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Using iCell® Microglia to Interrogate NLRP3-mediated Inflammation

iCell Lab Note

Introduction.

Activation of NLRP3 in microglia results in neuroinflammation that is hypothesized to be the driving force in many neurodegenerative diseases. NLRP3-mediated release of pro-inflammatory cytokines occurs via a two-step process: (1) "priming", triggered by LPS to promote NF-κB gene transcription, NLRP3 expression, and production of the pro-peptides for IL-1β and IL-18; and (2) "activation", which is prompted by molecules like ATP or Nigericin to start NLRP3 inflammasome assembly and subsequent activation of caspase-1. This in turn initiates the processing and release of IL-1β and IL-18 in their mature and active forms. This iCell Lab Note describes the use of iCell Microglia together with bioluminescent assay technologies from Promega to monitor NLRP3 inflammasome activity. This *in vitro* model system has high potential for evaluating the role of microglia in neuroinflammation, for interrogating the connections between inflammatory cascades and genetic risk factors (such as TREM2 or APOE), and for identifying new therapeutic drug targets.

Results.

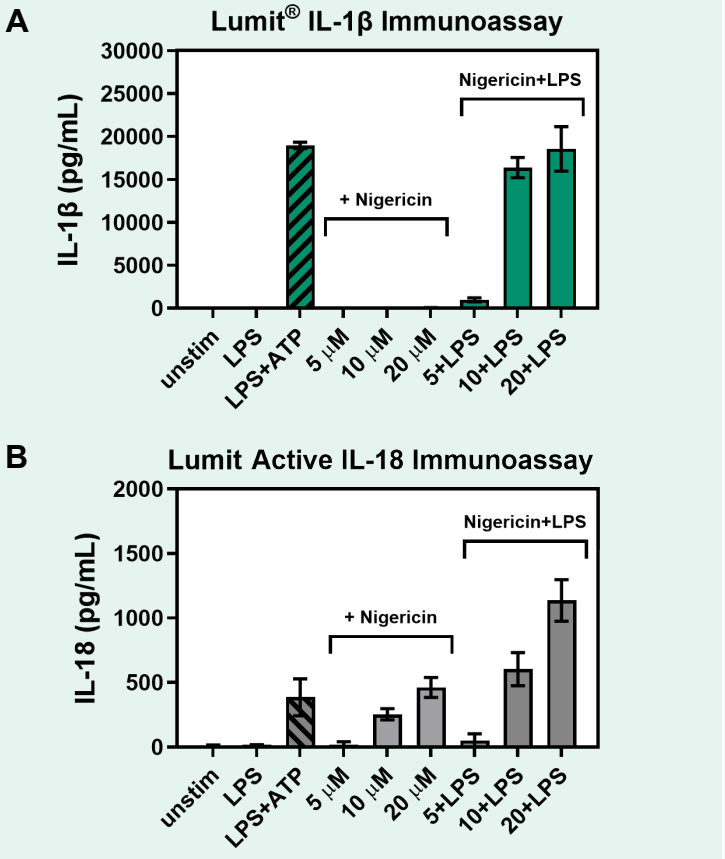


Figure 1. Immunoassay detection of IL-1β and IL-18. (A) Secretion of IL-1β by iCell Microglia is only detected following sequential stimulation with LPS (100 ng/ml) and ATP (1 mM) or Nigericin (10-20 μM), but not with either molecule alone. **(B)** IL-18 does not require LPS priming step as it is released into cell culture media following treatment with Nigericin alone. Cytokine levels are further increased in combination with LPS treatment. *Note:* The amount of IL-1β released (pg/ml) is ~10X greater than IL-18.

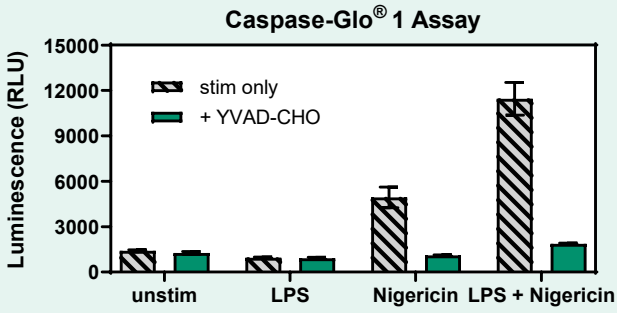


Figure 2. Detection of Caspase-1 Activity. Priming with LPS alone (100 ng/ml for 3 h) is not enough to measure activation of caspase-1 in iCell Microglia. Instead, cells require exposure to Nigericin (20 μM for 1 h) for robust caspase-1 activity. Importantly, "YVAD inhibitor" (in green) decreases the signal to show specific inhibition of cellular protease activity.

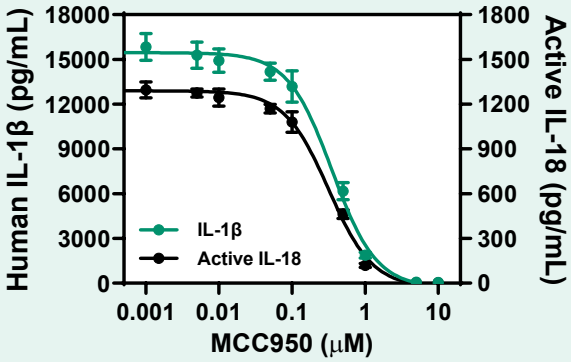
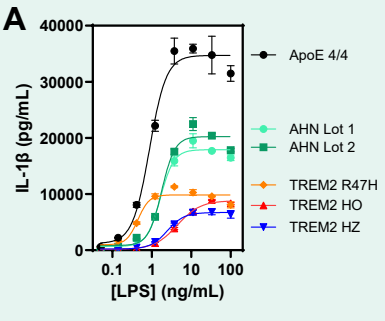


Figure 3. Inhibition of NLRP3 Inflammasome Activity. iCell Microglia were pre-treated with LPS (100 ng/ml for 2 h) prior to adding the NLRP3 inhibitor, MCC950 (1 h), followed by Nigericin (20 μM for 1 h). MCC950 inhibited NLRP3 activity, significantly reducing release of IL-1β and active IL-18 (IC_{50} values of 0.35 μM and 0.32 μM, respectively).



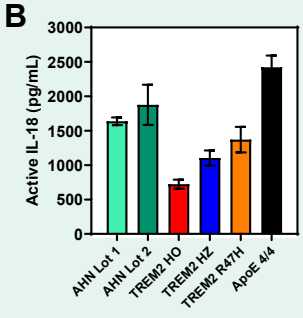


Figure 4. Impact of AD risk-associated genotypes on the NLRP3 pathway. iCell Microglia with various Alzheimer's Disease mutations (genetically-engineered or patient-derived) were tested for NLRP3-mediated inflammation readouts IL-1β and IL-18. Compared to AHN (apparently healthy normal) microglia, cytokine levels were increased dramatically in ApoE 4/4 cells and significantly decreased with all three TREM2 variants. Interestingly, R47H mutant was more sensitive to priming with LPS than the TREM2 knockout microglia (i.e., at 1 ng/mL LPS).

Methods.

Prepare complete iCell Microglia Maintenance Medium and thaw iCell Microglia according to the Quick Guide.

- On Day 0, seed iCell Microglia onto a PDL pre-coated 96-well plate at a density of 20,000 cells per well in 100 ul.
- Incubate cells at 37ºC / 5% CO_2 for 3 days (until assay).
- On Day 3, stimulate cells first with LPS (1-100 ng/ml) for 3 h and then with Nigericin (20 µM) for 1 h.
  - For IL-18 detection, the human IL-18 binding protein labeled with Peptide β is also added at the time of cell stimulation.
  - For inhibition assays, add compound after 2 h of LPS treatment.
- Collect cell culture supernatants and test immediately or store at -20ºC for future analysis.
  - Perform Lumit immunoassay for detection of IL-1β or IL-18 following the manufacturer's instructions.
  - Measure caspase-1 activity from either culture media or directly in cells.
- Determine the amount of cytokine released (pg/ml) using a standard curve.
- Refer to the Lumit immunoassay kit instructions for complete details.

Table 1. Materials Needed

Product	Vendor	Cat. #
iCell® Microglia, 01279 Kit	FCDI	R1131
• iCell Microglia (cryopreserved cells) *	(incl. in kit)	C1110
• iCell Glial Base Medium	(incl. in kit)	M1054
• iCell Microglia Supplement A	(incl. in kit)	M1036
• iCell Microglia Supplement B	(incl. in kit)	M1037
• iCell Microglia Supplement C	(incl. in kit)	M1055
LPS from E. coli O127:B8	Sigma	L4516
Nigericin Sodium Salt	InvivoGen	tlrl-nig
MCC950 – NLRP3 Inhibitor	InvivoGen	Inh-mcc
Caspase-Glo 1 Inflammasome Assay	Promega	G9951
Lumit IL-1β Human Immunoassay	Promega	W6010
Lumit Active IL-18 Immunoassay	Promega	Early Access
96-well Poly-D-Lysine (PDL) plate	Greiner	655946

* iCell Microglia with AD-relevant genotypes are available at fujifilmcdi.com.

Summary.

NLRP3 inflammasome engagement in CNS microglia is an emerging drug target in neurodegenerative disease. This iCell Lab Note presents an accessible human-relevant *in vitro* model system for interrogating NLRP3 inflammasome activation, subsequent inflammatory events, and compound testing for pathway modulation. A stimulation paradigm is provided for robust inflammasome activation and downstream events, specifically activation of caspase-1, and secretion of active IL-18 and IL-1β cytokines. In addition, iCell Microglia with TREM2 and APOE variants show differences in NLRP3 engagement, suggesting a connection between pathway activation and Alzheimer's Disease.

Highlights.

- iCell Microglia can be used as a human-relevant model to study NLRP3 inflammasome pathway activation as a therapeutic drug target.
- Stimulation paradigms that activate the inflammasome are provided. NLRP3-mediated cytokine release can be robustly detected via Lumit immunoassays.
- Multiplexing with Caspase-Glo 1 Inflammasome Assay offers mechanistic insights.
- Notable differences in NLRP3 pathway activation are observed in AD iCell Microglia.

Scan here to download the iCell Microglia Quick Guide

Contact **Technical Support** for more protocol details and supportive data.
FCDI-Support@fujifilm.com

www.fujifilmcdi.com
FUJIFILM Cellular Dynamics, Inc.
(608) 310-5100 | Toll-free US (877) 310-6688

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