FireCyte THERAPEUTICS

Validation of a Systems Biology Screening Platform for Neuroinflammation with Benchmark Pharmacological Agents

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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 neuropsychiatric 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 neuroinflammatory 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.

MATERIALS & METHODS

Cell culture, LPS stimulation & compound addition:
Cortices from E17.5 rat embryos (Wistar Han) were collected, dissociated, and plated in PDL-coated 384-well plates in B27 plus complete medium. 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 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 quantify total cell count, changes to microglial morphology, astrocyte density and MAP2 dendritic density, and to capture all cellular features necessary for multiparametric analysis.

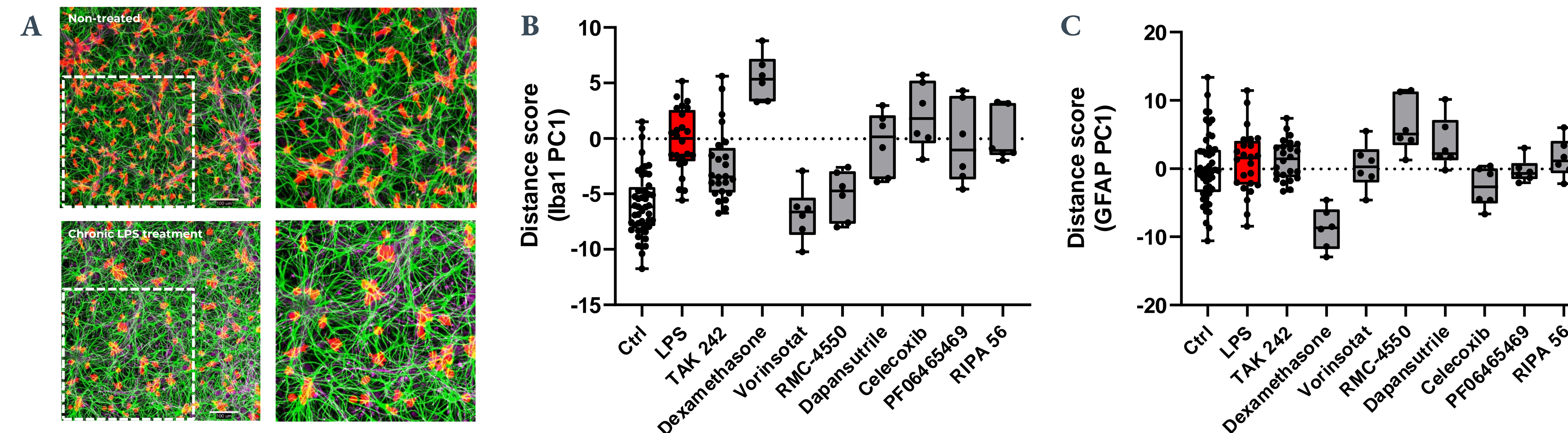
Multiparametric analysis:
Multiparametric analysis for phenotypic profiling was performed using StratoMineR™, a web-based tool for HCS data analytics.

This was used for normalization, transformation, dimensionality reduction, hit detection, clustering and potency calculations.

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

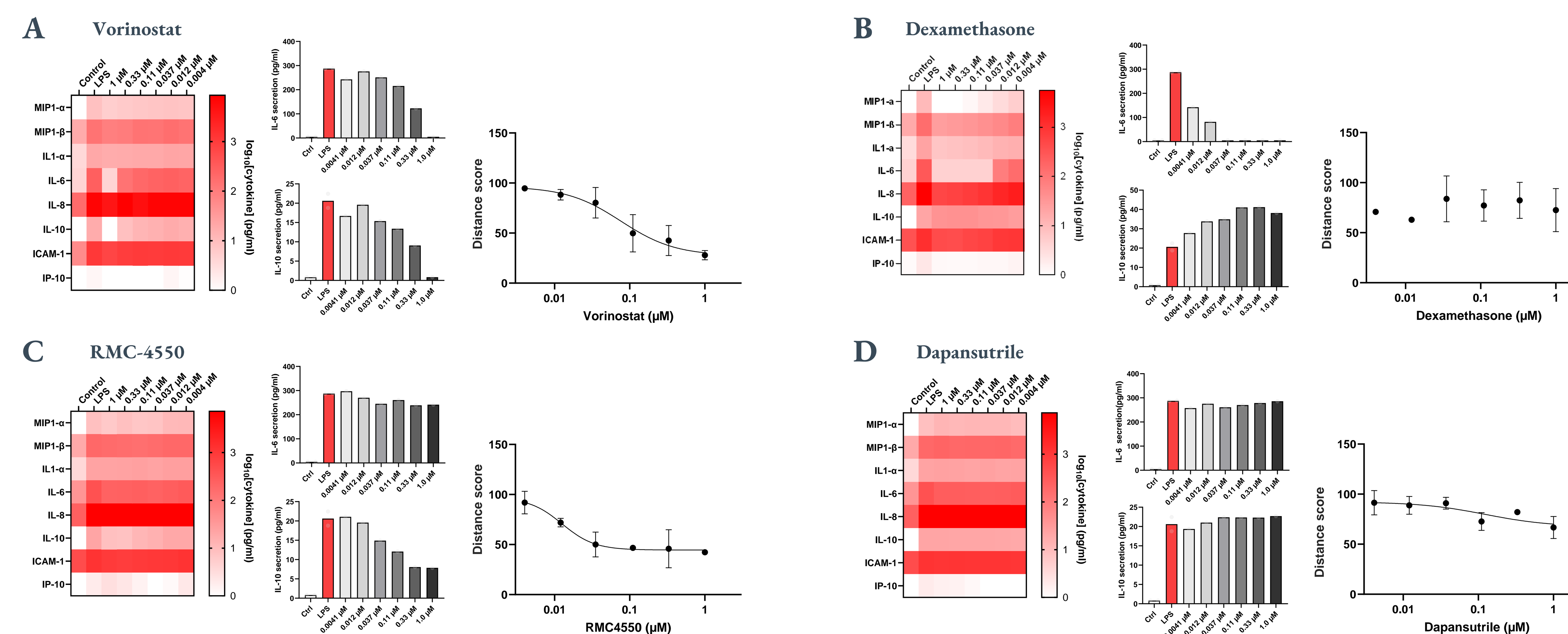
RESULTS

LPS induces an inflammatory cellular phenotype that is reversed by certain anti-inflammatory compounds



A. Stimulation of chimeric tri-cultures with LPS results in an activated microglia phenotype. Iba1 positive microglia (red), MAP2 dendritic network (magenta) and GFAP positive astrocytes (green).
B. Multiparametric PCA analysis of Iba1 parameters show an LPS-induced change in microglia that can be reversed with the TLR4 inhibitor TAK242, Vorinostat and RMC-4550. Other suggested anti-inflammatory compounds such as Dexamethasone and the RIP kinase inhibitor RIPA-56 does not reverse this phenotype.
C. Multiparametric PCA analysis of GFAP parameters show an effect of Dexamethasone (in combination with LPS) and a differential effect of RMC-4550 and Dapansutrile (also in combination with LPS).

The effects of anti-inflammatory compounds on cytokine release do not always coincide with a reversal of cellular microglia phenotype



A. The HDAC inhibitor Vorinostat inhibits the LPS-induced secretion of several pro- and anti-inflammatory cytokines including IL-6 and IL-10. Vorinostat also dose dependently reverses the LPS-induced cellular phenotype of microglia.
B. The glucocorticoid receptor antagonist Dexamethasone strongly inhibits the secretion of pro-inflammatory cytokines and increases secretion of anti-inflammatory cytokine IL-10. However, no effect on the LPS-induced microglia cellular phenotype is observed.
C. The PTPN11/SHP2 inhibitor RMC-4550 slightly inhibits the LPS-induced secretion of pro-inflammatory cytokines and inhibits the secretion of the anti-inflammatory cytokine IL-10. RMC-4550 partially reverses the LPS-induced microglia cellular phenotype.
D. The NLRP3 inflammasome inhibitor Dapansutrile does not inhibit the the LPS-induced secretion of several pro- and anti-inflammatory cytokines. A slight reversal of LPS-induced microglia cellular phenotype is observed.

Summary of compound effects

Compound	Target	Outcome
TAK-242	TLR4	Concentration-dependent reversal of cytokine release and cellular phenotype
Vorinostat	HDAC	Concentration-dependent reversal of cytokine release and cellular phenotype
RMC-4550	PTPN11/SHP2	Concentration-dependent partial reversal of cytokine release and cellular phenotype
Dexamethasone	GR	Potent inhibitor of proinflammatory cytokines, increase of anti-inflammatory cytokines, failed to reverse cellular phenotype
Dapansutrile	NLRP3	Potentially weak inhibition of some proinflammatory cytokines and a weak and partial reversal of cellular phenotype
Celecoxib	COX-2	No effect on cytokine release or cellular phenotype
PF06465469	ITK/BTK	No effect on cytokine release or cellular phenotype
RIPA-56	RIPK1	No effect on cytokine release or cellular phenotype
Pexidartinib	CSF1R	Concentration-dependent depletion of microglia in the tri-culture

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.
- LPS induces robust changes in microglia morphology and cytokine release.
- LPS-induced cytokine release was differently affected by the glucocorticoid receptor antagonist Dexamethasone, the HDAC inhibitor Vorinostat and the PTPN11/SHP2 inhibitor RMC-4550, but not the NLRP3 inhibitor Dapanustrile.
- LPS-induced changes in microglia cellular phenotype was dose dependently reversed by Vorinostat and partially by RMC-4550 and Dapanustrile, but not Dexamethasone.
- These results indicate that this chimeric culture system can be used to screen compounds in human microglia in a platform that recapitulates a comprehensive swath of neuroinflammatory systems biology.