Microglia regulate levels of extracellular, neuron-derived tau in an activation-dependent manner

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Introduction & objectives

- Genetic associations between multiple immune genes and risk of developing Alzheimer's disease highlight the role of neuroinflammation in dementia pathogenesis
- We aimed to develop a fully human iPSC-derived cortical neuron-microglia coculture system, enabling mechanistic studies of neuroinflammation, suitable for target identification and validation
- Given the importance of neuronal release and uptake of pathogenic forms of tau protein in dementia pathogenesis, this system was used to investigate whether microglial activation status affects levels of extracellular, neuron-derived tau

Methods

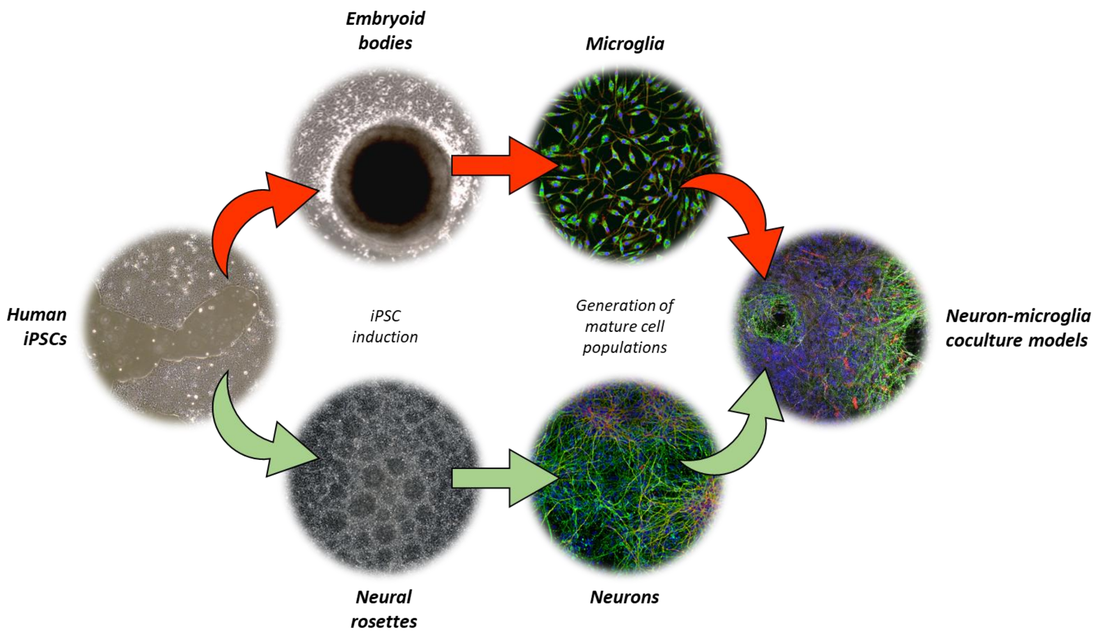

Figure 1. Human iPSCs underwent different induction processes to generate neural and macrophage progenitors. These cell populations were cultured together to establish a coculture system consisting of mature human neurons and microglia.

- Cortical neurons[1] and microglia[2] were generated from human non-demented control iPSCs and combined to form cocultures
- Cells were characterised using transcriptomic analysis (NanoString), immunofluorescence (Opera Phenix) and ELISA
- Compound profiling was performed by stimulating pro-inflammatory cytokine release from microglia (LPS-driven and inflammasome-driven) in the presence of a half-log compound dilution series, and quantifying relevant cytokine release by ELISA
- Levels of extracellular, neuron-derived tau and Aβ were measured by MSD ELISA

Results

Activation and inflammasome induction in microglia in a human neuron-microglia coculture system

- In coculture with neurons, microglia were functionally active and released cytokines (TNFα, IL-6) in response to pro-inflammatory stimuli (LPS or LPS/IFNγ), and inflammasome activation was robustly induced with LPS and nigericin or ATP, accompanied by IL-1β and IL-18 release

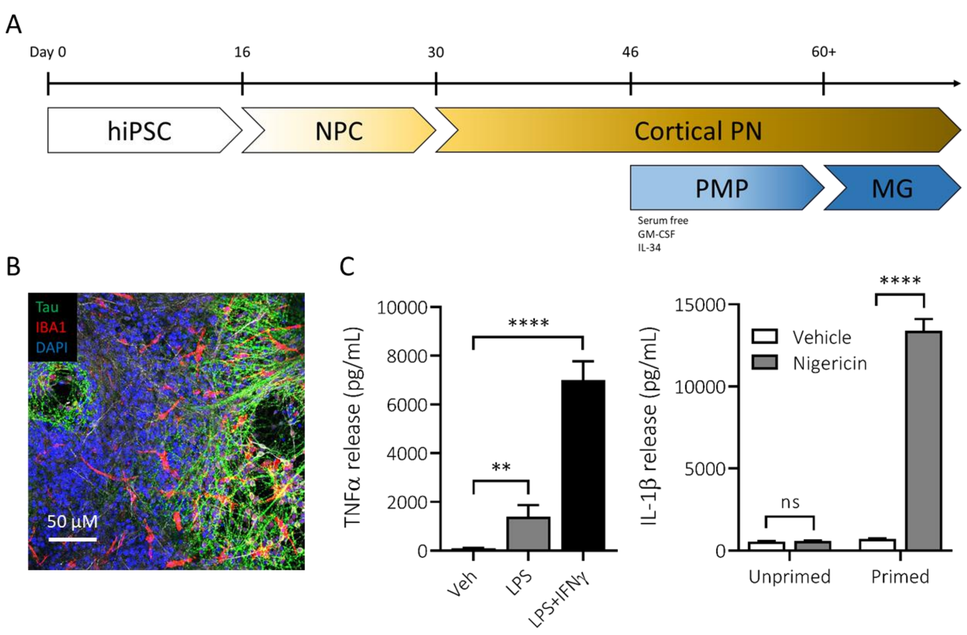

Figure 2. (A) Timeline detailing the differentiation process from hiPSC to glutamatergic cortical projection neurons (PNs) and the establishment of cocultures by adding primitive macrophage progenitors (PMPs) to neurons. (B) ICC of cocultures stained for IBA1 (microglia) and tau (neurons). (C) Quantification of cytokine release from cocultures following pro-inflammatory stimulation. *Left:* TNFα release from cocultures treated with either 10 ng/mL LPS or 10 ng/mL LPS + 20 ng/mL IFNγ for 24h. *Right:* IL-1β release from unprimed and LPS-primed (100 ng/mL, 3h) cocultures treated +/- 10 µM nigericin (30 min).

Identification of small molecule modulators of microglial activation in human cocultures

- Release of TNFα/IL-6 was dose-dependently blocked by a p38 inhibitor (CP-863187), a glucocorticoid (dexamethasone) and an IKK-2 inhibitor (TPCA-1), while NLRP3 inhibition (MCC950) blocked IL-1β/IL-18 release

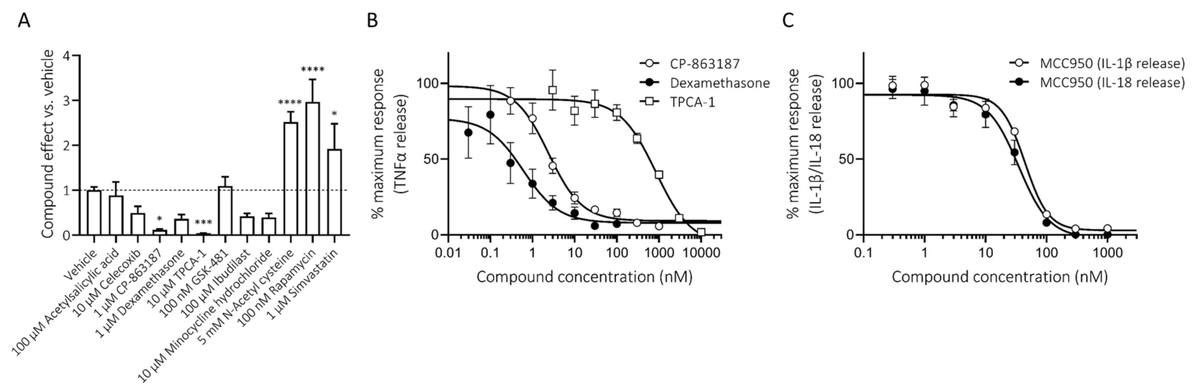

Figure 3. (A) Multiple compounds impact LPS/IFNγ-induced TNFα release. (B) Concentration-effect curves for three anti-inflammatory compounds showing TNFα levels measured in coculture supernatant 24h after treatment with LPS/IFNγ in the presence of compounds. (C) Concentration-effect curves for the NLRP3 inhibitor, MCC950, showing IL-1β and IL-18 levels measured in coculture supernatant following nigericin-mediated inflammasome activation in the presence of compound.

Microglial activation status alters steady-state levels of neuronally-secreted tau

- The presence of microglia reduced steady-state extracellular levels of neuron-derived tau under resting conditions, whilst levels of amyloid-β peptides were unaffected
- Inflammatory activation of microglia in cocultures resulted in increased levels of neuron-derived extracellular tau

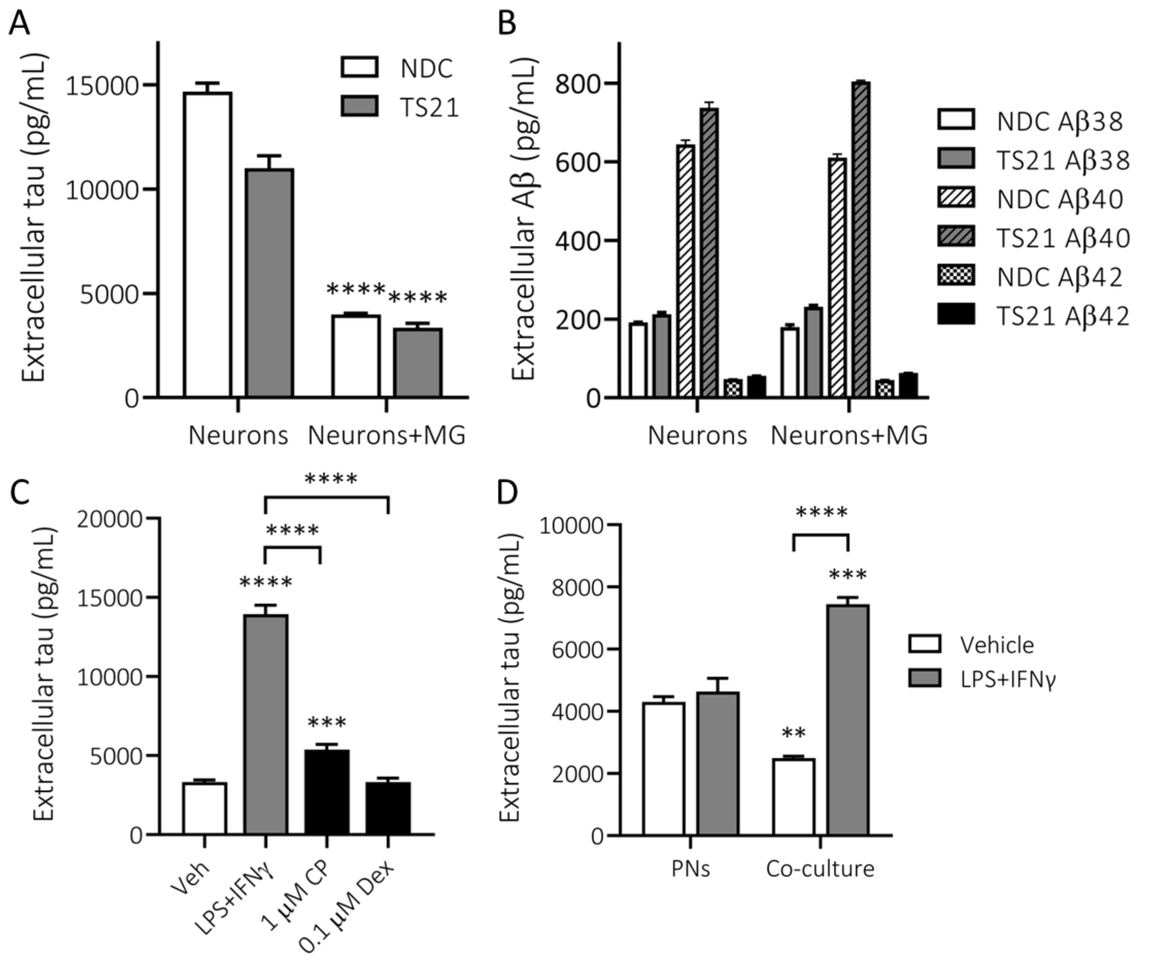

Figure 4. (A) Quantification of extracellular tau protein from human non-demented control (NDC) and trisomy 21 (TS21) projection neuron cultures in the presence and absence of microglia. (B) Quantification of extracellular Aβ38/40/42 peptides from human NDC and TS21 projection neuron cultures in the presence and absence of microglia. (C) Quantification of extracellular tau protein from human cocultures following pro-inflammatory stimulation with 10 ng/mL LPS + 20 ng/mL IFNγ for 24h in the presence and absence of anti-inflammatory compounds. (D) Quantification of extracellular tau protein from human projection neurons in the presence and absence of microglia following pro-inflammatory stimulation with 10 ng/mL LPS + 20 ng/mL IFNγ for 24h.

Conclusions

- We present a fully human *in vitro* iPSC-derived neuron-microglia coculture cell model that is amenable to high-throughput screening assays for drug discovery
- This fully human coculture system is scalable, robust and captures relevant neuroinflammatory outcomes
- Our model provides a more physiologically-relevant platform for therapeutic screening efforts to aid development of treatments for human neurodegenerative diseases and demonstrates that microglia regulate extracellular levels of neuron-generated tau in an activation-dependent manner

References:
[1]Shi *et al.* (2012) **Nature Neuroscience** 15:477-86
[2]Brownjohn *et al.* (2018) **Stem Cell Reports** 10:1294-1307