ImmunoTools *special* Award 2018

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The impact of retinal microglial exosomes in glaucoma – focus on neuroinflammation

Glaucoma is a leading cause of blindness worldwide, characterized by progressive loss of retinal ganglion cells and optic nerve damage[1]. One of the main risk factors for the development of glaucoma is elevated intraocular pressure. The onset of the disease is often accompanied by increased microglia reactivity and neuroinflammation[2-4]. Reactive microglia may have initially protective properties, but sustained and exacerbated inflammation contributes to cell death[5]. It is conceivable that a fine-tuned cell-to-cell communication plays a crucial regulatory role in maintaining retinal homeostasis and it. crucial to elucidate the mechanisms involved in the spreading of retinal inflammation by microglia.

Exosomes are small extracellular vesicles (30-150 nm) derived from the endosomal system that have arisen as important players in cell-to-cell communication[6]. Microglia release exosomes that are known to mediate inflammation in several brain disorders[7]. Hence, we hypothesize that microglia release exosomes that mediate an inflammatory response that amplify microglia reactivity and elicit a pro-inflammatory phenotype in the retina. We aim to disentangle the relevance of microglial exosomes in glaucoma neuroinflammation.
Microglial cells will be isolated from the retina (mouse or human) and maintained with rm GM-CSF or rh GM-CSF, which will be required for the culture of purified retinal microglial cells. Microglial cells will be stimulated with elevated hydrostatic pressure (EHP), mimicking the increased intraocular pressure[8]. The exosomes will be isolated from microglial cell supernatant by sequential ultracentrifugation. The relevance of microglial exosomes in the spreading of retinal neuroinflammation will be assessed by exposing naïve microglial cells to exosomes derived from control or EHP conditions. We will then quantify the production of inflammatory mediators TNF and IL-6 by ELISA using the mouse TNF-a and mouse IL-6 ELISA-sets. To evaluate the relevance of microglial exosomes to the human retina, human retinal organotypic cell cultures will be exposed to exosomes from control or EHP conditions. Retinal inflammation will be evaluated in culture media and tissue through ELISA using the human ELISA-sets for human IFN-gamma, human IL-1beta and human TNF-a. Microglia reactivity in human retinal tissue

will be evaluated by flow cytometry using the antibodies FITC-CD74, PerCP-CD11b, PE-HLA-DR.

The project will clarify the role of microglial exosomes in retinal degeneration and pro-inflammatory signaling focusing on microglia-microglial cell crosstalk in the context of glaucoma. This is an innovative project in the field of glaucoma, which may provide insights in the identification of therapeutic interventions or biomarkers, as little is known regarding exosomal cell-to-cell communication in the retina.

References:

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FITC - conjugated anti-human CD74

PE - conjugated anti-human HLA-DR

PerCP - conjugated anti-human CD11b

human ELISA-set (for one 96 plate): human IFN-gamma, human IL-1beta, human TNF-alpha

mouse ELISA-set (for one 96 plate): mouse IL-6, mouse TNF-alpha

recombinant human cytokines: rh GM-CSF

recombinant mouse cytokines: rm GM-CSF

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