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Sommario	ABSTRACT Neuronal survival in neurodegenerative diseases and brain damage is closely related to the cell populations of the environment and in particular to glial cells. Astrocytes, microglia and oligodendrocytes oversee brain homeostasis providing the intrinsic brain defence system. Damage to brain cells triggers a condition generally referred to as reactive gliosis, which includes astrogliosis and activation of microglia. Neuroglia is also thoroughly involved in pathogenesis of many chronic neurological disorders and in neurodegeneration. Endocannabinoids modulating the behaviour of microglia and astrocytes might act as possible targets for therapeutic intervention. Recent studies have indicated that endocannabinoid levels and metabolic enzymes change during the progression of Alzheimer's disease (AD) and that the inhibition of fatty acid amide hydrolase (FAAH), the main catabolic enzyme of anandamide (AEA), has beneficial effects in mice with AD. The aim of this study was to determine whether URB597, a FAAH inhibitor, targets microglia polarization by altering the cytoskeleton reorganization induced by amyloid-β peptide (Aβ) in BV-2 microglial cells. Evaluation of actin cytoskeleton showed that Aβ treatment increased the surface area of BV-2 cells, which acquired a flat and polygonal morphology. Although URB597 did not

affect cell morphology only, it partially rescued the control phenotype in BV-2 cells incubated with the combined treatment. Rho family proteins have a critical role in the plasticity of the actin cytoskeleton, influencing morphological changes, migration and phagocytic activity of cells. We observed an increase of Rho protein activation in Aβ samples and a decrease in samples treated with URB597 alone or in combination with Aβ compared to controls, while an increase of Cdc42 protein activation was observed in all samples with respect to control. Aβ induced the migration of BV-2 cells up to 2 h after stimulation. We also found that by reducing Rho protein activity, URB597 was able to reduce the migration rate. URB597 also increased the number of BV-2 cells performing phagocytosis. Taken together, these data suggest that an increase of anandamide (AEA), due to FAAH inhibition, may induce cytoskeleton reorganization, regulating phagocytosis and cell migration processes, and promote microglial polarization towards an anti-inflammatory phenotype. As most research worldwide has focused on neurons, there is a dearth of protocols to generate glial cells and to produce co-culture systems for biomedical research. The aim of this project has also been the generation of co-culture with neurons, astrocytes and microglia cells and the subsequent characterization of the resulting model, evaluating interspecies differences through the generation of co-cultures with murine microglia. We focused our interest on the repair functions during brain injury and on the interactions between microglia and astrocytes. The protective effect of astrocytes and microglia against neuronal cells in the presence of inflammatory and pro-apoptotic processes was investigated. Human astrocytes and human microglia cells were activated with TNF-, IL-1 and IFN- to evaluate the inflammatory response. The results showed an increase of inflammatory cytokines gene expression such as IL-6 and IL-8 in both cell lines examined. The astrocytes activation by TNF-, or by conditioned medium (CM) of activated microglia cells was confirmed by NF-kB nuclearization. Therefore, the arise of inflammatory process in astrocyte cells is driven not only by TNF- induction, but also by a synergic effect due to microglia activation. Neuroinflammation, oxidative stress, and progressive degeneration of specific brain regions is also driven by proteasomal impairment, promoting protein accumulations. Since LUHMES neurons are quite susceptible cells to proteotoxic stress and amino acid starvation, we investigated whether murine microglia and human astrocytes exerted a protective effect also when the cell lines were treated with URB597. The obtained data demonstrated that the astrocytes through the glutathione (GSH) release, were able to attenuate neuronal proteotoxic stress in LUHMES cells. URB597 contributed to GSH anti-oxidant effects modulating GSH metabolism. The overall data demonstrated that neuroglial cells play a pivotal role on neuronal protection from noxious stimuli.

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