Abstracts of papers presented at the 2012 meeting on

BLOOD BRAIN BARRIER

December 5–December 8, 2012

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2012 meeting on

BLOOD BRAIN BARRIER

December 5–December 8, 2012

Arranged by

Richard Daneman, *University of California, San Francisco*
Britta Engelhardt, *University of Bern, Switzerland*
Ryan Watts*, Genentech, Inc.*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Contributions from the following companies provide core support for the Cold Spring Harbor meetings program.

Corporate Benefactors

Astellas-OSI Oncology

Corporate Sponsors

Agilent Technologies
Bristol-Myers Squibb Company
Genentech, Inc.
GlaxoSmithKline
Life Technologies (Invitrogen & Applied Biosystems)
New England BioLabs, Inc.
Sanofi US

Plant Corporate Associates

Monsanto Company
Pioneer Hi-Bred International, Inc.

Cover: An immunofluorescence staining of the cerebellum in a brain section of a mouse suffering from and animal model for multiple sclerosis. The staining shows fibronectin (red), CD45+ inflammatory cells (green), cell nuclei (blue). In the center three inflamed microvessels can be distinguished due to the widening of their perivascular space, bordered on both sides by the fibronectin+ basement membranes and filled with CD45+ inflammatory cells. Photograph by Therese Périnat, Theodor Kocher Institute, University of Bern, Switzerland.

BLOOD BRAIN BARRIER

Wednesday, December 5 – Saturday, December 8, 2012

Wednesday	7:30 pm	Opening Remarks **1** Molecular Control of BBB Development and Function
Thursday	9:00 am	**2** Cellular and Acellular Elements
Thursday	2:00 pm	**3** Poster Session
Thursday	4:30 pm	*Wine and Cheese Party*
Thursday	7:30 pm	**4** Model Organisms
Friday	9:00 am	**5** Retinal Vasculature and Other Tissue Barriers
Friday	2:00 pm	**6** BBB in Inflammation and Disease
Friday	5:00 pm	Keynote Speaker
Friday	6:00 pm	Banquet
Saturday	9:00 am	**7** Crossing the BBB

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

Abstracts are the responsibility of the author(s) and publication of an abstract does not imply endorsement by Cold Spring Harbor Laboratory of the studies reported in the abstract.

These abstracts should not be cited in bibliographies. Material herein should be treated as personal communications and should be cited as such only with the consent of the author.

Please note that recording of oral sessions by audio, video or still photography is strictly prohibited except with the advance permission of the author(s), the organizers, and Cold Spring Harbor Laboratory.

Printed on 100% recycled paper.

PROGRAM

WEDNESDAY, December 5—7:30 PM

SESSION 1 MOLECULAR CONTROL OF BBB DEVELOPMENT AND FUNCTION

Chairpersons: **S. Liebner,** Goethe University Clinic, Frankfurt am Main, Germany
A. Prat, CHUM-Université de Montréal, Canada

Regulation of endothelial barrier properties in the CNS—Understanding from where the Wnt blows
Stefan Liebner.
Presenter affiliation: Goethe University Clinic, Frankfurt am Main, Germany. 1

Regulation of monocarboxylate transporter 1 (*Mct1*) by canonical Wnt/β-catenin signaling pathway in the blood-brain barrier
Zejian Liu, Mary Sneve, Lester R. Drewes.
Presenter affiliation: University of Minnesota Duluth, Duluth, Minnesota. 2

Role of endothelial microRNAs in leukocyte adhesion to cytokine-activated human brain microvascular endothelium
Camilla Cerutti, Patricia Soblechero, Laura Edwards, Basil Sharrack, David K. Male, Ignacio A. Romero.
Presenter affiliation: The Open University, Milton Keynes, United Kingdom. 3

Postnatal deletion of endothelial Rbp-J causes vascular defects resembling brain arteriovenous malformations
Corinne M. Nielsen, Henar Cuervo, Rong A. Wang.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 4

Molecular control of CNS inflammation at the BBB
Alex Prat.
Presenter affiliation: Université de Montréal, Montreal, Canada. 5

Retinoic acid signaling in the developing neurovasculature
Julie Siegenthaler, Youngshik Choe, Dan Li, Shou-Ching Jaminet, Marcus Fruttiger, Sam Pleasure.
Presenter affiliation: University of California, San Francisco, San Francisco, California; University of Colorado School of Medicine, Aurora, Colorado. 6

BBB disruption precedes neuroinflammatory pathology, demyelination and cognitive impairment in hyperhomocysteinemic mice
Shawn E. Bearden, Bryce C. Rhodehouse.
Presenter affiliation: Idaho State University, Pocatello, Idaho. 7

THURSDAY, December 6—9:00 AM

SESSION 2 CELLULAR AND ACELLULAR ELEMENTS

Chairpersons: **C. Betsholtz,** Karolinska Institute, Stockholm, Sweden
L. Sorokin, University of Münster, Germany

Christer Betscholtz.
Presenter affiliation: Karolinska Institute, Stockholm, Sweden.

Pericyte-derived scar formation following lesions to the central nervous system
Christian Göritz, David O. Dias, Daniel Holl, Jonas Frisén.
Presenter affiliation: Karolinska Institute, Stockholm, Sweden. 8

Astrocyte-derived VEGF-A drives blood-brain barrier disruption in CNS inflammatory disease
Azeb T. Argaw, Linnea Asp, Jingya Zhang, Trinh Pham, John N. Mariani, Dipankar J. Dutta, Elisabeth G. Kramer, Napoleone Ferrara, Michael V. Sofroniew, Gareth R. John.
Presenter affiliation: Mount Sinai School of Medicine, New York, New York. 9

Signaling at the neurovascular unit following transitory focal astrocyte loss and post-translational occludin modification
Colin L. Willis, Ryan Camire, Stephanie A. Brule, Courtney C. Wallace.
Presenter affiliation: University of New England, Biddeford, Maine. 10

Vascular basement membranes and their contribution to the blood-brain barrier
Lydia M. Sorokin.
Presenter affiliation: University of Muenster, Muenster, Germany. 11

Analyzing integrins and the extracellular matrix in neurovascular development and disease
Hye Shin Lee, Steve B. Reyes, Qian Liu, Shinya Hirota, Joseph H. McCarty.
Presenter affiliation: MD Anderson Cancer Center, Houston, Texas. 12

Astrocytic laminin regulates pericyte differentiation and blood brain barrier integrity
Yao Yao, Zu-Lin Chen, Erin H. Norris, Sidney Strickland.
Presenter affiliation: The Rockefeller University, New York, New York. 13

THURSDAY, December 6—2:00 PM

SESSION 3 POSTER SESSION

The role of JAML in promoting leukocyte migration across CNS barriers
Jorge I. Alvarez, Alexandre Prat.
Presenter affiliation: Universite de Montreal-CRCHUM, Montreal, Canada. 14

Obesity is associated with elevated plasma S100B which is reduced following weight-loss associated with Roux-en-Y gastric bypass surgery
Laura B. Buckman, Anna E. Garcia, Robyn A. Tamboli, Naji N. Abumrad, Kate L. Ellacott.
Presenter affiliation: Vanderbilt University Medical Center, Nashville, Tennessee. 15

Analgesic efficacy of systemically administered Dalargin conjugated with the blood-brain barrier carrier, FC5
Nadia Caram-Salas, Arsalan Haqqani, Eric Brunette, Christie Delany, Eve Boileau, Graham Farrington, John Eldredge, William Sisk, Wen Ding, Danica Stanimirovic.
Presenter affiliation: National Research Council of Canada, Ottawa, Canada. 16

The cavitation-dependent tight-junction integrity of ultrasound-induced blood-brain barrier opening
Yao-Sheng Tung, Cherry C. Chen, Oluyemi O. Olumolade, Shih-Ying Wu, Shu-Tao Wang, Elisa K. Konofagou.
Presenter affiliation: Columbia University, New York, New York. 17

Fibroblast growth factor binding protein 1 (FGFBP1) in blood brain barrier establishment and maintenance
Azzurra Cottarelli, Maria Grazia Lampugnani, Gianluca Deflorian, Dejana Elisabetta.
Presenter affiliation: IFOM Foundation, Milan, Italy. 18

Time-lapse in vivo two photon imaging of adult cerebral vasculature
Robert H. Cudmore, David J. Linden.
Presenter affiliation: Johns Hopkins School of Medicine, Baltimore, Maryland. 19

Fibrinogen-induced perivascular microglial clustering is required for the development of axonal damage in neuroinflammatory disease
Dimitrios Davalos, Jae Kyu Ryu, Mario Merlini, Kim M. Baeten, Natacha LeMoan, Mark A. Petersen, Thomas J. Deerinck, Dimitri S. Smirnoff, Catherine Bedard, Hiroyuki Hakozaki, Sara Gonias Murray, Jennie B. Ling, Hans Lassmann, Jay L. Degen, Mark H. Ellisman, Katerina Akassoglou.
Presenter affiliation: University of California, San Franciso, San Francisco, California. 20

Inflammatory upregulation of claudin-12 protein in brain endothelial tight junctions
Annika Deiss, Gesa Weise, Friederike Berberich-Siebelt, Lena Dietz, Guido Stoll, Mathias Buttmann.
Presenter affiliation: University of Wuerzburg, Wuerzburg, Germany. 21

Analgesic efficacy of systemically administered Dalargin conjugated with the blood-brain barrier carrier FC5 fused to the human Fc
Graham Farrington, Ellen Garber, John Eldredge, William Sisk, Nadia Caram-Salas, Danica Stanimirovic, Giovanna Antognetti, Eric Brunette.
Presenter affiliation: Biogenidec Corp., Cambridge, Massachusetts. 22

Incorporation of Transcend (melanotransferrin, MTf or p97), a protein vector, in a therapeutic antibody results in its transport in the brain at therapeutical concentration for the treatment of brain disorders
Reinhard Gabathuler, Timothy Z. Vitalis, Umar Iqbal, Maria Moreno, Paul R. Lockman, Quentin R. Smith.
Presenter affiliation: biOasis Technologies Inc., Vancouver BC, Canada. 23

Pervasive blood-brain/spinal cord barrier impairment in ALS patients
Svitlana Garbuzova-Davis, Paul R. Sanberg.
Presenter affiliation: University of South Florida Morsani College of Medicine, Tampa, Florida. 24

Integrin α8β1/Nephronectin interactions participate in Th lymphocytes transmigration through blood-brain barrier endothelial cells
Steve Gendron, Alexandre Prat.
Presenter affiliation: CRCHUM- University of Montreal, Montreal, Canada. 25

Lapatinib distribution and efficacy in a Her2-overexpressing brain metastasis preclinical mouse model of breast cancer
Brunilde Gril, Diane Palmieri, Yongzhen Qian, Paul R. R. Lockman, Quentin R. R. Smith, Patricia S. S. Steeg.
Presenter affiliation: National Cancer Institute, Bethesda, Maryland. 26

Interleukin 26—A Th17 cytokine which impacts on the blood brain barrier
Lamia N. Hachehouche, Hania Kebir, Jorge Alvarez, Simone Terouz, Lyne Bourbonnière, Alexandre Prat.
Presenter affiliation: CHUM, Montréal, Canada. 27

Comparison of blood-brain barrier transport of P-glycoprotein substrates in humanized, knock-out and wild-type mice
Muhammad Waqas Sadiq, C. Roland Wolf, Nico Scheer, Margareta Hammarlund-Udenaes.
Presenter affiliation: Uppsala University, Uppsala, Sweden. 28

Modulation of the blood-brain barrier in traumatic brain injury (TBI) and neuroinflammatory implications
Finnian Hanrahan, Matthew Campbell, James Keaney, Marian M. Humphries, Anna-Sophia Kiang, Peter Humphries.
Presenter affiliation: Trinity College Dublin, Dublin 2, Ireland. 29

Pericyte subpopulations of the CNS microvasculature
Daniel Holl, Christian Göritz.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden. 30

Non-invasive cell translocation to the Alzheimer's brain using focused ultrasound for blood-brain barrier disruption
Alison Burgess, Kullervo Hynynen.
Presenter affiliation: Sunnybrook Research Institute, Toronto, Ontario, Canada; University of Toronto, Ontario, Canada. 31

Evidences of tricellular tight junctions in brain endothelial cells
Noriko Iwamoto, Tomohito Higashi, Mikio Furuse.
Presenter affiliation: Graduate School of Medicine, Kobe University, Kobe, Japan. 32

Early increase in BBB permeability, hemorrhage and brain injury after transient cerebral ischemia in PGRN-deficient mice
Katherine Jackman, Timo Kahles, Karin Hochrainer, Lidia Garcia-Bonilla, Diane Lane, Ping Zhou, Aihao Ding, Josef Anrather, Costantino Iadecola.
Presenter affiliation: Weill Cornell Medical College, New York, New York. 33

Uptake and retention of bispecific antibodies in mouse brain by intravenous or intraperitoneal injection
Denise Karaoglu Hanzatian, Annette Schwartz, Farid Gizatullin, Jamie Erickson, Kangwen Deng, Ruth Villanueva, Christopher Stedman, Christina Harris, Andrew Goodearl, Tariq Ghayur.
Presenter affiliation: Abbott Bioresearch Center, Worcester, Massachusetts. 34

Methamphetamine-induced blood brain barrier dysfunction and cerebral vascular alterations: potential contributors to increased risk for developing Parkinson's disease
Sharanya M. Kousik, Steven M. Graves, T. Celeste Napier, Paul M. Carvey.
Presenter affiliation: Rush University Medical Center, Chicago, Illinois. 35

Developmental loss of blood brain barrier integrity between young and adult rats
Kuen-Bao Chen, Eva Yuhua Kuo, Kin-Shing Poon, Ka-Shun Cheng, Chia-Sheng Chang, Yu-Cheng Liu, Ted Weita Lai.
Presenter affiliation: China Medical University, Taichung, Taiwan. 36

Pharmacokinetics and regional distribution of Evans blue dye in blood-brain barrier research
Eva Yuhua Kuo, Lola Fenghuei Yen, Ted Weita Lai.
Presenter affiliation: China Medical University, Taichung, Taiwan. 37

Vascular-dependency of Evans blue dye delivery in circulation-paired rats
Eva Yuhua Kuo, Lola Fenghuei Yen, Ted Weita Lai.
Presenter affiliation: China Medical University, Taichung, Taiwan. 38

A genomic approach to identify sex-specific molecules in the blood brain barrier of *Drosophila melanogaster*
Chamala Lama, Brigitte Dauwalder.
Presenter affiliation: University of Houston, Houston, Texas. 39

MCAM promotes recruitment of encephalitogenic T lymphocytes across the blood brain barrier
Catherine Larochelle, Jorge I. Alvarez, Romain Cayrol, Hania Kebir, Marc-André Lécuyer, Olivia Saint-Laurent, Nathalie Arbour, Alexandre Prat.
Presenter affiliation: CRCHUM, Montreal, Canada; Multiple Sclerosis Clinic of the CHUM, Montreal, Canada. 40

Contribution of Pannexin1 to experimental autoimmune encephalomyelitis
Sarah E. Lutz, Estibaliz Gonzalez, Juan Carlos Chiara, Alberto Pérez-Samartín, Leonid Tarassishin, Naman K. Patel, Hiromitsu Negoro, Sylvia O. Suadicani, Sunhee C. Lee, Carlos Matute, Eliana Scemes.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York. 41

Loss of astrocyte connexins 43 and 30 does not significantly alter susceptibility or severity of acute experimental autoimmune encephalomyelitis in mice
Sarah E. Lutz, Cedric S. Raine, Celia F. Brosnan.
Presenter affiliation: Albert Einstein College of Medicine, Bronx, New York. 42

A zebrafish model of bacterial meningitis
Cressida A. Madigan, Lalita Ramakrishnan.
Presenter affiliation: University of Washington, Seeattle, Washington. 43

Studies on pericyte regulated functions at the neurovascular unit
Maarja A. Mäe, Annika Keller, Elisabeth Raschperger, Henrik Johansson, Janne Lehtiö, Christer Betsholtz.
Presenter affiliation: Karolinska Institute, Stockholm, Sweden. 44

Glutathione PEGylated liposomes—Towards enhanced drug delivery to the brain
David Maussang, Jaap Rip, Linda Chen, Susanne M. Van Der Pol, Marco De Boer, Helga E. De Vries, Pieter Gaillard.
Presenter affiliation: BBB technologies BV, Leiden, Netherlands. 45

The extracellular matrix protein laminin regulates the structure and function of the blood-brain barrier
Michael J. Menezes, Holly Colognato.
Presenter affiliation: Stony Brook University, Stony Brook, New York. 46

Mechanisms of lymphocyte transmigration across the blood-brain barrier studied in an *in vitro* model incorporating flow
Birgit Obermeier, Shumei Man, Yukio Takeshita, Anne C. Cotleur, Richard M. Ransohoff.
Presenter affiliation: Lerner Research Institute, Cleveland Clinic, Cleveland, Ohio. 47

Novel approaches to pathological BBB hyperpermeability—Targeting VEGFR2 phosphorylation sites
K. Elisabet Ohlin, Xiujuan Li, Lena Claesson-Welsh.
Presenter affiliation: Uppsala University, Rudbeck Laboratory, Uppsala, Sweden. 48

Behavioral and motor control studies of repeated FUS induced BBB openings in mice
Oluyemi O. Olumolade, Shutao Wang, Gesthmani Samiotaki, Elisa Konofagou.
Presenter affiliation: Columbia University, New York, New York. 49

Netrins enhance blood-brain barrier function and regulate immune responses at the blood-brain barrier
Cornelia Podjaski, Nathalie Lebeurrier, Lyne Bourbonniere, Sandra Larouche, Simone Terouz, Manon Blain, Catherine Larochelle, Lamia N. Hachehouche, Akiko Nakano, Mike Sabbagh, Jorge I. Alvarez, Jenea Bin, Pavel Gris, Peter Darlington, Jack P. Antel, Timothy E. Kennedy, Alexandre Prat.
Presenter affiliation: MNI, McGill University, Montreal, Canada; CHUM-Hospital Notre-Dame and CHUM Research Centre, Montreal University, Montreal, Canada. 50

Th17 cells enter into the brain parenchyma independently of α4 integrins but fail to control cerebral viral infection
Veit Rothhammer, Andreas Muschaweckh, Franziska Petermann, Sylvia Heink, Bernhard Hemmer, Ingo Drexler, Thomas Korn.
Presenter affiliation: Klinikum Rechts der Isar, Technical University Munich, Munich, Germany. 51

Permeability and reversibility timeline study of the focused-ultrasound induced blood-brain barrier opening at distinct pressures and pulse lengths *in vivo*
Gesthimani Samiotaki, Oluyemi Olumolade, Elisa Konofagou.
Presenter affiliation: Columbia University, New York, New York. 52

LSR, a new tight junction protein expressed at the blood-brain barrier
Fabien Sohet, Seo Yeon Lee, Nadine Ruderisch, Richard Daneman.
Presenter affiliation: University of California San Francisco, San Francisco, California. 53

Hetergeneous permeability of experimental brain metastases of breast cancer
Patricia S. Steeg, Brunilde Gril, Paul R. Lockman, Diane Palmieri, Quentin R. Smith.
Presenter affiliation: Women's Cancers Section, Bethesda, Maryland. 54

A new *in vitro* blood-brain barrier model under shear stress incorporating endothelial cells and astrocytes
Yukio Takeshita, Anne Cotleur, Birgit Obermeier, Richard M. Ransohoff.
Presenter affiliation: Cleveland Clinic, Cleveland, Ohio. 55

Initial contact of glioblastoma cells with existing normal brain endothelial cells strengthen the barrier function via fibroblast growth factor 2 secretion
Kunihiko Tanaka, Keisuke Toyoda, Shinsuke Nakagawa, Masami Niwa.
Presenter affiliation: Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan. 56

Endothelial apico-basal signal compartmentalisation shapes the microvascular response at blood-neural barriers
Natalie Hudson, Mosharraf H. Sarker, Kubala Ewa, Zoe K. Ockrim, Martha Betson, Roberta Martinelli, Clare E. Futter, Paul A. Fraser, John Greenwood, David T. Shima, Patric Turowski.
Presenter affiliation: UCL Institute of Ophthalmology, London, United Kingdom. 57

Microbubble distribution dependence of focused ultrasound induced blood-brain barrier opening
Shutao Wang, Gesthimani Samiotaki, Oluyemi Olumolade, Jameel A. Jameel, Elisa E. Konofagou.
Presenter affiliation: Columbia University, New York, New York. 58

Brain endothelial COX-2 is a key player in inflammatory fever
Daniel B. Wilhelms, Unn D. Kugelberg, David Engblom, Anders Blomqvist.
Presenter affiliation: Linköping University, Linköping, Sweden. 59

Leptin modified with amphiphilic block copolymers for enhanced CNS delivery and treatment of obesity
Xiang Yi, Sue A. Farr, William A. Banks, Alexander V. Kabanov.
Presenter affiliation: University of North Carolina, Chapel Hill, North Carolina. 60

β-Catenin mediates regulation of CYP1B1 in endothelial cells of the blood-brain barrier
Nicole Ziegler, Beate Fisslthaler, Cathrin J. Czupalla, Elisabetta Dejana, Ingrid Fleming, Stefan Liebner.
Presenter affiliation: Goethe University, Frankfurt, Germany. 61

THURSDAY, December 6—4:30 PM

Wine and Cheese Party

THURSDAY, December 6—7:30 PM

SESSION 4 MODEL ORGANISMS

Chairpersons: **B. Anand-Apte,** Cleveland Clinic Lerner College of Medicine, Ohio
R. Bainton, University of California, San Francisco

Regulation of the blood retinal barrier
Jing Xie, Alecia Cutler, Bela Anand-Apte.
Presenter affiliation: Cleveland Clinic Lerner College of Medicine, Cleveland, Ohio. 62

The role of glial cells in nutrient homeostasis of the *Drosophila* brain
Stefanie Limmer, Christian Klämbt.
Presenter affiliation: Institut für Neurobiologie, University of Münster, Münster, Germany. 63

Predicting efflux ratios and blood-brain barrier penetration from chemical structure: combining passive permeability with active efflux by P-glycoprotein
Elena Dolghih, Matthew P. Jacobson.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 64

Live imaging of blood-brain barrier development in zebrafish
Bensheng Ju, Robyn A. Umans, Hannah E. Henson, Chaithanyarani Parupalli, Jennifer L. Peters, Michael R. Taylor.
Presenter affiliation: St. Jude Children's Research Hospital, Memphis, Tennessee. 65

Discovery and interrogation of evolutionarily conserved blood brain barrier chemical protection pathways
Michael K. DeSavo, Samantha Hindle, Souvinh Orng, Roeben Munji, Fahima Mayer, Roland Bainton.
Presenter affiliation: University of California at San Francisco, San Francisco, California. 66

Blood-brain barrier on chip to study the effect of shear stress on barrier function
Lonneke M. Griep, Floor Wolbers, Bjorn de Wagenaar, Paul M. ter Braak, Andries D. van der Meer, Albert van den Berg.
Presenter affiliation: University of Twente, Enschede, Netherlands. 67

ABC transporters regulate CNS chemoprotection through endogenous steroid signaling at the BBB.
Samantha Hindle, Souvinh Orng, Michael DeSalvo, Elena Dolgikh, Hiroshi Ishimoto, Fahima Mayer, Toshihiro Kitamoto, Matt Jacobson, Roland Bainton.
Presenter affiliation: University of California San Francisco, San Francisco, California. 68

FRIDAY, December 7—9:00 AM

SESSION 5 RETINAL VASCULATURE AND OTHER TISSUE BARRIERS

Chairpersons: **C.Y. Cheng,** Rockefeller University, Population Council, New York, New York
H. Gerhardt, London Research Institute-Cancer Research UK

Blood-testis barrier, drug transporters and spermatogenesis
C. Yan Cheng, Dolores D. Mruk.
Presenter affiliation: Rockefeller University, Population Council, New York, New York. 69

Perivascular resident macrophage-like melanocytes in the inner ear are essential for intrastrial fluid-blood barrier integrity
Xiaorui Shi.
Presenter affiliation: Oregon Health & Science University, Portland, Oregon. 70

Novel mechanisms of brain response to immune signaling after peripheral surgery
Niccolò Terrando, Maarja Mäe, Daniel Gustavsson, Katerina Akassoglou, Lars I. Eriksson.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden. 71

Novel 3D analysis of Claudin-5 reveals significant endothelial heterogeneity among CNS microvessels
Debayon Paul, Ann Cowan, Shujun Ge, Joel S. Pachter.
Presenter affiliation: UConn Health Center, Farmington, Connecticut. 72

Holger Gerhardt.
Presenter affiliation: London Research Institute-Cancer Research UK, London, United Kingdom.

A high throughput screen for regulators of blood retina barrier permeability
Daniel L. Chao, Enrique L. Salero-Coca, Claude-Henry Volmar, Shaun Brothers, Claes Wahlestedt, Jeffrey L. Goldberg.
Presenter affiliation: University of Miami- Bascom Palmer Eye Institute, Miami, Florida. 73

Blood-brain barrier modulation in amyloid precursor protein (App-Tg2576) mice
James Keaney, Matthew Campbell, Finnian Hanrahan, Marian M. Humphries, Anna-Sophia Kiang, Paul F. Kenna, Pete Humphries.
Presenter affiliation: Trinity College Dublin, Dublin, Ireland. 74

FRIDAY, December 7—2:00 PM

SESSION 6 BBB IN INFLAMMATION AND DISEASE

Chairpersons: **E. Tournier-Lasserve,** Université Paris Diderot - Paris 7, France
E. de Vries, MS Center, Amsterdam, the Netherlands

Elisabeth Tournier-Lasserve.
Presenter affiliation: Université Paris Diderot, Paris, France.

Polymicrobial sepsis elicits a robust CNS transcriptional response which does not require MyD88-dependent signaling in endothelial cells
Gerard Honig, Betty Diamond.
Presenter affiliation: Feinstein Institute for Medical Research, Manhasset, New York. 75

Neuroimmunological blood brain barrier opening in experimental cerebral malaria
Alexandru Movila, Adela Nacer, Ute Frevert.
Presenter affiliation: NYU School of Medicine, New York, New York. 76

Visualizing the stepwise recruitment of paracellular and transcellular pathways during blood-brain barrier breakdown in stroke using two-photon imaging
Axel Nimmerjahn, Ahmet Arac, Daniel Knowland, Sarah E. Lutz, John Perrino, Gary K. Steinberg, Ben A. Barres, Dritan Agalliu.
Presenter affiliation: University of California, Irvine, Irvine, California. 77

The blood-brain barrier in multiple sclerosis
Elga de Vries.
Presenter affiliation: MS center Amsterdam, Amsterdam, the Netherlands. 78

Activated leukocyte cell adhesion molecule regulates BBB integrity and neuroinflammation
Marc-André Lécuyer, Olivia Saint-Laurent, Jorge I. Alvarez, Romain Cayrol, Lyne Bourbonnière, Alexandre Prat.
Presenter affiliation: CRCHUM-University of Montreal, Montreal, Canada. 79

ALCAM is not required for T cell invasion into the CNS and for the development of EAE in the C57BL/6 mouse
Ruth Lyck, Christoph Wyss, Michael Abadier, Claudia Blatti, Urban Deutsch, Britta Engelhardt.
Presenter affiliation: University of Bern, Bern, Switzerland. 80

FRIDAY, December 7—5:00 PM

KEYNOTE SPEAKER

N. Joan Abbott
King's College London, United Kingdom

"Evolution of the blood-brain barrier and neurovascular unit" 81

FRIDAY, December 7

BANQUET

Cocktails 6:00 PM Dinner 6:45 PM

SATURDAY, December 8—9:00 AM

SESSION 7 CROSSING THE BBB

Chairpersons: **M. Bynoe,** Cornell University Veterinary Medical College, Ithaca, New York
M. Wood, University of Oxford, United Kingdom

Central nervous system barriers under adenosine signaling control
Margaret S. Bynoe.
Presenter affiliation: Cornell University, Veterinary Medical College l, Ithaca, New York. 82

The challenge of engineered nanomaterial brain delivery
Robert A. Yokel, Eric A. Grulke, Michael T. Tseng, Younsoo Bae, Thomas D. Dziubla, Mo Dan.
Presenter affiliation: University of Kentucky, Lexington, Kentucky. 83

3D single molecule tracking of Tf transport at blood-CNS barriers using multifocal plane microscopy
Sripad Ram, Dongyoung Kim, E. Sally Ward, Raimund J. Ober.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas; UT Dallas, Richardson, Texas. 84

Defining the therapeutic profile of blood-brain barrier crossing TfR bispecific antibodies
Joy Yu, Jessica A. Couch, Yin Zhang, Jacqueline Tarrant, Reina N. Fuji, Raymond K. Tong, Kwame Hoyte, Wilman Luk, Yanmei Lu, Kapil Gadkar, Saileta Prabhu, Benjamin Ordonia, Yuwen Lin, William J. Meilandt, Kimberly Scearce-Levie, James A. Ernst, Mark S. Dennis, Ryan J. Watts.
Presenter affiliation: Genentech, South San Francisco, California. 85

Matthew Wood.
Presenter affiliation: University of Oxford, Oxford, United Kingdom.

Blood-brain or blood-retinal barrier disruption by focused ultrasound and microbubbles for targeted CNS drug delivery
Juyoung Park, Yong-Zhi Zhang, Natalia Vykhodtseva, Nathan J. McDannold.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts; Sungkyunkwan University, Seoul, South Korea. 86

Activation of signaling pathways following localized delivery of systemically-administered neurotrophic factors across the blood-brain barrier using focused ultrasound and microbubbles
Babak Baseri, James J. Choi, Thomas Deffieux, Mania Samiotaki, Yao-Sheng Tung, Oluyemi Omuolade, Scott A. Small, Barclay Morrison III, Elisa E. Konofagou.
Presenter affiliation: Columbia University, New York, New York. 87

AUTHOR INDEX

Abadier, Michael, 80
Abbott, N. Joan, 81
Abumrad, Naji N., 15
Agalliu, Dritan, 77
Akassoglou, Katerina, 20, 71
Alvarez, Jorge I., 14, 27, 40, 50, 79
Anand-Apte, Bela, 62
Anrather, Josef, 33
Antel, Jack P., 50
Antognetti, Giovanna, 22
Arac, Ahmet, 77
Arbour, Nathalie, 40
Argaw, Azeb T., 9
Asp, Linnea, 9

Bae, Younsoo, 83
Baeten, Kim M., 20
Bainton, Roland, 66, 68
Banks, William A., 60
Barres, Ben A., 77
Baseri, Babak, 87
Bearden, Shawn E., 7
Bedard, Catherine, 20
Berberich-Siebelt, Friederike, 21
Betsholtz, Christer, 44
Betson, Martha, 57
Bin, Jenea, 50
Blain, Manon, 50
Blatti, Claudia, 80
Blomqvist, Anders, 59
Boileau, Eve, 16
Bourbonnière, Lyne, 27, 50, 79
Brosnan, Celia F., 42
Brothers, Shaun, 73
Brule, Stephanie A., 10
Brunette, Eric, 16, 22
Buckman, Laura B., 15
Burgess, Alison, 31
Buttmann, Mathias, 21
Bynoe, Margaret S., 82

Camire, Ryan, 10
Campbell, Matthew, 29, 74
Caram-Salas, Nadia, 16, 22
Carvey, Paul M., 35
Cayrol, Romain, 40, 79
Cerutti, Camilla, 3
Chang, Chia-Sheng, 36
Chao, Daniel L., 73
Chen, Cherry C., 17
Chen, Kuen-Bao, 36
Chen, Linda, 45
Chen, Zu-Lin, 13
Cheng, C. Yan, 69
Cheng, Ka-Shun, 36
Chiara, Juan Carlos, 41
Choe, Youngshik, 6
Choi, James J., 87
Claesson-Welsh, Lena, 48
Colognato, Holly, 46
Cotleur, Anne, 47, 55
Cottarelli, Azzurra, 18
Couch, Jessica A., 85
Cowan, Ann, 72
Cudmore, Robert H., 19
Cuervo, Henar, 4
Cutler, Alecia, 62
Czupalla, Cathrin J., 61

Dan, Mo, 83
Daneman, Richard, 53
Darlington, Peter, 50
Dauwalder, Brigitte, 39
Davalos, Dimitrios, 20
De Boer, Marco, 45
De Vries, Helga E., 45, 78
de Wagenaar, Bjorn, 67
Deerinck, Thomas J., 20
Deffieux, Thomas, 87
Deflorian, Gianluca, 18
Degen, Jay L., 20
Deiss, Annika, 21
Dejana, Elisabetta, 61
Delany, Christie, 16
Deng, Kangwen, 34
Dennis, Mark S., 85
DeSalvo, Michael, 66, 68
Deutsch, Urban, 80
Diamond, Betty, 75

Dias, David O., 8
Dietz, Lena, 21
Ding, Aihao, 33
Ding, Wen, 16
Dolghih, Elena, 64, 68
Drewes, Lester R., 2
Drexler, Ingo, 51
Dutta, Dipankar J., 9
Dziubla, Thomas D., 83

Edwards, Laura, 3
Eldredge, John, 16, 22
Elisabetta, Dejana, 18
Ellacott, Kate L., 15
Ellisman, Mark H., 20
Engblom, David, 59
Engelhardt, Britta, 80
Erickson, Jamie, 34
Eriksson, Lars I., 71
Ernst, James A., 85
Ewa, Kubala, 57

Farr, Sue A., 60
Farrington, Graham, 16, 22
Ferrara, Napoleone, 9
Fisslthaler, Beate, 61
Fleming, Ingrid, 61
Fraser, Paul A., 57
Frevert, Ute, 76
Frisén, Jonas, 8
Fruttiger, Marcus, 6
Fuji, Reina N., 85
Furuse, Mikio, 32
Futter, Clare E., 57

Gabathuler, Reinhard, 23
Gadkar, Kapil, 85
Gaillard, Pieter, 45
Garber, Ellen, 22
Garbuzova-Davis, Svitlana, 24
Garcia, Anna E., 15
Garcia-Bonilla, Lidia, 33
Ge, Shujun, 72
Gendron, Steve, 25
Ghayur, Tariq, 34
Gizatullin, Farid, 34
Goldberg, Jeffrey L., 73
Gonias Murray, Sara, 20
Gonzalez, Estibaliz, 41
Goodearl, Andrew, 34
Göritz, Christian, 8, 30
Graves, Steven M., 35
Greenwood, John, 57
Griep, Lonneke M., 67
Gril, Brunilde, 26, 54
Gris, Pavel, 50
Grulke, Eric A., 83
Gustavsson, Daniel, 71

Hachehouche, Lamia N., 27, 50
Hakozaki, Hiroyuki, 20
Hammarlund-Udenaes, Margareta, 28
Hanrahan, Finnian, 29, 74
Haqqani, Arsalan, 16
Harris, Christina, 34
Heink, Sylvia, 51
Hemmer, Bernhard, 51
Henson, Hannah E., 65
Higashi, Tomohito, 32
Hindle, Samantha, 66, 68
Hirota, Shinya, 12
Hochrainer, Karin, 33
Holl, Daniel, 8, 30
Honig, Gerard, 75
Hoyte, Kwame, 85
Hudson, Natalie, 57
Humphries, Marian M., 29, 74
Humphries, Peter, 29, 74
Hynynen, Kullervo, 31

Iadecola, Costantino, 33
Iqbal, Umar, 23
Ishimoto, Hiroshi, 68
Iwamoto, Noriko, 32

Jackman, Katherine, 33
Jacobson, Matthew P., 64, 68
Jameel, Jameel A., 58
Jaminet, Shou-Ching, 6
Johansson, Henrik, 44
John, Gareth R., 9
Ju, Bensheng, 65

Kabanov, Alexander V., 60
Kahles, Timo, 33

Karaoglu Hanzatian, Denise, 34
Keaney, James, 29, 74
Kebir, Hania, 27, 40
Keller, Annika, 44
Kenna, Paul F., 74
Kennedy, Timothy E., 50
Kiang, Anna-Sophia, 29, 74
Kim, Dongyoung, 84
Kitamoto, Toshihiro, 68
Klämbt, Christian, 63
Knowland, Daniel, 77
Konofagou, Elisa, 17, 49, 52, 58, 87
Korn, Thomas, 51
Kousik, Sharanya M., 35
Kramer, Elisabeth G., 9
Kugelberg, Unn Ö., 59
Kuo, Eva Yuhua, 36, 37, 38

Lai, Ted Weita, 36, 37, 38
Lama, Chamala, 39
Lampugnani, Maria Grazia, 18
Lane, Diane, 33
Larochelle, Catherine, 40, 50
Larouche, Sandra, 50
Lassmann, Hans, 20
Lebeurrier, Nathalie, 50
Lécuyer, Marc-André, 40, 79
Lee, Hye Shin, 12
Lee, Seo Yeon, 53
Lee, Sunhee C., 41
Lehtiö, Janne, 44
LeMoan, Natacha, 20
Li, Dan, 6
Li, Xiujuan, 48
Liebner, Stefan, 1, 61
Limmer, Stefanie, 63
Lin, Yuwen, 85
Linden, David J., 19
Ling, Jennie B., 20
Liu, Qian, 12
Liu, Yu-Cheng, 36
Liu, Zejian, 2
Lockman, Paul R., 23, 26, 54
Lu, Yanmei, 85
Luk, Wilman, 85
Lutz, Sarah E., 41, 42, 77
Lyck, Ruth, 80

Madigan, Cressida A., 43
Mäe, Maarja, 44, 71
Male, David K., 3
Man, Shumei, 47
Mariani, John N., 9
Martinelli, Roberta, 57
Matute, Carlos, 41
Maussang, David, 45
Mayer, Fahima, 66, 68
McCarty, Joseph H., 12
McDannold, Nathan J., 86
Meilandt, William J., 85
Menezes, Michael J., 46
Merlini, Mario, 20
Moreno, Maria, 23
Morrison, Barclay, 87
Movila, Alexandru, 76
Mruk, Dolores D., 69
Munji, Roeben, 66
Muschaweckh, Andreas, 51

Nacer, Adela, 76
Nakagawa, Shinsuke, 56
Nakano, Akiko, 50
Napier, T. Celeste, 35
Negoro, Hiromitsu, 41
Nielsen, Corinne M., 4
Nimmerjahn, Axel, 77
Niwa, Masami, 56
Norris, Erin H., 13

Ober, Raimund J., 84
Obermeier, Birgit, 47, 55
Ockrim, Zoe K., 57
Ohlin, K. Elisabet, 48
Olumolade, Oluyemi, 17, 49, 52, 58, 87
Ordonia, Benjamin, 85
Orng, Souvinh, 66, 68

Pachter, Joel S., 72
Palmieri, Diane, 26, 54
Park, Juyoung, 86
Parupalli, Chaithanyarani, 65
Patel, Naman K., 41
Paul, Debayon, 72
Pérez-Samartín, Alberto, 41
Perrino, John, 77

Petermann, Franziska, 51
Peters, Jennifer L., 65
Petersen, Mark A., 20
Pham, Trinh, 9
Pleasure, Sam, 6
Podjaski, Cornelia, 50
Poon, Kin-Shing, 36
Prabhu, Saileta, 85
Prat, Alexandre, 5, 14, 25, 27, 40, 50, 79

Qian, Yongzhen, 26

Raine, Cedric S., 42
Ram, Sripad, 84
Ramakrishnan, Lalita, 43
Ransohoff, Richard M., 47, 55
Raschperger, Elisabeth, 44
Reyes, Steve B., 12
Rhodehouse, Bryce C., 7
Rip, Jaap, 45
Romero, Ignacio A., 3
Rothhammer, Veit, 51
Ruderisch, Nadine, 53
Ryu, Jae Kyu, 20

Sabbagh, Mike, 50
Sadiq, Muhammad Waqas, 28
Saint-Laurent, Olivia, 40, 79
Salero-Coca, Enrique L., 73
Samiotaki, Gesthimani, 49, 52, 58, 87
Sanberg, Paul R., 24
Sarker, Mosharraf H., 57
Scearce-Levie, Kimberly, 85
Scemes, Eliana, 41
Scheer, Nico, 28
Schwartz, Annette, 34
Sharrack, Basil, 3
Shi, Xiaorui, 70
Shima, David T., 57
Siegenthaler, Julie, 6
Sisk, William, 16, 22
Small, Scott A., 87
Smirnoff, Dimitri S., 20
Smith, Quentin R., 23, 26, 54
Sneve, Mary, 2
Soblechero, Patricia, 3
Sofroniew, Michael V., 9
Sohet, Fabien, 53
Sorokin, Lydia M., 11
Stanimirovic, Danica, 16, 22
Stedman, Christopher, 34
Steeg, Patricia S., 26, 54
Steinberg, Gary K., 77
Stoll, Guido, 21
Strickland, Sidney, 13
Suadicani, Sylvia O., 41

Takeshita, Yukio, 47, 55
Tamboli, Robyn A., 15
Tanaka, Kunihiko, 56
Tarassishin, Leonid, 41
Tarrant, Jacqueline, 85
Taylor, Michael R., 65
ter Braak, Paul M., 67
Terouz, Simone, 27, 50
Terrando, Niccolò, 71
Tong, Raymond K., 85
Toyoda, Keisuke, 56
Tseng, Michael T., 83
Tung, Yao-Sheng, 17, 87
Turowski, Patric, 57

Umans, Robyn A., 65

van den Berg, Albert, 67
van der Meer, Andries D., 67
Van Der Pol, Susanne M., 45
Villanueva, Ruth, 34
Vitalis, Timothy Z., 23
Volmar, Claude-Henry, 73
Vykhodtseva, Natalia, 86

Wahlestedt, Claes, 73
Wallace, Courtney C., 10
Wang, Rong A., 4
Wang, Shutao, 17, 49, 58
Ward, E. Sally, 84
Watts, Ryan J., 85
Weise, Gesa, 21
Wilhelms, Daniel B., 59
Willis, Colin L., 10
Wolbers, Floor, 67
Wolf, C. Roland, 28
Wu, Shih-Ying, 17

Wyss, Christoph, 80

Xie, Jing, 62

Yao, Yao, 13
Yen, Lola Fenghuei, 37, 38
Yi, Xiang, 60
Yokel, Robert A., 83
Yu, Joy, 85

Zhang, Jingya, 9
Zhang, Yin, 85
Zhang, Yong-Zhi, 86
Zhou, Ping, 33
Ziegler, Nicole, 61

REGULATION OF ENDOTHELIAL BARRIER PROPERTIES IN THE CNS: UNDERSTANDING FROM WHERE THE WNT BLOWS

Stefan Liebner

Goethe University Clinic, Institute of Neurology, Frankfurt am Main, 60528, Germany

Endothelial Wnt/β-catenin signaling is necessary for developmental angiogenesis of the central nervous system (CNS) and differentiation of the blood-brain barrier (BBB), but it appears to be inoperable in the adult. In particular, its relevance for BBB maintenance is largely unknown. Additionally, we currently don‘t understand how vascular heterogeneity in CNS is accomplished during development and how it is maintained in adulthood. In particular, the leaky vascular phenotype in the circumventricular organs (CVOs), conferring neurosecretory and -sensory function, is the best described example.
To investigate if the Wnt/β-catenin pathway is operable in CVO endothelial cells we analyzed these structures in the BAT-gal reporter mouse during embryonic and postnatal development. At no stage of embryonic development, nor during early postnatal stages we could detect activation of β-catenin signaling in CVO vessels. Interestingly, reporter gene activation was observed specifically in the ependymal layer adjacent to vessels for example in the subfornical organ (SFO), suggesting that the Wnt/β-catenin pathway contributes to ependymal cell differentiation towards the barrier-forming tanycyte phenotype in the CVOs.
Dominant activation of the β-catenin pathway (gain-of-function, GOF) in endothelial cells during early postnatal development lead to premature expression of claudin-3/-5 in vessels of the SFO, whereas Meca-32 immunoreactivity was reduced. Moreover, β-catenin GOF generated a thinner vascular phenotype within the SFO. These findings are in line with previous data, showing a vascular „normalizing“ function of endothelial β-catenin signaling in brain tumor angiogenesis.
Currently, we investigate in detail the circuitry of the Wnt pathway in the CVOs and its specific role in CVO differentiation.
In summary, we can show so far that in CVO differentiation activation of the Wnt pathway switches from the endothelial compartment to the ependymal, epithelial compartment, suggesting either a cell-specific interpretation of a common Wnt signal or secretion of specific Wnt factors. Further investigations are required to better understand vascular heterogeneity in the brain in general and in the CVOs in particular.

REGULATION OF MONOCARBOXYLATE TRANSPORTER 1 (*MCT1*) BY CANONICAL WNT/β-CATENIN SIGNALING PATHWAY IN THE BLOOD-BRAIN BARRIER.

Zejian Liu, Mary Sneve, Lester R Drewes

University of Minnesota Duluth, Biomedical Sciences, Duluth, MN, 55812

MCT1 is the prominent facilitator for monocarboxylates (e.g. lactates, pyruvates and ketone bodies) across cerebral endothelium, thus it plays an essential role in cellular metabolism and brain homeostasis. Canonical Wnt/β-catenin signaling pathway has been shown to be critical for rodent blood-brain barrier formation during embryonic development. Here we investigated the effects of Wnt/β-catenin signaling on the expression of *Mct1* in cultured RBE4 cells, an *in vitro* model of rat brain endothelium. As a transcription factor, β-catenin in resting cells is phosphorylated by glycogen synthase kinase beta (GSK-3β), followed by proteasomal degradation. Lithium is able to activate this pathway by inhibiting GSK-3β, thus to stabilize cytoplasmic β-catenin, which will then accumulate and translocate into the nucleus to promote target gene expression. We found that, compared with 20mM NaCl control, treatment of RBE4 cells with 20mM LiCl for 24 hours caused MCT1 protein level to be elevated by 55%. Accordingly, nuclear accumulation of β-catenin was increased by 300%. The specificity of Wnt/β-catenin signaling on these effects was confirmed by the observation that quercetin, an inhibitor to β-catenin signaling by blocking its entry into nucleus, completely reversed the increase of both MCT1 and nuclear β-catenin levels. Although LiCl treatment significantly increased the mRNA level of Cyclin D1 by ~4 fold, a well established target gene downstream of Wnt/β-catenin signaling pathway, it failed to induce *Mct1*'s transcription. In addition, MCT1 was shown here to be regulated by lysosomal rather than proteasomal degradation. All taken together, we propose that the canonical Wnt/β-catenin pathway regulates *Mct1* expression in brain endothelium through lysosomal degradation machinery. Further studies will be focused on understanding the mechanism of MCT1's regulation that is lysosome mediated and involves the canonical Wnt/β-catenin signaling pathway, e.g. ubiquitination.

ROLE OF ENDOTHELIAL microRNAs IN LEUKOCYTE ADHESION TO CYTOKINE-ACTIVATED HUMAN BRAIN MICROVASCULAR ENDOTHELIUM

Camilla Cerutti[1], Patricia Soblechero[1], Laura Edwards[2], Basil Sharrack[3], David K Male[1], Ignacio A Romero[1]

[1]The Open University, LHCS, Milton Keynes, MK7 6AA, United Kingdom, [2]University of Nottingham , Clinical Neurology, Nottingham, NG7 2UH, United Kingdom, [3]University of Sheffield, SiTraN, Sheffield, S10 2HQ, United Kingdom

MicroRNAs (miRs) are small non-coding regulatory RNAs that act through repression of protein translation and/or mRNA degradation at the post-transcriptional level. MiRs are critical players in the pathogenesis of many diseases, including neuroinflammatory disorders such as multiple sclerosis (MS). One of the main pathological features of MS is leukocyte adhesion to cerebrovascular endothelium and subsequent infiltration into the CNS. Leukocyte adhesion to cerebrovascular endothelial cells (CECs), the main cellular constituent of the blood brain barrier (BBB), is a complex multi-step process where expression of endothelial adhesion molecules such as VCAM1 and ICAM1 is increased. Our previous results showed that the pro-inflammatory cytokines TNFα and INFγ induced changes in the levels of at least 100 miRs in human CECs *in vitro*. Here we assessed the role of specific deregulated endothelial miRs on leukocyte adhesion to CECs with both static and flow-based leukocyte adhesion *in vitro* assays using monocytic (THP1), T-cell (Jurkat) and human brain endothelial (hCMEC/D3) cell lines. Following an initial screening of five cytokine-deregulated CEC miRS using this *in vitro* BBB model, endothelial mir-126 and -155 appeared to have the most significant effects on leukocyte adhesion to hCMEC/D3 cells. Decreased levels of miR-155 prevented both THP1 and Jurkat adhesion to endothelium both under basal and cytokine-stimulated conditions. Opposite effects were observed when miR-155 expression was increased in unstimulated hCMEC/D3 cells, but not under inflammatory conditions. Elevated levels of miR-126 and miR-126* significantly prevented Jurkat and THP1 cell adhesion to hCMEC/D3 cells under both unstimulated and cytokine-treated conditions, an effect that was associated with decreased VCAM1 expression by endothelial cells. A similar effect on adhesion to CECs was observed when using peripheral blood mononuclear cells isolated from MS patients. In contrast, decreased levels of miR-126*, but not of miR-126, increased THP1 adhesion on cytokine-treated hCMEC/D3 cells whereas THP1 and Jurkat adhesion to unstimulated CECs was increased by antagonizing each miR. Our data indicate that miR-155, miR-126 and miR-126* modulate leukocyte adhesion to human brain microvascular endothelium and suggest a role for these miRs in neuroinflammation at the BBB.

POSTNATAL DELETION OF ENDOTHELIAL RBP-J CAUSES VASCULAR DEFECTS RESEMBLING BRAIN ARTERIOVENOUS MALFORMATIONS

Corinne M Nielsen, Henar Cuervo, Rong A Wang

University of California, San Francisco, Surgery, San Francisco, CA, 94143

Arteriovenous malformations (AVMs) are enlarged vessels that directly connect arteries and veins without an intervening capillary network. Clinically, AVMs are characterized by an entangled nidus of vessels, delivering high-flow, non-nutritive blood and prone to rupture. While molecular and cellular mechanisms promoting AVM formation are beginning to be understood, the cause of AVMs remains unknown. Notch signaling has emerged as a critical mediator of arteriovenous (AV) specification during development, by promoting arterial over venous endothelial cell fate. We have previously shown that misexpression of constitutively active *Notch4* throughout the neonatal mouse vascular endothelium induces hallmarks of cerebral AVMs; however, whether loss of *Notch* gene function leads to brain AVMs is unknown.

Here, we show that postnatal, endothelial specific loss of Rbp-J, a transcriptional regulator of Notch signaling, causes vascular defects resembling AVMs in both immature (neonatal Rbp-J deletion) and mature (adult Rbp-J deletion) mouse brains. Vascular abnormalities include enlarged and tortuous vessels within the brain vasculature, which function as AV shunts. Deletion of Rbp-J from the immature endothelium results in neonatal lethality, and histopathology suggests patterned hypoxia and necrosis in the brain, accompanied by gross motor deficits. To determine whether deletion of Rbp-J alters endothelial AV specification, we are investigating expression of *ephrinB2* and *EphB4*, genes encoding downstream effectors of Notch signaling and essential for arterial and venous endothelial identity, respectively. *EphB4* expression is upregulated in Rbp-J-deficient AV shunts in the cerebrovasculature. We are currently examining *ephrinB2* expression. Postnatal deletion of Notch1 in the vascular endothelium also results in abnormal AV connections, a key feature of AVMs in the cerebrovasculature.

Our data demonstrate that loss-of-function mutations in Notch pathway members lead to vascular abnormalities resembling brain AVMs. This finding may inform on strategies for effective therapeutic application – as either loss or gain of *Notch* gene function leads to formation of cerebrovascular AV shunts, fine-tuned modulation of Notch signaling is critical to ensure safe and effective treatments for Notch-associated brain AVMs.

MOLECULAR CONTROL OF CNS INFLAMMATION AT THE BBB

Alex Prat

Université de Montréal, Neuroscience, Montreal, H2L 4M1, Canada

The Blood Brain Barrier (BBB) protects the central nervous system by regulating molecular and cellular exchanges between the brain and the blood. The BBB is made of a network of tightly adherent endothelial cells (ECs) surrounded by astrocytic processes and pericytes which provide factors that contribute to BBB maintenance. Several proteomic based-profiling of human and animal BBB endothelial cells have revealed the presence of unique regulatory proteins involved in BBB physiology and trans-endothelial leukocyte migration, including proteins involved in cellular adhesion, cell structure, BBB development, immunity and defense, transport and trafficking and signal transduction. Recent work, using animal models of MS and spinal cord contusion, as well as human in vitro, in situ and ex vivo analyses revealed that these new BBB candidate proteins, including the adhesion molecules ALCAM, MCAM and Ninjurin-1, as well as the brain morphogens Hedgehog and Wnt/beta-catenin pathways are involved in the regulation of immune cell trafficking across vascular structures of the CNS. This presentation will provide a short overview of the progresses that were made over the last 5 years to identify novel pathways that are involved in the selective recruitment of specific immune cells to the CNS and in the process of CNS immune quiescence. These molecules are currently seen as the basis for the development of future therapies in neuroinflammatory disorders, including multiple sclerosis.

RETINOIC ACID SIGNALING IN THE DEVELOPING NEUROVASCULATURE

Julie Siegenthaler[1,2], Youngshik Choe[1], Dan Li[3], Shou-Ching Jaminet[3], Marcus Fruttiger[4], Sam Pleasure[1]

[1]University of California, San Francisco, Neurology, San Francisco, CA, 94158, [2]University of Colorado, School of Medicine, Pediatrics, Aurora, CO, 80045, [3]Beth Isreal Deaconess Medical Center/Harvard School of Medicine, Pathology, Boston, MA, 02215, [4] University College London, Opthalmology, London, EC1V 9EL, United Kingdom

As neural structures grow in size and increase their metabolic demand, the neurovasculature undergoes extensive growth and remodeling. Angiogenic cues produced and secreted by neural cells, including VEGF and Wnt, are critical for both initial blood vessel ingression, vessel patterning and maturation. What have not been well-defined are brain-specific modulators of these angiogenic pathways, signals that may put the brakes on strong angiogenic cues in a specific developmental context. Using both mouse genetic and in vitro models, we have identified retinoic acid (RA) as an inhibitor of Wnt signaling in fetal brain endothelial cells. One consequence of loss of RA-mediated inhibition of Wnt signaling is up regulation in the transcription factor Sox17 in brain endothelial cells. In the brain vasculature, Sox17 is normally found at high levels in arterial blood vessels and at low or undetectable levels in venous blood vessels. Also, Sox17 expression appears to be regulated by Wnt signaling in the neurovasculature as conditional knockdown of β-catenin in endothelial cells leads to reduced expression of Sox17. Targeted disruption of RA signaling in brain endothelial cells leads to up-regulation of Sox17, most notably in veins though arterial-venous specification does not appear to be affected. Surprisingly, targeted disruption of RA signaling also causes a significant increase in brain pericyte number. We suspect that this is also due to increased Wnt signaling since conditional knockdown of β-catenin in endothelial cells leads to diminished brain pericyte coverage. Taken together, this data points toward a model in which RA and Wnt signaling interact to ensure normal expression of Sox17 and, possibly, proper pericyte coverage in the developing brain vasculature.

BBB DISRUPTION PRECEDES NEUROINFLAMMATORY PATHOLOGY, DEMYELINATION AND COGNITIVE IMPAIRMENT IN HYPERHOMOCYSTEINEMIC MICE

Shawn E Bearden, Bryce C Rhodehouse

Idaho State University, Biological Sciences, Pocatello, ID, 83209-8007

Hyperhomocysteinemia (HHcy) is associated with blood-brain barrier disruption, white matter lesions, and dementia. The etiology of this process is unknown. Using a mouse model of mild HHcy, heterozygous deficiency of the cystathionine beta-synthase gene, we have documented these phenotypes across the lifespan. Increased blood-brain barrier permeability is manifest in early adulthood (3-6 months of age) and appears to be highest through the hippocampus and non-cortical regions. Markers of inflammation (VCAM, CD45) increase with HHcy in early adulthood followed by progressive demyelination in the fimbriae of the hippocampus. In the second half of the lifespan (13-29 months of age), HHcy mice present with approximately twice the number of apoptotic hippocampal cells than age-matched wild type mice. Cognitive performance was assessed by Morris water maze tests of spatial memory. Cognitive impairment was evident on multiple measures in older, but not younger, HHcy mice compared with age-matched wild type mice. There was no difference in amyloid beta load (x-40 or x-42) between HHcy and wild type mice at any age. These data collectively support the idea that blood-brain barrier disruption precedes inflammation and neuronal loss/demyelination in HHcy and that this phenotype progresses to significant spatial cognitive impairment without Alzheimer-like pathology.

PERICYTE-DERIVED SCAR FORMATION FOLLOWING LESIONS TO THE CENTRAL NERVOUS SYSTEM.

Christian Göritz, David O Dias, Daniel Holl, Jonas Frisén

Karolinska Institute, Department of Cell and Molecular Biology, Stockholm, SE-17177, Sweden

Damage to the central nervous system (CNS) often leads to scar tissue formation that constitutes locally at lesions, causing permanent tissue alteration and functional impairment.
Studying scar formation after spinal cord injury, we became interested in the origin and function of the stromal, non-glial, component of the scar. Pericytes (perivascular cells lining most capillaries) have been suggested to be mesenchymal stem cells based on their in vitro differentiation potential and are abundant in CNS tissue. We asked whether pericytes react upon injury and participate in scar formation. In our recently published work (Göritz et al., 2011, Science), we identified a specific pericyte subpopulation, named "type A pericytes", as major source of scar contributing stromal cells. Type A pericytes are embedded in the vascular wall but proliferate and leave the blood vessel upon injury, differentiating into fibroblast-like cells that deposit extracellular matrix to seal the lesion and form the persistent stromal scar core. The subtype specific injury response demonstrates functional heterogeneity among pericytes.
Exploring the general nature of type A pericyte-mediated scarring, we will present data comparing spinal cord injury with lesions in the brain.

ASTROCYTE-DERIVED VEGF-A DRIVES BLOOD-BRAIN BARRIER DISRUPTION IN CNS INFLAMMATORY DISEASE

Azeb T Argaw[1], Linnea Asp[1], Jingya Zhang[1], Trinh Pham[1], John N Mariani[1], Dipankar J Dutta[1], Elisabeth G Kramer[1], Napoleone Ferrara[2], Michael V Sofroniew[3], Gareth R John[1]

[1]Mount Sinai School of Medicine, Friedman Brain Institute, New York, NY, 10029, [2]Genentech, Research Oncology, South San Francisco, CA, 94080, [3]UCLA, David Geffen School of Medicine, Los Angeles, CA, 90095

In inflammatory CNS conditions such as multiple sclerosis (MS), current options to treat clinical relapse are limited, and more selective agents are needed. Disruption of the blood-brain barrier (BBB) is an early feature of lesion formation that correlates with clinical exacerbation, leading to edema, excitotoxicity, and entry of serum proteins and inflammatory cells. Here, we identify astrocytic expression of VEGF-A as a driver of BBB permeability in mice. Inactivation of astrocytic *Vegfa* expression reduced BBB breakdown, decreased lymphocyte infiltration and neuropathology in inflammatory and demyelinating lesions, and reduced paralysis in a mouse model of MS. Knockdown studies in CNS endothelium indicated activation of the downstream effector eNOS as the principal mechanism underlying the effects of VEGF-A on the BBB. Systemic administration of the selective eNOS inhibitor cavtratin in mice abrogated VEGF-A–induced BBB disruption and pathology and protected against neurologic deficit in the MS model system. Collectively, these data identify blockade of VEGF-A signaling as a protective strategy to treat inflammatory CNS disease.

SIGNALING AT THE NEUROVASCULAR UNIT FOLLOWING TRANSITORY FOCAL ASTROCYTE LOSS AND POST-TRANSLATIONAL OCCLUDIN MODIFICATION.

Colin L Willis, Ryan Camire, Stephanie A Brule, Courtney C Wallace

University of New England, Biomedical Sciences, Biddeford, ME, 04005

Blood-brain barrier (BBB) dysfunction is a feature of multiple sclerosis, ischemic stroke and other neurological disorders. Signal transduction at the neurovascular unit regulating BBB integrity, is poorly understood. We have previously shown that 3-chloropropanediol induced transitory loss of astrocytes in the rat inferior colliculus with disruption of tight junction complexes and BBB integrity. However, barrier integrity to dextrans (10-70 kDa) was restored while paracellular claudin-5 and occludin were absent. We now show increased occludin phosphorylation and maintenance of VE-cadherin and beta-catenin expression as BBB integrity was lost. Extracellular matrix components laminin and fibronectin showed extensive remodeling and deposition while astrocytes were absent. Extracellular matrix receptors play a key role in regulating signal transduction between these cell–extracellular matrix and cell–cell adhesion events. Integrin subunit alpha-5 showed transitory increased expression at 2-3 days, while integrin beta-3 showed increased expression at days 1-2, followed by decreased expression before returning to control levels by 6 days. Integrin receptor antagonist RGDS attenuated the increased laminin and fibronectin expression. This study shows an important difference between the regulation of tight and adherens junction proteins. In addition, it supports the hypothesis that a combination of adherens junctional proteins and a remodeled basement membrane, mediated in part through integrin receptors, provide a temporary size-selective barrier until tight junction proteins are restored to paracellular domains.

VASCULAR BASEMENT MEMBRANES AND THEIR CONTRIBUTION TO THE BLOOD-BRAIN BARRIER

Lydia M Sorokin

University of Muenster, Physiological Chemistry and Pathobiochemistry, Muenster, D-48149, Germany

The biochemical compositional of basement membranes (BM) varies with both blood vessel and with tissue type. Of all BM components, the laminin family shows the greatest variability and represents the biological active component of BMs, interacting with a wide repertoire of integrin and non-integrin receptors to control functions such as vessel integrity and permeability. Focus will be on the central nervous system (CNS) microvessels, which have a unique composition of cellular and extracellular matrix layers that collectively constitute the blood-brain barrier. In addition to the endothelial cell monolayer and its underlying BM, cerebral microvessels are ensheathed by astrocyte endfeet and leptomeningeal cells, which contribute to a second BM, the so-called parenchymal BM as it delineates the border to the brain parenchyma. At the level of capillaries these two BMs fuse to form a single structure, which shares characteristics of both endothelial and parenchymal BMs. While considerable information is available on the cellular constitutents of the CNS microvessels and their contribution to the BBB, little is known about the BM layers. Our work has shown that endothelial and parenchymal BMs of CNS vessels are structurally and functionally distinct, and has highlighted their importance in the restricted permeability characteristic of the CNS microvessels. In particular, laminin isoforms are heterogeneously localized along the length of CNS microvessels and play an important role in defining sites of high and low penetrability by infiltrating cells, such as extravasating leukocytes during inflammation1. Data will be presented on the biochemical differences of BMs of CNS microvessels, and how vascular laminins provide cues that determine mechanisms of leukocyte penetration of CNS postcapillary venules (1).

1. Wu, C., F. Ivars, P. Anderson, R. Hallmann, D. Vestweber, P. Nilsson, H. Robenek, K. Tryggvason, J. Song, E. Korpos, K. Loser, S. Beissert, E. Georges-Labouesse, & L.M. Sorokin. 2009. Nat Med. 15, 519-27

ANALYZING INTEGRINS AND THE EXTRACELLULAR MATRIX IN NEUROVASCULAR DEVELOPMENT AND DISEASE

Hye Shin Lee, Steve B Reyes, Qian Liu, Shinya Hirota, Joseph H McCarty

MD Anderson Cancer Center, Cancer Biology, Houston, TX, 77030

Precise regulation of cell-cell and cell-extracellular matrix (ECM) adhesion and communication is essential for proper development and homeostasis of the neurovascular unit and blood-brain barrier (BBB). Integrins are receptors for ECM proteins and many integrins are expressed in neural and vascular cells. Roles for integrin-mediated adhesion and signaling pathways in BBB development and homeostasis, however, remain mostly uncharacterized. We have used Cre/lox strategies in mice to analyze the roles for αvβ8 integrin. This integrin is expressed in perivascular glial cells where is binds and activates ECM-associated latent TGFβs. Selective ablation of αv or β8 integrin genes in developing glial cells (via Nestin-Cre or GFAP-Cre) leads to severe neurovascular pathologies, including abnormal endothelial cell growth and sprouting, hemorrhage, and premature death associated with post-natal neurological decline. Genetic ablation of TGFβ receptor signaling in vascular endothelial cells during development using Alk1-Cre or PDGFBB-CreERT2 leads to similar neurovascular pathologies. In contrast, when integrin expression is inducibly ablated in astroglial cells (via GFAP-CreERT2), after blood vessels have reached quiescence and endothelial cells are no longer proliferating and sprouting, we do not detect acute neurovascular pathologies. However, if we initiate pathological angiogenesis in the adult brain by implanting a tumor or inducing vascular damage via cortical stab wounds, we find that αvβ8 integrin is essential for controlling new blood vessel growth and BBB permeability. These data reveal essential roles for αvβ8 integrin and its latent TGFβ ECM ligands in regulating cerebral blood vessel patterning and BBB permeability primarily during active stages of angiogenesis. Our results also identify the first paracrine signaling axis that couples perivascular glial cells to endothelial cells in the brain and retina.

ASTROCYTIC LAMININ REGULATES PERICYTE DIFFERENTIATION AND BLOOD BRAIN BARRIER INTEGRITY

Yao Yao, Zu-Lin Chen, Erin H Norris, Sidney Strickland

The Rockefeller University, Neurobiology and Genetics, New York, NY, 10065

Astrocytic endfeet generate brain-specific basement membrane (BM), which merges with endothelial BM, participating in the formation of blood-brain barrier (BBB). Pericytes, embedded in the BMs, signal to both astrocytes and endothelial cells. Among all extracellular matrix (ECM) proteins, laminin shows differential distribution between astroytic BM and endothelial BM. How this distribution pattern confers or affects BBB integrity remains largely unknown. Here, we demonstrated a direct role of astrocytic laminin in the maintenance of BBB integrity. Using conditional knockout mice and an acute model to abrogate astroytic laminin expression specifically in astrocytes, we showed that lack of astrocytic laminin promoted the differentiation of pericytes from the resting stage to the contractile stage. Additionally, it also decreased the expression of aquaporin-4 (AQP4) in astrocytic endfeet and tight junction proteins in endothelial cells. Our results indicate a novel and crucial role of astroytic laminin in the regulation of pericyte differentiation and BBB permeability.

THE ROLE OF JAML IN PROMOTING LEUKOCYTE MIGRATION ACROSS CNS BARRIERS

Jorge I Alvarez, Alexandre Prat

CRCHUM, Neurosciences, Montreal, H2L2W5, Canada

The earliest events leading to lesion formation in multiple sclerosis (MS) include blood-brain barrier (BBB) disruption and migration of leukocytes into the central nervous system (CNS). The engagement of cell adhesion molecules (CAMs) on endothelial cells (ECs) and their ligands on leukocytes is a well known step in the process of leukocyte infiltration to the brain; however the extent of players governing this process in the distinct CNS barriers remains to be established. Aside from CAMs, molecules of the junctional adhesion molecule (JAM) family are also known to promote leukocyte migration. In this study we found JAM-like protein (JAML) to be expressed in primary cultures of human BBB-ECs and to be upregulated under inflammatory conditions. These findings were paralleled in situ when analyzing the CNS of MS and experimental autoimmune encephalomyelitis animals. In human peripheral blood, JAML was predominantly expressed by monocytes and CD8 T cells and barely detected by flow cytometry on the surface of other immune cells. Analysis of ex-vivo blood leukocytes and CNS specimen obtained from MS patients demonstrated a significant upregulation of JAML expression in both cell types. In addition, we found expression of the Coksackie Adenovirus Receptor (CAR), a ligand of JAML, in the choroid plexus of human and mice. Our preliminary data indicate a potential role for JAML in supporting the interaction of monocytes and CD8 T cells with the BBB, but also with the Blood –cerebrospinal fluid Barrier (BCB) and thus highlight JAML as an important player in leukocyte migration across distinct CNS barriers.

OBESITY IS ASSOCIATED WITH ELEVATED PLASMA S100B WHICH IS REDUCED FOLLOWING WEIGHT-LOSS ASSOCIATED WITH ROUX-EN-Y GASTRIC BYPASS SURGERY

Laura B Buckman[1], Anna E Garcia[2], Robyn A Tamboli[2], Naji N Abumrad[2], Kate L Ellacott[1]

[1]Vanderbilt University Medical Center, Molecular Physiology and Biophysics, Nashville, TN, 37232, [2]Vanderbilt University Medical Center, Surgery, Nashville, TN, 37232

Obesity increases the risk of severe neurological disorders with a 74% increased risk of dementia, an 11% increased risk of mortality from traumatic brain injury, and a 6% higher incidence of stroke per unit of body mass index (BMI; kg/m^2) > 30. Neuroinflammation has been linked to obesity in rodent models, characterized by astrogliosis and increased pro-inflammatory cytokine production. However, there is limited information on obesity-associated neuroinflammation in humans. Plasma S100B is a putative biochemical marker of neuroinflammation and dysfunction; where elevated levels may indicate changes in permeability at the blood-brain barrier associated with neuropathological conditions such as stroke, Alzheimer's disease and psychiatric illness. Plasma S100B levels have been shown to be positively correlated with BMI; however, this has led to the suggestion that the increased plasma S100B in obesity is adipose derived. We investigated the influence of obesity and gastric bypass-induced weight loss on the regulation of plasma S100B. Plasma S100B protein was measured by ELISA in 21 non-obese (BMI <25), healthy adults and compared to 42 obese (BMI >35) adults before and 6 months after gastric bypass surgery. Obese patients had 62% higher plasma S100B than non-obese subjects. There was a significant decrease in plasma S100B after gastric bypass surgery. However, there was no statistically significant relationship between change in plasma S100B and any of the metabolic parameters examined including change in BMI, plasma C-reactive protein, weight, body fat, plasma leptin and HOMA-IR (homeostatic model assessment-insulin resistance). Studies in mice also indicate a significant increase in plasma S100B in diet-induced obese compared to lean animals. S100B expression was increased in the brain of the obese mice mirroring the pattern seen in the plasma. In contrast, the white adipose tissue of obese mice had decreased expression of S100B. Combined with the data from the human studies, this suggests that changes in plasma S100B in obesity are not solely due to increased adiposity. In summary, the significant increase in plasma levels of S100B in obese human subjects may reflect a link between obesity and neuroinflammation.

ANALGESIC EFFICACY OF SYSTEMICALLY ADMINISTERED DALARGIN CONJUGATED WITH THE BLOOD-BRAIN BARRIER CARRIER, FC5

Nadia Caram-Salas[1], Arsalan Haqqani[1], Eric Brunette[1], Christie Delany[1], Eve Boileau[1], Graham Farrington[2], John Eldredge[2], William Sisk[2], Wen Ding[1], Danica Stanimirovic[1]

[1]National Research Council of Canada, Human Health Therapeuthics, Ottawa, K1A0R6, Canada, [2]Biogen Idec, Corp., Cambridge, MA, 02142

The blood-brain barrier (BBB) effectively restricts the passage of molecules of >500 Da from the blood into the brain parenchyma. Delivery of (macro) molecules across the BBB potentially could be achieved by linking them to a ligand undergoing a receptor-mediated transcytosis. We have been evaluating the use of FC5, a camelid single-domain antibody fragment (V_HH; 15 kD) that has been shown to transmigrates the BBB in vitro, as a potential platform molecule that can efficiently transport therapeutic peptides across the BBB. In this study, we evaluated the efficacy of systemically administered FC5 conjugated to the analgesic enkephalin Dalargin [D-Ala2, Leu5]-enkephalyl-Arg (Dal). Dal produces analgesia by activation of μ opioid receptors located in the periaqueductal gray region of the brain. Systemically administered Dal has no efficacy due to the poor brain bioavailability, but is pharmacologically effective following either intracerebroventricular (icv) or intrathecal injection. Dal with a C-terminal cysteine was chemically conjugated to FC5 or a negative control V_HH antibody fragment, A20.1 (anti-C. Dificille toxin A) using a heterobifunctional NHS-maleimide cross-linker. The brain bioavailability of FC5-Dal and A20.1-Dal was assessed by the behavioral pain reflex evoked by a focused noxious heat stimuli in the Hargreaves pain model (intraplantar injection of Complete Freund Adjuvant). The maximum possible analgesic effect (%MPE; 20s cut off) after icv administration of Dal, FC5-Dal and A20.1-Dal showed a range of 35-47%. In contrast iv injections of Dal alone and A20.1-Dal showed no analgesic effect whereas iv administration of FC5-Dal (6-21 mg/kg) induced a dose response analgesic effect; 3 consecutive injections of 7 mg/kg FC5-Dal given one hour apart achieved a MPE of 40%, a magnitude similar to that observed after the icv injections. A highly sensitive and specific multiple reaction monitoring (MRM)-ILIS method to detect and quantify unlabeled V_HHs in multiplexed assays was used to quantify serum and CSF concentration of FC5 and A20.1, co-injected iv using the same dosing paradigm. The plasma pharmacokinetics of FC5 and A20.1 were identical. However, while A20.1 remained undetectable in CSF, FC5 was detected at levels>100ng/mL 15 min after the last injection, and showed a decay curve with slower kinetics compared to that in the blood. The results demonstrate that FC5 is pharmacologically efficacious vector for brain delivery of neuroactive peptides, including analgesic peptides.

THE CAVITATION-DEPENDENT TIGHT-JUNCTION INTEGRITY OF ULTRASOUND-INDUCED BLOOD-BRAIN BARRIER OPENING

Yao-Sheng Tung[1], Cherry C Chen[1], Oluyemi O Olumolade[1], Shih-Ying Wu[1], Shu-Tao Wang[1], Elisa K Konofagou[1,2]

[1]Columbia University, Biomedical Engineering, New York, NY, 10027,
[2]Columbia University, Radiology, New York, NY, 10032

Distinct from the general capillaries, the blood-brain barrier (BBB) consists of the tight junctions between endothelial cells in Central Nervous System capillaries that restrict the passage of solutes. Acoustic cavitation induced by the activation of microbubbles in an acoustic field is currently the only noninvasive approach to temporarily induce localized BBB opening without damaging the surrounding tissues. At lower acoustic pressures, acoustically driven microbubble oscillations have been shown to open the BBB, i.e., without requiring any microbubble collapse or fragmentation. At higher acoustic pressures, inertial cavitation, i.e., the collapse of microbubbles, releases high energy and may damage the surrounding structures. Here, the tight junction integrity, determined by immunofluorescence staining of the zonula occluden (ZO)-1 protein, under stable cavitation (SC) and inertial cavitation (IC) responsible for BBB opening was investigated for the first time. Using 1.5-MHz focused ultrasound (FUS) and 4-5-µm microbubbles, the BBB at the right hippocampus was opened using SC (acoustic pressure: 0.18 MPa) and IC (acoustic pressure: 0.45 MPa) in 33 mice through their intact scalp and skull, while the left hippocampus served as control. BBB opening was detected using T1-weighted magnetic resonance (MR) and fluorescence imaging. 3-kDa fluorescently-tagged dextran was shown capable of diffusing to the parenchyma after IC-induced BBB opening at 0.45 MPa but not after SC-induced BBB opening at 0.18 MPa. No tight junction rupture and no difference was noted in the expression of ZO-1 between the FUS and control sites or between the SC and IC cases, indicating that the tight junctions were left intact. Hence, either the tight junction became more permeable or the transcellular diffusion may constitute the main molecular diffusion route through the FUS-induced BBB opening at the acoustic parameters that were used in our study.

FIBROBLAST GROWTH FACTOR BINDING PROTEIN 1 (FGFBP1) IN BLOOD BRAIN BARRIER ESTABLISHMENT AND MAINTENANCE

Azzurra Cottarelli, Maria Grazia Lampugnani, Gianluca Deflorian, Dejana Elisabetta

IFOM Foundation, Vascular Biology Unit, Milan, 20139, Italy

Endothelial cells (ECs) in different tissues and organs show distinct morphological and functional traits that confer high levels of specialization suitable to each distinct vascular district. One of the most striking examples of this phenomenon is the brain microvasculature, which represents a component of the Blood Brain Barrier (BBB). BBB is a highly specialized vascular structure that tightly regulates the exchanges between the blood and the central nervous system (CNS). The canonical Wnt/β-catenin pathway that is specifically activated in CNS vessels during development regulates BBB initiation and maintenance. Moreover, inactivation of this pathway in vivo leads to angiogenic defects in the CNS and not in other vascular regions.
Affymetrix analysis previously performed in our group provided a list of genes whose transcription is selectively regulated upon Wnt3a stimulation in murine primary ECs isolated from brain (bMEC). One of the most upregulated transcripts is that of Fibroblast Growth Factor Binding Protein 1 (FGFBP1) gene. QPCR confirmed a 20 fold increase of FGFBP1 transcript in bMEC treated with Wnt3a. FGFBP1 is a cargo protein that, after being secreted in the extracellular matrix (ECM), is able to non-covalently bind the FGF immobilized in the ECM and to mobilize it, protecting it from degradation and presenting it to FGF tyrosine-kinase receptor on the cell membrane.
The aim of this project is to clarify the role of FGFBP1 in BBB function with an experimental approach based on both in vitro (primary cultures of murine brain ECs) and in vivo (zebrafish and the endothelial-specific tamoxifen-inducible knock out mice) models.
FGFBP1 knock down in zebrafish, using morpholinos, displayed a strong hemorrhagic phenotype at the level of the brain, together with a highly abnormal vascular pattern, suggesting a role for this protein in the vascular development. Moreover, also vessel permeability seems to be impaired in FGFBP1 morphants. Indeed, they show massive leakage of the tracer 10 kDa dextran-rhodamine into the nervous system in comparison to animals treated with control morpholino.
These results, together with the data coming from the other models, will allow defining the role of FGFBP1 in brain vascular development and in the acquisition and/or maintenance of the specific and peculiar barrier properties that blood vessels show in the brain.

TIME-LAPSE IN VIVO TWO PHOTON IMAGING OF ADULT CEREBRAL VASCULATURE

Robert H Cudmore, David J Linden

Johns Hopkins School of Medicine, Department of Neuroscience, Baltimore, MD, 21205

The formation, maintenance, and rewiring of the cerebral vasculature are critical processes that ensure healthy brain function during development, adulthood, and in response to injury. Ultimately, this vascular network of arteries, veins, and capillaries creates a healthy environment by providing a range of brain cell types with their required nutrients. The picture that has emerged is that in the healthy adult, the cerebral vascular network remains remarkably stable, ready to respond to injury or disease. The data in support of this view is primarily derived from observations of vascular networks using ex vivo, in vitro, and fixed tissue preparations. While these techniques are invaluable, they provide limited snapshots of the vascular network and often require comparisons across populations of animals. What is lacking is a real-time in vivo readout of the vascular network within an individual animal over long time scales.
Here we report time-lapse in vivo two photon imaging of adult cerebral vasculature in transgenic mice harboring endothelial cells that express membrane-anchored GFP (Tie2-Cre x mTmG mice). Following implantion of a cranial window and a recovery period we are able to repeatedly image cerebral vascular networks at micron (um) resolution over time-scales of weeks to months. Time-lapse imaging in the motor cortex from superficial arteries and veins down to fine capillary beds (300-400 um deep) corresponding to neuronal layer I through IV reveal a relatively stable vascular network over a 2-3 month period. If the vascular network is stable over long time-scales, an open question is whether there is an ongoing dynamic process of angiogenesis and pruning. We are addressing this by tracking the fine temporal evolution of vascular tube morphology (length, diameter, tortuosity) and network topology and have observed a process of ongoing structural plasticity including: angiogenesis, tip-cell formation, and vascular pruning.

FIBRINOGEN-INDUCED PERIVASCULAR MICROGLIAL CLUSTERING IS REQUIRED FOR THE DEVELOPMENT OF AXONAL DAMAGE IN NEUROINFLAMMATORY DISEASE

Dimitrios Davalos[1], Jae Kyu Ryu[1], Mario Merlini[1], Kim M Baeten[1], Natacha LeMoan[1], Mark A Petersen[1,2], Thomas J Deerinck[3,4], Dimitri S Smirnoff[1], Catherine Bedard[1], Hiroyuki Hakozaki[4], Sara Gonias Murray[1], Jennie B Ling[1], Hans Lassmann[5], Jay L Degen[6], Mark H Ellisman[3,4], Katerina Akassoglou[1,7]

[1]University of California, San Franciso, Gladstone Institute of Neurological Disease, San Francisco, CA, 94158, [2]University of California, San Francisco, Pediatrics, Division of Neonatology, San Francisco, CA, 94143, [3]University of California, San Diego, Neurosciences, La Jolla, CA, 92093, [4]University of California, San Diego, National Center for Microscopy and Imaging Research, La Jolla, CA, 92093, [5]Medical University of Vienna, Centre for Brain Research, Vienna, A-1090, Austria, [6]Cincinnati Children's Hospital Research Foundation and University of Cincinnati College of Medicine, Division of Experimental Hematology, Cincinnati, OH, 45229, [7]University of California, San Francisco, Neurology, San Francisco, CA, 94158

Blood brain barrier (BBB) disruption, microglial activation and neurodegeneration are hallmarks of multiple sclerosis. However, the initial triggers that activate innate immune responses and their role in axonal damage remain unknown. We show that the blood protein fibrinogen induces rapid microglial responses toward the vasculature and is required for axonal damage in neuroinflammation. Using in vivo two-photon microscopy, we demonstrate that microglia form perivascular clusters before myelin loss or paralysis onset and that, of the plasma proteins, fibrinogen specifically induces rapid and sustained microglial responses in vivo. Fibrinogen leakage correlates with areas of axonal damage and induces reactive oxygen species release in microglia. Blocking fibrin formation with anticoagulant treatment or genetically eliminating the fibrinogen binding motif recognized by the microglial integrin receptor CD11b/CD18 inhibits perivascular microglial clustering and axonal damage. Thus, early and progressive perivascular microglial clustering triggered by fibrinogen leakage upon BBB disruption contributes to axonal damage in neuroinflammatory disease.

INFLAMMATORY UPREGULATION OF CLAUDIN-12 PROTEIN IN BRAIN ENDOTHELIAL TIGHT JUNCTIONS

Annika Deiss[1], Gesa Weise[1], Friederike Berberich-Siebelt[2], Lena Dietz[2], Guido Stoll[1], Mathias Buttmann[1]

[1]University of Wuerzburg, Neurology, Wuerzburg, 97080, Germany, [2]University of Wuerzburg, Molecular Pathology, Wuerzburg, 97080, Germany

Brain endothelial cells protect the CNS by formation of tissue-specific tight junctions, which are composed of occludin, claudin-5 and claudin-3, and according to limited evidence also of claudin-12. Here we confirm that claudin-12 protein is indeed expressed at the blood-brain barrier. Furthermore, we demonstrate for the first time that this member of the claudin family, which acts as an intestinal calcium pore, is upregulated at the inflamed blood-brain barrier. Western blotting of protein extracts from the mouse brain endothelial cell line bEND.3 with well characterized antibodies to the N- and the C-terminus of claudin-12 revealed overlapping bands of expected size, which were abolished by shRNA knockdown of claudin-12. Claudin-12 immunocytochemistry demonstrated a tight junction staining pattern. Of note, pro-inflammatory stimulation of bEND.3 cells increased claudin-12 mRNA and protein expression as well as tight junction staining. Immunohistochemistry of CNS tissue from healthy mice revealed claudin-12 positive blood vessels of various sizes. An upregulation of claudin-12 protein particularly in those vessels showing albumin extravasation, was observed in two different experimental autoimmune encephalitis models. Furthermore, Western blotting of spinal cord protein extracts from EAE mice demonstrated a strong positive correlation between claudin-12 protein expression and neurological dysfunction. Finally, claudin-12 immunocytochemistry of cultured primary human brain EC revealed a weak tight junction staining pattern, which was increased by pro-inflammatory stimulation. In summary, our results confirm claudin-12 protein expression at the blood-brain barrier and demonstrate an upregulation in inflamed, leaky vessels, where it possibly contributes to increased tight junction permeability.

ANALGESIC EFFICACY OF SYSTEMICALLY ADMINISTERED DALARGIN CONJUGATED WITH THE BLOOD-BRAIN BARRIER CARRIER FC5 FUSED TO THE HUMAN FC

Graham Farrington[1], Ellen Garber[1], John Eldredge[1], William Sisk[1], Nadia Caram-Salas[2], Danica Stanimirovic[2], Giovanna Antognetti[1], Eric Brunette[2]

[1]Biogenidec, Molecular Discovery, Cambridge, MA, 02142, [2]National Research Council of Canada, Life Sciences, Ottawa, K1H 8M5 , Canada

We have shown recently that neuroactive peptides, including analgesic peptides, could be rendered systemically efficacious by the conjugation to FC5, a camelid VhH (15 kD) that transmigrates the blood-brain barrier (BBB) in vitro and in vivo. However, FC5 and its peptide conjugates have short plasma half-life (~10 min) that necessitates multiple injections to achieve maximum effect. To improve plasma half-life, FC5 was fused to the N-terminus of human IgG Fc domain (FC5Fc) creating a bi-valent FC5 molecule of ~80kD, with plasma half-life of ~40 h. Systemically ineffective analgesic enkephalin Dalargin [D-Ala2, Leu5]-enkephalyl-Arg (Dal) was chemically conjugated to Fc or FC5Fc via a C-terminal cysteine using a heterobifunctional NHS-maleimide cross-linker. Chronic pain in rats was induced by intraplantar injection of Complete Freund Adjuvant (CFA) and was measured as a decrease in the withdrawal threshold latency of the injected paw after applying a radiant-heat stimulus (thermal hyperalgesia). Analgesis effects of increasing single iv doses of FC5-Dal (2-21 mg/kg), Fc-Dal or FC5Fc-Dal (both at 0.5-6 mg/kg) were evaluated two days after the CFA injection. The icv administration of FC5-Dal and FC5Fc-Dal showed the same maximal possible effect (MPE AUC of 47% and 43%, respectively). Intravenously administered Fc-Dal showed no analgesic effect. In contrast, both FC5-Dal and FC5Fc-Dal were analgesic systemically; however, the same dose (6 mg/kg) of FC5Fc-Dal and FC5-Dal produced MPE AUC of 63% and 6%, respectively. A single dose of FC5-Dal at 21 mg/kg produced about the same level of pain suppression as FC5Fc-Dal at 0.5 mg/kg. Based on the molar ratio of Dalargin injected, the FC5Fc-Dal shows ~80 fold greater potency than FC5-Dal in suppressing pain in the Hargreaves model. The observed enhanced biological activity of the FC5Fc-Dal compared to FC5-Dal correlates with the prolonged plasma half-life and improved apparent binding affinity to its RMT target on brain endothelial cells.

INCORPORATION OF TRANSCEND (MELANOTRANSFERRIN, MTF OR P97), A PROTEIN VECTOR, IN A THERAPEUTIC ANTIBODY RESULTS IN ITS TRANSPORT IN THE BRAIN AT THERAPEUTICAL CONCENTRATION FOR THE TREATMENT OF BRAIN DISORDERS

Reinhard Gabathuler[1], Timothy Z Vitalis[1], Umar Iqbal[2], Maria Moreno[2], Paul R Lockman[3], Quentin R Smith[3]

[1]biOasis Technologies Inc., Research and Development, Vancouver BC, V6H 3V9, Canada, [2]NRC-IBS, Cerebrovascular Research, Ottawa ON, K1R 0R6, Canada, [3]TTU-HSC, Pharmaceutical Sciences, Amarillo , TX, 79106

The blood-brain barrier (BBB) is mainly formed by brain capillary endothelial cells characterized by tight junctions between cells and a high expression of efflux pumps only allowing brain access to nutrients necessary for cell survival and function. These properties of the BBB result in the incapacity of small and large therapeutic compounds to reach the brain in therapeutic concentrations. The research on new peptide and protein vectors able to cross the BBB and able to deliver therapeutic agents in therapeutic concentrations across the BBB has been necessary for the development of new therapeutics for the treatment of brain diseases.
Transcend (Melanotransferrin-MTf, p97) has been developed by biOasis Technologies Inc. as a vector for receptor mediated drug delivery into the brain and involves a receptor of the family of LDL receptor related protein (LRP). Using MTf-rhodamine, we show by fluorescence microscopy that MTf is rapidly transported in the brain parenchyma, colocalizes with markers of neurons and astrocytes and is endocytosed in endosomes and lysosomes. In a proof of concept study we demonstrate that antibodies labelled with rhodamine or other fluorescent dyes can be transported in the brain parenchyma after incorporation of Transcend. Using marker proteins labelled with fluorescent dyes and binding to lectins or to CD31 localized specifically on brain capillary endothelial cells separation of the proteins localized in the brain parenchyma and capillaries can be done. By quantitative confocal fluorescence microscopy we determined that 10 to 15 times more antibodies were delivered in the brain parenchyma when conjugated to Transcend.
Quantification and pharmaco-kinetic analysis of BT2111, the antibody against Her-2 (trastuzumab) conjugated to Transcend, was done using a mice model characterized by the formation of brain metastasis after intracardiac administration of MDA-MB 231BR. The analysis show that Transcend when incorporated in Trastuzumab (BT2111) is homogenously distributed in normal brain and can reach therapeutical concentration in the brain. These studies demonstrate that Transcend can be used as a vector for the transport of biologics such as antibodies across the BBB and capable of shuttling therapeutic levels of a variety of compounds from small anti-cancer agent to larger biologics such as antibodies across the BBB for the treatment of neurological disorders.

PERVASIVE BLOOD-BRAIN/SPINAL CORD BARRIER IMPAIRMENT IN ALS PATIENTS

Svitlana Garbuzova-Davis, Paul R Sanberg

University of South Florida Morsani College of Medicine, Center of Excellence for Aging and Brain Repair, Department of Neurosurgery and Brain Repair, Tampa, FL, 33612

Vascular pathology, including blood-brain/spinal cord barrier (BBB/BSCB) alterations, has recently been recognized as a key factor possibly aggravating motor neuron damage, identifying amyotrophic lateral sclerosis (ALS) as a neurovascular disease. The majority of findings on microvascular pathology in ALS have been established in mutant SOD1 rodent models, identifying barrier damage during disease development which might similarly occur in familial ALS (FALS) patients carrying the SOD1 mutation. However, BBB/BSCB competence in sporadic ALS (SALS) is still undetermined. In this study, BBB/BSCB structural and functional integrity in postmortem gray and white matter of medulla and spinal cord tissue from 25 SALS patients and 18 controls obtained from human tissue banks (Human Brain and Spinal Fluid Resource Center, Los Angeles, CA; NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD) was investigated. Our major findings: (1) endothelial cell damage and pericyte degeneration, (2) endothelial cells with numerous cytoplasmic vacuoles and membrane rupture, (3) severe intra- and extracellular edema, (4) reduced CD31 and CD105 expressions in endothelium, (5) significant accumulation of perivascular collagen IV and fibrin deposits, (6) significantly increased microvascular density in lumbar spinal cord, (7) IgG microvascular leakage, (8) reduced tight junction and adhesion protein expressions. Microvascular structural and functional barrier abnormalities determined in gray and white matter of the medulla, cervical, and lumbar spinal cord of SALS patients are novel findings. Since endothelial cell degeneration was the primary cause of observed CNS barrier impairment, our current focus is on establishing possible cause(s) of capillary endothelial cell alteration. Thus, complexity and pervasiveness of barrier damage discovered in ALS may have implications for disease pathogenesis and this damage should be considered as a novel therapeutic target.
Supported by the Muscular Dystrophy Association (Grant #92452).

INTEGRIN α8β1/NEPHRONECTIN INTERACTIONS PARTICIPATE IN TH LYMPHOCYTES TRANSMIGRATION THROUGH BLOOD-BRAIN BARRIER ENDOTHELIAL CELLS.

Steve Gendron, Alexandre Prat

CRCHUM, Neuroimmunology, Montreal, H2L4M1, Canada

During central nervous system (CNS) inflammation, transmigration of pathogenic Thelper (Th) lymphocytes through the blood-brain barrier (BBB) is a critical event that leads to infiltration of these cells into the CNS. Th lymphocyte transmigration is largely attributable to integrins of the β1 family. Integrins alpha4beta1 (α4β1/VLA-4) is known to be the most important β1 integrin for this process but its blockade do not completely abrogate Multiple Sclerosis (MS) and Experimental Autoimmune Encephalomyelitis (EAE) symptoms, suggesting the participation of other integrins of the β1 family in this process. Endothelial cells (EC) and extracellular matrix (ECM) proteins are major constituent of the BBB and data from our lab indicate that BBB-ECs express a new ECM protein called Nephronectin (NPNT). Integrin α8β1 is the only β1 integrin known to interact with NPNT. We looked at the expression of this integrin in T lymphocytes and found that it is not expressed by resting CD4CD45RO from peripheral blood of healthy donors. In contrast, integrin α8β1 expression is induced upon activation of these cells *in vitro*. We also found that integrin α8β1 is strikingly up-regulated on Th17 lymphocytes compared to Th1 and Th2 cells. Immunohistofluorescence analysis showed that NPNT is detected in the blood vessels structure in normal and inflamed CNS tissues, obtained from both human and mouse, and that, infiltrating α8β1-expressing Th lymphocytes are found to be in close contact with NPNT in these tissues. Our preliminary data indicate that α8β1 blockade reduces Th1 and Th17 cells migration across BBB-ECs *in vitro*. Our data highlight a potential new interaction between pathological T cells expressing integrin α8β1 and NPNT expressed by brain endothelial cells which could be important for the recruitment of encephalitogenic T lymphocytes to the CNS.

LAPATINIB DISTRIBUTION AND EFFICACY IN A HER2-OVEREXPRESSING BRAIN METASTASIS PRECLINICAL MOUSE MODEL OF BREAST CANCER.

Brunilde Gril[1], Diane Palmieri[1], Yongzhen Qian[1], Paul R. R Lockman[2], Quentin R. R Smith[2], Patricia S. S Steeg[1]

[1]National Cancer Institute, Laboratory of Molecular Pharmacology, Bethesda, MD, 20892, [2]Texas Tech University Health Sciences Center Amarillo, Department of Pharmaceutical Sciences, Cancer Biology Center, Amarillo, TX, 79106,

Central nervous system or brain metastases occur in 10-20% of all metastatic breast cancer patients, but upwards of 35% of patients whose tumors overexpress the Her-2 oncogene. No effective treatments exist for brain metastases with care relying on palliative surgery and radiation therapy. Herein, we determined the brain distribution and the efficacy of the FDA approved dual EGFR/Her-2 tyrosine kinase inhibitor lapatinib on the brain metastatic colonization of a human breast carcinoma cell line. An EGFP labeled brain-seeking derivative of the human MDA-MB-231 cell line (231-BR) endogenously overexpressed EGFR and was transfected with Her-2 cDNA (231-BR-Her-2).
The 231-BR-Her-2 cells produced 6.83 (5.86 to 7.79) clinical metastases per histological brain section after intracardiac injection into immunosuppressed mice. Treatment with 30 mg/kg lapatinib resulted in a 53% decline to 3.21 (2.31 to 4.11) clinical metastases ($p<0.0001$), while treatment with 100 mg/kg resulted in a 50% decline to 3.44 (2.55 to 4.32) metastases ($P<0.0001$). Immunohistochemical analysis of residual brain metastases showed significant reduced pHer-2 expression ($P< 0.0001$ at 100mg/kg). The data suggest that lapatinib may partially prevent the formation of Her-2+ brain metastasis, which is being tested in ongoing adjuvant clinical trials. In order to understand why 50% of the metastases remained unaffected by lapatinib treatment, we questioned the ability of lapatinib to penetrate the blood-brain barrier. The ability of lapatinib to reach therapeutic concentrations in the CNS following 14C lapatinib administration (100 mg/kg p.o. or 10 mg/kg, i.v.) was determined. 14C-Lapatinib concentration was very heterogenous among experimental brain metastases. On average, brain metastasis concentrations were 7–9-fold greater than surrounding brain tissue at 2 and 12 h after oral administration, but it was still only 10–20% of the concentrations reached in peripheral metastases. Only in 17% brain lesions did lapatinib concentration approach that of systemic metastases. The limited access of lapatinib to brain metastases likely underlies its partial preventive effects. These results highlight the blood-brain barrier as a major obstacle for drug efficacy in the brain.

INTERLEUKIN 26: A TH17 CYTOKINE WHICH IMPACTS ON THE BLOOD BRAIN BARRIER.

Lamia N Hachehouche, Hania Kebir, Jorge Alvarez, Simone Terouz, Lyne Bourbonnière, Alexandre Prat

CHUM, Neuroimmunology Unit, Montréal, H2L2W5, Canada

Multiple sclerosis (MS) is an autoimmune disease characterised by the disruption of blood brain barrier (BBB) integrity. Our laboratory has previously shown that Th17 lymphocytes are implicated in this pathology and that IL-17 has a particular ability to affect the permeability of the BBB. However, little is known about additional Th17-related mediators and their impact on BBB integrity. IL-26 is a pro-inflammatory cytokine, so far only known to regulate epithelial cells functions. In this study, we aim to investigate whether IL-26 is associated with the human Th17 phenotype and whether it could impact on BBB functions. We first performed a thorough characterization of IL-26 expression in human Th1, Th2 and Th17 lymphocytes using q-PCR and flow cytometry analysis. We found that IL-26 expression is linked to Th17 phenotype and that IL-26 expression correlated with levels of IL-17, IL-22, IL-23R and *RORc*. We also evaluated IL-26 receptor (IL-26R) expression on the BBB, using primary cultures of human brain-derived endothelial cells (BBB-ECs) and human brain tissue sections. We found that both IL-26R α (IL-20Rα) and β (Il-10Rβ) chains are expressed at the human BBB *in situ* and by BBB-ECs *in vitro*, suggesting that BBB endothelial cells can respond to IL-26. *In vitro* treatment of human BBB-ECs resulted in an increase in permeability and a down regulation of the tight junction molecule occludin. Therefore, the Th17-secreted cytokine IL-26 might play a role in impacting BBB integrity and has potential implications in MS pathology.

COMPARISON OF BLOOD-BRAIN BARRIER TRANSPORT OF P-GLYCOPROTEIN SUBSTRATES IN HUMANIZED, KNOCK-OUT AND WILD-TYPE MICE

Muhammad Waqas Sadiq[1], C. Roland Wolf[2], Nico Scheer[3], Margareta Hammarlund-Udenaes[1]

[1]Uppsala University, Department of Pharmaceutical Biosciences, Uppsala, 75124, Sweden, [2]CXR Biosciences, Limited, Dundee, DD1 9SY, United Kingdom, [3]TaconicArtemis, Translational ADMET Research, Cologne, 51063, Germany

The aim of the current study was to compare the BBB penetration of selected compounds and effects of P-pg inhibitors between C57BL/6 Mdr1a/1b(+/+) (C57BL/6 WT) and C57BL/6 humanized MDR1 mice (hMDR1) and in this context to evaluate the hMDR1 model for future use. The P-gp substrates digoxin, verapamil and docetaxel, were administered to male FVB Mdr1a/1b(+/+) (FVB WT), FVB Mdr1a/1b(-/-) (Mdr1a/1b(-/-), C57BL/6 WT and hMDR1 mice. Brain to plasma total concentration ratios (Kp) of the substances were measured for each animal. Inhibition of digoxin transport across the BBB by human and mouse P-gp was studied in C57BL/6 WT and hMDR1 mice, while oxycodone was studied in male C57BL/6 WT mice with and without the P-gp blockers. The ratio of unbound drug in brain to unbound drug in plasma (Kp,uu) was also calculated by using the Kp, volume of distribution of the unbound drug in brain (Vu,brain) and and unbound plasma fraction (fu,p) values. The Kp values for digoxin, verapamil, and docetaxel were 20, 30 and 4 times higher, respectively, in the Mdr1a/1b(-/-) mice than in the FVB WT controls, confirming the role of P-gp for the efflux of these drugs at the BBB. The Kp values for digoxin, verapamil and docetaxel were 2, 16 and 2 times higher in the hMDR1 compared to the C57BL/6 WT mice, respectively. Digoxin had a significantly higher Kp in hMDR1 mice as compared to the C57BL/6 WT controls, also when the mice were treated with different blockers (verapamil, elacridar and quinidine) with the exception of cyclosporine A. For all four compounds there was a trend towards a higher Kp,uu in hMDR1 mice compared to the C57BL/6 WT controls, showing differences in the function or expression level of human and mouse P-gp in these models. The results from the present study demonstrates clear differences between the hMDR1 mice and their C57BL/6 WT controls with regard to BBB penetration of various P-gp substrates and the effect of blockers on P-gp function. Quantitative measurements of the P-gp expression level at the BBB and a comparison with human data are crucial for the future use of the hMDR1 model.

MODULATION OF THE BLOOD-BRAIN BARRIER IN TRAUMATIC BRAIN INJURY (TBI) AND NEUROINFLAMMATORY IMPLICATIONS

Finnian Hanrahan, Matthew Campbell, James Keaney, Marian M Humphries, Anna-Sophia Kiang, Peter Humphries

Trinity College Dublin, Ocular Genetics, Dublin 2, 2, Ireland

Traumatic brain injury (TBI) is one of the leading causes of death in children and young adults. Cerebral edema that occurs post TBI plays a major role in the morbidity and mortality of the condition and it is also associated with acute stroke, hypoxic ischemic coma, neurological cancers and brain infection. Unfortunately, therapies directed against controlling brain swelling are limited and current approaches using hyperosmolar therapy are short-lived and can induce severe intravascular volume depletion, hypotension and hyperkalemia. The brain is a delicate organ which maintains a stable microenvironment and excludes harmful material and pathogens by means of the blood-brain barrier (BBB). However, the BBB is the primary hurdle to treating many conditions of the brain, and in the case of TBI and subsequent cerebral edema the BBB becomes highly dysfunctional. We have recently shown that a marginal and transient modulation of the BBB using RNAi directed against the tight junction protein claudin-5 can protect the brain from the development of malignant cerebral edema. This is likely due to a change in the osmotic gradients of the BBB as the paracellular pathway becomes modulated and holds tremendous therapeutic potential clinically. We propose that a temporary and size-selective modulation of the BBB allows enhanced movement of oedematous material from the brain to the blood and significantly impacts on the formation of cerebral edema. Moreover, we also show cognitive improvement in mice with focal cerebral edema following administration in these animals of siRNA directed against claudin-5. In the same regard however, we have also shown that modulation of the BBB using claudin-5 siRNA in an experimental model of TBI induces increased expression of inflammatory cytokines in the region of focal injury in particular levels of CXCL10 and IL-1β. Neuroinflammatory events post TBI can have a major bearing on the development of cerebral edema and novel therapeutic targets associated with the maturation of these cytokines will be explored. In particular, components associated with the sterile inflammatory environment of the brain post TBI, such as caspase-1, caspase-11, NLRP3 and ASC could be amenable to a range of therapeutic entities currently being developed for other non-neural conditions.

PERICYTE SUBPOPULATIONS OF THE CNS MICROVASCULATURE

Daniel Holl, Christian Göritz

Karolinska Institutet, Department of Cell and Molecular Biology,
Stockholm, SE-17177, Sweden

Pericytes are an essential component of the central nervous system (CNS) microvasculature and are important for the functioning of the blood brain barrier. While pericytes have been suggested previously to be a heterogeneous population of cells, a clear characterization of subpopulations is missing. Recently, we could demonstrate functional heterogeneity within the pericyte population, with a particular subpopulation (type A pericytes) participating in scar tissue formation following spinal cord injury. In order to better understand heterogeneity among pericytes we started an in situ characterization of pericyte subpopulations in the adult CNS. Using a combination of genetic labeling and marker expression we were able to distinguish different pericyte subpopulations. Our data provide further evidence for pericyte heterogeneity that could reflect functional differences.

NON-INVASIVE CELL TRANSLOCATION TO THE ALZHEIMER'S BRAIN USING FOCUSED ULTRASOUND FOR BLOOD-BRAIN BARRIER DISRUPTION

Alison Burgess[1], Kullervo Hynynen[1,2]

[1]Sunnybrook Research Institute, Physical Sciences, Toronto, ON, M4N 3M5, Canada, [2]University of Toronto, Medical Biophysics, Toronto, ON, M4N 3M5, Canada

Brain disorders are notoriously difficult to treat with pharmaceutical agents due to the presence of the blood-brain barrier (BBB). The use of focused ultrasound (FUS) is emerging as a non-invasive, reversible method for disrupting the BBB to promote enhanced targeted drug delivery to the brain. Combined with the use of microbubbles, FUS has been shown to be safe and effective for delivery of chemotherapeutics, antibodies, viral vectors and cells to the brain in various preclinical models. Here, we will discuss recent results demonstrating the ability of FUS to deliver stem cells to a transgenic mouse model of Alzheimer's disease and their wildtype controls. Enhanced green-fluorescent protein (EGFP) -expressing neural stem cells were delivered to hippocampus using MRI guidance FUS. Contrast enhanced T1 weighted MR images were used to confirm BBB opening post treatment. 24 hrs and 1 week later, the animals were sacrificed and immunohistochemistry was used to demonstrate that the cells survive post-FUS and that they locate to the targeted hippocampus. In parallel experiments, two-photon microscopy was used for real time imaging of the BBB disruption in the transgenic mice. We used methoxyX-04 to fluorescently label the plaques in vivo and injected dextran-conjugated Texas Red intravenously for detection of BBB disruption. We observed many similarities in BBB disruption between transgenic and wild type mice, however, we also demonstrated that the time required for the BBB to close following FUS exposure is longer in the transgenic mice. Together this data demonstrates the feasibility of FUS for non-invasive stem cell delivery in a preclinical model of Alzheimer's disease.

EVIDENCES OF TRICELLULAR TIGHT JUNCTIONS IN BRAIN ENDOTHELIAL CELLS

Noriko Iwamoto, Tomohito Higashi, Mikio Furuse

Division of Cell Biology, Department of Physiology and Cell Biology, Graduate School of Medicine, Kobe University, Kobe City, 650-0017, Japan

A major part of the blood-brain barrier (BBB) exists at the level of brain endothelial cells. BBB-forming endothelial cells have specific features: the expression of specialized membrane transporters for the efflux of toxins and influx of nutrients, low transcytotic activity, lack of fenestrae and developed tight junctions (TJs). TJs are one mode of epithelial and endothelial cell-cell junctions and prevent the leakage of solutes through the paracellular space, thereby contribute to the barrier function of cellular sheets. In addition to TJs located at bicellular cell-cell contacts, a tight seal at tricellular contacts, where the corners of three cells meet, is also essential to maintain the integrity of cellular sheets. Epithelial cells have a specialized mode of TJs at tricellular contacts, named tricellular tight junctions (tTJs). To date, two integral membrane proteins, tricellullin and LSR, are known as molecular components of tTJs. Depletion of tricellulin or LSR in cultured epithelial cells results in reduction of trans-epithelial electrical resistance, suggesting that tTJs are required for the full barrier function of epithelial cellular sheets (1, 2). However, tTJs have not been described in endothelial cells so far.
In this study, we examined whether endothelial cells possess tTJs by immunofluorescence staining of frozen sections of various mouse tissues using antibodies for two tTJ markers, tricellulin and LSR. As far as we examined, endothelial tricellular contacts in non-central nervous system (CNS) tissues were labeled with neither tricellulin nor LSR. On the contrary, the concentrations of these tTJ markers were observed specifically at tricellular contacts in endothelial cells in the brain and retina. These findings provide the first evidence of tTJs in endothelial cells and suggest that tTJs are specialized characteristics for BBB-forming endothelial cells.

References

(1) Ikenouchi et al. *J. Cell Biol.* 171:939-945 (2005)
(2) Masuda et al. *J. Cell Sci.* 124:548-555 (2011)

EARLY INCREASE IN BBB PERMEABILITY, HEMORRHAGE AND BRAIN INJURY AFTER TRANSIENT CEREBRAL ISCHEMIA IN PGRN-DEFICIENT MICE.

Katherine Jackman[1], Timo Kahles[1], Karin Hochrainer[1], Lidia Garcia-Bonilla[1], Diane Lane[1], Ping Zhou[1], Aihao Ding[2], Josef Anrather[1], Costantino Iadecola[1]

[1]Weill Cornell Medical College, Division of Neurobiology, Department of Neurology, New York, NY, 10065, [2]Weill Cornell Medical College, Department of Microbiology and Immunology, New York, NY, 10065

Progranulin (PGRN), a secreted protein involved in a wide array of biological processes, is implicated in the neurodegeneration associated with frontotemporal dementia. PGRN was recently linked to a key pathway regulating blood brain barrier (BBB) and tight junction (TJ) development. We tested the hypothesis that PGRN regulates TJ structure and protects the brain from injury following transient ischemia. First, we examined TJ ultrastructure using transmission electron microscopy. Compared to wild-type (WT) mice, PGRN deficient (-/-) mice have fewer cortical TJ (WT 2.1±0.1; KO1.6±0.1; P<0.05), reduced TJ length (WT 2.3±0.1; KO 1.6±0.1um; P<0.05) and decreased TJ tortuosity (WT 1.5±0.04; KO 1.1±0.001 ratio; P<0.05, n=3). Despite these differences, protein expression of TJ proteins zona occludens (ZO)-1 and occludin were similar in WT and PGRN-/- mice (n=5-7). We then subjected PGRN-/- and WT mice to cerebral ischemia by middle cerebral artery occlusion (MCAO). The reduction in cerebral blood flow produced by MCAO in the ischemic core (WT 12.0±0.5%; KO 11.9±0.7% max; P>0.05; n=13-14) and periphery (WT 49.3±4.0%; KO 40.6±1.8% max; P>0.05; n=13-14) was similar in WT and PGRN-/- mice. Post-ischemic BBB permeability, assessed using i.v. Evans blue (EB) dye, was greater in PGRN-/- mice than WT 6h after ischemia (WT 1.6±0.2; KO 8.5±2.75 EB ng (RH/LH); P<0.05, n=5-15). In addition, hemorrhagic transformation (HT) was greater in PGRN-/- mice 6h after ischemia (WT 0.1±0.04; KO 0.5±0.1 mm3; P<0.05, n=5-8). Interestingly, TJ protein (ZO-1 and occludin) expression and MMP-9 activity were not altered in PGRN-/-mice at this time point (n=5-8). 72h after induction of transient ischemia, PGRN-/- mice had greater brain injury and water content versus WT (infarct: WT 25±5; KO 41±6 mm3; brain water: WT: 80.2±0.7%; KO: 83.5±0.7%; P<0.05, n=5-6). Similarly, functional impairment was greater in PGRN-/- mice after ischemia (latency to fall: WT: 44±6; KO: 24±6 sec; P<0.05, n=10-13). Our findings suggest that PGRN deficiency alters TJ ultrastructure and enhances BBB permeability, HT and brain injury early after cerebral ischemia without altering TJ expression and MMP-9 activity. Thus, PGRN may be vital to the maintenance of BBB structure and post-ischemic BBB integrity and could provide new insights into the therapy of stroke and neurodegeneration.

UPTAKE AND RETENTION OF BISPECIFIC ANTIBODIES IN MOUSE BRAIN BY INTRAVENOUS OR INTRAPERITONEAL INJECTION

Denise Karaoglu Hanzatian[1], Annette Schwartz[2], Farid Gizatullin[1], Jamie Erickson[2], Kangwen Deng[1], Ruth Villanueva[1], Christopher Stedman[2], Christina Harris[1], Andrew Goodearl[1], Tariq Ghayur[1]

[1]Abbott Bioresearch Center, Biologics, Worcester, MA, 01605, [2]Abbott Bioresearch Center, Pharmacology, Worcester, MA, 01605

Blood–brain barrier (BBB), which consists mainly of brain capillary endothelial cells, is a physical and physiological barrier that controls very efficiently and selectively the entry of compounds from blood into the CNS, and protects nervous tissue from harmful substances and infectious agents present in circulating blood. More than 98% of all small organic molecule drugs do not cross the BBB to therapeutically-relevant concentrations. Nearly all large molecules (e.g. recombinant proteins, MAbs, peptides, siRNAs) do not cross the BBB.
Here, we will describe the generation and expression of bispecific antibodies which are capable of binding specific targets in the brain. The levels and localization of bispecific antibodies, which were injected systemically, were assessed by two orthogonal methods. Results showing the uptake and retention of antibodies up to 96 hours will be demonstrated.

METHAMPHETAMINE-INDUCED BLOOD BRAIN BARRIER DYSFUNCTION AND CEREBRAL VASCULAR ALTERATIONS: POTENTIAL CONTRIBUTORS TO INCREASED RISK FOR DEVELOPING PARKINSON'S DISEASE

Sharanya M Kousik[1], Steven M Graves[1], T. Celeste Napier[1], Paul M Carvey[1,2]

[1]Rush University Medical Center, Pharmacology, Chicago, IL, 60612, [2]Rush University Medical Center, Neurology, Chicago, IL, 60612

Methamphetamine (meth) causes excess dopamine (DA) release and promotes DA-induced neurotoxicity. With approximately 26 million users globally, meth addiction is made more alarming by reports that meth abuse is a risk factor for Parkinson's disease (PD); meth abusers display reduced DAergic function and motor deficits similar to PD patients. Potential mechanisms of meth-induced neurotoxicity include DA/quinone- and reactive oxygen species-aided neuroinflammation and blood brain barrier (BBB) dysfunction. Clinical studies also report reduced regional cerebral blood flow in the putamen and cortex of detoxified meth abusers. Our study used laboratory rats to determine how meth-induced changes in the BBB, tissue perfusion, and the DA system may contribute to the vulnerability of meth abusers to PD. Rats were acutely treated with saline, or 3 or 9 mg/kg meth, or trained to self-administer meth chronically (~2.2 mg/kg/day for 14 days), then perfused with fluorescein isothiocyanate-labeled albumin (FITC-LA), a vascular integrity marker. Additional rats acutely treated with 9 mg/kg meth were perfused with Microfil-MV for micro computed tomography (μCT). Rats given 3 or 9 mg/kg meth as well as rats sacrificed 24 hours after the last session of meth self-administration displayed FITC-LA leakage in the prefrontal cortex and nucleus accumbens shell, suggesting that BBB dysfunction was region-selective. Compared to controls, the dorsal striatum of all meth-treated rats uniquely exhibited a striking absence of FITC-LA ($F_{(3,106)}$=221.119) suggesting that meth induced region-specific hypoperfusion. μCT revealed that meth lowered striatal vascular volume and vessel thickness compared with controls. As hypoperfusion may damage DA terminals in striatal regions affected by PD, we assessed tyrosine hydroxylase (TH) using immunohistochemistry. In rats trained to self-administer meth, we observed significant reductions in striatal TH levels ($F_{(3,32)}$=14.777) and an 18% decrease in TH+ cells in the substantia nigra ($F_{(3,30)}$=10.964) suggesting that chronic meth may render this system more vulnerable to PD. This is the first preclinical study on meth-induced cerebral vascular changes as a new mechanism of neurotoxicity and may provide a novel model for meth-induced PD risk.
(Supported by NS052414, DA15760, DA024923, Kenneth Douglas Fdn, and the Daniel and Ada Rice Fdn.)

DEVELOPMENTAL LOSS OF BLOOD BRAIN BARRIER INTEGRITY BETWEEN YOUNG AND ADULT RATS

Kuen-Bao Chen[2], Eva Yuhua Kuo[1], Kin-Shing Poon[2], Ka-Shun Cheng[2], Chia-Sheng Chang[2], Yu-Cheng Liu[2], Ted Weita Lai[1,3]

[1]China Medical University, Graduate Institute of Clinical Medical Science, Taichung, 404, Taiwan, [2]China Medical University Hospital, Department of Anesthesiology, Taichung, 404, Taiwan, [3]China Medical University Hospital, Translational Medicine Research Center, Taichung, 404, Taiwan

The development of the blood-brain barrier (BBB) against permeability to inert tracers, such as Evans Blue dye (EBD), occurs quite early on at embryonic stages (before E13~E15), and the BBB remains resistant to EBD between E15 to early adulthood (P20-P30). Here, we sought to investigate changes in EBD permeability at a later stage in development, specifically comparing young rats (P20) to adult rats (P86). We found substantially higher EBD extravasation into the forebrains of adult rats, compared to those of the young rats ($p=0.0132$; student's t test). In contrast, there was no difference in EBD extravasation to the liver, suggesting no change in vascular permeability in peripheral tissues. Furthermore, EBD extravasation into the cerebellum was less prominent than that into the forebrain, suggesting that the disruption of the BBB was brain region-specific. In conclusion, we found a specific increase in EBD extravasation in the mature forebrain, and the protocol that we used may be a good template for studying developmental disruption of the BBB. (Supported by the National Research Council of Taiwan; presented by: E.Y.K., and correspondence to: T.W.L.)

PHARMACOKINETICS AND REGIONAL DISTRIBUTION OF EVANS BLUE DYE IN BLOOD-BRAIN BARRIER RESEARCH

Eva Yuhua Kuo[1], Lola Fenghuei Yen[1], Ted Weita Lai[1,2]

[1]China Medical University, Graduate Institute of Clinical Medical Science, Taichung, 404, Taiwan, [2]China Medical University Hospital, Translational Medicine Research Center, Taichung, 404, Taiwan

The blood-brain barrier (BBB) is a safeguard that prevents unwanted exchanges of chemicals between the blood and the brain, and research into the BBB can lead to important discoveries in the field of neuropathology, physiology, and pharmacology. The simplest way to study functional BBB integrity is to infuse an inert tracer into the circulation, and measuring its penetration into the brain. The Evans blue dye (EBD) is an inert tracer commonly used to study blood-brain barrier (BBB) permeability. Owing to its high affinity to serum albumin, almost all EBD becomes albumin-abound following intra-vascular infusion into laboratory animals. This property makes EBD an attractive tool for studying BBB penetration by serum albumin. One unique feature of EBD as a protein tracer is that it can be administered not only by i.v. injection but also by i.p. injection. In this study, we investigated the pharmacokinetics of EBD following i.v. and i.p. injections, and found that while little ip. EBD were uptaked into the circulation and became accumulated in peripheral tissues (compared to i.v. EBD), the amount of EBD accumulated into the brain tissues following i.p. injection was not much different from that following i.v. injection. This raised an intriguing concern on the mechanism of i.p. EBD delivery into the brain, which presumably occurred via the vascular route. (Supported by the National Research Council of Taiwan; presented by: E.Y.K., and correspondence to: T.W.L.)

VASCULAR-DEPENDENCY OF EVANS BLUE DYE DELIVERY IN CIRCULATION-PAIRED RATS

Eva Yuhua Kuo[1], Lola Fenghuei Yen[1], Ted Weita Lai[1,2]

[1]China Medical University, Graduate Institute of Clinical Medical Science, Taichung, 404, Taiwan, [2]China Medical University Hospital, Translational Medicine Research Center, Taichung, 404, Taiwan

Functional blood-brain-barrier (BBB) integrity can be determined in laboratory animals by way of tracer infusion into the circulation, and measuring its penetration into the brain parenchyma. An ideal tracer mimics an endogenous protein that normally does not cross the BBB, and could be administered via i.p. injection (rather than direct vascular infusion) so as to avoid confounding effects due to animal anesthesia and/or immobilization/surgical stress. The Evans blue dye (EBD) is unique in that it can be administered by i.p. injection, and once inside the circulation, it binds strongly to serum albumin to become an endogenous protein tracer. To investigate whether i.p. EBD enters the brain via vascular delivery, we designed a de novo animal model in which rats were hemodynamically-paired via ex vivo catheters, such that they would share the same vascular circulation system. Expectedly, i.p. EBD injection into one rat of each pair resulted in equivalent rise in blood EBD concentration in both rats of the same pair. More importantly, photospectrometric analysis of their brain tissues revealed that i.p. EBD injection into only one rat of each pair resulted in equivalent EBD brain accumulation between the two rats that shared the same circulation. In conclusion, we have validated a circulation-pairing animal model and demonstrated its use in delineating vascular-dependency for chemical/tracer delivery into central and peripheral tissues. Using this model, we found unequivocal evidence that i.p. EBD enters the brain primarily via vascular delivery, and thus by crossing the BBB.
(Supported by the National Research Council of Taiwan; presented by and correspondence to: T.W.L.)

A GENOMIC APPROACH TO IDENTIFY SEX-SPECIFIC MOLECULES IN THE BLOOD BRAIN BARRIER OF *DROSOPHILA MELANOGASTER*

Chamala Lama, Brigitte Dauwalder

University of Houston, Biology and Biochemistry, Houston, TX, 77004

Drosophila male mating behavior is a well characterized stereotyped ritual. It is well established that this behavior is mainly controlled by the sex specific transcription factors FruM and DsxM, which define male neuronal circuits inside the brain. In our lab, we have shown that male factors that are present in the circulating system are also required for normal mating behavior. How these endocrine factors interact with the CNS is unknown. We have evidence that the blood brain barrier (bbb) plays an important role in this communication. Specific feminization of the bbb in otherwise normal males, by the expression of the female specific protein TraF, severely reduces their courtship. This suggests that male specific factors in the bbb play an important role in mating behavior. To identify these factors we have performed several genomic screens on dissected brains and isolated bbb cells. mRNA sequencing and microarray studies have identified several male-specific candidate genes with possible novel roles in courtship. In addition, we have also identified bbb-specific microRNAs that are present in the bbb. Using these approaches, we hope to identify novel genes/microRNAs in the bbb that are important for male courtship behavior.

This work was supported by NSF grant IOS- 0919697

MCAM PROMOTES RECRUITMENT OF ENCEPHALITOGENIC T LYMPHOCYTES ACROSS THE BLOOD BRAIN BARRIER

Catherine Larochelle[1,2], Jorge I Alvarez[1], Romain Cayrol[1], Hania Kebir[1], Marc-André Lécuyer[1], Olivia Saint-Laurent[1], Nathalie Arbour[1], Alexandre Prat[1,2]

[1]CRCHUM, Neuroimmunology, Montreal, H2L 4M1, Canada, [2]Multiple Sclerosis Clinic of the CHUM, Neurology, Montreal, H2L 4M1, Canada

Encephalitogenic CD4+ lymphocytes require adhesion molecules to cross the blood brain barrier (BBB) and accumulate into CNS inflammatory lesions, an early phenomenon in Multiple Sclerosis (MS). Using proteomic techniques, we identified expression of melanoma cell adhesion molecule (MCAM), a molecule that can display both homotypic and heterotypic interactions, on BBB endothelial cells (ECs) and on a subset of human effector memory CD4+ lymphocytes. Herein, we demonstrate that MCAM is a stable surface marker that refines the identification of IL-17+, IL-22+, GM-CSF+, granzyme B+, RORγ+ and IL-23Receptor+ memory CD4+ lymphocytes within the CD161+CCR6+ subset. Furthermore, MCAM expression is significantly upregulated at the BBB within inflammatory MS and experimental autoimmune encephalomyelitis (EAE) lesions, and the proportion of MCAM+ CD4+ lymphocytes is significantly increased in the blood and in the CNS of MS patients and EAE animals as compared to controls. Moreover, blockade of MCAM or depletion of MCAM+ CD4+ T lymphocytes both restrict the migration of TH17 lymphocytes across BBB-ECs and decrease the severity of EAE. Our findings indicate that MCAM could serve as a potential biomarker for MS and represents a valuable target for restricting the entry of encephalitogenic T lymphocytes into the CNS.

CONTRIBUTION OF PANNEXIN1 TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

Sarah E Lutz[1], Estibaliz Gonzalez[2], Juan Carlos Chiara[2], Alberto Pérez-Samartín[2], Leonid Tarassishin[3], Naman K Patel[1], Hiromitsu Negoro[1], Sylvia O Suadicani[1,4], Sunhee C Lee[3], Carlos Matute[2], Eliana Scemes[1]

[1]Albert Einstein College of Medicine, Dominick P. Purpura Department of Neuroscience, Bronx, NY, 10461, [2]Universidad del País Vasco, Departamento de Neurociencias, Leioa, 48940, Spain, [3]Albert Einstein College of Medicine, Department of Pathology, Bronx, NY, 10461, [4] Albert Einstein College of Medicine, Department of Urology, Bronx, NY, 10461

Pannexin1 (Panx1) is a member of the gap junction family of pro¬teins. Panx1 forms 500pS plasma membrane channels permeable to relatively large molecules, such as ATP and fluorescent dyes. When activated, Panx1 functions as an ATP release channel. Panx1 is ubiquitously expressed; in the central nervous system (CNS) Panx1 is found in neuro¬ns and glia and in the immune system in macro¬phages and T-cells. Inhibition of the ATP receptor $P2X_7$ is known to protect against CNS demyelination and inflammation. We tested the hypothesis that Panx1-mediated ATP release contributes to expression of Experimental Autoimmune Encephalomyelitis (EAE), an animal model for multiple sclerosis, using WT and Panx1 KO mice. Panx1 KO mice displayed a delayed onset of clinical signs of EAE compared to WT mice. Spinal cord inflammatory lesions were also reduced in Panx1 KO EAE mice during acute disease. ATP-induced IL-1b release was impaired in Panx1 KO macrophages. Additionally, pharmacologic inhibition of Panx1 channels with mefloquine reduced severity of acute and chronic EAE when administered before or after onset of clinical signs, respectively. To further test whether Panx1 channels contribute to neuroinflammation, activity of Panx1 channels in EAE was assessed by measuring ATP release and by uptake of the dye YoPro in freshly isolated spinal cord tissues from normal and chronic EAE animals. ATP release and YoPro uptake were significantly increased in WT mice with EAE as compared to WT non-EAE, and reduced in tissues of EAE Panx1 KO mice. These studies show that Panx1 contributes to ATP release in the diseased CNS, and that inhibition of Panx1 using pharmacology or gene disruption delays and attenuates clinical signs of EAE.

LOSS OF ASTROCYTE CONNEXINS 43 AND 30 DOES NOT SIGNIFICANTLY ALTER SUSCEPTIBILITY OR SEVERITY OF ACUTE EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS IN MICE.

Sarah E Lutz, Cedric S Raine, Celia F Brosnan

Albert Einstein College of Medicine, Pathology, Bronx, NY, 10461

We showed previously that mice deficient in astrocyte gap junctions Cx43 and Cx30 exhibit white matter vacuolation and hypomyelination. In this study we tested the hypothesis that loss of astrocytic gap junction proteins leads to exacerbation of the primary demyelinating diseases, using experimental autoimmune encephalomyelitis (EAE) as a model system. To test for this, Cx43 floxed mice were crossed with GFAP:Cre, Cx30 null mice to generate mice lacking astrocytic expression of both Cx43 and Cx30 (dKO). EAE was induced using myelin oligodendrocyte glycoprotein (MOG(35-55)) peptide, and mice were monitored for acute expression of disease. No statistically significant difference in clinical or pathological expression of EAE was observed. Lesion load and susceptibility of different areas of the CNS to inflammation were similar in all genotypes. Moreover, no differences were noted in blood-brain barrier (BBB) permeability, tissue wet weight, axonal pathology, gliosis or demyelination during acute disease. These data show that loss of the astrocytic connexins, Cx43 and Cx30, and the white matter pathology observed in these mice does not statistically affect clinical or pathological expression of EAE and show that astrocyte gap junctions do not regulate autoimmune inflammation and associated BBB disruption in acute EAE.

A ZEBRAFISH MODEL OF BACTERIAL MENINGITIS

Cressida A Madigan, Lalita Ramakrishnan

University of Washington, Microbiology, Seattle, WA, 98195

Central nervous system (CNS) invasion by fungal, eukaryotic and bacterial pathogens remains a major cause of mortality and morbidity despite appropriate antimicrobial therapy. To enter the CNS, blood-borne bacteria first must cross brain microvascular endothelial cells, a component of the blood-brain barrier (BBB). Due in part to the paucity of BBB models, we lack a detailed mechanistic understanding of how pathogens penetrate the BBB, for all but a handful of pathogens. Zebrafish, often used to study neural development, are an increasing popular infection model, due to their genetic tractability, optically transparent embryos, and immune system containing all major cell types found in the human immune system. Like mammals, zebrafish have an intact BBB by a few days after fertilization. Integrity of the zebrafish BBB requires specialized endothelial tight junctions, including protein Claudin5, a core component of the mammalian BBB. The zebrafish BBB can be interrogated using dyes, chemical inhibitors, and gene knockdown with morpholinos. When infected by the caudal vein with a natural fish pathogen, *Mycobacterium marinum* (*Mm*), zebrafish develop an infection that spreads hematogenously to the CNS in an inoculum dependent fashion. We will present our ongoing studies that take advantage of microscopy, chemical and genetic modulators of BBB permeability, and zebrafish and *Mm* mutants to perturb CNS infection.

STUDIES ON PERICYTE REGULATED FUNCTIONS AT THE NEUROVASCULAR UNIT

Maarja A Mäe[1], Annika Keller[1], Elisabeth Raschperger[1], Henrik Johansson[2], Janne Lehtiö[2], Christer Betsholtz[1]

[1]Karolinska Institute, Vascular Biology, Stockholm, 17177, Sweden,
[2]Karolinska Institute, Clinical Proteomics, Stockholm, 17121, Sweden

Blood brain barrier (BBB) is an effective barrier restricting transport of molecules from blood to the brain parenchyma. The main physical barrier between blood and the brain is formed by endothelial cells. Other supporting components of the BBB include basement membrane, pericytes, astrocytes, microglia and neurons and all these components together form a so called neurovascular unit (NVU). The interplay of these components is highly important to control the passage of the blood-born molecules into the brain and also remaining proper brain homeostasis. However, in some circumstances it is desired to transiently open this barrier in order to let small molecules, antibodies, siRNA and other biomolecules pass into the brain to treat various brain diseases. Others, and we have previously shown that pericytes regulate at least one aspect of that barrier, namely they inhibit the transcytotic transport of molecules from blood to brain. However, the mechanism(s) how pericytes regulate the vesicular transport of molecules are not known. We are using mass spectrometry based proteomics to study the differences of the proteome of the microvascular fragments purified from control and pericyte-deficient mice brain and comparing these results with previously published transcriptional profiling data.
Additionally, we previously showed that administration of broad-spectrum tyrosine kinase inhibitor Imatinib abolishes the extravasation of tracers with various sizes into the brain parenchyma in pericyte-deficient animals. Instead, the tracers accumulate in large vesicles in the endothelium. We are investigating what is the target for Imatinib with immunohistochemistry, transmission electron microscopy and phospho-proteomics.

GLUTATHIONE PEGYLATED LIPOSOMES: TOWARDS ENHANCED DRUG DELIVERY TO THE BRAIN

David Maussang[1], Jaap Rip[1], Linda Chen[1], Susanne M Van Der Pol[2], Marco De Boer[1], Helga E De Vries[2], Pieter Gaillard[1]

[1]to-BBB technologies BV, Leiden, 2333 CA, Netherlands, [2]VU University Medical Center, Department of Molecular Cell Biology and Immunology, Amsterdam, 1007 MB, Netherlands

Several promising drug candidates for the treatment of central nervous system (CNS) disorders are available. However, in order to achieve CNS effects they must be able to effectively cross the blood-brain barrier (BBB). Glutathione PEGylated liposomes (G-Technology®) were found to enhance delivery of encapsulated drugs to the brain. To strengthen the G-Technology as a brain drug delivery platform we have encapsulated several fluorescent small molecules and have investigated the brain uptake; for this we have used *in vitro* cell uptake and transport experiments as well as an *in vivo* microdialysis study.

Glutathione PEGylated (GSH-PEG) liposomes (HSPC:Cholesterol:PEG-DSPE) were prepared by post-insertion of GSH-PEG-DSPE micelles (4 mol%) into preformed vesicles that already contained fluorescein isothiocyanate–dextran (FD4) or carboxyfluorescein (CF). PEG-DSPE micelles were used for non-targeted control liposomes. FD4-containing liposomes demonstrated a significant higher uptake of GSH-PEG versus non-targeted liposomes in the human endothelial brain cell line hCMEC/D3. Incubation of the cells at 4°C abolished the uptake of FD4 GSH-PEG liposomes suggesting an active process. The GSH-specific mechanism of internalization of our G-Technology® was demonstrated by competing the uptake of FD4-containing liposomes with non-fluorescent GSH-PEG liposomes. Furthermore, several endocytosis pathways (e.g. clathrin, dynamin) and cytoskeleton (e.g. actin, tubulin) inhibitors allowed for the determination of the mechanism of liposome internalization. The uptake of liposomes through the blood-brain barrier *in vivo* was investigated using CF-loaded liposomes in a brain microdialysis study in rats. In line with our *in vitro* findings, GSH-PEG liposomes entered the brain and released CF in the parenchymal area in a time-dependent manner and to a greater extent than control liposomes.

In conclusion, using in vitro and in vivo mechanistic studies we were able to demonstrate that our G-Technology® offers a promising platform for safely enhancing the delivery of drugs to the brain.

THE EXTRACELLULAR MATRIX PROTEIN LAMININ REGULATES THE STRUCTURE AND FUNCTION OF THE BLOOD-BRAIN BARRIER.

Michael J Menezes[1,2], Holly Colognato[1]

[1]Stony Brook University, Dept. of Pharmacology, Stony Brook, NY, 11794, [2]Stony Brook University, Dept. of Physiology and Biophysics, Stony Brook, NY, 11794

The blood-brain barrier (BBB) protects the cerebral parenchyma from plasma components, thus ensuring correct neural function. Several major elements contribute to barrier properties: endothelial cell tight junctions, pericytes, astrocytic end feet that surround endothelial cells, and a basal lamina composed of extracellular matrix (ECM) produced by both astrocytes and endothelia. Members of the laminin family of ECM proteins are major structural components of the BBB basal lamina, however, the role of laminins in the regulation of BBB formation and function are largely unknown. In the current study we report that LAMA2, the gene that encodes the astrocyte-specific laminin α2 subunit, regulates both the structure and function of the blood brain barrier. LAMA2 -/- mice had significant leakage of Evans Blue dye into the cerebral parenchyma, indicative of a faulty BBB. At the gliovascular interface, LAMA2 -/- mice had decreased immunoreactivity for AQP4 and Kir4.1, both of which contribute to cortical fluid homeostasis. In cultured astrocytes, laminin induced clustering of Aqp4 and Kir4.1 and is dependent on interactions between laminin and the adhesion receptor dystroglycan. Immunohistochemical analysis indicated that the BBB remained immature in LAMA2-/- mice, as mutant mice both had abnormally low levels of glucose transporter 1, which typically increases with BBB maturity. At postnatal day 21 and beyond, LAMA2 -/- mice had a subset of larger vessels that remained positive for MECA32, an immature endothelial cell marker that is typically lost upon BBB maturation. These immunohistochemical findings suggested that LAMA2 -/- brains have at least a subpopulation of blood vessels that are immature. Furthermore, ultrastructural analysis by TEM indicated that larger, but not smaller, blood vessels in LAMA2 -/- brains are surrounded by an abnormal, i.e. discontinuous basal lamina. Finally, preliminary data indicate that LAMA2 -/- mice have elevated levels of activated microglia, as well as perivascular CD45+ T cell accumulation and extravasation. Together these data suggest that astrocytic α2-containing laminins interact with the developing postnatal cerebral vasculature to promote appropriate BBB formation and/or maintenance.

MECHANISMS OF LYMPHOCYTE TRANSMIGRATION ACROSS THE BLOOD-BRAIN BARRIER STUDIED IN AN *IN VITRO* MODEL INCORPORATING FLOW

Birgit Obermeier, Shumei Man, Yukio Takeshita, Anne C Cotleur, Richard M Ransohoff

Lerner Research Institute, Cleveland Clinic, Department of Neurosciences, Neuroinflammation Research Center, Cleveland, OH, 44195

Multiple sclerosis (MS) is a common neurological disease with about 2,000,000 people affected worldwide. The infiltration of autoreactive immune cells into the central nervous system (CNS) is a crucial step in its pathogenesis as these cells cause injury to the brain tissue. Proof of principle comes from drugs like Natalizumab that prevent immune cells from crossing the blood-brain barrier (BBB) and provide benefit for patients. However, the mechanisms of cell trafficking across the BBB remain incompletely resolved. In order to study cellular transmigration, we developed an *in vitro* model of the BBB using a monolayer of cytokine-activated human brain microvascular endothelial cells and incorporating shear forces mimicking physiological blood flow. Studies using this model revealed that monocytes, but not lymphocytes, adhered efficiently on the inflamed endothelium upon encounter with the chemokine CXCL12 although both cell types expressed the chemokine receptor CXCR4 (Man S *et al.*, 2012). We propose that this selective response might be triggered by the chemokine's physical state, which requires more detailed characterization. Unexpectedly, monocyte arrest gave rise to the transmigration of T and B cells. We further hypothesize that monocyte-endothelial interactions promote secretion of factors (chemokines, cytokines, or proteolytic enzymes) which render the endothelial surface permissive for interaction with lymphocytes. We will examine our hypothesis using the novel flow-based *in vitro* BBB model developed in our lab. Results arising from that project may reveal new perspectives on the development of selective therapeutics that prevent CNS infiltration of autoreactive immune cells in MS.

NOVEL APPROACHES TO PATHOLOGICAL BBB HYPERPERMEABILITY: TARGETING VEGFR2 PHOSPHORYLATION SITES

K. Elisabet Ohlin, Xiujuan Li, Lena Claesson-Welsh

Uppsala University, Rudbeck Laboratory, Department Immunology, Genetics and Pathology, Uppsala, 751 85, Sweden

Background: Vascular endothelial growth factor (VEGF) is a pivotal regulator of vascular permeability. The permeability effects of VEGF are mainly mediated via the VEGF receptor 2 (VEGFR2) through phosphorylation of tyrosine residues on the intracellular domains and their downstream signals. One of the intracellular amino acid residues (Y949 in the mouse, Y951 in the human) is coupled to the adaptor molecule T cell specific adapter (TSAd). TSAd specifically regulates VEGF-induced vascular permeability while being insensitive to other permeability-inducing stimuli, e.g. histamine.
Glioblastoma multiforme (GBM) is the most frequent form of primary malignant brain tumor in adults. Effective treatment is currently lacking, thus anything that might hamper the aggressive development and growth of the tumor is relevant to improve clinical outcome in patients. Human GBM expresses higher levels of VEGF than other forms of brain tumors, making it a suitable target for therapy. The orthotopic mouse glioma model (GL261) has several features reminiscent of human GBM.
In this project, we specifically target the VEGFR2-TSAd-Src/Yes signaling pathway using the GL261 model in transgenic mice with alterations at phosphorylation sites that govern permeability effects of VEGF signaling. The relative contribution of VEGF-dependent BBB hyperpermeability to outcome measures of the glioma will be evaluated.
Methods: Mutant C57BL/6 mice that have a single amino acid residue exchange at the Y949 position (Y949F) of the VEGFR2. To induce glioma, GL261 cells are stereotactically injected to the medial striatum. Tumors are allowed to grow for 21 days. Tracers of BBB compromise (Alexa 555-Cadaverine and FITC-labeled microspheres) are injected via the tail-vein 60 minutes before euthanization. Brains are perfusion-fixed and sectioned through the affected region. Passage of tracers is quantitated using fluorescence microscopy, densitometry and distribution maps of diffusion. Tumor size is estimated with hematoxylin staining of representative series of sections throughout the brain region.
Preliminary data/results: Effects on VEGF-permeability in the periphery has shown acute reduction in permeability, but also reduced rates of tumor growth (B16 melanoma). The methods described above have been established in the laboratory. Results obtained thus far show clear a size-dependent diffusion distance of tracers in the affected brain region. Results from the first experiment using transgenic mice and wild type littermates will be available and presented at the meeting.

BEHAVIORAL AND MOTOR CONTROL STUDIES OF REPEATED FUS INDUCED BBB OPENINGS IN MICE

Oluyemi O Olumolade[1], Shutao Wang[1], Gesthmani Samiotaki[1], Elisa Konofagou[1,2]

[1]Columbia University, Biomedical Engineering, New York, NY, 10027,
[2]Columbia University, Radiology, New York, NY, 10032

Background

Focused Ultrasound (FUS) paired with microbubble ultrasound contrast agents has been demonstrated to non-invasively disrupt the blood brain barrier (BBB) while causing no structural damage to cerebral tissue or vasculature. Little focus has been shed on the ability of FUS to consistently and reversibly open the BBB at serial applications. In addition, behavioral assessment after BBB opening has yet to be established. The main goal of this study was to examine behavioral changes that could be attributed to consecutive BBB openings in mice.

Methods

A total of 56 C57BL/6 mice were divided into 11 groups (n=5, positive control, n=1) and studied for varying durations, up to 6 months. Monthly treated groups survived for a period of 2, 4, or 6 months, and bi-weekly survival was 1, 3, or 5 months. Parameters for FUS sonication are as follows: The transducer frequency was set to 1.5 MHz, peak rarefractional pressure was 0.45 MPa for 60 s, the burst had a rate of 10 Hz and a length of 500 cycles. The positive control had a peak negative pressure of 1.5 MPa. FDA approved microbubbles (Definity™) were intravenously injected prior to sonication. Mice received treatment to the left caudate putamen. MRI confirmed BBB opening and volume. 24 hours following FUS, behavioral testing was conducted. The mice were placed in a custom chamber and were free to explore the field. Visual tracking software recorded the data. Following the open field, the mice were placed on the rotating rod (rotarod), where they were required to maintain balance and motor coordination for a fixed period.

Results and Conclusion

In the open field, two indicative factors of potential brain damage were the total distance traveled and rotation direction. There was no difference in distance traveled between the BBB-opened and control groups. Similarly, there was no affinity for a certain turn angle in the sonicated and control animals. On the other hand, the positive control exhibited a decrease in locomotor activity, as well as rotation contralateral to the left hemisphere. In the rotarod, animals were able to complete the test successfully. The positive control was unable to complete the task. The assessment of the mice using open field and rotarod suggests that repeated opening of the BBB in the caudate putamen using FUS paired with microbubbles under the parameters of BBB opening used do not cause motor impairment. This indicates the potential safety of repeated and/or long-term drug delivery using the FUS methodology.

Acknowledgements

The study was supported by the Kinetics Foundation.

NETRINS ENHANCE BLOOD-BRAIN BARRIER FUNCTION AND REGULATE IMMUNE RESPONSES AT THE BLOOD-BRAIN BARRIER

Cornelia Podjaski[1,2,3], Nathalie Lebeurrier[1], Lyne Bourbonniere[3], Sandra Larouche[3], Simone Terouz[3], Manon Blain[1], Catherine Larochelle[3], Lamia N Hachehouche[3], Akiko Nakano[3], Mike Sabbagh[3], Jorge I Alvarez[3], Jenea Bin[2], Pavel Gris[2], Peter Darlington[1], Jack P Antel[1], Timothy E Kennedy[2], Alexandre Prat[3]

[1]MNI, McGill University, Neuroimmunology, Montreal, H3A 2B4, Canada, [2]MNI, McGill University, Neuroscience, Montreal, H3A 2B4, Canada, [3]CHUM-Hospital Notre-Dame and CHUM Research Centre, Montreal University, Neuroscience, Montreal, H2L 4M1, Canada

During development, netrin guidance cues control cell motility and cell adhesion. Cell-adhesion between endothelial cells at the BBB makes the endothelium impermeable to blood-derivatives and immune cells. To establish and maintain this barrier and protect it during disease, brain endothelial cells must develop and sustain these strong adhesive contacts, through expression of tight junction molecules. However, we do not know whether netrin supports inter-endothelial cell adhesion at the BBB. Given this, we hypothesize that netrin tightens the BBB during development, adulthood, and protects it during disease.
To test this, we used human adult primary brain-derived endothelial cells and newborn netrin-1 knockout mice and evaluated netrin's effect on inter-endothelial cell adhesion and barrier permeability. We also assessed netrins' therapeutic potential to maintain the barrier and limit immune cell infiltration into the central nervous system (CNS) during experimental autoimmune encephalomyelitis (EAE).
Our results demonstrate that brain endothelial cells express netrins where they help to form a tighter BBB during development and maintain and protect the adult barrier by increasing the expression of endothelial junction molecules. Thus netrins promote inter-endothelial adhesion and reduce protein leakage across the barrier. Netrins also reduce BBB breakdown and diminish initial myeloid cell infiltration into the brain and spinal cord during EAE, which delays disease onset and ameliorates disease severity. However, during the chronic phase of EAE, netrin-1 treated mice have higher numbers and more activated T cells in their CNS and exhibit an ataxic gait and limb spasticity.
We conclude that netrins enhance BBB stability, but have dual functions on immune responses during neuroinflammatory disease. These findings favour the hypothesis that if netrin function was to be manipulated as a therapeutic, early short-term approaches would likely be the most effective.

TH17 CELLS ENTER INTO THE BRAIN PARENCHYMA INDEPENDENTLY OF α4 INTEGRINS BUT FAIL TO CONTROL CEREBRAL VIRAL INFECTION

Veit Rothhammer[1], Andreas Muschaweckh[2], Franziska Petermann[1], Sylvia Heink[1], Bernhard Hemmer[1], Ingo Drexler[3], Thomas Korn[1]

[1]Klinikum rechts der Isar, Technical University Munich, Dept. of Neurology, Munich, 81675, Germany, [2]Technical University Munich, Institute of Virology, Munich, 81675, Germany, [3]Universitätsklinikum Düsseldorf, Dept. of Molecular Virology, Düsseldorf, 40225, Germany

Objective: The integrin α4β1 (VLA-4) is used by T helper cells to enter into the CNS. We have shown that in experimental autoimmune encephalomyelitis, Th1 cells but not Th17 cells depend on α4 integrins in order to enter into the CNS. However, in infectious encephalitis, integrin mediated T cell infiltration into the CNS may be altered due to functional changes of the blood brain barrier. Here, we developed a T cell dependent model of acute viral encephalitis in order to study the role of α4 integrins for immune cell infiltration and pathogen clearance in the CNS.
Methods and Results: T cell conditional α4 deficient C57BL/6 mice (CD4Cre+ α4flox/flox and CD4Cre- controls) were immunized with a replication deficient strain of Vaccinia Virus (MVA) in complete Freund's adjuvant and subsequently challenged by intrathecal injection of a replication competent strain of VV. To dissect the role of CD4+ and CD8+ T cells, animals were treated with depleting antibodies to CD8 or rIgG control before and every other day after VV injection. When CD8+ T cells were depleted, α4 integrin deficient mice died of encephalitis at day 6 while their α4 integrin competent counterparts efficiently cleared the infection. Although the numbers of antigen specific CD4+ T cells in the CNS were comparable, we found that the expression profile of CNS derived CD4+ T cells resembled Th17 cells in α4 integrin deficient mice while wild type mice had predominantly recruited Th1 cells into the infected brain. Regarding potential effector molecules for CD4+ T cell mediated killing, perforin-1 was expressed in Th1 cells but significantly reduced in Th17 cells.
Conclusion: We have generated a novel model of T helper cell dependent immunoprotection against acute viral infection of the CNS. While antigen specific Th1 cells alone clear the infection, α4 integrin deficiency in T cells leads to a relative enrichment of Th17 cells that are recruited into the CNS indepently of of α4 integrin expression but fail to control the infection most likely due to their lack of perforin-1 expression. We will further exploit this model to better understand the induction and effector functions of T helper cells in immunity to viral infections of the CNS in order to prevent the break-down of immunosurveillance under conditions of T cell directed immunotherapy for chronic inflammation and autoimmunity.

PERMEABILITY AND REVERSIBILITY TIMELINE STUDY OF THE FOCUSED-ULTRASOUND INDUCED BLOOD-BRAIN BARRIER OPENING AT DISTINCT PRESSURES AND PULSE LENGTHS *IN VIVO*

Gesthimani Samiotaki[1], Oluyemi Olumolade[1], Elisa Konofagou[1,2]

[1]Columbia University, Biomedical Engineering, New York, NY, 10027,
[2]Columbia University, Radiology, New York, NY, 10032

Impermeability of Blood-Brain Barrier (BBB) remains the biggest challenge in the brain drug delivery. Focused Ultrasound (FUS) in conjunction with microbubbles has been shown to open the BBB locally, non-invasively and reversibly. In this study the FUS pulse length (PL) and peak-negative pressure (PNP) are studied as two major parameters for the BBB opening. Prediction and control of the BBB opening permeability and its recovery timeline using MRI are also investigated.

The FUS (1.5 MHz) was used together with Definity® microbubbles and its PL ranged from 67 μs to 6.7 ms while the PNP varied between 0.30 MPa and 0.60 MPa, with a PRF of 10 Hz. T1-weighted and Dynamic Contrast Enhanced (DCE) MRI using gadodiamide was used to quantify the volume of opening and the transfer rate from blood to the brain (K_{trans}). The reversibility timeline was also obtained, and safety was assessed using H&E staining 7 days later.

In this longitudinal study, the dependence of the BBB opening permeability, volume and duration on the PL and PNP was established. At the lowest PNP (0.30 MPa), a longer PL was required to induce BBB opening, indicating that the FUS needs to be applied over a minimum period of time to induce BBB opening. At the lowest (67 μs) PL, the opening PNP threshold was higher, the average Ktrans was equal to 0.010 ± 0.002 min-^{1}, the opening volume was 5.38 ± 5.34 mm^3, and closing occurred within 4 h from opening. Above the 0.67ms PL threshold, there is no significant difference in opening among different PNPs, indicating that the most significant interaction of the FUS with the microbubbles occurs in the first few FUS cycles. At 0.67- and 6.7- ms PLs, the K_{trans} reached the same plateau of 0.025 min^{-1} at both 0.45 MPa and 0.60 MPa, with opening volumes of 13.39 ± 5.94 mm^3 and 18.56 ± 11.12 mm^3, and 16.10 ± 5.98 mm^3 and 22.16 ± 5.38 mm^3, respectively.Longer PLs and higher pressures within the ranges studied have an effect on the BBB opening volume and duration, but not on the safety of the methodology within the pressure range studied. The BBB opening volume was measured to have reduction rate of 11.36 ± 4.02 mm^3 per day. Correlation between the time required for closing and the K_{trans} on the day of opening was shown to follow an exponential relationship, while the opening duration monotonically increased with the opening volume. It was therefore shown that the time required for closing could be predicted from the volume of opening and the permeability change.

LSR, A NEW TIGHT JUNCTION PROTEIN EXPRESSED AT THE BLOOD-BRAIN BARRIER

Fabien Sohet, Seo Yeon Lee, Nadine Ruderisch, Richard Daneman

University of California San Francisco, Anatomy, San Francisco, CA, 94143

Central Nervous System (CNS) Endothelial Cells (ECs) are very different compared to ECs in non-neural tissues in that they are held together by tight junctions (TJs) in order to create a paracellular barrier. Moreover, they exhibit a transcellular barrier to hydrophilic molecules, express specific efflux and influx transporters to protect and nourish the CNS, respectively and finally they limit the entry of immune cells into the CNS. Recently, the lipolysis stimulated lipoprotein receptor (LSR) has been shown to be part of the new family of tricellular tight junctions (tTJ) proteins that restrict the free diffusion of molecules where three epithelial cells meet. In a microarray analysis, we recently showed that both members of tTJs family, LSR and Tricellulin, were enriched in the mouse CNS ECs compared to peripheral ECs. Our current results confirmed that LSR is expressed at tricellular TJs in blood vessels specific of the CNS. Moreover, the expression pattern of LSR during embryogenesis seems to follow the maturation of the BBB suggesting its critical role for the BBB function. To confirm this hypothesis, we are screening the *Lsr* knockout mouse for a BBB phenotype. Finally, we are developing a transgenic mouse model to inducibly knockdown the *Lsr* gene expression in the mouse ECs and therefore we will be able to study the role of the protein in the formation, maintenance and function of the BBB.

HETERGENEOUS PERMEABILITY OF EXPERIMENTAL BRAIN METASTASES OF BREAST CANCER.

Patricia S Steeg[1], Brunilde Gril[1], Paul R Lockman[2], Diane Palmieri[1], Quentin R Smith[2]

[1]Women's Cancers Section, Laboratory of Molecular Pharmacology, Bethesda, MD, 20892, [2]Department of Pharmaceutical Sciences, Cancer Biology Center, Amarillo, TX, 79106

Brain metastases of breast and lung cancers, and melanoma, outnumber primary brain tumors by 10-fold. In breast cancer, brain metastases are increasing in incidence.This alarming trend is thought to be due in large part to the development of effective systemic chemotherapy and molecular therapy regimens, which are partially excluded from the brain, creating a sanctuary site. We developed five experimental models of brain metastasis of breast cancer, injecting selected brain tropic cell lines into the left cardiac ventricle. Brain metastases are quantified in histologic sections approximately one month later.

The patency of brain metastases has been debated. To resolve this debate, we used two preclinical models of brain metastasis of breast cancer (231-BR-HER2 and 4T1-BR5) and quantified the uptake of markers and chemotherapeutics. Mice that harbored experimental brain metastases were injected with a 3000 mw Texas red dextran, a smaller radiolabeled AIB peptide, or radiolabeled paclitaxel or doxorubicin; the mice were perfused and tissue uptake in brain sections quantified. While most brain metastases showed greater permeability than normal brain, heterogeneous and limited permeability of markers and drugs was observed. Permeability was heterogeneous between and within metastatic lesions in the same brain. Permeability was poorly correlated with lesion size ($r^2 = 0.54$). For paclitaxel, only 9% of brain lesions showed >50-fold greater uptake than normal brain, with 15% of lesions indistinguishable from normal brain. To ask how much drug is "enough", tumor cell apoptosis was measured by cleaved caspace 3 expression. Only the 9% of lesions with the highest fold-increase in permeability showed any apoptotic response to paclitaxel. Immunoflourescence was conducted to ascertain structural differences in the BBB between permeable and non-permeable lesions within a single brain. Enhanced brain metastasis permeability was correlated with increased desmin expression, suggestive of greater pericyte expression, and vasular remodeling. The data demonstrate that the BBB remains partially intact in mice harboring experimental brain metastases of breast cancer. The data also confirm the remnants of the BBB as contributors to the lack of chemotherapeutic efficacy. Heterogeneous BBB permeability may be linked to specific changes in the BBB structure and composition. Current investigations are focusing on the identification of BBB-permeable drugs with preventive or therapeutic efficacy, which will be discussed.

A NEW *IN VITRO* BLOOD-BRAIN BARRIER MODEL UNDER SHEAR STRESS INCORPORATING ENDOTHELIAL CELLS AND ASTROCYTES.

Yukio Takeshita, Anne Cotleur, Birgit Obermeier , Richard M Ransohoff

Cleveland Clinic, Neurosicence, Cleveland, OH, 44195

Neuromyelitis optica (NMO), an inflammatory disease of the central nervous system, is characterized by antibodies to aquaporin 4 in Astrocyte (AQP4). One central event in NMO is blood brain barrier (BBB) dysfunction where AQP4 antibodies (AQP4-abs) cross the BBB and engage AQP4. As there are no *in vitro* BBB models that enable us to address *in vivo* pathogenesis, the mechanisms by which AQP4-abs act across the BBB have not been clarified. We propose four useful properties for *in vitro* models to enable studying BBB interactions with inflammatory elements such as leukocytes and pathogenic antibodies during NMO (Takeshita and Ransohoff 2012) First, cells that demonstrate consistent BBB properties are utilized. Second, the model incorporates co-culture of human brain microvascular endothelial cells (HBMEC) and astrocytes. Third, it incorporates shear stress. Fourth, it allows the transmigration of leukocytes which can be recovered for further analysis. Our aims in this study are 1) to generate a BBB model to examine NMO pathogenesis with these properties; and 2) using this model, to evaluate whether BBB function is influenced by AQP4 of astrocyte expression of AQP4 in co-cultures of cytokine-activated HBMEC- and astrocytes. [Methods] Three conditional immortalized human cell lines are used: endothelial cells (TY10/HBMEC) (Sano et al. 2010); astrocytes which do (hASTAQP4) or do not (hAST) express AQP4. These cells proliferate at 33°C; at 37°C proliferation ceases and they differentiate. HBMEC were cultured atop a membrane at 33°C with either hAST or hASTAQP4 co-cultured on the membrane bottom. Cells were activated with empirically-determined optimal concentrations of TNF-α and IFN-γ for 24hr at 37°C. The membrane was placed in a Bioflux3D chamber. Peripheral blood mononuclear cells (PBMC) flowed through the chamber over HBMEC with shear force (1.6 dyn/cm^2) for one hr at 37°C. Numbers of migrated cells were quantified. Transendothelial electrical resistance (TEER) and solute permeability with 10k dextran were measured. [Results] The TEER and solute permeability of HBMEC/hASTAQP4 co-culture were not significantly difference from HBMEC/hAST co-culture, but different from HBMEC alone. Numbers of PBMC which migrated across either HBMEC/hASTAQP4 or HBMEC/hAST were equal, and both were significantly higher than in HBMEC monoculture. [Conclusion] We developed the BBB model incorporating HBMEC/astrocyte co-cultures under flow. These results position us well to introduce AQP4-abs to determine their effect on leulocyte-endothelial interactions at the BBB.

INITIAL CONTACT OF GLIOBLASTOMA CELLS WITH EXISTING NORMAL BRAIN ENDOTHELIAL CELLS STRENGTHEN THE BARRIER FUNCTION VIA FIBROBLAST GROWTH FACTOR 2 SECRETION.

Kunihiko Tanaka[1], Keisuke Toyoda[2], Shinsuke Nakagawa[1,3], Masami Niwa[1,3]

[1]Nagasaki University Graduate School of Biomedical Sciences, Department of Pharmacology, Nagasaki, 852-8523, Japan, [2]Nagasaki Kawatana Medical Center, Department of Neurosurgery, Kawatana, 859-3615, Japan, [3]PharmaCo-Cell Company Limited, BBB laboratory, Nagasaki, 852-8523, Japan

Glioblastoma multiforme (GBM) is one of the most malignant brain tumors. Although GBM is known to be highly invasive in a brain, it is unable to metastasize by invading into the blood vessels. The blood-brain barrier (BBB) function when GBM cells invade along normal brain capillaries is not well-studied. Thus we constructed the in vitro BBB models and investigated the influence of the GBM cells on the BBB function. At first, we examined which extracellular matrix components blocked the invasion of GBM cells, and found that laminin was the responsible protein to block the invasion.
Next, to investigate whether GBM cells modulate the BBB function of normal endothelial cells, we developed a new in vitro BBB model with primary cultures of rat brain endothelial cells (RBECs), pericytes and astrocytes. Cells were plated on a membrane with 8 µm pores, either as a monolayer or as a triple culture model of the BBB. The BBB model consisted with RBEC on the luminal side as a bottom, and pericytes and astrocytes on the abluminal side as a top of the chamber. Human GBM cell line, LN-18 cells or lung cancer cell line, NCI-H1299 cells placed on either the RBEC monolayer or the BBB model increased the transendothelial electrical resistance (TEER) values against the model, which peaked within 72 hours after the tumor cell application. The TEER value gradually returned to the baseline with LN-18 cells, whereas the value quickly dropped to the baseline in 24 hours with NCI-H1299 cells. NCI-H1299 cells invaded into the RBEC layer through the membrane, but LN-18 cells didn't. Fibroblast growth factor 2 (FGF-2) strengthens the endothelial cell BBB function by increased occludin and ZO-1 expression. In our model, LN-18 and NCI-H1299 cells secreted FGF-2, and a neutralization antibody to FGF-2 inhibited LN-18 cells-enhanced BBB function. These results suggest that FGF-2 would be a novel therapeutic target for GBM in the perivascular invasive front.

ENDOTHELIAL APICO-BASAL SIGNAL COMPARTMENTALISATION SHAPES THE MICROVASCULAR RESPONSE AT BLOOD-NEURAL BARRIERS

Natalie Hudson[1], Mosharraf H Sarker[1,2], Kubala Ewa[3], Zoe K Ockrim[1], Martha Betson[1], Roberta Martinelli[1], Clare E Futter[1], Paul A Fraser[4], John Greenwood[1], David T Shima[3], Patric Turowski[1]

[1]UCL Institute of Ophthalmology, Cell Biology, London, EC1V 9EL, United Kingdom, [2]University of Teesside, School of Science and Engineering, Middlesbrough, Tees Valley, TS1 3BA, United Kingdom, [3]UCL Institute of Ophthalmology, Ocular Biology and Therapeutics, London, EC1V 9EL, United Kingdom, [4] King's College London, Cardiovascular Division, London , SE1 9NH , United Kingdom

Besides being the central angiogenic factor in health and disease, vascular endothelial growth factor A (VEGF-A) is a potent vascular permeability factor and is linked to interstitial fluid accumulation in tumours and oedema formation in the brain and retina. Accordingly, targeting VEGF-A has become a major treatment focus. However, VEGF-A also acts as a trophic factor and regulates normal vascular tone and these seemingly opposing observations illustrate the existence of mechanisms that distinguish between the pathological and homeostatic functions of VEGF-A. Here, we show that the permeability-enhancing action of VEGF-A at blood-neural barriers is restricted to the abluminal side of the microvascular endothelium. We found that activity and localisation of VEGF receptors 1 and 2 were confined to the luminal and abluminal face of endothelial cells, respectively. Abluminal VEGFR2 activation mediated rapid p38 and ERK activation, significant reduction in junctional claudin-5 and increased permeability. In contrast, luminal VEGFR1 mediated AKT activation and cytoprotection. Significantly, the exquisite sidedness of VEGF receptors and their response was found in microvascular endothelium of the brain and retina but not the skin, indicating that this represents another example of functional specialisation of blood-neural barriers. Lastly, differential apical-basal responses were also observed with histamine, LPA and IGFBP3. Taken together these data show that endothelial cells at blood-neural barriers exhibit sophisticated functional signalling polarity, which constitutes a general strategy to compartmentalise the response to vasoactive stimuli.

Supported by the British Heart Foundation

Microbubble Distribution Dependence of Focused Ultrasound Induced Blood-Brain Barrier Opening

Shutao Wang[1], Gesthimani Samiotaki[1], Oluyemi Olumolade[1], Jameel A Jameel [1], Elisa E Konofagou[1,2]

[1]Columbia University, Biomedical Engineering, New York, NY, 10027,
[2]Columbia University, Department of Radiology, New York, NY, 10032

Blood-Brain Barrier (BBB) plays an important role in regulating movements of molecules in the brain. Focused Ultrasound (FUS) in the presence of microbubbles has been used to non-invasively induce reversible BBB opening in both rodents and non-human primates. This study aimed at investigating the dependence of BBB opening on the clinically approved ultrasound contrast agent Definity™ and custom-made polydisperse (CMP) microbubbles in mice.

The concentration and volume distribution of both the CMP microbubbles (phosphocholine lipid and perfluorobutane gas) and activated Definity™ (lipid-shelled, gas content: octofluoropropane) were measured by a multisizer. It was observed that the volume distribution of Definity™ peaked around the diameter of 3 μm, while the volume percentage of CMP microbubbles reached a plateau around a diameter of 4.5 μm. Both types of microbubbles were diluted to 6×10^8 #/ml prior to injection. A total of 18 black wild-type mice (3 per group) were used in this study, and each mouse received either Definity™ or CMP microbubbles (1 μl/g body weight) via tail vein injection. Immediately after the microbubble administration, FUS sonications were given with the following parameters: frequency of 1.5 MHz, pulse length of 0.67 ms, pulse repetition frequency of 10 Hz, peak rarefactional acoustic pressures of 0.3, 0.45 MPa, and 0.6 MPa, and a sonication duration of 60 s. The opening of BBB was confirmed using contrast enhanced T1-weighted MRI. A general kinetic model (GKM) was used to analyze brain tissue permeability. The volume of BBB opening using 3D MRI was quantified for the different pressures with both types of microbubbles. Student's t tests were used to analyze the difference between the two microbubble groups. Finally, the closing timeline was established and histology (H&E) was performed to evaluate the safety of the treatments one week post treatment.

The permeability of treated region using the two types of microbubbles did not show significant difference ($P > 0.05$) for FUS pressure levels of 0.45 MPa and 0.6 MPa, while CMP microbubbles showed significantly high permeability ($P < 0.01$) at the lower pressure of 0.3 MPa. The volumes of BBB opening showed similar trends at all pressure levels across the two types of microbubbles. The results from this study indicate that the gas and shell material of microbubbles may have significant effects on FUS induced BBB opening at low pressure levels, possibly due to higher levels of stable cavitation of the CMP microbubbles. This difference became less significant for high FUS pressure levels where inertial cavitation typically occurs.
Acknowledgement (funding): This study was supported in part by NIH R01EB009041, NIH R01AG038961, NIH MH059244, and the Kinetics Foundation.

BRAIN ENDOTHELIAL COX-2 IS A KEY PLAYER IN INFLAMMATORY FEVER

Daniel B Wilhelms, Unn Ö Kugelberg, David Engblom, Anders Blomqvist

Linköping University, Department of Clinical and Experimental Medicine, Division of Cell Biology, Linköping, SE-581 85, Sweden

Fever is a common response to inflammatory and infectious challenge and many aspects of fever seem to be critically dependent on prostaglandin synthesis at the interface between the periphery and the brain. Specifically, it is known that prostaglandin E2 production catalyzed by the enzymes cyclooxygenase-2 (COX-2) and mPGES-1 is an essential step in the signaling upon injection of bacterial lipopolysaccharide (LPS). However, the cellular origin of COX-2 for the febrile response still remains a subject of debate. To address this question, we crossed *Cox-2*$^{fl/fl}$ mice with the *Slco1c1/Oatp14 CreERT2* mouse to selectively delete COX-2 in the cerebrovascular endothelium. Upon i.p. injection of LPS (100 µg/kg), the resulting offspring *(COX-2* $^{Oatp14\ CreERT2}$*)* showed a significantly attenuated febrile response as compared to *Cox-2*$^{fl/fl}$ littermates. In line with this phenotype, immunoreactivity of COX-2 at the blood brain barrier was strongly attenuated, however not completely ablated, in *COX-2* $^{Oatp14\ CreERT2}$ animals. These findings point to the cerebrovascular endothelium as the main cellular origin of COX-2 upon LPS challenge, providing new understanding of the mechanisms underlying inflammatory fever.

LEPTIN MODIFIED WITH AMPHIPHILIC BLOCK COPOLYMERS FOR ENHANCED CNS DELIVERY AND TREATMENT OF OBESITY

Xiang Yi[1], Sue A Farr[2], William A Banks[3], Alexander V Kabanov[1]

[1]Division of Molecular Pharmaceutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC, 27599, [2]St. Louis University School of Medicine, Department of Internal Medicine, St. Louis, MI, 63106, [3]University of Washington School of Medicine, Department of Internal Medicine, Seattle, WA, 98108

The concept of modulating the ability of hydrophilic proteins to penetrate biological membranes such as blood-brain barrier (BBB) by hydrophobization existed decades ago. We focus on modification of proteins with an amphiphilic block copolymer, Pluronic (poly(ethylene oxide)-b-poly(propylene oxide)-b-poly(ethylene oxide). Leptin, a candidate for the treatment of epidemic obesity has failed in part because of impairment in transport across the BBB that develops with obesity. We posit that modification of leptin with Pluronic copolymer might enhance leptin permeability at the BBB and therefore provide potential therapy for obesity treatment. To achieve this goal, three leptin-Pluronic P85 conjugate were synthesized and characterized. They were compound A: a mixture of unmodified leptin and leptin attached to 1 or more Pluronic P85 chains; compound B: leptin attached by 2 and more Pluronic P85 chains; compound C: leptin attached by a single Pluronic P85 chain. In contrast to leptin, all three compounds circulated longer in blood (half-life in blood of A, B and C was 32 min, 75 min and 41 min respectively vs. leptin of 11 min) and more stable in both serum and brain following i.v injection. The influx rate at the BBB and entry mechanism however varied among all three compounds. Compound A penetrated the BBB at a rate similar to native leptin but the entry was non-saturable and independent of the leptin transporter. Compound B and C were able to enter the brain but at a slower entry rate in comparison to leptin. The entry mechanism of compound B was similar to that of A, non-saturable and independent of the leptin transporter; whereas compound C was more similar to native leptin, and transported across the BBB using the leptin transporter. The biological activity of compound A was then tested in normal mice, ob/ob mice and diet-induced obese (DIO) mice. Compared to leptin, compound A decreased food intake in normal mice at a higher dose but produced a more reliable dose-response curve after i.c.v. injection. It significantly reduced food intake and body weight in ob/ob mice after i.p administration. More importantly, feeding was inhibited in a DIO mouse model when compound A was given by both i.c.v and i.v routes. This work suggests that Pluronic modified leptins are able to penetrate the BBB, preserve biological activity, overcome leptin peripheral resistance and therefore provide potential therapeutics for the treatment of obesity.

β-CATENIN MEDIATES REGULATION OF CYP1B1 IN ENDOTHELIAL CELLS OF THE BLOOD-BRAIN BARRIER

Nicole Ziegler[1], Beate Fisslthaler[2], Cathrin J Czupalla[1], Elisabetta Dejana[3], Ingrid Fleming[2], Stefan Liebner[1]

[1]Goethe University, Institute of Neurology (Edinger Institute), Frankfurt, 60528, Germany, [2]Goethe University, Institute for Vascular Signaling, Department of Molecular Medicine, Frankfurt, 60596, Germany, [3]IFOM, The FIRC Institute of Molecular Oncology, Milan, 20139, Italy

Cytochrome P450 (CYP) proteins, including CYP1B1, are enzymes participating in the metabolism of various endogenous compounds as well as in the detoxification of xenobiotics. As it has been shown that especially CYP1B1 is highly expressed in brain endothelial cells (BECs), the drug-metabolizing properties of this enzyme are considered to play a role in BBB function.
However, neither its impact on BBB properties nor the transcriptional regulation specifically in ECs has been evaluated so far.
Endothelial Wnt/β-catenin signalling is known to be indispensable for the induction of BBB characteristics during brain angiogenesis. In order to identify BBB-relevant genes that are dependent on β-catenin, we compared β-catenin-deficient (KO) endothelial cell lines to wild-type (WT) controls by an Affymetrix® transcriptome analysis, revealing decreased expression of Cyp1b1 in the KO that could be confirmed by quantitative RT-PCR. To test if this effect can also be observed under physiological conditions, we treated mouse brain endotheliomas as well as primary mouse brain microvascular ECs (MBMECs) with Wnt-conditioned medium (CM), revealing increased Cyp1b1 expression upon activation of the Wnt pathway. Interestingly, transcriptionally active β-catenin was sufficient to induce Cyp1b1, but not aryl hydrocarbon receptor (AHR) expression, a ligand-dependent transcription factor known to regulate several CYP genes including CYP1B1.
It has been shown in non-ECs that disruption of adherens junctions by subconfluent cell growth leads to upregulation of CYP1B1. To test if a similar effect could be observed in ECs, VE-cadherin(VEC)-deficient endothelioma cells were analyzed for CYP1B1 expression. Indeed, CYP1B1 was highly upregulated in VEC-/- ECs. Along this line EC activation by shear stress upregulated CYP1B1 in WT, VEC-/- but not in β-catenin deficient ECs.
In conclusion, this preliminary data suggest that β-catenin participates in the regulation of CYP1B1 in ECs via AHR and via direct transcriptional control independent of the AHR. These data lead to the assumption that the Wnt/β-catenin pathway contributes to the regulation of a broad range of BBB-specific genes including Cyp1b1.

REGULATION OF THE BLOOD RETINAL BARRIER

Jing Xie[1], Alecia Cutler[1], Bela Anand-Apte[1,2]

[1]Cleveland Clinic Lerner College of Medicine, Ophthalmology, Cleveland, OH, 44195, [2]Cleveland Clinic Lerner College of Medicine, Molecular Medicine, Cleveland, OH, 44195

Diabetic retinopathy is the most severe ocular complication of diabetes and is the leading cause of vision loss in the western hemisphere. Progression of diabetic retinopathy often results in diabetic macular edema, which is a consequence of the breakdown of the blood-retinal barrier, increased retinal vascular permeability and leakage of plasma from small blood vessels in the macula leading to loss of central vision. Vascular Endothelial Growth Factor (VEGF) is believed to play a major role in this process and recent clinical efforts are underway to determine the efficacy of VEGF blocking agents in proliferative diabetic retinopathy and macular edema. A relationship between hyperglycemia, VEGF and vascular permeability has not been established and has led to the hypothesis that other as yet unidentified permeability factors might play a role in the development of macular edema.

Despite decades of research on the blood-brain/blood-retinal barrier, our knowledge on its formation and maintenance is very limited. This may be due to the fact that research on BRB has been based mainly on in vitro models of cultured cells and in situ vascular perfusion. The blood- retinal barrier (BRB) is comprised of an extensive network of endothelial cells, pericytes and astrocytes in which tight junctions between endothelial cells seal off the vascular lumen. Because of its tissue transparency, external development and genetic amenability, we hypothesized that the zebrafish would be an excellent model system to examine the BRB. To develop an unbiased genetic approach to identify molecules involved in regulating retinal vascular permeability and the blood-retinal barrier, we have generated a transgenic zebra fish line that expresses Green Fluorescent Protein (GFP) in the blood plasma whose retinal vasculature can be visualized in vivo. Using this model we have determined that the BRB is completely developed at 3 dpf in zebrafish and maintained up to 60 dpf. The broad long-term goal will be to use the zebrafish model developed in this study to identify molecular pathways involved in regulating the development and maintenance of the BRB.

THE ROLE OF GLIAL CELLS IN NUTRIENT HOMEOSTASIS OF THE DROSOPHILA BRAIN

Stefanie Limmer, Christian Klämbt

Institut für Neurobiologie, Biology, Münster, 48149, Germany

Neuronal function consumes a large amount of energy and thus the brain requires a constant but regulated supply of metabolites. In invertebrates, such as Drosophila, the nervous system is floating in the hemolymph and all metabolites that enter the brain have to be transported across the blood brain barrier (BBB). As in primitive vertebrates, the Drosophila BBB is generated by glia. A layer of so-called subperineurial glial cells completely encapsulates the nervous system and thus, all metabolites reach the neurons en route through glia.
The main energy supply of the inner organs of Drosophila is provided by trehalose that is found in high concentrations in the hemolymph. Trehalose is a non-reducing disaccharide, in which two D-glucose units are linked via a α,α-1,1-glycosidic linkage. The uptake of trehalose is mediated by two trehalose transporters, which are members of the SLC2A gene family. The subperineurial glial cells then either secrete C6 sugars to the neurons - or alternatively supply energy to neurons by secreting C3 metabolites as described for the bee retina. To discriminate between these possibilities we have followed a number of different approaches. The relevance of trehalose transporters has been determined by mutant analysis and RNAi studies. Furthermore, we have studied the role of all other putative sugar and monocarboxylate transporters in glial cells by RNAi knockdown. To determine whether C6 or C3 carbohydrates are secreted by glial cells we suppressed the expression of core enzymes regulating glycolysis or enzymes involved in the citrate cycle in either glial cells or in neurons. Moreover, we have ablated mitochondria specifically in glia or in neurons following expression of a restriction enzyme targeted to mitochondria. We will summarize our results and discuss approaches towards identifying neuronal signals that control glial carbohydrate secretion during energy homeostasis of the brain.

PREDICTING EFFLUX RATIOS AND BLOOD-BRAIN BARRIER PENETRATION FROM CHEMICAL STRUCTURE: COMBINING PASSIVE PERMEABILITY WITH ACTIVE EFFLUX BY P-GLYCOPROTEIN

Elena Dolghih, Matthew P Jacobson

University of California, San Francisco, Department of Pharmaceutical Chemistry, San Francisco, CA, 94158

One of the unique challenges of developing drugs for the central nervous system is overcoming the blood-brain barrier, a cellular and enzymatic barrier that tightly regulates passage of molecules from blood into the brain. Herein we report a new computational approach that combines two mechanism-based models, for passive permeation and for active efflux by P-glycoprotein, to provide insight into the multi-parameter optimization problem of designing small molecules able to access the CNS. Our results indicate that this approach is capable of distinguishing compounds with high/low efflux ratios as well as CNS+/CNS- compounds and provides advantage over estimating P-glycoprotein efflux or passive permeability alone when trying to predict experimental measurements. We also demonstrate that this method could be useful for rank-ordering chemically similar compounds and that it can provide detailed mechanistic insight into which property specifically, passive permeability or interaction with P-glycoprotein, affects the efflux ratios and/or CNS penetration, offering limited guidance as to how compounds could be modified to improve their access into the brain.

LIVE IMAGING OF BLOOD-BRAIN BARRIER DEVELOPMENT IN ZEBRAFISH

Bensheng Ju[1], Robyn A Umans[1,2], Hannah E Henson[1,2], Chaithanyarani Parupalli[1], Jennifer L Peters[3], Michael R Taylor[1]

[1]St. Jude Children's Research Hospital, Chemical Biology & Therapeutics, Memphis, TN, 38105, [2] St. Jude Children's Research Hospital, Cell and Tissue Imaging, Memphis, TN, 38105

The blood-brain barrier (BBB) plays a vital role in both normal and pathological processes within the central nervous system (CNS). Yet, a comprehensive understanding of the BBB has been hampered by difficulties in observing the BBB in vivo and in performing unbiased genetic and small molecule screens. Here we show that the optically transparent zebrafish provide a valuable model for imaging BBB formation and dissecting BBB signaling pathways. We generated a transgenic zebrafish line to serve as an in vivo reporter of the BBB. We show that our transgenic line drives expression specifically in brain endothelial cells (BECs) and not in the vasculature of peripheral tissues or circumventricular organs. Using time-lapse confocal microscopy, we find that barriergenesis (i.e. the initiation of BBB development) occurs immediately as new vessels sprout into the brain parenchyma. Using a combination of genetic and small molecule tools, we demonstrate that canonical Wnt signaling, but not VEGF signaling, is essential for barriergenesis. Our results indicate that CNS angiogenesis and barriergenesis occur simultaneously, but require distinct signals for proper formation.

DISCOVERY AND INTERROGATION OF EVOLUTIONARILY CONSERVED BLOOD BRAIN BARRIER CHEMICAL PROTECTION PATHWAYS.

Michael K DeSavo, Samantha Hindle , Souvinh Orng , Roeben Munji, Fahima Mayer, Roland Bainton

University of California at San Francisco, Department of Anesthesia, San Francisco, CA, 94158

Nervous system function requires special physiologic support systems to maintain optimal performance. The blood brain barrier (BBB) is the primary conduit for the brain with the rest of the body and maintains tight regulation over the ingress and egress of endogenous small molecules and drugs. We hypothesize that an evolutionarily conserved set of molecular functions is required to maintain BBB chemical protection physiology and metabolic homeostasis. In order to parse the complex genetic integration of these processes, we have combined genomic, bioinformatic and physiologic methods to discover the most highly BBB-conserved molecular systems between flies and vertebrates. Using gene set enrichment analyses and sequence-based comparisons, we compared the Drosophila BBB (surface glia) to the vertebrate BBB cell layers – the vascular endothelia, pericytes, and astrocytic glia. The conserved BBB transcriptomes contain many orthologous ABC and SLC transporters, cell adhesion molecules, xenobiotic metabolism pathways, metabolic enzymes, and signaling molecules. Furthermore we compare BBB transcriptomes to genetic modules, built by independent component analysis of large species-specific genomic data sets, to discover new brain-specific pharmacokinetic regulatory pathways. These analyses lay out a roadmap for determining the pharmacokinetic logic of the BBB that is conserved and divergent across phyla. Thus bioinformatic analysis of BBB transcriptomes can be used by vertebrate and fly biologists alike to derive specific and testable hypotheses for interrogation of chemical protection pathways of the brain

BLOOD-BRAIN BARRIER ON CHIP TO STUDY THE EFFECT OF SHEAR STRESS ON BARRIER FUNCTION

Lonneke M Griep, Floor Wolbers, Bjorn de Wagenaar, Paul M ter Braak, Andries D van der Meer, Albert van den Berg

University of Twente, BIOS/Lab on a Chip group, MESA+ Institute for Nanotechnology, Enschede, 7500 AE, Netherlands

Organs-on-chip is an emerging research field and gives the opportunity to create realistic biomimetic micro environments and integrate an array of analysis strategies. In addition, shear stress can be introduced as an important physiological factor. Our focus is on the development of a representative model of the human blood-brain barrier (BBB). Especially for the BBB, it is known that shear stress positively affects endothelial cell physiology and tight junction formation. Therefore, it is essential to include shear stress in a realistic in vitro model of the BBB. However, most of the BBB studies are performed in static Transwell cell culture systems. An improved and more realistic in vitro model is required for representative drug transport studies and better understanding of the processes involved in BBB functionality.

We have developed a BBB on chip and included physiological relevant shear stress, ranging from 0 to 15 dyne/cm^2. This microfluidic device comprises two microfluidic channels separated with a 0.4 µm polycarbonate membrane. Moreover, the integration of platinum electrodes at the cross section enables the analysis of the transendothelial electrical resistance (TEER) of the BBB. Human brain microvascular endothelial cells (hCMEC/D3) were cultured in the BBB chip and confluent monolayers were formed at day 1 of culture. Furthermore, hCMEC/D3 expressed the tight junction protein ZO-1 at day 4. Tightness of the monolayer was analyzed every day up to day 7 in both the BBB chip and Transwell and showed average TEER values (± SEM) of 36.9 Ω.cm^2 (± 0.9 Ω.cm^2) and 28.2 Ω.cm^2 (± 1.3 Ω.cm^2) respectively. Exposure of the cells to a shear stress of 5.8 dyne/cm^2 for 18h resulted in a 10-fold increase of the TEER value, hence the barrier tightness was enhanced.

To gain more insight in the processes involved in BBB function, we will focus on the effect of shear stress on both paracellular permeability and P-gp activity in future experiments. Paracellular permeability for different sized dextrans and P-gp transporter activity of the barrier will be analyzed with absorbance spectroscopy and the rhodamine 123 efflux assay respectively. Overall, this realistic microfluidic platform integrates TEER analysis, optical analysis and permeability analysis to study BBB functionality under physiologically relevant conditions.

ABC TRANSPORTERS REGULATE CNS CHEMOPROTECTION THROUGH ENDOGENOUS STEROID SIGNALING AT THE BBB.

Samantha Hindle[1], Souvinh Orng[1], Michael DeSalvo[1], Elena Dolgikh[2], Hiroshi Ishimoto[4], Fahima Mayer[1], Toshihiro Kitamoto[3], Matt Jacobson[2], Roland Bainton[1]

[1]University of California San Francisco, Department of Anesthesia and Perioperative Care, San Francisco, CA, 94158, [2]University of California San Francisco, Department of Pharmaceutical Chemistry, San Francisco, CA, 94158, [3]University of Iowa, Department of Anesthesia, Iowa City, IA, 52242, [4]RIKEN Center for Developmental Biology, Kobe, 650-0047, Japan

Maintenance of homeostasis in the central nervous system (CNS) requires tight regulation over the metabolites and toxins entering the brain space; this function is performed by the blood-brain barrier (BBB). Understanding how the BBB orchestrates this is fundamental for both disease prevention and efficient targeting of therapeutics into the brain space. The major challenge in understanding the BBB is being able to analyze it within the context of intact animals and under physiological conditions. We have pioneered the use of *Drosophila* (Dm) BBB surface glia layers for this purpose. P-glycoprotein (Pgp), a broad-specificity ABC transporter, is well known for its role as a xenobiotic efflux transporter. However, its role in controlling the localization of endogenous molecules is unclear. BBB knock-down of Mdr65, the *Drosophila* homolog of Pgp, allowed us to investigate the endogenous substrate specificity of Mdr65 and the physiological effect on the animal. **We revealed that Mdr65 is important for controlling steroid partitioning between the humoral and brain space.** Manipulations of Mdr65 function also impacted upon steroid-regulated behaviors, including sleep and longevity. Our findings that Mdr65 is required for both xenobiotic and endogenous molecule partitioning suggest a central role for Pgp-like transporters in communicating exogenous chemical threats through their effect on endogenous molecule partitioning.

BLOOD-TESTIS BARRIER, DRUG TRANSPORTERS AND SPERMATOGENESIS

C. Yan Cheng, Dolores D Mruk

Population Council, Center for Biomedical Research, New York, NY, 10065

In the mammalian testis, the blood-testis barrier (BTB) is constituted by specialized junctions between adjacent Sertoli cells near the basement membrane of the seminiferous tubule, not involving the endothelial tight junction (TJ)-barrier of microvessels, which are restricted to the interstitial space between seminiferous tubules. In short, the BTB is different from other blood-tissue barriers ultrastructurally, such as the blood-brain barrier and the blood-retinal barrier since these latter tissue barriers are constituted almost exclusively by endothelial TJ-barrier of the microvessel. More important, while TJs between Sertoli cells play a crucial role to confer the barrier function at the BTB, these TJs coexist with basal ectoplasmic specialization (basal ES, a testis-specific atypical adherens junction), which together with gap junction and desmosome contribute the functional BTB in mammalian testes. The BTB also segregates the seminiferous epithelium into the basal and the adluminal compartment. Thus, post-meiotic spermatid development can take place in a unique microenvironment at the adluminal compartment via spermiogenesis by sequestering many cancer-testis antigens, many of which are oncogenes that express transiently during spermatogenesis, from the systemic circulation to avoid the production anti-sperm antibodies. While the BTB is one of the tightest blood-tissue barriers, it is a dynamic ultrastructure since it undergoes extensive restructuring at stage VIII of the s epithelial cycle of spermatogenesis, involving its transient “opening” and “closing” to facilitate the transit of preleptotene spermatocytes residing at the basal compartment, traversing the BTB to enter the adluminal compartment to prepare for meiosis I and II. Recent studies have shown that these various junction types at the BTB are working in concert to maintain the BTB homeostasis and to modulate the transient “opening” and “closing” of the barrier during spermatogenesis. Furthermore, studies have shown that drug transporters are playing some important roles in conferring the opening and closing of the BTB, and surprisingly, some of these drug transporters are not restricted to the Sertoli cell but expressed almost exclusively by developing germ cells. All this information will be critically reviewed and evaluated in this seminar in order to provide some insightful thoughts in studying other blood-tissue barriers. [Supported by NIH grants R01 HD056034 and U54 HD029990, Project 5]

PERIVASCULAR RESIDENT MACROPHAGE-LIKE MELANOCYTES IN THE INNER EAR ARE ESSENTIAL FOR INTRASTRIAL FLUID-BLOOD BARRIER INTEGRITY

Xiaorui Shi

Oregon Health & Science University, Department of Otolaryngology/Head & Neck Surgery, Portland , OR, 97006

The microenvironment of the cochlea is maintained by the barrier between the systemic circulation and the fluids inside the stria vascularis. However, the mechanisms that control intrastrial fluid-blood barrier permeability remain largely unknown. The barrier comprises endothelial cells connected to each other by tight junctions and an underlying basement membrane. In a recent study, we discovered that the intrastrial fluid-blood barrier also includes a large number of perivascular cells with both macrophage and melanocyte characteristics. The perivascular resident macrophage-like melanocytes (PVM/Ms) are in close contact with vessels through cytoplasmic processes. Here we demonstrate that PVM/Ms have an important role in maintaining intrastrial fluid-blood barrier integrity and hearing function. Using a new cell culture-based in vitro model and a genetically-induced PVM/M-depleted animal model, we show that absence of PVM/Ms increases the permeability of the intrastrial fluid-blood barrier to both low- and high- molecular-weight tracers. The increased permeability is caused by decreased expression of pigment epithelial-derived factor (PEDF), which regulates expression of several tight junction-associated proteins instrumental to barrier integrity. When tested for endocochlear potential (EP) and auditory brainstem response, PVM/M-depleted animals show substantial drop in EP with accompanying hearing loss. Our results demonstrate a novel and critical role for PVM/Ms in the regulation of intrastrial fluid-blood barrier permeability for establishing a normal hearing threshold.

NOVEL MECHANISMS OF BRAIN RESPONSE TO IMMUNE SIGNALING AFTER PERIPHERAL SURGERY.

Niccolò Terrando[1], Maarja Mäe[2], Daniel Gustavsson[1], Katerina Akassoglou[3,4], Lars I Eriksson[1]

[1]Karolinska Institutet, Physiology and Pharmacology, Section for Anesthesiology and Intensive Care Medicine, Stockholm, 17177, Sweden, [2]Karolinska Institutet, Medical Biochemistry and Biophysics, Stockholm, 17177, Sweden, [3]University of California, San Francisco, Gladstone Institute of Neurological Disease, San Francisco, CA, 94158, [4] University of California, San Francisco, Neurology, San Francisco, CA, 94158

Impairment of cognition is a frequent complication of acute illness, infection or surgical trauma. Aseptic injury initiates local inflammation *via* release of pro-inflammatory molecules that result in neuroinflammation and cognitive impairment. Herein we describe the immune-to-brain signaling underlying CNS changes after peripheral surgery.

Adult male wildtype, LysM-Cre/$Ikk\beta^{F/F}$ and Ccr2$^{RFP/-}$ Cx3cr1$^{GFP/-}$ mice were randomly assigned as follows: 1) untreated animals, 2) surgery (open tibia fracture of the left hind leg with intramedullary fixation) under isoflurane anesthesia and postoperative analgesia. Animals were assessed for plasma cytokines, blood-brain barrier (BBB) permeability, neuroinflammation and hippocampal-dependent memory using trace fear conditioning.

Peripheral surgery causes distinct changes in systemic pro-inflammatory cytokines leading to subsequent BBB disruption and CNS impairments. At 12h we found a reduction in the expression of claudin-5 in the hippocampus of operated mice compared to controls. Fibrinogen deposition was observed 24h after surgery and associated with enhanced GFAP and CD11b immunoreactivity, indicative of astrocyte and microglial activation. While targeting NF-κB signaling in myeloid cells using LysM-Cre/$Ikk\beta^{F/F}$ after surgery, we found no signs of BBB disruption and neuroinflammation compared to $Ikk^{F/F}$. To distinguish between monocyte-derived macrophages and resident microglia activation we then used CCR2-RFP knock-in peripheral macrophage reporter mice. CCR2-positive monocytes were observed within the hippocampus and perivascular space of the operated mice, suggesting an influx of peripheral cells through permeable areas of the BBB. Modulation of this surgical response *via* stimulation of endogenous α7 nAChR cholinergic anti-inflammatory pathway prevents BBB damage, migration of peripheral macrophages into the hippocampus, and subsequent cognitive decline.

Surgery causes changes in BBB permeability, which underlie neuroinflammation and memory dysfunction. Prophylactic strategies aimed to limit postoperative inflammation, thus preventing BBB disruption, may represent a feasible target to prevent cognitive decline in the susceptible population.

NOVEL 3D ANALYSIS OF CLAUDIN-5 REVEALS SIGNIFICANT ENDOTHELIAL HETEROGENEITY AMONG CNS MICROVESSELS

Debayon Paul[1], Ann Cowan[2], Shujun Ge[1], Joel S Pachter[1]

[1]UConn Health Center, Blood-Brain Barrier Laboratory, Dept. of Cell Biology, Farmington, CT, 06030, [2]UConn Health Center, Center for Cell Analysis and Modeling, Dept. of Molecular, Microbial & Structural Biology, Farmington, CT, 06030

The integrity of the blood-brain barrier (BBB) and central nervous system (CNS) homeostasis critically rely on an intricate network of tight-junctions (TJs) between the specialized endothelial cells of CNS microvessels. Significant disruption of TJs accompanies neuroinflammatory disease, during which expression and/or distribution of claudin-5 (CLN-5), a prominent TJ protein in CNS microvessels, has been shown to parallel pathophysiological changes at the BBB. However, conventional two-dimensional (2D) immunohistochemical analysis of thin sections to quantify CLN-5 in situ is encumbered by the tortuosity of capillaries and distorted diameter of inflamed venules. There is also increasing evidence that endothelial cells display remarkable heterogeneity in structure and function among the different microvascular tributaries - a situation that might relate to intercellular TJ density. To address this possible relationship, we have developed a novel contour-based 3D image visualization and quantification method, employing high-resolution confocal z-stacks from thick immunofluorescently stained spinal cord sections, to analyze interendothelial CLN-5 in “different-sized” CNS microvascular tributaries. Imaris® software-based image segmentation allows a single tributary to be optically segregated from a complex microvascular network in a confocal z-stack, thus enabling focal TJ analysis. Specifically, a contour surface is first created to isolate a tributary from rest of the dataset, and to approximate the “surface area” defined by the endothelium. A measure of CLN-5 ‘density’ (CLN-5 intensity/endothelial surface area), can then be determined within the tributary. Using this method, we analyzed spinal cords of healthy mice and mice with experimental autoimmune encephalomyelitis (EAE). In healthy mice CLN-5 density was greatest in the smallest capillaries, and least in the larger venules. This heterogeneity was exacerbated during EAE, as lumbar spinal venules revealed a significant loss of CLN-5 staining that was associated with focal leukocyte extravasation, while adjacent capillaries exhibited neither CLN-5 loss nor infiltrating leukocytes. However, both capillaries and venules evidenced leakage of IgG during disease, underscoring the complexity of microvascular response during CNS inflammation. This method is adaptable to analyzing other junctional proteins, and will serve to highlight their respective role(s) within specific microvascular tributaries during health and disease.

A HIGH THROUGHPUT SCREEN FOR REGULATORS OF BLOOD RETINA BARRIER PERMEABILITY

Daniel L Chao[1], Enrique L Salero-Coca[1], Claude-Henry Volmar[2], Shaun Brothers[2], Claes Wahlestedt[2], Jeffrey L Goldberg[1]

[1]University of Miami- Bascom Palmer Eye Institute, Ophthalmology, Miami, FL, 33134, [2]University of Miami, Center for Therapeutic Innovation, Miami, FL, 33134

The inner blood retina barrier is composed of tight junctions on retinal endothelial cells, which act as a molecular sieve to regulate flow of molecules in and out of the retinal blood vessels. Disruption of this blood retina barrier is central to a variety of retinal vascular diseases such as diabetic retinopathy and age related macular degeneration. Although some of the key regulators of vascular permeability such as VEGF have been uncovered, the regulation of the retinal vascular permeability remains poorly understood. We have adapted an *in vitro* assay to assess blood retina barrier permeability to utilize an unbiased high throughput chemical genetic approach to identify novel regulators of blood retina barrier. RBE4 cells, a well studied rat brain endothelial cell line are grown on transwell plates in a 96 well format, and permeability to 70kDa dextrans is used as readout for blood retina barrier permeability. RBE4 cell monolayers are able to form tight junctions *in vitro* via both structural and functional assays. Addition of VEGF, TNF-alpha, and IL-1 beta, molecules which have been shown to be implicated in blood retina barrier breakdown in ocular disease, increase the permeability of RBE4 monolayers. Preliminary results of the screen will be discussed and future directions will be outlined to validate candidates in our screen.

BLOOD-BRAIN BARRIER MODULATION IN AMYLOID PRECURSOR PROTEIN (APP-TG2576) MICE

James Keaney*, Matthew Campbell*, Finnian Hanrahan, Marian M Humphries, Anna-Sophia Kiang, Paul F Kenna, Pete Humphries

Trinity College Dublin, Ocular Genetics Unit, Smurfit Institute of Genetics, Dublin , 2, Ireland

*These authors contributed equally

The blood-brain (BBB) and inner blood-retina (iBRB) barriers are essential for maintaining brain/retina homeostasis and protecting delicate neural tissue from damaging blood-borne agents. The BBB and iBRB of the CNS vasculature is characterized by tight junctions that limit the paracellular diffusion of polar solutes and macromolecules between the blood plasma and brain extracellular fluid, thus maintaining a microenvironment for optimum neural function. Previous studies have demonstrated that remodeling of neural barriers via RNAi-mediated claudin-5 downregulation renders the BBB and iBRB controllably and reversibly permeable to molecules up to approximately 1 kDa in size. More recently BBB modulation has been shown to enhance the movement of edematous material from the brain to the blood and decrease brain swelling in a model of focal cerebral oedema (1). Here, we show that down-regulation of occludin, a second major tight junction protein at the BBB, in combination with claudin-5 suppression, can modulate BBB permeability to a 3 kDa dextran tracer molecule. This has direct relevance to the etiology of Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA) in particular where failure to eliminate the pathogenic soluble amyloid-β (Aβ) peptide has been implicated as a key factor in disease progression. Transfection of mouse brain microvascular endothelial cells with tight junction-directed siRNAs increased endothelial cell permeability to a synthetic FITC-conjugated soluble Aβ1-40 peptide. Furthermore, in a long term study, we have shown that Aβ1-40 plasma levels are increased following systemic administration of occludin and claudin-5 siRNAs in APP-Tg2576 mice, a transgenic mouse model of AD. Improvements in cognitive function were observed with increasing soluble Aβ1-40 detected in plasma samples of mice receiving periodic systemic injection of siRNA's directed against occludin and claudin-5. These results indicate enhanced paracellular clearance of Aβ1-40 across the BBB in response to occludin and claudin-5 suppression and highlight a role for occludin in tandem with claudin-5 at the BBB in the molecular etiology of AD. Therapeutic modulation of the BBB for the purposes of removing synaptotoxic Aβ could hold real promise as an adjuvant to current antibody based clinical trials.

1. Campbell M, Hanrahan F, Gobbo OL, Kelly ME, Kiang AS, et al. (2012) Targeted suppression of claudin-5 decreases cerebral oedema and improves cognitive outcome following traumatic brain injury. Nature Communications 3: (2012), May 22;3:849

POLYMICROBIAL SEPSIS ELICITS A ROBUST CNS TRANSCRIPTIONAL RESPONSE WHICH DOES NOT REQUIRE MYD88-DEPENDENT SIGNALING IN ENDOTHELIAL CELLS.

Gerard Honig, Betty Diamond

Feinstein Institute for Medical Research, Center for Autoimmune and Musculoskeletal Disease, Manhasset, NY, 11050

Bacterial sepsis is a common and life-threatening syndrome in which a blood-borne infection causes a massive innate immune response, leading to dysfunction of multiple organs including the central nervous system (CNS). Patients who survive sepsis are at greater risk for cognitive decline; mice subjected to cecum ligation and puncture (CLP), a standard model of sepsis, also exhibit sustained cognitive deficits. Sepsis-induced CNS pathophysiology is thought to involve acute activation of cerebrovascular endothelial cells with blood-brain barrier dysfunction and subsequent induction of pro-inflammatory gene expression in the CNS. Stimulation of MyD88-dependent signaling within CNS endothelial cells (through Toll-Like Receptor ligands and/or IL-1) has been proposed to be critical, but the cellular physiology of this process is not well defined. Using the CLP model, we tested the role of MyD88-dependent signaling in endothelial cells in sepsis-induced CNS effects. Expression of Cre recombinase from the *Tie2* promoter led to deletion of a conditional *MyD88* allele with the expected endothelial pattern. Following CLP, we observed concentrations of CxCL-1 protein in perfused hippocampal tissue approaching those observed in serum, consistent with acute BBB dysfunction. CLP induced the transcription of multiple inflammatory pathway genes within the hippocampus, notably those encoding the IL-1 receptor and the chemokine CCL-2. Following CLP, mice with MyD88-deficient endothelial cells mounted a comparable transcriptional response in the hippocampus. These results demonstrate that polymicrobial infection elicits a pronounced and specific transcriptional response in the CNS and that MyD88-dependent signaling within endothelial cells is dispensable for this response. Thus, bacterial and immune signals elicited during polymicrobial sepsis may be transduced through MyD88-independent pathways in the inflamed CNS endothelium; alternatively, other neurovascular cell types or specialized neurosensory organs may mediate CNS responses to infection. Future experiments will address these possibilities.

NEUROIMMUNOLOGICAL BLOOD BRAIN BARRIER OPENING IN EXPERIMENTAL CEREBRAL MALARIA

Alexandru Movila, Adela Nacer, Ute Frevert

NYU School of Medicine, Microbiology, New York, NY, 10010

Plasmodium falciparum malaria is responsible for nearly one million annual deaths worldwide. Because of the difficulty in monitoring the pathogenesis of cerebral malaria in humans, we conducted a study in various mouse models to better understand disease progression in experimental cerebral malaria (ECM). We compared the effect on the integrity of the blood brain barrier (BBB) and the histopathology of the brain of *P. berghei* ANKA, a known ECM model, and *P. yoelii* 17XL, a model for lethal hyperparasitemia. *P. berghei* ANKA infection caused neurological signs, cerebral hemorrhages, and BBB dysfunction in CBA/CaJ and Swiss Webster mice, while Balb/c and A/J mice were resistant. *P. yoelii* 17XL caused lethal hyperparasitemia in all mouse strains; histopathological alterations, BBB dysfunction, or neurological signs were not observed. Intravital imaging revealed that infected erythrocytes containing mature parasites passed slowly through capillaries making intimate contact with the endothelium, but did not arrest. Except for relatively rare microhemorrhages, mice with ECM presented no obvious histopathological alterations that would explain the widespread disruption of the BBB. Intravital imaging did reveal, however, that postcapillary venules, but not capillaries or arterioles, from mice with ECM, but not hyperparasitemia, exhibit platelet marginalization, extravascular fibrin deposition, CD14 expression, and extensive vascular leakage. Blockage of LFA-1 mediated cellular interactions prevented leukocyte adhesion, vascular leakage, neurological signs, and death from ECM. The endothelial barrier-stabilizing mediators imatinib and FTY720 inhibited vascular leakage and neurological signs and prolonged survival to ECM. Thus, it appears that neurological signs and coma in ECM are due to regulated opening of paracellular-junctional and/or transcellular-vesicular fluid transport pathways at the neuroimmunological BBB.

VISUALIZING THE STEPWISE RECRUITMENT OF PARACELLULAR AND TRANSCELLULAR PATHWAYS DURING BLOOD-BRAIN BARRIER BREAKDOWN IN STROKE USING TWO-PHOTON IMAGING

Axel Nimmerjahn[2], Ahmet Arac[3], Daniel Knowland[1], Sarah E Lutz[1], John Perrino[4], Gary K Steinberg[3], Ben A Barres[5], Dritan Agalliu[1,5]

[1]University of California, Irvine, Developmental and Cell Biology, Irvine, CA, 92697, [2]The Salk Institute, Waitt Advanced Biophotonics Center, La Jolla, CA, 92037, [3]Stanford University, Neurosurgery, Stanford, CA, 94305, [4] Stanford University, Cell Science Imaging Facility, Stanford, CA, 94305, [5]Stanford University, Neurobiology, Stanford, CA, 94305

Endothelial cells (ECs) in the central nervous system (CNS) form a barrier that regulates solute permeability. The barrier properties of ECs are mediated by three strategies: a) tight junctions (TJs); b) few endocytotic vesicles (caveolae); and c) selective transport systems. The blood-brain barrier (BBB) breaks down during many CNS insults including stroke. During BBB breakdown following stroke, TJs become leaky and the rate of transcytosis increases, thereby allowing serum proteins to enter the CNS. This contributes directly to cerebral vasogenic edema, hemorrhagic transformation and increased mortality. However, few mechanisms that regulate the dynamic behavior of TJs or the rate of transcytosis in the ECs have been identified, and the roles of paracellular (TJs) and transcellular (transcytosis) pathways in stroke pathogenesis remain unclear.

In vivo analysis of dynamic structural and functional BBB changes in stroke is crucial to understanding their role in pathogenesis. We have created a novel transgenic mouse strain where TJs are labeled with eGFP (Tie2p::eGFP::Claudin-5). This allows real-time analysis of TJ dynamics in vivo. We have imaged TJs in brain capillaries from eGFP::Claudin-5 transgenic mice in vivo using two-photon microscopy, and combined it with quantitative analysis of fluorescent tracer leakage, in order to correlate changes in TJ protein localization with diffusion of tracers across the BBB in stroke. We have found that, although BBB function is impaired as early as 6 hours after stroke, TJs break down only 48 hours after occlusion in the transient Middle Cerebral Artery Occlusion model (tMCAO). These observations are supported also by transmission electron microscopy studies. On the contrary, we have found that the number of transcytosis vesicles found within endothelial cells is increased as early as 6 hours after occlusion in the tMCAO mouse model. Therefore these findings suggest that there is a stepwise recruitment of two distinct pathways that contribute to blood-brain barrier impairment in stroke. The increased rate of transcytosis precedes tight junction breakdown and may be the major pathway that impairs BBB function in the initial phases of stroke.

THE BLOOD-BRAIN BARRIER IN MULTIPLE SCLEROSIS.

Elga de Vries

MS center Amsterdam, VU medical center, Department of Molecular Cell Biology and Immunology, Amsterdam, The Netherlands

In patients suffering from the neuro-inflammatory disorder multiple sclerosis (MS), the BBB looses its protective function already in the early stages of the disease. This results in the activation of an inflammatory cascade which triggers the trafficking of immune cells and subsequent formation of demyelinating lesions ultimately leading to progressive neurological deficits.
Before invading the CNS, activated immune cells need to cross the BBB, which normally limits transendothelial leukocyte migration. Astrocytes play an important role in the function of the barrier, together with other surrounding cells forming the neurovascular unit. However, to date the role of astrocytes in barrier regulation during a neuroinflammatory attack remains undefined. In recent years, it has become clear that the sphingomyelin metabolism plays a key role in biological processes. Our recent data indicate that during MS pathogenesis, an increasing amount of pro-inflammatory lipids are produced. In particular, enhanced levels of ceramide are detected which are produced by reactive astrocyte and their endfeet that contact the brain vasculature. Using in vitro assays, it became evident that ceramide shift the barrier phenotype of the endothelium towards a more pro-inflammatory phenotype, allowing cells of the immune system to traverse the endothelial layer. Subsequent treatment of reactive astrocytes with the S1P receptor agonist FTY-720P, currently used as novel anti-inflammatory agents in MS treatment, reduced the neuro-inflammatory events. Together these data indicate that during pathology an altered signalling occurs between astrocytes and endothelial cells leading to neuro-inflammatory events.
In the past we have shown that structural and functional alterations occur at the level of the neurovasculature. Yet, little is known regarding the molecular mechanisms that control the neuroprotective features of brain endothelial cells forming the BBB or those that underlie the BBB alterations which lead to lesion formation in MS patients.
We recently have discovered that microRNAs, small regulators of gene transcription, are associated with inflammation at the BBB. Moreover, recent evidence suggests that the barrier phenotype is under the control of a number of nuclear receptors, including ones involved in the signaling of retinoic acid. Recent findings and subsequent implications for the treatment of disorders which are marked by a malfunction of the BBB will be discussed.

ACTIVATED LEUKOCYTE CELL ADHESION MOLECULE REGULATES BBB INTEGRITY AND NEUROINFLAMMATION

Marc-André Lécuyer, Olivia Saint-Laurent, Jorge I Alvarez, Romain Cayrol, Lyne Bourbonnière, Alexandre Prat

CRCHUM-University of Montreal, Neuroimmunologie, Montreal, H2L 2W5, Canada

The loss of blood-brain barrier (BBB) integrity is a hallmark of multiple sclerosis (MS) and is associated with increased leukocyte trafficking into the central nervous system (CNS). In MS, BBB endothelial dysfunction correlates with an upregulation of cell adhesion molecules (CAMs) as well as chemokines and cytokines production. We recently described the role of ALCAM as an important mediator of leukocytes migration into the CNS. In this study, using an ALCAM knock-out (KO) mouse strain, we assessed the specific role of ALCAM on BBB endothelial cells (BBB-ECs) and its effect on leukocyte transmigration during the course of experimental autoimmune encephalomyelitis (EAE).

Using an adhesion assay under flow, we demonstrated a 60% reduction in adhesion of human monocytes following ALCAM steric inhibition under shear stress, while increasing their velocity. We also observed a 50% reduction in human $CD14^+$ monocyte migration across a monolayer of human BBB-endothelial cells when blocking ALCAM with a specific anti-ALCAM monoclonal antibody. In addition, following ALCAM neutralization using MAB656, the migration of Th17 and Th1 lymphocytes was reduced by 70% and 45% respectively. *In vivo*, ALCAM neutralization using the anti-ALCAM monoclonal antibody reduced leukocyte infiltration and EAE severity. However, we observed a more severe active EAE in ALCAM KO animals associated with a significant increase in perivascular infiltration and demyelination, as compared to WT controls. In addition, EAE transfer experiments in which ALCAM KO deficient animals received WT MOG reactive splenocytes suggest that the effects observed are linked to the absence of ALCAM at the level of BBB-ECs. Phenotypic characterization of un-immunized ALCAM KO mice revealed a reduced expression of most BBB junctional proteins, which contributes to increased BBB leakage, serving as the basis to explain more severe EAE clinical course in these animals.

Collectively, our data provide evidence of the implication of ALCAM in leukocyte adhesion and migration across the brain endothelium in human and point to a biologically relevant function of ALCAM in BBB integrity *in vivo* in mouse.

ALCAM IS NOT REQUIRED FOR T CELL INVASION INTO THE CNS AND FOR THE DEVELOPMENT OF EAE IN THE C57BL/6 MOUSE

Ruth Lyck, Christoph Wyss, Michael Abadier, Claudia Blatti, Urban Deutsch, Britta Engelhardt

University of Bern, Theodor Kocher Institut, Bern, 3012, Switzerland

Extravasation of T cells from the vasculature to the site of inflammation is a tightly regulated multi-step process involving rolling, arrest, crawling and – finally - diapedesis across the endothelium. Using live cell imaging of T cell interaction with primary mouse brain microvascular endothelial cells (pMBMECs) - an in vitro model for the parenchymal blood brain barrier (BBB) - under flow conditions, we have recently shown a sequential involvement of endothelial ICAM-1 and VCAM-1 in mediating shear resistant arrest of encephalitogenic T cells on the inflamed BBB, whereas endothelial ICAM-1 and ICAM-2 were essential for subsequent T cell polarization and crawling to sites permissive for diapedesis. Endothelial ALCAM has recently been described to promote the migration of human CD4+ T cells across a human model of the BBB in vitro and to be involved in the development of experimental autoimmune encephalomyelitis (EAE) in vivo. Here, we aimed to identify the precise step of T cell extravasation across the BBB mediated by endothelial ALCAM. After having confirmed expression of the ALCAM ligand CD6 on mouse encephalitogenic CD4+ T helper 1 cells, we investigated T cell interaction with pMBMECs isolated from ALCAM-/- C57BL/6 mice. Surprisingly, under static conditions the rate of T cell diapedesis across ALCAM-/- pMBMECs was comparable to wild type pMBMECs. Under flow conditions, T cell arrest and crawling on and diapedesis across inflamed ALCAM-/- pMBMECs was without any differences to wt pMBMECs when visualized with time lapse video microscopy. Moreover, encephalitogenic T cells failed to adhere to recombinant ALCAM protein. Investigating the expression of ALCAM in brain and spinal cord tissue sections of C57BL/6 mice by immunohistology showed that ALCAM immunostaining was restricted to meninges and not detectable on parenchymal microvessels in healthy mice and in mice suffering from EAE. Finally, we asked if EAE pathogenesis is altered in the absence of ALCAM. However, an equal onset of actively induced EAE in ALCAM-/- C57BL/6 and wild-type C57BL/6 mice was followed by a more severe disease course during the chronic phase in ALCAM-/- C57BL/6 mice. Taken together, our data underline that in the C57BL/6 mouse ALCAM is not involved in the migration of encephalitogenic T cells across the parenchymal BBB and in the initiation of neuroinflammatory pathogenesis of actively induced EAE. ALCAM expression in the meninges and the worsened disease course in ALCAM-/- mice leave open additional yet not identified functions for ALCAM in rodents.

EVOLUTION OF THE BLOOD-BRAIN BARRIER AND NEUROVASCULAR UNIT.

N. Joan Abbott

King's College London, Institute of Pharmaceutical Science, London, SE1 9NH, United Kingdom

The brain endothelium forming the modern mammalian blood-brain barrier is surrounded by cells of the neurovascular unit, chiefly pericytes, astrocyte end-feet and microglia, together with their extracellular matrices, subserving the needs of the 'client' cell population, the neurons. Current research is continuing to reveal details of the many ways in which these cells interact and support each other, in the critical developmental stages, in maintenance of healthy barrier function, and in repair. Further barrier layers are present at the choroid plexus epithelium secreting the cerebrospinal fluid (CSF) and the arachnoid layer of the meninges. Within the CNS, the interstitial fluid (ISF) and CSF create a dynamic fluid environment which helps to maintain homeostasis of the brain microenvironment; the ependyma acts as a key interface for ISF-CSF exchange. Further dynamic processes connecting the immune system of the systemic circulation with that of the brain are responsible for immune surveillance and intervention while minimising disturbance of neuronal networks. Specializations of the perivascular space not only provide a pathway for ISF circulation but also create a specialized antechamber regulating the entry of leukocytes into the CNS.

Examination of the CNS barrier organisation of modern invertebrates and non-mammalian vertebrates gives insights into the evolution of the mammalian system, and suggests some of the features that gave selective advantage in the evolutionary process. Advances in genomic, proteomic and signalling information are also contributing to this understanding. This talk will review the key features of the evolutionary process and their implications for understanding development of CNS barriers and homeostasis of the CNS microenvironment.

CENTRAL NERVOUS SYSTEM BARRIERS UNDER ADENOSINE SIGNALING CONTROL

Margaret S Bynoe

Cornell University, Veterinary Medical College l, Microbiology and Immunology, Ithaca, NY, 14853

The blood brain barrier (BBB) is a tight knit layer of endothelial cells that blocks the entry of potentially harmful substances, including pathogenic immune cells and toxic substances, from the circulation into the central nervous system (CNS). However, by virtue of its protective function, the blood brain barrier hampers the entry of therapeutic drugs into the CNS needed to treat myriad neurological diseases ranging from HIV to brain tumors. We have recently made the novel observation that the purine nucleoside, adenosine, regulates the closing and opening of central nervous system barriers.

Adenosine is a primordial molecule with multiple functions. Most notable is its role in curtailing the immune response to limit damage to healthy tissues. In addition to its role in downregulating inflammation, it signals the migration of immune cells to sites of tissue damage. Increase in extracellular adenosine and activation of adenosine receptor signaling promotes cellular chemotaxis, induce cell migration to sites of injury to augment tissue repair. We have pioneered work demonstrating that adenosine receptor signaling is required for lymphocytes to gain access into the CNS and cause experimental autoimmune encephalomyelitis, the animal model for MS. We demonstrated that mice that lack the enzyme, CD73 (CD73-/-) which produces extracellular adenosine are protected from EAE. Adenosine mediates its effects via four G-protein coupled receptors, two of which are expressed on CNS barrier cells (endothelial cells and choroid plexus). We further demonstrated that both CD73 and the A2A AR are abundantly expressed at the choroid plexus, which is a structure that forms the blood to CSF barrier. We demonstrated that the presence or absence of CD73 and /or alteration of the A2A adenosine receptor significantly alter the outcome of EAE by altering the ability of cells to get into the CNS. Recently, we determined that adenosine receptor signaling regulates immune cell entry into the CNS via the choroid plexus by modulating the chemokine, fractalkine (CX3CL1).

We next asked whether adenosine also regulates brain endothelial barrier permeability to molecules. We observed that adenosine can dose-dependently regulate the permeability of the BBB by permitting or blocking entry molecules across the BBB of a variety of sizes, both in vitro and in vivo. Further, we demonstrate that BBB modulation by adenosine receptor signaling is tunable.

THE CHALLENGE OF ENGINEERED NANOMATERIAL BRAIN DELIVERY

Robert A Yokel[1], Eric A Grulke[2], Michael T Tseng[3], Younsoo Bae[1], Thomas D Dziubla[2], Mo Dan[1]

[1]University of Kentucky, Pharmaceutical Sciences, Lexington, KY, 40536, [2]University of Kentucky, Chemical and Materials Engineering, Lexington, KY, 40506, [3]University of Louisville, Anatomical Sciences & Neurobiology, Louisville, KY, 40202

Considerable effort is ongoing to use engineered nanomaterials (ENMs) to deliver agents across the BBB into the brain. *In vivo* studies of brain distribution of metal-based ENMs typically report ENM in brain samples that contain the blood in the brain vasculature, brain microvascular endothelial cells (BMECs), and brain parenchyma. This does not inform whether the ENM distributed across the BBB into brain parenchyma. There is little evidence that metal-based ENMs achieve significant brain parenchymal concentration. Using nanoceria as a model insoluble metal-based ENM, rat brain cerium was very low 1 hour to 90 days after iv administration of 5 to 55 nm cubic/polyhedral citrate-coated nanoceria or ceria nanowires. The *in situ* brain perfusion technique showed ceria ENM on the luminal surface of BMECs after 2 min intra-carotid infusion of 5 nm ceria. The capillary depletion method showed a very high percentage of the ceria associated with the BMECs. This may be due to insufficient time for brain parenchymal uptake. However, electron microscopy revealed very little nanoceria in brain parenchyma up to 90 days after iv ceria ENM infusion. Two approaches were taken to try to increase brain uptake of metal-containing ENMs. In a passive targeting approach, iron oxide nanoparticle (IONP) loaded-cross-linked nanoassemblies (~ 30 nm) were designed to minimize cell interaction to capitalize on fluid-mediated endocytosis. They preferentially accumulated in rat brain glioma compared to other brain regions, shown by MRI. In an active targeting approach, IONPs were coated with PECAM-1 antibody (primary particle size ~ 25 nm) to target BMEC tight junctions. They showed greater brain accumulation than control (IgG-coated) IONPs. These passive and active targeting approaches can enhance the ability to deliver metal-containing ENMs, and presumably their drug payloads, to the brain, but we do not yet know if they result in uptake into brain parenchyma. Support: US EPA STAR Grant RD-833772 and NCI Cancer Nanotechnology Training Center grant R25CA153954.

3D SINGLE MOLECULE TRACKING OF TF TRANSPORT AT BLOOD-CNS BARRIERS USING MULTIFOCAL PLANE MICROSCOPY

Sripad Ram[1,2], Dongyoung Kim[1,2], E. Sally Ward[1], Raimund J Ober[1,2]

[1]UT Southwestern Medical Center, Immunology, Dallas, TX, 75390, [2]UT Dallas, Electrical Engineering, Richardson, TX, 75080

The study of the transport pathways at the blood-CNS barriers represents an important problem with significant implications for CNS homeostasis since many neurological disorders are associated with the accumulation of toxic substances in the CNS. Moreover, these pathways are of relevance to the delivery of therapeutics, which remains a major challenge for the treatment of CNS cancers and neurodegenerative disorders. To date, however, efforts to understand these pathways at the subcellular level have been severely limited due to the lack of cellular imaging approaches to enable rapid 3D imaging of the intracellular trafficking processes. Towards overcoming this limitation, we have made significant advances in a 3D imaging modality, multifocal plane microscopy (MUM) [1-3], to enable 3D single molecule tracking of transferrin (Tf) molecules in an in vitro model of the blood-CSF barrier (BCSFB).

The use of MUM has led to the discovery of a new cellular process, intercellular transfer, which involves the rapid exchange of Tf between adjacent cells at the BCSFB. We also report 3D single molecule tracking of endocytosis and exocytosis at the lateral plasma membrane of cells in the BCSFB. This lateral membrane has been notoriously difficult to image with other cellular imaging modalities. A detailed characterization of these events based on the temporal and 3D intracellular spatial behavior of Tf molecules has been made. Central to this study has been the validation of an in vitro model of the BCSFB, which opens up the possibility to investigate BCSFB transport pathways through imaging approaches. The approaches used in this study should have general relevance to defining 3D trafficking pathways in other cell systems.

1. P. Prabhat, S. Ram, E. S. Ward and R. J. Ober. IEEE Trans. Nanobiosci., 2004, 3:237-242.

2. P. Prabhat, Z. Gan, J. Chao, S. Ram, C. Vaccaro, S. Gibbons, R. J. Ober and E. S. Ward. Proc. Natl. Acad. Sci. USA, 2007, 104:5889-5894.

3. S. Ram, P. Prabhat, J. Chao, E. S. Ward and R. J. Ober. Biophysical Journal, 2008, 95:6025-6043.

DEFINING THE THERAPEUTIC PROFILE OF BLOOD-BRAIN BARRIER CROSSING TFR BISPECIFIC ANTIBODIES

Joy Yu*[1], Jessica A Couch*[2], Yin Zhang[3], Jacqueline Tarrant[2], Reina N Fuji[2], Raymond K Tong[5], Kwame Hoyte[6], Wilman Luk[6], Yanmei Lu[6], Kapil Gadkar[2], Saileta Prabhu[2], Benjamin Ordonia[2], Yuwen Lin[2], William J Meilandt[1], Kimberly Scearce-Levie[1], James A Ernst[5], Mark S Dennis[3], Ryan J Watts[1]

[1]Genentech, Neuroscience, South San Francisco, CA, 94080, [2]Genentech, Developmental Sciences, South San Francisco, CA, 94080, [3]Genentech, Antibody Engineering, South San Francisco, CA, 94080, [4] Genentech, Neuroscience, South San Francisco, CA, 94080, [5]Genentech, Protein Chemistry, South San Francisco, CA, 94080, [6]Genentech, Biochemical and Cellular Technology, South San Francisco, CA, 94080

Receptor-mediated transcytosis is a promising approach for boosting the delivery of therapeutic antibodies to the brain. We have shown that reducing the affinity of an antibody targeting the transferrin receptor (TfR) can substantially increase its uptake in brain. Bispecific antibodies that target both TfR and β-secretase (BACE1), an enzyme that cleaves amyloid precursor protein leading to the generation of beta amyloid peptides associated with Alzheimer's disease, demonstrate the potential therapeutic utility of this approach. Nevertheless, there is limited data on the desired antibody affinity, optimal pharmacokinetics, and safety implications for utilizing TfR to transverse the blood-brain barrier (BBB). Here we evaluate additional TfR/BACE1 bispecific antibody variants and show that reducing antibody affinity to TfR not only improves uptake and biodistribution in brain, but also improves peripheral exposure and the safety profile of these antibodies. In particular, we address liabilities of targeting TfR with antibodies, namely acute clinical signs and decreases in circulating reticulocytes observed after dosing. We show that by eliminating Fc effector function we can completely ameliorate the acute clinical signs, and partially rescue the decreases in reticulocytes. Furthermore, we show that complement mediates the residual reticulocyte decreases observed after Fc effector function is eliminated. These data provide essential insights for the development of BBB-crossing therapies utilizing TfR.

*These authors contributed equally to this work.

BLOOD-BRAIN OR BLOOD-RETINAL BARRIER DISRUPTION BY FOCUSED ULTRASOUND AND MICROBUBBLES FOR TARGETED CNS DRUG DELIVERY.

Juyoung Park[1,2], Yong-Zhi Zhang[1], Natalia Vykhodtseva[1], Nathan J McDannold[1]

[1]Harvard Medical School, Radiology, Boston, MA, 02115, [2]Sungkyunkwan University, Radiology, Seoul, 138-225, South Korea

Drug delivery to the central nervous system is a challenge because currently an estimated 98% of clinically validated drugs cannot cross the blood-brain barrier (BBB) and blood-retinal barrier (BRB). This study explored permeabilization of the BBB, the blood-tumor barrier (BTB), and the BRB using focused ultrasound (FUS) and an IV injected microbubbles (Definity) using MRI and functional evaluation.
Sprague Dawley rats were sonicated at the targets in the brain or on the retina using a 690 kHz focused transducer. 10ms bursts were applied at 1Hz for 60s with peak rarefactional pressure amplitudes from 0.5 up to 1.1 MPa combined with IV injection of Definity (10-20 μl/kg). For a glioma model, we implantated 9L glioma cells in both hemispheres of the brain. Targets in the tumor and in a normal brain region were sonicated; the contralateral hemisphere served as a control. An MRI contrast agent (Magnevist) and a chemotherapy agent, doxorubicin (DOX), were injected immediately after the last sonication. The BBB permeability was assessed by calculating of the transfer coefficient (Ktrans) for the Magnevist using dynamic contrast enhanced MRI. The permeability was compared to the DOX concentrations (measured by fluorometric analysis). For the BRB disruption study, Magnevist was administered immediately after the last sonication, and serial T1-weighted MR images were acquired every 5 min for 25 min to confirm the BRB disruption. Electroretinograms (ERG) were performed after the sonications to evaluate retinal function.
After sonication in normal brain, the mean Ktrans was 0.0142±0.006 min^{-1} at 30 min and was two or more orders of magnitude higher than the non-sonicated targets. DOX concentrations at targeted area were significantly greater than in the non-sonicated area. The mean Ktrans in the tumor was 0.0163±0.0069 min^{-1} before sonication and 0.0316±0.0085 min^{-1} 30 min after sonication. DOX concentrations in the sonicated tumor were 2.5-fold higher than in the non-sonicated tumor. Also, a linear correlation was found between the DOX concentration and the Ktrans measured 30 min after sonication (R: 0.8). For the BRB disruption study, we observed extravasation of a systemically injected MRI contrast in the targeted retinal area, which then leaked into the vitreous. Sonication at 0.6 MPa produced no evident changes in the ERG.
These results demonstrate that this FUS method can enhance drug delivery to the brain and brain tumors and that evaluation of an MRI contrast agent can predict drug concentrations. The method also can be applied in the retina while maintaining retinal function.

ACTIVATION OF SIGNALING PATHWAYS FOLLOWING LOCALIZED DELIVERY OF SYSTEMICALLY-ADMINISTERED NEUROTROPHIC FACTORS ACROSS THE BLOOD-BRAIN BARRIER USING FOCUSED ULTRASOUND AND MICROBUBBLES

Babak Baseri[1], James J Choi[1], Thomas Deffieux[1], Mania Samiotaki[1], Yao-Sheng Tung[1], Oluyemi Omuolade[1], Scott A Small[2], Barclay Morrison III[1], Elisa E Konofagou[1,3]
[1]Columbia University, Biomedical Engineering, New York, NY, 10027, [2]Columbia University, Neurology, New York, NY, 10027, [3]Columbia University, Radiology, New York, NY, 10027

Background/Introduction
Neurotrophic factors have been shown to have broad neuroprotective effects in addition to its therapeutic role in neurodegenerative disease. In this study, the efficacy of delivering exogenous BDNF, GDNF and NTN to the left hippocampus was assessed in wild-type mice through the noninvasively disrupted blood-brain barrier (BBB) using FUS and microbubbles.

Methods
A total of 20 C57Bl6 male mice were used for this study. A single-element spherical segment FUS transducer (center frequency: 1.525 MHz; focal depth: 90 mm) was driven by a function generator (Agilent Technologies) through a 50-dB power amplifier (ENI) to generate therapeutic ultrasound waves (Choi et al., 2007b). A pulse-echo transducer (center frequency: 7.5 MHz; focal length 60 mm) was positioned through a center hole of the FUS transducer so that the foci of the two transducers were aligned. It was driven by a pulser-receiver system (Panametrics) connected to a digitizer (Gage Applied Technologies) and was used for imaging. Definity® microbubbles were injected intravenously prior to sonication (peak-rarefactional pressure: 0.46 MPa; pulse repetition frequency: 10 Hz; pulse length: 20 ms). Each of the four target locations was sonicated twice, resulting in a total of 8 sets of 30s sonication with a 30s delay between each set. Three different neurotrophic factors, the Brain-Derived Neurotrophic Factor (BDNF) (40-90mg/kg in 0.15 ml PBS, n=8), the Glia-Derived Neurotrophic Factor (GDNF) (40-90mg/kg in 0.15 ml PBS, n=10) (20mg/kg in 0.2 ml PBS, n=2) and Neurturin (NTN) were conjugated to Alexa Fluor® 594 dye (Invitrogen Corp, Carlsbad, CA, USA) and injected intravenously prior to FUS. Upon sacrifice, fluorescence imaging and immunohistochemistry were performed to confirm molecular diffusion and triggered downstream effects, respectively.

Results and Conclusions Both the BDNF and NTN were found to have significantly higher fluorescence in the sonicated hippocampus and putamen, respectively. The GDNF, however, was found not to cause any fluorescence in the sonicated region, as the GDNF was found to be rapidly broken down in circulation (within the first 45 s). The BDNF bioactivity was found to be preserved following delivery as assessed quantitatively by immunohistochemical detection of the pTrkB receptor and activated pAkt, pMAPK, and pCREB in the hippocampal neurons. Neurturin behaved similarly to BDNF permeating through the opened barrier and into the parenchyma); however, GDNF did not. It was therefore shown for the first time that systemically administered neurotrophic factors can cross the noninvasively disrupted BBB and trigger neuronal downstream signaling effects in a highly localized region in the brain but also that not all factors when administered systemically will successfully cross the opened BBB.

Acknowledgements (Funding) This study was supported in part by NIH R01EB009041, NIH R01AG038961 and the Kinetics foundation.

Participant List

Dr. Joan Abbott
King's College London
joan.abbott@kcl.ac.uk

Dr. Jorge Alvarez
Universite de Montreal-CRCHUM
jorge.alvarez@umontreal.ca

Dr. Bela Anand-Apte
Cleveland Clinic Lerner College of Medicine
anandab@ccf.org

Dr. Matthew Andrews
University of Minnesota Duluth
mandrews@d.umn.edu

Dr. Roland Bainton
University of California at San Francisco
baintonr@anesthesia.ucsf.edu

Dr. Joel Bard
Pfizer
joel.bard@pfizer.com

Dr. Shawn Bearden
Idaho State University
bearshaw@isu.edu

Dr. Ayal Ben-Zvi
Harvard medicl school
ayal_ben-zvi@hms.harvard.edu

Dr. Christer Betsholtz
Karolinska Institute
christer.betsholtz@ki.se

Dr. Nga Bien-Ly
Genentech
bienlyn@gene.com

Prof. Anders Blomqvist
Linkoping University
anders.blomqvist@liu.se

Ms. Laura Buckman
Vanderbilt University Medical Center
Laura.B.Hunt@vanderbilt.edu

Dr. Margaret Bynoe
Cornell University, Veterinary Medical College
msb76@cornell.edu

Dr. Matthew Campbell
Trinity College Dublin
matthew.campbell@tcd.ie

Dr. Nadia Caram-Salas
National Research Council-Canada
nadia.caram-salas@nrc.gc.ca

Dr. Daniel Chao
University of Miami/ Bascom Palmer Eye Institute
dchao@med.miami.edu

Dr. Cherry Chen
Columbia University
ccc2136@columbia.edu

Prof. Si Chen
Hospital
yechao9527@sina.com

Dr. C. Yan Cheng
Rockefeller University, Population Council
Y-Cheng@popcbr.rockefeller.edu

Dr. Azzurra Cottarelli
IFOM Foundation
azzurra.cottarelli@ifom.eu

Dr. Jessica Couch
Genentech
couch.jessica@gene.com

Dr. Robert Cudmore
Johns Hopkins School of Medicine
cudmore@jhu.edu

Dr. Richard Daneman
UCSF
richard.daneman@ucsf.edu

Dr. Bridgitte Dauwalder
University of Houston
bdauwalder@uh.edu

Dr. Dimitrios Davalos
UCSF
dimdavalos@gmail.com

Dr. Elga de Vries
MS center Amsterdam, VU medical center
he.devries@vumc.nl

Dr. Annika Deiss
University of Wuerzburg
annika.deiss@web.de

Dr. Elena Dolghih
UCSF
ldolghih@gmail.com

Dr. Lester Drewes
University of Minnesota
ldrewes@d.umn.edu

Dr. Kate Ellacott
Vanderbilt University Medical Center
kate.ellacott@vanderbilt.edu

Dr. Britta Engelhardt
University of Bern
bengel@tki.unibe.ch

Dr. James Ernst
Genentech
jernst@gene.com

Dr. Graham Farrington
Biogenidec Corp.
graham.farrington@biogenidec.com

Dr. Ute Frevert
NYU School of Medicine
ute.frevert@nyumc.org

Dr. Reinhard Gabathuler
biOasis Technologies Inc.
reinhard@bioasis.ca

Dr. Min Gao
Regeneron Pharmaceutical
min.gao@regeneron.com

Dr. Svitlana Garbuzova-Davis
University of South Florida
sgarbuzo@health.usf.edu

Dr. Steve Gendron
CRCHUM- University of Montreal
steve.gendron@umontreal.ca

Dr. Holger Gerhardt
London Research Institute - Cancer Research UK
holger.gerhardt@cancer.org.uk

Dr. Holger Gerhardt
London Research Institute - Cancer Research UK
holger.gerhardt@cancer.org.uk

Dr. Christian Göritz
Karolinska Institute
christian.goeritz@ki.se

Ms. Lonneke Griep
University of Twente
l.m.griep@utwente.nl

Dr. Brunilde Gril
National Institues of Health/ NCI
grilbrun@mail.nih.gov

Ms. Lamia Naouel Hachehouche
Université de Montréal
lamia.naouel.hachehouche@umontreal.ca

Prof. Margareta Hammarlund-Udenaes
Uppsala University
mhu@farmbio.uu.se

Mr. Finnian Hanrahan
Trinity College Dublin
hanrahfe@tcd.ie

Dr. Samantha Hindle
UCSF
hindles@anesthesia.ucsf.edu

Mr. Daniel Holl
Karolinska Institutet
daniel.holl@stud.ki.se

Dr. Gerard Honig
Feinstein Institute for Medical Research
ghonig@nshs.edu

Dr. Huaiyu Hu
SUNY Upstate Medical University
huh@upstate.edu

Dr. Kullervo Hynynen
University of Toronto
khynynen@sri.utoronto.ca

Dr. Noriko Iwamoto
Division of Cell Biology
niwamoto@med.kobe-u.ac.jp

Dr. Katherine Jackman
Weill Cornell Medical College
kaj2009@med.cornell.edu

Dr. Gareth John
Mount Sinai School of Medicine
gareth.john@mssm.edu

Dr. Denise Karaoglu Hanzatian
Abbott Laboratories
denise.karaoglu@abbott.com

Mr. James Keaney
Trinity College Dublin
jkeaney@tcd.ie

Dr. Julianna Kele Olovsson
LICR
Julianna.Kele@licr.ki.se

Prof. Christian Klämbt
University of Muenster
klaembt@wwu.de

Dr. Elisa Konofagou
Columbia university
ek2191@columbia.edu

Ms. Sharanya Kousik
Rush University Medical Center
sharanya_m_kousik@rush.edu

Ms. Eva Yuhua Kuo
China Medical University
yulovekuan@yahoo.com.tw

Prof. Ted Weita Lai
China Medical University
ted.weita@me.com

Ms. Chamala Lama
University of Houston
chamalalama@hotmail.com

Ms. Catherine Larochelle
CRCHUM
cath_larochelle@yahoo.com

Mr. Marc-André Lécuyer
CRCHUM Montreal University
marc-andre.lecuyer@mail.mcgill.ca

Dr. Stefan Liebner
Goethe University Clinic
stefan.liebner@kgu.de

Mr. Zejian Liu
University of Minnesota Duluth
liux0585@umn.edu

Ms. Ileana Lorenzini
Johns Hopkins University
ilopez2@jhmi.edu

Dr. Sarah Lutz
University of California Irvine
sarah.lutz@uci.edu

Dr. Ruth Lyck
University of Bern
ruth.lyck@tki.unibe.ch

Dr. Cressida Madigan
University of Washington
cressida@uw.edu

Dr. Maarja Mäe
Karolinska Institute
maarja.mae@ki.se

Dr. David Maussang
BBB Technologies BV
davidmaussang@tobbb.com

Dr. Joseph McCarty
MD Anderson Cancer Center
jhmccarty@mdanderson.org

Mr. Michael Menezes
Stony Brook University
menezes@pharm.stonybrook.edu

Dr. Roeben Munji
University of California - San Francisco
Roeben.Munji@ucsf.edu

Dr. Corinne Nielsen
University of California, San Francisco
corinne.nielsen@ucsfmedctr.org

Dr. Birgit Obermeier
Lerner Research Institute, Cleveland Clinic
obermeb@ccf.org

Dr. Elisabet Ohlin
Uppsala University
elisabet.ohlin@igp.uu.se

Ms. Oluyemi Olumolade
Columbia University
ooo2113@columbia.edu

Dr. Souvinh Orng
UCSF
orngs@anesthesia.ucsf.edu

Dr. Juyoung Park
Harvard medical school
jypark@bwh.harvard.edu

Mr. Debayon Paul
University of Connecticut Health Center
Paul@student.uchc.edu

Ms. Cornelia Podjaski
McGill University / CHUM-Hospital Notre-Dame
cornelia.podjaski@mail.mcgill.ca

Dr. Jan Powell
Shire HGT
jpowell@shire.com

Dr. Alexandre Prat
CHUM-Université de Montréal
a.prat@umontreal.ca

Dr. Jenny Presto
Karolinska Institutet
jenny.presto@ki.se

Dr. Sripad Ram
University of Texas Southwestern Medical Center
sripad.ram@utsouthwestern.edu

Dr. Ignacio Romero
The Open University
i.romero@open.ac.uk

Dr. Veit Rothhammer
TU Munich
rothhammer@lrz.tum.de

Dr. Nadine Ruderisch
UCSF
nadine.ruderisch@ucsf.edu

Dr. Lynn Rutkowski
Tufts
l.rutkowski@hotmail.com

Ms. Gesthimani Samiotaki
Columbia University
gs2496@columbia.edu

Dr. Jennifer Schiltz
NIH/CSR
schiltzjc@csr.nih.gov

Dr. Xiaorui Shi
Oregon Health & Science University
shix@ohsu.edu

Dr. Julie Siegenthaler
University of Colorado, School of Medicine
julie.siegenthaler@ucdenver.edu

Dr. William Sisk
Biogen Idec
william.sisk@biogenidec.com

Dr. Fabien Sohet
UCSF
fabien.sohet@ucsf.edu

Dr. Lydia Sorokin
University of Muenster
sorokin@uni-muenster.de

Dr. Patricia Steeg
National Cancer Institute
steegp@mail.nih.gov

Dr. Yukio Takeshita
Cleveland Clinic
miyukio0606@gmail.com

Dr. Kunihiko Tanaka
Nagasaki University Graduate School of Biomedical
kunny-ta@nagasaki-u.ac.jp

Dr. Michael Taylor
St. Jude Children's Research Hospital
michael.taylor@stjude.org

Dr. Niccolo Terrando
Karolinska Institutet
niccolo.terrando@ki.se

Dr. Binu Tharakan
Texas A&M Health Science Center/Scott and White Ho
btharakan@yahoo.com

Dr. Elisabeth Tournier-Lasserve
Universite Paris Diderot - Paris 7
tournier-lasserve@univ-paris-diderot.fr

Dr. Patric Turowski
University College London
p.turowski@ucl.ac.uk

Dr. Benoit Vanhollebeke
UCSF
Benoit.Vanhollebeke@ulb.ac.be

Dr. Shutao Wang
Columbia University
sw2735@columbia.edu

Dr. Ryan Watts
Genentech, Inc.
rwatts@gene.com

Dr. Daniel Wilhelms
Linkoping University
daniel.wilhelms@liu.se

Dr. Colin Willis
University of New England
cwillis1@une.edu

Dr. Matthew Wood
University of Oxford
matthew.wood@dpag.ox.ac.uk

Dr. Jenny Xie
Bristol Myers Squibb
jenny.xie@bms.com

Dr. Yao Yao
The Rockefeller University
yyao@rockefeller.edu

Dr. Xiang Yi
University of North Carolina Chapel Hill
xiang_yi@unc.edu

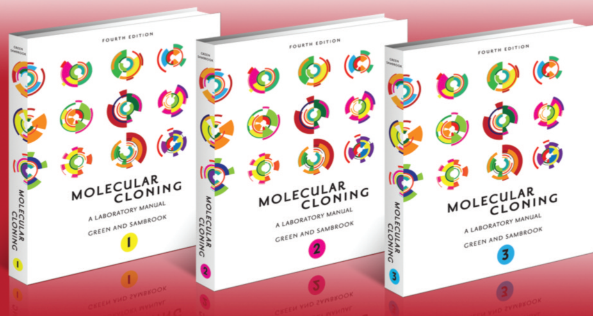
Prof. Robert Yokel
University of Kentucky
ryokel@email.uky.edu

Dr. Joy Yu
Genentech
yu.joy@gene.com

Ms. Nicole Ziegler
Goethe University clinic
nicole.ziegler@kgu.de

Now Available!

MOLECULAR CLONING 4



By **Michael R. Green,** *Howard Hughes Medical Institute, University of Massachusetts Medical School* and **Joseph Sambrook,** *Peter MacCallum Cancer Institute, Melbourne, Australia*

Molecular Cloning: A Laboratory Manual has always been the one indispensable molecular biology laboratory manual for protocols and techniques. The fourth edition of this classic manual preserves the detail and clarity of previous editions as well as the theoretical and historical underpinnings of the techniques presented. Ten original core chapters reflect developments and innovation in standard techniques and introduce new cutting-edge protocols. Twelve entirely new chapters are devoted to the most exciting current research strategies, including epigenetic analysis, RNA interference, genome sequencing, and bioinformatics. This manual is essential for both the inexperienced and the advanced user.

2012 2,028 pp., illus., appendices, index
Cloth (three-volume set) $475 — **ISBN 978-1-936113-41-5**
Paperback (three-volume set) $365 — **ISBN 978-1-936113-42-2**

Contents

VOLUME 1

Part 1 Essentials

1. Isolation and Quantification of DNA
2. Analysis of DNA
3. Cloning and Transformation with Plasmid Vectors
4. Gateway Recombinational Cloning
5. Working with Bacterial Artificial Chromosomes and Other High-Capacity Vectors
6. Extraction, Purification, and Analysis of RNA from Eukaryotic Cells
7. Polymerase Chain Reaction
8. Bioinformatics

VOLUME 2

Part 2 Analysis and Manipulation of DNA and RNA

9. Quantification of DNA and RNA by Real-Time Polymerase Chain Reaction
10. Nucleic Acid Platform Technologies
11. DNA Sequencing
12. Analysis of DNA Methylation in Mammalian Cells
13. Preparation of Labeled DNA, RNA, and Oligonucleotide Probes
14. Methods for In Vitro Mutagenesis

Part 3 Introducing Genes into Cells

15. Introducing Genes into Cultured Mammalian Cells
16. Introducing Genes into Mammalian Cells: Viral Vectors

VOLUME 3

Part 4 Gene Expression

17. Analysis of Gene Regulation Using Reporter Systems
18. RNA Interference and Small RNA Analysis
19. Expressing Cloned Genes for Protein Production, Purification, and Analysis

Part 5 Interaction Analysis

20. Cross-Linking Technologies for Analysis of Chromatin Structure and Function
21. Mapping of In Vivo RNA-Binding Sites by UV-Cross-Linking Immunoprecipitation (CLIP)
22. Gateway-Compatible Yeast One-Hybrid and Two-Hybrid Assays

Appendices

1. Reagents and Buffers
2. Commonly Used Techniques
3. Detection Systems
4. General Safety and Hazardous Material

Index

WWW.CSHLPRESS.ORG

VISITOR INFORMATION

EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **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 62575 (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 Center	x 5170

New York City

Helpful tip -
Take Syosset Taxi to Syosset Train Station ($9.00 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**)
To head west of CSHL - Syosset train station	
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