were used: goat anti-mouse CD13 antibody (1:100; R&D Systems, catalog no. AF2335); rabbit anti-mouse CD31 (vascular endothelial cell marker, 1:100, Abcam, catalog no. ab28364); Alexa Fluor 488 and Alexa Fluor 568 conjugated to isotype-specific antibodies (1:500, Invitrogen); and streptavidin-conjugated Alexa Fluor 488 (1:500, Invitrogen).

In a separate set of studies, cryosections of 30 μm in thickness were blocked and permeabilized with 10% swine serum containing 0.1% Triton X-100. Sections were incubated with antibodies against rabbit anti-mouse cleaved caspase-3 and biotinylated anti-mouse PDGFRβ overnight at 4 °C and then incubated with Alexa Fluor 568 conjugated to anti-rabbit IgG (1:500, Invitrogen) and streptavidin-conjugated Alexa Fluor 488 (1:500, Invitrogen).

To visualize vascular endothelial cells using lectin, sections were incubated with DyLight 594-labeled *Lycopersicon esculentum* (tomato) lectin (vascular endothelial cell marker, 1:100; Vector Laboratories, catalog no. DL-1177) according to the manufacturer’s instructions. The length of lectin vasculature in the dorsal column of the spinal cord was measured using ImageJ software. The average length was calculated from three sections per sample.

**Surgical Procedure**—Anesthetized mice were subjected to laminectomy at thoracic level Th8 and injected with 2 μl of 1% (w/v) LPC (Sigma) dissolved in PBS over a 1-min period (0.5 mm, depth at the midline) into the dorsal column of the thoracic spinal cord. Pharmacological treatment started immediately after LPC injection. For administration of pharmacological reagents, an osmotic pump (model no. 1007D; ALZET Corp) was filled with vehicle solution (saline) or iloprost (20 mg/kg/day, Cayman Chemical). The delivery tube connected to an osmotic pump was placed close to the lesion just after LPC injection. The pump was implanted subcutaneously in the dorsal skin.

**Immunohistochemical Analysis**—One day after operation, mice were transcardially perfused with 4% PFA in PBS, and the spinal cords were postfixed for 24 h at 4 °C, followed by immersion in 30% sucrose/PBS for 24 h at 4 °C. Because the pericyte coverage varies depending on the level of the spinal cord (21), we carefully dissected the spinal cord from one segment rostral to one segment caudal to the lesion center (Th7-9). Spinal cord tissue was embedded in Tissue-Tek OCT compound (Sakura Finetek) and stored at −80 °C. We prepared cryosections of 30-μm thickness and mounted them on adhesive silane-coated slides (Matsunami Glass). Sections were dried in air at room temperature for 1 h. After washing in PBS, sections were incubated with PBS containing 0.1% Triton X-100 and 10% swine serum (Vector Laboratories) for 1 h at room temperature. The sections were incubated with primary antibodies overnight at 4 °C and then incubated with fluorescent secondary antibodies for 2 h at room temperature. Th following antibodies were used: goat anti-mouse CD13 antibody (1:100; R&D Systems, catalog no. AF2335); rabbit anti-mouse CD31 (vascular endothelial cell marker, 1:100, Abcam, catalog no. ab28364); Alexa Fluor 488 and Alexa Fluor 568 conjugated to isotype-specific antibodies (1:500, Invitrogen); and streptavidin-conjugated Alexa Fluor 488 (1:500, Invitrogen).

To evaluate the pericyte coverage of endothelial cells, the CD13- or PDGFRβ-positive area in the dorsal column of spinal cord was determined using the ImageJ area measurement tool and divided by the lectin-positive capillary area (20). The average ratio was calculated from three sections per sample.

To evaluate the pericyte damage, the number of cleaved caspase-3/PDGFRβ double-positive cells was counted and
divided by the number of PDGFRβ-positive cells. We calculated the average length from three sections per sample.

**Western Blot Analysis**—The tissues or cells were homogenized in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 0.1% SDS) containing protease inhibitors (Roche Applied Science). The lysates were clarified by centrifugation at 13,000 × g at 4 °C for 20 min, and the supernatants were collected and normalized for protein concentration. Proteins were separated by 8% SDS-PAGE and transferred onto polyvinylidene difluoride membranes (Immobilon-P, Millipore). After blocking with PBS containing 5% skim milk and 0.05% Tween 20, the membranes were incubated with primary antibodies.

For detection, a fluorescence-conjugated secondary antibody and an electronegated chemiluminescence system (GE Healthcare) were used. The membrane was exposed to an imaging system (LAS-3000, Fujifilm) according to the manufacturer's specifications. The protein bands were quantified using ImageJ 1.44p software. The following antibodies were used: mouse anti-α-tubulin (1:1000, Santa Cruz Biotechnology, catalog no. sc-5286); rabbit anti-occludin (1:1000, Invitrogen, catalog no. 71-1500); mouse anti-ZO-1 (1:1000, Invitrogen, catalog no. 33-9100); and mouse anti-claudin-5 (1:1000, Invitrogen, catalog no. 35-2500). Horseradish peroxidase-conjugated mouse IgG-specific (1:5000, Cell Signaling Technology, catalog no.7076S) and rabbit IgG-specific (1:5000, Cell Signaling Technology, catalog no.7074S) were used for secondary antibodies.

**Measurement of Evans Blue Leakage**—Evans blue dye (2% in saline, 4 ml/kg; Sigma) was injected intravenously into the tail vein in adult mice under anesthesia. One hour after the injection, mice were transcardially perfused with saline to remove the intravascular dye. The spinal cord tissues (T7-9) was placed on slides and fixed in 30% sucrose overnight at 4 °C. Low magnification images of cadaverine leakage were obtained using fluorescent microscopy (SZX-16, Olympus). To observe cadaverine leakages under high magnification, tissues were immersed in 30% sucrose/PBS for 24 h at 4 °C and embedded in Tissue-Tek OCT compound. We prepared cryosections with a thickness of 30 µm and performed immunohistochemical analysis using a rabbit anti-mouse CD31 antibody (1:100, Abcam, catalog no. ab28364).

**Kluver-Barrera Staining**—One day after the operation, mice were perfused with Bouin fixture containing 9% PFA and 7% picric acid. The spinal cord tissues were removed and postfixed with Bouin solution for 2 h. The samples were washed in 70% ethanol, followed by decolorization with 70% ethanol containing lithium carbonate. Subsequently, the samples were dehydrated in an ascending ethanol series and then embedded in paraffin (tissue preparation, T580, FALMA) through Clear Plus (FALMA). The paraffin-embedded tissues were cut into 7-µm thick sections using a microtome (RM2145, Leica), and sections were mounted on glass slides. After deparaffinization using Clear Plus, the slides were stained with 0.1% luxol fast blue (LFB) in distilled water at 60 °C overnight. The slides were decolorized using both 0.05% lithium carbonate in distilled water and 70% ethanol, followed by Nissl staining with 0.1% LFB in distilled water. After dehydration, coverslips were placed on slides and sealed with Entellan (Merck). All samples were analyzed using a light microscope (BX61; Olympus). The demyelinated area was calculated by subtracting the blue area from the total area in the dorsal horn of the spinal cord using ImageJ 1.44p software. The average demyelinated area was calculated from five samples per groups.

**Basso Mouse Scale Score**—Recovery of hindlimb motor function was scored by the Basso Mouse Scale open-field locomotor rating scale, which was developed specifically for mice, and it results in scores ranging from 0 (complete paralysis) to 9 (normal mobility) (22). The number of errors at each step was measured during 100 steps. Mice were observed individually for 2 min in an open field.

**Behavioral Data Recording**—Mice were allowed to freely explore the open field. In total, 50 steps were video recorded for each hindlimb (23). Scores were given to every step according to the flowing scheme: 0, normal step; 1, minor error (slight insecurity of foot placement); 2, major error (foot slipped completely from beam surface). The behavioral score is defined as the sum of the score of 50 steps.

**Phagocytosis Assay**—Phagocytosis activity was evaluated using a phagocytosis assay kit (IgG FITC) (Cayman Chemical) according to the manufacturer's instructions. Phagocytosis activity was measured by a plate reader (SpectraMax M2, Molecular Device).

**Data Analysis and Statistics**—The data are presented as the means ± S.E. The statistical analyses were performed with Student’s unpaired t tests, one-way analysis of variance with post hoc Tukey's tests, Scheffe’s tests, or two-way repeated measures analysis of variance. A value of p < 0.05 was considered as statistically significant.

**RESULTS**

**LPC Perturbs Vascular Barrier Integrity and Induces Pericyte Loss in Vitro**—We first investigated the effect of LPC accumulation on the vascular barrier in vitro; the in vitro BBB consisted of cultured purified astrocytes, brain capillary endothelial cells, and pericytes. To determine whether LPC compromises the integrity of the BBB, we treated these cells with various concentrations of LPC for 24 h and assessed the TEER. LPC treatment
significantly reduced the electrical resistance of the BBB (Fig. 1A). We next examined which cell types in the BBB are reactive to LPC. We cultured the cells in the presence of 50 μM LPC for 24 h and counted the number of endothelial cells, pericytes, and astrocytes. The number of pericytes was significantly reduced after LPC treatment for 24 h (Fig. 1, B and C). However, LPC did not change the number of endothelial cells or astrocytes compared with control groups (Fig. 1, B, D, and E). We confirmed that LPC treatment does not decrease the number of neurons and microglia (Fig. 1, F and G), whereas LPC treatment reduces the number of MBP+ oligodendrocytes (Fig. 1H). These results suggest that pericyte damage is involved in LPC-induced disruption of vascular barrier integrity.

**Iloprost Attenuates LPC-mediated Pericyte Loss and Vascular Barrier Dysfunction**—We next wished to assess whether prostacyclin signaling can mitigate LPC-induced pericyte dam-
Prostacyclin Prevents Pericyte Damage

We first prepared pericyte cultures obtained from rat brain and examined IP receptor expression on pericytes. Immunocytochemical analysis revealed IP receptor expression in PDGFRβ+ or CD13+ pericytes (Fig. 2A). To determine whether IP receptor activation modulates LPC-mediated pericyte damage, we pretreated pericytes with or without iloprost, an analog of prostacyclin, and then stimulated the cells with LPC. Immunocytochemical analysis revealed that LPC treatment increases the ratio of PDGFRβ+ caspase-3+ cells compared with the control and that iloprost treatment inhibits the LPC-mediated increase in the number of caspase-3+ pericytes (Fig. 2B). As a control, we found that

FIGURE 2. Iloprost rescues LPC-induced pericyte loss. A, representative images of cultured pericytes stained with antibody against the IP receptor. Upper panels, PDGFRβ (pericyte marker, green), IP receptor (red), and DAPI (blue); lower panels, CD13 (pericyte marker, green), IP receptor (red), and DAPI (blue). Scale bars, 50 μm. B, representative images of cultured pericytes stained with an antibody against PDGFRβ (pericyte marker, green) and cleaved caspase-3 (red). Pericytes were pretreated with or without iloprost (1 μM) for 30 min, and LPC was then added to the culture at a final concentration of 50 μM. Left panels, low magnification images. Right panels, high magnification images. Scale bars, 200 μm. Quantification of the ratio of pericytes positive for caspase-3 after 24 h of treatment with the indicated reagents. Values represent the mean ± S.E. of three independent experiments. **, p < 0.01 using Scheffé’s test. C, graph shows changes in the TEER in rat in vitro BBB model treated with LPC in the presence or absence of iloprost. n = 4–5. NS, not significant. D, graph shows changes in the TEER in rat vascular endothelial cells (solo-culture) treated with LPC in the presence or absence of iloprost. The ratio of TEER values was calculated based on the TEER value of control in the BBB kit. Values represent the mean ± S.E. of four independent experiments. E, Representative images of cultured oligodendrocytes treated with LPC and/or iloprost. Scale bar, 50 μm. Cells were stained with antibody against MBP (oligodendrocyte marker, green) and DAPI (blue). The graph shows the number of MBP+ cells after 24 h of treatment with the indicated reagents. Values represent the mean ± S.E. of three independent experiments. **, p < 0.01 using Scheffé’s test. F, representative images of cultured oligodendrocytes that were treated with LPC and/or iloprost. Scale bar, 50 μm. The graph shows the MBP expression in oligodendrocytes after 24 h of treatment with the indicated reagents. Values represent the mean ± S.E. of three independent experiments.
recently, we detected a significant difference in the MBP expression in oligodendrocytes is not affected by treatment with LPC and/or iloprost (Fig. 2F). These data indicate that prostacyclin signaling rescues LPC-mediated pericyte damage and consequently protects LPC-mediated vascular barrier dysfunction in vivo.

Prostacyclin Signaling Prevents LPC-mediated Vascular Barrier Disruption and Degradation of Tight Junction Proteins in Vivo—As our in vitro evidence positions prostacyclin signaling as an important player for vascular barrier integrity, we next studied in vivo effects of LPC and prostacyclin signaling. Specifically, we examined whether LPC disrupts vascular barrier integrity in the spinal cord of adult mice in vivo. We intravenously injected Alexa Fluor 555-conjugated cadaverine to observe the leakage of blood component to the CNS. Mice with LPC injection showed a fluorescent cadaverine signal in the spinal cord (Fig. 3A), suggesting that LPC injection leads to vascular barrier disruption in the spinal cord. To examine the role of prostacyclin in vascular barrier disruption, we intrathecaled administered iloprost or saline to mice immediately after LPC injection. Treatment with iloprost inhibited LPC-mediated cadaverine leakage into the spinal cord. Obser-
The degree of pericyte coverage, based on staining for CD13 or change in pericyte coverage after LPC injection. Immunohistochemical analysis showed that LPC causes the leakage of serum IgG through the vasculature in the spinal cord (Fig. 3A) and that iloprost treatment inhibits LPC-mediated IgG leakage into the spinal cord (Fig. 3A).

To quantitatively assess the disruption of vascular barrier integrity, we measured the volume of Evans blue dye in the spinal cord after LPC injection. In particular, we detected a large amount of Evans blue dye in the spinal cord tissue after LPC injection (Fig. 3B) and observed that Evans blue leakage in the spinal cord of mice was decreased by treatment with iloprost (Fig. 3B). Because the vascular barrier is maintained by the interendothelial tight junctions that form impermeable seals between the cells (4), we further analyzed whether LPC injection dephosphorylates tight junction proteins in the mouse spinal cord tissue. Western blot analysis revealed that LPC injection decreases the protein expression of claudin-5, occludin, and ZO-1 in the spinal cord tissue around the lesion site (Fig. 3, C and D). These findings support the result that LPC injection disrupts vascular barrier integrity. To investigate whether prostacyclin also prevents LPC-mediated vascular barrier disruption and decreased expression of tight junction-related proteins, we analyzed the expression of proteins related to tight junction formation in the spinal cord and found that iloprost treatment significantly prevented LPC-induced decreases of protein expression 1 day after the operation (Fig. 3, C and D). These data indicate a protective role of prostacyclin-mediated signaling in LPC-dependent disruption of the vascular barrier in the CNS.

Prostacyclin Signaling Prevents LPC-mediated Loss of Pericyte Coverage in the Spinal Cord—We next assessed whether prostacyclin inhibits pericyte loss in vivo. We investigated the change in pericyte coverage after LPC injection. Immunohistochemical analysis revealed that LPC injection decreases the degree of pericyte coverage, based on staining for CD13 or PDGFRβ on vascular endothelial cells (Fig. 4, A–C). Intrathecal administration of iloprost abolished the LPC-mediated reduction of pericyte coverage (Fig. 4, A–C), suggesting that iloprost treatment prevents LPC-mediated pericyte loss in the CNS. We examined the total length of lectin-positive neovessels in the sections and found that there were no significant differences between the groups (Fig. 3D). These results indicate that the change in pericyte coverage does not result from the loss of vascular endothelial cells.

Because LPC induces pericyte apoptosis in vitro, we examined whether LPC injection increases the number of caspase-3-positive pericytes in vivo. Mice that received LPC injection presented increased numbers of caspase-3-positive pericytes (Fig. 4, E and F). Intrathecal administration of iloprost inhibited the LPC-mediated increase in the number of caspase-3-positive pericytes. These results demonstrate that prostacyclin signaling reduces LPC-mediated pericyte damages.

DISCUSSION

LPC damages the peripheral vasculature by causing cytoskeletal changes in vascular endothelial cells and by inducing cell death in vascular smooth muscle cells (26). Although LPC injection into the adult CNS also disrupts the vasculature, this effect was attributed to immunocyte activation (17). However, our experiment using an in vitro BBB model suggests that LPC directly disrupts the vascular barrier independent of immune cells. More specifically, we found that LPC leads to pericyte apoptosis in vitro and also causes pericyte loss without disruption of vascular endothelial cells in vivo. These observations suggest that LPC at least partly acts directly on the vasculature, especially pericytes, and disrupts the vascular barrier. The signal transduction mechanism of LPC-mediated disruption of the vascular barrier and pericyte loss remains an open question. Ovarian cancer G protein-coupled receptor 1 (also known as G
Protein-coupled receptor-68 (GPCR), GPR4, and G2A have been identified as receptors for LPC. Although GPR4 is critically involved in the impairment of endothelial barrier function by LPC (27), it is still unknown which receptor subtypes are associated with the LPC-mediated pericyte death. Further studies are required to identify the receptor and the downstream signal transduction of LPC-mediated pericycle damage.

Under pathological conditions in the CNS, intraventricular treatment of the stable analog of prostacyclin has neuroprotective effects by increasing blood flow, reducing platelet aggregation, and directly protecting neurons (28, 29). In addition, we previously revealed that prostacyclin treatment directly enhanced axon regeneration and remyelination in the injured adult CNS (30, 31). Designing therapies that enhance prostacyclin signaling is thus considered beneficial for CNS disorders, and clarifying the effect of prostacyclin on the homeostasis of CNS vasculature is required to ensure the therapeutic potential of prostacyclin. This study describes a previously unidentified role of prostacyclin that can potentially preserve the vascular integrity so often undermined in the pathological CNS. We should note that endogenous prostacyclin expression is considered abundant in the vascular endothelium, and this context provides the possibility that constitutive expression of prostacyclin in endothelial cells supports the homeostasis of the vasculature. This hypothesis is consistent with our previous findings that prostacyclin secreted by neovessels acts on IP receptors on both injured axons (30) and oligodendrocyte precursor cells (31) by facilitating axon regeneration and remyelination in the injured spinal cord. The novel aspects of the present findings are as follows: prostacyclin protects against pericycle...
damage but prostacyclin does not have play a protective role in oligodendrocytes at the start of demyelination.

Recent studies have shown multiple functions of pericytes, including BBB formation and maintenance, vascular stability and angio-architecture, regulation of capillary blood flow, and clearance of toxic cellular by-products necessary for normal functioning of the CNS (3–8). Pericyte degeneration is identified in neurodegenerative disorders such as sporadic amyotrophic lateral sclerosis (32) and Alzheimer disease (33). Although evidence for the protective role of pericytes on CNS homeostasis in humans is incomplete, a study using an Alzheimer disease model mouse demonstrated that pericyte loss leads to progression of an Alzheimer disease-like neurodegeneration pathogenic cascade (34), suggesting a direct link between pericyte loss and the progression of neurodegeneration. With regard to other pericyte functions that are not limited to vascular-related events, it has been demonstrated that pericytes can sense inflammation and promote leukocyte transmigration from the vessel and behave as a stem cell population capable of commitment to neuronal cells (35).

Another role of pericytes under pathological states is a tissue repair response to CNS injury, the scar tissue that forms at injuries seals lesions in the CNS. Blocking pericyte generation failed to seal the injured tissues, showing that pericytes are

FIGURE 5. Treatment with iloprost prevents LPC-mediated demyelination and progression of motor dysfunction. A, representative thoracic spinal cord cross-sections with Klüver-Barrera staining (myelin staining) 1 day after surgery. Scale bars, 500 μm for left panel and 200 μm for right panel. B, graph shows demyelinated areas in the dorsal horn of the spinal cord. The demyelinated area was calculated by subtracting the blue area from the total area in the dorsal horn of the spinal cord. Values represent means ± S.E. (control: n = 3; LPC: n = 4; LPC + iloprost: n = 4). **, p < 0.01 compared with control; #, p < 0.01 compared with LPC using Scheffe’s test. C and D, graphs show the score of behavioral motor recording (C) and Basso mouse scale score (D) in mice injected with LPC into the spinal cord treated with or without iloprost. Values represent means ± S.E. (control: n = 6; LPC: n = 7; LPC + iloprost: n = 7). **, p < 0.01; *, p < 0.05 compared with LPC using Scheffe’s test. E and F, graphs show the ratio of the phagocytic activity of macrophages (E) and microglia (F) treated with LPC and/or iloprost. Values represent the mean ± S.E. of three independent experiments.
involved in the formation of spinal cord scar tissue (36). Thus, pericytes contribute to tissue connection and repair of the injured spinal cord. Continued search for the molecular cues that prevent pericyte loss in CNS diseases may ultimately lead to the discovery of new therapies to control pericyte loss and the progression of neurodegeneration.

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