Abstracts of papers presented at the 2010 meeting on BLOOD-BRAIN BARRIER

December 8–December 11, 2010

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Cover: Aquaporin 4+ Astrocyte endfeet (red) contacting blood vessels (green).
<table>
<thead>
<tr>
<th>Day</th>
<th>Time</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wednesday</td>
<td>7:30 pm</td>
<td>Opening Remarks</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 Modeling of the BBB</td>
</tr>
<tr>
<td>Thursday</td>
<td>9:00 am</td>
<td>2 Model Organisms for BBB Analysis</td>
</tr>
<tr>
<td>Thursday</td>
<td>2:00 pm</td>
<td>Keynote Speaker</td>
</tr>
<tr>
<td>Thursday</td>
<td>3:00 pm</td>
<td>3 Poster Session</td>
</tr>
<tr>
<td>Thursday</td>
<td>4:30 pm</td>
<td>Wine and Cheese Party</td>
</tr>
<tr>
<td>Thursday</td>
<td>7:30 pm</td>
<td>4 Cellular and Molecular Regulation of the BBB</td>
</tr>
<tr>
<td>Friday</td>
<td>9:00 am</td>
<td>5 Angiogenesis and BBB Development</td>
</tr>
<tr>
<td>Friday</td>
<td>2:00 pm</td>
<td>6 Disease States of the BBB</td>
</tr>
<tr>
<td>Friday</td>
<td>5:00 pm</td>
<td>Keynote Speaker</td>
</tr>
<tr>
<td>Friday</td>
<td>6:00</td>
<td>Banquet</td>
</tr>
<tr>
<td>Saturday</td>
<td>9:00 am</td>
<td>7 Mechanisms of BBB Crossing</td>
</tr>
</tbody>
</table>

Mealtimes at Blackford Hall are as follows:
- Breakfast  7:30 am-9:00 am
- Lunch      11:30 am-1:30 pm
- Dinner     5:30 pm-7:00 pm

Bar is open from 5:00 pm until late
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PROGRAM

WEDNESDAY, December 8—7:30 PM

OPENING REMARKS
Richard Daneman

SESSION 1  MODELING OF THE BBB

Chairpersons:  E. Shusta, University of Wisconsin-Madison
               M. Jacobson, University of California, San Francisco

Human blood-brain barrier endothelial cells derived from pluripotent stem cells
Eric V. Shusta, Ethan S. Lippmann, Samira M. Azarin, Sean P. Palecek.
Presenter affiliation: University of Wisconsin, Madison, Wisconsin.  1

Development of genetic methods to image dynamic structural and functional changes in the mammalian blood brain barrier in health and disease
Dritan Agalli, Axel Nimmerjahn, Ahmet Arac, Mark Schintzer, Ben A. Barres.
Presenter affiliation: Stanford University, Stanford, California.  2

Wnt signaling in the development of BBB models in vitro
Roberta Paolinelli, Monica Corada, Noemi Rudini, Luigi Maddaluno, Maria Grazia Lampugnani, Elisabetta Dejana.
Presenter affiliation: IFOM, Milan, Italy.  3

Targeted blood-brain barrier disruption in nonhuman primates using focused ultrasound and microbubbles
Nathan McDannold, Costas D. Arvanitis, Natalia Vykhodtseva, Margaret S. Livingstone.
Presenter affiliation: Brigham & Women's Hospital, Harvard Medical School, Boston, Massachusetts.  4

Predicting small molecule permeation across the blood-brain barrier
Matthew P. Jacobson.
Presenter affiliation: UCSF, San Francisco, California.  5
Inverse relationship between affinity and brain uptake of receptor-mediated transcytosis targeting antibodies
Joy Yu, Yin Zhang, Jasvinder Atwal, Yan Wu, Kwame Hoyte, Yanmei Lu, Margaret Kenrick, Saileta Prabhu, Ryan J. Watts, Mark Dennis.
Presenter affiliation: Genentech, Inc., South San Francisco, California.

Imaging *Borrelia* in a murine model
Alexandru Movila, Star M. Dunham-Ems, Melissa J. Caimano, Nico van Rooijen, Justin D. Radolf, Ute Frevert.
Presenter affiliation: NYU School of Medicine, New York, New York.

THURSDAY, December 9—9:00 AM

SESSION 2  MODEL ORGANISMS FOR BBB ANALYSIS

Chairpersons:  I. Blasig, Leibniz-Institut for Molekulare Pharmakologie, Berlin, Germany  
H. Gelbard, University of Rochester Medical Center, New York

Establishment of a neuroepithelial barrier by claudin-5a is essential for zebrafish brain ventricular lumen expansion
Ingolf E. Blasig.
Presenter affiliation: FMP, Berlin-Buch, Germany.

A genetic approach for the organization and function of the blood-brain barrier in *Drosophila*.
Hiroshi Kanda, Rieko Shimamura, Hideyuki Okano.
Presenter affiliation: Keio University School of Medicine, Tokyo, Japan.

Developing transgenic zebrafish for high-throughput genetic and small molecule screening of the blood-brain and blood-CSF barriers
Chaithanyarani Parupalli, Robyn A. Umans, Hannah E. Henson, Bensheng Ju, Michael R. Taylor.
Presenter affiliation: St. Jude Children's Research Hospital, Memphis, Tennessee.

The cAMP effector PKA mediates GPCR signaling in blood-brain barrier development in *Drosophila*
Xiaoling Li, Ulrike Gaul.
Presenter affiliation: University of Munich, Munich, Germany; The Rockefeller University, New York, New York.
Development of centrally acting novel kinase inhibitors for neuroAIDS
Presenter affiliation: University of Rochester Medical Center, Rochester, New York

Group B Streptococcus exploits integrin machinery for activation of neutrophil signaling pathways and efficient blood-brain barrier penetration
Anirban Banerjee, Ellese Carmona, Michael A. Gurney, Christopher Carlos, Kelly S. Doran.
Presenter affiliation: San Diego State University, San Diego, California.

Ecr4 gene in the central nervous system—A role for augurin in neuroprogenitor cell growth and CSF hydrodynamics
Presenter affiliation: University of California-San Diego, San Diego, California.

THURSDAY, December 9—2:00 PM
KEYNOTE SPEAKER
“Central nervous system infection and blood-brain barrier (BBB)—Lessons learned from E. coli translocation of the BBB”
Kwang Sik Kim
Johns Hopkins Children’s Center

THURSDAY, December 9—3:00 PM
SESSION 3 POSTER SESSION

Transcriptional profiling of the blood-brain barrier
Presenter affiliation: University of California, San Francisco, California.
Pericytes regulate blood-brain barrier formation during embryogenesis
R Daneman, L Zhou, A A. Kebede, B A. Barres.
Presenter affiliation: University of California, San Francisco, California.

Moody signaling in the blood brain barrier is required for Drosophila male courtship behavior
Valbona Hoxha, Brigitte Dauwalder.
Presenter affiliation: University of Houston, Houston, Texas.

Molecular imaging of drug penetrance through the blood-brain barrier using MALDI mass spectrometry
Murat N. Karabacak, Charles D. Stiles, Nathalie Y. Agar.
Presenter affiliation: Brigham and Women's Hospital and Dana Farber Cancer Institute, Harvard Medical School, Boston, Massachusetts.

Chemokine receptor modulation during leukocyte transmigration across a blood-brain barrier model in vitro—Effect of shear forces
Shumei Man, Barbara Tucky, Anne Cotleur, Richard M. Ransohoff.
Presenter affiliation: The Cleveland Clinic, Cleveland, Ohio.

Transcriptome analysis of the ependymal barrier during murine neurocysticercosis
Pramod K. Mishra, Judy M. Teale.
Presenter affiliation: University of Texas Health Science Center at San Antonio, San Antonio, Texas.

RAF/MEK/ERK signalling controls peripheral nerve regeneration switching Schwann cell state in vivo
Presenter affiliation: University College London, London, United Kingdom.

Tight junction complex modification at the blood-brain barrier in TBI—Mechanisms of injury
Presenter affiliation: Temple University School of Medicine, Philadelphia, Pennsylvania.

Mechanisms of endothelial barrier penetration by Listeria monocytogenes
Michelle Rengarajan, Julie A. Theriot.
Presenter affiliation: Stanford University, Stanford, California.
Expression of Toll-like receptors in the choroid plexus after neonatal hypoxia-ischemia in mice
Linnea Stridh, Carina Mallard.
Presenter affiliation: Gothenburg University, Gothenburg, Sweden.

Brain microvascular endothelial cell hyperpermeability induced by caspase-3 activation
Binu Tharakan, Devendra A. Sawant, Felicia A. Hunter, Ed W. Childs.
Presenter affiliation: Texas A&M Health Science Center College of Medicine & Scott and White Hospital, Temple, Texas.

THURSDAY, December 9—4:30 PM
Wine and Cheese Party

THURSDAY, December 9—7:30 PM

SESSION 4 CELLULAR AND MOLECULAR REGULATION OF THE BBB

Chairpersons: E. Dejana, Fondazione IFOM, Milano, Italy
A. Reijerkerk, VU University Medical Center, Amsterdam, the Netherlands

Elisabetta Dejana.
Presenter affiliation: Fondazione IFOM, Milano, Italy.

Pericytes are important for the blood-brain barrier integrity in vivo
Annika Armulik, Maarja Mäe, Guillem Genové, Maya H. Nisancioglu, Bengt R. Johansson, Christer Betsholtz.
Presenter affiliation: Karolinska Institute, Stockholm, Sweden.

Regulation of endothelial barrier maintenance at the blood-brain barrier
Marco Reis, Cathrin J. Czupalla, Nicole Ziegler, Kavi Devraj, Sascha Seidel, Sonja Thom, Ernesto Bockamp, Stefanie Dimmeler, Karl H. Plate, Stefan Liebner.
Presenter affiliation: Goethe University Clinic, Frankfurt/Main, Germany.
Ontogeny and the effects of exogenous and endogenous glucocorticoids on the blood-brain barrier of sheep

Barbara S. Stonestreet
Presenter affiliation: Women & Infants Hospital of Rhode Island, Providence, Rhode Island; The Warren Alpert Medical School of Brown University, Providence, Rhode Island.

microRNAs control brain endothelial cell barrier function

Arie Reijerkerk, Reuven Agami, Alex Prat, Anton J. van Zonneveld, Anton J. Horrevoets, Helga E. de Vries.
Presenter affiliation: VU University Medical Center, Amsterdam, Netherlands.

Role of microRNAs in inflammation at the blood brain barrier

Presenter affiliation: The Open University, Milton Keynes, United Kingdom.

Monoclonal antibody binding to unique structures (loops D and F) of BACE1 inhibits β-secretase activity in vivo

Presenter affiliation: VIB, Leuven, Belgium; KULeuven, Leuven, Belgium.

FRIDAY, December 10—9:00 AM

SESSION 5 ANGIOGENESIS AND BBB DEVELOPMENT

Chairpersons: N. Saunders, University of Melbourne, Parkville, Australia
C. Gu, Harvard Medical School, Boston, Massachusetts

How well is the internal environment of the brain controlled in the embryo?

Norman R. Saunders.
Presenter affiliation: University of Melbourne, Parkville, Australia.

Molecular mechanisms of CNS angiogenesis and barrier genesis

Stephen J. Tam, David Richmond, Josh Kaminker, Zora Modrusan, Baby Martin-McNulty, Nick van Bruggen, Marc Tessier-Lavigne, Watts J. Ryan.
Presenter affiliation: Genentech Inc., South San Francisco, California.
Macromolecular transfer across the blood-CSF interface
Shane A. Liddelow, Katarzyna M. Dziegielewska, Natassya M. Noor, Norman R. Saunders.
Presenter affiliation: University of Melbourne, Parkville, Australia.

Loss of αvβ8-mediated TGFβ activation, and subsequent loss of TGFβ signaling in endothelial cells or pericytes, causes vascular dysplasia and germinal matrix hemorrhage in mice
Thomas Arnold, Louis Reichardt, Julie Siegenthaler.
Presenter affiliation: University of California-San Francisco, San Francisco, California.

Vascular patterning and blood-brain barrier formation during development
Ayal Ben-Zvi, Sarah Pease, Chenghua Gu.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts.

Analysis of integrin-mediated adhesion and signaling pathways in neurovascular biology
Joseph H. McCarty, Aaron K. Mobley.
Presenter affiliation: MD Anderson Cancer Center, Houston, Texas.

Notch gene can convert veins to arteries and sustain conduit vessel structure in mice
Patrick A. Murphy, Tyson N. Kim, Gloria Lu, Andrew W. Bollen, Chris B. Schaffer, Rong A. Wang.
Presenter affiliation: University of California-San Francisco, San Francisco, California.

FRIDAY, December 10—2:00 PM

SESSION 6 DISEASE STATES OF THE BBB

Chairpersons: K. Akassoglou, Gladstone Institutes/UCSF, San Francisco, California
A. Flugel, University Medical Center Göttingen, Germany

Fibrinogen in neuroinflammatory disease
Katerina Akassoglou.
Presenter affiliation: Gladstone Institutes, University of California, San Francisco, California.
Paracellular diffusion and tight junctions are better preserved after neonatal than after adult acute stroke
Joel Faustino, Nikita Derugin, Michael Wendland, Baomei Liu, Zinaida Vexler.
Presenter affiliation: University California San Francisco, San Francisco, California.

Expression of the multi-drug resistant efflux transporter P-glycoprotein increases in spinal cord astrocytes throughout disease progression in the ALS mouse
Dena Jacob, Michael Jablonski, Alexey Bogush, Michael Goffredo, Piera Pasinelli, Davide Trotti.

Impairment of BBB by exposure to neurotoxins—Effects of LPS and unconjugated bilirubin on rat brain microvascular endothelial cells
Filipa L. Cardoso, Szilvia Veszelka, Inês Palmela, Boglárka Csiszár, Ana R. Vaz, Andrea Tóth, Loránd Kiss, Dora Brites, Mária A. Deli, Maria A. Brito.
Presenter affiliation: iMed.UL, Lisbon, Portugal.

Alexander Flugel.
Presenter affiliation: University Medical Center Göttingen, Germany.

Tumor necrosis factor-α triggers a cytokine cascade yielding postoperative cognitive decline
Niccolo Terrando, Claudia Monaco, Marc Feldmann, Mervyn Maze.
Presenter affiliation: University of California San Francisco, San Francisco, California; Imperial College London, London, United Kingdom.

Gene expression analyses of pial vessels of the blood brain barrier during murine neurocysticercosis
Pramod K. Mishra, Judy M. Teale.
Presenter affiliation: University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Enhancement of blood brain barrier (BBB) is the most important factor in preventing animals from developing rabies
Zhen F. Fu, Hualei Wang.
Presenter affiliation: University of Georgia, Athens, Georgia; Huazhong Agricultural University, Wuhan, China.
FRIDAY, December 10—5:00 PM

KEYNOTE SPEAKERS

“Crossing biological barriers—Yeast and worm chemical biology”

Corey Nislow
University of Toronto

Guri Giaever
University of Toronto

FRIDAY, December 10

BANQUET

Cocktails 6:00 PM  Dinner 6:45 PM

SATURDAY, December 11—9:00 AM

SESSION 7  MECHANISMS OF BBB CROSSING

Chairpersons:  B. Engelhardt, University of Bern, Switzerland
               X. Nassif, INSERM U570, Hopital Necker Enfants Malades,
               Paris, France

The blood-brain and the blood-cerebrospinal fluid barriers
provide different molecular cues for T cell entry into the CNS
Britta Engelhardt.
Presenter affiliation: University of Bern, Switzerland. 46

Localised, inducible and size-selective modulation of neuronal
barriers—An AAV approach
Matthew Campbell, Marian Humphries, Anh Nguyen, Sophie Kiang,
Paul Kenna, Lawrence Tam, Jane Farrar, Pete Humphries.
Presenter affiliation: Trinity College Dublin, Dublin, Ireland. 47
A novel cannabinoid receptor-2 agonist attenuates leukocyte-endothelial interactions and blood-brain barrier (BBB) dysfunction under systemic inflammatory conditions
János Haskó, Ming Zhang, Ronald Tuma, Servio H. Ramirez, Yuri Persidsky.
Presenter affiliation: Temple University School of Medicine, Philadelphia, Pennsylvania; Biological Research Center, Szeged, Hungary. 48

Lipoprotein particles cross the blood brain barrier in Drosophila
Marko Brankatschk, Wilhelm Palm, Suzanne Eaton.
Presenter affiliation: MPI-CBG, Dresden, Germany. 49

Neisseria meningitides, a bacterial pathogen, crosses the blood brain barrier by hijacking the b2-adrenoceptor-b-arrestin pathway
Mathieu Coureuil, Xavier Nassif, Stefano Marullo.
Presenter affiliation: INSERM U1002, Paris, France. 50

Diagnostic ultrasound and SonoVue open the blood-brain barrier and its effect on cognitive function.
Lei Zhang, Qiang Wang, Xiaodong Zhou, Haili Su, Zan Zhang, Shiquan Wang, Lize Xiong
Presenter affiliation: Xijing Hospital of the Forth, Xi'an, China. 51

Physiology-based screens for small molecules that alter metabolism and target drug permeation into the brain
Presenter affiliation: University of California-San Francisco, San Francisco, California. 52
AUTHOR INDEX

Agalliu, Dritan, 2, 15
Agami, Reuven, 29
Agar, Nathalie Y., 18
Akassoglou, Katerina, 39
Annaert, Wim, 31
Arac, Ahmet, 2
Armulik, Annika, 26
Arnold, Thomas, 35
Arvanitis, Costas D., 4
Ashraf, Kaveh, 52
Atwal, Jasvinder, 6
Azarin, Samira M., 1
Bainton, Roland J., 52
Baird, Andrew, 14
Baker, David, 30
Banerjee, Anirban, 13
Barres, Ben A., 2, 15, 16
Ben-Zvi, Ayal, 36
Betsholtz, Christer, 26
Blasig, Ingolf E., 8
Bockamp, Ernesto, 27
Bogush, Alexey, 41
Bollert, Andrew W., 38
Brankatschk, Marko, 49
Brites, Dora, 42
Brito, Maria A., 42
Cahoy, John D., 15
Caimano, Melissa J., 7
Campbell, Matthew, 47
Cardoso, Filipa L., 42
Carlos, Christopher, 13
Carmona, Ellese, 13
Chávez-Gutiérrez, Lucia, 31
Childs, Ed W., 25
Corada, Monica, 3
Cotleur, Anne, 19
Coureuil, Mathieu, 50
Csiszár, Boglárka, 42
Czupalla, Cathrin J., 27
Daneman, Richard, 15, 16
Dauwalder, Brigitte, 17
De Strooper, Bart, 31
de Vries, Helga E., 29
Dejana, Elisabetta, 3
Deli, Mária A., 42
Dennis, Mark, 6
Derugin, Nikita, 40
DeSalvo, Michael K., 52
Devraj, Kavi, 27
Dewhurst, Stephen, 12
Dimmeler, Stefanie, 27
Doran, Kelly S., 13
Dunham-Ems, Star M., 7
Dziegielewska, Katarzyna M., 34
Eaton, Suzanne, 49
Eliceiri, Brian P., 14
Elliot, Melanie B., 22
Engelhardt, Britta, 46
Farrar, Jane, 47
Faustino, Joel, 40
Feldmann, Marc, 43
Frevert, Ute, 7
Fu, Zhen F., 45
Gallo, Jack I., 22
Gaul, Ulrike, 11
Gelbard, Harris A., 12
Genové, Guillaume, 26
Goffredo, Michael, 41
Gonzalez, Ana, 14
Goodfellow, Val, 12
Gu, Chenghua, 36
Gurney, Michael A., 13
Harrisingh, Marie, 21
Haskó, János, 48
Henson, Hannah E., 10
Hirst, Mark C., 30
Horevets, Anton J., 29
Hoxha, Valbona, 17
Hoyte, Kwame, 6
Humphries, Marian, 47
Ribeiro, Sara, 21
Richmond, David, 33
Romero, Ignacio A., 30
Rosenberg, Laura, 21
Rudini, Noemi, 3
Ryan, Watts J., 33
Saunders, Norman R., 32, 34
Sawant, Devendra A., 25
Schaffer, Chris B., 38
Schintzer, Mark, 2
Seidel, Sascha, 27
Sharrack, Basil, 30
Shimamura, Rieko, 9
Shusta, Eric V., 1
Siegenthaler, Julie, 35
Stiles, Charles D., 18
Stonestreet, Barbara S., 28
Stopa, Edward, 14
Stridh, Linnea, 24
Su, H., 51

Tam, Lawrence, 47
Tam, Stephen J., 33
Taylor, Michael R., 10
Teale, Judy M., 20, 44
Terrando, Niccolò, 43
Tessier-Lavigne, Marc, 33
Tharakan, Binu, 25
Theriot, Julie A., 23
Thom, Sonja, 27
Tóth, Andrea, 42
Trotti, Davide, 41
Tucky, Barbara, 19
Tuma, Ronald, 48

Umans, Robyn A., 10

van Bruggen, Nick, 33
van Rooijen, Nico, 7
van Zonneveld, Anton J., 29
Vaz, Ana R., 42
Veszelka, Szilvia, 42
Vexler, Zinaida, 40
Vykhodtseva, Natalia, 4

Wang, Hualei, 45
The blood-brain barrier (BBB) plays an important role in brain health and disease by selectively trafficking compounds to and from the brain across its endothelial interface. For decades, researchers have cultured brain microvascular endothelial cells (BMECs) ex vivo to serve two main purposes: to elucidate the molecular mechanisms responsible for BBB differentiation and regulation, and to establish a platform for high throughput screening of brain-penetrating pharmaceuticals. Unfortunately, while much success has been achieved in the isolation and in vitro culture of animal BMECs, progress in the establishment of a robust human in vitro BBB model has been more limited. Human BMEC platforms either possess poor barrier properties that do not appropriately represent the BBB or are derived from sources with insufficient yield to allow widespread use of the model. As such, we have devised a novel method for high-yield differentiation of human pluripotent stem cells to endothelial cells with specific properties of the BBB, including selective responsiveness to astrocyte co-culture, high trans-endothelial electrical resistance, low passive permeability, and polarized transport protein activity. Importantly, we provide evidence that the BBB specification process occurs in concert with Wnt/β-catenin signaling, which is the pathway identified for BBB differentiation in transgenic rodent studies. Thus, this system has the potential to provide a robust human BMEC platform for high throughput screening of neuropharmaceuticals and will be useful for studies of BBB development and maturation that were previously intractable in humans.
DEVELOPMENT OF GENETIC METHODS TO IMAGE DYNAMIC STRUCTURAL AND FUNCTIONAL CHANGES IN THE MAMMALIAN BLOOD BRAIN BARRIER IN HEALTH AND DISEASE

Dritan Agalliu*¹, Axel Nimmerjahn*², Ahmet Arac³, Mark Schintzer², Ben A Barres¹

¹Stanford University, Neurobiology, Stanford, CA, 94305, ²Stanford University, Biological Sciences & Applied Physics, Stanford, CA, 94305, ³Stanford University, Neurosurgery, Stanford, CA, 94305

The endothelial cells in the Central Nervous System (CNS) form a barrier to blood-borne molecules that is essential for CNS function by three mechanisms: a) tight junctions (TJs); b) few caveolae with a low rate of transcytosis; and c) transporter systems. Abnormalities in blood brain barrier (BBB) permeability are found in many CNS diseases such as stroke or multiple sclerosis. However, it is unclear which structural components of the BBB are predominantly affected in these CNS diseases. The timing and extent of BBB structural changes during disease progression are also unknown.

To address these questions, we have generated two novel lines of transgenic mice that label two components necessary for BBB integrity namely, TJs and caveolae. These lines express in all endothelial cells either a fusion protein of eGFP with the TJ protein Claudin-5 or the red fluorescent protein pmKate2 with the caveolar transmembrane protein Caveolin-1. We have imaged TJs in the CNS endothelium of eGFP::Claudin-5 transgenic mice using a two-photon laser microscope, and combined this with fluorescent molecular tracers to correlate structural changes in TJ protein localization with diffusion of tracers across the BBB in healthy mice and those suffering from stroke. We have found that TJs are very stable structures in the CNS endothelium of healthy transgenic mice and the tracers are retained within the CNS capillaries. Surprisingly, although BBB functional integrity is impaired in transgenic mice that have undergone middle cerebral artery occlusion - a mouse model for stroke - as early as 12-14 hours after the disease, TJs seem very stable at least within 24-30 hours post-lesion at the ischemic core region by light microscopy. However, TJs were abnormal between 48-56 hours post stroke at the ischemic core region. We also plan to image pmKate2::Caveolin-1 transgenic mice to analyze changes in transcytosis rates across CNS capillaries in healthy and disease states. This will allow us to elucidate the mechanisms that impair BBB integrity during various CNS diseases and develop therapies to restore it.

*equal contribution
The blood brain barrier (BBB) maintains the homeostasis of the brain microenvironment, which is crucial for neuronal activity and function. The main cellular basis of its restrictive nature are the brain microvascular endothelial cells (BMECs), responsible for the transport of metabolites, precursors and nutrients from the blood to the brain, along with the association of other cell types of the neurovascular unit. BMECs are characterized by the absence of fenestrations, a low level of pinocytic vesicles and an elaborate junctional complex formed by both tight junctions (TJs) and adherens junctions (AJs). The Wnt canonical signaling pathway regulates fundamental aspects of development including cell fate specification, proliferation, survival and overall organogenesis. Endothelial Wnt/β-catenin signaling is one of the major pathway that regulates induction and maintenance of BBB characteristics during embryonic and postnatal development. Stabilization of β-catenin in vivo enhances BBB maturation by inducing expression of TJ protein Claudin3 (Cldn3), which is predominantly present in brain ECs, and down-regulation of the plasmalemma vesicle-associated protein (Plvap), which is usually only expressed on immature brain endothelium. Most in vitro BBB models proposed so far are based on primary co-cultures of brain ECs and astrocytes or glial cells. An easier and reliable in vitro model of BBB is still missing, limiting the possibility to perform drugs screenings able to cross or modify the barrier. An important advancement in the development of an alternative optimal and reproducible screening assay is the use of immortalized endothelial cell lines. We focused on a widely usable-in-the-field BBB in vitro system with no need of co-culture, by culturing freshly isolated brain endothelium (primary mouse endothelial cells-MBECs), or bEnd5, a polyoma middle T-oncogen-immortalized mouse brain endothelioma), in contact with Wnt3a, which acts through β-catenin, or with chemicals known to stabilize β-catenin such as inhibitors of glycogen synthase kinase-3β (GSK3β). Interestingly, these treatments resulted in the activation of β catenin signaling (Axin2 upregulation) and induction of barrier characteristics (Cldn3 upregulation and Plvap downregulation). Importantly, as readout of BBB properties, we have defined an identikit of the most important genes known to be expressed in BMECs. Our models will be instrumental not only for further studies on the molecular mechanisms which induce BBB properties, but also for the screening of agents and drugs to modify BBB permeability.
TARGETED BLOOD-BRAIN BARRIER DISRUPTION IN NONHUMAN PRIMATES USING FOCUSED ULTRASOUND AND MICROBUBBLES

Nathan McDannold¹, Costas D Arvanitis¹, Natalia Vykhodtseva¹, Margaret S Livingstone²

¹Brigham & Women's Hospital, Harvard Medical School, Radiology, Boston, MA, 02115, ²Harvard Medical School, Neurobiology, Boston, MA, 02115

Small animal experiments have shown that ultrasound bursts combined with a microbubble agent can locally and temporarily disrupt the blood-brain barrier (BBB), providing a potential noninvasive means for targeted drug delivery in the CNS. This work was a safety study of targeted BBB disruption (BBBD) with a clinical MR-guided focused ultrasound (MRgFUS) system in nonhuman primates.

Methods: Our Institutional Animal Care and Use Committee approved the experiments. Sonication was performed transcranially in 4 anesthetized adult male rhesus macaques with a clinical MRgFUS system (ExAblate 4000 brain, InSightec), which uses a 1024-channel phased array (30 cm hemisphere; 220 kHz) integrated with a 3T MRI. Multiple locations in each brain were targeted via electronic beam steering with 70s sonications (10 ms bursts; 1 Hz PRF). In one animal, the beam was steered dynamically during sonication to disrupt a ~1 cm³ volume with a single exposure. At the start of each sonication, ultrasound contrast agent (Definity, 10 µl/kg) was injected intravenously. BBBD was evaluated in contrast-enhanced T1-weighted (Magnevist, 0.2 ml/kg). The presence of edema and erythrocyte extravasation was evaluated in T2 and T2*-weighted imaging, respectively. Targets included the thalamus, putamen, cerebellum, cortex, and white matter. In this preliminary parametric study, acoustic power ranged from 0.5-10 W.

Results and Conclusions: BBBD was observed at 27/38 targeted locations. Eleven sonications at 0.5-1W in one animal did not produce BBBD; sonication a week later at different targets at 1.5-2W resulted in BBBD. Changes in T2/T2*-weighted imaging, suggesting tissue damage, were evident for sonication at 4W and above (N=6), and for two locations (1.5, 2W) in the thalamus. BBBD without MRI-evident damage was achieved in 19 locations (1-3W). In one location targeted at 10W, secondary damage was evident between the target and the transducer. Otherwise, the sonication effects appeared to be fully contained to the target location and did not appear to affect any other brain region. Targeting multiple overlapping locations via dynamic steering produced contiguous volumes of BBBD.

Overall, these data demonstrate that it is possible to use a clinical MRgFUS system for targeted BBBD in multiple brain structures in a large animal model without producing other MRI-evident effects on surrounding tissues. Future work will evaluate histological and functional effects produced by these exposures and investigate methods to guide the procedure.
PREDICTING SMALL MOLECULE PERMEATION ACROSS THE BLOOD-BRAIN BARRIER

Matthew P Jacobson

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I will describe my group's efforts to model key processes underlying the partitioning of drugs across the blood-brain barrier. The first key process is passive permeation across membranes, and I will discuss the key physical aspects of this process and how they can be computed, and a hypothesis that the lipid composition in the blood-brain barrier may account for differences in passive membrane permeability relative to, e.g., intestinal membrane permeation. The second key process is active efflux by P-glycoprotein. We are exploiting the recently published structures of P-gp to better understand the physico-chemical determinants of this process and reconcile previously confusing structure-activity relationships. Finally I will discuss preliminary efforts to address other aspects of partitioning across the BBB, including serum protein binding and interactions with other transporters.
INVERSE RELATIONSHIP BETWEEN AFFINITY AND BRAIN UPTAKE OF RECEPTOR-MEDIATED TRANSCYTOSIS TARGETING ANTIBODIES

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Monoclonal antibodies have vast therapeutic potential for treatment of central nervous system (CNS) diseases, but their passage into the brain is restricted by the blood-brain barrier (BBB). Here we explore receptor-mediated transcytosis (RMT) pathways of brain endothelial cells as a potential mechanism to deliver antibodies across the BBB. We demonstrate that trace levels of anti-transferrin receptor (TfR) antibodies accumulate in the brain after a single systemic dose in mice; however, distribution of anti-TfR appears to remain localized to brain vasculature. At therapeutically relevant dose levels, however, we observe that antibody distribution after systemic administration changes from vascular to neuronal 24 hours after administration, suggesting that a significant amount of antibody had transcytosed through brain endothelial cells to reach the parenchyma. Additionally, we discovered that the magnitude of antibody uptake was inversely related to its binding affinity to TfR. This novel insight into the inverse relationship between affinity and brain uptake suggests that the ability of bound antibody to dissociate from the receptor is integral to its successful transcytosis through the vasculature. Proof of this BBB transport was achieved using a bispecific antibody that binds both TfR and the amyloid precursor protein (APP) cleavage enzyme, β-secretase (BACE1). Systemic administration of anti-TfR/BACE1 produced a greater than 5-fold increased accumulation in the brain, compared to monospecific anti-BACE1. Localization of anti-TfR/BACE1 was found to be distinctly neuronal 24 hours after injection. Given that inhibition of neuronal BACE1 activity has been shown to reduce amyloid beta production, this bispecific anti-TfR/BACE1 antibody introduces a promising and novel therapeutic candidate for the treatment of Alzheimer’s disease. Furthermore, this RMT-based bispecific targeting technology opens the door for a wide range of potential therapeutics for CNS diseases.
Intravital microscopy has had a major impact on our understanding of the behavior of a broad range of microorganisms within the intact host. By confirming some hypotheses, refuting others, and revealing unexpected new mechanisms, *in vivo* imaging has provided an entirely new insight into the dynamics of infectious disease pathogenesis. The mouse has been extensively used to study *Borrelia burgdorferi* induced heart and joint disease, but there is currently no reliable small animal model for neuroborreliosis. Under normal circumstances, spirochetes entering the bloodstream are rapidly eliminated by Kupffer cells, the resident macrophages of the liver. To extend *Borrelia* circulation within the cardiovascular system, thus enhancing exposure of the cerebral microvasculature to the spirochetes, we depleted mice of all hepatic and splenic macrophages with liposome-encapsulated clodronate prior to infection. Analysis of the integrity of the blood brain barrier revealed transient Evans blue leakage in clodronate liposome-treated infected mice; neither control liposome-treated infected mice nor clodronate liposome-treated uninfected mice exhibited vascular impairment. The effect of *B. burgdorferi* on the cortical microvasculature was studied by dynamic imaging of mice inoculated with fluorescent spirochetes and markers for inflammation and coagulation.

The work was supported by a grant from the Neurology Research Foundation
Establishment of a neuroepithelial barrier by claudin-5a is essential for zebrafish brain ventricular lumen expansion

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Lumen expansion driven by hydrostatic pressure occurs during many morphogenetic processes. It is well established that members of the claudin family of transmembrane tight junction proteins determine paracellular tightness of epithelial and endothelial tissue barriers. However, functional evidence for a role of claudins in the morphogenesis of lumenized organs has been scarce. Here, we identify claudin-5a as a core component of an early cerebral-ventricular barrier system that is required for ventricular lumen expansion in the zebrafish embryonic brain before the establishment of the embryonic blood–brain barrier. Loss of claudin-5a or expression of a tight junction-opening claudin-5a mutant reduces brain ventricular volume expansion without disrupting the polarized organization of the neuroepithelium. Perfusion experiments with the electron-dense small molecule lanthanum nitrate reveal that paracellular tightness of the cerebral-ventricular barrier decreases upon loss of claudin-5a. Genetic analyses show that the apical neuroepithelial localization of claudin-5a depends on epithelial cell polarity and provide evidence for concerted activities between claudin-5a and Na,K-ATPase during luminal expansion of brain ventricles. These data establish an essential role of a barrier-forming claudin in ventricular lumen expansion, thereby contributing to brain morphogenesis.
A GENETIC APPROACH FOR THE ORGANIZATION AND FUNCTION OF THE BLOOD-BRAIN BARRIER IN DROSOPHILA

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In Drosophila, Blood-Brain Barrier (BBB) is provided by a type of glia, subperineurial glia (SPG), that form paracellular barrier through Septate Junctions (SJs) that have functional and molecular similarities to vertebrate Tight Junctions (TJs). It has also been revealed that Drosophila SPG also has the xenobiotic exclusion system which is achieved by conserved fly ATP-binding cassette (ABC) transporter(s). We thus aimed to use Drosophila as a model organism to study the conserved molecular mechanisms for regulating the BBB functions in vivo using its powerful genetics.

In order to address this issue, we have set up a genetic system with a combination of dye-injection assay to perform a large-scale screen for identifying novel genes that are involved in the regulation of the integrity of paracellular barrier function. In this system, double-stranded RNAs of single genes are overexpressed under the control of SPG-specific driver to induce the ‘BBB-specific RNAi’ in vivo. In our system, the knockdown of a G-protein coupled receptor, Moody, which is previously reported to be required for the integrity of SJs, or Kune-kune, a Drosophila Claudin family protein, resulted in the diffusion of dye into central nervous system. Among candidates identified in the screen, we have picked up several genes and reconfirmed that the mutant animals for these genes also showed the dye-penetration phenotype when they were examined the BBB function. We are currently further performing the screen, as well as studying the molecular functions of genes identified in it.
DEVELOPING TRANSGENIC ZEBRAFISH FOR HIGH-THROUGHPUT GENETIC AND SMALL MOLECULE SCREENING OF THE BLOOD-BRAIN AND BLOOD-CSF BARRIERS

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The field of brain barrier biology could greatly benefit from the creation of animal models suitable for high-throughput genetic and small-molecule screens. To address this problem, we are exploiting the strengths of zebrafish as a model organism. Zebrafish provide many advantages for the study of complex biological systems like the BBB and BCB. They are vertebrates; transparent; small; develop rapidly outside of the mother; and are amenable to high-throughput screening strategies. Our preliminary studies and recent work of others demonstrate that zebrafish possess the structural and functional properties of the brain barriers, further indicating the utility of this animal model. For this study, we are creating two transgenic zebrafish lines that “report” upon the developmental and functional status of the brain barriers in vivo. These transgenic lines will allow for rapid, in vivo examination of brain barrier integrity without having to inject, dissect, or sacrifice the animal. Thus, instead of analyzing a few animals at a time using antiquated techniques, we will be able to examine thousands of animals daily, significantly increasing the rate of discovery. Furthermore, we have generated an additional transgenic line that expresses GFP within the choroid plexus of the BCB. Our long-term goals are to use these transgenic zebrafish to screen for genes and small molecules that modulate brain barriers, ultimately leading to the discovery of drugs that allow controlled access of therapeutic agents into the brain.
The blood-brain barrier (BBB) of Drosophila is established by a thin layer of surface glia, which ensheath the nervous system and insulate it against the potassium-rich hemolymph by forming intercellular septate junctions (SJs). We have previously identified Moody, a novel G protein-coupled receptor (GPCR), is required in the development and maintenance of the BBB. However, the precise mechanism underlying GPCR signaling during BBB development has remained obscure. Here we show that cAMP-dependent protein kinase A (PKA), a major effector of cAMP, is required for the insulation of the nervous system and plays a critical role in the Moody signaling pathway. At the cellular level, PKA not only regulates cell shape by reorganization of the actin and microtubule cytoskeleton, but also affects the expression and proper localization of SJ components to the interface with neighboring surface glia, which are required for effective insulation. Strikingly, the PKA catalytic subunit DC0 shows highly polarized localization in the surface glia, and both over- and underactivity of PKA result in severe insulation defects but totally opposite cell biological phenotype, indicating that the cAMP/PKA pathway acts downstream of Moody and produces a local signal within the cell. Our study demonstrates a novel role of cAMP/PKA signaling in the regulation of the intercellular SJs organization that generates BBB integrity, and provides new insights into the molecular and cellular mechanism of Moody-regulated BBB development and maintenance.
Combination antiretroviral therapy has dramatically changed the phenotype of HIV-associated neurocognitive disease (HAND), but has neither eradicated it or decreased its prevalence. Thus we hypothesize there is a reversible metabolic component of HAND with the molecular etiologic target of mixed lineage kinase type 3 (MLK3) that we can define in laboratory models, design rational adjunctive neuroprotective drugs for using a series of lead compounds (URMC-099c*), then test in our in vitro and in vivo models of NeuroAIDS, with the goal of advancing the most efficacious development compound to the FDA for IND filing. In previous studies, we have validated MLK3 as an enzyme pathologically activated by HIV-1 neurotoxins, then successfully developed a small molecule MLK3 inhibitor, URMC-099, capable of achieving therapeutic concentrations in the CNS. Using our industry partner to synthesize a series of small molecule MLK3 inhibitors with drug-like properties and a favorable profile for blood-brain barrier penetration, we have used URMC-099 and -099c* in our models of HAND to investigate their ability to reverse neuroinflammation and synaptotodendritic damage. Thus, we initially investigate URMC-099c* in in vivo models of HAND, using irradiation chimera of mice transgenic for XFP to identify both peripheral and central mononuclear phagocytes and neurons, while cerebral microvessels are identified by infusion of fluorophores. After defining pathologic phenotypes for peripheral and central immune effector cells inside and outside cerebral microvessels, as well as neuronal synapses, we quantitate the ability of URMC-099c* to ameliorate these phenotypes, accompanied by ex vivo or in vitro dissection of relevant signaling pathways upstream and downstream of MLK3. Ultimately, the most promising lead compound is further evaluated for neuroprotective efficacy in a humanized CD34+ murine model of HAND and finally in a rhesus macaque model of SIV. In aggregate, this defines a testing funnel for further structure activity relationship (SAR) work relevant to advancement of URMC-099c* to development compound status, with the goal of applying to the NIH Rapid Access for Interventional Development (RAID) program for good laboratory practice (GLP) scale-up and manufacture, as well as remaining safety and toxicity studies of our development compound, prior to filing an IND with the FDA.
GROUP B STREPTOCOCCUS EXPLOITS INTEGRIN MACHINERY FOR ACTIVATION OF NEUTROPHIL SIGNALING PATHWAYS AND EFFICIENT BLOOD-BRAIN BARRIER PENETRATION

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Group B Streptococcus (GBS) is the leading cause of meningitis in newborn infants and also increasingly associated with infections in adults. We have demonstrated previously that GBS clinical isolate NCTC10/84 express pili and that PilA, which is thought to be located at the tip of the pilus, contributes to GBS adherence to brain endothelium. In this study, we sought to examine the global transcriptional response of the human brain microvascular endothelial cells (hBMEC), the single cell layer which comprises the blood-brain barrier (BBB), to GBS PilA. Using microarray analysis we found that GBS infection of hBMEC resulted in a significant induction of genes acting to orchestrate neutrophil chemotaxis including IL-8, CCL-20, IL-6, CXCL-1 and CXCL-2. In contrast infection with an isogenic GBS PilA-deficient mutant resulted in a marked reduction in expression of these genes. These results were confirmed using real time RT-PCR and protein based assays. Recombinant PilA proteins purified from various GBS serotypes also elicited chemokine expression. Additionally, neutrophil chemotaxis to the site of infection in vivo was found to be dependent on PilA; decreased neutrophil recruitment and tissue damage in the brain were also observed in a murine model of hematogenous meningitis following infection with the PilA-deficient GBS strain. We further demonstrated that PilA binds to collagen, which acts as a molecular bridge, promoting GBS interaction with the α2β1 integrin expressed in BBB endothelium. This interaction involves focal adhesion kinase which results in both bacterial internalization and proinflammatory cytokine release. Our studies suggest that GBS PilA hijacks integrin machinery to promote efficient penetration of BBB endothelium and characteristic neutrophilic inflammation during acute bacterial meningitis.
ECRG4 GENE IN THE CENTRAL NERVOUS SYSTEM: A ROLE FOR AUGURIN IN NEUROPROGENITOR CELL GROWTH AND CSF HYDRODYNAMICS

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The choroid plexus (CP) is largely responsible for the content and composition of cerebrospinal fluid (CSF) either through the translocation of hormones from blood, or via their de novo synthesis in, and secretion by, choroid epithelia. Because these CSF hormones can potentially affect cell function throughout the central nervous system (CNS), it is critical to determine their role in normal CNS homeostasis. One newly recognized factor is augurin, a 14,000 Dalton secreted protein that is encoded by a candidate, and epigenetically regulated, tumor suppressor gene called esophageal cancer related gene-4 (ecrg4). Ecrg4 gene expression in the CP was studied by in situ hybridization and quantitative RT-PCR. Augurin was detected by immunoblotting, immunohistochemistry and ELISA. The biological consequence of transient augurin over- and under-production in the CNS was studied by transduction of CP epithelium and ependyma after intracerebroventricular injection of an adenovirus containing the ecrg4 gene and by gene knockdown in the developing Zebrafish, respectively. Gene expression analyses reveal that, in the CNS, ecrg4 mRNA predominantly localizes to choroid plexus epithelial (CPE), ventricular ependymal and aqueductal canal cells. This distribution was confirmed by immunohistochemical analyses quantitative PCR. Immunoblotting and ELISA show that transduced CPE cells synthesize and secrete augurin. Over expression of ecrg4 in vivo decreased subependymal progenitor cell proliferation that is observed after injury and its knock down in developing zebrafish embryos caused increased cell proliferation and dose-dependent ventriculomegaly that was reversible by co-injection of ECRG4 mRNA.
The blood-brain barrier (BBB) maintains brain homeostasis and limits the entry of toxins and pathogens into the brain. Despite its importance, little is known about the molecular mechanisms regulating its development and function. In this study we have developed novel methods to separate the cellular components of the BBB, brain endothelial cells and pericytes, allowing me to highly purify and gene profile these cells. By comparing the transcriptional profile of brain endothelial cells with those purified from the liver and lung, we have generated a comprehensive resource of transcripts that are specific to the BBB. Through this comparison we have identified novel tight junction molecules, transporters, metabolic enzymes, signaling components, and unknown transcripts that are expressed specifically by CNS endothelial cells. Wnt and RXRα signaling cascades are specifically enriched at the BBB, implicating these pathways in regulating this vital barrier. These endothelial and pericyte transcriptomes provide a useful resource for the neuroscience community to better understand brain angiogenesis and the blood-brain barrier.
Although the properties of the BBB are manifested within endothelial cells, transplantation studies have demonstrated that these properties are induced by signals from the microenvironment of the CNS. Due to the close spatial relationship between astrocytes and endothelial cells, astrocytes have been thought to induce the BBB during postnatal development, however the timing of BBB formation remains controversial. Here we demonstrate that the BBB is formed antigenically and functionally during early embryogenesis, days before glia are generated. This demonstrates that astrocytes are not involved in BBB induction. We further explore the role of CNS pericytes in regulating the barrier, examining BBB function, structure and gene expression in pericyte-deficient mice, as well as the ability of pericytes to induce barrier properties in endothelial cells in co-culture experiments. We find that pericytes are required for BBB formation during development, and that these cells regulate functional aspects of the BBB, including tight junction structure, rates of transcytosis, and limiting CNS immune infiltration. We further show that pericytes accomplish this not by inducing BBB-specific gene expression, but by inhibiting the expression of genes which increase the permeability of the CNS vessels.
MOODY SIGNALING IN THE BLOOD BRAIN BARRIER IS REQUIRED FOR DROSOPHILA MALE COURTSHIP BEHAVIOR

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Drosophila male courtship consists of a sequence of stereotyped steps of behavior that can be studied and quantified in the lab. It has been known for some time that specific regions in the brain of a male are important for successful courtship and that the cells in these regions are characterized by the presence of two major male regulators. However, we have recently shown that male factors produced by a non-neuronal secretory tissue that surrounds the brain (the fat body) are also required and that courtship is significantly compromised when these factors are missing. Efficient courtship may therefore require the interaction of endocrine factors with specific parts of the nervous system. However, it is unclear how these factors either pass through or communicate through the blood brain barrier (BBB).

We have performed genetic studies to examine the role of the BBB in the control of male courtship. We found that mutations in moody, a BBB-specific G-protein coupled receptor (GPCR) reduce courtship, and we have identified a Ga subunit that is required for courtship in the BBB. Furthermore, when we specifically feminized the BBB in otherwise normal males by expression of the female specific TRAF protein, we found significantly reduced male courtship. Together, these data suggest that male-specific proteins and G-protein mediated signaling in the blood brain barrier are important for Drosophila male mating behavior.
MOLECULAR IMAGING OF DRUG PENETRANCE THROUGH THE BLOOD-BRAIN BARRIER USING MALDI MASS SPECTROMETRY

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The ability of a drug or its metabolites to leave the lumen of brain capillaries and penetrate the brain parenchyma is a general problem during drug development for disorders of the central nervous system. Chemical analyses of brain homogenates and cerebral spinal fluid do not yield complete spatial information required for accurate assessment of a molecule’s penetration capability. Visualization of drug related molecules using radioisotopes is a powerful method, but requires labeling and lacks the ability to resolve possible metabolites. Matrix-assisted laser desorption ionization (MALDI) mass spectrometry imaging can directly report the locations of drug and related molecules in tissue sections of model organisms using solely their accurate mass. MALDI mass spectrometry imaging does not require labeling of the drug molecules and can resolve the studied drug and its metabolites. The presented study uses mass spectrometry for imaging drug penetrance through the blood-brain barrier. We find heme signal from mass spectrometry imaging to be a marker for visualizing the vasculature and validate this using a fluorescent molecule that localizes in the lumen and walls of blood vessels. We use high-resolution MALDI mass spectrometry for imaging the tumor penetration of erlotinib, a model drug, administered at therapeutic levels in mouse xenografts of human U87 gliomas.

Brain, liver, and kidney from mice treated for 4 hours with 100 mg/kg erlotinib were imaged at up to 50 μm spatial resolution with a high-field MALDI Fourier transform ion cyclotron resonance mass spectrometer. A total of eight metabolites were observed in liver tissue, representing more than half of the reported erlotinib metabolism. The blood-tumor barrier was mapped by rendering of the heme molecular ion across the tissue section, and the image was compared to an H&E staining of an adjacent section to delineate the U87 tumor bulk. The penetration of erlotinib and its active metabolites through the blood-tumor barrier were observed, and their distribution was assessed. For validation of heme as a marker for blood, fluorescein sodium salt (400 mg/kg) was injected in the tail vein of a mouse, and the animal was sacrificed after 5 minutes. Tissue sections were imaged using a fluorescent scanner, and a MALDI-TOF/TOF mass spectrometer. Co-localization of fluorescein and heme from the mass spectrometry image suggests that heme is a valid marker of the vascular system, and could be used in studies for the development of drugs needing to cross the blood-brain barrier.
Chemokine receptor modulation during leukocyte transmigration across a blood-brain barrier model in vitro: effect of shear forces

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Inflammation initiates the lesions of multiple sclerosis (MS) and the accumulation of hematogenous inflammatory cells in the central nervous system parenchyma is crucial for MS pathogenesis. Chemokines and adhesion molecules orchestrate leukocyte accumulation in inflamed tissues. Chemokine receptors on defined cell populations (such as monocytes or CD45RO+/CD4+ memory T cells) differ strikingly when circulating cells are compared with infiltrating cells, suggesting the need of in vitro studies to disclose the dynamic modulation of chemokine receptors during transmigration. A novel in vitro flow-based BBB model was developed to enable bridging the gap between descriptive brain tissue immunohistochemistry and target identification for treatment of neuroinflammatory diseases. Here, we describe initial studies using this model to assay chemokine receptors on T cells, B cells and monocytes undergoing BBB transmigration in vitro under flow. Results showed that “luminal” CXCL12 (applied to the apical surface of a brain microvascular endothelial cell monolayer) stimulated CD4+, CD8+ lymphocyte and CD14+ monocyte transmigration, which was susceptible to anti-CXCR4 neutralizing antibody. Unexpectedly, luminal CXCL12 selectively downregulated CXCR4 on monocytes during migration. We also found that “abluminal” CCL2 (in the bottom well of the chemotaxis chamber) promoted migration of CD4+, CD8+ and CD14+ cells. Monocyte cell-surface CCR2 protein was downregulated by ligand CCL2. Independent of CCL2, monocyte CCR2 mRNA was reduced during the process of transmigration, suggesting an explanation for the lack of CCR2 protein on tissue-infiltrated monocytes. Results also showed that migrated monocytes were CCR5+, compatible with our findings in analyzing MS brain lesions. In conclusion, data from tissue staining and BBB models in vitro, taken together, will be instrumental for selecting therapeutic targets to modulate leukocyte brain infiltration and achieve neuroinflammatory disease control.
TRANSCRIPTOME ANALYSIS OF THE EPENDYMAL BARRIER DURING MURINE NEUROCYSTICERCOSIS

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Central Nervous System (CNS) barriers play a pivotal role in the protection and homeostasis of the CNS by enabling the exchange of metabolites while restricting the entry of xenobiotics, blood cells and blood borne macromolecules. While the blood-brain barrier and blood-cerebrospinal fluid barrier (CSF) control the interface between the blood and CNS, the ependyma acts as a barrier between the CSF and parenchyma and regulates hydrocephalic pressure and metabolic toxicity. Neurocysticercosis (NCC) is an infection of the CNS caused by the metacestode (larva) of Taenia solium and a major cause of acquired epilepsy worldwide. The common clinical manifestations of NCC are seizures, hydrocephalus and symptoms due to increased intracranial pressure. The majority of the associated pathogenesis is attributed to the immune response against the parasite. The properties of the CNS barriers, including the ependyma, are affected during infection resulting in disrupted homeostasis and infiltration of leukocytes that correlates with the pathology and disease symptoms of NCC patients. In order to characterize the role of the ependymal barrier in the immunopathogenesis of NCC, we isolated ependyma cells using laser capture microdissection from mice infected or mock-infected with the closely related parasite Mesocestoides corti and analyzed the genes that were differentially expressed using microarray analysis. The expression of 400 genes was altered. Immune response related genes were verified by realtime RT-PCR. Ingenuity Pathway Analysis (IPA) software was used to analyze the biological significance of the differentially expressed genes and revealed that genes known to participate in innate immune responses, antigen presentation and leukocyte infiltration were affected along with the genes involved in carbohydrate, lipid and small molecule biochemistry. Further, MHC class II and chemokines, including CCL12, were found to be upregulated at the protein level using immunofluorescence microcopy. This is important, because these molecules are members of the most significant pathways by IPA analyses. Thus our study indicates that ependyma cells actively express immune mediators and likely contribute to the observed immunopathogenesis during infection. Of particular interest is the major upregulation of antigen presentation pathway-related genes and chemokines/ cytokines. This could explain how the ependyma is a prominent source of leukocyte infiltration into ventricles through the disrupted ependymal lining by way of pial vessels present in the internal leptomeninges in murine NCC.
Glial cells are important for the formation and maintenance of the Blood Brain Barrier in the Central Nervous System (CNS). Much less is known however, about the role glial cells play in the regulation of the Blood Nerve Barrier in the Peripheral Nervous System (PNS). Following injury or demyelinating disease, these barriers break down and inflammatory cells are recruited at the injury site. However the molecular mechanisms regulating these processes are poorly understood.

In contrast to the CNS, the PNS can successfully regenerate after injury. While Schwann cells, the glia of PNS, are essential to drive the regeneration process through their ability to dedifferentiate into progenitor-like cells, the recruitment of inflammatory cells is also crucial.

We have previously shown that the Ras/Raf/Erk pathway is able to induce the dedifferentiation of Schwann cells in vitro (Harrisingh et al., Embo J, 2004). To address whether ERK activation is also sufficient to drive Schwann cell dedifferentiation in vivo and investigate the effect of this specific signal on the biology of the peripheral nerve, we have generated a transgenic mouse model in which Raf kinase can be activated in myelinating Schwann cells in the adult nerve. We found that activation of Raf in these mice drives demyelination of peripheral nerves in vivo and results in severe impairment of motor function. Moreover, we show that ERK activation also induces the break down of the Nerve Blood Barrier and is consistently accompanied by an inflammatory response and macrophage infiltration along the entire nerve despite the absence of injury. Importantly, the phenotype of peripheral nerve degeneration is reversible with the period of dedifferentiation determined by the period of ERK activation. Moreover as part of this regeneration process, the Blood Nerve Barrier is reformed.

This mouse model provides a powerful system to study the regulation of PNS degeneration and regeneration.
TIGHT JUNCTION COMPLEX MODIFICATION AT THE BLOOD-BRAIN BARRIER IN TBI: MECHANISMS OF INJURY

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As the gate keeper of the CNS, the BBB is an important physiological structure in regulating and maintaining the environment that is optimal for neuronal communication. It is known that in instances of neuroinflammation such as in traumatic brain injury (TBI), alteration of the BBB is evident. However, what still lacking is a characterization of the BBB dysfunction in TBI through evaluation of the tight junctions (TJ). The goals of this study were to determine whether alteration to the BBB could be detected by morphological changes in the integrity of the TJ as a function of injury severity, time and brain region location. Using the controlled cortical impact (CCI) mouse model of TBI, mice (n=5 per group) were exposed to the following conditions: sham (craniotomy only), mild CCI-TBI (1.5 m/s to a depth of 1mm) and moderate CCI-TBI (3.5m/s to a depth of 1mm). After seven days post-injury the brain tissue was collected and immunofluorescence was performed. First, the pathophysiology of the CCI-TBI was confirmed by the presence of gliosis and microglial reactivity. Neurodegeneration was evaluated using MAP-2 and NeuN which indicated decreased neuronal staining in and around the lesion area. Also, Greater neurodegeneration was evident in the moderate CCI-TBI along with cavitation. Of note, the sham group had no detectable TBI related pathology. Assessment of the TJ integrity by ZO-1, occludin and claudin-5 staining revealed morphological disruptions. As expected, the sham group had the characteristic intense and continuous ZO-1 strand-like staining pattern. However, a significant number of TJs on the ipsilateral and contralateral side of the injury were identified as having discontinuous/punctate patterns of staining. Furthermore, many microvessels clearly identified by P-glycoprotein and showing no apparent gross vessel damage, were found to have little to no ZO-1 staining. As in the case with ZO-1, occludin and claudin-5 also showed similar patterns of TJ disruption. Recent studies suggest that phosphorylation of occludin and claudin 5 are important in contributing to BBB permeability. Using phosho-specific antibodies, our results also showed that phosphorylated forms of occludin and claudin-5 were apparent at TJs proximal and distal from the site of injury. In sum, our study provides a comprehensive analysis of the degree of tight junction modification throughout the brain in a mouse model of traumatic brain injury.
MECHANISMS OF ENDOTHELIAL BARRIER PENETRATION BY 
LISTERIA MONOCYTOGENES

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The ubiquitous food-borne bacterium *Listeria monocytogenes*, though ordinarily an agent of gastroenteritis, causes meningitis in immune-compromised hosts and, rarely, encephalitis in previously healthy hosts. The interaction of *L. monocytogenes* with the mammalian gut epithelium has been extensively studied, but less is understood about how *L. monocytogenes* spreads from the digestive tract to infect distal sites, such as the central nervous system, and very little is known about how this organism is able to breach the blood-brain barrier.

Generally, upon entering a host cell, the bacterium replicates in the cytoplasm and expresses the protein ActA, which activates Arp2/3 to polymerize actin at the bacterial surface; polarized actin polymerization propels the bacterium through the cytoplasm. Eventually, a motile bacterium at the membrane of an infected cell protrudes into and is taken up by an adjacent uninfected cell; this process, referred to as cell-to-cell spread, allows *L. monocytogenes* to spread through a sheet of epithelial cells, without exposure to the extracellular space.

We are examining how *L. monocytogenes* subverts the barrier properties of the vascular endothelium during systemic infection. The most likely mechanisms of bypassing the barrier include: direct infection of endothelial cells by free bacteria in the bloodstream, infection of endothelial cells via cell-to-cell spread from infected circulating immune system cells, and transmigration of infected immune system cells across an uninfected endothelium. Using fluorescence microscopy, we have confirmed that *L. monocytogenes* can directly infect and spread through a monolayer of human umbilical vein endothelial cells (HUVEC) in tissue culture. We have demonstrated that *L. monocytogenes* in infected macrophages and macrophage-like cell lines can robustly infect monolayers of HUVEC via direct heterotypic cell-to-cell spread, in an ActA-dependent manner. Using time-lapse microscopy, we have determined that, in infected macrophages, *L. monocytogenes* form protrusions that may extend for more than ten microns before being taken up by an endothelial cell. We are currently investigating how astrocyte coculture and blood-brain barrier-like differentiation influence the behavior of *L. monocytogenes* at the endothelium. We are also exploring whether macrophage/endothelial cell adhesion facilitates bacterial transfer at the endothelium.
EXPRESSION OF TOLL-LIKE RECEPTORS IN THE CHOROID PLEXUS AFTER NEONATAL HYPOXIA-ISCHEMIA IN MICE

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Hypoxia-ischemia (HI) is a major cause of brain damage in the newborn, where inflammation plays a key role in the damaging processes. Growing evidence suggest that a group of innate immune receptors, Toll-like receptors (TLRs), are involved in the inflammatory response to ischemia. We have shown that LPS-induced HI brain injury is dependent on MyD88, a TLR adaptor protein, and that systemic stimulation with a TLR2 agonist impairs mouse brain development, suggesting that TLRs also play an important role in neonatal brain damage. The choroid plexus is damaged after HI and responds with an altered gene expression after peripheral inflammation induced by LPS. However, data is lacking on the role of TLRs in the choroid plexus after neonatal brain damage. The purpose of this study was to examine the TLR expression and the downstream signaling pathway in choroid plexus after HI alone or in combination with different TLR agonists.

Wild type C57/Bl6 mice were subjected to left carotid artery ligation and 10% O2 for 50min (HI) on postnatal day 9. The animals were sacrificed 24h after HI (n=6-12) and compared to mice not subjected to HI (n=5-8). The brains or the choroid plexus from the left lateral ventricles were dissected out and prepared for mRNA analysis. The mRNA expression was determined by a TLR pathway-specific PCR array (SABiosciences). Results are shown as fold regulation where the threshold was set to a fold regulation of ±1.5.

At 24h after HI, TLR 3 (1.64), TLR 5 (1.95) and TLR 6 (1.51) were up regulated whereas TLR1 (-1.73) was down regulated in the choroid plexus. TLR 2, 4, 7, 8 and 9 were not regulated. There was also altered gene expression in the NF\(\kappa\)B signaling pathway downstream of the TLRs (Tirap/mal (-1.56), MEKK1 (1.50), NF\(\kappa\)B2 (1.55), NF\(\kappa\)Bib (2.00), Rel (1.64), IL1\(\alpha\) (2.10), Ptg2/Cox-2 (3.04), and Ccl2 (5.37)). In the brain, TLR 1 (3.35), TLR 2 (2.27) and TLR 7 (2.16) were upregulated, while TLR 5 was down regulated (-1.76) and TLR 3, 4, 6, 8 and 9 did not change. Changes in gene expression in the choroid plexus after stimulation with different TLR agonists with or without HI are under investigation.

Conclusion: The mRNA expression of several TLRs and their downstream signaling molecules was altered in the neonatal brain and in choroid plexus after HI. However, the response in choroid plexus was completely different to the changes in the brain. This is the first demonstration of TLR regulation in the choroid plexus after neonatal HI. The data suggest that specific TLRs may be functioning in choroid plexus after HI, which differ from those in the rest of the brain.
BRAIN MICROVASCULAR ENDOTHELIAL CELL
HYPERPERMEABILITY INDUCED BY CASPASE-3 ACTIVATION

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The blood brain barrier (BBB) integrity depends mainly on the integrity of the tight junction proteins (TJPs) of the brain microvascular endothelial cells. We hypothesized that the increased mitochondrial ROS formation following ischemic-stroke reperfusion and subsequent activation of caspase-3 will lead to the proteolytic cleavage of the TJPs, resulting in barrier disruption, hyperpermeability and brain edema. Our objective was to study if oxygen and glucose deprivation followed by reoxygenation (OGD-R) would disrupt the TJPs, ZO-1 and occludin and lead to brain microvascular endothelial cell hyperpermeability under in vitro conditions. Rat brain microvascular endothelial cell (RBMEC) monolayers grown either on Transwell plates, chamber slides or on regular dishes were subjected to OGD-R in presence or absence of a capase-3 inhibitor Z-DEVD-FMK. The OGD-R was achieved by replacing the regular medium with a glucose free medium and exposing the cells to an anaerobic chamber at 37°C. The OGD-R period was terminated by replacing the glucose free medium with the regular medium and placing the cells in a 5%CO2/95% air incubator. The monolayer permeability was studied by using FITC-dextran flux across the monolayer. The mitochondrial ROS formation was studied using dihydorhodamine 123 and the mitochondrial transmembrane potential (MTP) by the JC-1 method. The integrity of the tight junctions was determined by immunofluorescence of ZO-1 and occludin. The caspase-3 activity was determined using a fluorometric assay. OGD-R induced, hyperpermeability of the monolayer that was inhibited by the caspase-3 inhibitor Z-DEVD-FMK (p<0.05). OGD-R, induced mitochondrial ROS formation, decreased the MTP, and increased caspase-3 activity significantly (p<0.05). The control cells showed junctional continuity of ZO-1 and occludin whereas the cells exposed to OGD-R showed discontinuity of junctional staining and intercellular gap formations that was prevented by Z-DEVD-FMK. Our results suggests that, the increased mitochondrial ROS formation following OGD-R and subsequent activation of caspase-3 led to the proteolytic cleavage of ZO-1 and/or occludin resulting in barrier disruption and hyperpermeability. This might serves as a mechanism for ischemic-stroke reperfusion induced BBB disruption and brain edema.
PERICYTES ARE IMPORTANT FOR THE BLOOD-BRAIN BARRIER INTEGRITY IN VIVO

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In the mammalian brain the passage of molecules from the blood to the brain parenchyma is tightly controlled in order to maintain an environment necessary for proper neuronal function. In the central nervous system the main physical barrier is formed by endothelial cells. Other suggested components of the blood-brain barrier include extracellular matrix components, astrocytes, and pericytes. However, the relative contribution of the different blood-brain barrier building blocks remains largely unknown. In this study, we have analyzed the role of pericytes at the neurovascular unit in the adult animals. In order to do this we have generated two viable pericyte-deficient mouse models. We show that pericyte-deficiency does not affect the endothelial cell differentiation specific for the central nervous system, and polarization of endothelial cells. Formation of endothelial adherens and tight junctions does take place in pericyte-deficient mice. However, pericyte-deficient animals show accumulation of intravenously injected tracers (with different molecular weights) in the brain parenchyma and leakage of tracers correlates with the degree of pericyte-deficiency. Administration of broad spectrum tyrosine kinase inhibitor Imatinib abolishes the extravasation of tracers into the brain parenchyma in pericyte-deficient animals. We also show that pericytes are important for astrocyte end-feet polarization. In conclusion, we demonstrate the importance of pericytes for the integrity of the blood-brain barrier and organization of the astrocyte end-feet in vivo.
REGULATION OF ENDOTHELIAL BARRIER MAINTENANCE AT THE BLOOD-BRAIN BARRIER.

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Tight control of vascular permeability is essential for normal brain function but little has been known about the molecular basis of blood-brain barrier (BBB) formation and maintenance. It is well established however, that during developmental brain angiogenesis and in the mature brain the specific brain milieu, and in particular neural progenitors and astrocytes, is responsible for the induction and maintenance of BBB characteristics in endothelial cells (ECs). Only recently others and we provided first evidence that endothelial Wnt/beta-catenin signaling is necessary for brain angiogenesis and the induction of BBB characteristics. Endothelial specific beta-catenin signaling was observed during embryonic BBB development and its postnatal maturation but becomes largely silenced in the adult. Endothelial specific stabilization of beta-catenin in vivo and in primary brain ECs in vitro enhanced barrier maturation, and induced claudin-3 expression and BBB-type tight junction formation evidenced by freeze-fracture preparations. In turn, endothelial deletion of beta-catenin led to a rapid loss of barrier properties in vivo and in vitro. Presumably, brain endothelial cells, once their barrier properties have been established, continuously need by now unknown „cues“ to maintain BBB characteristics. This becomes particularly apparent under pathological conditions in the brain when BBB function is disturbed. Specifically, in glioblastoma (astrocytoma grade IV) the significant loss of BBB function in brain capillaries promote the severe symptoms of brain edema, ultimately leading to clinical complications and death of the patient. We now provide evidence that in a murine brain tumor model, beta-catenin stabilization in angiogenic glioblastoma vessels decreased tumor angiogenesis and growth. In turn, inhibiting Wnt signaling in these tumors significantly increased vascularity and size of the tumors. Additionally, augmented Wnt/beta-catenin signaling in the tumor endothelium prevented vascular hyper-permeability, suggesting a new potential therapeutic avenue for limiting the devastating effects of tumor induced BBB breakdown.
We quantified blood-brain barrier (BBB) permeability with the rate constant for influx (K_i) across the BBB with α-aminoisobutyric acid (AIB) in fetal, neonatal and adult sheep. Although the permeability of the BBB measured with AIB is relatively low during fetal and neonatal development, the ovine fetus and neonate exhibited ontogenic decreases in barrier permeability. The low permeability measured with AIB suggests that a functionally tight barrier is present from very early in gestation. We also examined the developmental regulation of tight junction (TJ) proteins in sheep and found that occludin, claudin-1, claudin-5, ZO-1, and ZO-2 were expressed very early in fetal life and throughout ovine development. The patterns of expression varied amongst TJ proteins. In general, the transmembrane protein was higher in the fetuses than in newborn and adult sheep, and ZO-1 was highest in newborn sheep and ZO-2 higher in newborn and adult sheep than fetuses. The expression patterns of individual TJ proteins do not necessarily reflect ontogenic changes in BBB permeability with development, rather, the proteins of the TJ complex work together to form an effective barrier. The pituitary-adrenal cortical axis matures during fetal development. We postulated that the pituitary-adrenal cortical axis functions as a physiological regulator of BBB function in the fetus. We showed that both increases in gestation and in plasma cortisol contributed to the decreases in BBB permeability during fetal development. Maternally administered antenatal glucocorticoids are used to accelerate fetal maturation. Antenatal glucocorticoids reduce the incidence of intraventricular hemorrhage and may enhance brain microvascular integrity in the fetus. We showed that antenatal glucocorticoids reduce BBB permeability in the ovine fetus at 60%, 70%, and 80% of gestation, but not at 90% of gestation or in newborn lambs. The age-related differential responsiveness of the fetal and neonatal barrier to exogenous glucocorticoids are most likely to related to the effects of increases in endogenous steroids on BBB maturation. We also showed that maternal glucocorticoid treatment increases in claudin-1, claudin-5 and claudin-5 expression in select fetal brain regions and that decreases in permeability correlate with the increases in claudin-1 and claudin-5, but not occludin. Therefore, this common maternal treatment affects both the functional and molecular characteristics of the fetal BBB. We conclude that BBB function develops early in fetal gestation, the structural proteins of tight junctions are present very early in ovine development, and endogenous increases in glucocorticoids are associated with decreases in BBB permeability, and that maternal glucocorticoid treatment decreases BBB permeability and increases TJ protein expression in the fetus.
MICRORNAS CONTROL BRAIN ENDOTHELIAL CELL BARRIER FUNCTION

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Endothelial cells in the body display a remarkable diversity and are perfectly adapted to the needs of the underlying tissue. One of the best examples of specific endothelial cell function is found in the blood–brain barrier (BBB) which is required for optimal brain homeostasis and neuronal performance. Brain capillaries are surrounded by and closely associated with several other cell types, including the perivascular endfeet of astrocytes, pericytes, microglia and neuronal processes that have been shown to contribute to barrier function. In particular, astrocytic endfeet are in close proximity to the endothelial cell plasma membrane, are separated only by the basal lamina and have been implicated in the induction of the BBB.

Perturbations of BBB function are hallmarks of a variety of brain diseases. On the other hand, multiple efforts are focused on developing strategies to overcome the BBB and to effectively deliver active drugs to the brain. Understanding the mechanisms involved in the regulation of the blood-brain barrier may therefore open novel therapeutic avenues for treatment of neurological diseases. Yet, little is known regarding the molecular mechanisms that support the function of the BBB.

Using a combined genetic and bioinformatics approach, we uncovered a novel mechanism by which astrocytes mediate BBB function, i.e. through the activity of a new class of gene regulators called microRNAs. microRNAs regulate gene expression by binding to partially complementary sites in the 3' untranslated regions (UTR) of target genes, thereby causing degradation or translational repression. The role of microRNAs in endothelial biology is emerging and several microRNAs have been implicated in peripheral endothelial cell function in vitro and in vivo. It was not known whether microRNAs through their potential target genes play a role in blood-brain barrier function.

We believe that detailed knowledge about these genes will allow us to design in the future novel therapeutic approaches for several neurological diseases in which blood-brain barrier repair is required or controlled opening is needed for effective treatment in for example brain cancer. Our latest findings will be presented.
ROLE OF MICRONAS IN INFLAMMATION AT THE BLOOD BRAIN BARRIER

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During inflammation, increased production of cytokines by activated leukocytes and/or central nervous system (CNS) resident cells has been shown to alter the phenotype and function of cerebral microvascular endothelial cells leading to blood-brain barrier (BBB) dysfunction. MicroRNAs (miRs) are endogenous non-coding small RNAs recognised as potent post-transcriptional regulators of gene expression by silencing their mRNA targets. Deregulated miR levels have been demonstrated in several pathologies although their role in the pathogenesis of CNS inflammatory disorders remains to be elucidated. We hypothesised that endothelial miRs regulate CNS inflammation, by modulating blood-brain barrier permeability. To investigate this, we have determined changes in the pattern of both miR and mRNA expression induced by pro-inflammatory cytokines in an immortalized human cerebral microvascular endothelial cell line, hCMEC/D3 by microRNA and mRNA microarray respectively. Treatment with TNF-α and IFN-γ altered mRNA levels of 706 genes more than 2 fold over control levels, with the highest increases (>30 fold) observed for chemokines and adhesion molecules. We then identified 13 up-regulated and 122 down-regulated miRs in hCMEC/D3 cells, following treatment with cytokines. We selected miR-155 for further analysis, as it showed a rapid increase following cytokines stimulation. Transfection of brain endothelial cells with miR-155 induced a moderate increase in leukocyte adhesion and paracellular permeability which was concurrent with altered mRNA levels of genes associated with tight junctions and regulation of the actin cytoskeleton. We are currently investigating the putative mRNA targets of miR-155 in human brain endothelium implicated in BBB dysfunction following inflammation. Understanding the role of microRNAs in inflammation might help to identify novel therapeutic targets for CNS inflammatory disorders.
MONOCLONAL ANTIBODY BINDING TO UNIQUE STRUCTURES (LOOPS D AND F) OF BACE1 INHIBITS B-SECRETASE ACTIVITY IN VIVO

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β-Secretase (BACE1) is an attractive drug target for Alzheimer’s disease. The design of clinical useful inhibitors targeting the active-site of BACE1, has however been extremely challenging. To identify potentially alternative drug targeting sites we have generated a panel of BACE1 monoclonal antibodies (mAb) that interfere with BACE1 activity in various assays and determined their binding sites. We identified mAb 5G7 as a potent BACE1 inhibitor in enzymatic assays (IC50 ~0.47nM), that displayed no inhibitory effect in primary neurons. The mAb 5G7 binds to a surface helix comprising residues 299-312 and the epitope is “hidden” when BACE1 is membrane-anchored. Mutagenesis of this helix strongly reduced BACE1 ectodomain shedding. A second mAb, 1A11, inhibited BACE1 activity potently in vitro (IC50 ~0.76nM), and in primary neurons (EC50 ~1.8nM). It blocked BACE1 activity in vivo in mouse brain after stereotaxic injection. Paradoxically mAb 1A11 increased the activity of BACE1 towards small substrates in vitro. It binds to the adjacent loops D and F of BACE1, which together with helix A, distinguish BACE1 from BACE2 and other aspartic proteases. These structures are highly flexible and flank the active-site cleft of BACE1. Mutagenesis of loop F and helix A decreased or increased BACE1 activity, suggesting that these regions are modulating BACE1 activity. Our work suggests alternative promising drug targeting sites for BACE1 inhibition and should stimulate alternative development of small compounds that target these sites.
HOW WELL IS THE INTERNAL ENVIRONMENT OF THE BRAIN CONTROLLED IN THE EMBRYO?

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Even early in development, the brain and spinal cord show evidence of function e.g. breathing and limb movements. Still earlier, complex processes are involved in cell differentiation from precursor cells to form neurons, pericytes & glia. However, it has been widely believed for decades that brain develops in an environment not much different from the embryo as whole, exemplified by terms such as “leaky” blood-brain barrier. Although more a matter of philosophy than science, it is worth considering why this belief has persisted and what are the consequences for understanding the relation between specific features of the local environment of brain and particular features of its development and pathologies that may result from impaired brain barrier function. The belief seems to lie in a teleological argument that the developing brain would not “need” a protective mechanism as provided by the blood-brain barrier in adults, because of protection by the placenta, as explicitly stated by Barcroft (1938, The brain and its environment. Yale Univ Press) and supported by careful selection of experimental evidence. I shall briefly review some older evidence, which should have led to acceptance of the hypothesis that the brain develops and functions within its own well-defined internal environment. I shall deal mainly with key experiments performed recently, which provide convincing evidence that the main structural and functional features of blood-brain barrier mechanisms develop as soon as cerebral blood vessels (blood-brain barrier) and choroid plexuses (blood-CSF barrier) differentiate (Ek et al 2006, J Comp Neurol 496, 13-26; Daneman et al 2010, Nature. Oct 13. doi:10.1038/nature09513). This recent work also lays to rest the misconception that astrocytes are involved in tight junction formation in the early stages of vascularisation of the brain. There are several serious consequences of this longstanding belief in a leaky or immature blood-brain barrier. It has inhibited serious research on properties of the real internal environment of developing brain. Until recently there has been little consideration of the possibility that pathological conditions in embryos might affect blood-brain barrier properties and lead to abnormal brain development (Stolp & Dziegielewska, 2009, Neuropath Appl Neurobiol 35, 132-146). It seems likely that this belief has delayed serious consideration of whether the protective mechanisms against drugs and toxins so prominent in adult brain, may exist in embryos (Ek et al 2010). This has important implications for clinical practice and formal regulation of use of drugs in pregnant women and newborn infants.

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MOLECULAR MECHANISMS OF CNS ANGIOGENESIS AND BARRIERGENESIS

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The blood-brain barrier (BBB) is an effective gatekeeper that tightly regulates trafficking of molecules between the bloodstream and neuronal parenchyma. Although many substances are restricted from crossing the BBB, specific molecules can traverse the BBB through both receptor- and carrier-mediated transport mechanisms. Accordingly, the BBB gene expression profile is markedly distinct from that of the peripheral vasculature, specifically expressing molecules that drive both low paracellular/transcellular movement and exchange of essential nutrients and neurotoxic waste. While many of these BBB-specific transporters/molecules have been functionally characterized, the distinct signaling events that drive central nervous system (CNS) angiogenesis and barriergenesis are just beginning to be understood. To this end, we have utilized two complementary strategies towards a better understanding of BBB development, maturation, and maintenance: (i) Expression profiling of the murine BBB to identify new molecules enriched at the BBB along the developmental timeline, and (ii) a subsequent morpholino-based zebrafish screen to determine the functional relevance of these BBB-specific molecules. To date, we have identified several signaling receptors that drive BBB-specific development in both zebrafish and mouse models, including members of the TNFR family. These findings allow us to add novel molecular components to the known signaling mechanisms that drive CNS angiogenesis and barriergenesis.
MACROMOLECULAR TRANSFER ACROSS THE BLOOD-CSF INTERFACE

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Early in development cerebrospinal fluid (CSF) in the fetal brain contains very high concentrations of protein generated by their transfer from plasma across choroid plexus epithelial cells (Johansson et al., Bioessays 2008, 30:237). Previous studies demonstrated that about 10% of plexus cells in neonatal opossum (Monodelphis domestica) are involved in unidirectional transfer of plasma proteins from blood to CSF (Liddelow et al., Eur J Neurosci 2009, 29:253). We have investigated the sensitivity of this transfer mechanism by altering circulating levels of individual proteins in the plasma. Control of protein transfer across plexus epithelial cells was investigated by intraperitoneal injection of adult plasma into opossum pups at different postnatal (P) ages. Concentration of total and individual plasma proteins were estimated and volumes of the lateral ventricular system measured. Injections of adult plasma resulted in increased total protein concentration in the CSF within 6-24 hours at P65 and P110, but not at P9 due to ventricular expansion at this age only. Protein content in CSF increased at all 3 ages. Albumin and hemopexin increased in CSF at P9 and P65, but not in adults. The number of choroid plexus cells immunopositive for individual proteins mirrored these changes. AFP concentration did not change at P9 (the only age it was detected), however the number of cells decreased. Injection of a foreign protein, bovine fetuin, also resulted in an increase in CSF protein content and the exogenous protein was detected both in the CSF and within protein-transferring plexus cells. Bovine fetuin appeared to displace some endogenous proteins – hijacking the native protein transport system of the choroid plexus. These results suggest that there may be specific, developmentally regulated, transporters responsible for protein transfer across the blood/CSF barrier which may be important for some features of brain development. This raises the possibility of designing delivery systems from the blood to CSF and thence the brain via the choroid plexus epithelial cells.

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LOSS OF $\alpha V\beta 8$-MEDIATED TGFβ ACTIVATION, AND SUBSEQUENT LOSS OF TGFβ SIGNALING IN ENDOTHELIAL CELLS OR PERICYTES, CAUSES VASCULAR DYSPLASIA AND GERMINAL MATRIX HEMORRHAGE IN MICE

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Integrins are heterodimeric cell-surface proteins that regulate cell growth, migration and survival. It was previously shown that glial-derived integrin $\alpha V\beta 8$ binds and activates latent transforming growth factor-$\beta$ (TGF$\beta$), and that loss of integrin $\beta 8$ from the developing brain neuroepithelium ($\beta 8$nescre mice) leads to brain hemorrhage. Based on this work, we analyzed TGF$\beta$ activation and vascular phenotypes preceding hemorrhage in $\beta 8$nescre mice, TGF$\beta 1$ ligand knockout mice, and mice with conditional deletion of TGF$\beta$ receptor in endothelial cells and pericytes. We report that TGF$\beta$ activation is spatially restricted to the ventral germinal matrix neuroepithelium, and that $\beta 8$;nescre mice develop vascular dysplasia and hemorrhage coincident with loss of domain-specific TGF$\beta$ activation. Furthermore, we show that mice deficient in TGF$\beta$ signaling in endothelial cells or pericytes recapitulate key aspects of the $\beta 8$nescre phenotype. We have therefore uncovered a pathway in which the loss of integrin-mediated activation of latent TGF$\beta$ causes vascular specific morphogenic changes leading to germinal matrix hemorrhage. The remarkable similarity between the phenotypes in $\beta 8$ and TGF$\beta$ mutants and human infants with germinal matrix hemorrhage suggests that a functional alteration in the TGF$\beta$ activation pathway may affect susceptibility to this disease.
VASCULAR PATTERNING AND BLOOD-BRAIN BARRIER FORMATION DURING DEVELOPMENT

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Normal brain function depends upon the proper structure and function of the vascular system. One unique feature of the blood vessels in the central nervous system compared to the blood vessels in the rest of the body is the blood brain barrier (BBB), a highly specialized brain endothelial structure composed of tight junctions and specialized transporters. In concert with pericytes and astrocytes, the BBB protects the brain from various toxins and pathogens and provides the proper chemical composition for synaptic transmissions. Emerging evidence demonstrates that the BBB is formed much earlier than previously thought. However, the timing of its formation and the mechanisms governing the initial formation of the BBB are still largely unknown. Here we focus on the developmental process of blood vessel network formation in the CNS, to investigate the relationship between the BBB formation and vascular network formation. Most of the extensive vessel networks are formed through angiogenesis, a process where new vessels form by sprouting from existing vessels. This process begins when select endothelial cells called “tip cells” arise from a distinct site on the mother vessel and sprout towards angiogenic cues in the environment. Neighboring “stalk cells” proliferate and add to the new sprout. When two new sprouts meet, a connection is formed, augmenting the existing network. Reiteration of the tip cell selection, sprouting, and connecting events is the basis for building a sophisticated vascular network. We hypothesize that BBB begins to form as soon as two neighboring tip cell filopodia connect, i.e. vascular anastomosis occurs. We test this hypothesis using mouse genetics and in vitro assays. We aim to understand the temporal, spatial, and molecular interactions between BBB formation and angiogenesis process.
Precise regulation of cell-cell and cell-extracellular matrix (ECM) adhesion and communication within neurovascular units is essential for proper development and homeostasis of the blood-brain barrier (BBB). Integrins are cell surface receptors for ECM proteins and many integrins are expressed in neural and vascular cell types; however, their adhesion and signaling roles in neurovascular unit development and physiology remain mostly uncharacterized. We have used Cre/lox technology to analyze the roles for one integrin, αvβ8, which is expressed in perivascular radial glial cells and astrocytes where it binds to the ECM-associated forms of latent TGFβs. Selective ablation of αv or β8 integrin genes in glial cells leads to CNS-specific vascular pathologies, including abnormal endothelial cell growth and sprouting in the brain and retina, hemorrhage and premature death. Knockout mice that survive to adulthood display multiple vascular abnormalities in the brain and retina, including defective vascular basement membrane integrity, compromised BBB permeability and perivascular astrogliosis. Adult mutants also develop hemorrhage-induced hydrocephalus and die by six months of age due to related neurological deficits. These neurovascular phenotypes are due, in part, to impaired αvβ8 integrin-mediated adhesion to latent TGFβs in vascular basement membranes and subsequent activation of TGFβ signaling pathways in endothelial cells. Collectively, these data reveal essential roles for αvβ8 integrin in regulating neurovascular unit development and physiology in the embryonic and post-natal mouse brain.
NOTCH GENE CAN CONVERT VEINS TO ARTERIES AND SUSTAIN CONDUIT VESSEL STRUCTURE IN MICE

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Reduction in blood flow is known to induce blood vessel regression. However, genetic perturbations that are effective in inducing the regression of high flow conduit vessels are largely unknown. Here we demonstrate rapid regression of high-flow large vessels upon repression of constitutively active Notch4 (Notch4*) in endothelial cells, as revealed by recurring high-resolution two-photon imaging in live mouse brains. Notch4* arterialized vein segments, which then abnormally connected to arteries, resulting in arteriovenous shunting. Turning off Notch4* led to specific regression of the established high-flow arteriovenous connections. Notch repression initiated prompt reorganization of endothelial cells, vessel constrictions, and blood flow reductions in regressing vessels. The involution of arteriovenous shunts returned shunted blood to perfusing vessels, reversing tissue hypoxia and neuronal dysfunction. Our data provide direct, in vivo evidence that Notch signaling can exert dominant effects over hemodynamics in regulating arterial structure. Our findings imply that Notch inhibition may potentially lead to degeneration of arteriovenous shunts, whereas Notch activation may improve arterialization of vein grafts in the treatment of arterial occlusive diseases.
The blood protein fibrinogen as a ligand for integrin receptors functions as the molecular nexus of coagulation, inflammation and immunity. Fibrinogen extravasates in the nervous system after injury or disease associated with vascular damage or blood-brain barrier (BBB) disruption. In multiple sclerosis (MS), perivascular demyelination is accompanied by increased vascular permeability resulting to extensive deposition of fibrin. Our studies in animal models for MS have demonstrated that fibrinogen is not merely a marker of BBB disruption, but a mediator of neuroinflammation. Fibrinogen mediates pro-inflammatory functions in the nervous system by activating the Mac-1 integrin receptor (also known as CD11b/CD18 and complement receptor 3) in microglial cells. In vivo imaging in the mouse spinal cord using two-photon microscopy shows that microglia perform constant surveillance of blood vessel walls in myelinated areas. Pharmacologic or genetic disruption of the fibrinogen/Mac-1 interaction suppresses neurologic symptoms, inflammation and demyelination in Experimental Autoimmune Encephalomyelitis (EAE), a model of MS. Because blocking fibrinogen/Mac-1 interaction affects the proinflammatory but not the procoagulant properties of fibrinogen, strategies to target fibrinogen receptors within the tissue microenvironment could reveal selective and disease-specific agents for therapeutic intervention in neuroinflammatory diseases.
PARACELLULAR DIFFUSION AND TIGHT JUNCTIONS ARE BETTER PRESERVED AFTER NEONATAL THAN AFTER ADULT ACUTE STROKE

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Arterial ischemic stroke in the neonate occurs at a rate similar to that in adults but immaturity affects both injury and recovery. The blood-brain barrier (BBB) may contribute to the age-related injury patterns but little is known about BBB after neonatal stroke. Emerging evidence suggests that the early postnatal BBB is not as permeable as commonly thought. Tight junctions (TJ) are present early in embryonic development, restricting entrance of proteins into the brain, and by birth BBB is functional with no fenestrations. Objective: Using an age-appropriate rodent model that mimics arterial stroke in term babies, we asked if BBB is disrupted by cerebral ischemia in immature animals. Methods: Postnatal day 7 (P7) and adult rats were subjected to a transient 3hr middle cerebral artery occlusion (MCAO). Diffusion-weighted MRI was used to identify injured animals and guide in tissue dissection. TJ protein expression (Western Blot; fold increase, injured/contralateral), paracellular diffusion (Evans Blue assay), Gd-enhanced T1W MRI, cytokine levels (multiplex), and histological outcome were determined 24hr after reperfusion. A neutralizing anti-CINC-1 antibody was administered before MCAO. Results: In adult, Evans Blue showed a 14-fold increase in albumin extravasation in the injured caudate and a 5-fold increase in the cortex whereas in the neonates increase was 2-fold. The pattern of TJ expression changes differed between neonatal and adult rats. A significant 2 fold increase in claudin-5 expression was seen in injured cortex of neonates whereas expression remained unchanged in adults. Occludin expression tended to increase in neonatal but decreased in adult rats. ZO-1 expression was unaffected in neonatal but was significantly reduced (0.5 fold) in adult rats. In P7 rats, Gd-enhancement (T1W-MRI) was <10%, and neutrophil extravasation was low 1-24hr after reperfusion despite the marked accumulation of inflammatory cytokines, including IL-1β and CINC-1. CINC-1 neutralization exacerbated injury and affected TJ protein expression. Conclusions: Paracellular diffusion is markedly disrupted in adult but not in neonatal acute stroke. The better preserved neonatal BBB is associated with more preserved TJ and low neutrophil extravasation. Ischemia-induced increase of neutrophil chemoattractants likely contributes to endogenous defenses. Understanding the mechanisms that contribute to BBB integrity after neonatal stroke is critical in the search for new therapeutic targets.

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Despite numerous animal drug trials targeting different pathogenic pathways, pharmacological approaches to cure the mutant mouse that models amyotrophic lateral sclerosis (ALS) have so far failed. This suggests either yet undefined crucial pathogenic mechanisms or a frank pharmacoresistance to treatments in these mice. Drug transporters influence pharmacokinetics and pharmacodynamics; among different drug transporter systems, the multi-drug resistant efflux transporter P-glycoprotein (P-gp; \textit{mdr1}) extrudes a broad range of xenobiotics from cells and confers chemoresistance and a poor clinical outcome. In addition to normal expression in several peripheral tissues, P-gp is expressed in the central nervous system with predominant localization to capillary endothelial cells and, to a lesser extent, parenchymal and perivascular astrocytes of the blood brain/blood cerebrospinal fluid barriers (BBB/BCSF). P-gp is up-regulated under certain neuropathological conditions such as intractable epilepsy and in rodent models of temporal lobe epilepsy, and this appears to be regulated by the excessive glutamate release occurring in these seizure episodes. Kainate-triggered seizures induce neuronal P-gp expression and up-regulate P-gp in BBB endothelial cells and astrocytes, but P-gp expression, localization, and distribution under pathological conditions of the spinal cord are virtually unexplored. Although glutamate excitotoxicity and neuroinflammation are also components of ALS, drugs that increase glutamate clearance in the ALS mouse have been largely unsuccessful. We previously reported consistent yet transient effects of nordihydroguaiaretic acid (NDGA), a glutamate uptake enhancer, as well as a disease-driven up-regulation of spinal cord P-gp in ALS mice, suggesting that this and other pharmacotherapeutic failures may result from acquired, P-gp-mediated pharmacoresistance. Here we investigate P-gp cellular localization and expression in the spinal cord of ALS mice throughout disease progression. We find that P-gp is expressed in motoneurons and that P-gp expression increases in ventral spinal cord astrocytes throughout disease. We also examined P-gp expression in spinal cord homogenate from humans and report that P-gp is elevated in ALS patients. Thus, P-gp expression in spinal cord parenchyma may impart overt pharmacoresistance to ALS affected cells and account for the poor therapeutic effect observed in multiple ALS clinical drug trials. P-gp up-regulation in diseased CNS parenchyma may be a cellular mechanism to extrude increased levels of endogenous toxins resulting from the disease.
IMPAINMENT OF BBB BY EXPOSURE TO NEUROTOXINS: EFFECTS OF LPS AND UNCONJUGATED BILIRUBIN ON RAT BRAIN MICROVASCULAR ENDOTHELIAL CELLS

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Sepsis and jaundice, by elevated levels of unconjugated bilirubin (UCB) are two common conditions in neonatal disease. There are studies on endothelial cells showing that lipopolysaccharide (LPS) modifies the integrity of confluent monolayers. UCB is known for its potential neurotoxicity, although the mechanisms underlying its access to the brain remain unclear. In fact, there is only one study on the effects of UCB on bovine brain endothelial cells where it was shown that UCB induces apoptosis. Furthermore, the effects of both compounds on endothelial cells have also never been investigated. In this study we used a monolayer of rat brain microvascular endothelial cells (RBMEC) as a simplified model of the BBB in order to investigate whether these compounds (i) alter RBMEC viability; (ii) compromise BBB integrity; (iii) and impair BBB function.

Primary cultures of RBMEC were exposed to 1 µg/ml LPS with or without 50 µM purified UCB in the presence of 100 µM human serum albumin, used to solubilize UCB, for 4 h and 24 h at 37°C. Cell viability was evaluated by determining lactate dehydrogenase (LDH) release. Barrier integrity was studied in terms of permeability to sodium fluorescein (Na-F) (MW: 376 Da), transendothelial electrical resistance, as well as changes in tight junction proteins by immunostaining. In addition, we evaluated P-glycoprotein (P-gp) activity. Exposure of RBMEC to LPS appeared to have no significant effect on cell viability, whilst incubation with UCB already induced death upon 4 h treatment. Transendothelial electrical resistance was affected by LPS mostly at 4 h treatment, while UCB caused a major decrease after 24 h. Accordingly, LPS-induced leakiness to Na-F was more marked at 4 h, whereas the increased permeability due to UCB augmented along exposure. Tight junctions were altered both by LPS and UCB. As for P-gp activity, LPS did not appear to have any effect, whereas UCB impaired its activity for the time studied (24 h). In conclusion, the observed effects produced by UCB and LPS on brain endothelium may have significant implications in the first days of life by potentially allowing the influx of the neurotoxins to the brain and consequent injury.

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TUMOR NECROSIS FACTOR-A TRIGGERS A CYTOKINE CASCADE YIELDING POSTOPERATIVE COGNITIVE DECLINE

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Surgical trauma may lead to cognitive decline. Activation of the innate immunity, cytokines, and neuroinflammation are putative mechanisms to underlie cognitive dysfunction. Yet, no successful therapies exist to prevent this complication after surgery. Here we report on the key role of tumor necrosis factor-\(\alpha\) (TNF) as an early initiator and possible therapeutic target for the prevention of postoperative cognitive dysfunction (POCD).

Adult C56BL/6J, MyD88\textsuperscript{-/-}, IL-1\textsuperscript{-/-}, and TLR4\textsuperscript{-/-} male mice were randomly assigned into groups: 1) untreated animals; 2) general anesthesia (GA) with isoflurane + buprenorphine for analgesia; 3) stabilized tibial fracture under GA and analgesia; 4) anti-TNF prophylaxis 18 h prior to surgical intervention. Separate cohorts of mice per group were assessed for plasma and hippocampal cytokines (ELISA), microglial activation, and hippocampal dependent memory using trace fear conditioning.

Preemptive targeting of TNF with the administration of a blocking monoclonal antibody (ab) before surgery significantly reduced systemic IL-1\(\beta\) and IL-6 at 6 and 24 hours following tibial surgery. TNF ab also reduced surgery-induced expression of IL-1\(\beta\) in the hippocampus and subsequent microglial activation. Postoperative decline in the contextual fear response was significantly ameliorated following TNF ab prophylaxis (\(p<0.05\) vs surgery). Delaying administration of ab until 1 h postoperatively was ineffective. As anti-TNF prophylaxis did not fully abolish the inflammatory response, we explored alternative pathways in innate immunity. MyD88\textsuperscript{-/-} and IL-1\textsuperscript{-/-} mice showed elevation in TNF similarly to wild-types, however they were both protected from cognitive decline. Combination of anti-TNF prophylaxis in MyD88\textsuperscript{-/-} completely abrogated the systemic inflammatory response. Addressing the role of pattern recognition receptors, TLR4\textsuperscript{-/-} were not protected from postoperative cognitive decline, and resulted in elevations of IL-1\(\beta\) and IL-6 both systemic and centrally.

These data demonstrate that preemptive targeting of TNF as an early marker during post-surgical inflammation suppresses generation of IL-1, preventing neuroinflammation and cognitive decline. Systemic cytokines appear as key mediators in mediating further neuroinflammation both through MyD88-dependent and independent pathways.
GENE EXPRESSION ANALYSES OF PIAL VESSELS OF THE BLOOD BRAIN BARRIER DURING MURINE NEUROCYSTICERCOSIS

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Neurocysticercosis (NCC) is one of the most common parasitic infections of the Central Nervous System (CNS) caused by the metacestode (larva) of the tapeworm Taenia solium. The majority of the associated pathogenesis is attributed to the immune response against the metacestode stage of the parasite. In the murine model of NCC, immunopathogenesis leads to differential breakdown of the Blood Brain Barrier (BBB) resulting in leakage of blood borne molecules and leukocytes that contribute to CNS pathology. The pial vessels of the BBB are most affected in comparison to the BBB associated with the vasculature of other CNS compartments, particularly parenchymal vessels. In this study, we describe a comprehensive study to compare the genome wide gene expression of pial vessels in the brains of mice that were infected or mock-infected with the closely related parasite Mesocestoides corti that mimics the human form of the disease. Endothelial cells from pial vessels were labeled in-vivo by injecting Ricinus communis agglutinin conjugated with rhodamine dye, and isolated by utilizing laser capture microdissection. Differentially expressed genes were assessed by microarray. Overall 397 genes were found to be differentially regulated by infection including several angiostatic and angiogenic chemokines implicated in the trafficking of leukocyte subsets observed during NCC. Realtime RT-PCR analyses validated the pattern of gene expression obtained from microarray analyses. In addition, the protein expression profile of several chemokines, including CCL5 and CCL9, was examined by immunofluorescence staining and found to be upregulated in pial vessels from infected mice which correlated with the increased transcripts observed in microarray and realtime RT-PCR. Analyses for the biological significance of differentially expressed genes using Ingenuity Pathway Analysis software suggested their roles in immune response, cell death and development, connective tissue disorder and metabolism. Our data delineate infection-induced changes in the expression of genes associated with both immunity and disease, and collectively provide insight into the dysfunction of the BBB and mechanisms associated with leukocyte infiltration during murine NCC.
Rabies remains a public health threat with more than 70,000 fatalities each year around the world. Vaccination has been proven to be the best way to prevent rabies. However, current vaccines are too expensive for people who need the most. Once symptoms occur, the disease is almost always fatal. There was only one reported survival in the history in a patient without any immunological intervention. It was found that this patient had high levels of virus neutralizing antibodies in the CSF at the time of clinical onset. Thus it is hypothesized that immune effectors in the CNS might be important in recovery from clinical rabies. To allow immune effectors to enter the CNS, the enhancement of the BBB has been found to be the most important. In our study, we have constructed recombinant rabies viruses expressing chemokines/cytokines and investigated the possibility of using these constructs to clear rabies virus from the CNS and to prevent animals from developing rabies. It was found that administration by direct intracerebral injection of these viruses can prevent mice from developing rabies as late as 5 days after infection with wild-type rabies virus. These recombinant viruses can induce expression of chemokines/cytokines, enhance the BBB permeability, allow infiltration of inflammatory and other immune cells into, and clear not only the recombinant virus, but also wild-type rabies virus from the CNS. Thus these viruses may have potential to be developed as therapeutics for clinical rabies.
THE BLOOD-BRAIN AND THE BLOOD-CEREBROSPINAL FLUID BARRIERS PROVIDE DIFFERENT MOLECULAR CUES FOR T CELL ENTRY INTO THE CNS

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Central nervous system (CNS) homeostasis is a prerequisite for the proper communication of neuronal cells. To this end, the endothelial blood-brain barrier (BBB) and the epithelial blood-cerebrospinal fluid barrier (BCSFB) tightly seal off the CNS from the continuously changing milieu within the blood stream. It is now well established that in spite of the presence of these barriers the CNS is subject to immune surveillance and immune mediated pathogenic events. Numerous studies in an animal model for multiple sclerosis, experimental autoimmune encephalomyelitis (EAE), have elucidated that memory T cells can cross the non-inflamed BBB or BCSFB using specific molecular keys and gain access to the cerebrospinal fluid (CSF) drained ventricular, subarachnoidal and perivascular spaces. If these pioneer T cells encounter their specific antigen on antigen presenting cells strategically localized immediately behind the brain barriers, reactivation of the T cells will trigger a local inflammatory response leading to the stimulation of the BBB. The activated BBB will then provide novel traffic signals allowing for the entry of large numbers of circulating inflammatory cells into the perivascular spaces and finally across the glia limitans into the CNS parenchyma, where they progress to initiate tissue injury.
LOCALISED, INDUCIBLE AND SIZE-SELECTIVE MODULATION OF NEURONAL BARRIERS; AN AAV APPROACH

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Systemic access of low molecular weight drugs to the brain and retina is normally blocked, to a large degree, by the tight junctions of the endothelial cells lining the microvasculatures supplying these organs—the blood-brain and inner blood-retina barriers (BBB and iBRB). We describe a procedure for controlled, periodic, reversible modulation of selected regions of the BBB or the iBRB based on incorporation into an AAV2/9 vector of a doxycycline-inducible gene encoding shRNA targeting claudin-5, one of 30 or so proteins constituting the tight junctions of the BBB and iBRB. The vector may be introduced stereotaxically into pre-selected regions of the brain or into the retina, rendering these regions permeable to low molecular weight compounds up to approximately 1kDa for the period of time during which the inducing agent, doxycycline, is administered in drinking water, but excluding potentially toxic higher molecular weight materials such as anaphylatoxins, soluble enzymes, pathogens etc. The process does not induce neuronal edema and has no significant impact on neuronal transcription. Using the retina as a model for functional assays, we report on the use of barrier modulation in tandem with systemic drug therapy to prevent retinal degeneration and to suppress laser-induced choroidal neovascularisation (CNV), the latter being the hallmark pathology associated with wet age-related macular degeneration (AMD). These observations constitute the basis of a minimally invasive approach to low molecular weight drug therapy of neuroretinal and neurodegenerative disorders while also providing a platform for the screening of compounds currently deemed unsuitable for neuronal indications.
A NOVEL CANNABINOID RECEPTOR-2 AGONIST ATTENUATES LEUKOCYTE-ENDOTHELIAL INTERACTIONS AND BLOOD-BRAIN BARRIER (BBB) DYSFUNCTION UNDER SYSTEMIC INFLAMMATORY CONDITIONS

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Studies have shown that modulation of the receptor-mediated and endogenous cannabinoid system is neuroprotective in various settings of neuroinflammation. However, the selective activation of the cannabinoid type-2 receptor (CB2R) lacks further characterization in whether agonist of this type can affect cerebral vascular inflammatory response and barrier function. We used lipopolysaccharide (LPS from E. coli 0127:B8), released from gram-negative bacteria, to induce systemic endothelial activation. Cranial window and intravital microscopy were used to directly observe leukocyte-endothelial interactions in the mouse brain after LPS (6 mg/kg) injection. Animals were treated either with the CB2R agonist JWH-133 (10 mg/kg) or the novel CB2R agonist, O-1966 (5 mg/kg), concurrently with LPS. Our results revealed that LPS exposure induced a marked increase in leukocyte rolling and adhesion (6-fold by 4 hours, p<0.05) on brain endothelium after LPS inoculation. Leukocyte rolling and adhesion were both significantly reduced by JWH-133 and O-1966 in the LPS-treated animals (2-fold, p<0.05). Analysis of BBB function was also evaluated using Evans blue extravasation ex-vivo and Transendothelial Electrical Resistance (TEER) in-vitro. Permeability of the BBB (by Evans blue) was increased after LPS injection (2.2-fold, p<0.05) and these changes were prevented by treatment with O-1966. Barrier integrity monitored by TEER in human brain endothelial cells showed that CB2R agonists, O-1966 or JWH133, increased barrier tightness and restored barrier integrity. Thus, our findings demonstrate that under inflammatory insult, selective CB2R activation greatly attenuates leukocyte-endothelium engagement and provides protection to the BBB. CB2R activation could be a new strategy for BBB protection during neuroinflammation.
Circulating lipoprotein particles are systemic lipid and protein carriers that may also represent a communication link between different tissues. The CNS however, is wrapped by the blood–brain barrier (BBB) which regulates the passage of nutrients and signaling molecules from the periphery into the brain. Whether lipoproteins cross the BBB in vivo has been controversial, and no clear requirement for circulating lipoproteins in brain development has been shown.

We address these issues in Drosophila, which has a functionally conserved BBB, and lipoproteins that functionally resemble those of vertebrates. We show that the Drosophila lipoprotein Lipophorin (Lpp) exists in two isoforms. Both isoforms cross the BBB, but accumulate on distinct subsets of cells within the brain. Further, Lpp carries both sterol-linked and GPI-linked proteins into the circulation and transports them across the BBB. Finally, Lpp promotes neuroblast proliferation by a mechanism that does not depend on delivery of dietary lipids.

In addition, a second lipoprotein, LTP (lipid transfer protein), also crosses the BBB and accumulates on an even more restricted set of neurons by a mechanism that depends on the LDL receptor homologues LPR1 and LPR2. These results suggest that lipoproteins may represent an important vehicle for communication between the central nervous system and peripheral tissues.
NEISSERIA MENINGITIDES, A BACTERIAL PATHOGEN, CROSSES THE BLOOD BRAIN BARRIER BY HIJACKING THE B2-ADRENOCEPTOR-β-ARRESTIN PATHWAY

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The tropism of \textit{N. meningitidis} for the meninges reveals the efficiency with which the interactions between this pathogen and the brain microvasculature modify the BBB function.

Adhesion of the meningococcus is mediated by type IV pili that induce a localized remodeling of the sub cortical cytoskeleton, leading to the formation of endothelial membrane protrusions that anchor bacterial colonies at the endoluminal face of the endothelial cell membrane, allowing a better resistance to blood flow. \textit{N. meningitidis} is also able to recruit the polarity complex Par3/Par6/aPKC that re-routes junctional molecules at the site of bacterial cell interaction thus opening a paracellular route for bacteria to cross the endothelial barrier.

The signaling receptor activated by the pathogen remained unknown. We report that \textit{N. meningitidis} specifically stimulates a β2-adrenoceptor β-arrestin signaling pathway in human brain endothelial cells (hCMEC/D3), which ultimately traps β-arrestin interacting partners, such as cytoskeletal and junctional proteins under bacterial colonies. These molecules are progressively depleted from endothelial cell junctions resulting in anatomical gaps likely used by bacteria to penetrate into tissues.

Pharmacological activation of β2-adrenoceptors with specific agonists that induce their endocytosis prevents signaling events downstream of \textit{N. meningitidis} adhesion and inhibits bacterial crossing of endothelial cell monolayers. These results reveal a novel strategy used by a pathogen to hijack host cell signaling machineries and open the blood brain barrier.
DIAGNOSTIC ULTRASOUND AND SONOVUE OPEN THE BLOOD-BRAIN BARRIER AND ITS EFFECT ON COGNITIVE FUNCTION.

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Purpose: The objective of this work was to investigate the recovery time of the change of the blood-brain barrier’s permeability by diagnostic ultrasound and contrast agent in rat, and determine whether the opening could affect the cognitive ability.

Materials and Methods: All animals were anesthetized by phenobarbital, the hair of the right eye to ipsilateral ear to the top of skull was removed before treatment. The SonoVue was administrated intravenously at the dosage of 7 ml/kg, and the ultrasound was delivered to rat brain at 1.3 mechanical indexes (MI) for 10 minutes, then the permeability of the BBB was investigated at different times after the contrast imaging by evans blue dye exudation. Morris water maze was used for assessing the spatial learning and memory 24 hours after the treatment. Arterial blood gas samples were obtained at three time points: before the SonoVue injection, right at 5 mins of the ultrasound exposure, after the treatment. The cerebral blood flow was measured before ultrasound exposure with SonoVue and after the treatment.

Result: Evans blue could be detected in the local brain after the contrast imaging and the permeability’ change recovered in 6 hours (P<0.05). The escape latency and pathlength to the platform decreased compared to the control groups (P<0.05). Ph, PaO2, PaCO2 were no different among the three time points (P>0.05). Cerebral blood flow increased after contrast imaging (P<0.05).

Conclusion: Blood-brain barrier could open after diagnostic ultrasound and contrast agent in rat. The cognitive function was improved after ultrasound with SonoVue. Arterial blood gas is not affected. Cerebral infusion improves after the treatment.
PHYSIOLOGY-BASED SCREENS FOR SMALL MOLECULES THAT ALTER METABOLISM AND TARGET DRUG PERMEATION INTO THE BRAIN

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The maintenance of organism-wide metabolic and chemical homeostasis is a biological process central to life that is impossible to fully reconstitute in vitro. In metazoans, small molecule utilization is regulated by networks that operate at cellular, tissue and organism-wide levels to modulate both physiology and behavior. A major challenge is to understand how organisms integrate these diverse pathways to control small molecule localization. All eukaryotes manifest chemical partition physiology to protect and maintain susceptible organ systems like the CNS, promote the best of use of energy stores, and to deliver the building blocks of biology. The nematode C. elegans is a useful model system to understand how small molecule partition is regulated in multicellular organisms. Many of the known mammalian pathways that regulate feeding, nutrient uptake, transport, energy storage and utilization in mammals are conserved in C. elegans. Notably, the robust genetic tools available in C. elegans have facilitated the identification of pathways as well as the targets of compounds that induce phenotypes in this organism. Pharmacological tools can complement the many genetic tools that exist in C. elegans in that they allow better temporal control, their dose-response variation gives an effect a continuous allelic series, they can deal effectively with functional redundancy, and their ease of implementation facilitates genetic interaction studies. Furthermore, pharmacological tools are easily portable between systems to discover special application to highly integrated and protected barriers like the blood brain interface (BBI). To systematically discover compounds that modulate small molecule localization in C. elegans, we developed a microscopy-based screen that utilizes the vital dye Nile Red to visualize chemical compartments in the worm. These small molecules are vetted by several genetic and ex vivo systems and tested at the fly BBI for special modulation function at the humoral/CNS interface of Drosophila.
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VISITOR INFORMATION

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<thead>
<tr>
<th>EMERGENCY</th>
<th>CSHL</th>
<th>BANBURY</th>
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<tbody>
<tr>
<td>Fire</td>
<td>(9) 742-3300</td>
<td>(9) 692-4747</td>
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<tr>
<td>Ambulance</td>
<td>(9) 742-3300</td>
<td>(9) 692-4747</td>
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<tr>
<td>Poison</td>
<td>(9) 542-2323</td>
<td>(9) 542-2323</td>
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<tr>
<td>Police</td>
<td>(9) 911</td>
<td>(9) 549-8800</td>
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<tr>
<td>Safety-Security</td>
<td>Extension 8870</td>
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Emergency Room
Huntington Hospital
270 Park Avenue, Huntington
631-351-2300 (1037)

Dentists
Dr. William Berg 631-271-2310
Dr. Robert Zeman 631-271-8090

Doctor
MediCenter
234 W. Jericho Tpke., Huntington Station
631-423-5400 (1034)

Drugs - 24 hours, 7 days
Rite-Aid
391 W. Main Street, Huntington
631-549-9400 (1039)

Free Speed Dial
Dial the four numbers (****) from any tan house phone to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office
to enter Library after hours.  See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room
Messages, Mail, Faxes
Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Russell Fitness Center
Dolan Hall, east wing, lower level
PIN#: Press 64580 (then enter #)

Concierge
On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones
Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

Cshl’s Green Campus
Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a “single stream waste management” system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory’s efforts can be improved. This book, for example, is printed on recycled paper.
1-800 Access Numbers

AT&T  9-1-800-321-0288
MCI  9-1-800-674-7000

Local Interest
Fish Hatchery  631-692-6768
Sagamore Hill  516-922-4447
Whaling Museum  631-367-3418
Heckscher Museum  631-351-3250
CSHL DNA Learning x 5170

New York City
Helpful tip -
Take Syosset Taxi to Syosset Train Station
($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue).
Train ride about one hour.

TRANSPORTATION

Limo, Taxi
Syosset Limousine  516-364-9681 (1031)
Super Shuttle  800-957-4533 (1033)
Syosset Taxi  516-921-2141 (1030)
To head east of CSHL - Huntington Village
Orange & White Taxi  631-271-3600 (1032)
Executive Limo  631-696-8000 (1047)

Trains
Long Island Rail Road  822-LIRR
Schedules available from the Meetings & Courses Office.
Amtrak  800-872-7245
MetroNorth  800-638-7646
New Jersey Transit  201-762-5100

Ferries
Bridgeport / Port Jefferson  631-473-0286 (1036)
Orient Point/ New London  631-323-2525 (1038)

Car Rentals
Avis  631-271-9300
Enterprise  631-424-8300
Hertz  631-427-6106

Airlines
American  800-433-7300
America West  800-237-9292
British Airways  800-247-9297
Continental  800-525-0280
Delta  800-221-1212
Japan Airlines  800-525-3663
Jet Blue  800-538-2583
KLM  800-374-7747
Lufthansa  800-645-3880
Northwest  800-225-2525
United  800-241-6522
US Airways  800-428-4322