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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 (Aβ) proteins induce neuroinflammation, synapse loss, and neuronal cell death. Microglia play an especially important role in Aβ clearance, and alterations in microglial function due to aging or disease may result in Aβ 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 Aβ clearance) and proteomic response to exogenous Aβ.

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 Aβ (FITC-Aβ) particles for varying durations. Images were analyzed to determine the number of FITC-Aβ particles after specific lengths of exposure. A group of cells were stained for βIII-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-Aβ particles were more effectively cleared in the tri-culture compared to the co-culture. This was attributed to microglia engulfing FITC-Aβ particles, as confirmed via epifluorescence and confocal microscopy. Adding FITC-Aβ 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-Aβ addition revealed a significant increase in proinflammatory cytokines (TNF-α, IL-1α, IL-1β, IL-6) in tri-culture, but not co-culture. In addition, Aβ 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 Aβ clearance) and the utility of the tri-culture model to further investigate microglia dysfunction in AD.

Full Text Availability