Development of Cytokine Release Assays for Human iPSC-derived Microglia

Michelle L. Curtis, Sarah Burton, Christie A. Savic, Madelyn E. Donegan, Rebecca K. Fiene, Scott Schachtele, and Coby B. Carlson

FUJIFILM Cellular Dynamics, Inc., Madison, WI USA

Board # B56
Abstract # 5530

Abstract

OBJECTIVE / RATIONALE: Microglia are the resident immune cells of the central nervous system (CNS) and are essential for maintenance of normal brain function. However, impaired or dysregulated microglia can contribute to a variety of neurodegenerative diseases. Thus, deciphering microglia functional states and understanding how they modulate inflammation during CNS infection holds great promise to identify key mechanisms of development, disease, and opportunities for therapeutic intervention. The differentiation of human induced pluripotent stem cells (iPSC) into microglia offers a reliable and functional source of cells for understanding human microglia function in health and disease.

METHODS/RESULTS: Human iPSC-derived microglia (iCell® Microglia; MGL), medium, and supplements were from FUJIFILM Cellular Dynamics. These cells were differentiated from an apparently healthy normal (AHN) male donor based on the protocol published by *Abud et al.* in 2017 (PMID: 28426964). Cytokine release and cell signaling assays were performed on iCell Microglia plated at either 45,000 cells/well (96-well plate) or 15,000 cells/well (384-well plate). Microglia were cultured for 3-days post-thaw to allow the cells to recover from cryopreservation. In this poster, we developed three different assay endpoints for microglia in mono-culture. First, we evaluated IL-6 secretion from microglia treated overnight with LPS (100 ng/ml) using an HTRF Kit (Revvity; 62HIL06PET). Stimulated microglia consistently yielded >1000 pg/ml of IL-6 with an EC50 value for LPS from 1-5 ng/ml. This assay was optimized and validated across multiple lots of iCell Microglia. Second, we measured IL-1β release using the Lumit Immunoassay (Promega; W6030). To trigger processing and secretion of mature IL-1β analyte, cells were sequentially treated with LPS (3 hours) and ATP (30 minutes). Third, a p-Syk assay was used to evaluate the TREM2-mediated signaling pathway using the THUNDER™ p-Syk (Y525/Y526) TR-FRET Assay Kit (BioAuxilium; KIT-SYKP-100). Robust signal was achieved following exposure to sodium pervanadate (0.1 mM for 30 minutes).

CONCLUSIONS / FUTURE DIRECTIONS: These data establish a foundation for studying cytokine release and cell signaling following inflammatory activation of AHN human iPSC-derived microglia. This provides a baseline for future studies on neuroinflammation, co-culture with iPSC-derived neurons and astrocytes, and disease modeling (i.e., iPSC-derived microglia expressing Alzheimer's Disease-relevant TREM2 or APOE mutations). Additionally, these assays provide robust readouts for cell activation that will enable discovery of new microglia modulating compounds with more physiological relevance and therapeutic potential.

Keywords: Microglia, iPSC, Cytokine

Characterization of iCell Microglia

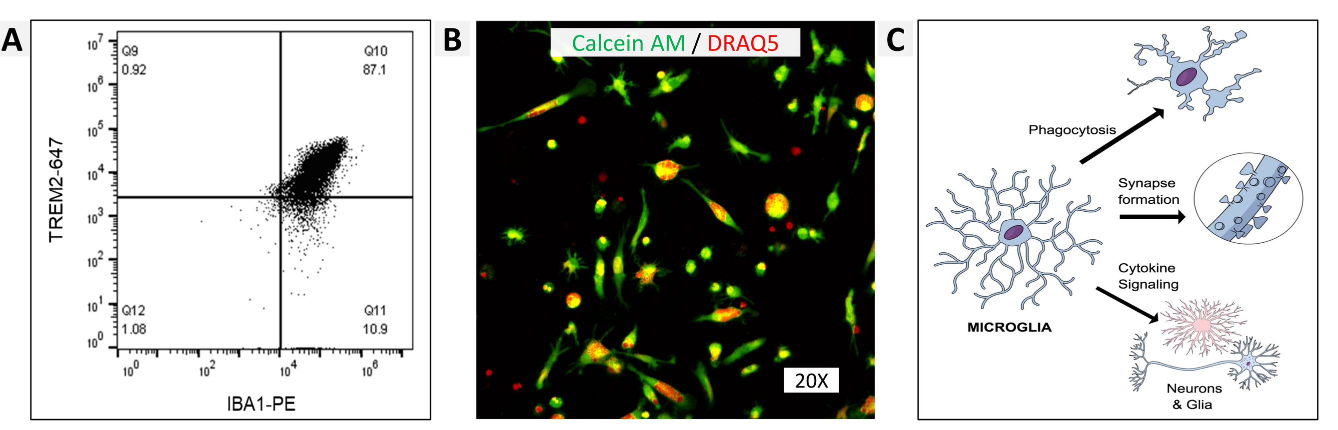
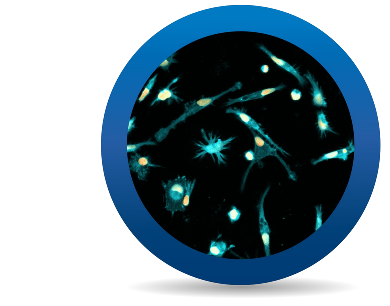


Figure 1. **(A)** iCell Microglia are a highly pure population of microglia derived from human induced pluripotent stem cells (iPSC). These MGL are differentiated based on technology developed by the Blurton-Jones laboratory (Abud et al. *Neuron* 2017) for which FUJIFILM Cellular Dynamics holds the exclusive license from UC-Irvine. **(B)** Cells are cryopreserved and provided as a product kit with optimized media with supplements that support cell viability and functionality. **(C)** iCell Microglia are a biologically relevant cell type amenable to research in the following areas: neuroinflammation and cytokine signaling, modeling Alzheimer's Disease, drug screening, and co-culture systems w/ neurons and astrocytes.



Key Features of iCell Microglia (Catalog # C1110)	
Quantity	≥1.0 M viable cells/vial
Morphology	Semi-adherent translucent cells with processes upon post thaw
Population	Mixture of amoeboid and ramified cells
Purity (at thaw)	CD33, CD45, TREM2 (Cell surface) P2RY12, TMEM119, CX3CR1, IBA1 (Intracellular)

Background on Cytokine Release

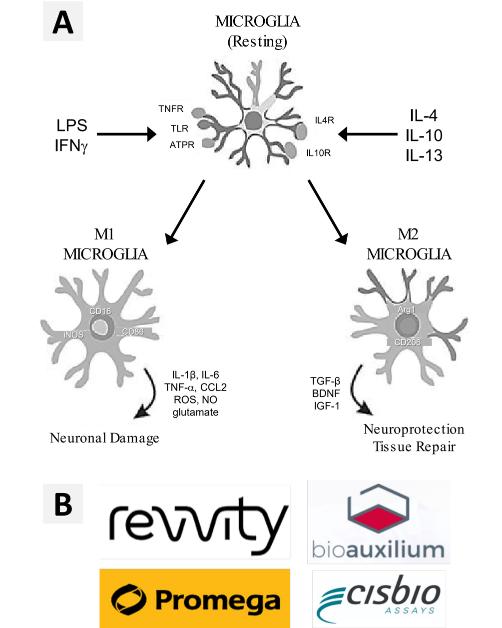


Figure 2. (A) Schematic of M1/M2 microglia polarization. M1 activation is induced by pro-inflammatory cytokines (LPS, IFNγ) and cells secrete cytokines like IL-6, IL-1β, and TNFα. M1 microglia favor the neuroinflammatory and oxidant state, which can lead to neuronal damage and death. Alternative M2 activation with IL-4, IL-10, and/or IL-13 increases expression of mannose receptor (CD206) and results in secretion of anti-inflammatory cytokines and other neurotrophic factors. M2 microglia exert both a tissue repairing/remodeling and neuroprotective function. **(B)** Various assay technology formats enable the detection and quantitation of released cytokines, including HTRF or THUNDER TR-FRET technology (cisbio, bioauxilium), Lumit (Promega), and AlphaLISA (Revvity).

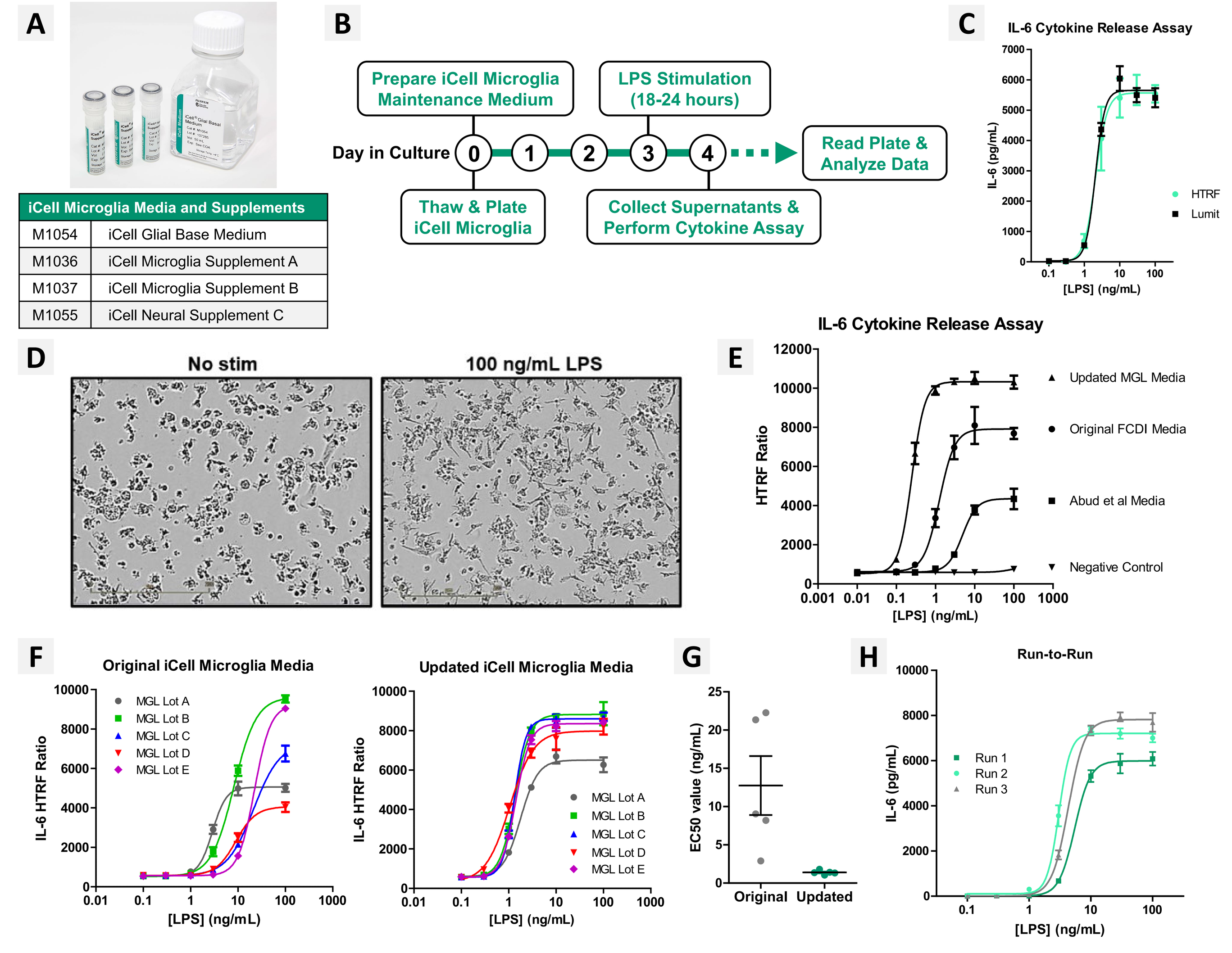
	W6030	W6032	W6031
Promega	100	500	1000
Price	$597	$2,234	$3,887
$/well	$5.97	$4.47	$3.89

	62HIL06PET	PEG	PEH
cisbio	96	500	10,000
Price	$652	$1,103	$10,290
$/well	$6.80	$2.21	$1.03

	KIT-IL6-100	-500	-2500	-5000	-10000
THUNDER	100	500	2,500	5,000	10,000
Price	$404	$707	$3,002	$5,104	$6,862
$/well	$4.04	$1.41	$1.20	$1.02	$0.69

	AL3160HV	AL3160C	AL3160F
Alpha	100	500	5,000
Price	$897	$2,261	$14,900
$/well	$8.97	$4.52	$2.98

Impact of Media Configuration on Cytokine Release



iCell Microglia Media and Supplements	
M1054	iCell Glial Base Medium
M1036	iCell Microglia Supplement A
M1037	iCell Microglia Supplement B
M1055	iCell Neural Supplement C

Figure 3. (A) Catalog info for iCell Microglia media with supplements. **(B)** Workflow schematic for the cytokine release assay. Note: timing may differ depending on the target analyte. **(C)** Both HTRF (Revvity) and Lumit (Promega) cytokine assay kits were used in this study. **(D)** Phase contrast images of iCell Microglia on Day 4 in culture after ~24 hours of LPS stimulation. Cells were plated on a PDL-coated plate at 15,000 cells/well. Images were acquired on an Incucyte SX5 instrument with a 10X objective. **(E)** Evolution of the IL-6 cytokine response to LPS for iCell Microglia in different media. **(F)** The original media provided by FCDI supported robust cytokine release, but recent adjustments to the media and supplements have resulted in higher degree of assay consistency across lots of iCell Microglia as demonstrated with IL-6 cytokine release. **(G)** More consistent EC_{50} values with LPS are observed with the updated media and **(H)** Optimized conditions for this assay were performed across three independent runs with the same lot of iCell Microglia. Secreted IL-6 present in the supernatants from LPS-stimulated cells were detected with HTRF assay. Quantifying the amount of IL-6 (pg/ml) is accomplished by using a standard curve. Importantly, the iCell media and supplements provided with the cells are key to good assay performance.

Optimization of IL-6 Cytokine Release Assay

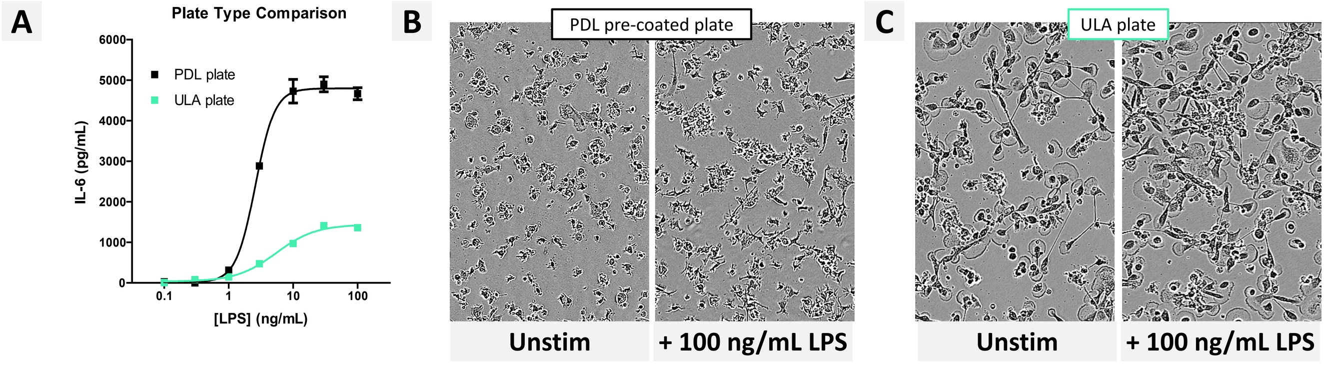


Figure 4. Plate type comparison. (A) The plate type and/or ECM can influence cytokine release responses. Phase contrast images of iCell Microglia on Day 4 in culture after ~24 hours of LPS stimulation plated at 15,000 cells/well using a (B) CELLCOAT® Poly-D-Lysine (PDL) pre-coated plate (Greiner 655946) or a (C) ultra-low attachment (ULA) plate (Corning 3474). Images were acquired on an Incucyte SX5 with a 20X objective.

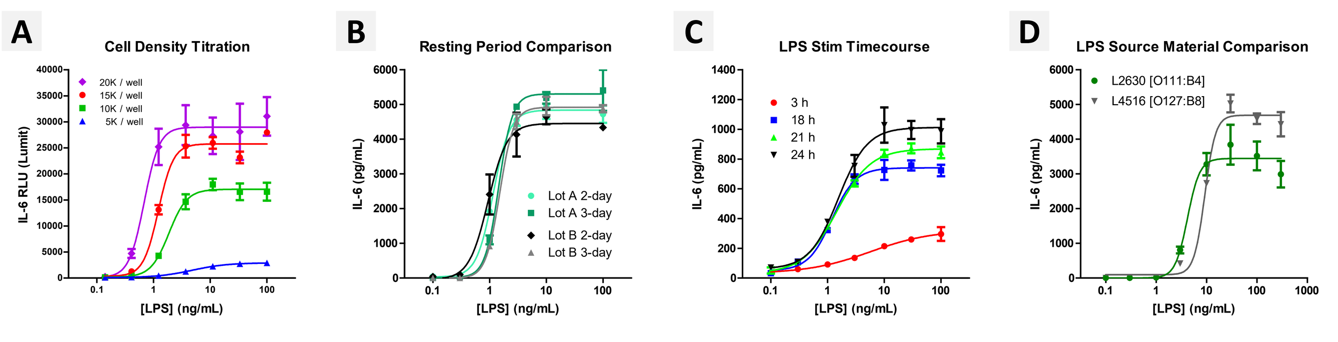


Figure 5. Cell-based Assay Variables. IL-6 cytokine release assay responses from LPS-stimulated iCell Microglia when varying: (A) cell density, (B) cell rest period prior to stimulation (comparing two different lots of iCell Microglia), (C) LPS stimulation time, and (D) LPS source material (both from Sigma-Aldrich, but different *E. coli* strains).

Different Cytokines, Stimulation Conditions, and Assay Media

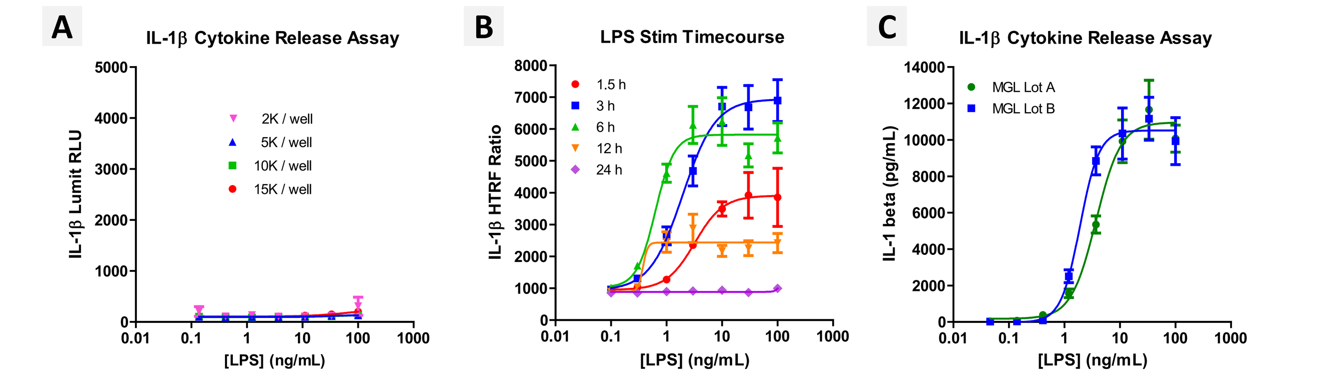


Figure 6. IL-1β Cytokine Release Assay. LPS stimulation conditions are not universal across all cytokines. (A) The 24-hour stimulation time optimized above for the IL-6 assay does not support IL-1β release, no matter the cell density. (B) A shorter LPS stimulation time (3-6 hours) is recommended for this cytokine. Moreover, addition of ATP (1 mM; 30 min prior to assay) on top of the LPS stim is required to release IL-1β from the cells. (C) Under these optimized stimulation conditions, IL-1β cytokine release assay results are consistent across lots of iCell Microglia.

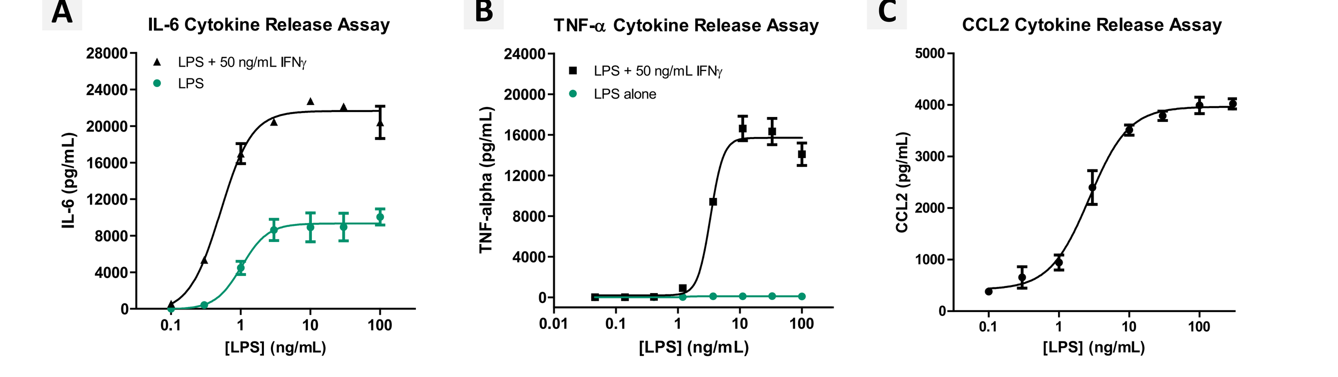


Figure 7. Additional Assay Conditions. Stimulation of iCell MGL with LPS in the presence of 50 ng/mL IFNγ (A) increases the release of IL-6 (Thunder) and (B) is required for TNFα release (Lumit). (C) CCL2/MCP-1 can also be detected upon pro-inflammatory LPS stimulation (Revvity). (D) Response of microglia to LPS and subsequent IL-6 release (Revvity) differs when cells are cultured in alternative medias that are designed to support co-culture experiments. MGL function fine in tri-culture (blue), but not neuron media (green).

Cytokine Release Profiles from AD TREM2 Microglia

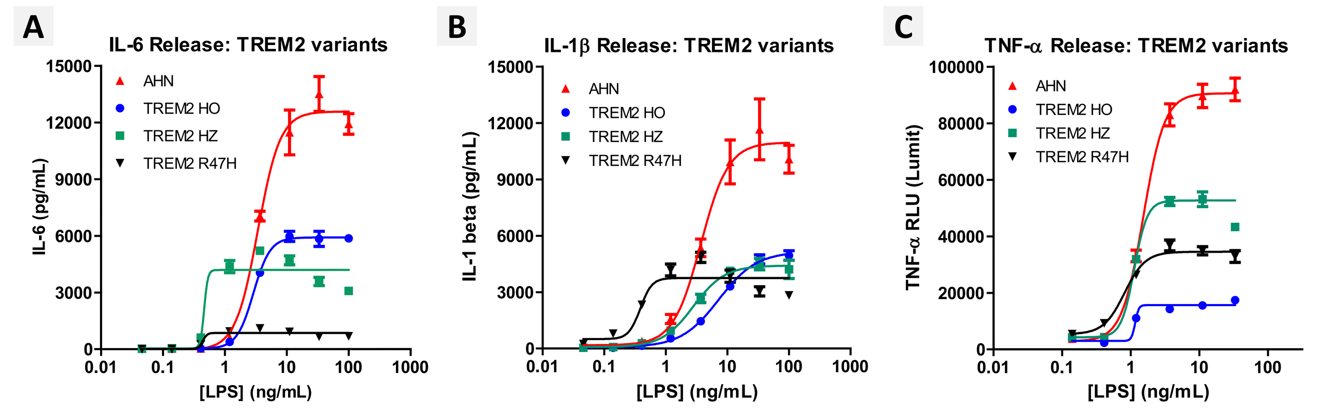


Figure 7. Alzheimer's Disease Modeling. Microglia were differentiated from iPSC with either engineered TREM2 mutations to create functional HO and HZ knockouts or from donor-derived iPSC cells harboring TREM2 R47H mutation. These TREM2 lines were tested separately against TREM2 WT (AHN) iCell Microglia in the cytokine release assays presented here: (A) IL-6 (24 h LPS stim), (B) IL-1β (3 h LPS stim + 30 min ATP incubation), and (C) TNF-α (24 h LPS stim with fixed 50 ng/mL IFNγ). TREM2 variants all displayed reduced cytokine release overall compared to AHN, but the dose-dependent LPS response profiles differed significantly for a given cytokine and did not follow a consistent rank ordering. Take the R47H mutant for example, which barely released any IL-6 but was the most sensitive to IL-1β. The ability to measure and quantify cytokines in this assay format is a powerful approach to understanding cell function.

Anti-inflammatory Cytokine Secretion

Figure 8. M2 activation. LPS treatment can also stimulate release of the anti-inflammatory cytokine, IL-10. As expected, iCell Microglia secreted IL-10 upon overnight stimulation with LPS (Revvity). While these cells should also release IL-10 (and other anti-inflammatory cytokines) when treated with M2 activating agonists such as IL-4 and IL-13, initial attempts to capture this response have not yet been successful. Further optimization steps (beyond cell density, media, plate type, timing, day of assay, stim conditions, etc.) are ongoing.

Detection of p-Syk in iPSC-derived Microglia

Figure 9. Cell Signaling. In addition to cytokine release data, interrogating cell signaling events in iCell Microglia is also of importance. Specifically, spleen tyrosine kinase (SYK) is a central intracellular regulator of microglial activation and has been identified as the central kinase that instructs effector functions downstream of TREM2. We have screened many immunoassay kits for detection of phospho-Tyr525/526 and have explored different methods for inducing phosphorylation. This project is still ongoing, but we have found that treating cells with a sodium pervanadate solution for 30 min results in robust p-Syk TR-FRET signal, which serves as a positive control.

Summary and Future Directions

Microglia are responsible for surveillance in the brain and can respond to a number of different homeostatic or pathological states. Microglia can be stimulated to produce pro-inflammatory cytokines like IL-1β, IL-6, and TNF-α. Recent findings suggest that microglial activation and cytokine release are important targets in the treatment of Alzheimer's Disease. Human iPSC-derived microglia are a unique cell type and critical for the study of neuroinflammation in the brain. iCell Microglia from FUJIFILM Cellular Dynamics, Inc. are an excellent option for performing such experiments. Data presented here showcase the development of physiologically-relevant assays to investigate the role that healthy microglia and TREM2 variants might play with respect to neuroprotection or neuro-degeneration. The next step is to establish more complex co-culture systems with iPSC-derived neurons and astrocytes for deeper insight into the biology.