ImmunoTools IT-Box-Cy55M-Award 2013

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Development and characterization of two novel animal model for autism (ASD)

A growing body of evidence suggests that microglial activation in CNS is involved in the pathophysiology of neuropsychiatric disorders (such as Autism Spectrum Disorder, ASD) and neurodegenerative disorders (such as Alzheimer's Disease, AD), (Patterson 2002; Griffin et al., 1989; Rogers et al., 1988). Upon exposure of the brain to any form of insult such as infection, trauma, or ischemia, microglia are rapidly activated and produce neurotoxic substances, including pro-inflammatory cytokines; their biological effects include stimulation or inhibition of cell proliferation, cytotoxicity/apoptosis, antiviral activity, cell growth and differentiation, inflammatory responses, and upregulated expression of surface membrane proteins.

Chronic inflammatory diseases and abnormal response to infection by different blood cell populations have been described in ASD children and adults. On the other hand, in AD the intractable nature of Aβ plaques and tangles stimulates a chronic inflammatory reaction (Town et al., 2005). These plaques contain dystrophic neurites, activated microglia, and reactive astrocytes (Akiyama et al., 2000; Rogers et al., 1988; Dickson et al., 1988). Aggregated amyloid fibrils and inflammatory mediators secreted by microglia and astrocytes contribute to neuronal dystrophy (Nussbaum and Ellis, 2003; Findeis, 2007)

Organotypic Cerebellar Culture slices (OCCs) are a useful *in vitro* model characterized by: conserved phenotypic and physiological features of cells *in vivo*; homogenous cellular population; easy manipulation. They represent an optimal model for studying cellular and molecular correlates of survival/apoptosis and neurodegeneration/neuroprotection.

Caspase family activation represents an irreversible endpoint and plays a central role in apoptosis, useful for detection of dying cells. A novel approach to the detection of caspase-3 activation in living cells is represented by the use of confocal fluorescence imaging and FRET technology: this functional analysis of caspase 3 activation and regulation offers the possibility of qualitative enzyme function monitoring and also gives quantitative information about its activation. To this purpose, neurons in OCCs are transfected by a biolistic (gene gun) approach with a plasmid DNA codiyfing a FRET probe. This consists of a donor (enhanced cyan fluorescent protein, ECFP) and an acceptor (Venus, a mutant of yellow fluorescent protein) (Takemoto et al., 2007). The donor and the acceptor are linked with a caspase-3 recognition and

cleavage sequence (DEVD) (Wu et al., 2006). The activated caspase-3 cleaves the linker DEVD, and then induces a marked decrease in the FRET efficiency and a significant increase in the lifetime of ECFP. Currently, monitoring the cleavage of SCAT3 is mostly based on the altered fluorescence intensity ratio of Venus to ECFP (Wu et al., 2007; Liu et al., 2008).

Thanks to confocal microscopy associated with FRET technology and live imaging we can monitor caspase 3 activation following different environmental stimuli, in a living system.

Our proposal is to monitor caspase 3 activation in different neurons and glial cells subsequent to cytokine stimulation. Therefore, we will test a variety of recombinant cytokines in our OCC model and the effect on neuronal apoptosis will be assessed by FRET. The aim testing how the balance between pro- and anti-inflammatory cytokines influences cell fate in CNS.

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