Objective: Cerebral cavernous malformation type III (CCM3) is associated with null mutations in programmed cell death 10 (PDCD10 or CCM3) and is characterized by vessel dilation and increased blood–brain barrier (BBB) permeability, leading to cerebral hemorrhage. Our lab demonstrated that the absence of CCM3 in a mouse brain microvascular endothelial cell (mBEC) line leads to increased connexin 43 (Cx43) expression, gap and tight junction (TJ) remodeling, and increased permeability. Absence of CCM3 also leads to increased expression of a 20 kDa Cx43 isoform (20-Cx43). Our study objectives include analyzing the role of 20-Cx43 in increased permeability and TJ complex reorganization in CCM3 knockdown (CCM3KD) mBECs and dissecting signaling pathways involved in 20-Cx43 expression.
Methods: The role of 20-Cx43 in brain endothelial barrier permeability was analyzed in CCM3KD mBECs, transfected with CCM3 siRNA, and mBECs overexpressing 20-Cx43. Morphological and functional alterations of CCM3KD and 20-Cx43-overexpressing mBECs were analyzed through immunoblotting and immunofluorescent staining, FRET analysis, and transendothelial electrical resistance (TEER) assays. To assess whether the 20-Cx43 is a product of cleavage or internal translation, CCM3KD mBECs were treated with Batimastat, an MMP inhibitor, or transfected with mitogen-activated protein kinase (MAPK)-interacting kinase (MNK) or mammalian target of rapamycin (mTOR) siRNA. Following inhibitor treatment or transfection, 20-Cx43 expression was determined through immunoblotting.
Results: 20-Cx43 overexpression causes increased brain endothelial permeability and TJ reorganization, as 20-Cx43-overexpressing mBECs had decreased Claudin-5 and zonula occludens-1 (ZO-1) expression, disrupted ZO-1 incorporation into TJ complexes, and decreased Claudin-5/ZO-1 interaction. Treatment with the MMP inhibitor did not decrease 20-Cx43 expression in CCM3KD cells, indicating that 20-Cx43 is not a cleavage product. 20-Cx43 expression, however, was altered when signaling pathways regulating internal translation were inhibited. While inhibiting mTOR signaling upregulates 20-Cx43 expression, MNK inhibition decreases 20-Cx43 expression, suggesting 20-Cx43 is an internal translation product.
Conclusion: Generated through internal translation, 20-Cx43 is directly involved in TJ complex reorganization and brain endothelial barrier permeability in CCM3KD mBECs.

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Contrasting intranasal IgG delivery to the CNS in wild-type and transgenic mice

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Objective: Intranasal route can non-invasively transport IgG antibodies to the central nervous system (CNS) via olfactory and trigeminal nerve pathways in the nasal mucosae (1,2), bypassing CNS barriers to access perivascular spaces around cerebral blood vessels (3). Here, we investigated the variations in intranasal (IN) antibody distribution in the CNS with time and effect on pathology which remain largely undiscussed. We further examined expression differences of neonatal Fc receptor (FcRn) on primary cell cultures and brain sections of rats, mice and transgenic murine models.
Methods: Radiolabeled IgG was intranasally applied to similar-aged wild-type C57BL6/J (WT) and transgenic APPswe/PS1dE9 (AD) mice over 30 min or 6 h. A separate group of AD mice were subjected to 14 weeks of IN exposure of a targeted antibody (6E10). Animals were euthanized at 30 min/6 h/15 weeks post first IN dose by saline perfusion. Whole brains from acute and semi-chronic IgG exposure were post-fixed and processed for gamma-counting/autoradiography/immunohistochemistry (IHC). Hemibrains from chronic IgG exposure were either post-fixed for sectioning and Thioflavin-S (ThioS) staining or processed for ELISA. Images were quantified using a custom Fiji/ImageJ program. Cell cultures underwent IHC and fluorescence imaging to validate anti-FcRn antibodies (1G3, M-255, H-4) prior to ex vivo IHC.
Results: Rapid IgG exposure was observed in WT and AD cortical regions, albeit higher for AD mice. IgG exposure spread to subcortical regions over 6 h. Chronic IN exposure of a targeted IgG led to a significant reduction in Thioflavin-S staining in the olfactory bulb and frontal cortex regions. No significant differences were observed in Aβ levels with ELISA. In the parenchyma, FcRn immunoreactivity was mostly neuronal and astrocytic in rats, non-neuronal, non-astrocytic in WT mice and perivascular in AD mice. In vitro FcRn immunolabeling was negative for rat and mouse endothelial cells, negative for mouse astrocytes and inconclusive for rat astrocytes.
Conclusion: IgG distribution in the murine CNS increases with time and potentially leads to reduction in pathology via target engagement and redistribution. IgG exposure can be variable across species and transgenic models, potentially due to the alteration of Fc receptors in the CNS
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A36

Decreased migration of Mycobacterium-infected dendritic cells facilitates cellular aggregate formation and bacterial dissemination in an in vitro model of the blood brain barrier

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Objective: Mycobacterium tuberculosis (Mtb) can disseminate from the lung to the central nervous system (CNS) leading to tuberculous meningitis, which causes serious neurological damage with a high mortality rate. The mechanism of bacterial dissemination into the CNS is not known. Previously we showed that infected dendritic cells (DCs) mediate systemic dissemination and subsequent granuloma formation of Mycobacterium bovis bacillus Calmette-Guérin (BCG) and Mtb. The objective of this study was to test the role of mycobacterium-infected DC driven cell aggregation and bacterial dissemination across the BBB in vitro.
Methods: Primary bone marrow-derived DCs from CD11c-eYFP mice were used to study the migration capacity of mycobacterium-infected DCs in real time with fluorescent confocal microscopy. To investigate the dissemination of BCG and Mtb into the brain we developed a unique in vitro granuloma model combined with an in vitro double BBB co-culture model consisting of primary mouse brain endothelial cells and astroglia. In this model, BCG or Mtb-infected DCs were co-cultured with peripheral blood mononuclear cells (PBMCs) isolated from P25 Mycobacterium antigen-specific T-cell receptor transgenic mice. To reveal the mechanism of cell migration and cellular aggregate formation on brain endothelial cells iNOS, MMP9 and TNFα-KO mice were used.
Results: First we show that BCG infection decreases the migration capacity of DCs. Co-culture of mycobacterium-infected DCs and P25 transgenic mouse-derived PBMCs leads to cellular aggregate formation on brain endothelial cells which facilitate transmigration of infected cells across the BBB. Barrier properties of the BBB are impaired in the vicinity of cell clusters and endothelial ICAM-1 and VCAM-1 adhesion molecule expression is elevated. We revealed a double role of iNOS in the system: it causes BBB damage through reactive oxygen stress but by itself it slows down DC cell migration through the endothelial monolayer.

Conclusion: This novel and unique in vitro BBB-granuloma combined model suggests that mycobacterium-infected DC induced cell aggregation on the surface of brain vessels promotes Mtb dissemination into the CNS.
Grant Support: NIH grants 3R01NS076946; R01HL128778-01; HC128778-01 and T32GM081061.

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A37

Dendrimers-new hope for the treatment of Alzheimer's disease

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Objective: The lack of effective treatment for Alzheimer's disease (AD) stems mainly from the incomplete understanding of AD causes. Currently there are several hypothesis which try to explain the early molecular mechanisms of AD pathogenesis.
Methods: Considering that AD is a multi-factorial disease with several pathogenic mechanisms and pathways, a multifunctional nanotechnology approach may be needed to target its main molecular culprits. To very early diagnosis of AD we need to have an affordable, ultrasensitive and selective molecular detection methods. Ultra-low concentrations of protein biomarkers (e.g. ADDL-amyloid-Beta-derived diffusible ligands) which have been implicated in the pathogenesis of AD, is possible to detect, owing to carrier dendrimers -polymeric molecules chemically synthesized with well-defined shape size and nanoscopic physicochemical properties reminiscent of proteins
Results: Our studies have revealed that dendrimers have ability to prevent aggregation and fibrillation of proteins involved in AD. Some of dendrimers were demonstrated to cross blood–brain barrier, which legitimized research research on these compounds as potential drugs for neurological disorders like AD. Recent our studies have revealed that dendrimers possess the intrinsic ability to localize in cells associated with neuroinflammation (activated microglia and astrocytes) and thus can be used in neuroinflammation therapy
Conclusion: Above/mentioned findings may be significance in the context of potential application of dendrimers as drug carriers or active compounds per se. According to opinion the authors of this presentation, they are promising macromolecules for further investigations on their applicable in neurodegenerative disorders, for instance AD
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A38

Design of experiments: an efficient method to guide sub-culture and maturation of human stem cell-derived brain microvascular endothelial cells

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Objective: To optimize post-differentiation freeze, thaw, and culture conditions for induced pluripotent stem cell-derived brain microvascular endothelial cell (iPSC-BMEC) maturation, functionality, and reproducibility.
Methods: Human iPSCs were spontaneously differentiated into BMECs (protocol based on Lippmann et al. Nat Biotech 2012). Dissociation and sub-culture conditions were investigated using Design of Experiments (DoE), an unbiased method based on structured statistical analysis of variance (ANOVA). DoE efficiently identifies interactions amongst experimental variables and predicts responses. Our design focused on maximizing trans-endothelial electrical resistance (TEER). We incorporated a Mantis liquid handler to execute precise culture conditions in 96-well transwell systems and measured TEER over time. Top hit conditions were validated on multiple batches of iPSC-BMECs, these included post-thaw cell viability, hourly TEER reads, and immunocytochemistry (ICC).
Results: We have identified a robust cryopreservation method, time course and medium formulation for sub-culturing spontaneously differentiated iPSC-BMEC. The optimized cryopreservation protocol yields high cell viability recovery allowing the ability to produce bulk batches of iPSC-BMECs, thereby minimizing interexperimental variability. Surprisingly, we found that the removal of retinoic acid and the extension to at least 7 days in culture reproducibly resulted in prolonged high TEER, approximately 500 ohms * cm^2 greater than TEER peak at 48 h, in iPSC-BMEC mono-culture transwells. Our sub-culture method generates iPSC-BMECs that express the endothelial surface marker PECAM1, relevant blood–brain barrier (BBB) tight junction proteins (claudin-5, ZO-1, occludin), and transporters enriched on brain microvessels (Glut-1, transferrin receptor, insulin receptor).
Conclusion: We demonstrate the power of applying DoE to fine-tune complex culture conditions to maximize cell performance. Our cryopreservation and sub-culture protocols robustly produce functional endothelial cells of the BBB that are suited to address basic cerebral vascular biology questions, study the vascular phenotype of neurological disorders, and enable high-throughput screening for drug discovery.

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Developing a human in vitro blood–brain barrier model for predictive safety

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The blood brain barrier (BBB) protects the brain from toxic agents in the blood(Abbott and Friedman 2012). From a drug safety perspective, disruption to the BBB integrity or functionality by a therapeutic agent has been shown to be associated with the risks of neuroinflammation and neurodegeneration. For example, the administration of the CAR-T therapy has been shown to potentially increase BBB permeability, a risk factor for neurotoxicity (Gust, Hay et al. 2017). Therefore, it is important to have a functional in vitro human BBB model to understand and assess the effects of a drug candidate which potentially trigger immune cell infiltration and direct interactions of the endothelial cells in the BBB.
We have developed a human in vitro BBB model from the differentiation of human pluripotent stem cells (hiPSCs). When compared with the primary human brain endothelial cells, the human iPSC derived BBB model showed robust structural and functional characteristics. The in vitro BBB model exhibited barrier integrity through sustained trans endothelial electrical resistance (TEER) greater than 1500 Ohms. cm2, closely reflecting the in vivo BBB.
We are further investigating the effects of normoxia and hypoxia conditions, on the functionality and integrity of the BBB. Effects of inflammatory cytokines on BBB equilibrium is also being tested. The development of the human in vitro BBB model will provide a valuable tool to study drug related disruption of BBB and help in selecting compounds that are safe to BBB function.

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