Final report on the project entitled: "Microglia activation and neuroinflammation in chronic stress-induced depression"

In human, chronic stress is a major environmental factor which precipitates depression in genetically and epigenetically vulnerable patients. In the framework of this OTKA project, we have established a mouse model compatible with the ***"two hits hypothesis" of depression*** by combining early life adversity (maternal separation, MS) with 3 weeks long chronic variable stress (CVS) paradigm in the adulthood. Using C57Bl6 mice, we found this paradigm repeatedly ***recapitulates some, but not all features of stress-induced depression***. For instance, decreased body weight (especially in the first weeks), enlargement of the adrenal glands (corresponding to increased hypophyseal drive), decreased sucrose consumption (sign of anhedonia) anxiety and passive coping strategy in behavior tests have been revealed. However, the normalized thymus weight (indicative of long term glucocorticoid load), hypothalamic and amygdala corticotropin-releasing hormone (CRH) expression was not significantly different in chronically stressed mice. Recently, increased inflammatory mediators that are frequently found in depressed human patients, favor a neuroinflammatory background of the disease. To assess the role of ***microglia*** (the resident macrophage-like cells in the CNS) in chronic stress-induced neuroinflammation, we used quantitative morphological assays (MATLAB application which has been developed in our laboratory) on Iba-1 immunostained sections. The number of Iba-1 positive microglial cells decreased in the hypothalamic paraventricular nucleus of mice exposed to MS+CVS. Signs of microglia activation (decreased process length and nearest neighbor distance) have not been significant. By contrast, morphological activation of microglia was reported in other brain areas, such as the prefrontal cortex and hippocampus, which have already been implicated in stress regulation. Because microglia is the major source of various cytokines, next, we measured expression of pro-and anti-inflammatory cytokines in these stress-related brain areas. In spite of morphological activation, we could not detect increased mRNA levels of IL-1a,IL-1b, TNFa, IL-6, MCP-1 levels in the hypothalamus, but IL-1b mRNA expression was significantly higher in the prefrontal cortex of chronically stressed animals.

The fractalkine-fractalkine receptor chemokine signaling has been implicated as a major regulator of microglia activity. Fractalkine is synthesized by neurons and acts locally when expressed in membrane bound form or recruits fractalkine receptor-expressing microglial cells or monocytes as a chemokine when released from the cell surface. To reveal the role of this signaling system in stress-induced microglia activation and behavioral changes we used mice lacking functional fractalkine receptor (CX3CR1) due to the genetic insertion of the fluorescent marker protein gene *gfp*.

When compared to the background C57Bl6 strain, CX3CR1-/- mice displayed ***active coping*** strategy in behavioral tests with significant stress component, exaggerated hormonal and activational (cFos) responses to acute stress. Analysis of metabolic parameters of control and fractalkine receptor deficient mice under basal and stress situation, we have concluded that active escape behavior of CX3CR1-/- animals might be related to their improved metabolic performance.

In the MS+CVS chronic stress paradigm CX3CR1-/- mice do not became anhedonic, suggesting critical involvement of neuron-microglia communication in stress-induced

depression. These results have been published in Behavior Brain Research (Winkler et al. BBR 334:119-128, 2017).

In addition to the CNS effects, we have found significant ***stress-induced changes at the periphery***. Chronic variable psychogenic stress combined with maternal separation resulted in changes in the ***gut microbiome***, which could be corrected by oligomannan prebiotics (Ferenczi et al. Sci Rep. 2016) or by non-absorbable antibiotic, rifaximine (Kuti et al. Eur Neuropsychopharm. 2017).

Based on the constitutive expression of green fluorescent protein (GFP) in monocytes of hetero- and homozygous CX3CR1 KO mice, we have developed a formula with which to estimate stress-induced recruitment of macrophages, monocytes and NK cells in various peripheral organs, such as liver, gut or adipose tissues. Chronic psychogenic (MS+ CVS) and metabolic (high fat diet, HFD) stress resulted in significant increase of normalized GFP mRNA in the ***liver*** as well as in white and brown ***adipose tissue***, raising the involvement of these sites in stress-induced immune responses.

Diet-induced obesity and related peripheral and central inflammation are major risk factors for metabolic,neurological and psychiatric diseases. Long term high fat dieting (HFD) as a ***chronic metabolic stress***, results in a significant weight gain which is accompanied by adipose- and liver inflammation in C57Bl6 animals. We have provided a critical involvement of the fractalkine-fractalkine receptor signaling in the development of ***HFD-induced systemic inflammation***, since CX3CR1-/-) mice gain significantly less weight on fat-enriched diet and have smaller amount of white adipose tissue (WAT) in the visceral compartment than heterozygote controls. Furthermore, Cx3CR1 gfp/gfp mice fed a fat-enriched diet do not develop glucose intolerance, recruit proportionally less number of gfp-positive cells and express significantly less proinflammatory cytokines in the WAT than control animals with fat-enriched diet induced obesity (Polyák et al, Brain Behavior and Immunity, 38:25-35, 2014). In addition to its pivotal role in thermogenesis, ***brown adipose tissue*** (BAT) has recently been implicated in the regulation of glucose tolerance and obesity. We were the first to reveal BAT inflammation in obese mice, which was accompanied by increased expression of proinflammatory cytokines and “whitening” of BAT. Fractalkine receptor-mediated recruitment of monocytes in the BAT during high fat dieting significantly affects genes involved in lipogenesis and lipolysis as well as in the thermogenesis (Polyák et al, BBA, 1861(11):1614-1622.
Intriguingly, in HFD mice, we found microglia morphological activation selectively in the ***arcuate nucleus/median eminence region***, the hypothalamic center implicated in food intake and energy metabolism. However, this microglia activation was not accompanied with upregulation of mRNA expression of typical proinflammatory cytokines.

We also found -for the first time- that insulin-induced ***hypoglycemia, an acute metabolic stressor*** also ***activates hypothalamic microglia***. This activation is specific for the arcuate nucleus and is not seen in any other hypothalamic region (such as the stress-related paraventricular nucleus) or any cortical or limbic structures involved in stress regulation. Activated microglia was revealed in the close proximity of hypoglycemia activated, c-Fos positive, NPY expressing neurons in the arcuate nucleus. Based on the physiological measurements it is likely that microglia activity inhibits the full expression of hypoglycemia induced counter-regulatory responses. Inhibition of microglia activity by minocycline, absence of neuron-to-microglia communication in fractalkine receptor deficient CX3CR1-/- mice, depletion of microglia by colony stimulating factor receptor 1 (CSF R1) antagonists

PLX 3397 and PLX 5622 and inhibition of proinflammatory polarization of microglia by ibudilast, an anti-inflammatory drug all alleviated hypoglycemia induced by insulin. Using IL-1a,b KO mice, IL-1 receptor antagonist, we also provided evidence favoring the involvement of microglial IL-1 as a confounding factor, which is responsible for the inhibition of hormonal and activational responses to hypoglycemia or neuroglycopenia.

Together, results obtained during this OTKA grant revealed novel role of microglia in the CNS and monocytes in the periphery in central and peripheral regulation of acute- and chronic-; psychological- physiological- and metabolic stressors.

PhD thesis in the framework of this OTKA project

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Diploma thesis in the framework of this OTKA project

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