

ImmunoTools *special* Award 2014



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Role of adenosine A2A receptor in pressure-induced retinal microglia reactivity

Glaucoma is a progressive and non-curable retinal degenerative disease and the second cause of vision loss in the world, affecting 67 million people worldwide [1]. The degeneration of retinal ganglion cells (RGCs) in glaucoma is accompanied by a neuroinflammatory response.

Adenosine is a neuromodulator in CNS involved in inflammatory responses, central and peripheral neural function, and neuroprotection. Adenosine A2A receptor (A2AR) antagonists confer robust neuroprotection, without peripheral effects [2,3], justifying the interest in A2AR antagonists as neuroprotective agents. Several studies carried out using different brain insults and focusing in different brain regions have systematically found that pharmacological blockade or genetic inactivation of A2ARs afford protection [4]. Increasing evidence show that neuroprotection afforded by A2ARs blockade may result from the control of microglia cell-mediated neuroinflammation. However, the putative protective role of A2AR blockade in glaucoma remains to be evaluated. We will study the effect of A2AR inactivation on microglia reactivity using *in vitro* models exposed to elevated hydrostatic pressure. We expect to demonstrate that A2AR blockade, through the control of neuroinflammation, protects retinal ganglion cells in models of glaucoma.

We will investigate the effects mediated by A2AR on microglia reactivity and on RGC death in retinal neuronal cell cultures and retinal explants exposed to elevated hydrostatic pressure. Retinal explants have the advantage of maintenance of cellular diversity as well as anatomical arrangements, since all the retinal cell types are present.

Retinal neuronal cell cultures and retina explants will be challenged with hyperbaric conditions for 24h by exposure to +70 mmHg above atmospheric pressure (described previously to activate retinal microglia, see Ref. 5). Cultures will be pre-treated with the A2AR antagonist SCH58261 before challenging cells with elevated pressure. Cultures of retinal explants will be prepared from A2AR knockout (A2AR-KO) mice and wild-type (WT) mice. The retinas will be cultured on inserts and maintained for 4 days *in vitro*. Pharmacological blockade of A2AR will be also performed in WT mice by incubating the retinal explants with A2AR selective antagonist (SCH58261) prior challenging cultures with elevated pressure.

The expression of A2AR will be assessed by qPCR, immunocytochemistry, and Western blotting. Microglia activation will be assessed by morphology assessment counting with anti-CD11b antibody, and by immunostaining for the markers of microglia activation, i.e., MHC-II or ED-1. NO production will be quantified with DAF-FM staining. The levels of cytokines TNF, and IL-1 β will be measured by ELISA. Phagocytosis will be quantified using fluorescent latex beads. In vitro chemotaxis assay will be performed using 24-well transwell inserts (8 μ m diameter holes), to allow the migration of cells from the upper chamber into the bottom chamber, in the presence of monocyte chemoattractant protein-1 (CCL2/MCP-1).

The RGC layer contains RGCs, glial cells, and other cell types. Therefore, in addition to microglia reactivity, in the cultures of retinal explants, we will also assess the loss of RGCs. RGCs will be identified by immunohistochemistry with an antibody against Brn-3a, a transcription factor that is specific for RGCs [6]. Co-localization of Brn-3a and TUNEL-positive cells will be also assessed.

References

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human IL-4 ELISA-set, IL-6 ELISA-set, IL-8 ELISA-set, human TNF α ELISA set,
(each 3 reagents)

recombinant mouse cytokines rm IL-1beta; rm IL-10, rm MCP1 / CCL2; rm M-CSF;
rm TNF α ;

FITC - conjugated anti-mouse CD11b

PE - conjugated anti-mouse CD11b

recombinant mouse cytokines rr IL-1beta; rr IFN γ ; rr MCP1 / CCL2; rr M-CSF;
rr TNF α

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