project title: MOLECULAR BASIS OF THE BLOOD-BRAIN BARRIER FUNCTION UNDER INFLAMMATORY CONDITIONS

PROJECT SUMMARY

By forming a single cell layer lining the blood vessels of the brain, cerebral endothelial cells (CECs) constitute the principal component of the blood-brain barrier (BBB). Tight junctions and adherens junctions play a key role in the maintenance of the barrier function. Despite considerable experimental efforts, regulation of cerebral interendothelial junctions in pathological conditions is less well understood. Using an in vitro model of the BBB and molecular, biochemical and immunofluorescent techniques changes in the expression, localization, interaction and posttranslational modifications of the junctional proteins will be investigated in CECs in response to inflammatory stimuli.

BACKGROUND OF THE STUDY

CECs fulfill several important functions in the central nervous system (CNS). By forming a single-cell layer lining the blood vessels of the brain they constitute the principal component of the blood-brain barrier (BBB). They form an active interface between blood and neural tissue and play a key role in the maintenance of the homeostasis of central nervous system (CNS). Pathological conditions of the brain, including cerebral ischemia, brain tumors, trauma or neurodegenerative disorders, can often lead to an increased BBB permeability which may severely influence the outcome of the disease. Inflammatory processes are often associated to the above mentioned CNS disorders and these can significantly contribute to the increase in BBB permeability, which could further disturb the homeostasis of the CNS with severe consequences. The innate immune system plays an important role in inflammatory processes, because activation of pattern recognition receptors leads to the production of inflammatory mediators. Sensing of infectious agents or different other – potentially dangerous – molecular structures by the innate immunity relies on a limited number of germline encoded receptors, known as pattern recognition receptors (PRRs). Pattern recognition receptors consist of at least four major families. Members of the Toll-like receptor (TLR) and the C-type lectin receptor (CLR) families can recognize extracellular and intracellular pathogen associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs) as well, while the retinoid acid-inducible gene-1 (RIG-1)-like receptors (RLRs) and the NOD-like receptors (NLRs) detect intracellular patterns. Ligand recognition by several members of the NLR family leads to the activation of a multiprotein complex, the inflammasome. The most important NLRs in this respect are NLRP1, NLRP3, and NLRC4. In addition, AIM2 (absent in melanoma 2) can also be part of inflammasomes. Activated inflammasomes cleave and thus activate interleukin IL-1 beta and IL-18, which are the effector components of the activated inflammasomes. Pattern recognition receptors are mainly expressed on macrophages, neutrophils, dendritic cells and
other innate immune cells. However, there is increasing evidence that these receptors are expressed in non-immune cells as well, including epithelial and endothelial cells with yet largely uncharacterized role.

RELEVANT RESEARCH IN THE HOST LABORATORY

CECs are in the front of the defense line of the CNS. We have recently revealed that CECs are able to express a large number of pattern recognition receptors - including Toll-like and NOD-like receptors - which are key components of the innate immune system. We have shown that expression of Toll-like receptors is regulated by oxidative stress and inflammatory stimuli as well. Furthermore, we have shown that activation of TLR2/6 leads to an increased permeability of the BBB which is accompanied by a downregulation of occludin and claudin-5 expression and disappearance of these tight junction proteins from the cell membrane. Changes in occludin expression and localization could be inhibited by the ERK1/2 inhibitor U0126. Recently we detected the expression of several NOD-like receptors in CECs as well of which NLRP3 was significantly induced by inflammatory stimuli. Our results suggest a significant role of the cerebral endothelium in mediation of the neural effects of different inflammatory processes. Currently we are investigating the role of endothelial NOD-like receptors in inflammatory processes of the brain.

SPECIFIC AIMS

Our investigations will be focused 1) on the regulation of NOD-like receptors and inflammasome components in CECs and pericytes in response to inflammatory stimuli and oxidative stress and 2) on the elucidation of the effect of NOD-like receptor activation on the barrier functions of the BBB. Furthermore, 3) signalling pathways leading to possible junctional damage and permeability increase will be investigated as well.

MATERIAL AND METHODS

Both in vitro and in vivo model systems will be necessary to be applied. In in vitro experimental setups besides cerebral endothelial cell and pericyte cultures in Petri dishes we will use an in vitro model of the barrier. This is based on the culture of cerebral endothelial cells on filter inserts with different pore size in coculture with pericytes on the bottom side of the filter allowing a direct contact between the two cell types. In addition, in order that the model contains all the major cellular constituents of the NVU, astrocytes are cultured on the bottom of the wells or alternatively astrocyte conditioned medium can be used. The in vitro model of the NVU is a well established technique in our laboratory. The model, besides mimicking very closely the in vivo situation, allows the investigation of the role of individual cellular components and makes possible different biochemical and molecular biological investigations as well. Both primary mouse cerebral endothelial cells and a human endothelial cell line will be used. Similarly, we will use primary mouse pericytes isolated according to a protocol adapted by our laboratory and well characterized human pericytes will also be used. In addition basic molecular and biochemical techniques will be applied for the study of junctional protein expression and immunofluorescent techniques for their subcellular localization. Signaling pathways will be investigated using antibody arrays with antibodies which recognize phosphorylated and thus activated proteins of key signalling pathways. Barrier characteristics will be monitored using measurements of
transendothelial electrical resistance (TEER). The role of individual inflammasome components will be investigated using gene silencing.

In vivo experiments will be based on two photon microscopy. We will use intravital microscopy via cranial window to allow the direct observation of cerebral vascular changes in real-time.

SUGGESTED READINGS


SNAPSHOTS FROM THE HOST LABORATORY

Significant publications


Representative recent research grants


“The role of the interaction between metastatic cells and brain endothelium in the development of brain metastases” (OTKA, 2012-2015)

Some of the latest students in the laboratory

Fazakas C, Ph.D., 2008-2014, “Role of the BBB in brain metastasis formation”

Haskó J, Ph.D., 2010-present, “Role of CB2 receptors in cellular adhesion and transmigration through the BBB”

Nyúl-Tóth A, Ph.D., 2011-present, “Role of NOD-like receptors in the regulation of the BBB”

Molnár J, Ph.D., 2011-present, “Signaling pathways involved in the transmigration of metastatic cells through the BBB”

Kozma M M.Sc., 2014-present, “Expression of NOD-like receptors in cerebral endothelial cells”