The impact of prenatal inflammatory priming on the neuron-microglia proteines expression in young offspring brain: immunohistochemical study in neurodevelopmental model of schizophrenia

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Introduction: A number of studies suggest that in brain microglia are held in a surveillant and quiescent state of activation through several inhibitory signaling dyads. Recently the fractalkine (CX3CL1) and its receptor (CX3CR1) as well as CD200 and CD200 receptor (CD200R) are particularly noteworthy, because disruption of these networks extended the duration of pro-inflammatory response, mainly via malfunction of a unique communication system between neuron-microglia cells.

Objective: To explore the possibility that maternal inflammatory priming based on the bacterial endotoxin (lipopolisaccharide, LPS) administration might serve as a trigger to disturbances in CX3CL1-CX3CR1 and CD200-CD200R signaling in the brain of young offspring.

Material and Methods: Pregnant females were injected subcutaneously with LPS at a dose of 2 mg/kg every second day from the seventh day of pregnancy until the delivery. Control pregnant rats were left undisturbed in their homecages. At 7 days of age, control and prenatally LPS-treated rats were decapitaded and hippocampi and frontal cortices were dissected. Immunohistofluorescent staining was performed using primary antibodies: anti-CX3CL1, anti-OX2 and anti-CX3CR1 as well as anti-OX2R. Moreover proteins were co-stained with neuronal (anti-MAP2), astroglial (anti-GFAP) and microglial markers (anti-Iba1), to clearly demonstrate the co-localization of CX3CL1, CD200, CD200R and CX3CR1 with brain cells in selected areas of hippocampus and frontal cortex of young offspring.

Results: First we demonstrated the localization of CX3CL1 and CD200 mainly on neuronal cells, while CX3CR1 and CD200R on microglial cells in hippocampus and frontal cortex of young offspring. Analyzing both ligands localization we observed that

maternal LPS administration tender to increase the expression of neuronal CX3CL1 in hippocampus, while CD200 in both examined brain areas. On the other hand, maternal immune challenge in the opposite way affects the microglial expression of CX3CR1 and CD200R. Prenatal exposure to LPS have been found to increase the CX3CR1 expression in hippocampus regions, while decrease a staining of CD200R in hippocampus and also in the frontal cortex of young offspring.

Conclusions: These immunohistofluorescent data provide evidence that maternal exposure to immune challenges induce malfunction of neuron-microglia proteines staining in brain areas of young offspring. Thus phenomenon, termed prenatal neuroinflammatory priming, via disruption of homeostatic mechanisms regulating brain immune system, may be a key factor leading to the occurrence of schizophrenia-like behavioral deficits observed in adult animals in commonly accepted neurodevelopmental model of this disease.

Keywords: experimental model,rats, schizophrenia, brain microglia, receptors

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Therefore it is belived that fractalkine is responsabile for the regulation of proper microglial cell function and their activation

In the present study, using microglial cultures we investigated whether tianeptine modifies microglial activation after lipopolysaccharide (LPS) stimulation.

Our study shows that tianeptine attenuated the LPS-evoked inflammatory activation of microglia by decreasing the expression of pro-inflammatory cytokines such as IL-1β, IL-18, IL-6 and TNF-α as well as nitric oxide (NO) release and the expression of inducible nitric oxide synthase (iNOS). Analyses of signaling pathways demonstrate that tianeptine led to the suppression of LPS-induced ERK1/2 phosphorylation....(kaspaza).
Furthermore, this study demonstrates the inhibitory impact of this antidepressant on the degradation of IκB and the phosphorylation of p65/NF-κB in microglial cells.

Taken together, our results show anti-inflammatory properties of tianeptine in microglial cultures stimulated by LPS. This study provides evidence that the beneficial effect of tianeptine may be partially mediated by the suppression of microglial overactivation.

Several studies indicate that dysfunction of the brain immune system is a crucial factor in the pathogenesis of psychiatric disorders, including schziophrenia.

In the central nervous system, the primary component of the immune system and the first line of defense are the microglial cells. In the physiological state, the microglia exist in a ramified, “quiescent” form, but dynamically monitor their surroundings, controling brain homeostasis. However, in response to pathological stimuli, the microglia rapidly transform from “resting” cells to “activated“ ones. They undergo not only morphological changes, but also different biochemical shifts. Upon activation microglia up-regulate a number of surface proteins, (CD40, MHC II), cytokines (IL-18, IL-1β, TNF-α, and IL-6) and neurotoxic mediators, such as nitric oxide (NO), prostaglandins (PG) as well as superoxide anions (Hanisch et al, Marco and Prinz, 2013; Slusarczyk et al., 2015, 2016). Importantly these processes are crucial in return the brain back to the homeostasis. However, excessive or prolonged glial activation results in a more severe and chronic neuronal damage that eventually propagates neuroinflammation and neurodegeneration (Tian et al., 2012). Moreover studies postulate

(Zujovic et al., 2001; Corona et al., 2010; Slusarczyk et al., 2016).

Interestingly a link between changes in CX3CL1 signaling and behavioral changes, especially disturbances related to the animals’ responses after stress stimuli has been found. Evidence from CX3CR1 knock-out mice indicates that impairment of CX3CL1 signaling is expressed as memory deficits in adult animals (Roger et al. 2011). In CX3CR1-deficient mice, acute inflammatory stimuli amplified not only microglial activation and the release of toxic factors but also lead to prolonged depressive-like symptoms (Corona et al. 2013), as well as different resilience to stress (Milior et al. 2015).

Taking into account the mentioned above data it is plausible that the disturbances in the regulation of fractalkine-fractalkine receptor signaling may be a potential mechanism to counteract changes occuring in depressive disorders.

Aim:

During my short-term stay in the Neuroscience Center at the Univeristy of Helsinki, Finland at the research group under the supervision of Li Tian, Ph.D, involved in the studies of neuroimmune interaction in immune-related brain disorders, I would like to learn modern research techniques like isolation of microglial cells from adult rodents and flow cytometry analysis using various cell markers for identification both nervous as well as brain immune cells. What is more, during my stay in Helsinki I would like to learn the deep brain imaging/recording/manipulation technique such as two-photon microscopy, which allows monitor and activate individual brain cells.

Operational objectives and working plan:

Our previous findings (Slusarczyk et al., 2015) demonstrated changes in the morphology and activation of primary microglia cell obtained from 1-2-day old neonates in animal model of depression based on the prenatal stress procedure. Furthermore we described in the animal model of depression the prolonged inflammatory activation in frontal cortex and hippocampus, brain structures involved in pathogenesis of depression (Slusarczyk et al.), which pointed to the link between excessive microglia activation and depression. Importantly our results for the first time showed that the activation of microglial cells was accompanied by prolonged reduction of the level of fractalkine and its receptor in both examined brain areas. Interestingly, a single intracerebroventricular administration of exogenous fractalkine not only attenuated the pro-depressive and anxiety-like behavioral disturbances, but also has anti-inflammatory property expressed as normalization of enhanced neuroinflammatory status, mainly in the frontal cortex of adult animals.

Since, the exact mechanisms of fractalkine action in the brain, especially in terms of microglial cell function requires further investigation, I am convinced that my stay at the Li Tian, Ph.D laboratory give me an apportunity to extend my previous study and would allow me in great detail to explain the role of neuron-microglia crosstalk in the pathogenesis of depression.

In particular the knowledge of the microglia isolation technique from adult rats will help me to explain the role of malfunction of fractalkine signaling in neuroinflammation observed in adult animals in model of depression, as well as after my return to the Institute in Cracow will be useful in realization of two projects financially supported by National Science Center in Poland, in which I am a main performer.

Furthermore it will be especially important for me in terms of my interest and research to become familiar with flow cytometry, because labeling of microglia markers can explain whether where it is a link between the shift of microglia phenotype from M1 to M2 and fractalkine action in adult animals. Furthermore a new technologie - two-photon microscopy not available in the Institute of Pharmacology PAS, while widely used in the Neuroscience Center at the University of Helsinki would bring me to answer the question about the impact of fractalkine released by neurons on the morphology of microglia and fractalkine receptor expression in adult animals, and thuse will be crucial for my future studies.

Summary:

I would like to emphasize that a short-term fellowship at the Univeristy of Helsinki, Neuroscience Center in Helsinki is in my opinion relevant to my professional expertise, therefore, I would highly appreciate to be given an opportunity to obtain the scholarship I apply for, which is for me an exceptional chance for fruitful exchange of experience. I perceive this as an occasion to familiarize with good practices as a channel for my personal development. This fellowship funded by EFIS-IL will greatly help me to acquire knowledge of new experimental techniques which would much enrich my research and establish scientific collaboration in the field of neuro-immune interactions.

SMIECI

In microglial or mixed glial cultures treatment with soluble CX3CL1 down-regulates lipopolysaccharide (LPS)-induced production of cytokines (Zujovic et al. 2000, Mizuno et al. 2003). Furthermore, in an *in vivo* study, pretreatment with neutralizing anti-CX3CR1 antibody was shown to potentiate TNF-α and 8-isoprostane production after intracerebroventicular (icv) injection of LPS (Zujovic et al. 2001), whereas in animals lacking CX3CR1 up-regulated IL-1β expression in the brain after repeated systemic administration of LPS were found (Corona et al. 2010).

For decades, mechanistic studies on brain circuits had to be performed in reduced preparations: acute brain slices, organotypic slice cultures, dissociated neurons and glial cells. While these *in vitro* and *ex vivo* approaches may greatly simplify the research and yield a wealth of valuable data, they may also introduce a variety of artifacts because brain cells behave very differently in the intact brain as compared to the reduced preparations.

With recent advances in, multichannel electrophysiology and optogenetic stimulation), it has finally become possible to, and even individual synapses, within the brain of anesthetized rodents. Furthermore, since anesthesia is known to distort the brain function, the most recent trend is to maximize the relevance of *in vivo* research by performing imaging and recording without anesthesia, i.e. in awake and behaving mice. In order to provide access to these advanced techniques for its research groups, the Neuroscience Center has established in 2013 a new facility - In Vivo Microscopy Unit (IVMU).
The IVM Unit has a chief purpose of enabling advanced research of NC groups by providing expertise in the methodologies of *in vivo* microscopy (primarily, two-photon microscopic imaging), animal surgery and data analysis. The Unit is organized as a collaborative core facility, whereby the IVMU personnel provides consultancy and training to NC personnel and closely supervises their *in vivo* microscopy experiments. The Unit is coordinated by a part-time Principal Investigator (Leonard Khiroug) and is staffed with a full-time Animal Surgery Technician (Marina Tibeikina) and a part-time Postdoctoral Researcher (Evgeny Pryazhnikov). Key equipment of the IVMU consists of a two-photon microscopy setup, an animal surgery setup, rodent housing rigs (Scantainers) and supporting equipment for standard lab procedures (biochemistry and molecular biology).

Neuroimmune interaction in immune-related brain disorders

Parallel advances in neuroscience and immunology highlight the importance of bidirectional interactions between the nervous and immune systems in maintenance of the physiological homeostasis of the brain and mind, as well as to control immune functions. Abnormal proinflammatory activation of innate and adaptive immune cells may be detrimental for the brain development and functions and propagate the onset or progression of mental disorders and neurological diseases. Vice versa, mood disorders and abnormal development of the nervous system may also affect immune cell development and functions, which jeopardizes defensive and restorative capabilities of a host towards bodily diseases such as infections, cancers, and autoimmune diseases.

The research of my group follows two major lines. Our bottom-up research line focuses on the role of immune activation in controlling the brain development, behaviors and neuropsychiatric diseases. Our top-down research line focuses on the role of the nervous

system in regulation of immune cell development and activation, e.g. the importance of mind-body connection in somatic diseases, such as inflammation, autoimmune disease, and neoplasia. We study both human patients and rodent disease models and use cutting-edge genetic and laboratorial approaches to holistically evaluate the brain-immune crosstalk. Our current major focuses are:

1. The role of peripheral and central immune activation in the brain development and behaviors.
2. Development and activation of innate and adaptive immune cells in the primary and secondary lymphoid organs regulated by the autonomic nervous system.

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Teaching activity: We organize the course **"Immunity in the central nervous system and its role in neuropsychiatric and neurological diseases"** in the University of Helsinki (3 ECT; Syllabus: CellImmCNS: www.helisci.fi/fgsn-courses/immunology). The course is selected and financed as a FENS-IBRO training course for international PhD students in 2016