ImmunoTools *special* Award 2014

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Effects of lysophosphatidic acid on microglia function

Microglia, the immunocompetent cells of the CNS, rapidly respond to brain injury and disease, altering their morphology and phenotype to adopt an activated state [1]. Under chronic inflammatory as observed in many neurodegenerative diseases, microglia are characterized by overactivation and secretion of proinflammatory and neurotoxic factors that can induce neuronal damage [2]. It is still under debate whether this state of microglia activity exacerbates pathology or aids in tissue repair. Due to their high „functional plasticity“, microglial activation can originate different phenotypes and according to macrophage nomenclature M1, M2a, M2b, and M2c-like polarization phenotypes were identified [3, 4]. Characterization of signaling cascades that drive microglia polarization could open new (therapeutic?) possibilities to modulate microglia function.

In my project I aim to characterize the contribution of the lysophosphatidic (LPA)-LPA receptor-protein kinase D (PKD) axis during microglia polarization. In vivo, lysophosphatidic acid (LPA) species are produced via the autotaxin or phospholipase A-mediated pathways. LPA has diverse biological functions mediated by downstream signaling through different G-protein-coupled LPA receptors (LPAR1-4, GPR87 and P2Y5). These receptors play a central role in the developing and adult brain and signaling is amplified at sites of inflammation where LPA concentrations are increased. LPA - via its cognate receptors - is a potently activates PKD family members that are centrally involved in the immune response.

During ongoing, preparatory experiments I have observed pronounced changes in morphology (towards an amoeboid morphology compared to the basal ramified cytoskeletal architecture) of LPA-stimulated microglia, an event that was associated with rapid phosphorylation of PKDs and activation of the MAPK pathways.

In response to injury microglia are able to polarize towards an M1-like (classical) or M2-like (alternative) phenotype in order to produce neurotoxic or neurotrophic factors respectively. Using flow cytometry, I have obtained preliminary evidence that LPA can drive microglia polarization towards an M1-like phenotype. LPA treatment of permanent BV-2 microglia increased CD40 and CD86 (M1 markers) and reduced CD206 and CD163 (M2 markers) expression. Western blot analysis demonstrated that LPA increased protein expression of iNOS and COX-2 (both M1). On the contrary, the basal expression of arginase I and RELMa(FIZZ) was gradually suppressed (both M2). Immunofluorescence for iNOS, COX2, Arginase I and RELMa supported these results. These findings were accompanied by increased IL-6 secretion and nitric oxide (NO) production.

To be able to obtain biologically more meaningful data I will switch to primary mouse microglia (isolation procedure is established) and test different LPA species for their potential to drive microglia polarization. During these experiments M1/M2 polarization markers will be analyzed by flow cytometry, immunofluorescence microscopy and western blotting. Secreted cytokines will be quantitated by ELISA.

As a next step to my research, I plan to examine the impact of LPA on primary cells that are already primed for an M1 or M2-like phenotype. LPS, IL4/IL13 and IL10 will be used in order to induce M1, M2a and/or M2c phenotypes. After treatment M1/M2 marker analysis will be performed (flow cytometry, Western blotting). The levels of IL-6 will be measured using ELISA.

Finally, I plan to investigate the role of PKD signaling on microglia phenotype adaptation and shifting. Lentiviral particles will be used to silence the expression of PKD1, PKD2 and PKD3. Non polarized, M1 and M2 polarized cells will be treated with inflammatory levels of LPA and using the same techniques as described above I

will investigate the contribution of this downstream pathway on functional plasticity of microglia.

The results of my study could contribute to a better understanding of the role of bioactive lipid mediators in microglia physiology.

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