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ITMO Neurosciences, Sciences Cognitives, Neurologie, Psychiatrie

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Workshop

The neurovascular unit in health and disease

May 26th 2015

Abstracts

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Workshop : The neurovascular unit in health and disease

26 mai 2015

Espaces Diderot
10, rue Traversière PARIS 12e

ATELIER : THE NEUROVASCULAR UNIT IN HEALTH AND DISEASE

- 9h-9h15 — Introduction
  Étienne Hirsch, Bernard Poulain, Élisabeth Tournier-Lasserve, Denis Vivien
- 9h15-11h15 — **SESSION I - Regulation of Brain Endothelial homeostasis**
  - 9h15-10h15 Pericytes and the NVU, **Annika Armulik-Keller** (Switzerland)
  - 10h15 -11h15 Endothelial TAK1 and NEMO safeguard the neurovascular unit, **Markus Schwaninger** (Germany)
- 11h15-11h30 — Coffee break
- 11h30-12h30 — **SESSION II - Neurovascular coupling**
  - 11h00-12h30 Neurovascular coupling, **Serge Charpak** (Paris)
- 12h30-14h00 — Lunch
- 14h00-16h15 — **SESSION III - Translational NVU**
  - 14h00-14h30 *NVU et infections bactériennes*, **Sandrine Bourdoulous** (Paris)
  - 14h30-15h00 *NVU et maladies cérébrovasculaires*, **Anne Joutel** (Paris)
  - 15h00-15h30 Spatiotemporal characterization of blood-spinal cord barrier perturbations in animal models of multiple sclerosis, **Fabian Docagne** (Cyceron)

- 15h30-16h00 Physiologically based pharmacokinetic modeling at the level of BBB/NVU, **Kathryn Ball** (Orléans)

- 16h00-16h15 Discussion
Pierre-Olivier Couraud (Paris)

- 16h15-17h15 Round table
Étienne Hirsch, Bernard Poulain, Élisabeth Tournier-Lasserve, Denis Vivien et les intervenants de la journée

- 17h15-18h00 Cocktail

FINANCEMENT DE PROJETS

Afin de favoriser les rencontres transdisciplinaires et translationnelles, l'Institut Thématique Multi-Organisme (ITMO) Neurosciences, Sciences Cognitives, Neurologie, Psychiatrie organise le mardi 26 Mai 2015 un atelier sur la thématique **« The neurovascular unit in health and disease ».**

La thématique de cet atelier s'inscrit dans les **priorités stratégiques** de l'ITMO Neurosciences, Sciences Cognitives, Neurologie, Psychiatrie qui ont été définies dans sa contribution au document de **Stratégie Nationale de Recherche** que vous pouvez consulter sur notre site web. En Europe, avec 800 Milliards d'€/an, les maladies du cerveau et du système nerveux représentent le premier poste de dépense de santé toutes pathologies confondues. Les enjeux médicaux sont donc immenses et en particulier dans la thématique de cet atelier puisque les conséquences des accidents vasculaires cérébraux constituent la première cause de handicap acquis de l'adulte et la deuxième cause de démence.

Cet atelier « The neurovascular unit in health and disease » réunira une communauté de praticiens et de chercheurs engagés dans des travaux de recherche cliniques et biologiques de ce domaine à l'interface de deux disciplines majeures, la recherche cardiovasculaire et la recherche en neurosciences. Au travers des présentations scientifiques, les communications/interactions entre circulation sanguine et système nerveux central via l'unité neurovasculaire illustreront l'impact de ce système sur la santé et les maladies. L'enjeu de cet atelier est de dégager des axes de recherche innovants et de nouvelles pistes thérapeutiques.

Pour initier cette démarche, l'**ITMO Neurosciences Sciences Cognitives, Neurologie, Psychiatrie** propose de financer soit **deux projets de recherche** interdisciplinaire/translationnelle d'un montant de 15 000 euros chacun soit un projet de structuration de la communauté **« Neurovasculaire».** La décision du type de financement sera prise lors de la table ronde qui clôturera cet atelier.

Si l'option financement de projet est adoptée, vous trouverez les conditions et les renseignements pratiques pour répondre à cet appel à financement de projet sur le site de l'ITMO au lien suivant à partir du 26 juin 2015 : https://itneuro.aviesan.fr/index.php?pagendx=866

M. Etienne Hirsch & M. Bernard Poulain

Co-directeurs de l'ITMO Neurosciences, Sciences Cognitives, Neurologie, Psychiatrie

SESSION 1

REGULATION OF BRAIN ENDOTHELIAL HOMEOSTASIS

PERICYTES AND THE NEUROVASCULAR UNIT

ANNIKA ARMULIK-KELLER

Division of Neurosurgery, Zürich University Hospital, Zürich, Switzerland

Arterial calcification is common in aging and often occurs in patients with chronic kidney disease or type II diabetes. In addition, primary arterial calcification is associated with several human genetic diseases. Soft tissue calcification is caused by an imbalance between anti- and pro-mineralization signals, yet the molecular mechanism underlying ectopic mineralization is largely not understood. Interestingly, capillary calcification in the CNS can lead to neurodegeneration (e.g., primary familial basal ganglia calcification) or is associated with neurodegenerative diseases (e.g., Parkinsonism, Alzheimer's).

The CNS vasculature differs from that in other organs. It is largely impermeable to blood-born molecules due to specific characteristics of endothelial cells and is referred to as the BBB. The BBB is a collective term for several brain endothelial cell characteristics that render these cells impermeable to blood-borne molecules (i.e., closed endothelial cell-cell junctions, low rate of vesicular transport, expression of ATP-binding cassette efflux transporters), but allows the entry of essential nutrients via facilitated influx (SLC transporters). The entire abluminal surface of the endothelium is covered by astrocyte end-feet, which are specialized structures that regulate water and potassium homeostasis. In fact, CNS vasculature has the highest longitudinal pericyte coverage, which reaches almost 100%, meaning that all endothelial cells and astrocyte end-feet have a cellular surface faced by a pericyte. In addition, blood vessels interface with nerve-endings and microglial processes. The term neurovascular unit (NVU) is used to describe the spatial orientation of neurons, astrocytes, and brain vascular cells that underlie the integrated control of neural tissue and blood vessels in brain homeostasis and function. While the importance of the NVU as a modulator of brain homeostasis is still emerging as an accepted concept, there is only limited understanding of the interactions at the molecular level between the acellular and cellular components of the NVU in healthy brain, and the mechanisms underlying deregulation in the diseased state. Since pericytes are strategically positioned between endothelial cells and astrocytes, they may coordinate signalling at the NVU. We have recently demonstrated that pericytes control brain endothelial permeability at the level of transcytosis. Furthermore, pericytes regulate astrocyte end-feet polarization and the localization of the principal brain water channel Aqp4. Pericyte-deficent animals, generated by modifying PDGF-B/PDGFR-□ signalling axis develop age-dependent small vessel calcifications in deep brain regions. Thus, pericyte-deficiency and BBB-impairment correlate with the severity of calcifications . We have also shown that endothelial expression of PDGF-B is protective against vessel calcification. Accordingly, these data support the role of endothelial PDGF-B and pericytes in small vessel calcification.

Loss-of-function mutations in PDGF-B and PDGFR-□ have recently been associated with a human disease – primary familial basal ganglia calcification (PFGC). PFGC is a rare neurodegenerative disease with a dominant inheritance, characterized by calcifications in basal ganglia. Neurological symptoms include parkinsonism, migrane, pschychosis. Our current research has been directed towards understanding how diminished PDGFB/PDGFR□ signalling in pericytes causes small vessel calcification. We also investigated the role of enviromental factors (e.g. oxidative stress) and other cell types as modifiers of small vessel calcification at the NVU (e.g. microglia). Although PFGC is a rare disease, small vessel calcification in the CNS is very common and is associated with numerous brain pathologies with various etiologies (infections, metabolic, vasculopathies, tumors, toxic injury).

ENDOTHELIAL TAK1 AND NEMO SAFEGUARD THE NEUROVASCULAR UNIT

MARKUS SCHWANINGER

Markus Schwaninger, Germany

Inactivating mutations of NEMO, a key component of NF-κB signaling, cause the genetic disease incontinentia pigmenti (IP). Patients frequently suffer from neurological manifestations, such as acute encephalopathy, epileptic seizures, mental retardation and others. However, the mechanisms underlying brain involvement were unclear. To uncover the basis of CNS involvement in this disease we investigated Nemo-/+ mice, an animal model of IP. In these animals we found evidence for endothelial cell death in the brain. Capillaries degenerated to so-called string vessels that consisted only of basement membrane strands without inside endothelial cells. A vascular pathology in this disease was confirmed by the cell-specific deletion of Nemo in brain endothelial cells (NemobeKO) that mimicked the neurological phenotype of Nemo-/+ mice. Deleting Nemo or the upstream kinase Tak1 selectively in brain endothelial cells of mice also resulted in a disrupted blood-brain barrier (BBB) and epileptic seizures. Further experiments indicated that TAK1 and NEMO protect the BBB by activating the transcription factor NF-κB, which stabilizes the tight junction protein occludin, but they prevent brain endothelial cell death independent of NF-κB by reducing oxidative damage. Overall, the data identify crucial functions of inflammatory TAK1-NEMO signaling in protecting the BBB and maintaining normal brain function. Disruption of this pathway in brain endothelium explains the neurological symptoms of IP.

SESSION 2

NEUROVASCULAR COUPLING

NEUROVASCULAR COUPLING

SERGE CHARPAK

Paris

SESSION 3

TRANSLATIONAL NVU

THE BLOOD BRAIN BARRIER:
FROM BACTERIAL MENINGITIS TO DRUG DELIVERY INTO THE BRAIN

SANDRINE BOURDOULOUS

Paris

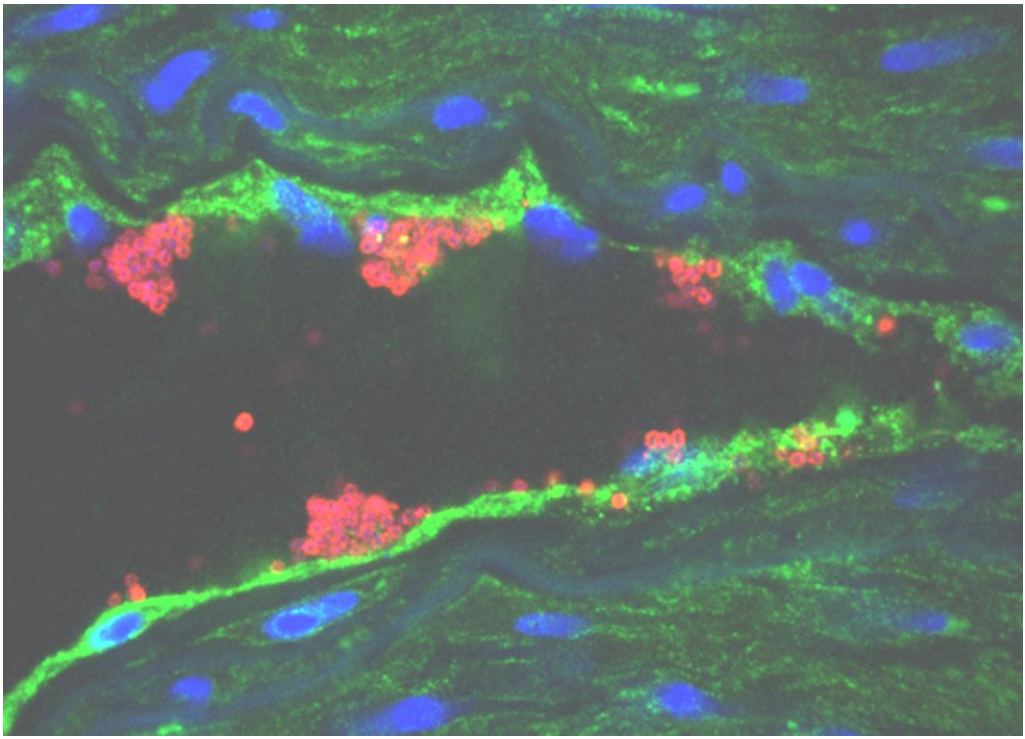
The Blood-brain barrier (BBB) is a complex biological system ensuring the proper function of the brain. As a result of its restricted permeability, only 2% of therapeutic compounds in clinical use get access to the brain. Although advances in deciphering properties of the neurovascular unit components have been made in the past years, our lack of suitable models of human BBB clearly contribute to the major difficulties currently encountered in designing efficient drug-based treatments in brain diseases, including some with a paramount socio-economic impact (stroke, Alzheimer's disease, epilepsy, multiple sclerosis).

Among the microorganisms that are pathogenic to humans and capable of invading the bloodstream, only a few have gained the ability to colonize human brain tissues after crossing the BBB. Among those Neisseria meningitidis (meningococcus) is the one that achieves this task most frequently and efficiently. This bacterium has selected efficient strategies to modulate and subvert fundamental properties of the BBB to colonize the brain with a minimal loss of BBB integrity. As such, this microorganism represents a powerful tool to probe for complex pathways that are involved in the control of BBB homeostasis, and to identify novel strategies for the delivery of therapeutic compounds to the brain.

In collaboration with the group of Pr Xavier Nassif (INEM, Paris), we recently precise some of the key events of meningococcal interaction with brain endothelial cells leading to bacterial crossing of the BBB. Taking advantage of a unique human brain endothelial cell line (hCMEC/D3) engineered in our laboratory, that recapitulate most of the unique properties of the brain endothelium [Patent WO/2006/056879, now distributed in more than 200 laboratories worldwide and under license with several pharmaceutical companies] and in situ meningococcal infection model of fresh human front brain tissues, we recently highlighted that CD147 is a critical host receptor for the initial adhesion of N. meningitidis to human brain endothelial cells, through a direct interaction with two meningococcal ligands contained within their type IV pili (Bernard et al, Nature Medicine, 2014). Furthermore, the expression of the G-protein-coupled β2-adrenergic receptor (β2AR) is essential for menincococcal type IV pili to trigger endothelial cell signalling events (Coureuil et al, Cell 2010). The activation of β2-AR/ βarrestin pathway by N. meningitidis initiates signalling events leading to the remodelling of the brain endothelial intercellular junctions allowing the bacterial crossing of the BBB by potentially both a transcellular and a paracellular route (Mikaty et al, PloS Path 2009; Coureuil et al, Science, 2009).

Our current objective is to translate this knowledge to novel models and approaches for the delivery of large molecular drugs across the BBB into the brain, based on a molecular mimicry of the mechanism used by this bacterial pathogen.

Illustration: In situ infection of fresh human brain section by N. meningitidis. CD147 (green), meningococcus colonies (red) and nucleus (blue) (Bernard et al, Nature Med 2014).



EXCESS TIMP3 MEDIATES CEREBROVASCULAR DYSFUNCTION IN A MENDELIAN PARADIGM OF SMALL VESSEL DISEASE OF THE BRAIN

ANNE JOUTEL

Carmen Capone1,2, Céline Baron-Menguy1,2, Fabrice Dabertrand3, Athena Chalaris4, Valérie Domenga-Denier1,2, Heidi Stöhr5, Stefan Rose-John4 , Mark Nelson3, Anne Joutel1,2

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CADASIL is a genetic paradigm of small vessel disease of the brain caused by dominant mutations in the NOTCH3 receptor. Using TgNotch3R169C mice, a well-established preclinical model of CADASIL, we previously demonstrated that cerebrovascular regulation is impaired early during disease progression. Abnormal deposition of NOTCH3 ectodomain (Notch3ECD) around vessels is suspected to be a key pathogenic factor in this disease, however, the mechanisms linking Notch3ECD deposition and cerebrovascular dysfunction have not been identified. We recently reported that Notch3ECD accumulation results in increased TIMP3 levels in the brain vessels of patients and mice with CADASIL. Here, we show that transgenic TIMP3 overexpression mimics Notch3R169C-induced cerebrovascular defects. Topical application of TIMP3 onto the neocortex produced a similar effect and also impaired myogenic responses of isolated pressurized pial arteries. Conversely, a haploinsufficiency of Timp3 (Timp3+/-) in TgNotch3R169C mice normalized in vivo cerebrovascular responses and ex vivo myogenic responses, despite unchanged Notch3ECD deposition. Mice with genetic reduction or pharmacological inhibition of tumor necrosis factor-α–converting enzyme (TACE), a specific target of TIMP3, have strongly attenuated CBF responses and arterial tone. Conversely, topical application of TACE restored cerebrovascular function and myogenic responses in TgNotch3R169C mice.
We conclude that Notch3ECD accumulation triggers a cascade that leads to reduced arterial tone and cerebrovascular dysfunction via increased TIMP3 repression of TACE.

SPATIOTEMPORAL CHARACTERIZATION OF BLOOD-SPINAL CORD BARRIER PERTURBATIONS IN ANIMAL MODELS OF MULTIPLE SCLEROSIS

FABIAN DOCAGNE

(Cyceron)

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING AT THE LEVEL OF THE BBB/NVU

KATHRYN BALL

(Orléans)

It is notoriously difficult to predict the rate and extent of the penetration of drugs across the blood-brain barrier (BBB) and into the central nervous system (CNS). Physiologically based pharmacokinetic modeling (PBPK) is being used more and more frequently within the pharmaceutical industry to predict the pharmacokinetics in various tissues of the body of drugs under development. Key components of PBPK models are their anatomically realistic model structures, which allow the use of known physiological values as species-specific model input parameters, and facilitate inter-species scaling. Furthermore, the integration of in vitro data as drug-specific input parameters within the model reduces the need to obtain in vivo data in impractical or inaccessible areas such as the human brain. In vitro systems such as brain microvessel endothelial cells can be used to measure BBB permeability, and brain slices or brain tissue homogenate to measure distribution and binding within the brain. These in vitro systems are therefore prime candidates for which to obtain input parameters for PBPK models of the CNS. It is very important to test the in vitro-in vivo extrapolation (IVIVE) by using in vivo methods such as brain microdialysis in the rat, which provides the unbound drug concentrations within the brain extracellular fluid and the blood. A good IVIVE in preclinical PBPK models gives confidence in the subsequent human PBPK model predictions. The potential applications of CNS-PBPK modeling are exciting. The physiological parameters of PBPK models can be adapted to enable simulations in different populations or disease states, and pharmacodynamic measures of efficacy or toxicity can be integrated into the model to enable PK/PD modeling using the free drug concentrations at the target site as the PK input.