ImmunoTools *special* Award 2018

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Neuroinflammation and neuroprotection in Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disorder, characterized by the degeneration of dopaminergic neurons in the *Substantia nigra pars compacta*, and intracellular inclusions of aggregated a-synuclein, resulting into severe motor symptoms. About 90% of PD cases have a sporadic origin, and only 10% of the cases are familial forms of PD.

Although the pathogenic mechanisms are not entirely understood, neuroinflammation seems to play a major role in PD. Microglia are brain-resident myeloid cell population, normally present in a surveillant nonpolarized state but rapidly become activated in the presence of neuronal injuries, and were found near dopaminergic neurons in PD. Activation of microglia is characterized by morphological changes, increased expression of surface molecules, secretion of pro-inflammatory cytokines, up-regulation of pro-inflammatory enzymes, and phagocytosis. Thus, once activated by injured neurons microglia can itself become a donor source of toxic factors causing injuries to nearby neurons. These neuronal injuries, in turn, will induce additional microglial activation and may be an important process that drives and exacerbates the progressive neurodegeneration after the initial stimulus. Additionally, infiltration of T cells and direct access to dopaminergic neurons is facilitated blood-brain barrier dysfunctions and increased permeability, that allow T cell mediated responses leading to neurotoxicity.

Thus, understanding the neuroinflammatory response in PD is of outmost importance in order to find adequate therapeutic strategies to reduce degeneration. Using a toxin-induced models of PD, we showed activation of microglia, increased expression of IL-1beta and cyclooxigenase-2 and decreased expression of the anti-inflammatory protein Annexin A1 (ANXA1). Interestingly, we found that a neuroprotective bile acid decreased motor symptoms in parkinsonian mice, and reduced neuroinflammation.

We are now trying to understand the ability of other compounds to diminish neuroinflammation and prevent neurodegeneration in toxin-based and familial cellular models of the disease. For that, ImmunoTools provides a large set of reagents that are essential for this work:

1) Primary mice microglia and neurons

1.1) Neuronal conditioned medium used in microglia cultures
Microglial activation will be evaluated by flow cytometry using mouse CD11b-FITC. The pro-inflammatory phenotype of microglia will be evaluated by mouse IL-6 and mouse TNF-a release in the culture supernatants by ELISA.

1.2) Microglia conditioned medium used in neuron cultures
Microglia will be activated in the presence of rm IFN-gamma, rm IL-1beta, rm IL-10, rm IL-6, and/or rm TNF-a. Neuronal cell death will be determined by flow cytometry using mouse Annexin-V-FITC.

2) Human SH-SY5Y cells over-expressing a-synuclein and BV2 mice microglia cell lines

2.1) Understanding if exposure to the pro-inflammatory stimuli rh IFN-gamma, rh TGF-beta3, rh IL-1beta /IL-1F2, rh TNFα and/or rh IL-6 affect neuronal viability by staining with human Annexin V-FITC.

2.2.) Conditioned medium of SH-SY5Y cells treated as in #2.1 will be used to evaluate BV2 microglia activation as in #1.1.

2.3) BV2 cells will be activated as in #1.2 and the conditioned media will be used to treat SH-SY5Y cells concomitantly exposed to vehicle, rh BDNF, rh IL-1RA/IL1F3, rh sIL-6rec (sCD126), or rh sTNFrec / CD120b.

3) The expression of ANXA1, and other markers of inflammation will be also checked in all the experimental conditions. The best results obtained will be recapitulated in cells genetically modified to over-express/null for key modulators of inflammation and/or in the presence of selected neuroprotective compounds.

With these in vitro experiments we expect to obtain results that can be translatable to pre-clinical studies using in vivo models of PD hopping to contribute to the elucidation of the mechanisms underlying this disease and to find new therapeutic strategies.

ImmunoTools *special* AWARD for **Margarida Castro Caldas**

includes 25 reagents

FITC - conjugated anti-human Annexin V-FITC

recombinant human cytokines: rh BDNF, rh IFNgamma, rh IL-1beta /IL-1F2,

rh IL-1RA/IL1F3, rh IL-6, rh sIL-6rec (sCD126), rh sTNFrec / CD120b, rh TGF-beta3, rh TNFα

FITC - conjugated anti-mouse CD11b, Annexin V-FITC

recombinant mouse cytokines: rm IFN-gamma, rm IL-1beta, rm IL-10, rm IL-6,

rm TNFa

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

DETAILS more AWARDS