Establishing a robust platform for investigating the pro- and anti-inflammatory response of iPSC-derived microglia in drug discovery

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Abstract

Microglia are the main inflammatory cell of the brain and have been implicated in the development and progression of several neuroinflammatory and neurodegenerative conditions such as Alzheimer's disease (AD), Frontotemporal Dementia (FTD) and Amyotrophic Lateral Sclerosis (ALS). iPSC-derived microglia provide a scalable, reproducible and physiological relevant model with which to study this role. Axol Bioscience has developed a robust protocol to generate microglia, with over 30 lines successfully differentiated, including those derived from healthy and patient donors and gene-edited lines.

Microglia are highly plastic cells influenced by environmental cues to drive both pro- and anti-inflammatory responses. As such, any dysregulation in their activation status can drive an exaggerated and sustained state of inflammation. axoCells™ iPSC-derived microglia provide a reliable platform to study phenotypes of healthy and diseased microglia and assess the ability of novel compounds to decrease inflammation. In this study, Axol used a range of assays to investigate the activation state of microglia in response to different stimuli, as well as assessing inter batch and inter assay variability of our control (ax0664) microglia.

HTRF assays for IL8, IL6 and TNFα release demonstrated consistent effects across batches of microglia, when stimulated with a concentration range of lipopolysaccharide (LPS). In addition, HTRF and ELISAs were used to assess activation states of microglia induced by IL-4. Further characterization by flow cytometry, investigated the effect of different stimuli and incubation times on CD80, CD83 and CD206 receptor expression. Additional profiling of ax0664 investigated the release of a broad range of cytokines, using O-link technology. Furthermore, TempO-Seq analysis was used to investigate transcriptomic profiles driven by different stimuli. Overall, this supports the ability of Axol's human iPSC-derived microglia to polarise towards different activation states. axoCells iPSC-derived microglia can be used as a relevant platform for human drug discovery where they can help predict the action of new drugs and identify mechanisms involved in neuroinflammation.

Methods

Human iPSC lines were differentiated to monocyte-like cells by mesoderm induction via embryoid-body formation [1]. After maturation towards microglia-like cells, cryopreservation of the cells was performed.

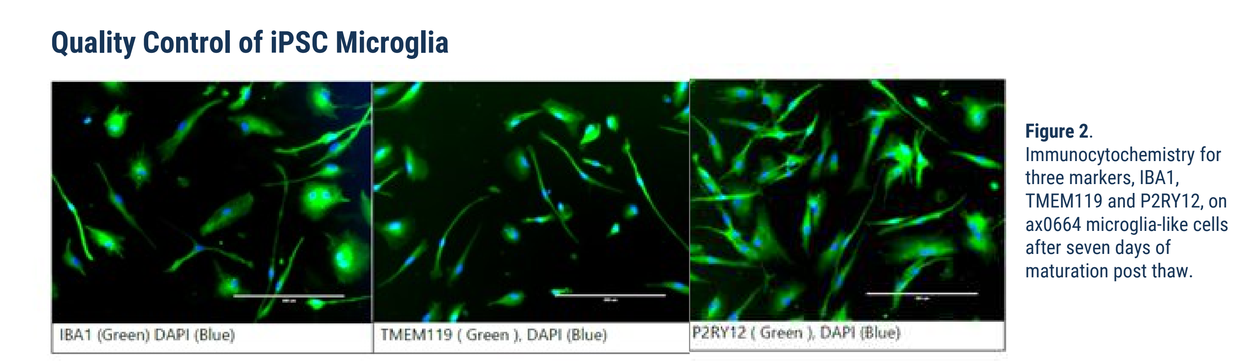


Figure 1. Flow diagram of differentiation from iPSC through a haemangioblast generation to cryopreserved microglial-like cells.

Upon thaw, quality control via immunocytochemistry staining for three key markers was performed to determine the identity of the cells.

Once the cells had passed QC, the microglia were treated with pro- / anti-inflammatory stimuli before measuring the IL8, IL6, TNFα and MMP12 concentration in the cell culture medium via HTRF or proteomics. In addition, TNFα, IL6 and IL8 release was measured following incubation of the cells with TLR4 antagonists + LPS.

Transcriptomics analysis, via TempoSeq, was performed on stimulated microglia. Finally, Flow cytometry following stimulation of the microglia was also performed to investigate the expression of three cell surface markers, CD80, CD83 and CD206.

Results

Quality Control of iPSC Microglia

Figure 2. Immunocytochemistry for three markers, IBA1, TMEM119 and P2RY12, on ax0664 microglia-like cells after seven days of maturation post thaw.

Microglia Proteomics

	LPS	INFγ	IL4
CCL8/IL8			
IL33			
CXCL12			
OLR1			
IL27			
IL2			
CXCL9			
TGFa			
IL1b			
IL6			
TNFSF12			
TSLP			
CCL11			
HGF			
FLT3LG			
IL17F			
IL7			
IL13			

	LPS	INFγ	IL4
IL18			
TNFSF10			
CXCL10			
IL10			
CCL19			
TNF			
IL15			
CCL3			
MMP12			
CSF2			
CSF3			
VEGFa			
IL17C			
EGF			
IL17A			
OSM			
CSF1			
CXCL11			
LTA			
CCL7			
MMP1			
INFγ			

Microglia Transcriptomics

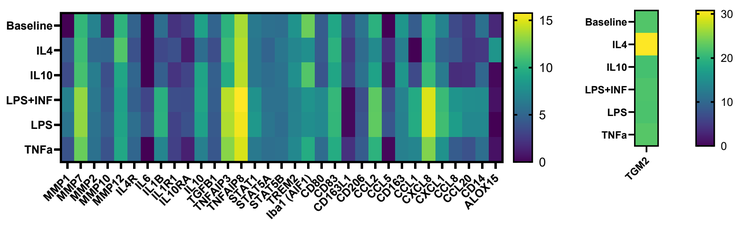
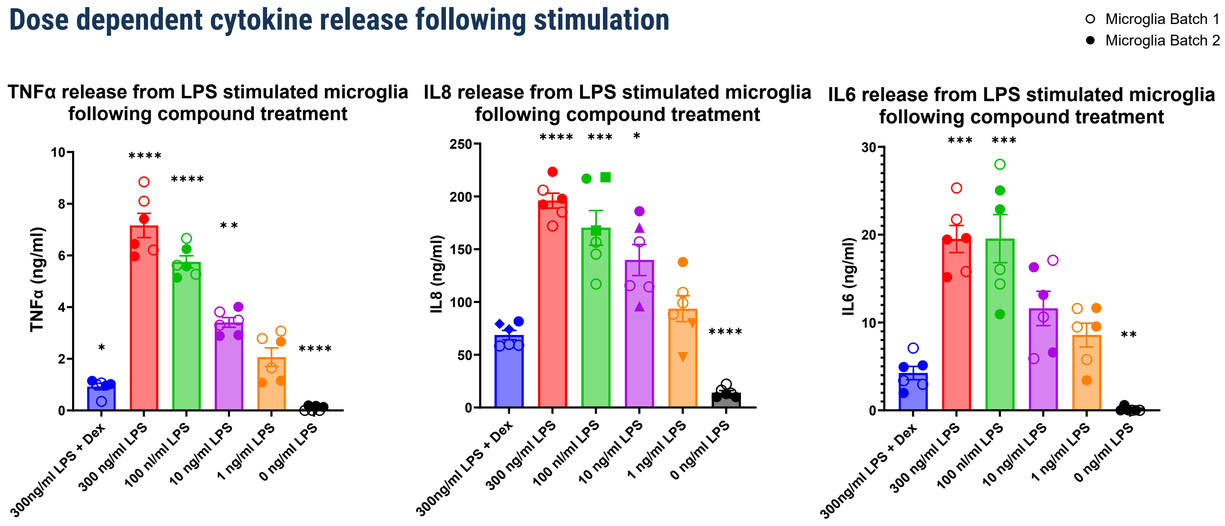


Figure 3. Proteomics analysis of cytokines in the cell culture supernatant (left) was measured using O-link technology on wildtype microglia following stimulation with LPS, IFNγ or IL-4 (red: no change, orange: moderate increase, green: increase in cytokine release).Transcriptomics was performed using TempoSeq sequencing (right) after stimulating wildtype microglia with pro/anti-inflammatory stimuli.

Dose dependent cytokine release following stimulation



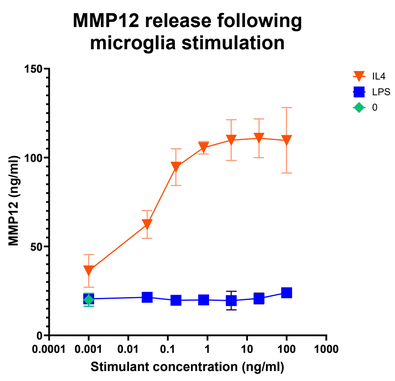


Figure 4 (Above). Two batches of wildtype microglia were stimulated over six experiments (each point is the mean of one experiment) with 0, 1, 10, 100 and 300ng/ml of LPS for 21 hours. A pretreatment with Dexamethasone (+Dex) was included to inhibit cytokine release. TNFα, IL6 and IL8 release was measured in the cell culture medium using HTRFs. Each point is the mean of one independent experiment, error bars = SEM. A one-way ANOVA with multiple comparisons was performed with each treatment being compared to the 1ng/ml LPS stimulation, * = P ≤ 0.05 , ** = P ≤ 0.01, *** = P ≤ 0.001 and **** = P ≤ 0.0001.

Figure 5 (Left): Treatment of wildtype microglia with IL-4 and LPS demonstrated that increasing concentrations of IL-4 increased MMP12 concentration in the cell culture media, measured using HTRF. Mean of two independent experiments, error bars = SEM.

High reproducibility of a cytokine release assay

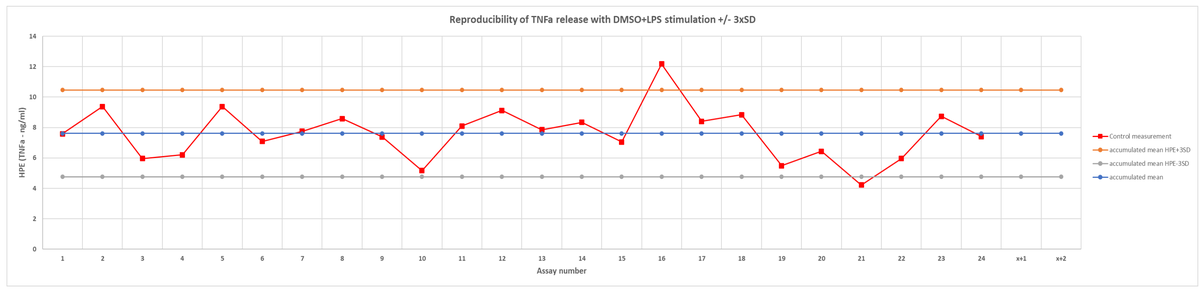


Figure 6. Levy-Jennings plot of twenty-four experiments measuring the TNFα concentrations in cell culture medium after a 21 hour, 300ng/ml LPS stimulation. If the average TNFα concentration of the experiment (red boxes) is larger than +/- 3SD (orange and grey lines) than the mean (blue line) then the experiment is rejected, and a repeat is performed.

Inhibition of LPS stimulation

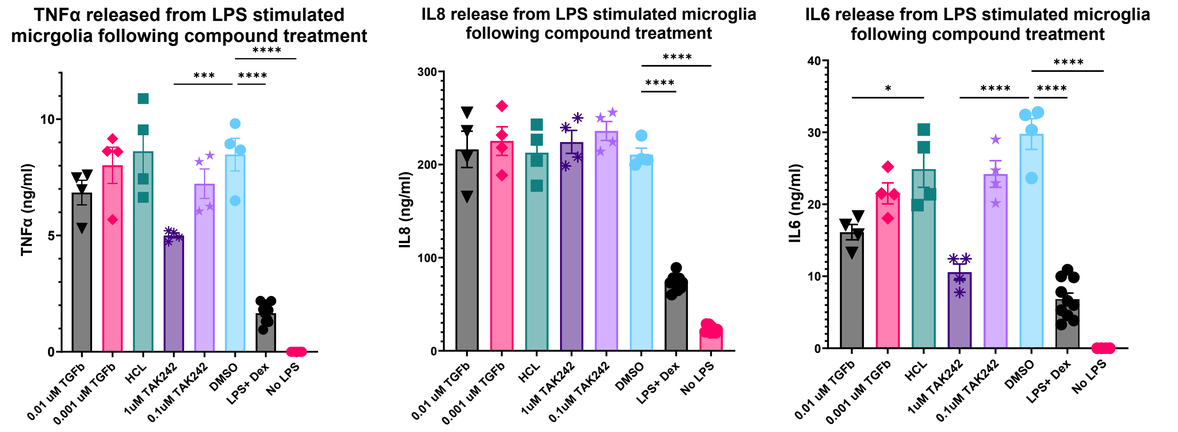
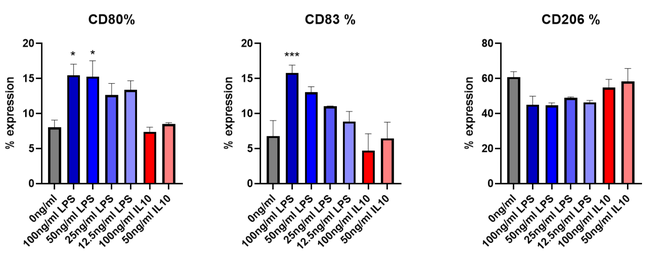


Figure 7. Wildtype microglia were pretreated with TGF-b, TAK242, dexamethasone, HCl or DMSO before stimulating with 300ng/ml LPS for 21 hours. TNFα, IL8 and IL6 concentrations in the cell culture medium were measured via HTRF. TGF-b , TAK242 (both known TLR4 antagonists) and dexamethasone decreased the concentration of IL6 and TNFα in the cell culture medium suggesting an inhibition effect of the M1 pro-inflammatory state via TLR4 inhibition. IL8 concentrations remain high with TGF-b and TAK242 treatment, suggesting that IL8 expression isn't dependent on TLR4 activation. Mean of two technical repeats across two independent experiments, error bars = SEM. A one-way ANOVA with multiple comparisons was performed with each treatment being compared to its vehicle control, * = P ≤ 0.05 , *** = P ≤ 0.001 and **** = P ≤ 0.0001.

Surface receptor expression changes following stimulation with pro/anti-inflammatory stimuli



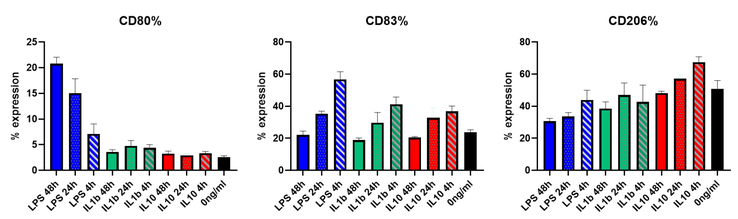


Figure 8:
Top: Expression of three surface receptors (CD83, CD80 and CD206), measured using flow cytometry, after stimulating wildtype microglia with IL-10 or LPS for 24 hours. Mean of three independent experiments, error bars = SEM, Ordinary one-way ANOVA with multiple comparisons compared to 0ng/ml, * = P ≤ 0.05 , *** = P ≤ 0.001

Bottom: Time course of the expression of three cell surface receptors (CD83, CD80 and CD206), measured using flow cytometry, after stimulating wildtype microglia with IL-10, IL1b or LPS (top). Mean of two independent experiments, error bars = SEM.

Conclusion

Increased IL8, IL6 and TNFα concentrations were observed, via proteomics and HTRF, in the cell culture supernatant following LPS stimulation. This increase in LPS induced IL6 and TNFα was reduced when pre-treating the microglia with TLR4 antagonists. Additionally, LPS stimulation increased IL6 mRNA, observed via TempoSeq, as well as increasing receptors that are indicative of M1 microglia, CD80 and CD83 [2, 3] expression. Together this data suggests that the iPSC-derived microglia can be induced towards an M1, pro inflammatory phenotype [2, 3, 4].

Conversely stimulation with IL-10 or IL-4, known anti-inflammatory cytokines, resulted in increased CD206 expression after 4hrs of stimulation, detected via flow cytometry. In addition, MMP12 release is increased in response to IL-4 stimulation, observed in the TempoSeq, proteomics and HTRF, and has been demonstrated to act to aid resolution of inflammation through cleavage of IFNγ by M2 macrophages [5].

This data demonstrates that the axoCells iPSC-derived microglia display a robust and reproducible response to pro- / anti-inflammatory stimuli across multiple assays and differentiation batches.

References

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