CONCEPT LIFE SCIENCES

Astrocyte Polarisation Assay

Introduction

Astrocytes are glial cells in the central nervous system (CNS) that support and protect neurons. Their numerous functions include maintaining the blood-brain barrier, providing nutrients to nervous tissue, regulating the extracellular ion balance and neurotransmission. In response to CNS injury, disease, or infection, microglia (the immune cells of the brain) become activated and release pro-inflammatory cytokines, inducing a reactive state in astrocytes, which lose neuroprotective functions and acquire neurotoxic properties by releasing substances like saturated lipids (Figure 1).

Neurotoxic astrocytes are involved in chronic neuroinflammation, a common feature of many neurodegenerative diseases, exacerbating neuronal damage and disease severity. Identifying and targeting the signaling pathways that lead to the formation of neurotoxic astrocytes could provide novel therapeutic approaches to halt or reverse neurodegeneration.

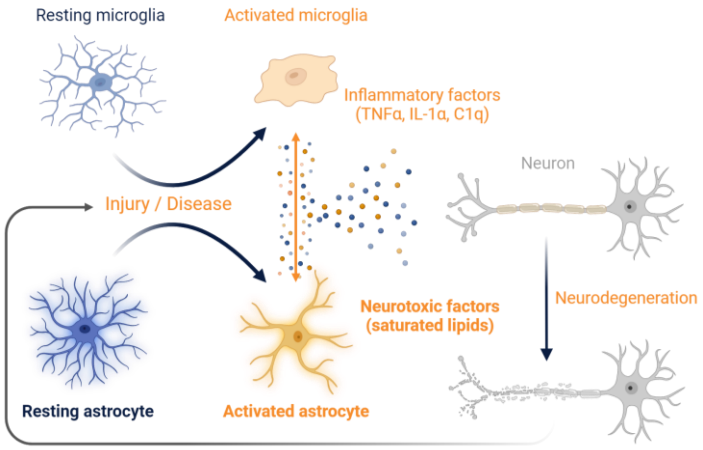

Figure 1. Pathological stimuli (e.g. disease or injury) induce microglial activation and the release of cytokines such as TNFα, IL-1α and C1q. These factors, in turn, polarise astrocytes towards a reactive neurotoxic phenotype, which potentiates neuronal degeneration, further exacerbating neuroinflammation and disease progression.

Methods

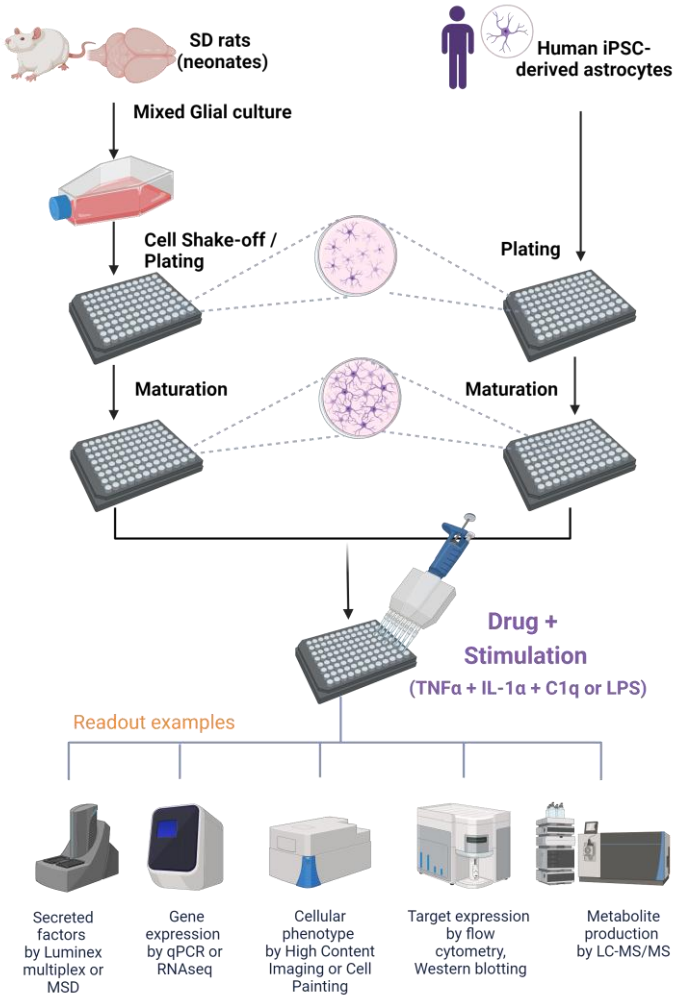

Figure 2. Following generation of mixed glial culture from neonatal Sprague Dawley (SD) rat brains, sequential shake-off of microglia and OPCs, astrocytes were isolated and plated into a 96-well plate. Human iPSC-derived astrocytes were thawed and plated into a 96-well plate. After a period of cell maturation, astrocytes were treated with Dexamethasone (Dex) and polarised with TNFα, IL-1α and C1q (TIC) or LPS. In these experiments, cytokine secretion profile was assessed via Luminex, gene expression was analysed by qPCR.

CONCEPT LIFE SCIENCES

CONCEPT LIFE SCIENCES

Results

Cytokine Secretion Profile

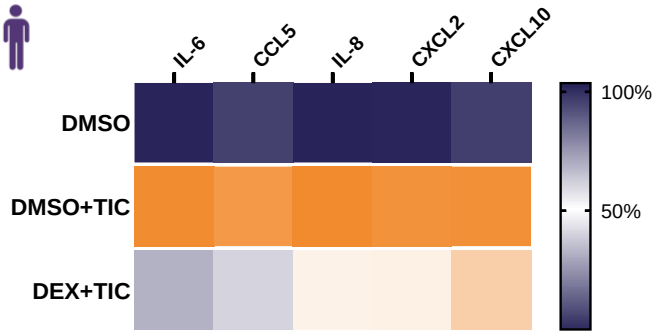

Figure 3. Cytokine secretion profile in human iPSC-derived astrocytes pre-treated with Dex and polarised with the TIC cocktail. The heatmap represents the median percentage of polarised vehicle control (3 experimental repeats).

Lot Consistency

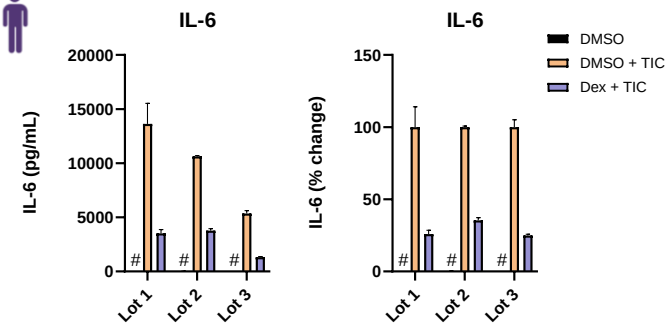

Figure 4. IL-6 secretion in human iPSC-derived astrocytes of 3 different lots pre-treated with Dex and polarised with the TIC cocktail. Data presented as mean + SEM concentration of percentage of polarised vehicle control (3 lot repeats).

Induction of Pro-Inflammatory Genes

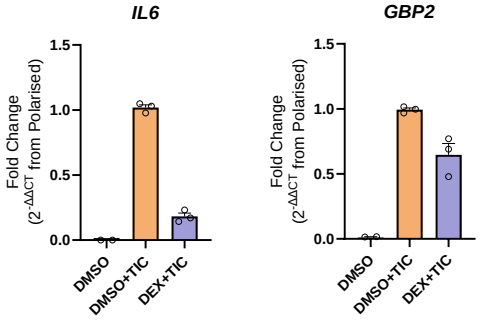

Figure 5. Gene expression analysis in human iPSC-derived astrocytes pre-treated with Dex and polarised with the TIC cocktail. Data presented as mean + SEM fold-change ($2^{-\Delta\Delta Ct}$) calculated relative to the polarised vehicle control (3 experimental repeats).

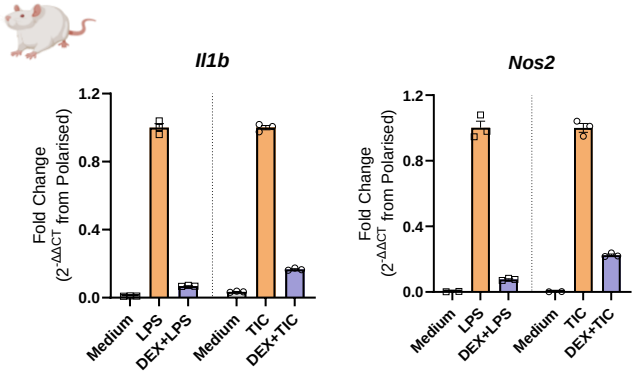

Figure 6. Gene expression analysis in rat primary astrocytes treated with Dex and polarised with LPS or the TIC cocktail. Data presented as mean + SEM fold-change ($2^{-\Delta\Delta Ct}$) calculated relative to the polarisation condition (3 technical replicates).

Conclusions

In neurodegenerative diseases, astrocytic activation is not only a consequence of the damaging inflammatory processes, but also their key driver, perpetuating a vicious cycle of neuroinflammation and neurotoxicity.

There is the potential to treat a range of neurodegenerative diseases, including Alzheimer's and Parkinson's diseases. Here we show that astrocytic activation can be reliably modelled in both human and rodent cells, enabling testing drug candidates for potential anti-inflammatory and neuroprotective properties.

Key Features

- ✓ A versatile model applicable to a range of neurodegenerative diseases and neurological conditions.
- ✓ A reproducible assay with robust induction of pro-inflammatory markers and neurotoxic phenotype.
- ✓ A flexible tool that can be tailored around the species and target of interest.
- ✓ A validated building block of complex multi-cellular systems (in combination with neurons and/or microglia).

Frith Knoll Road, Chapel-en-le-Frith, Derbyshire, SK23 0PG, United Kingdom
www.conceptlifesciences.com • 01298 816700 • LinkedIn • Contact Us