Transferrin Receptor 1 Facilitates Poliovirus Permeation of Mouse Brain Capillary Endothelial Cells*

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Taketoshi Mizutani1, Aya Ishizaka, and Coh-ichi Nihei
From the Institute of Microbial Chemistry, Microbial Chemistry Research Foundation (BIKAKEN), Tokyo, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141-0021, Japan

As a possible route for invasion of the CNS, circulating poliovirus (PV) in the blood is believed to traverse the blood-brain barrier (BBB), resulting in paralytic poliomyelitis. However, the underlying mechanism is poorly understood. In this study, we demonstrated that mouse transferrin receptor 1 (mTfR1) is responsible for PV attachment to the cell surface, allowing invasion into the CNS via the BBB. PV interacts with the apical domain of mTfR1 on mouse brain capillary endothelial cells (MBEC4) in a dose-dependent manner via its capsid protein (VP1). We found that F-G, G-H, and H-I loops in VP1 are important for this binding. However, C-D, D-E, and E-F loops in VP1-fused Venus proteins efficiently penetrate MBEC4 cells. These results imply that the VP1 functional domain responsible for cell attachment is different from that involved in viral permeation of the brain capillary endothelium. We observed that co-treatment of MBEC4 cells with excess PV particles but not dextran resulted in blockage of transferrin transport into cells. Using the Transwell in vitro BBB model, transferrin co-treatment inhibited permeation of PV into MBEC4 cells and delayed further viral permeation via mTfR1 knockdown. With mTfR1 as a positive mediator of PV-host cell attachment and PV permeation of MBEC4 cells, our results indicate a novel role of TfR1 as a cellular receptor for human PV receptor/CD155-independent PV invasion of the CNS.

Poliovirus (PV)2 is an enterovirus belonging to the family Picornaviridae and is the causative agent of poliomyelitis (1, 2). Generally, PV enters the stomach via oral ingestion and invades the alimentary mucosa in an unidentified manner, and PV then proliferates in the alimentary mucosa (1, 2) and moves to the bloodstream. The circulating virus invades the CNS and replicates in motor neurons (MNs). Poliomyelitis is known to involve accumulated damage to the MNs by PV replication (3). The human PV receptor (hPVR/CD155) facilitates PV infection of cells; however, PV replication is restricted by host immune activities (e.g. IFN-α/β) (4–6). Although wild-type mice are not sensitive to PV (7), hPVR-expressing transgenic (Tg) mice were susceptible to PV via intravenous and intramuscular routes but not the oral route (7–12). Further, an IFN-α/β-deficient hPVR-Tg mouse was found to be susceptible to PV via the oral route (13).

As a possible route for invasion of the CNS, PV enters the CNS via axonal transport through the skeletal muscle in an hPVR-dependent manner (14). Endocytic vesicles at the synapse take up intact PV, which is passively transported to the CNS. Interestingly, PV has been shown to invade the CNS via hPVR-independent axonal transport in hPVR-Tg and non-Tg mice (15), indicating that other unidentified pathways for PV transport may be present. Furthermore, we previously showed that PV promptly invades the CNS from the blood in non-Tg mice, which supports this speculation (16). In that study, intravenously injected PV permeated the brain as fast as cationized rat serum albumin, which is BBB-permeable (16). Therefore, PV is thought to efficiently permeate the CNS by overcoming the BBB.

The BBB is composed of a multilayer barrier composed of vascular endothelial cells with tight junctions filling the gaps between cells (17). Although the BBB was discovered over a century ago, its transport mechanisms are not fully understood. It restricts transport of substances between the CNS and blood by maintaining a strictly regulated microenvironment for high integrity neuronal response in the CNS (18, 19). Certain substances are permitted transmission via the BBB from the bloodstream to the brain, facilitated by specific transporters on the cell membrane (e.g. glucose, amino acids, transferrin, and insulin) (20–25). For example, transferrin is known to facilitate iron transport from the blood to the cells (26). Iron uptake increases transferrin affinity for the transferrin receptor on the cell membrane. The iron-transferrin complex is transported into the cells by receptor-mediated transcytosis, followed by the release of iron into the cytoplasm; transferrin then goes back to the outer cell membrane for recycling. This mechanism is sometimes exploited by viruses for entry during infection (27–31).

Given that transferrin receptor is a transporter in brain capillary endothelial cells and can be used as an entry receptor for several viral infections, we hypothesized that PV similarly invades the CNS via the BBB by using transferrin receptor as a vehicle. We examined this possibility in this study and demonstrated the interaction of PV with mouse transferrin receptor 1 (mTfR1) in vitro. Furthermore, we identified that VP1, a PV capsid protein, is responsible for the physical interaction...
between PV and mTfR1. Co-incubation with transferrin or knockdown of mTfR1 resulted in delayed PV transcytosis via the brain capillary endothelial cells in the BBB in vitro model. We identified the domain of mTfR1 responsible for attachment to mTfR1 and permeation of the brain capillary endothelial cells. In summary, we provide convincing evidence to support the direct involvement of mTfR1 in PV permeation into the CNS via BBB by using an in vitro model.

**Experimental Procedures**

**Cell Culture**—Mouse brain capillary endothelial cells (MBEC4) isolated from BALB/c mice brain cortices and immortalized by SV40 transformation were maintained (32) in DMEM containing 10% fetal calf serum at 37 °C and 5% CO₂. African green monkey kidney (AGMK) cells were grown in DMEM supplemented with 5% newborn calf serum.

**Plasmid Construction**—The pCold-6His vector was generated by inserting GST fragment (0.3 kb at DraIII-BamHI sites) in pCold-I vector (Takara Bio Ltd., Shiga, Japan), then a His₆ fragment was inserted between the XhoI and NotI restriction sites (33). To generate pCold-GST-6His-VP1, -VP2, and -VP3 vectors, all the coding fragments of capsid proteins (VP1, VP2, and VP3) were amplified by PCR, and these fragments were introduced at the BamHI/Xhol site in the pCold-GST-6His vector. The pCold-GST-Venus-6His-VP1 and each VP1 loop peptide (cDNA) were constructed as follows. To generate the pCold-GST-Venus-6His-empty vector, the PCR fragment of 0.7-kb Venus cDNA was inserted into the BamHI/Xhol site in the pCold-GST-6His vector. The linker sequence (SRG[SGGGG]₂SSG) was introduced at Xhol sites in the pCold-GST-Venus-6His vector to generate pCold-GST-Venus-L-6His. The whole cDNA or peptide sequences of VP1 were inserted after linker sequence at the EcoRI/Xhol site in pCold-GST-L-6His. FLAG-mTfR1 and permeation of the brain capillary endothelial cells in the BBB.

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**Titration of Virus Infectivity**—The number of plaque-forming unit in AGMK cells were determined by the plaque assay. For the measurement of plaque-forming unit, AGMK cells on 6-cm dishes were inoculated with the viral suspension and then incubated at 37 °C for 2–5 days for the observation of plaques.

**Transwell in vitro blood brain barrier (BBB) permeation assay**—Alexa 488-conjugated mouse transferrin was purchased from Jackson ImmunoResearch Laboratories. FITC-labeled dextran (2,000 K) was purchased from Sigma-Aldrich. Mouse brain capillary endothelial cells (MBEC4) were seeded (5 × 10⁴ via incubation of the cells with 0.1 mm isopropyl 1-thio-β-D-galactopyranoside overnight at 15 °C and then suspended in E. coli using His tag binding/wash buffer (20 mm Tris–HCl, pH 8.0, 600 mm NaCl, 1 mm MgCl₂, 10% glycerol, 0.1% Nonidet P-40, 20 mm imidazole, and phosphatase inhibitors). E. coli was sonicated on ice using ELESTAIN035SD (ELECON Science, Corp, Chiba, Japan). Purification of GST-His-tagged proteins was performed sequentially using Profinity IMAC nickel-charged resin (Bio-Rad) and glutathione-Sepharose 4B (GE Healthcare). The target proteins were eluted from these columns with His tag elution buffer (20 mm Tris–HCl, pH 8.0, 600 mm NaCl, 1 mm MgCl₂, 10% glycerol, 0.1% Nonidet P-40, 250 mm imidazole, and protease inhibitors) and GST elution buffer (100 mm Tris–HCl, pH 8.0, 12 mm NaCl, 20 mm glutathione, and protease inhibitors), respectively. [³⁵S]Methionine-labeled full-length mTfR1 or its derivative constructs were translated using a reticulocyte lysate system in vitro (Promega Corporation, Madison, WI), and incubated with PV particles or respective GST fusion proteins at 4 °C on a rotating platform for 2 h. The precipitated materials were washed for five times with TNE buffer (10 mm Tris–HCl, pH 7.8, 150 mm NaCl, 1 mm EDTA, 1% Nonidet P-40, and protease inhibitors) and analyzed by SDS-PAGE followed by autoradiography.

**Immunoprecipitation Assay**—Immunoprecipitation assays were performed as previously described (34). Briefly, cells were lysed with TNE buffer (10 mm Tris–HCl, pH 7.8, 150 mm NaCl, 1 mm EDTA, 1% Nonidet P-40, and protease inhibitors), and cell lysates were incubated overnight on a rotating platform at 4 °C with respective antibodies (5 μg each), which were previously bound to Dynabeads® Protein G (Dynal, Oslo, Norway). After washing, precipitates were purified, subjected to SDS-PAGE, and analyzed by Western blotting or autoradiography.

**PV Purification and Fluorescent Label of PV**—PV was purified from a protocol described previously (36). HeLa cells were infected with Mahoney virus at a multiplicity of infection of 10. The cells were harvested at 8 h postinfection, and the virus was purified from cytoplasmic extracts of the infected cells by using DEAE-Sepharose CL-6B, followed by sucrose density gradient and CsCl equilibrium centrifugation. Purified virus was desalted by gel filtration on a PD-10 column equilibrated with PBS(−). The PV concentration was determined by measuring the absorbance at 260 nm; 1.0 optical density unit was regarded as equivalent to 9.4 × 10¹² virions. Virus labeling was based on a protocol, which was described previously (13, 37). Briefly, PV was labeled with Alexa Fluor-succinimidyl ester according to the manufacturer’s instructions (Life Technologies). The labeled virus was purified on NAP5 columns (GE Healthcare), dialyzed against PBS(−) without loss of the specific infectivity.

**Trituration of Virus Infectivity**—The number of plaque-forming unit in AGMK cells were determined by the plaque assay. For the measurement of plaque-forming unit, AGMK cells on 6-cm dishes were inoculated with the viral suspension and then incubated at 37 °C for 2–5 days for the observation of plaques.
cells/well) into collagen-coated 24-well Transwell plates (#3496; Corning) and cultured for 48 h at 37 °C under 5% CO₂ (7). In vitro monolayer integrity was evaluated as trans-endothelial electrical resistance (TEER) using a Millicell® ERS voltohmeter (Millipore, Bedford, MA). The average TEER value of the cellular monolayer was 50 ± 10 Ω·cm² after background subtraction of the TEER value of a cell empty (blank) control well. Preparing 126.55 µg/ml purified PV particle, PV permeation was calculated as clearance volume in each time point as below: clearance volume (µl) = permeated apical side fluorescent intensity (µg)/input PV fluorescent intensity volume (µg/µl).

**FACS Analysis**—MBEC4 cells were assessed by single-color flow cytometric analysis. The following antibodies were used for the flow cytometric analysis: FITC anti-mouse CD71/TfR1 antibody (113805) (BioLegend, San Diego, CA). Dead cells were excluded with the propidium iodide staining (Sigma-Aldrich). Samples were analyzed on iCyte flow cytometer (Sony Biotechnology Inc., San Jose, CA).

**Statistical Analyses**—We performed t test. All statistical tests were two-sided. We considered p values less than 0.05 to be statistically significant.

**Results**

**PV Interacts with TJR1 on Mouse Brain Capillary Endothelial Cells**—To elucidate the interaction between PV particles and the transferrin receptor, we generated mouse brain capillary endothelial cell line (MBEC4) expressing FLAG-mTfR1. Because it is known that in PV-sensitive cells, the association between PV capsid and hPV receptor promptly leads to PV capsid conformational change followed by cell entry, we used PV-insensitive mouse cell lines to avoid this conformational change. We conducted immunoprecipitation using anti-PV antibody on total lysate obtained from FLAG-mTfR1-MBEC4 cells exposed to PV for 1 h, followed by blotting with anti-FLAG antibody (Fig. 1A). FLAG-mTfR1 was clearly detected by Western blotting using an antibody against FLAG peptide. Next, we evaluated the interaction between PV and endogenous TJR1 on MBEC4 cells. The MBEC4 cells were then exposed to various concentrations of PV for 1 h, following which the supernatant was discarded, and the whole cell extract was collected. Following immunoprecipitation using anti-PV antibody, endogenous mTfR1 was detected by Western blot using anti-mTfR1 antibody in accordance with the PV dose (Fig. 1B). These results suggested that PV interacts with mTfR1 in vitro.

We speculated that if PV requires mTfR1 for cell permeation, PV might compete against transferrin during transport into cells. To examine this possibility, we assessed FITC-labeled transferrin transport into MBEC4 cells in the presence of pretreated PV or dextran. Because dextran is known to permeate into arterial endothelial cells (39) through the intercellular gap (40) by virtue of its size (molecular mass, >15 kDa), dextran (molecular mass, 2 × 10⁴ kDa) was used as a control for a different pathway of permeation. It was presumably of a size similar to that of the PV particle, which is ~8.6 × 10³ kDa (41, 42).

Co-treatment with purified PV effectively inhibited FITC-labeled transferrin transport into MBEC4 cells (Fig. 1C). However, an excess amount of dextran as compared with that of PV showed no effect, even with increasing doses (Fig. 1D). This observation confirmed the interaction between PV and mTfR1 in vitro.

**PV Interacts with mTfR1 through the Ectodomain**—To identify the domains of PV and mTfR1 that are responsible for their
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interaction, we identified the region of mTrf1 necessary for PV interaction. Because mTrf1 contains three identical extracellular domains (Fig. 2A), we divided mTrf1 into four regions and generated in vitro translated 35S-labeled, FLAG-fused, and full-length mTrf1, as well as three derivative mTrf1 truncations using reticulocyte lysate (Fig. 2B). Subsequently, we tested their interaction with purified PV. The results showed that PV particles directly interact with full-length mTrf1 (Fig. 2C). Each truncated mTrf1 (mTrf1–609 and -388) also interacted with PV particles, except for the mTrf1–186 construct (Fig. 2C). According to the predicted structure of Trf1, the helical domain of Trf1 is folded into its homodimeric receptor (26). This hinders the natural interaction between PV and the helical domain. Together, these results indicated that the PV particle may interact with the apical domain and/or secondary part of protease-like domain of mTrf1.

PV Efficiently Interacts with the Apical Domain of mTrf1—We further identified the mTrf1 domain that associates with the PV particle. We constructed and generated in vitro translated, 35S-labeled apical domain (AD) and second region of protease-like domain (PD2) of mTrf1 (Fig. 2D). The result showed that both mTrf1–AD and -PD2 are efficiently precipitated with PV particle (Fig. 2E), although the background signal was slightly higher in the precipitation using mTrf1–PD2. Together with the result shown in Fig. 2C, this result implied that the apical domain of mTrf1 is important for PV association. Given that the outer side of the PV capsid is composed of three polypeptides (VP1, VP2, and VP3), these proteins are candidates for interaction with mTrf1. To identify the PV capsid protein responsible for mTrf1 association, we constructed GST fusion VP1, VP2, and VP3 plasmids and generated the corresponding recombinant proteins in E. coli. We then conducted GST pull-down assays using purified GST alone, GST-VP1, GST-VP2, and GST-VP3 and in vitro translated, 35S-labeled full-length mTrf1, as well as the three truncated proteins (Fig. 3A). VP1 clearly bound to all mTrf1 constructs, except for the mTrf1–186 mutant (Fig. 3B). VP2 and VP3 showed weak or no binding to any of the truncated mutants, although they bound to full-length mTrf1. This result is possibly due to conformational changes by truncation. Together, these results suggested that VP1 is the capsid protein with the highest affinity for mTrf1.

F-G, G-H, and H-I Loops on VP1 Are Responsible for Interaction with the Apical Domain of mTrf1—VP1 contains eight large β-sheet structures, and these antiparallel strands are compactly packed into the PV particle, similar to VP2 and VP3 (Fig. 4A) (43). VP1 contains seven loop domains that are considered as the outer exposed domains of the VP1 structure. To identify the VP1 loop responsible for interaction with the AD or PD2 domain of mTrf1, we constructed seven GST-Venus-fused
proteins, each containing VP1 loops under Venus protein (Fig. 4B and Table 1). We performed GST pulldown assays using these purified GST-Venus fusion proteins and in vitro translated AD or PD2 of mTfR1 (Fig. 4C). F-G, G-H, and H-I loops showed GST-Venus-fused protein clearly bound to the AD of mTfR1. GST-Venus-fused protein containing F-G loops was the only protein that interacted with PD2 of mTfR1 (Fig. 4C). These results showed that F-G, G-H, and H-I loops of VP1 are the domains mainly responsible for the interaction between PV and mTfR1.

**PV Permeates Mouse Brain Capillary Endothelial Cells and Competes against Transferrin for Transport into Cells**—We determined whether PV has the ability to permeate brain capillary endothelial cells in vitro. We performed cell permeation assay and quantified the cell-permeating ratio into MBEC4 cells using purified GST-Venus-VP1 and the 7 GST-Venus-VP1-loop proteins (Fig. 4D). The Venus fusion proteins were incubated with MBEC4 cells for 24 h, followed by preparation of cell lysate and measurement of fluorescence intensity of the permeated protein. As shown in Fig. 4D, full-length VP1 and B-C loops of VP1 connected with GST-Venus proteins exhibited marginal permeation into MBEC4 cells compared with control fusion protein. C-D, D-E, and E-F loops of VP1 connected with GST-Venus protein showed >2-fold permeable efficiency into MBEC4 cells, whereas F-G, G-H, and H-I loops of VP1, responsible for mTfR1 attachment, showed poor permeation into MBEC4 cells. These results suggest that the VP1 loops responsible for attachment to mTfR1 and permeation into MBEC4 cells are indeed different.

**Knockdown of Transferrin Receptor Prevents PV Transmission in the in Vitro BBB Model**—Our group previously observed that circulating PV promptly invaded the brain from the blood via the BBB in a non-hPVR-Tg mouse model (16). To confirm whether PV uses the transferrin receptor to invade the CNS, we conducted Transwell BBB in vitro model assay using MBEC4 cells (38), wherein MBEC4 cells were confluent seeded on a microporous membrane in the upper chamber of the Transwell. MBEC4 cells were exposed to fluorescein-labeled PV with or without transferrin, and then fluorescence intensity of the medium collected from the lower chamber was assessed to monitor PV that had permeated the MBEC4 cells (Fig. 5A). Because dextran is known to permeate into arterial endothelial cells (39) through the intercellular gap (40) by virtue of its size (molecular mass, >15 kDa), we used dextran as the permeation control. To validate the Transwell BBB in vitro model assay system, we first compared the permeation ratio of FITC-labeled transferrin to that of FITC-labeled dextran (70 kDa) as a similar size control. The permeation ratio of transferrin was 2.5-fold higher than that of dextran (Fig. 5B). Given that transferrin permeates into cells via its association with the transferrin receptor in the endocytic pathway, whereas dextran passively permeates through the intercellular gap, this result suggested that the assay system is functional. In a time course experiment, PV was shown to efficiently permeate through MBEC4 cells into the lower chamber (Fig. 5C). However, this PV transport was delayed by the addition of transferrin (Fig. 5C).
transferrin is known to interact with the protease-like domain (PD2) of TfR1 (44, 45), PV capsids and transferrin would compete for this TfR1 domain, which is in agreement with the result shown in Fig. 2C. To validate whether unmodified PV particles also require mTfR1 to permeate through MBEC4 cells, we constructed retroviral vector expressing shRNA against mTfR1 (sh-mTfR1). Stable transductants were obtained using appropriate antibiotics (Fig. 5D). We confirmed knockdown of mTfR1 expression on these cells by flow cytometric analysis (Fig. 5E). Using mTfR1 or control knockdown MBEC4 cells, we performed the Transwell BBB in vitro model assay. The number of permeated PVs was estimated by colony forming assay using AGMK cells. **, p < 0.01; *, p < 0.05.

Discussion

Given that PV permeates the brain as fast as cationized rat albumin with a high permeation rate in non-hPVR-Tg mice (16), it is believed that PV can directly invade the CNS via BBB transmission in an hPVR-independent manner. In this study, we suggest a novel pathway where PV invades the CNS in an hPVR-independent manner and that mTfR1 is a key receptor on brain capillary endothelial cells, responsible for PV permeation of the CNS via the BBB. Transferrin receptor is known to be a crucial molecule for cellular homeostasis during oxygenation (26). Because the CNS also requires active oxygenation and the antibody against the transferrin receptor stains brain capillary cells in the rat brain after intravenous administration (46, 47), transferrin receptor is a plausible candidate for the receptor of PV permeation of the brain.

PV clearly interacted with mTfR1 proteins in vitro (Figs. 1 and 2) in this study, and we found that VP1 is responsible for this binding (Fig. 3B). VP1 is also known to play a role in the interaction with hPVR/CD155. hPVR/CD155 inserts into the deep pocket “canyon” on the surface of the PV particle, which is composed of capsid proteins (43). After the association between the canyon and hPVR/CD155, the uncoating process promptly occurs in the PV particle. For PV proliferation in the brain after BBB transport, PV needs to retain its infectivity after transcytosis. The in vitro BBB model assay showed that permeated materials contained intact infectious particles (Fig. 5F). This result implies that PV permeates through brain capillary cells without loss of infectivity; a similar observation has been previously noted for the brain of hPVR-Tg and non-Tg mice (16).

Our in vitro binding experiment showed that F-G, G-H, and H-I loops are the VP1 regions responsible for mTfR1 interaction (Fig. 4C). The three-dimensional prediction analysis for PV particles showed that the G-H loop of VP1 faces the outer side and is located near the canyon, although the F-G and H-I loops of VP1 face the inside of the PV capsid. Considering this struc-
nurial analysis, G-H loop of VP1 is expected to be a probable candidate for mTfR1 binding. Further analysis by inserting a mutation at this loop on VP1 in PV particle or using the specific antibody against the identified loop of VP1 is an effective approach for understanding this binding mechanism. Homology between the sequence of Tfr1 in mice and humans is 77%, and both proteins play a role in iron delivery. Considering this, it is possible that PV also interacts with human Tfr1 as a mediator for BBB permeation.

The in vitro BBB model using MBEC4 cells showed that in vitro translated GST-Venus proteins fused with C-D, D-E, and E-F loops of VP1 permeate to a comparatively greater extent than other loops (Fig. 4D). All of these loops face the outer side of the PV particle, as revealed by the three-dimensional prediction analysis. This result shows that the VP1 loops responsible for mTfR1 binding and for permeation into MBEC4 cells are clearly separated. However, co-incubation with transferrin or knocking down of mTfR1 inhibited the transcytosis of PV through MBEC4 cells (Fig. 5); therefore, we speculated that mTfR1 plays a role in attachment to the cell membrane, followed by PV permeation of the brain capillary endothelial cells. This also means that additional unidentified molecules would mediate this transcytosis. The expression of transferrin receptor is known to be ubiquitous, although the expression level is comparatively higher in brain capillary endothelial cells. If this PV permeation is a specific event in the BBB, the existence of additional molecules that are expressed only in brain capillary endothelial cells is plausible. Therefore, further studies are required to identify these molecules to better understand the BBB permeation mechanism.

It has been shown that in the case of hPVR-dependent endocytosis, the route for PV transmission into the brain is via peripheral MNs from the skeletal muscle (14). However, PV can slowly transmit via MNs in non-hPVR Tg mice in vivo as well, although PV-containing endocytic vesicles were not observed in MNs cultured in vitro (15). These observations indicate that PV also potentially moves to the CNS by an unknown, hPVR-independent endocytic pathway (16). This discrepancy between in vitro and in vivo results may be explained by the decreased Tfr1 expression in the extending neurites of MNs in culture (48). It cannot be excluded that PV uses transferrin receptor as a positive mediator for transportation to the CNS from the skeletal muscles.

In this study, we showed that PV permeates mouse brain capillary endothelial cells through the BBB using the Transwell in vitro BBB model. Our results showed that mTfr1 is a positive mediator for BBB permeation of PV. To elucidate the complete mechanism for BBB permeation of PV, identification of the cellular counterpart for C-D, D-E, and E-F loops of VP1 is required. This study contributes to understanding the mechanism of viral transmission into the CNS, indicating its importance in the biology and pathogenesis of PV. Further, there is a growing recognition of the importance to develop CNS drug delivery systems. To this end, a BBB-permeable agent and/or a better understanding of delivering drug agents to the CNS is highly desired. Knowledge of the BBB permeation mechanism of PV will contribute to overcoming the challenge posed by the current inability to transport drugs to the CNS via the BBB.

References
1. Bordin, E. S. (1955) Ambiguity as a therapeutic variable. J. Consult. Psychol. 19, 9–15
2. Sabin, A. B. (1956) Pathogenesis of poliomyelitis; reappraisal in the light of new data. Science 123, 1151–1157
3. Groenemeyer, M., and Wimmer, E. (1998) Mechanism of injury-provoked poliomyelitis. J. Virol. 72, 5056–5060
4. Muñoz, A., and Carrasco, L. (1984) Action of human lymphoblastoid interferon on HeLa cells infected with RNA-containing animal viruses. J. Gen. Virol. 65, 377–390
5. Aoki, J., Koike, S., Ise, I., Sato-Yoshida, Y., and Nomoto, A. (1994) Amino acid residues on human poliovirus receptor involved in interaction with poliovirus. J. Biol. Chem. 269, 8431–8438
6. Ida-Hosounuma, M., Iwasaki, T., Yoshikawa, T., Nagata, N., Sato, Y., Sata, T., Yoneyama, M., Fujita, T., Taya, C., Yonekawa, H., and Koike, S. (2005) The α/β interferon response controls tissue tropism and pathogenicity of poliovirus. J. Virol. 79, 4460–4469
7. Koike, S., Taya, C., Kurata, T., Abe, S., Ise, I., Yonekawa, H., and Nomoto, A. (1991) Transgenic mice susceptible to poliovirus. Proc. Natl. Acad. Sci. U.S.A. 88, 951–955
8. Horie, H., Koike, S., Kurata, T., Sato-Yoshida, Y., Ise, I., Ota, Y., Abe, S., Hioki, K., Kato, H., and Taya, C. (1994) Transgenic mice carrying the human poliovirus receptor: new animal models for study of poliovirus neuroinfection. J. Virol. 68, 681–688
9. Okh, S., Igarashi, H., Nagata, N., Sakai, M., Koike, S., Nochi, T., Kiyono, H., and Nomoto, A. (2007) Establishment of a poliovirus oral infection model in human poliovirus receptor-expressing transgenic mice. J. Virol. 81, 7902–7912
10. Ohka, S., Yang, W. X., Terada, E., Igarashi, H., and Nomoto, A. (1998) Retrograde transport of intact poliovirus through the axon via the fast transport system. Virology 250, 67–75
11. Ohka, S., Nakada, M., Ohara, K., Igarashi, H., Deinhardt, K., Schiavo, G., and Nomoto, A. (2009) Receptor-dependent and -independent axonal transport of poliovirus in motor neurons. J. Virol. 83, 4995–5004
12. Yang, W. X., Terasaki, T., Shiroyo, K., Ohka, S., Aoki, J., Tanabe, S., Nomura, T., Terada, E., Sugiyama, Y., and Nomoto, A. (1997) Efficient delivery of circulating poliovirus to the central nervous system independently of poliovirus receptor. Virology 229, 421–428
13. Ballabh, P., Braun, A., and Nedergaard, M. (2004) The blood-brain barrier:...
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an overview: structure, regulation, and clinical implications. Neurobiol. Dis. 16, 1–13
18. Brightman, M. W. (1977) Morphology of blood-brain interfaces. Exp. Eye Res. 25, 1–25
19. Reese, T. S., and Karnovsky, M. J. (1967) Fine structural localization of a blood-brain barrier to exogenous peroxidase. J. Cell Biol. 34, 207–217
20. Shah, K., Desilva, S., and Abbruscato, T. (2012) The role of glucose transporters in brain disease: diabetes and Alzheimer’s Disease. Int. J. Mol. Sci. 13, 12629–12655
21. Pardridge, W. M., Eisenberg, J., and Yang, J. (1987) Human blood-brain barrier transferrin receptor. Metabolism 36, 892–895
22. Fishman, J. B., Rubin, J. B., Handragan, J. V., Connor, J. R., and Fine, R. E. (1987) Receptor-mediated transcytosis of transferrin across the blood-brain barrier. J. Neurosci. Res. 18, 299–304
23. Pardridge, W. M. (1986) Receptor-mediated peptide transport through the blood-brain barrier. Endocr. Rev. 7, 314–330
24. Pardridge, W. M., Yang, J., and Eisenberg, J. (1985) Blood-brain barrier protein phosphorylation and dephosphorylation. J. Neurochem. 45, 1141–1147
25. Pardridge, W. M. (1983) Brain metabolism: a perspective from the blood-brain barrier. Physiol. Rev. 63, 1481–1535
26. Wang, J., and Pantopoulos, K. (2011) Regulation of cellular iron metabolism. Biochem. J. 434, 365–381
27. Ross, S. R., Schofield, J. J., Farr, C. J., and Bucan, M. (2002) Mouse transferrin receptor 1 is the cell entry receptor for mouse mammary tumor virus. Proc. Natl. Acad. Sci. U.S.A. 99, 12386–12390
28. Flanagan, M. L., Oldenburg, J., Reignier, T., Holt, N., Hamilton, G. A., Martin, V. K., and Cannon, P. M. (2008) New world clade B arenaviruses can use transferrin receptor 1 (TfR1)-dependent and -independent entry pathways, and glycoproteins from human pathogenic strains are associated with the use of TfR1. J. Virol. 82, 938–948
29. Martin, D. N., and Uprichard, S. L. (2013) Identification of transferrin receptor 1 as a hepatitis C virus entry factor. Proc. Natl. Acad. Sci. U.S.A. 110, 10777–10782
30. Palermo, L. M., Hueffer, K., and Parrish, C. R. (2003) Residues in the apical domain of the feline and canine transferrin receptors control host-specific binding and cell infection of canine and feline parvoviruses. J. Virol. 77, 8915–8923
31. Radoshitzky, S. R., Abraham, J., Spiroupolou, C. F., Kuhn, J. H., Nguyen, D., Li, W., Nagel, J., Schmidt, P. J., Nunberg, J. H., Andrews, N. C., Farzan, M., and Choe, H. (2007) Transferrin receptor 1 is a cellular receptor for New World haemorrhagic fever arenaviruses. Nature 446, 92–96
32. Tatsuta, T., Naito, M., Oh-hara, T., Sugawara, I., and Tsuruo, T. (1992) Functional involvement of P-glycoprotein in blood-brain barrier. J. Biol. Chem. 267, 20383–20391
33. Mizutani, T., Ishizaka, A., and Furuichi, Y. (2015) The Werner protein acts as a coactivator of nuclear factor κB (NF-κB) on HIV-1 and interleukin-8 (IL-8) Promoters. J. Biol. Chem. 290, 18391–18399
34. Ishizaka, A., Mizutani, T., Kobayashi, K., Tando, T., Sakurai, K., Fujihara, T., and Iba, H. (2012) Double plant homeodomain (PHD) finger proteins DPF3a and -3b are required as transcriptional co-activators in SW1/SNF complex-dependent activation of NF-κB RelA/p50 heterodimer. J. Biol. Chem. 287, 11924–11933
35. Mizutani, T., Ishizaka, A., Suzuki, Y., and Iba, H. (2014) 7SK small nuclear ribonucleoprotein complex is recruited to the HIV-1 promoter via short viral transcripts. FEBS Lett. 588, 1630–1636
36. Kajiyama, S., Arakawa, H., Kuge, S., Koi, T., Imura, N., and Nomoto, A. (1985) Isolation and characterization of defective-interfering particles of poliovirus Sabin 1 strain. Virology 142, 307–316
37. Pelkmans, L., Kartenbeck, J., and Helenius, A. (2001) Caveolar endocytosis of simian virus 40 reveals a new two-step vesicular-transport pathway to the ER. Nat. Cell Biol. 3, 473–483
38. Doheu, S., Kataoka, Y., Ikesue, H., Naito, M., Tsuruo, T., Oishi, R., and Sawada, Y. (2000) Involvement of glial cells in cyclosporine-increased permeability of brain endothelial cells. Cell. Mol. Neurobiol. 20, 781–786
39. Hashara, R., Anamizu, C., Yagyu-Mizuno, Y., Ohkuma, S., and Takano, T. (1986) Transcellular transport of fluorescein dextran through an arterial endothelial cell monolayer. Cell Struct. Funct. 11, 343–349
40. Choi, J. J., Wang, S., Tung, Y. S., Morrison, B., 3rd, and Konofigou, E. E. (2010) Molecules of various pharmacologically-relevant sizes can cross the ultrasound-induced blood-brain barrier opening in vivo. Ultrasound Med. Biol. 36, 58–67
41. Putnak, J. R., and Phillips, B. A. (1981) Picornaviral structure and assembly. Microbiol. Rev. 45, 287–315
42. Scraba, D. G., Kay, C. M., and Colter, J. S. (1967) Physico-chemical studies of three variants of Mengo virus and their constituent ribonucleotides. J. Mol. Biol. 26, 67–79
43. Levy, H. C., Bostina, M., Filman, D. J., and Hogle, J. M. (2010) Catching a virus in the act of RNA release: a novel poliovirus uncoating intermediate characterized by cryo-electron microscopy. J. Virol. 84, 4426–4441
44. Cheng, Y., Zak, O., Aisen, P., Harrison, S. C., and Walz, T. (2004) Structure of the human transferrin receptor-transferrin complex. Cell 116, 565–576
45. Eckenroth, B. E., Steere, A. N., Chasteen, N. D., Everse, S. J., and Mason, A. B. (2011) How the binding of human transferrin primes the transferrin receptor potentiating iron release at endosomal pH. Proc. Natl. Acad. Sci. U.S.A. 108, 13089–13094
46. Fraden, P. M., Walus, L. R., Musso, G. F., Taylor, M. A., Malfroy, B., and Starzynski, R. M. (1991) Anti-transferrin receptor antibody and antibody-drug conjugates cross the blood-brain barrier. Proc. Natl. Acad. Sci. U.S.A. 88, 4771–4775
47. Jeffries, W. A., Brandon, M. R., Hunt, S. V., Williams, A. F., Gatter, K. C., and Mason, D. Y. (1984) Transferrin receptor on endothelium of brain capillaries. Nature 312, 162–163
48. Nakamura, Y., Nakamichi, N., Takarada, T., Ogita, K., and Yoneda, Y. (2012) Transferrin receptor-1 suppresses neurite outgrowth in neuroblastoma Neuro2A cells. Neurochem. Int. 60, 448–457