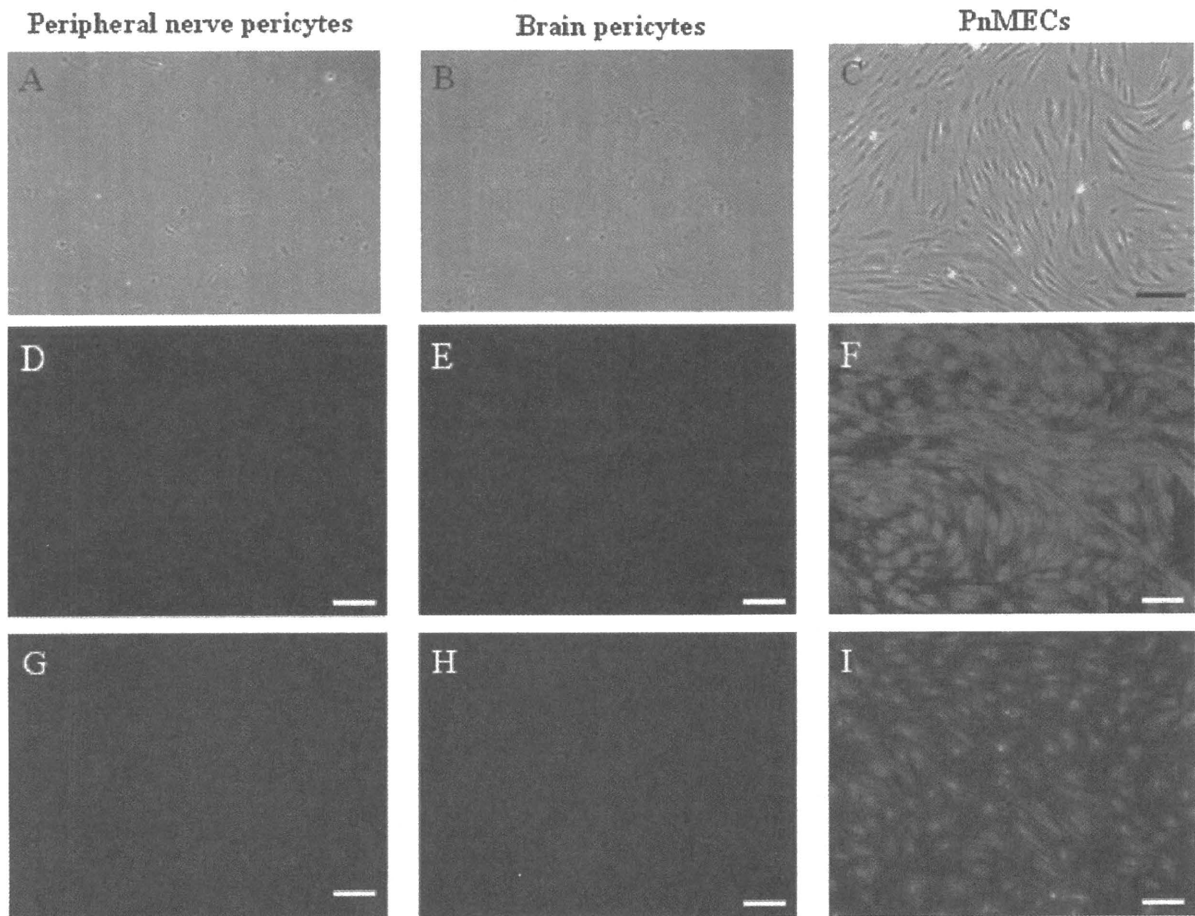


Fig. 1. A phase microscopic image of human peripheral nerve pericytes (A,D,G), brain pericytes (B,E,H), and PnMECs (C,F,I). Pericytes appeared to have ruffled-border morphology (A,B). Anti-vWF antibody did not stain peripheral nerve (D) or brain pericytes (E). PnMECs were used as a positive control (F). No uptake of Dil-Ac-LDL was observed in peripheral nerve (G) or brain pericytes (H). PnMECs were used as a positive control (I). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Paracrine growth factor and nerve growth factor secreted from the brain and peripheral nerve pericytes

The expression levels of the mRNA of pericyte paracrine growth factors such as *Ang-1*, *TGF-β1*, *VEGF*, and *bFGF* in the isolated pericytes are shown in Figure 3A.

In addition, the expression of Ang-1, TGF-β1, VEGF, and bFGF proteins in these pericyte cell lines was determined by a Western blot analysis (Fig. 3B). The Ang-1, TGF-β, VEGF, and bFGF bands, corresponding to 60-, 25-, 38-, and 16-kDa single bands, respectively, were detected in the brain and peripheral nerve pericytes, and astrocytes. The Ang-1, TGF-β, and bFGF bands, corresponding to this band, were not detected in PnMECs. Interestingly, the levels of Ang-1 (Fig. 3C), TGF-β (Fig. 3D), and bFGF (Fig. 3F) proteins in peripheral nerve pericytes were significantly higher than those in brain pericytes ($P < 0.01$; Fig. 3C,D,F). Their levels were almost equivalent to those in astrocytes. In contrast, the expression of VEGF protein did not show a significant difference between these cell lines (Fig. 3E). This study also investigated whether these pericyte cell lines expressed neurotrophic factors to promote nerve regeneration. The mRNA expression levels of nerve growth factors such as *GDNF*, *NGF*, and *BDNF* were determined in brain and peripheral nerve pericytes and shown in Figure 4A. These *GDNF* and *NGF* were not detected in PnMECs (Fig. 4A). Interestingly, the *GDNF* (Fig. 4B) and *NGF* (Fig. 4C) mRNA expression levels in peripheral nerve pericytes were significantly higher than those in brain pericytes ($P < 0.01$). Their levels were almost equivalent to those in astrocytes. Furthermore, the *BDNF* (Fig. 4D) mRNA expression level in the brain and peripheral nerve pericytes was also significantly higher than that in PnMECs ($P < 0.01$) and it was also equivalent to that in astrocytes. In addition, the expression of GDNF and BDNF proteins in the isolated pericytes was determined by a Western blot analysis (Fig. 4E). The GDNF and BDNF bands, corresponding to 15- and 14-kDa single bands, respectively, were detected in the brain and peripheral nerve pericytes, and astrocytes (Fig. 4E). The GDNF and BDNF bands were not detected in PnMECs. Interestingly, the levels of GDNF (Fig. 4F) and BDNF (Fig. 4G) protein in peripheral nerve pericytes were significantly higher than those in brain pericytes ($P < 0.01$) and astrocytes ($P < 0.01$). The change in *GDNF*, *BDNF*, and *NGF* mRNA in peripheral nerve pericytes after treatment with TNF-α (100 ng/ml) was measured using relative quantification with real-time RT-PCR (Fig. 4H–J). The *GDNF* (Fig. 4H), *BDNF* (Fig. 4I), and *NGF* (Fig. 4J) mRNA expression levels in the peripheral nerve pericytes significantly increased after treatment with TNF-α ($P < 0.01$).

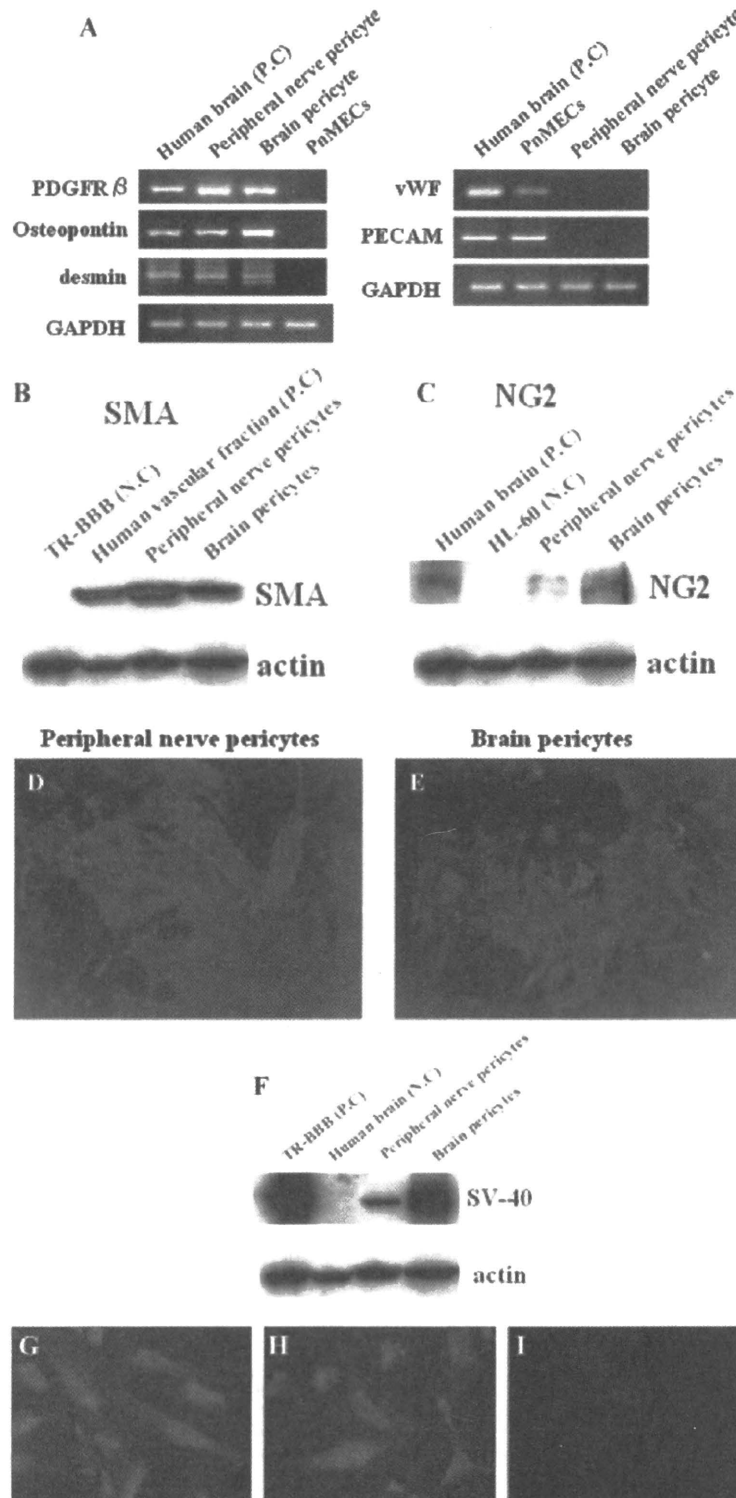


Fig. 2. A: The expression of pericyte markers, including PDGF-Rβ, osteopontin, and desmin, and of vWF and PECAM as endothelial cell markers by RT-PCR analysis. Human brain tissue was used as a positive control, and PnMECs were used as a negative control. Each mRNA expression level was normalized with respect to the GAPDH mRNA expression. A Western blot analysis of αSMA (B) and NG2 (C) proteins in pericyte cell lines. Human vascular fraction and human brain tissue were used as positive controls, and TR-BBB and HL-60 cells were used as negative controls. Immunocytochemistry using anti-αSMA antibody against peripheral nerve (D) and brain pericytes (E,F) The expression of a large SV40 T-antigen in peripheral nerve and brain pericytes cultured at 33°C. TR-BBB cultured at 33°C was used as a positive control and human brain tissue was used as a negative control. G,H,I: The presence of nuclear and perinuclear hTERT was confirmed by immunocytochemistry using anti-hTERT antibody in peripheral nerve (G) and brain (H) pericyte cell lines. HUVEC was used as a negative control (I). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

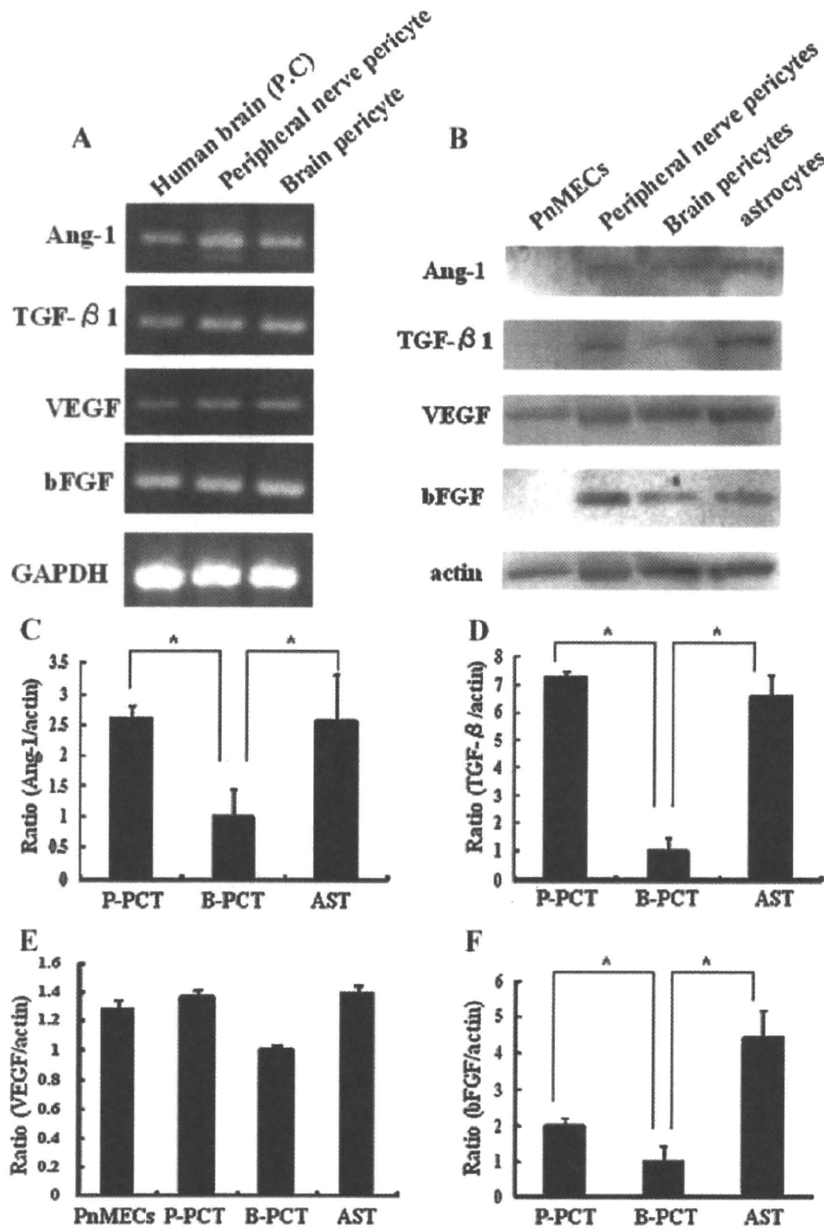


Fig. 3. A: The expression of the paracrine growth factors *Ang-1*, *TGF-β*, *VEGF*, and *bFGF* by RT-PCR analysis. Human brain tissue was used as a positive control. Each mRNA level was normalized with respect to the expression of *GAPDH* mRNA. Human brain served as a positive control (P.C). B: A Western blot analysis of Ang-1, VEGF, TGF-β, and bFGF in peripheral nerve and brain pericyte, PnMECs, and astrocyte. The Ang-1, TGF-β, VEGF, and bFGF bands, corresponding to 60-, 25-, 38-, and 16-kDa single bands, respectively, were detected in the brain and peripheral nerve pericytes, and astrocytes. The Ang-1, TGF-β, and bFGF proteins corresponding to this band were not detected in PnMECs. C–F: The bar graph reflects the combined densitometry data from three independent experiments (mean ± SEM, n = 3, $^*P < 0.01$). B,C: The Ang-1, TGF-β, and bFGF protein levels in peripheral nerve pericytes were significantly higher than those in brain pericytes (mean ± SEM, n = 3, $^*P < 0.01$).

The change of TEER and transendothelial permeability of PnMECs by pericyte-conditioned medium

The TEER and permeability for paracellular diffusion of [carboxyl-C^{14}]-inulin across the layer of PnMECs or TY08 in response to treatment with NCM, BPCT-CM, and PPCT-CM (Fig. 5A–E) was measured to determine whether soluble factors secreted by pericytes strengthen the barrier function of endothelial cells in the BBB or BNB. The TEER value of TY08 was not changed after treatment with fibroblast (FB)-CM, but significantly increased after incubation with AST-CM or BPCT-CM ($P < 0.01$; Fig. 5A). There was no significant difference in the TEER value after treatment of TY08 with AST-CM and BPCT-CM. When cultured with BPCT-CM or PPCT-CM, the TEER value of PnMECs was also significantly elevated ($P < 0.01$) in comparison to those treated with NCM, although it was not changed after exposure of FB-CM (Fig. 5B). The transfilter co-culture of TY08 with brain pericytes or astrocytes could

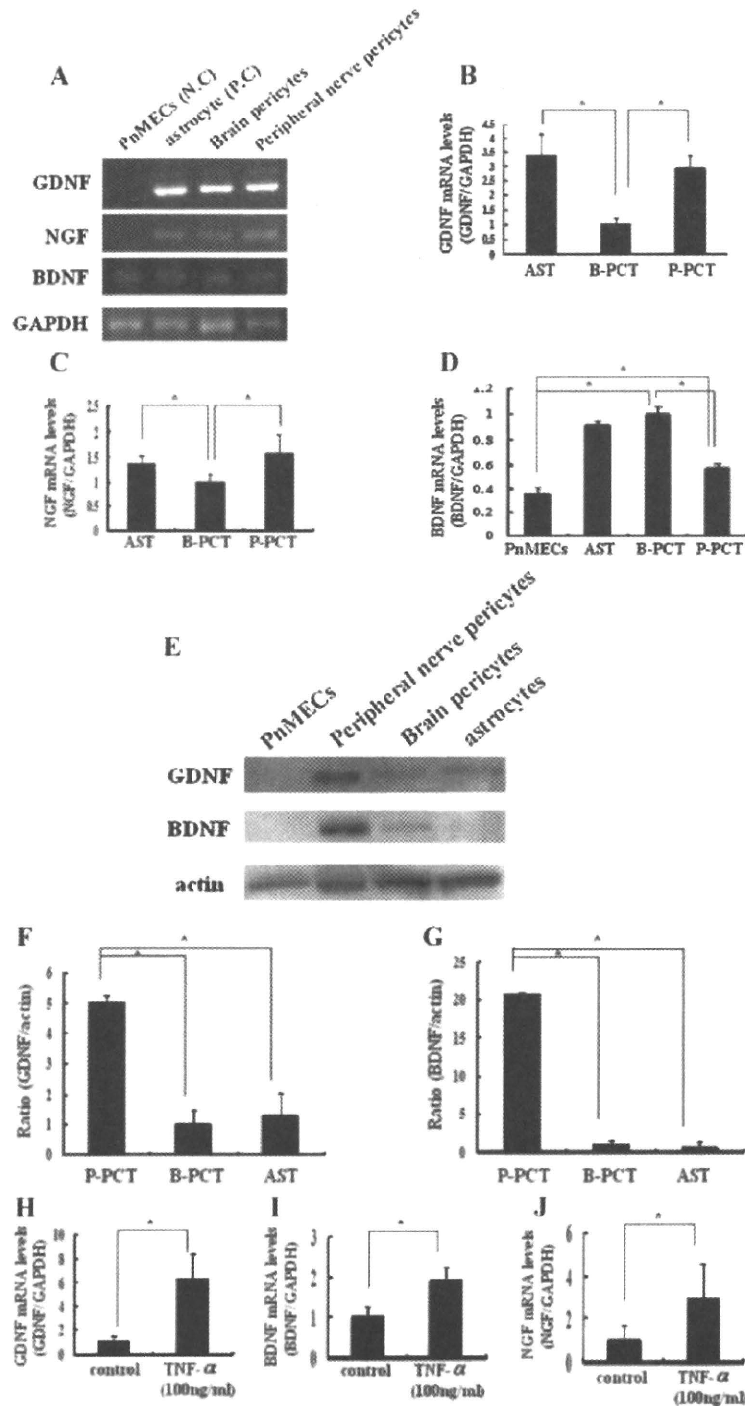


Fig. 4. A: The expression of paracrine growth factors, including *GDNF*, *NGF*, and *BDNF*, by RT-PCR analysis. Human brain tissue was used as a positive control. The mRNA expression levels were normalized with respect to the expression of *GAPDH* mRNA. The expression of *GDNF* and *NGF* mRNA was not detected in PnMECs. Astrocytes served as a positive control (P.C) and PnMECs as a negative control (N.C). B–D: *GDNF*, *NGF*, and *BDNF* mRNA levels were quantified in brain pericytes, peripheral nerve pericytes, PnMECs, and astrocytes by real-time RT-PCR expressed as the ratio of target gene/GAPDH. B,C: The *GDNF* and *NGF* mRNA expression levels in peripheral nerve pericytes were equivalent to those in astrocytes and significantly higher than those in brain pericytes (mean ± SEM, n = 3, $^*P < 0.01$). D: The *BDNF* mRNA expression levels in brain and peripheral nerve pericytes were also equivalent to that in astrocytes and significantly higher than those in PnMECs (mean ± SEM, n = 3, $^*P < 0.01$).
E: A Western blot analysis of GDNF and BDNF protein in peripheral nerve and brain pericytes, PnMECs, and astrocytes. The GDNF and BDNF bands, corresponding to 15- and 14-kDa single bands, respectively, were detected in the brain and peripheral nerve pericytes, and astrocytes. F,G: The bar graph reflects the combined densitometry data from three independent experiments (mean ± SEM, n = 3, $^*P < 0.01$). The GDNF and BDNF protein levels in peripheral nerve pericytes were significantly higher than those in brain pericytes and astrocyte (mean ± SEM, n = 3, $^*P < 0.01$). The GDNF and BDNF proteins corresponding to this band were not detected in PnMECs. H–J: The *GDNF*, *BDNF*, and *NGF* mRNA expression levels in peripheral nerve pericytes were significantly increased after treatment with TNF-α ($P < 0.01$).

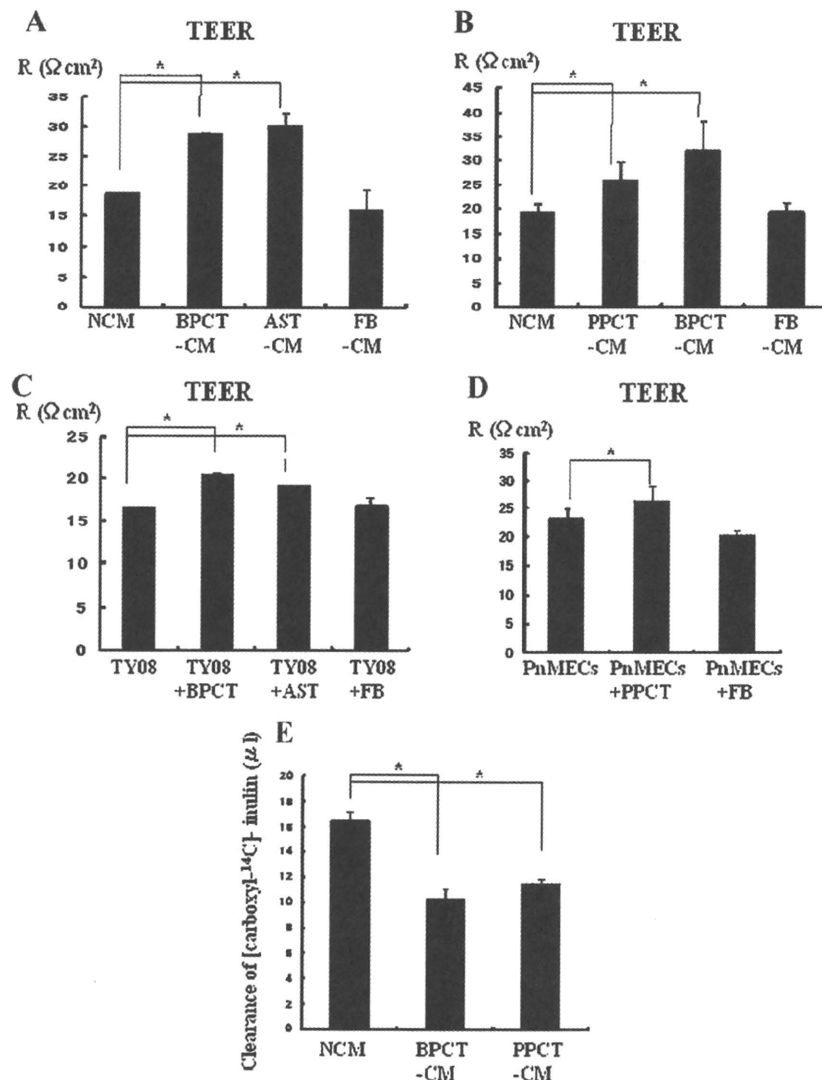


Fig. 5. **A: The effect of brain pericytes and astrocytes-conditioned media on the TEER values across TY08 monolayer (mean ± SD, n = 6 in each condition). The TEER value of TY08 was not changed after treatment with fibroblast (FB)-CM, but significantly increased after incubation with AST-CM and BPCT-CM ($P < 0.01$). B: The effect of brain and peripheral nerve pericyte-conditioned media on the TEER values across PnMEC monolayer (mean ± SD, n = 6 in each condition). The TEER values of PnMECs were significantly increased when cultured with brain or peripheral nerve pericyte-conditioned media. C: The effect of co-culture on induction of TEER in TY08 with brain pericytes or astrocytes. High TEER was observed in the co-culture of TY08 with brain pericytes or astrocytes in comparison to endothelial cell monolayer ($P < 0.01$). The TEER value of TY08 was not changed by co-culture with fibroblasts. D: The effect of co-culture on induction of TEER in PnMECs with peripheral nerve pericytes. The TEER value of PnMECs was not changed after co-culture with fibroblast, but significantly increased after co-culture with peripheral nerve pericytes ($P < 0.01$). E: The [carboxyl-^{14}C]-inulin clearance of the PnMEC monolayer treated with brain or peripheral nerve pericyte-conditioned media at 30 min (mean ± 6 in each experiment). PnMECs demonstrated a significantly lower inulin clearance when cultured with brain or peripheral nerve pericyte-conditioned media ($^*P < 0.01$). NCM, Non-conditioned medium was prepared by the same procedure using DMEM with 10% FBS; BPCT-CM, conditioned medium of brain pericytes; PPCT-CM, conditioned medium of peripheral nerve pericytes; AST-CM, conditioned medium of astrocytes; FB-CM, conditioned medium of fibroblasts. TY08, Monoculture of TY08; TY08 + BPCT, co-culture of TY08 with brain pericytes; TY08 + AST, co-culture of TY08 with astrocytes; TY08 + FB, co-culture of TY08 with fibroblasts; PnMECs, monoculture of PnMECs; PnMECs + PPCT, co-culture of PnMECs with peripheral nerve pericytes; PnMECs + FB, co-culture of PnMECs with fibroblasts.**

significantly increase the tightness of endothelial monolayers ($P < 0.01$; Fig. 5C). High TEER was observed in the co-culture of PnMECs with peripheral nerve pericytes in comparison to endothelial cell monolayer ($P < 0.01$; Fig. 5D). The TEER values of TY08 and PnMECs were unchanged by co-culture with fibroblasts in comparison to the single culture (Fig. 5C,D). Furthermore, PnMECs treated with BPCT-CM or PPCT-CM demonstrated significantly lower inulin clearance than those treated with NCM ($P < 0.01$; Fig. 5E).

Induction of claudin-5 and occludin in PnMECs following treatment with the pericyte-conditioned medium

Changes in the expression of *claudin-5* and *occludin* mRNA in PnMECs following treatment with NCM, BPCT-CM, and PPCT-CM were examined to determine whether soluble factors secreted from pericytes up-regulate the expression of tight junctional molecules (Fig. 6A,B). The *claudin-5* and *occludin*

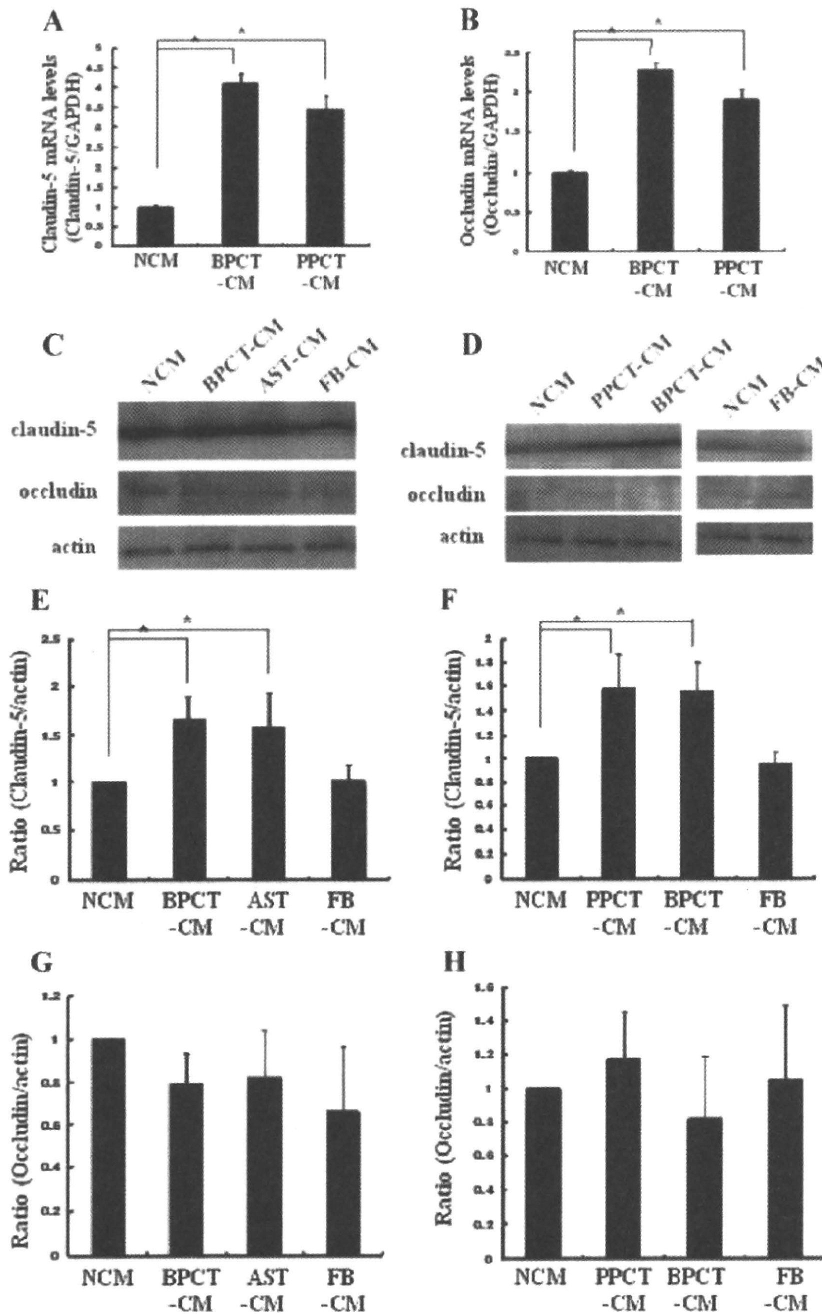
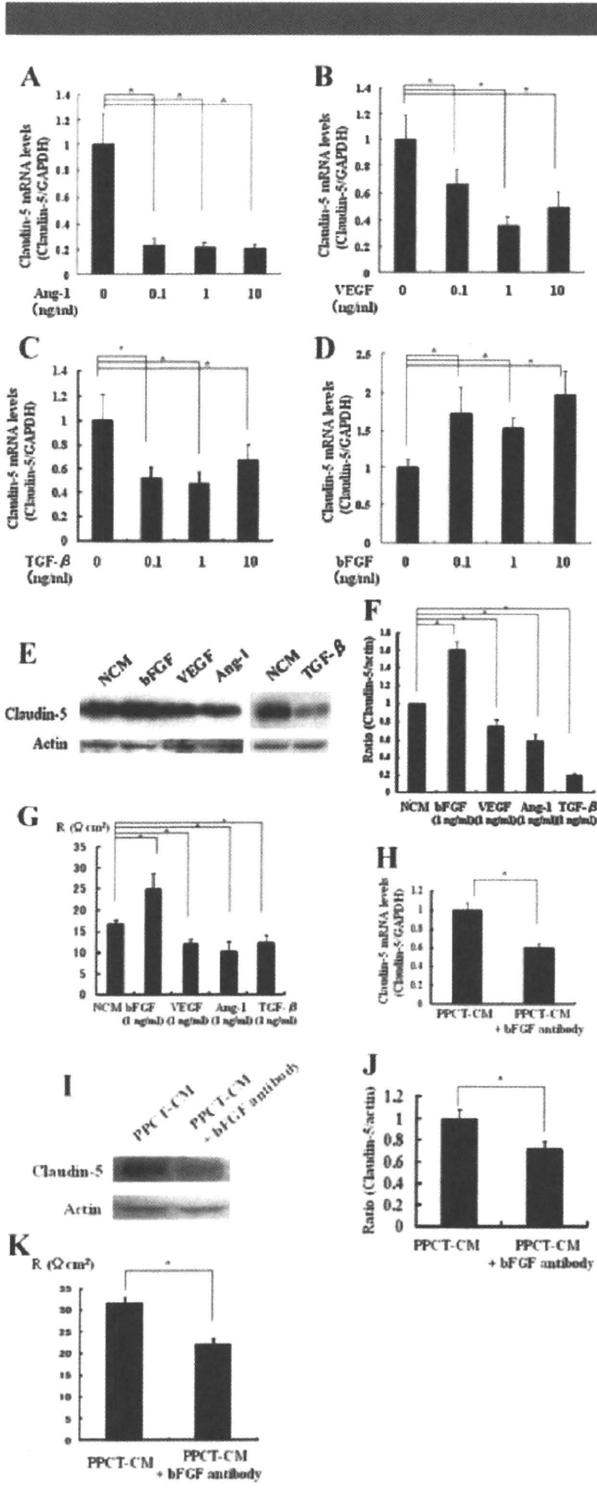


Fig. 6. Effect of brain and peripheral nerve pericyte-conditioned media on the mRNA expression of claudin-5 (A) and occludin (B) by real-time RT-PCR expressed as the ratio of target gene/GAPDH. The expression of claudin-5 and occludin in PnMECs was significantly increased when cultured with brain or peripheral nerve pericyte-conditioned medium. The change of claudin-5 (C) and occludin (D) expression in TY08 or PnMECs treated with BPCT-CM, PPCT-CM, AST-CM, or FB-CM. E–H: The bar graph reflects the combined densitometry data from three independent experiments (mean ± SEM, n = 3, *P < 0.01). E: Claudin-5 of TY08 was not changed following treatment with FB-CM, but those of TY08 was induced after incubation with AST-CM or BPCT-CM (P < 0.01). F: The expression of claudin-5 of PnMECs was significantly increased when cultured with BPCT-CM or PPCT-CM, although it was unchanged after application with FB-CM (mean ± SEM, n = 3, *P < 0.01). G,H: The expression of occludin in TY08 or PnMECs was unchanged following treatment with BPCT-CM, PPCT-CM, or AST-CM. NCM, Non-conditioned medium was prepared by the same procedure using DMEM with 10% FBS; BPCT-CM, conditioned medium of brain pericytes; PPCT-CM, conditioned medium of peripheral nerve pericytes; AST-CM, conditioned medium of astrocytes; FB-CM, conditioned medium of fibroblasts.

mRNA expression levels in PnMECs were increased significantly after treatment with BPCT-CM or PPCT-CM ($P < 0.01$; Fig. 6A,B). In addition, the change in claudin-5 and occludin proteins in TY08 and PnMECs treated with NCM, BPCT-CM, PPCT-CM, AST-CM, and FB-CM was determined by a Western blot analysis (Fig. 6C,D). Claudin-5 of TY08 was not changed following treatment with FB-CM but was induced after incubation with AST-CM or BPCT-CM ($P < 0.01$)

(Fig. 6E). There was no significant difference in the amount of claudin-5 after treatment of TY08 with AST-CM and BPCT-CM. When cultured with BPCT-CM or PPCT-CM, the expression of claudin-5 of PnMECs was significantly increased (Fig. 6F). In contrast, occludin was not affected following treatment with BPCT-CM, PPCT-CM, or AST-CM (Fig. 6G,H).



Regulation of claudin-5 by Ang-1, VEGF, TGF-β, and bFGF and the effect of anti-bFGF antibody on the induction of claudin-5 mRNA in PCT-CM-treated PnMECs

The effects of 24 h treatment of Ang-1, VEGF, TGF-β, and bFGF on the expression of claudin-5 mRNA in PnMECs were investigated to determine which soluble factors secreted from pericytes strengthened barrier function in the BBB and BNB. The *claudin-5* mRNA level was significantly reduced following treatment with Ang-1, VEGF, and TGF-β in a dose-dependent manner (Fig. 7A–C). Conversely, *claudin-5* mRNA was increased following treatment with bFGF (Fig. 7D). Furthermore, claudin-5 protein in PnMECs following treatment with Ang-1 (1 ng/ml), VEGF (1 ng/ml), TGF-β (1 ng/ml), and bFGF (1 ng/ml) was quantified using a Western blot analysis (Fig. 7E). Claudin-5 protein was significantly increased after treatment with bFGF ($P < 0.01$; Fig. 7E,F). In contrast, claudin-5 protein was significantly reduced following treatment with VEGF, Ang-1, or TGF-β ($P < 0.01$; Fig. 7E,F). The TEER value of PnMECs by treatment with bFGF was significantly higher ($P < 0.01$) in comparison to those treated with NCM (Fig. 7G). Conversely, the TEER value of PnMECs was significantly reduced following treatment with VEGF, Ang-1, or TGF-β (Fig. 7G). To clarify the contribution of bFGF to the induction of claudin-5 in PnMECs by PPCT-CM, bFGF activities were neutralized using anti-bFGF antibody. The *claudin-5* mRNA expression was inhibited by 42% after incubation with PPCT-CM pretreated by anti-bFGF antibody (Fig. 7H). In addition, claudin-5 protein in PnMECs following treatment with anti-bFGF neutralizing antibody was quantified using a Western blot analysis (Fig. 7I,J). Claudin-5 protein expression was inhibited after pretreatment with anti-bFGF neutralizing antibody (Fig. 7I,J). The TEER value of PnMECs was also significantly reduced following treatment with PPCT-CM pretreated with anti-bFGF neutralizing antibody (Fig. 7K).

Fig. 7. The *claudin-5* mRNA level after a 24-h application of VEGF, Ang-1, TGF-β, and bFGF in PnMECs. A–D: The *claudin-5* mRNA levels in PnMECs were quantified by real-time RT-PCR and expressed as the ratio of target gene/GAPDH. Data are presented as the mean (±SEM) of three independent PCR runs. *P*-values were calculated using unpaired *t*-test. The *claudin-5* mRNA level was significantly reduced following treatment with Ang-1, VEGF, or TGF-β in a dose-dependent manner. However, the claudin-5 mRNA level was increased following bFGF treatment. E: The effect of VEGF (1 ng/ml), Ang-1 (1 ng/ml), TGF-β (1 ng/ml), and bFGF (1 ng/ml) on the claudin-5 protein level in PnMECs after a 3-day treatment. F: The bar graph reflects the combined densitometry data from three independent experiments. G: The effect of VEGF, Ang-1, TGF-β, and bFGF on the TEER values across the PnMEC monolayer (mean ± SD, n = 6 in each condition). The TEER values of PnMECs were significantly increased when cultured with bFGF (1 ng/ml). In contrast, the TEER values of PnMECs were significantly reduced after treatment with VEGF, Ang-1, or TGF-β. H: The effect of anti-bFGF neutralizing antibody on the *claudin-5* induction following treatment with PPCT-CM. The PnMECs were cultured with PPCT-CM or PPCT-CM pretreated with bFGF antibody for 24 h. The level of *claudin-5* mRNA in PnMECs was assayed by real-time RT-PCR and expressed as the ratio of the target gene/GAPDH. The *claudin-5* mRNA was inhibited by 42% following PPCT-CM pretreated with anti-bFGF antibody in comparison to that treated with only PPCT-CM. I: The change in the level of claudin-5 protein in PnMECs following treatment with anti-bFGF neutralizing antibody using a Western blot analysis. Claudin-5 protein was inhibited after pretreatment with anti-bFGF neutralizing antibody. J: The bar graph reflects the combined densitometry data from three independent experiments. K: The TEER value of PnMECs was significantly reduced by the treatment of PPCT-CM pretreated with anti-bFGF antibody in comparison to that treated with PPCT-CM. NCM, Non-conditioned medium was prepared by the same procedure using DMEM with 20% FBS; PPCT-CM, conditioned medium of peripheral nerve pericytes; PPCT-CM + bFGF antibody, conditioned medium of peripheral nerve pericytes pretreated with bFGF neutralizing antibody.

Discussion

This study successfully established brain and peripheral nerve pericyte cell lines of human origin. Various human immortalized brain endothelial cell lines have been successfully established (Weksler et al., 2005) including ours, and one brain and peripheral nerve endothelial cell line has recently been developed (Sano et al., 2007, 2010). However, neighboring pericytes have long been ignored as barrier-forming cells. Pericytes are an important component of the BBB and BNB and participate in the maintenance of vascular stability (Hellstrom et al., 2001) and in the supply of some cytokines and growth factors to endothelial cells in paracrine manner (Armulik et al., 2005). Therefore, the human BBB- or BNB-derived pericyte cell lines, combined with BBB- or BNB-derived endothelial cells, could shed a novel light on the future studies of BBB and BNB.

Pericyte are morphologically, biochemically, and physiologically heterogeneous and they may have distinctive characteristics in different organs (Armulik et al., 2005). Pericytes are localized at the abluminal side of the microvascular endothelium and are completely enveloped by a basement membrane (Shepro and Morel, 1993). Pericytes increase vascular stability (Hellstrom et al., 2001) and regulate the BBB by secretion of paracrine growth factors (Armulik et al., 2005). However, the molecular mechanisms by which pericytes regulate the barrier function of the BBB are unknown. The BBB comprised endothelial cells, astrocytes, and pericytes of microvascular origin, whereas the BNB comprised endothelial cells and pericytes of endoneurial microvascular origin (Poduslo et al., 1994; Sano et al., 2007). Astrocytes strengthen the barrier function of BMECs via the secretion of soluble factors in the in vitro BBB model (Hori et al., 2004; Kim et al., 2006). The current study also showed that brain pericytes as well as astrocytes have property of increasing barrier integrity of BMECs through claudin-5 up-regulation. Unlike the BBB, the BNB lacks cells that correspond to astrocytes. We therefore hypothesized that peripheral nerve pericytes, which are the only cells composing in endoneurial microvessels other than PnMECs, might strengthen the barrier function of the BNB and play a similar role as that of astrocytes in the BBB. Astrocytes have been reported to regulate the BBB by secretion of paracrine growth factors such as TGF-β, VEGF, and bFGF (Abbott et al., 2006; Kim JH et al., 2006). The current study initially analyzed whether these factors are also secreted by brain and peripheral nerve pericytes. These results demonstrated that brain and peripheral nerve pericytes also expressed several soluble factors such as Ang-1, VEGF, TGF-β, and bFGF, which are secreted by astrocytes in the BBB (Abbott et al., 2006; Fig. 3A,B). In particular, the level of Ang-1, TGF-β, and bFGF in peripheral nerve pericytes was significantly higher than those in brain pericytes and PnMECs and were equivalent to those in astrocytes (Fig. 3C,D,F). Peripheral nerve pericytes secrete these soluble factors and might exert a beneficial effect on endothelial cells maintenance in BNB, thus playing a role similar to that of astrocytes in the BBB.

Pericytes could possibly strengthen the barrier properties of PnMECs by secreting several soluble factors because pericyte-conditioned media significantly increase the TEER value and decrease inulin clearance in PnMECs (Fig. 5B,D,E). It is well known that claudin-5 is a major component of TJs, and the expression level of claudin-5 is important for TJ maintenance in the mature BBB (Nitta et al., 2003). Ohtsuki et al. (2007) reported that exogenous expression of claudin-5 induces barrier properties in cultured rat brain capillary endothelial cells line which do not express claudin-5. Several reports previously demonstrated that the expression of claudin-5 was increased by humoral factors such as adrenomedullin (Honda et al., 2006) and bFGF (Bendfeldt et al., 2007), or reduced by VEGF (Argaw et al., 2009). The current results demonstrated that claudin-5 expression of PnMECs was not changed after incubation with fibroblasts-conditioned media but significantly increased by treatment with pericyte-conditioned media (Fig. 6A,D,F), thus suggesting that the soluble factors secreted from pericytes affect the barrier property of the endothelial cells in a paracrine manner through the up-regulation of claudin-5 in vivo. Furthermore, this study investigated which soluble factors secreted from pericytes increased claudin-5 expression. These results indicated that the barrier properties of PnMECs in the BNB were augmented by bFGF, and then decreased by VEGF, Ang-1, or TGF-β released from pericytes through change of claudin-5 (Fig. 7A,B,D,E,G–J). Recently, the breakdown of the BNB has been considered to be a key initial step in many autoimmune neuropathies such as Guillain–Barré syndrome and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP) (Lach et al., 1993; Kanda et al., 1994, 2000, 2004). Kanda et al. (2004) reported that the number of claudin-5-positive microvessels in the BNB in cases of CIDP was significantly decreased in comparison to that of patients with non-inflammatory neuropathies. This finding suggests that the modification of the integrity of tight junctions in the BNB may provide novel therapeutic avenues for many autoimmune peripheral neuropathies. The present findings demonstrated that the regulation of these soluble factors secreted from pericytes might have therapeutic potential in repairing and modifying the barrier properties of the BNB in autoimmune peripheral neuropathies.

Next, this study was the first to demonstrate that brain and peripheral nerve pericytes expressed several neurotrophic factors including NGF, GDNF, and BDNF (Fig. 4A,E). The inflammatory mediator TNF-α plays a key role in the pathological processes of Guillain–Barré syndrome and CIDP (Radhakrishnan et al., 2004; Deng et al., 2008; Yang et al., 2008). TNF-α also induces cell-specific damage to Schwann cells in vitro (Boyle et al., 2005) and thus contributes to the development of inflammatory neuropathy. The current study demonstrated that the expression of *GDNF* (Fig. 4H), *BDNF* (Fig. 4I), and *NGF* (Fig. 4J) mRNA in peripheral nerve pericytes was significantly increased after exposure of TNF-α. The physiological role of TNFα in the BNB is unclear, but these results suggested that neurotrophic factors secreted from the peripheral nerve pericytes might have a neuroprotective effect against axonal loss mediated by TNF-α in inflammatory neuropathies such as Guillain–Barré syndrome and CIDP. Interestingly, the GDNF and BDNF protein in peripheral nerve pericytes were significantly higher than those in brain pericytes and astrocytes (Fig. 4F,G). Many studies have shown that astrocytes produce neurotrophic factors such as GDNF and BDNF, which protect against neuronal loss in the central nervous system (Mizuta et al., 2001; Wang et al., 2002). Neurotrophic factors secreted from peripheral nerve pericytes might prevent axonal loss and promote axonal regeneration in the peripheral nervous system (PNS). Although neurotrophic factors such as GDNF, BDNF, and NGF cannot be used for neuroprotection following intravenous administration because these proteins do not cross the BNB, the neurotrophic factors secreted from peripheral nerve pericytes in the endoneurial space may be useful for neuroprotection in the PNS. The modification of these neurotrophic factors released from pericytes may have therapeutic potential for intractable peripheral neuropathies.

In conclusion, BBB or BNB-derived pericytes modify BNB functions through various soluble factors. The regulation of soluble factors secreted from pericytes may thus provide novel therapeutic strategies to modify the BNB functions and promote peripheral nerve regeneration. Further research is thus necessary to elucidate the characteristics of pericytes because knowledge concerning the molecular mechanisms by which pericytes regulate BBB and BNB function under both

physiological and pathological conditions could lead to the development of new therapies for various neurological disorders including diabetic neuropathy and stroke.

Acknowledgments

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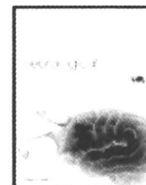

A serological analysis of viral and bacterial infections associated with neuromyelitis optica

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ABSTRACT

To evaluate the role of infections in the development of neuromyelitis optica (NMO), 19 patients positive for anti-aquaporin-4 antibody were screened for 24 viral and bacterial infections. Serological evidence of recent viral infection was found in 7 of 15 patients screened during the acute phase of the neurologic illness, which was a significantly more frequent rate of infection than seen in the control group of 33 patients with neurodegenerative, metabolic, or vertebral diseases (47% *versus* 15%). Mumps virus and human herpes viruses were the frequent causal agents, although there was no statistical difference in frequency between the two groups. Most patients with identified recent infection had monophasic or recurrent myelitis without evidence of optic nerve involvement and small number of total clinical relapses. Disease history tended to be shorter in patients with identified recent infection than those without, and an expanded long spinal cord lesion in magnetic resonance imaging was rarely found in patients with identified recent infection, although statistical significance could not be shown. These findings indicate that, not single, but various viral infections, can be associated with the development of NMO during the early stages of the illness, although the exact pathogenesis of NMO has yet to be clarified.

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1. Introduction

Neuromyelitis optica (NMO) is a disease entity that has recently been distinguished from multiple sclerosis (MS) based on clinical and immunological characteristics, especially the presence of serum anti-aquaporin-4 (AQP4) IgG antibody [1]. Although anti-AQP4 antibody appears to play the pathogenic role in the development of NMO, the exact pathogenesis of NMO remains unknown. Case analyses have demonstrated that in the Mayo Clinic, 25% of patients with monophasic or relapsing NMO had antecedent viral illness and that 15% of patients in Italy with relapsing NMO had a history of fever or infectious disease within four weeks before their clinical attack [2,3]. These findings strongly suggest that acute infections are related to the onset and the relapse of NMO, at least in a part of the patients.

Single case reports have suggested that human cytomegalovirus (CMV), varicella-zoster virus (VZV), Epstein–Barr virus (EBV), hepatitis A virus, human immunodeficiency virus (HIV), dengue virus, *Mycoplasma pneumoniae*, and *Mycobacterium tuberculosis* infections are related to the development of NMO and anti-AQP4 antibody-related myelitis [4–11]. However, there has been no comprehensive analysis of infections in NMO. We previously reported a case of NMO, in which chronic infection with human T lymphotropic virus type 1 (HTLV-1) was also evident in serum and cerebrospinal fluid analyses [12]. T cells infected by retroviruses, including HTLV-1, can induce polyclonal B cell activation [13], indicating the possibility that chronic HTLV-1 infection is a risk factor for the development of NMO. In this study, we serologically screened for various viral and bacterial infections in patients with NMO and related conditions to identify antecedent infectious agents, as well as chronic persistent infections, associated with risk for NMO development.

2. Patients and methods

2.1. Patients

Nineteen patients with various neurological deficits (median age, 53 [range, 16–71]; male/female, 2/17), all of whom were seen at the Yamaguchi University Hospital and judged positive for anti-AQP4 IgG antibody in a cell based assay [14], were included as patients with "NMO-related conditions." Their clinical diagnoses were as follows: NMO fulfilling proposed diagnostic criteria [15] ($N = 10$), monophasic or recurrent myelitis ($N = 7$), monophasic optic neuritis ($N = 1$), and medial longitudinal fasciculus syndrome followed by acute myelitis

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($N=1$). Five (26%) of the 19 patients with NMO-related conditions had been treated with interferon β-1b ($N=3$) or oral low dose prednisolone ($N=2$) to prevent the recurrence of neurological deficit at the time of sampling, whereas the other 14 received neither immunosuppression nor immunomodulation therapies. Serum samples were taken within one month after the onset or recurrence of the disease in 15 of the 19 patients, all of the 15 patients did not receive the treatments for the acute attack (high dose steroid or plasmapheresis) before blood sampling. A control group of patients (median age, 59 [range, 17–77]; male/female, 20/13), who were receiving neither immunosuppression nor immunomodulation drugs, were those with neurodegenerative, metabolic, or vertebral diseases ($N=33$). Informed consent was provided by all participants for serological analyses.

2.2. Infectious serology

Serum antibodies or antigen for 24 viral and bacterial infections were screened using the following methods: virus-specific IgM antibodies (for herpes simplex virus [HSV], VZV, CMV, EBV [against viral capsid antigen], parvovirus B19, rubella virus, measles virus, and mumps virus) by commercial enzyme-linked immunosorbent assay (ELISA) kits (Denka Seiken, Tokyo, Japan); IgG/IgM/IgA antibodies specific for *Campylobacter jejuni* and *Haemophilus influenzae* by the ELISA systems established for determination of antecedent infection in Guillain–Barré syndrome and its clinical variants [16]; *M. pneumoniae* (Serodia-Mycon II test kit, Fujirebio, Tokyo, Japan) and HTLV-1 (SRL, Inc, Tokyo, Japan) by particle agglutination assays; adenovirus, influenzae viruses A and B, respiratory syncytial virus, and rotavirus by complement fixation assays (SRL, Inc, Tokyo, Japan); human parainfluenza viruses A/B/C by hemagglutination inhibition assays (SRL, Inc, Tokyo, Japan); *Treponema pallidam* by hemagglutination assay; and hepatitis B virus (surface antigen), hepatitis C virus, and HIV by chemi-luminescent immunoassays.

2.3. Data analysis

Differences in frequency of infections between the groups were analyzed using the Fisher exact test. Differences in medians were examined by the Mann–Whitney *U* test. All statistical analyses were performed using SPSS 12.0 J software (SPSS Inc., Chicago, IL). Differences were considered significant for 2-sided *P* values less than 0.05.

3. Results

Statistical analysis did not identify any agent which was significantly more common in the group of patients with NMO-related conditions compared to the control group (Table 1). However, virus-specific IgM antibodies, which indicate recent infection, were seen in 7 (47%) of 15 patients with NMO-related conditions in whom serum samples were taken within 1 month after the onset or recurrence of the disease, which was significantly more common than in the control group (5 [15%] of 33; $P=0.03$; odds ratio = 4.9; 95% confidence interval = 1.2–19.7). None of the 4 patients in whom sera were taken during the remission phase of the illness had virus-specific IgM antibodies ($P=0.54$; compared to the control group). The most frequent infectious agent in patients with anti-AQP4 antibody was mumps virus ($N=3$; 20%). In 1 of the 3 patients with serological evidence of mumps virus, mumps-specific IgM antibody was confirmed to have disappeared within 3 months after the neurological attack. Viruses identified in the 4 other patients positive for IgM antibodies were human herpes viruses (HSV, VZV, EBV, and CMV), although 2 of these patients were each concomitantly positive for two viruses (HSV and VZV, or HSV and EBV) and the other 2 each for VZV and CMV. No patients were positive for anti-HTLV-1 antibody, except for the case we have previously reported [12]. Antibody titer against influenza B virus was elevated (16 or more) in 2 (13%) of the 15 patients with acute NMO-related conditions, which was more frequent than in the control group (1 of 32 subjects; 3.1%), although the difference did not reach significance ($P=0.24$).

The clinical diagnoses of all 3 patients with mumps-specific IgM antibody (26-year-old female, 53-year-old female, and 59-year-old male) were recurrent myelitis without other neurological deficits, including optic neuritis, and these patients had low Expanded Disability Status Scores (0.0, 2.0, and 3.0, respectively). Similarly, 2 of the 4 patients with IgM antibodies against human herpes viruses (median

Table 1
Infectious serology in the patients with neuromyelitis optica (NMO)-related conditions.

Agents	Methods	NMO-related conditions: Total ($N=19$)	NMO-related conditions: Acute phase[a] ($N=15$)	Controls[b] ($N=33$)	*P* value[c]
Acute infections					
Herpes simplex virus	ELISA (IgM)	2 (11%)	2 (13%)	1 (3.0%)	0.23
Varicella-zoster virus	ELISA (IgM)	2 (11%)	2 (13%)	0	0.09
Cytomegalovirus	ELISA (IgM)	1 (5.3%)	1 (6.7%)	1 (3.0%)	0.53
Epstein–Barr virus	ELISA (VCA-IgM)	1 (5.3%)	1 (6.7%)	1 (3.0%)	0.53
Parvovirus B19	ELISA (IgM)	0	0	NE	
Rubella virus	ELISA (IgM)	0	0	NE	
Measles virus	ELISA (IgM)	0	0	NE	
Mumps virus	ELISA (IgM)	3 (16%)	3 (20%)	2 (6.1%)	0.31
Campylobacter jejuni	ELISA (IgG/IgM/IgA)	0	0	NE	
Haemophilus influenzae	ELISA (IgG/IgM/IgA)	0	0	NE	
Mycoplasma pneumoniae	PA	1 (5.3%)	1/14 (7.1%)	2 (6.1%)	1.0
Chronic infections					
HTLV-1	PA	1 (5.3%)[d]	1 (6.7%)[d]	NE	
Hepatitis B	CLIA (HBsAg)	0/12	0/10	NE	
Hepatitis C	CLIA (Ab)	1/12 (8.3%)	1/10 (10%)	NE	
Human immunodeficiency virus	CLIA	0/12	0/10	NE	
Treponema pallidum	HA (TPHA)	1/12 (8.3%)	1/10 (10%)	NE	

HTLV-1 = human T lymphotropic virus type 1; NE = not examined; Ag = antigen; Ab = antibody;
ELISA = enzyme-linked immunosorbent assay; PA = particle agglutination;
CLIA = chemi-luminescent immunoassay; HA = hemagglutination
[a] Serum samples available within 1 month after the onset or the recurrence of the neurological disease.
[b] Patients with neurodegenerative, metabolic, or vertebral diseases.
[c] Two-tailed *P* value (acute NMO-related conditions *vs* controls).
[d] Previously reported case[12].

age, 52 years; all females) were diagnosed with myelitis (monophasic or recurrent) without optic neuritis, and only 1 received a clinical diagnosis of NMO. In contrast, 6 (75%) of 8 patients without virus-specific IgM antibodies received a clinical diagnosis of NMO, significantly more frequent than the 7 patients with virus-specific IgM antibodies ($P = 0.04$; odds ratio = 2.9; 95% confidence interval = 1.3–255.7).

The number of total clinical attacks at time of blood sampling was significantly smaller in the 7 patients with NMO-related conditions who were positive for virus-specific IgM antibodies (median, 2; range, 1–4) than in those who were negative (median, 4; range, 1–8; $P = 0.02$). Similarly, the median time from the onset of disease to serum sampling was 11 months (range, 16 days to 3 years) in the patients with the IgM antibodies, which was shorter than the median of 4 years (range, 14 days to 16 years) in those without the antibodies, although the difference did not reach significance ($P = 0.07$). An expanded long spinal cord lesion (3 or more vertebral segments in length) seen on spinal magnetic resonance imaging (MRI) was somewhat less common in patients with virus-specific IgM antibodies (50%, 3 of 6 patients in whom MRI was performed) than in those without (75%, 6 of 8 patients) ($P = 0.33$). Other neurological features, cerebrospinal fluid data (cell counts and protein level), and brain imaging did not differ between the groups of patients.

4. Discussion

This study is the first to comprehensively screen for infections in patients with NMO and its related conditions. Results revealed serological evidence of acute viral infections in about half of the patients with anti-AQP4 antibody detected during an acute phase of a neurological attack. The patients with acute infections were characterized by short disease history and acute myelitis without evidence of optic nerve involvement, which is an incomplete neurological presentation that does not satisfy the criteria for the diagnosis of NMO. By contrast, there were several patients in whom recent infection was not identified, and they commonly met the neurological and radiological criteria of NMO, and had long disease histories. These findings indicate that acute viral infections could be associated with the development of NMO during an early stage of the illness, whereas this seems not to be true in the late stages of the illness.

Various infectious agents, including EBV, have been suggested as triggers of the onset of MS, but there has not been enough evidence to draw conclusions [17]. The difficulty in collecting sufficient evidence may be due to infrequent antecedent infectious symptoms, complex pathogenesis including both genetic and environmental etiologies, and, in particular, the fact that MS is a heterogeneous disorder. In contrast, the high prevalence of serum anti-AQP4 IgG antibody indicates that NMO and its related conditions are relatively homogenous disorders, urging us to examine the possible contribution of specific infectious agents to the development of the disease. In the present study, immunosuppression/modulation therapies were given only in 5 (26%) of the 19 patients with NMO-related conditions before the serum sampling. Therefore, the close association of NMO-related conditions with acute virus infections does not appear to be due to the preceding therapies.

We identified the mumps virus, an enveloped RNA virus in the paramyxovirus family, as the most frequent agent of infections related to acute attacks of NMO-related illness. This study did not intend to investigate whether there was a history of antecedent infectious symptoms, and retrospective analysis of the patients' records found no patients who reported any antecedent symptoms, including parotid gland swelling. However, one third of patients who are infected by mumps virus are asymptomatic, and often in symptomatic patients, the symptoms are not specific, resembling upper respiratory tract infections [18]. In general, the elevation of virus-specific IgM antibody titers indicates acute primary viral infection or acute reactivation, and in this serological study we measured the IgM antibodies for screening mumps and some other virus infections. It is also known that several patients have persistent IgM antibodies specific for viruses including mumps virus and human herpes viruses, for a long time after infection, even after 3 years [19,20]. In this study, however, IgM anti-mumps antibody in 1 patient with NMO-related condition was confirmed to have disappeared 3 months after the neurological attack. Moreover, the commercially available ELISA system we used in this study had very low seropositive rate (0.3%) in 336 healthy persons, and was confirmed to show that anti-mumps IgM antibody had disappeared within 5 months after the acute infection in 14 patients with mumps-related parotitis [21]. We therefore believe that close association of NMO-related conditions with mumps virus was not due to limited methodology in this serological study.

Mumps virus infection can be accompanied by various neurological disorders, including encephalitis, sensorineural deafness, facial palsy and Guillain–Barré syndrome [18]. Interestingly, a case report of a 10-year-old boy developing mumps during acute myelitis also described a very long spinal cord lesion (from C3 to T12) on MRI, which is characteristic of NMO, although anti-AQP4 antibody was not mentioned [22]. Moreover, the optic chiasma was primarily perturbed in a case of acute optic neuritis following mumps virus infection [23]. Although this paper did not mention the presence of anti-AQP4 antibody, this clinical picture suggests that this patient also had an anti-AQP4 antibody-related disorder [24]. A population-based case-control study did not reveal the association of histories of mumps infection and measles–mumps–rubella vaccination with risk for MS [25], but these histories, as well as antecedent mumps infection, should be clarified in patients with anti-AQP4 antibody-related disorders.

A healthy patient positive for anti-AQP4 antibody for more than 10 years was reported to have suffered a clinical attack of acute myelitis following skin eruption suggestive of viral infection [26]. This case report raises the possibility that, in patients with NMO, viral infections play a role in increased blood–brain barrier permeability, which allows the autoantibody to cross the blood–brain barrier, although we have no data regarding leakage from blood to cerebrospinal fluid in the patients included in this study. In contrast, it remains unclear whether viral infection can trigger NMO-associated autoimmunity. In general, viruses can trigger autoimmunity through molecular mimicry and its adjuvant effects during the initiation of disease, and can promote autoimmune responses through bystander activation with or without antigen spreading [17]. Several cases have been reported in which mumps infection appeared to precede the onset of diabetes mellitus type 1, and it has been hypothesized that infection with mumps virus may induce autoimmunity via increased release of IL-1 and IL-6 and upregulated expression of HLA molecule in mumps virus-infected pancreatic beta cells [27]. Our study showed that the patients identified with acute viral infection had a short disease history of the illness, and therefore we might hypothesize that acute viral infection activates the immune system, causing initiation of autoimmunity, and thereby the development of NMO and its related disorders.

Conflict of interest statement

The authors report no conflicts of interest.

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≪トピックス≫
血液脳関門・血液神経関門

神田　隆*

要旨

- 近年，MRI の普及により血液脳関門の破綻が目にみえる形で呈示されるようになったことと，血液脳関門での炎症細胞浸潤を直接的にコントロールする薬物である natalizumab の出現により，自己免疫性神経疾患における血液脳関門についての関心は飛躍的に増したといってよい．
- 本稿では，まず血液脳関門・血液神経関門の基本的な構造とその分子背景について簡単に言及したあとに，血液脳関門・血液神経関門の破綻と自己免疫性神経疾患の病態について，最近得られた知見を中心に概説する．
- 血液脳関門・血液神経関門は単なる障壁ではない．神経系に対する物質透過を選択するシステムであり，また，新たな治療法の標的でもある．

はじめに

循環器系の最終目的は，組織での物質交換である．血液成分から組織への必要物質の移行と老廃物の回収を能率よく円滑に行うため，多くの組織での微小血管内皮細胞は有窓であり，多数の pinocytotic vesicle を有して細胞間・細胞内の物質往来を自由にしている．一方，体内の限られた部位においては，微小循環系構成内皮細胞は隣接する細胞間で tight junction を構成し，細胞間の物質の自由な通過を制限している．pinocytotic vesicle は極端に少なく，細胞内を経由する物質移送もむずかしくなっている．これを blood-tissue barrier といい，blood-testis barrier，blood-retinal barrier など体内には多数のバリアーシステムが存在する．

本稿の主題である血液脳関門(blood-brain barrier：BBB)と血液神経関門(blood-nerve barrier：BNB)は，いずれも神経系の内部環境を維持するうえできわめて重要な構造物である．中枢神経系のバリアーシステムは，① 脳実質内の微小血管内皮細胞，② くも膜上皮細胞，③ 脈絡叢上皮細胞の3ヵ所に局在しており，通常 BBB という名称を用いる際は ① を意味する．一方，末梢神経系のバリアーシステムは，④ 末梢神経実質内の微小血管内皮細胞と ⑤ 神経周膜の最内層の2ヵ所に局在し，BNB という名称は ④，⑤ のどちらにも用いられる．

“関門”または“バリアー”という語感からは，ただ単に物質の透過を妨害する装置(すなわち壁)という印象を受けがちである．しかしながら，たとえば中枢神経系で多量を必要とするグルコースを

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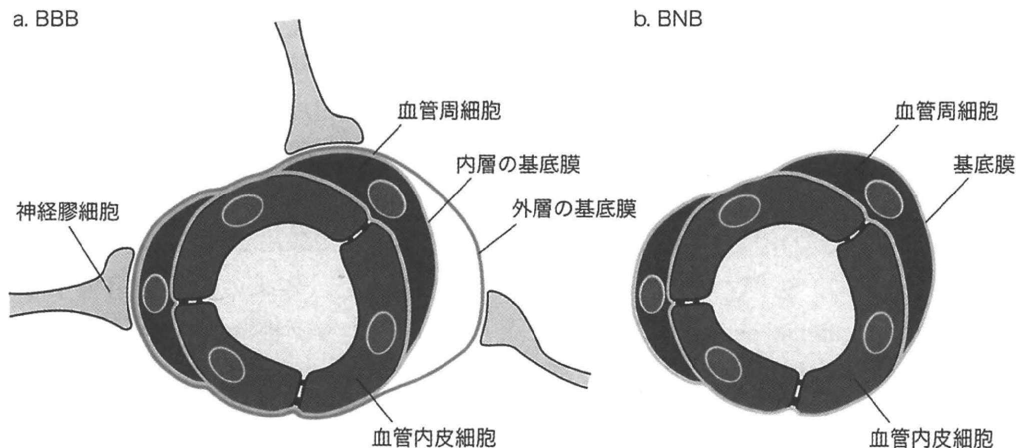

Fig. 1. BBBとBNBの基本構造

とってみると，BBBはD-glucoseのみを特異的に血管内から中枢神経実質へと移送し，その光学異性体であるL-glucoseに対する透過性はほとんど0に等しい．ここで例にあげた選択的なD-glucoseの脳内移行は，BBBを構成する内皮細胞に局在するglucose transporter type 1(GLUT1)が司っており，このような選択的物質透過はグルコース以外にも多数の物質で観察される．したがって，BBB/BNBは単なる障壁ではなく，神経系に対する物質透過を選択するシステムと捉えるのが妥当と考えられる．

近年急速に普及したMRIは，多発性硬化症(MS)の早期診断や活動性の評価を可能とし，“BBBの破壊”という現象を簡単かつ明瞭にわれわれの前に提示してくれるようになった．MSは中枢神経系髄鞘を標的とする自己免疫疾患であり，その病因論が病的T細胞の免疫原性を中心に論じられてきたのはある意味では当然というべきかもしれない．しかし，natalizumabなどBBB機能を直接コントロールする薬物が第一線の臨床で使われている今，病的T細胞の脳内侵入・増殖からMSの発症や増悪にいたる過程は，BBBの存在を抜きに議論ができない．また，Guillain-Barré症候群や慢性炎症性脱髄性多発神経炎(CIDP)などの自己免疫性末梢神経疾患も，“BNBの破綻”をキーワードとして論じられる機会が増えてきている[1]．

本稿では，BBB/BNBの概要について現在得られている知識を整理するとともに，自己免疫性神経疾患の病態・治療を考えるヒントになる最近の成果を紹介することとする．ただし，現時点ではBNB固有の知見はきわめて少なく，多くはBBBまたは一般臓器の微小血管をもとにした研究で得られた成果であることを最初にお断りしておく．

BBB/BNBの構造◯

BBBとBNBの基本構造をFig. 1に示す．BBBは脳の毛細血管に存在し，管腔に面した内層から順に血管内皮細胞(青色)，血管周細胞(赤色)，神経膠細胞(黄色)の3種類の細胞から成る．末梢神経系には神経膠細胞に相当する細胞はなく，BNBは血管内皮細胞と血管周細胞の2者から構成される．

実際のバリアーとしての機能をもちバリアーの本体として働くのは，最内層で血液成分と直接接触する血管内皮細胞であるが，BBBでは，同細胞がバリアーとして機能するためには神経膠細胞を欠くことができないことが，古くから指摘されていた．現在，血管内皮細胞のバリアー機能維持には血管周細胞と神経膠細胞から放出される液性因子が必須であることが明らかになっており，とくに近年，血管周細胞の神経系バリアーシステムで

の役割[2]に注目が集まっている．血管周細胞は血管内皮細胞と非常に近接した位置に存在し，両者は共通の基底膜(水色)に覆われている．BBBではその外側にもう一枚基底膜があり(黄緑色)，そこに神経膠細胞の足突起(黄色)が接する．

BBB/BNBを構成する血管内皮細胞は，①タイトジャンクションをもち，強固な細胞間接着によって細胞間経路(paracellular pathway)を遮断する，②pinocytotic vesicleが極端に少ないため，細胞内経路(transcellular pathway)が制限される，③特殊なtransporterを介した物質流入系，排出系が存在する，の3点において一般末梢臓器の内皮細胞と異なっている．

①，③の分子的背景については近年目覚ましい進歩がある．タイトジャンクションを構成する蛋白は次々に明らかになっており，BBBではclaudin-5とoccludin，とくに前者がタイトジャンクションの強度を決定する因子としてもっとも重要と考えられている．神経膠細胞に由来するVEGF-Aはclaudin-5の発現を低下させ，BBB破綻に導く[3]．③のバリアー特異的transporterについては後述する．

内皮細胞と白血球の接着プロセス――炎症細胞はどのようにしてBBB/BNBを乗り超えるか○

中枢神経・末梢神経実質への白血球の浸潤にいたるまでのプロセスとそれぞれに関与する分子は，基本的には一般臓器への白血球浸潤機序と大きな違いはない．大きく分けて①緩やかな接着(rolling)，②強固な接着(attachment)，③内皮下への遊走(migration)の三つの連続した過程があり，①にはselectin familyが，②にはVLA-4/VCAMとLFA-1，Mac-1/ICAMの二つの接着系が，③にはPECAMのほかIL-8，CD99，MCP-1，MMP-9などの多数の分子が関与している．IL-1β，TNF-α，IFN-γなどの炎症性サイトカインは，いずれもこれらの接着因子の発現に参画して炎症細胞浸潤に寄与する[4]．

いったん侵入した炎症細胞は再び局所で炎症性サイトカインをparacrineに放出し，さらなる接着因子発現の増強を引き起こす．しかし，内皮細胞上あるいは白血球上に表現された接着因子はいつまでも膜上に留まるわけではなく，蛋白質分解酵素の作用で切離(shedding)され，血中に可溶性接着因子として放出される．この可溶成分はリガンドと結合し，本来の接着を阻害するネガティブフィードバック機構の一端を担う．

接着因子の操作でBBB/BNBの破壊を制御するには，接着因子の発現そのものを抑制する方法と，可溶性接着因子の放出を促進する方法の二つの戦術が考えられる．rolling，attachment，migrationのいずれのプロセスをブロックしても白血球の浸潤は抑制可能である[5]が，現在MSに対し広く使用されているnatalizumab(日本では未認可)はVCAM-1のリガンドであるVLA-4に対する抗体製剤であり，細胞接着カスケードの中でもっとも強力な接着過程と考えられているVCAM-1/VLA-4の接着を阻害する．本剤はMSの病因そのものである中枢神経系ミエリンに対する自己免疫現象の抑制ではなく，BBBでの細胞侵入をターゲットとするだけでMSの増悪が抑制できるということを証明した画期的な薬剤といえる．

しかし，病的T細胞だけでなくimmunosurveilanceに関与するであろうT細胞なども含め，すべての白血球の中枢神経内へのアクセスをブロックするゆえ，2010年1月の段階で全世界で20人以上の進行性多巣性白質脳症(PML)患者[6]が発生していることは看過できない．natalizumabがきわめて有用な薬物であることは認めたうえで，今後はよりきめ細かく，たとえば病的T細胞のみを選択的に侵入阻止できる薬物の開発が待たれる．BBB/BNBにおける分子細胞生物学の発展により，おそらく近い将来には実用可能となるものと思う．

液性因子の神経実質内流入は必ずしもT細胞によるBBB/BNB破綻の二次的現象ではない○

サイトカインは大部分が8-26 kDaのサイズを

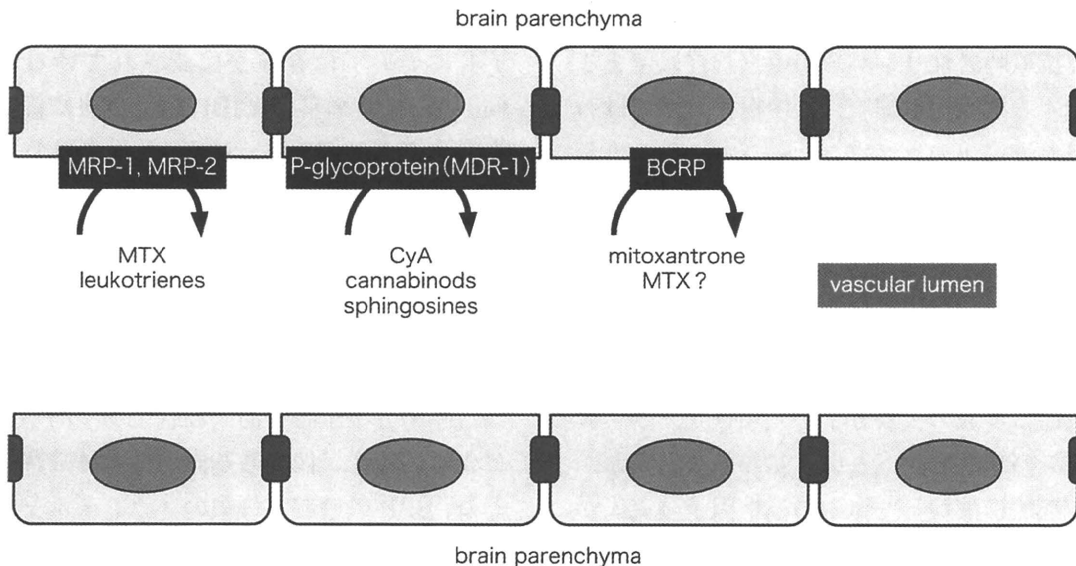

Fig. 2. BBBでの主要なABC transporterとそれぞれの基質

もつポリペプチドであり，健常なBBB/BNBが，単純拡散の形でサイトカインを通過させるとは考えにくい．このため，かつてはサイトカインの流入はT細胞によって破壊されたBBB/BNBの隙間から二次的に起きると考えられていた．現在では，T細胞の大部分は内皮細胞をすり抜ける際に細胞間(paracellular)ではなく，内皮細胞細胞体の真ん中を突っ切る形(transcellular)で入っていくとする考えが有力で[7]，したがって，T細胞の浸潤と液性因子の漏れは必ずしも並行しない．いくつかの炎症性サイトカインは，BBB/BNB構成内皮細胞に直接的に働いてその透過性を増し，自らの神経実質内流入をも促すことが実験的に知られるようになっている．

BBB/BNBの透過性を増加させるサイトカインは，TNF-α，IFN-γ，IL-1βなどの炎症性サイトカインと上述の血管内皮増殖因子(vascular endothelial growth factor：VEGF)が代表的なものである．MSをはじめとする免疫性神経疾患の病態と深く関わるのは前者の炎症性サイトカインであり，NF-κBの活性化とそれに引き続くNO放出が透過性亢進に関与しているものと考えられている．

BBBを乗り超えたあとも白血球はバリアーからのコントロールを受ける

バリアーを乗り超えた活性化T細胞は，神経実質内へ留まるのを促進する抗原呈示細胞と出会わない限り，① 神経系外へ出ていく，あるいは② アポトーシスによって死滅する，といった経過をとり，中枢神経系・末梢神経系全体へ拡散してはいかない．ここでT細胞を血管周囲につなぎ止める役割をするのが，BBB構成毛細血管に発現しているケモカインCXCL12であるという興味深い論文が最近発表された[8]．

このケモカインは，正常者やMS患者脳でも非炎症部では内皮細胞のbasolateral側に発現しているのに対し，MS脳の活動性炎症部位では管腔側に発現が異動する．非炎症時にbasolateralに局在しているCXCL12は，そのレセプターであるCXCR4をもつリンパ球を血管のすぐ外側に囲い込んで，中枢神経内全体へリンパ球が拡がるのを食い止める．一方，病変部では内皮細胞の活性化とともにCXCL12が管腔側へ移動，解放されたCXCR4陽性リンパ球は中枢神経内へ拡散して炎症に参画し，一方では，移動したCXCL12によって血流中のCXCR4陽性リンパ球がさらにリク

ルートされるという悪循環ができあがる．

ここで抗原特異性リンパ球が中枢神経に留まるのを促進する抗原提示細胞は，中枢神経系ではおそらく小血管周辺のマクロファージ，あるいはミクログリア細胞であろうといわれている．両細胞とも，外的刺激に反応して速やかにMHC抗原をupregulateさせる[9]．中枢神経由来抗原を認識するT細胞はここで刺激を受けて増殖，蓄積して炎症性サイトカインを放出し，内皮細胞を活性化してその表面に多数の接着分子を表現させ，さらに多数のリンパ球を脳内へと引き込む結果となる．バリアー機能は破綻して血中の液性因子の流入が促進され，これがcontrast-enhanced MRIでのガドリニウム増強効果として現れる．

MSの治療を考えるうえでBBB上のtransporterは重要である

BBBには脳に必要な物質を血液から効率よく取り込み，また，不要物を速やかに血中へ排泄する働きがあることはすでに述べた．この機構の主役をなすのは，血管内皮細胞の脳側，または管腔側の細胞膜に局在する各種transporterである[10]が，最近，ABC transporterと呼ばれる，管腔側細胞膜に局在する排出系のtransporterが注目されている．中でも臨床的に重要と考えられるのはMRP 1～3/ABCC 1～3，P-glycoprotein/ABCB 1，BCRP/ABCG 2の3者で，それぞれ，methotrexate(MTX)，mitoxantroneなど重要な薬物の神経系実質内への流入を妨げている(Fig. 2)．

近年，進行型MS患者脳の脳軟膜に異所性B-cell follicleが形成されることが話題になっているが[11]，二次進行型MS(SPMS)や一次進行型MS(PPMS)など，BBBが閉じてしまっている患者でBBBの向こう側に活動性の自己免疫現象が起きているとすれば，ABC transporterによる免疫抑制薬の脳外への強制排泄は今後，克服しなければならない重要な課題であろう．

BNBでのトランスポーター解析は緒に就いたばかりであるが，少なくともMRP 1～3/ABCC 1～3，P-glycoprotein/ABCB 1，BCRP/ABCG 2の3者については存在が確認されている[12]．Guillain-Barré症候群やCIDPの治療法に新たな示唆を与えるだけでなく，vincristineをはじめとするvinca alkaloids(P-glycoproteinの重要な基質の一つである)の末梢神経毒性の解明・克服にも大きな寄与をすると思われる．

おわりに

近年の分子生物学的・細胞生物学的手法の発展により，BBBに関する基礎的知識は飛躍的に増加した．BBBの破綻・修復のメカニズムを知ることはMS再発の予防に直結しており，いったん起こってしまったMS病変のさらなる増悪を防ぐためにも，常に考慮の対象とすべき重要な課題である．

一方，BNBに焦点を絞った基礎研究は緒に就いたばかりであり，BNBでの細胞浸潤，バリアー構成分子の動態などの多くは，BBBまたは全身血管系での知見から"おそらくBNBもこうであろう"という想像のもとに語られているだけにすぎない．Guillain-Barré症候群やCIDPのよりきめ細かい治療法の開発のためには，BNB固有の知見のさらなる蓄積が必須であろう．BNB研究の動向については，筆者の最近の総説[13]を参照されたい．

BBB/BNBは単なる神経系と全身循環系を隔てる障壁ではなく，難治性神経疾患の治療法開発のkeyとなる臓器である．今後のいっそうの研究の発展と，新たな若手研究者の参入を望みたい．

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血液脳関門の分子機構
—内皮細胞を中心に—

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血液脳関門（blood-brain barrier：BBB）の主役は脳微小血管内皮細胞であり，その破綻は脳血管障害，多発性硬化症をはじめとする自己免疫性神経疾患，脳腫瘍など多彩な中枢神経疾患の発症・増悪とかかわっている．本稿ではBBBの分子的基盤とBBB破綻にかかわる2つの分子メカニズムにつき，最近の知見を中心に概説する．BBBは中枢神経系と全身循環系を隔てる単なる“壁”ではなく，難治性神経疾患の新規治療法開発のkeyとなる臓器である．

Key Words
血液脳関門，血管内皮細胞，神経膠細胞，claudin-5，インテグリン

はじめに

内皮細胞は血液成分と直接に接する唯一の細胞である．血漿から組織への必要物質の移行と老廃物の回収を能率よく円滑におこなうため，体内のほとんどの臓器の微小循環系内皮細胞は有窓であり，多数のpinocytotic vesicleを有して細胞間・細胞内の物質往来を自由にしている．一方，体内の限られた部位では，臓器の内部環境を維持することを目的として，内皮細胞を介した物質のやりとりを制御するメカニズムが存在する．この機構はblood-tissue barrierと総称され，体内にはblood-testis barrier（血液精巣関門），blood-retinal barrier（血液網膜関門）など多数のバリアーシステムが存在する．なかでも，中枢神経系のバリアーシステムは脳のホメオスタシスを維持するうえできわめて重要な構造物であり，①脳実質内の微小血管内皮細胞，②くも膜上皮細胞，③脈絡叢上皮細胞の3ヵ所に局在している．本稿の主題である血液脳関門（blood-brain barrier：BBB）は①に局在する．

BBBの存在は脳の内部環境維持のうえで重要であることは論を待たない．しかし一方で，BBBが存在するがゆえに，たとえば脳腫瘍に対する抗癌剤など，治療に必要な物質が脳に届かないなどの問題点は古くから指摘されてきた．近年になって，各種神経栄養因子，siRNAなど神経変性疾患を治癒しうる薬剤候補が続々と出てきているが，健常なBBBを越える脳内へのこれら薬物のトランスファーは克服すべき大きな課題として立ちふさがっている．また，多発性硬化症（multiple sclerosis：MS）をはじめとする自己免疫性神経疾患や脳血管障害では，臓器傷害性Tリンパ球の脳内浸潤が増悪因子であり，そ