A systems biology platform that models patient-relevant neuroinflammation signature of progressive glaucoma

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INTRODUCTION

Dysfunctional microglia activation often manifests as a neurotoxic and inflammatory microenvironment that, if not resolved, can lead to chronic neuroinflammation associated with many pathological conditions including neuro-psychiatric and neurodegenerative diseases. Despite the longstanding recognition of their central role in chronic and degenerative diseases of the CNS, there is still a critical need for assay platforms that recapitulate the systems biology of microglia in a way that meaningfully facilitates neuro-inflammatory drug discovery.

We have previously developed a rodent-human chimeric *in vitro* model where phenotypes relevant to microglial and neuronal function could be quantified in the presence and absence of neuroinflammation. In this study, we used the model to assess the effect of nine compounds, in different ways suggested to have an impact on neuroinflammation, to validate our platform.

In addition, data from cell models were compared with clinical samples from patients suffering from Glaucoma to validate the translatability of the neuroinflammatory response in the chimeric tricultures.

MATERIALS & METHODS

Chimeric tricultures, LPS stimulation & compound addition: Cortices from E17.5 rat embryos (Wistar Han) were dissociated and plated in 384-well plates. At 7 days in vitro (DIV) human iPSC-derived microglia were added to the cultures along with supplements promoting microglial survival. Tricultures were stimulated with LPS at 10 DIV. Tool compounds were added 1 hour prior to LPS addition. Assay readouts were performed 24 h or 5 days post LPS stimulation.

High content imaging and analysis:
Cultures were fixed and stained for Hoechst, GFAP, IBA1 & MAP2, and imaged using a Molecular Devices ImageXpress micro confocal HT.ai in confocal mode using 20x magnification. Custom segmentation & image analysis pipelines were developed to extract as many cellular parameters as possible for neurons (MAP2), astrocytes (GFAP), microglia (IBA1) and nuclei for all cells (Hoechst).

Multiparametric analysis:
Multiparametric analysis for phenotypic profiling was performed using an in-house developed multiparametric platform. This was used for normalization, transformation, dimensionality reduction, hit detection, clustering and potency calculations.

Microglia monoculture:
Human iPSC-derived microglia were cultured as per manufacturer's instructions with reduced TGF-β supplement. On 7 DIV, LPS was added with readout 24 h later.

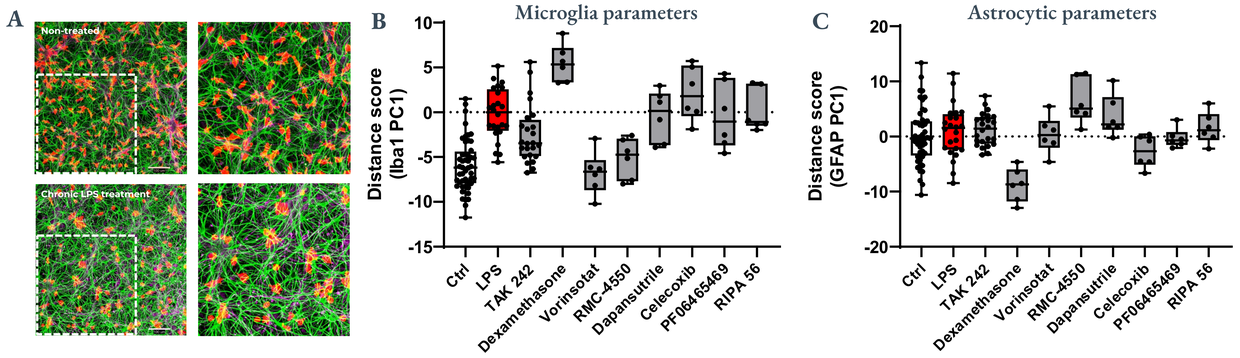
Cytokine profiling:
Conditioned media was diluted 1:4 and 2 technical replicates per sample were analyzed using the ProcartaPlex Human Inflammation 20-plex Panel.

Aqueous humor collection:
Surgical patients were recruited for participation at the Wilmer Eye Institute of Johns Hopkins Medical School. Groups included Progressive glaucoma (n=22), Stable glaucoma (n=20), and Cataract (n=20). Aqueous humor was collected at the Bendann Surgical pavilion on the day of the scheduled surgery. After processing, samples were stored at −80°C until analysis.

Aqueous humor proteomics:
Samples were sent to Somalogic for proteomic analysis. Aqueous humor samples were analyzed blind using the SomaScan assay (V. 4.1). Samples were analyzed in 2 batches.

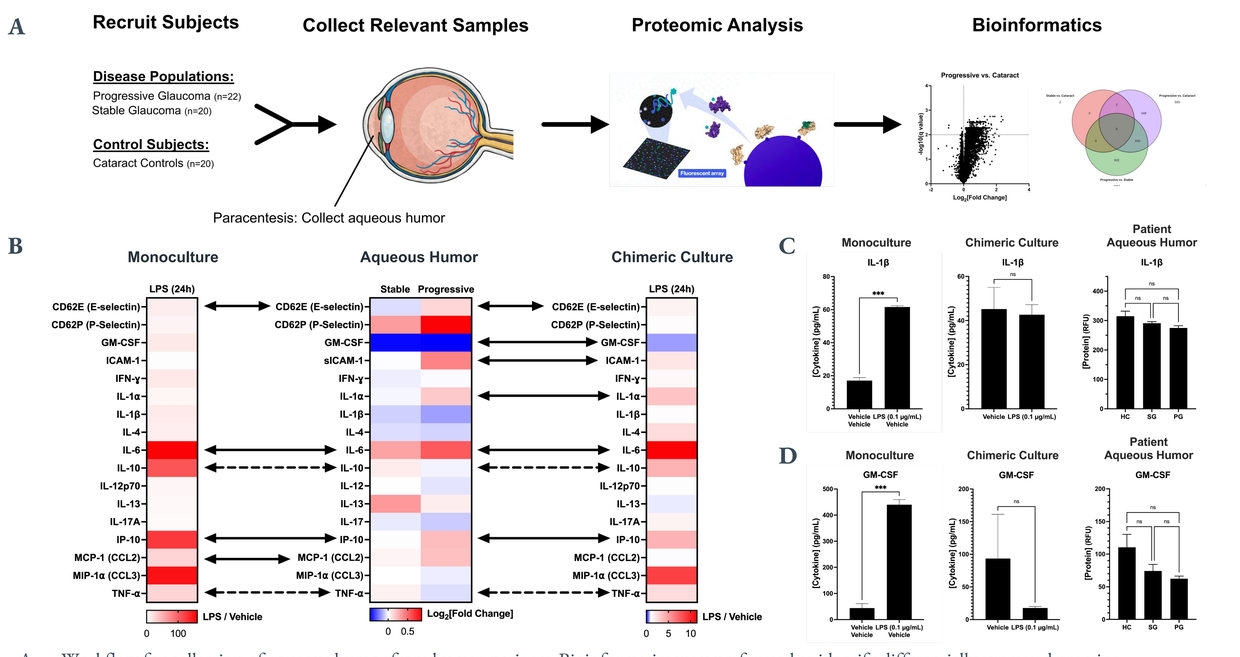
RESULTS

LPS-induced inflammatory cellular phenotype is reversed by certain anti-inflammatory compounds



A. Stimulation of chimeric tri-cultures with LPS resulted in an activated microglia phenotype. Iba1 positive microglia (red), MAP2 dendritic network (magenta) and GFAP positive astrocytes (green).
B. Multiparametric PCA analysis of Iba1/microglia parameters showed an LPS-induced change in microglia parameters that was reversed with the TLR4 inhibitor TAK242, Vorinostat and RMC-4550. Other suggested anti-inflammatory compounds did not reverse this phenotype. In fact, Dexamethasone in combination with LPS appeared to alter the microglia parameters differently to that observed in control or LPS-treated cultures.
C. Multiparametric PCA analysis of GFAP/astrocytic parameters revealed no alteration in presence of LPS. However, Dexamethasone and RMC-4550 (both in combination with LPS) appeared to differentially alter the astrocytic morphology parameters.

The chimeric culture more faithfully models changes in microglial cytokines measured in aqueous humor from patients with progressive glaucoma



A. Workflow for collection of aqueous humor from human patients. Bioinformatics were performed to identify differentially expressed proteins.
B. Comparison of cytokines measured from hiPSC-derived microglial monoculture, aqueous humor collected from patients, and from chimeric tricultures. Solid arrows indicate alignment with Progressive glaucoma, dashed arrows indicate alignment with Stable glaucoma. Chimeric tricultures were better aligned with Progressive glaucoma, an active neurodegenerative disease, compared with monocultures.
C,D. Exemplar cytokines: IL-1β (C) and GM-CSF (D). Both cytokines were robustly increased by LPS in monoculture but showed trends for decreases in both the chimeric cultures and progressive glaucoma patients, further indicating that chimeric tricultures were better aligned with the cytokine profile for Progressive glaucoma, compared with monocultures.

Summary of compound effects in chimeric tricultures

Compound	Target	Outcome on LPS-induced cytokine release and microglial phenotype
TAK-242	TLR4	Concentration-dependent reversal of cytokine release and microglial cellular phenotype
Vorinostat	HDAC	Concentration-dependent partial reversal of cytokine release and microglial cellular phenotype
RMC-4550	PTPN11/ SHP2	Concentration-dependent partial reversal of cytokine release and microglial cellular phenotype
Dexamethasone	GR	Potent inhibition of pro-inflammatory cytokines, increase of anti-inflammatory cytokines e.g. IL-10, but failed to reverse microglial cellular phenotype
Dapansutrile	NLRP3	Potentially weak inhibition of some pro-inflammatory cytokines and a weak and partial reversal of microglial cellular phenotype
Celecoxib	COX-2	No effect on cytokine release or microglial phenotype
PF06465469	ITK/BTK	No effect on cytokine release or microglial cellular phenotype
RIPA56	RIPK1	No effect on cytokine release or microglial cellular phenotype
Pexidartinib	CSF1R	Concentration-dependent depletion of microglia in the tri-culture

- LPS-induced changes in microglial cellular phenotype was concentration-dependently reversed by the HDAC inhibitor Vorinostat and partially by the PTPN11/SHP2 inhibitor RMC-4550.
- Only moderate effects were observed by the NLRP3 inhibitor Dapanustrile. However, LPS treatment alone might not fully activate the NLRP3 inflammasome, which could account for this partial/weak effect.
- LPS-induced cytokine release was differently modulated by Dexamethasone, Vorinostat and RMC-4550.

CONCLUSIONS

- hiPSC-derived microglia can be co-cultured with rat primary neurons and astrocytes. Microglia fully integrate and adopt morphologies suggestive of homeostatic and/or surveillant microglia.
- The chimeric triculture model can be used to differentiate compound effects on LPS-induced alteration of microglial morphology and cytokine release.
- Comparison of the cytokine release pattern indicates that the chimeric triculture system aligned well with that observed in aqueous humor from patients with Progressive glaucoma, an active neurodegenerative disease, thus indicating good clinical transability of the neuroinflammatory response in the chimeric tricultures.
- Taken together, these results indicate that this chimeric triculture system can be used to screen compounds in a platform that recapitulates a comprehensive swath of neuroinflammatory systems biology.