Generation and characterization of cryopreserved axoCells™ human iPSC-derived microglia

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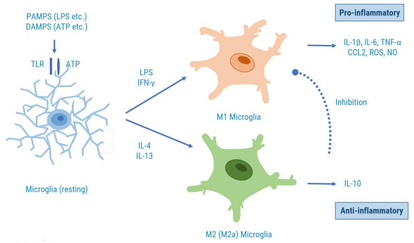
Introduction

Microglia are tissue-resident macrophages, making up 10 – 15% of the cells in the brain. Their key role in homeostasis is to support neurons, through actions such as synaptic pruning, as well as responding to inflammatory signals. As microglia play an important role in neuroinflammation they are a key target for drug discovery.

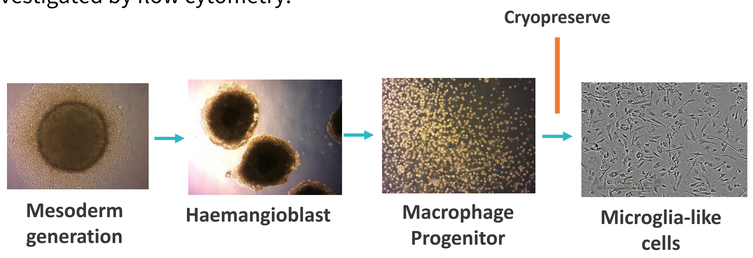


Stimulation of microglia towards M1 and M2 polarized cells [1].

Current models do not provide a physiologically relevant background or are limited by a finite amount of material, making it difficult to reliably produce a model that recapitulates the disease in a human background. Therefore, induced pluripotent stem cells (iPSCs), alongside robust differentiation methodologies can provide a human model with the large-scale production required for drug discovery. Here, we show the initial characterization of cryopreserved axoCells iPSC-derived microglial cells and demonstrate their use for studying functional activity.

Methods

Human iPSC lines were differentiated to monocyte-like cells by mesoderm induction via embryoid-body formation[2]. Surface expression of relevant markers was investigated by flow cytometry.



Flow diagram of cell differentiation to cryopreserved microglial-like cells

After maturation towards microglia-like cells, cryopreservation of the cells was performed. Upon thaw of the cells, marker expression, via immunocytochemistry, was performed for three key markers. Functional activity of the matured microglia-like cells was assessed by measuring phagocytosis of pHrodo labelled bait, IL-6 release in response to LPS stimulation and chemotaxis activity.

Results

Flow Cytometry

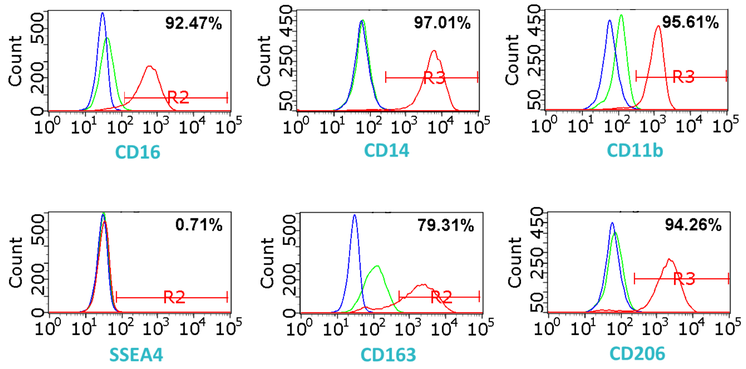


Figure 1. Flow cytometry of cell surface receptors on macrophage progenitor cells prior to maturation towards microglia-like cells. Blue: unstained, green: isotype, red: marker of interest

Immunocytochemistry

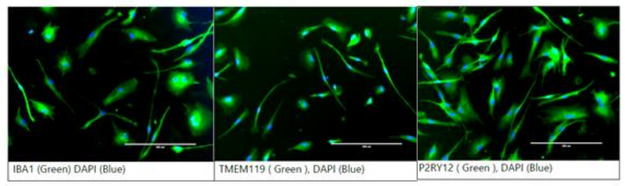


Figure 2. Immunocytochemistry for three markers, IBA1, TMEM119 and P2RY12, on axoCells microglia-like cells after seven days of maturation post thaw.

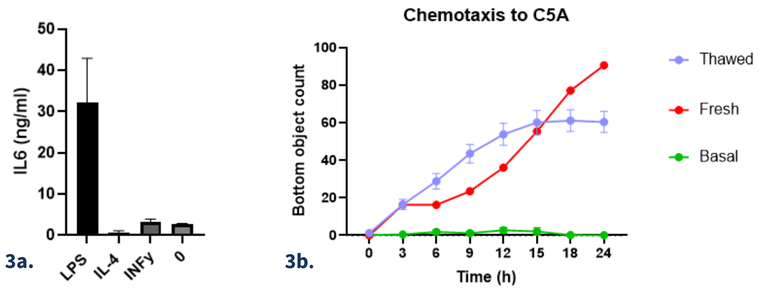


Figure 3. Functional analysis of axoCells microglia-like cells after seven days of maturation post-thawing.
3a. IL-6 response of axoCells microglia-like cells after 24-hour stimulation with either a proinflammatory stimuli (LPS) or anti-inflammatory cytokine (IL-4) (n = 3). IL-6 was measured via HTRF assay.
3b. Chemotaxis of fresh (n = 1) and cryopreserved (n = 3) microglia-like cells towards C5A was measured on an IncuCyte S3.

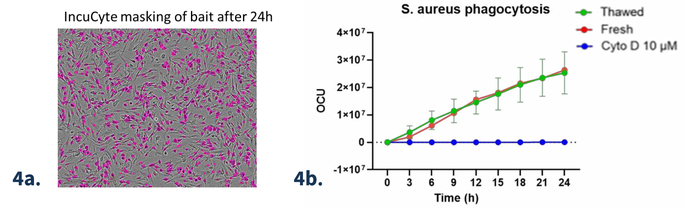


Figure 4. Phagocytosis of pHrodo labelled bait by axoCells microglia-like cells after seven days of maturation post-thawing.
4a. Phagocytosis of pHrodo-labelled *S.aureus* was measured on thawed (n = 3) and fresh (n = 1) microglia using an IncuCyte S3. **4b.** Phagocytosis was inhibited by treating microglia cells with the cytoskeletal inhibitor, cytochalasin D.

Disease modelling

Differentiation of patient-derived iPSC towards microglia-like cells allows the study of functional impairment in microglia in neurodegenerative disorders, replicating previously reported observations[3].

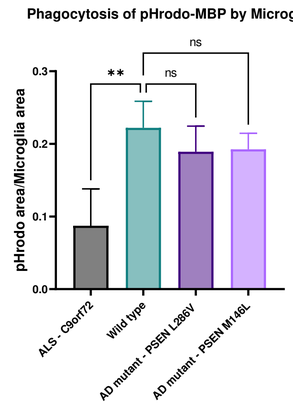


Figure 5. Microglia-like cells from an ALS (C9orf 72) patient line display reduced phagocytosis of pHrodo-labelled myelin basic protein (MBP) compared to the healthy line (axoCells microglia) and two Alzheimer's Disease patient lines (PSEN mutants). Statistical test performed One-way Anova, ** p<0.01.

Concluding remarks

Mouse models have extensively been used to investigate neurodegenerative diseases but do not directly recapitulate human neurological diseases[4], with some human genes not having mouse orthologues.

Here we have characterised a methodology to derive microglia-like cells from human iPSC lines that can be cryopreserved. The microglial-like cells demonstrate key marker expression as well as functional activity. In addition, differentiation of a patient iPSC line resulted in reduced phagocytosis, a phenotype that has previously been observed in C9orf72 microglia derived from iPSCs[3]. These cells provide a relevant disease model for advances in drug discovery.

References

1) Nakagawa, Y. *et al.* Role of Microglial M1/M2 Polarization in Relapse and Remission of Psychiatric Disorders and Diseases. Pharmaceuticals (2014) **7**, 1028-1048.
2) Karlsson, KR. *et al.* Homogeneous monocytes and macrophages from human embryonic stem cells following coculture-free differentiation in M-CSF and IL-3. Exp Hematol (2008) **36**(9):1167–75.
3) Banerjee, P. *et al.* Cell-autonomous immune dysfunction driven by disrupted autophagy in C9orf72-ALS iPSC-derived microglia contributes to neurodegeneration. Neuroscience (2023) **9**(16): DOI:10.1126/sciadv.abq0651
4) Friedman, BA. *et al.* Diverse Brain Myeloid Expression Profiles Reveal Distinct Microglial Activation States and Aspects of Alzheimer's Disease Not Evident in Mouse Models. Cell Reports (2018) **22**, 832–847.