Master de Sciences et Technologies
Mention Biologie Intégrative et Physiologie
Parcours : Neurosciences
Responsable : Professeur Régis Lambert

Internship Proposal
Academic Year 2019-2020

1. Host team :

Research Unit (e.g. Department or Institute) : Centre de recherche saint-antoine
Research Unit Director : Bruno Fève
Research Team Director : Guillaume Dorothée
Team name : Système immunitaire et neuroinflammation

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2. Internship project title:

Involvement of neutrophils in the pathophysiology of epilepsy

3. Internship Description :

Epilepsy is characterized by neuronal death, synaptic plasticity, hyperexcitabilty of neurons as well as a strong and permanent neuroinflammation which is known to contribute to neuronal hyperexcitability in both human patients and animal models. Importantly, this inflammatory environment could be also acquired from systemic inflammation via the migration of peripheral leucocytes through the blood brain barrier disruption (BBB). The presence of aberrant angiogenesis and a leaky blood-brain barrier (BBB) was observed in human epileptic brain and in animal models. The BBB controls the exchange between systemic circulation and the brain maintaining the homeostasis. Disruption of BBB is now admitted as an epilegonic conditions (Marchi N et al, 2011)

Innate immune system associated to macrophages or monocytes is found presently and highly activated in human and rodent model and could exacerbate neuronal damage in rodent model. Neutrophils (PMNs) constitute also key cells of the innate immune system. PMNs act as a first line of host defense and kill pathogens by various strategies, including phagocytosis, degranulation, rapid production of reactive oxygen species (ROS) in oxidative burst, and release of neutrophil extracellular traps (NETs), a process called NETosis. In rodent model, PMN infiltration in parenchyma is observed rapidly after seizures(Kim Je et al, 2012) and depletion of granulocytes reduced the severity of seizures (Fabene PF et al, 2008). In addition, we recently found that IL-8, a strong chemoattractant and activator of PMNs, is released by activated microglia 4 hours after epileptic seizures (Morin-Brureau M et al, 2018) induced on human slices. PMNs are able to modulate the BBB permeability (Joice SL et al 2009) and their depletion reduces the BBB breakdown in model of hemorrhage (Moxom-Emre I, 2011)

Despite some evidence of their possible impact on neuropathology as stroke or Alzheimer Disease (Dong Y et al, 2018) and their impact on BBB permeability, an epileptogenic condition, little attention has been focused on the

role of PMNs in epilepsy. **The project will be i) to characterize the infiltration of PMNs in brain of epileptic patient; ii) activation of PMNs in blood of epileptic patient iii) Impact of neutrophil on BBB integrity**

i) Infiltration of PMNs in the epileptic brain will be assessed by immunohistochemistry on tissue providing of the epilepsy surgery. PMNs will be identified using CD15 marker. In parallel adhesion molecules a integrin and selectin responsible of cells migration across blood vessels will be studied.

ii) We will analyze the major steps of neutrophil functional activity as i) adhesion molecules to endothelial cells (VLA-4, LFA-1, CD11b, CD62L); ii) Distribution of hyperactive senescent subset of PMNs (CXCR4) or immunosuppressive subset (CD16 bright /CD62L dim) iii) PMNs function as oxidative burst, survival, degranulation of proteolytic enzymes such as MMP9 and NETosis. This study will be performed in whole-blood conditions by flow cytometry

iii) Contributions of circulating PMNs on BBB alterations will be assessed using in vitro model of BBB: monolayer of hCMEC/D3. PMNs will be isolated from control and epileptic patient as already described (Dong Y et al, 2018) and immediately added to HCMEC/D3 culture (Cowan KM et al 2010; Joice Sl et al 2009). We will evaluate by immunocytochemistry i) endothelial cells activation (VCAM-1, ICAM-1 or selectin) ii) BBB integrity via the expression as and tight (ZO-1, Occludin, Claudin-5) and adherent junction (VE-cadherin). HCMEC/D3 will be seeded on transwell to evaluate i) BBB permeability using FITC-Dextran (4kDa) or Lucifer Yellow (400Da) ii) Migration of PMNs across the BBB.