Could the orphan receptor GPR18 modulate the spectacular plasticity of microglia?

Glial cells make up more than half of brain cells, among which microglial cells deserve special attention. Microglia are immune cells that are constantly present in the central nervous system (CNS) forming the first active immune barrier and are involved in processes of reparation of damaged tissue or removal of dying neurons. When infectious agents cross the brain-blood barrier or when there is an injury, this triggers microglia to undergo directed migration toward dead or dying neurons. This process is called microglia activation and is associated with the production and secretion of a variety of mediators such as cytokines, reactive oxygen species (ROS), or prostaglandins. Activated microglia are desirable in regulating tissue repair and recovery. However, excessive or chronic activation can cause harmful effects. Activation of microglia in response to inflammation is a hallmark of brain pathology. Microglia cells are activated in two different ways, leading to two distinct phenotypes. The first state, M1, is a classical activation of microglia that depicts a pro-inflammatory phenotype and is characterized by the release of pro-inflammatory cytokines. The second, M2, is an alternative activation of microglia that shows an anti-inflammatory phenotype and is associated with neurogenesis and anti-inflammatory effects. That is the explanation of why microglia can be neuroprotective or neurotoxic. Given the importance of microglia in neurodegenerative diseases, a new field of microglial therapeutics has recently emerged, ranging from pharmacological manipulation of existing microglia by switching their M1 and M2 status to inhibiting microglial activation. Therefore, modulation of microglia phenotypes seems to be an attractive research direction for the potential therapy of neuroinflammation.

The GPR18 receptor is an orphan receptor. Orphan receptors are receptors for which neither their function and role in pathophysiological conditions have been described nor their endogenous ligands have been identified so far. They may represent new therapeutic targets and offer the opportunity to introduce innovative therapies with a new mechanism of action. The GPR18 receptor is highly expressed in cells and tissues of the immune system. The expression pattern of GPR18 in tissues that is immunological or hematological in function may suggest immunomodulatory activity of this receptor. Furthermore, the expression of GPR18 has been confirmed in microglia, which is the subject of the investigation of the presented project.

Therefore, the main objective of this project is to improve understanding of the the role of GPR18 receptor in the basic mechanisms of regulation of microglial polarization (M1/M2). Faced with this challenge, we will assess the anti-inflammatory activity of GPR18 ligands. 3 structures will be investigated. These are newly discovered small peptide-like agonists from our research team (Department of Technology and Biotechnology of Drugs, Jagiellonian University Medical College) coordinated by Professor Katarzyna Kieć-Kononowicz in cooperation with Professor Christa Mueller research group from the Pharmaceutical Institute University of Bonn. One of these ligands has shown anti-inflammatory activity in an animal model of intestinal inflammation in previous studies, and the other has shown a significant antioxidant effect, which confirms the validity of its further investigation in an *in vivo* study. Commercially available GPR18 agonist structures, Δ9-THC and/or NAGly, will also be investigated for activity comparison. It is planned to use murine and rat microglial cells with confirmed expression of the GPR18 receptor and a rat model of neuroinflammation and dementia induced by intraventricular injection of streptozotocin. The expression of genes and proteins that are markers of M1/M2 microglia will be investigated. Additionally, using the latest Luminex xMAP® technology, a panel of pro-inflammatory and anti-inflammatory cytokines will be tested and the change in the level and activity of proteins involved in key cellular signal transduction pathways will be analysed in an *in vitro* and *in vivo* model of neuroinflammation after the administration of GPR18 ligands. This will allow us to describe the mechanism of microglia activation by GPR18 ligands.

The unique subject of this project involves pharmacological research linking the GPR18 receptor signaling system with microglial polarization. Suppressing M1 and / or improving the secretion of beneficial anti-inflammatory molecules from M2-polarized microglial cells could be a potential therapeutic approach for neuroinflammation. Our research will allow, for the first time, to develop novel therapeutic strategies that may greatly benefit public health. This knowledge will allow the use of GPR18 ligands as a pharmacological target in neuroinflammation.