ImmunoTools *special* Award 2021

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Identifying the trigger in microglia that mediates retinal neuroinflammation in glaucomatous neurodegeneration

Glaucoma, a leading cause of global irreversible blindness, is a progressive chronic retinal neurodegenerative disease characterized by retinal ganglion cell loss and damage of the optic nerve (retinal ganglion cell axons). Elevated intraocular pressure (IOP) and ageing are important risk factors for disease onset and progression. As the population in developing countries ages, the number of individuals with glaucoma will increase, likely worsening the socio-economic burden of the disease. Glaucoma progresses without causing symptoms, delaying diagnosis until substantial amounts of neural damage. The biological basis of glaucomatous neurodegeneration is poorly understood and the factors contributing to its progression have not been fully characterized. It is urgent to elucidate the pathophysiological mechanisms that contribute to glaucomatous neurodegeneration, because more effective therapeutic strategies might be envisaged (Boia et al., 2020a).

Chronic neuroinflammation plays an important role in glaucoma pathogenesis. We have been systematically reporting that microglia become reactive when challenged with elevated pressure, releasing cytotoxic factors that promote retinal neuronal death, including retinal ganglion cells (Aires et al., 2019; Aires et al., 2020; Ferreira-Silva et al., 2020; Madeira et al., 2016a; Madeira et al., 2015; Rodrigues-Neves et al., 2018). Retinal microglia reactivity also occurs in animal models of glaucoma, and the control of microglia-mediated neuroinflammation is sufficient to protect retinal ganglion cells from damage (Boia et al., 2017; Madeira et al., 2016a; Madeira et al., 2016b), reinforcing the crucial role of microglia to disease. Despite all these evidences showing microglia as active players in glaucomatous neurodegeneration, the sensor in microglia for elevated pressure that triggers the inflammatory phenotype was not identified yet.

Piezo1 is a mechanosensitive ion channel that senses pressure and shearing stress and plays an important role in mechanotransduction (Coste et al., 2012). Moreover, Piezo1 activation promotes inflammation (Ma et al., 2021; Solis et al., 2019) and has been identified in microglia (Liu et al., 2021). Interestingly, the expression of

Piezo1 is increased in the brain of aged mice (Velasco-Estevez et al., 2018), implicating Piezo1 in ageing-associated diseases, like glaucoma. We found Piezo1 in the ganglion cell layer (where microglia are located) and Piezo1 mRNA is upregulated in microglia exposed to elevated pressure. There are currently no reports regarding the role of Piezo1 in retinal microglia and we hypothesized that Piezo1 plays an important role in microglia-mediated neuroinflammation in glaucoma.

The project aims to identify the link between elevated IOP and the inflammatory response of microglia in glaucomatous conditions. Piezo1 channel will be inhibited either by pharmacological means or by genetic depletion using siRNA. In these conditions, microglial cells will be exposed to elevated pressure. Pro-inflammatory cytokines will be quantified by ELISA (**mouse TNF-a and human IL-1beta**).

Since retinal ganglion cells are lost in glaucoma and microglia reactivity has a role, we will determine the effects of Piezo1 in microglia to the death of retinal ganglion cells. Microglia cell cultures, in the presence and absence of pharmacological inhibitor or siRNA for Piezo1, will be exposed to elevated pressure. Cultures of purified retinal ganglion cells will be prepared by sequential immunopanning (Boia et al., 2020a, medium containing **rr CNTF**). The conditioned medium from microglia will be transferred to retinal ganglion cells and cell dysfunction and death assays will be performed.

An animal model of glaucoma with ocular hypertension to study the contribution of microglial Piezo1 to glaucomatous neurodegeneration and the presence of immune cells in the retina will be determined by flow cytometry (**FITC - conjugated anti-mouse CD11b**).