Characterisation of Human Derived Microglial Cells

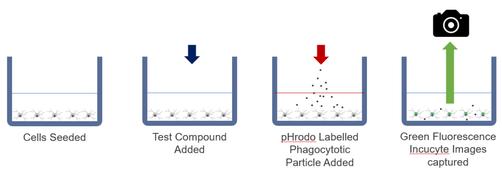
Kerry Shea, Gayle Marshall, Andrzej Rutkowski, Sally Price and Peter Simpson.
Medicines Discovery Catapult, Block 35, Alderley Park, Cheshire, SK10 4TG

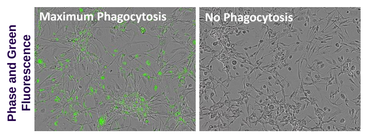
Introduction

- Healthy functioning of cells within the Central Nervous System (CNS) requires a homeostatic microenvironment. This homeostasis can be disrupted by bodies such as protein aggregates (E.g. β-Amyloid plaques, α-Synuclein) and apoptotic neurons, leading to both acute and chronic inflammation, or neuroinflammation.
- Microglia make up 10-20% of the total CNS cell population and are the resident immune cell responsible for the CNS immune response. In a resting state they survey their microenvironment for threats to homeostasis. Once activated they employ a number of phenotypic responses to protect neuronal function (migration towards perceived threats, proliferation, cytokine release and phagocytosis), all of which can be measured to determine a compound's effect on neuroinflammation.
- This work describes development of a phagocytosis assay using a variety of phagocytotic particles (E. coli or Zymosan bioparticles, β-Amyloid or α-Synuclein peptides or apoptotic cells) labelled with the pH sensitive pHrodo dye that exhibits an increase in fluorescence when within the acidic environment of the phagosome. The assay was used to functionally profile microglia cells from a variety of sources, including both mouse and human immortalised cell lines and human primary and iPSC derived cells.

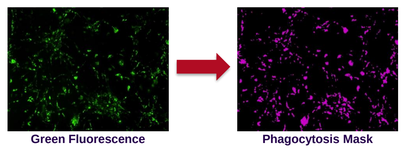
Principles of Assay

- A panel of microglial cells were seeded into transparent bottomed 96 or 384 well plates and incubated at 37°C/ 5% CO_2
- Test compounds (either DMSO or aqueous solution) were acoustically dispensed using an Echo555 and pre-incubated with the cells for 0.5 – 1 hour
- The appropriate pHrodo labelled particle was dispensed into the well containing cells and compound and the plate placed on an IncuCyte® S3 Live Cell Analysis System
- Phase and either green or red fluorescent images were captured at intervals for later analysis





- An algorithm was constructed to generate a mask defining regions of high fluorescence.
- The total fluorescent area was calculated per image and used as a measure of the level of phagocytosis



Profiling of Human Microglia Cell Models

Summary of Phagocytosis Functionality

- The following table illustrates the various combinations of microglial cell types and pHrodo labelled particles/ cells assayed for phagocytotic activity:

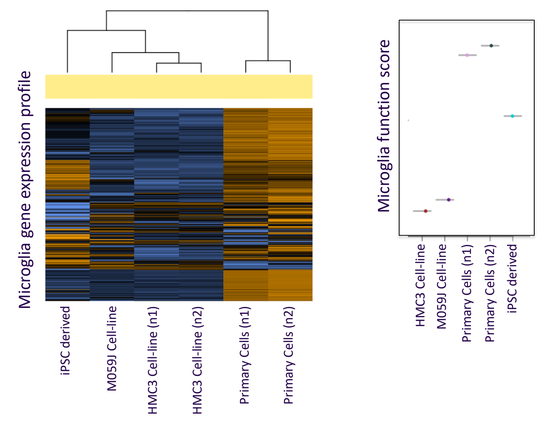
Microglial Cell Type	E.Coli Bio-particles	Zymosan Bio-particles	β-Amyloid Peptide	α-Synuclein Peptide	Apoptotic Cells
C8-B4 (Mouse Immortalised Cell Line)	Yes	ND	ND	ND	ND
HMC3 (Human Immortalised Cell Line)	No	No	ND	ND	No
Human iPSC Derived Microglia	Yes	Yes	Yes	Yes	Yes
Human Primary Cells	Partial	ND	Partial	Partial	ND

ND = Not determined

- Of the human models examined the iPSC derived microglia were the most useful for compound screening purposes with high levels of phagocytosis of all particles tested.
- The HMC3 cell line was not functional in this assay and did not phagocytose any of the particles tested.
- The primary cells were a heterogeneous population with only a small subset of them phagocytosing the test particle.

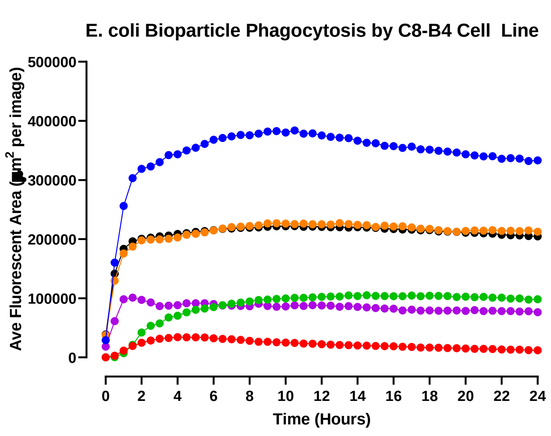
Nanostring Neuroinflammation Pathway Analysis

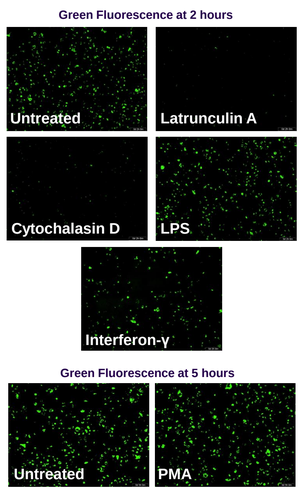
- mRNA was profiled in the human microglial cells and a glioblastoma cell line (M059J).
- Nanostring gene expression analysis exhibited a high microglia score in the primary cell lines, but low levels in all the immortalised cell lines. The profile of the iPSC derived microglia was closer to that of the primary cells than either of the immortalised cell lines.



E. Coli Bioparticle Phagocytosis in a Mouse Model

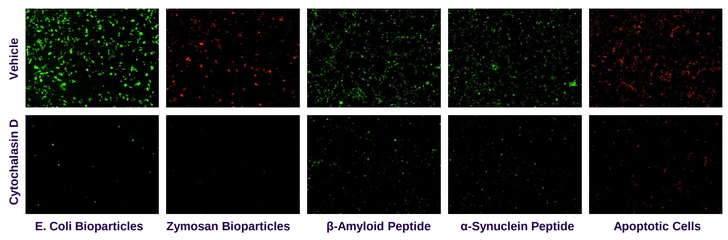
- Phagocytosis of E. coli bioparticles was examined in the mouse immortalised cell line, C8-B4, in response to a selection of compounds: 100nM Phorbol 12-myristate 13-acetate (PMA), 1μM Latrunculin A, 1μM Cytochalasin D, 0.01ng/mL Interferon-γ and 10μg/mL Lipopolysaccharide (LPS).

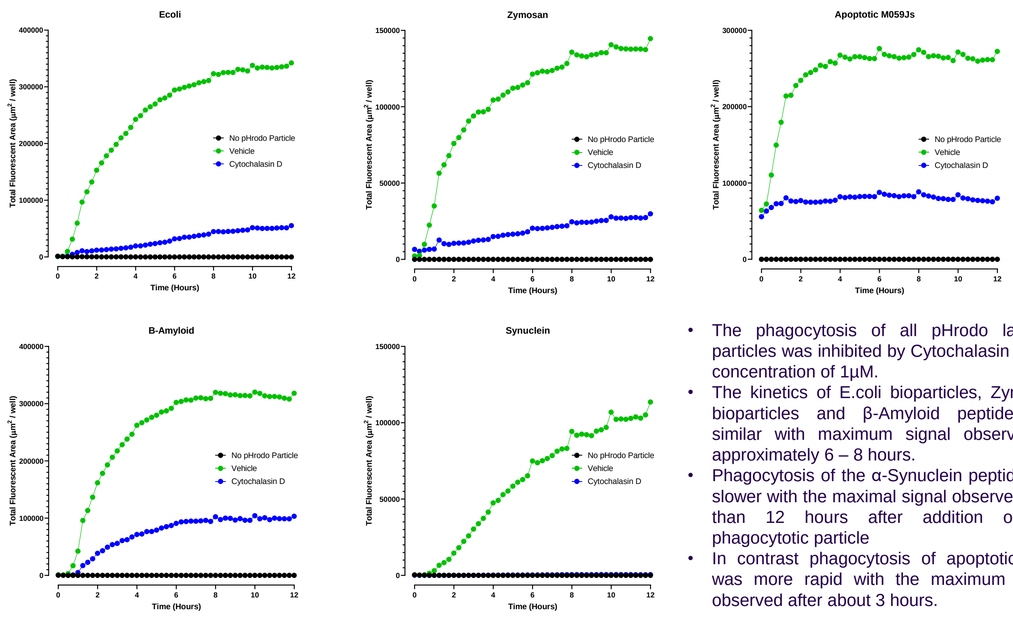




Phagocytosis in a Human Model

- Human iPSC derived microglia were assayed with the following pHrodo labelled particles for phagocytotic activity over 12 hours.
  - E. coli bioparticles, β-Amyloid peptide and α-synuclein peptide was labelled with pHrodo Green
  - Zymosan bioparticles or apoptotic M059J cells (Glioblastoma cell line treated with doxorubicin) were labelled with pHrodo Red
- Phagocytosis was inhibited using the actin polymerisation inhibitor, Cytochalasin D (1μM), to ensure the specificity of the signal for phagocytosis activity.

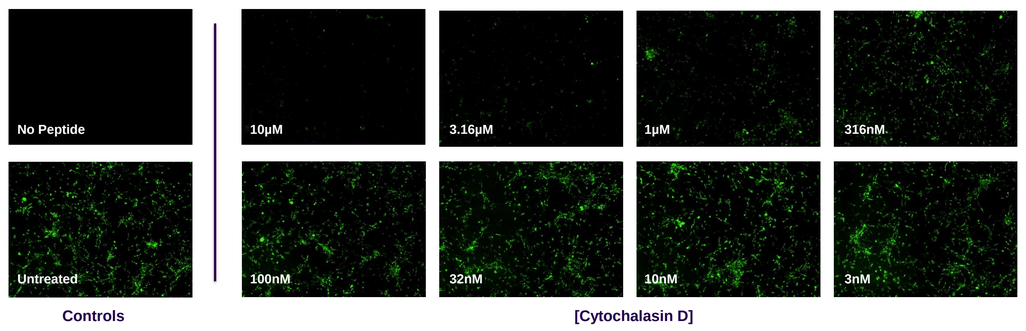


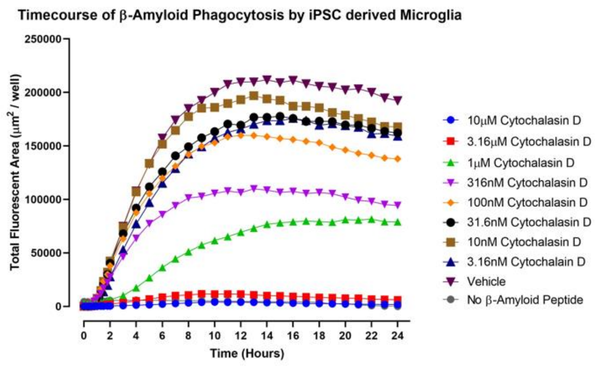


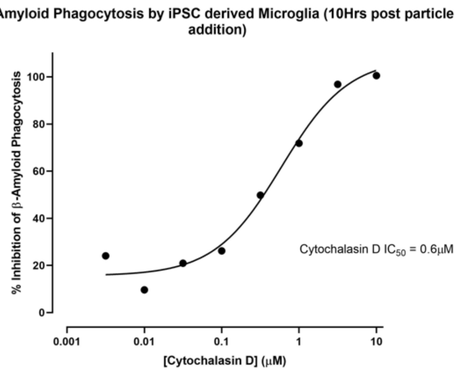
- The phagocytosis of all pHrodo labelled particles was inhibited by Cytochalasin D at a concentration of 1μM.
- The kinetics of E.coli bioparticles, Zymosan bioparticles and β-Amyloid peptide was similar with maximum signal observed at approximately 6 – 8 hours.
- Phagocytosis of the α-Synuclein peptide was slower with the maximal signal observed later than 12 hours after addition of the phagocytotic particle
- In contrast phagocytosis of apoptotic cells was more rapid with the maximum signal observed after about 3 hours.

Inhibition of β-Amyloid Phagocytosis in Human iPSC derived Microglia

- Phagocytosis of β-Amyloid peptide was examined in human iPSC derived microglia cells over a 24 hour time period where the maximal signal was detected at approximately 10 hours post addition of the β-Amyloid peptide.
- The potency (as determined by it's IC_{50}) of the prototypical inhibitor of phagocytosis, the actin depolymerisation inhibitor Cytochalasin D, was determined at this 10 hour time point.







- Phagocytosis of β-Amyloid peptide was inhibited in a dose dependent manner by Cytochalasin D with an IC_{50} of approximately 0.6μM.

Summary/ Future Direction

- An assay to measure inhibition (or stimulation) of phagocytosis has been developed in an iPSC derived microglial cell model using a variety of different pHrodo labelled phagocytotic particles (E. coli bioparticles, Zymosan bioparticles, β-Amyloid peptides, α-synuclein peptides and apoptotic cells).
- iPSC derived microglia are more microglia like than typically used cell lines but are practically more suitable for running assays than primary cells and therefore provide a superior model for studying neuroinflammation.

- Future plans include the following:
  - Further developing this assay in a multiple cell type co-culture and/ or 3D environment and to examine whether this moves the microglia profile closer to that of physiological microglia.
  - Comparing different amyloid peptides and forms for toxicity and phagocytosis.
  - Measuring cytokine release and microglial migration.
  - Examining expression of microglial markers by IHC and comparing to human microglia in both normal and diseased brains.

Medicines Discovery Catapult

- The Medicines Discovery Catapult (MDC), funded by Innovate UK, is a recently established national centre set up to help UK SMEs, biotechs, academics and innovators with access to lab facilities, knowledge, technologies, data and networks they need to progress their drug discovery programs.
- Through collaborative programmes of R&D we are tackling the most challenging issues in drug discovery, addressing systemic problems and bottlenecks and using innovative technologies to enable "fast-to-patient" medicines discovery.

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