Evaluation of human induced pluripotent stem cell (hiPSC)-derived tri-culture as in vitro model for neuroinflammation

Isabel Onofre, Chiara Formica, Leo Smit, Joyson Dcosta and Shushant Jain
Ncardia BV Leiden, The Netherlands

No conflicts of interest

Background

The interplay between the immune and nervous systems is a critical aspect of various neurodegenerative diseases (NDD), but the development of physiologically relevant models mimicking the intercellular interactions remains a challenge. To overcome this, human induced pluripotent stem cell technology can be leveraged to build multicellular models and bring human pathophysiology into early drug discovery to develop new therapeutics targeting neuroinflammation.
The development and validation of models of relevant biological disease processes, such as microglia-neuron communication provides insight on cellular interactions that play a role in recognizing apoptotic neurons and modulating neuronal activity which are crucial events in disease progression. Targeting these pathways in human models with a combination of readouts allows interrogation and evaluation of the ability of therapeutics on rescuing primary, secondary and tertiary neuro-pathological signatures.

In this study, Ncardia used the tri-culture approach to developed an *in vitro*, iPSC-derived tauopathy assay by inducing phosphorylation (phospho-Tau) and aggregation of Tau, with recombinant mutant Tau PFFs (pre-formed fibrils) and Tau seeds extracted from AD patient brain tissue. This approach enabled a multi-parametric readout of neuronal and glial phenotypes including activation of microglia and astrocytes in the tri-culture.

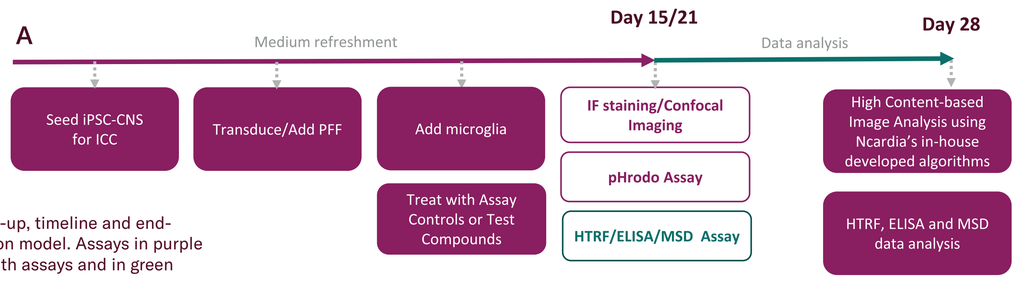
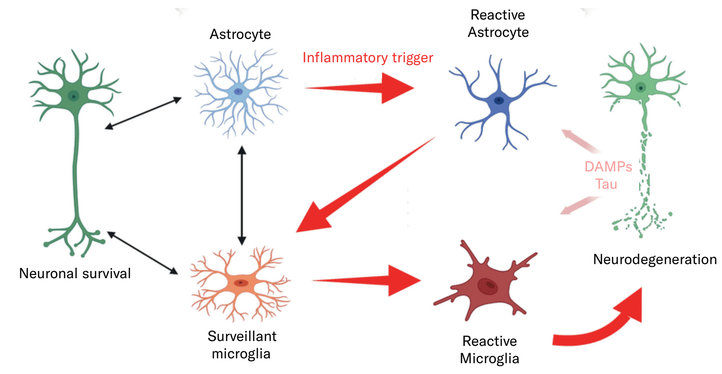


Figure A shows the experimental set-up, timeline and end-points of Ncardia's neuroinflammation model. Assays in purple can be combined and multiplexed with assays and in green



Cross-talk between neurodegeneration and neuroinflammation
- Neuronal homeostasis relies on astrocytes and microglia activation status, that is disturbed in pathogenic conditions as neurodegeneration
- Diseased neurons secrete factors that activate microglia and astrocytes, initiating a cascade of inflammatory triggers that induce further neurodegeneration and neuroinflammation

Adapted from:
Ullah, F., Gamage, R., Sen, M.K. et al. The Effects of Modified Curcumin Preparations on Glial Morphology in Aging and Neuroinflammation. Neurochem Res 47, 813–824 (2022). https://doi.org/10.1007/s11064-021-03499-4

1. Tri-culture model: neurons, astrocytes and microglia

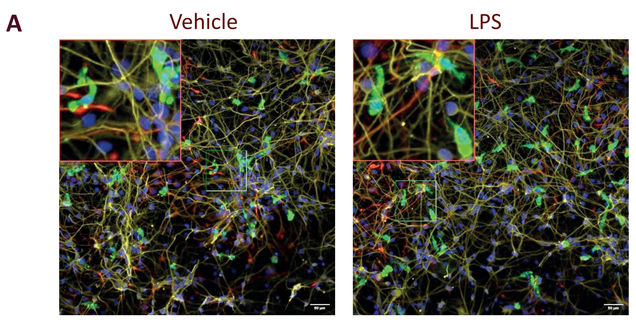


Figure A Fluorescent images characterizing human iPSC derived tri-cultures; IBA1 (green), MAP2 (yellow), GFAP (red) and DAPI (blue). 20x magnification

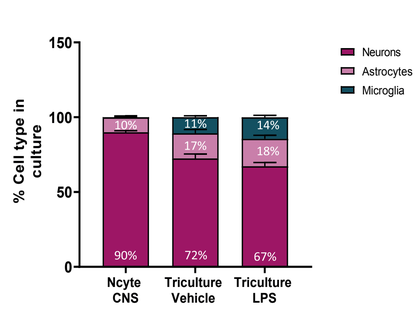


Figure B Tri-culture is composed of neurons (~70%), astrocytes (~17%) and microglia (~11%) at day 15. Microglia proliferate upon treatment with LPS, duplicating the population up to 14%.

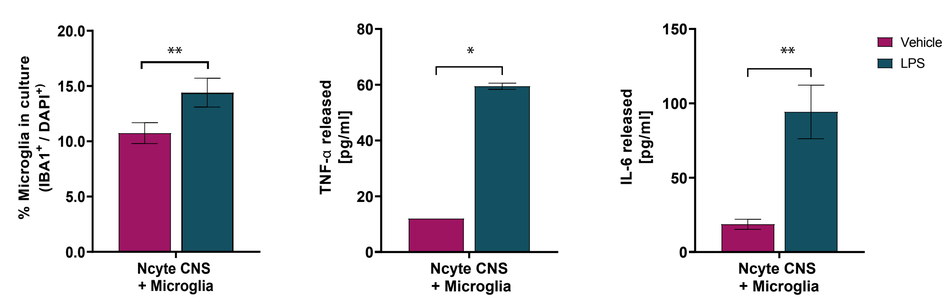


Figure C Microglia in tri-culture model are activated by treatment with LPS, becoming proliferative and releasing pro-inflammatory cytokines such as TNF-α and IL-6 measured by Homogeneous Time Resolved Fluorescence (HTRF) (n=3, *p<0.05, **p<0.01 unpaired t-test).

Ncardia's tri-culture model showed functional activation of microglia upon stimulation with LPS

2. Induction of neuroinflammation as a model for AD using TAU PFFs

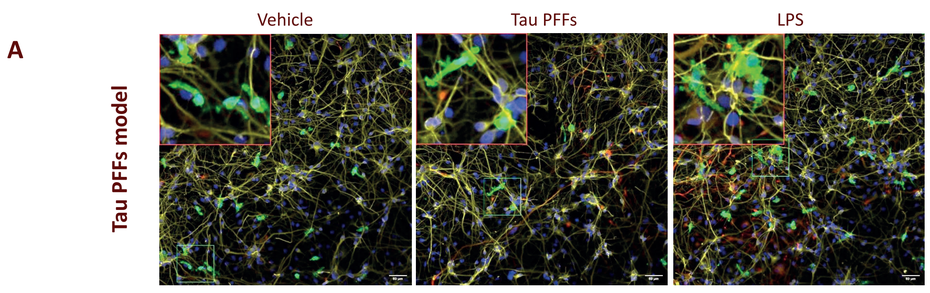


Figure A Fluorescent images of transduced day 15 post-thaw tri-cultures stained for IBA1 (green), GFAP (red), MAP2 (yellow) and DAPI (blue), treated with Tau PFF or LPS (20x magnification)

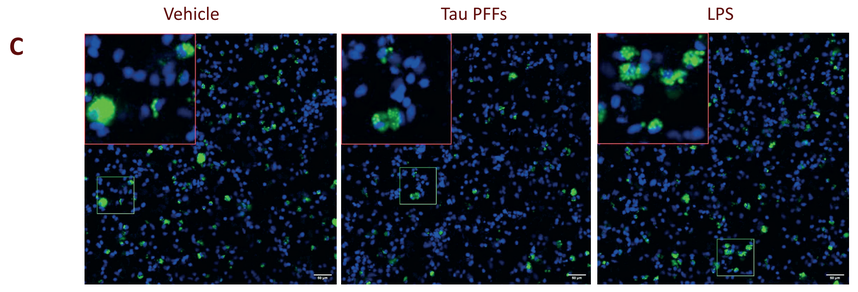


Figure C Fluorescent images from phagocytosis assay : tri-cultures were incubated with pHrodo beads and Hoechst (blue), upon treatment with Tau PFF or LPS. Phagocytic microglia uptake pHrodo beads that become green in contact with acidic content of lysosomal vesicles (20x magnification)

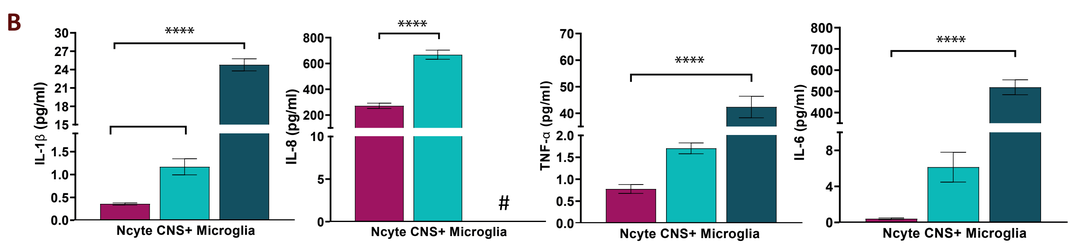


Figure B Quantification of cytokine release by tri-culture using MSD platform: microglia becomes activated upon treatment with LPS, releasing higher levels of proinflammatory cytokines (IL-1β, IL-8, TNF-α and IL-6); treatment with Tau PFF induce release of IL-1β and IL-8, compared with basal condition (n=6, Ordinary one-way ANOVA, Dunnett's multiple comparisons test to Tri-culture vehicle condition*p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001). # indicates level of cytokine was detected above upper limit of quantification.

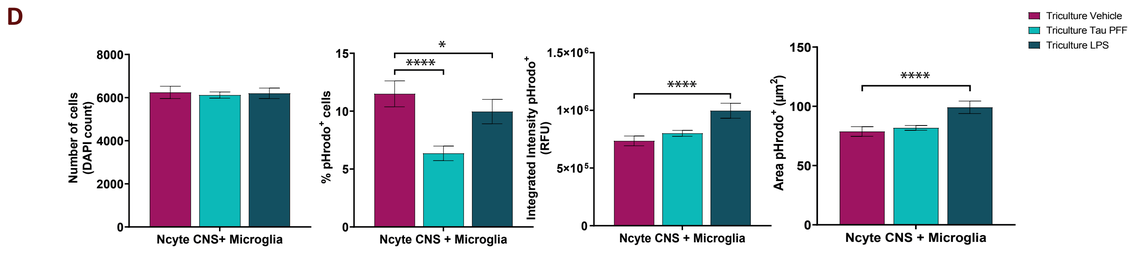


Figure D High content-based quantification of phagocytosis assay parameters: number of cells is similar across conditions, with a decrease of phagocytic microglia (pHrodo+) for tri-culture conditions treated with Tau PFF. Internalization of pHrodo beads assessed by Integrated intensity and area increase upon treatment with LPS (n=6, Ordinary one-way ANOVA, Dunnett's multiple comparisons test to Tri-culture vehicle condition*p < 0.05 **p < 0.01 ***p < 0.001 ****p < 0.0001).

Ncardia's tri-culture model showed functional activation of microglia upon stimulation with Tau PFF

3. Induction of neurodegeneration and neuroinflammation as a model for AD with human brain extracts

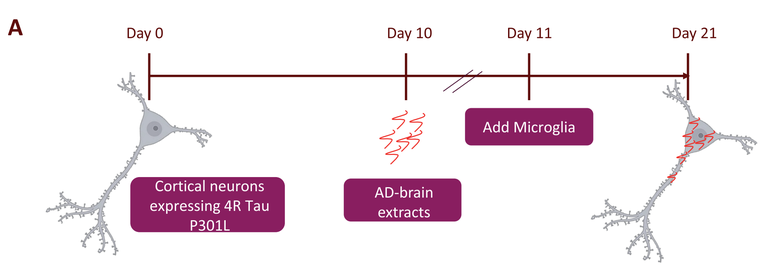


Figure A Schematic of AD model using iPSCs derived cortical neurons treated with AD brain extracts. Day 10 post thaw cortical neurons expressing 4R Tau P301L were treated with AD patient brain extracts (seeds) for 24h before setting the co-culture with microglia at day 11. Co-culture of neurons and microglia was kept for 10 days for endpoint analysis of phospho-Tau (pTau) levels in neurons (by ICC-IF) and culture supernatants (by ELISA).

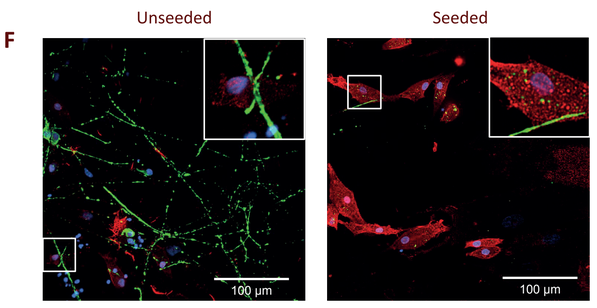


Figure F shows microglia in co-culture with seeded neurons treated with AD-insoluble fractions, phospho-Tau (green), IBA1 (red), DAPI (blue). Phospho-Tau was absorbed by either engulfing neurons affected by the disease (which expressed phospho-Tau) or by capturing it from the culture medium (40x magnification).

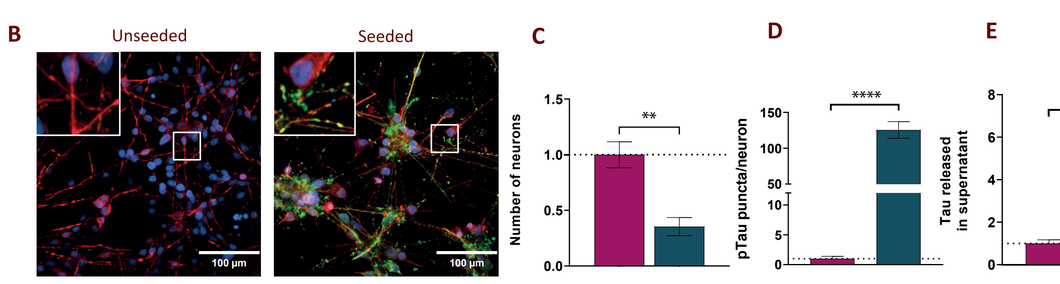


Figure B Fluorescent images of transduced day 21 post-thaw tri-cultures stained for phospho-Tau (green), βIII-Tubulin (red) and DAPI (blue), unseeded or seeded with AD brain Tau seeds (40x magnification).

Figure C-D High content-based quantification of the normalized parameters (number of neurons and pTau puncta counts) of unseeded neurons (in purple) compared to neurons treated with AD brain seeds (in turquoise), normalized to unseeded condition, set to 1 (n=6, **p<0.01 and ****p<0.0001 unpaired t-test). Figure E Quantification of Tau release in supernatant by ELISA (normalized to unseeded condition, set to 1) n=6, **p<0.01 unpaired t-test).

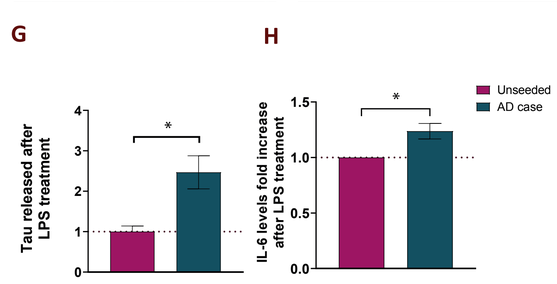


Figure G Quantification of Tau release in supernatant by ELISA (normalized to unseeded condition, set to 1). Cultures exposed to AD insoluble fraction release more Tau than controls following LPS stimulation (n=6, *p<0.05 unpaired t-test).

Figure H Homogeneous Time Resolved Fluorescence (HTRF) assay for IL-6 release (normalized to unseeded condition, set to 1) in conditioned medium from neuronal/-microglia coculture. Microglia exposed to neurons seeded with AD insoluble fraction release more IL-6 than controls following LPS stimulation (n=4, *p<0.05 unpaired t-test)

Ncardia's tri-culture model showed neurodegeneration and neuroinflammation phenotype upon treatment with AD Tau seeds

Conclusions

- We have established a physiological relevant human in vitro tri-culture model, composed by neurons, astrocytes and microglia suitable to study the effect of drug candidates on neuroinflammation and neurodegeneration.
- Microglia is activated upon stimulation with LPS and Tau PFF, increasing the release of pro-inflammatory cytokines, a hallmark of neuroinflammation. Neurons treated with Tau PFFs expressed and accumulated phospho-Tau, exhibiting a neurodegenerative phenotype.
- Upon the addition of Tau seeds extracted from AD brain tissue, it was observed increased levels of expression of phospho-Tau (pTau) in neurons and released Tau in supernatants. Microglia phagocyted neurons expressing phospho-Tau and released higher levels of Tau and IL-6. Together, these observations support a neurodegenerative phenotype, typical of tauopathies in which secreted or engulfed phospho-Tau activates microglia initiating and contributing to the neuroinflammatory cascade.
- These models are available on-demand to help drug developers select the best candidates earlier in the drug discovery process, increasing confidence and reducing the need for animal models.

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