Generation and Functional Characterization of iCell® Microglia From Human Induced Pluripotent Stem Cells

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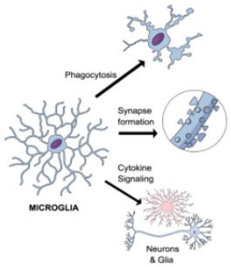
Abstract

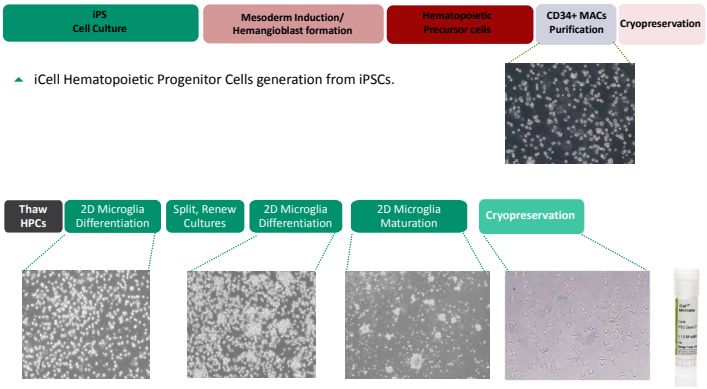
Microglia maintain immunological balance within the central nervous system by decreasing inflammation due to injury and buildup of cytotoxic substances and infectious material. Microglia research has been largely confined to rodents because human primary microglia are difficult to acquire and stably maintain in culture conditions. Here, we describe the generation, functional characterization and cryopreservation of human iCell Microglia from episomally reprogrammed iCell Hematopoietic Progenitor Cells (HPCs; proprietary technology) under defined conditions based on technology developed by the Blurton-Jones laboratory exclusively licensed to FUJIFILM Cellular Dynamics, Inc. from the University of California-Irvine. iCell Microglia maintain purity, secrete immunomodulatory cytokines and phagocytose pHrodo-labelled bacterial bioparticles and amyloid beta (Aβ) fibrils. The ability to produce essentially limitless quantities of iCell Microglia holds great promise for accelerating human neuroscience research into the role of microglia in normal and diseased states.

Microglia

Origin	From a mesodermal yolk sac progenitor
Residence	10-15% of brain
Appearance	Small cell body and extended processes
Personality	Territorial 15 - 30 µm wide
Movement	1.5 µm/min and represent a sophisticated scanning system in the brain
Function	Professional phagocytosis
Renewal	28% per year or 0.08% per day. A self sustaining population in the brain
Life span	4.2 years



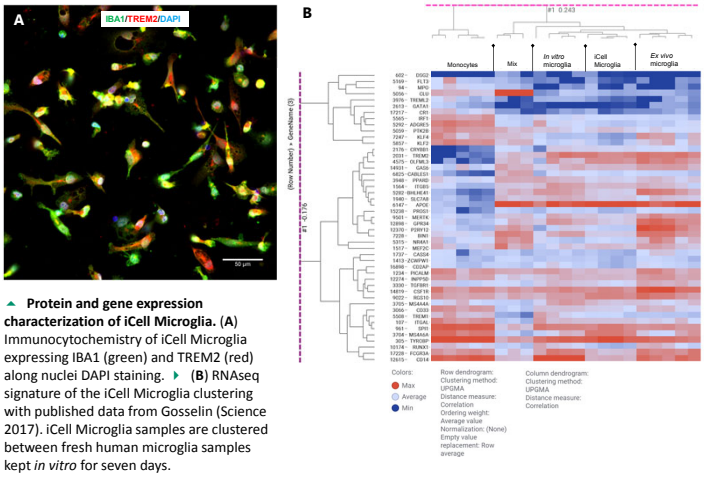
Generation of iCell Microglia



▲ iCell Hematopoietic Progenitor Cells generation from iPSCs.

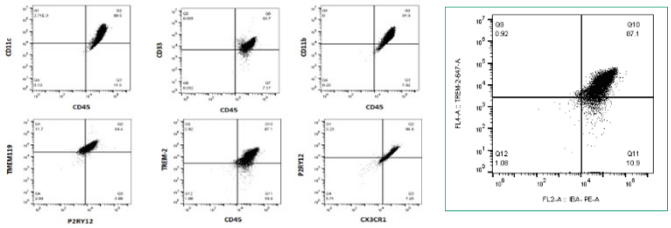
▲ iCell Hematopoietic Progenitor Cells are differentiated into iCell Microglia according to Abud *et al.* (2017).

Characterization of iCell Microglia

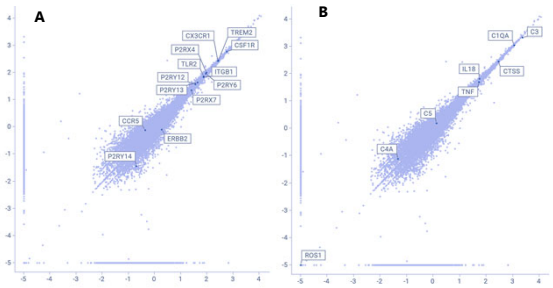


▲ **Protein and gene expression characterization of iCell Microglia.** (**A**) Immunocytochemistry of iCell Microglia expressing IBA1 (green) and TREM2 (red) along nuclei DAPI staining. ▶ (**B**) RNAseq signature of the iCell Microglia clustering with published data from Gosselin (Science 2017). iCell Microglia samples are clustered between fresh human microglia samples kept *in vitro* for seven days.

Purity of Cryopreserved iCell Microglia

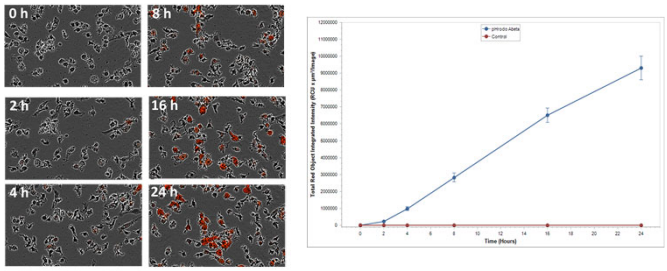


▲ **Purity of iCell Microglia.** Cryopreserved cells were stained for the presence of cell surface (CD45, CD11β and CD33) and intracellular (P2RY12, TREM-2, CX3CR1, IBA) antigens by flow cytometry. The specific staining is compared against matched isotype controls.



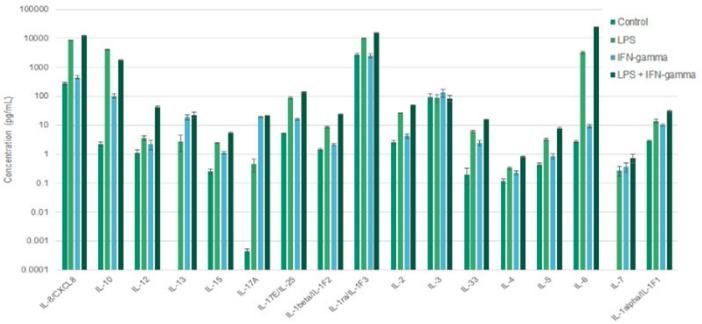
◀ **Expression of neuro-inflammation related genes.** (**A**) iCell Microglia gene expression of cell surface pain markers (Inoue & Tsuda, Nature Reviews 2018), and (**B**) iCell Microglia gene expression of diffusible factors. Samples were taken from iCell Microglia after 3 days post-thaw; graphs are made comparing different lots.

Phagocytic Function of Cryopreserved iCell Microglia



▲ **iCell Microglia can phagocytose amyloid beta particles.** Phagocytosis of pHrodo Red dye-labeled amyloid beta (1-42) particles was monitored over time using an IncuCyte S3 live-cell analysis system.

Cytokine Profile of Cryopreserved iCell Microglia



▲ **Cytokine Simulation of iCell Microglia**. Cells were thawed, plated in maintenance media and allowed to recover for 48 hr. The cells were starved for 12 hr prior to stimulating them with LPS, interferon gamma or a combination of LPS and interferon gamma for 24 hr. Cell culture supernatants were collected and assayed on a multiplex Luminex system.

Summary

- iCell Hematopoietic Progenitor Cells can be successfully differentiated to microglia according to the protocol outlined by Abud, *et al.*, Neuron 2017.
- Successful cryopreservation and recovery of end stage mature microglia was achieved.
- Cryopreserved microglia retain high purity and phagocytosis function.
- Generation of large-scale batches of iCell Microglia enable cell-based applications.

iCell Microglia	
Purity	>80% P2RY12+/ IBA+/ TREM2+/ CX3CR1+
Other markers	CD45+/ CD33+/ CD11b+/ PU.1+
Function	Phagocytosis of bacteria, Neuro2A and Aβ particles
Cytokine profile	Anti- and Pro-Inflamamtory