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Rat primary cortical cell tri-culture to study effects of amyloid-beta on microglia function

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Abstract

Introduction

The etiology and progression of sporadic Alzheimer's Disease (AD) have been studied for decades. One proposed mechanism is that amyloid-beta (A β) proteins induce neuroinflammation, synapse loss, and neuronal cell death. Microglia play an especially important role in A β clearance, and alterations in microglial function due to aging or disease may result in A β accumulation and deleterious effects on neuronal function. However, studying these complex factors *in vivo*, where numerous confounding processes exist, is challenging, and until recently, *in vitro* models have not allowed sustained culture of microglia, astrocytes and neurons in the same culture. Here, we employ a tri-culture model of rat primary neurons, astrocytes, and microglia and compare it to co-culture (neurons and astrocytes) and mono-culture enriched for microglia to study microglial function (i.e., motility and A β clearance) and proteomic response to exogenous A β .

Methods

We established cortical co-culture (neurons and astrocytes), tri-culture (neurons, astrocytes, and microglia), and mono-culture (microglia) from perinatal rat pups. On days *in vitro* (DIV) 7 – 14, the cultures were exposed to fluorescently-labeled A β (FITC-A β) particles for varying durations. Images were analyzed to determine the number of FITC-A β particles after specific lengths of exposure. A group of cells were stained for β III-tubulin, GFAP, and Iba1 for morphological

analysis via quantitative fluorescence microscopy. Cytokine profiles from conditioned media were obtained. Live-cell imaging with images acquired every 5 minutes for 4 hours was employed to extract microglia motility parameters (e.g., Euclidean distance, migration speed, directionality ratio).

Results and discussion

FITC-A β particles were more effectively cleared in the tri-culture compared to the co-culture. This was attributed to microglia engulfing FITC-A β particles, as confirmed via epifluorescence and confocal microscopy. Adding FITC-A β significantly increased the size of microglia, but had no significant effect on neuronal surface coverage or astrocyte size. Analysis of the cytokine profile upon FITC-A β addition revealed a significant increase in proinflammatory cytokines (TNF- α , IL-1 α , IL-1 β , IL-6) in tri-culture, but not co-culture. In addition, A β addition altered microglia motility marked by swarming-like motion with decreased Euclidean distance yet unaltered speed. These results highlight the importance of cell-cell communication in microglia function (e.g., motility and A β clearance) and the utility of the tri-culture model to further investigate microglia dysfunction in AD.

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