Development of assay systems to aid in the fight against chronic neuroinflammation

Domainex
Enrich your Medicines Pipeline

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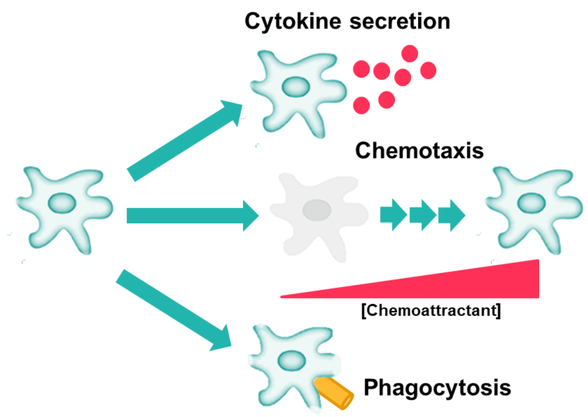
Background

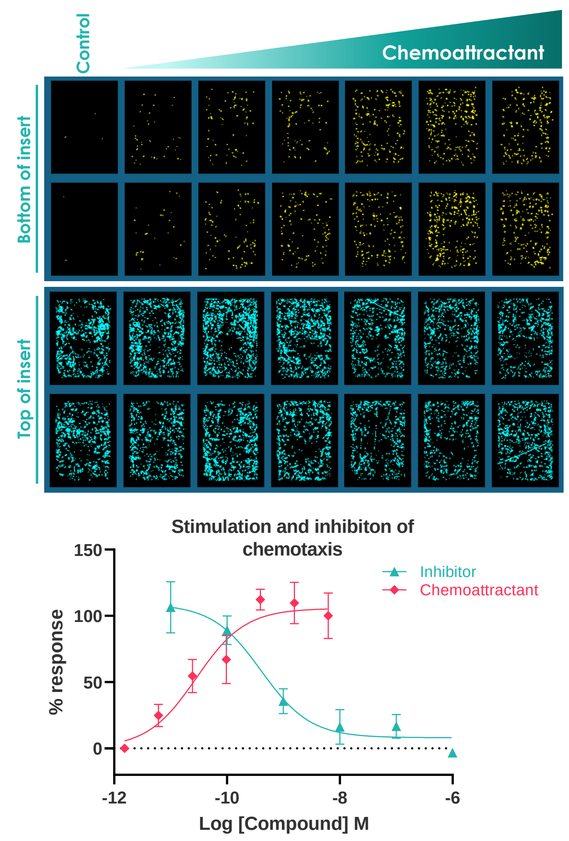
- Inflammatory responses are critical for survival; however, aberrant inflammation is implicated in a variety of neurodegenerative, cardiovascular, autoimmune, and metabolic diseases
- The CNS has a separate immune state to the rest of the body, initiating its own neuro-inflammatory response via microglia
- Due to the CNS's poor repair mechanisms, immune responses must be terminated to maintain homeostasis
- Persistent insult, or inadequate termination, leads to chronic neuroinflammation, a continuous and self-exacerbating cycle
- Chronic neuroinflammation has been identified as a driving force in many brain pathologies including dementias, traumatic brain injury and stroke
- Drugs that inhibit abnormal inflammatory responses (*e.g.* NLRP3 inflammasome inhibitors) have thus become highly sought after, due to their potential to modulate multiple disease-states



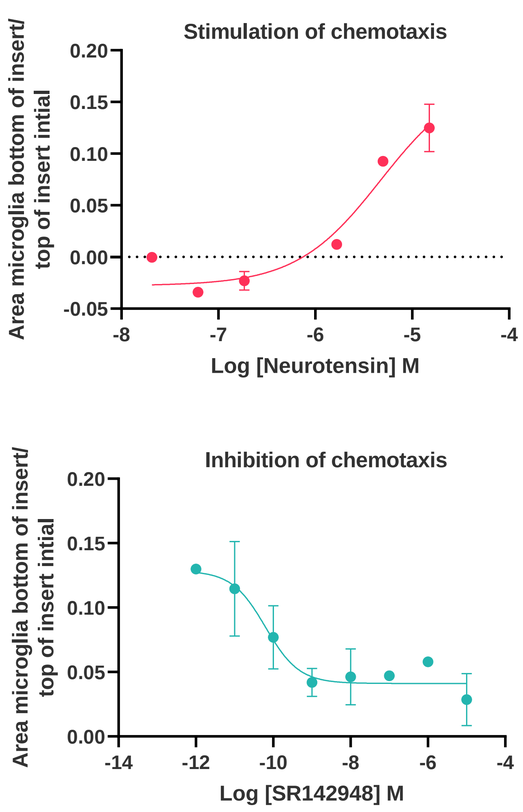
Chemotaxis

- Macrophages and microglia both possess an inherent ability to migrate towards a chemoattractant (e.g. at the site of injury)
- Domainex has developed Incucyte-based assays allowing the measurement of the induction and inhibition of chemotaxis in both THP-1 cells, differentiated into M0 macrophages and iPSC-derived microglia

a) THP-1 cells



b) iPSC-derived microglia

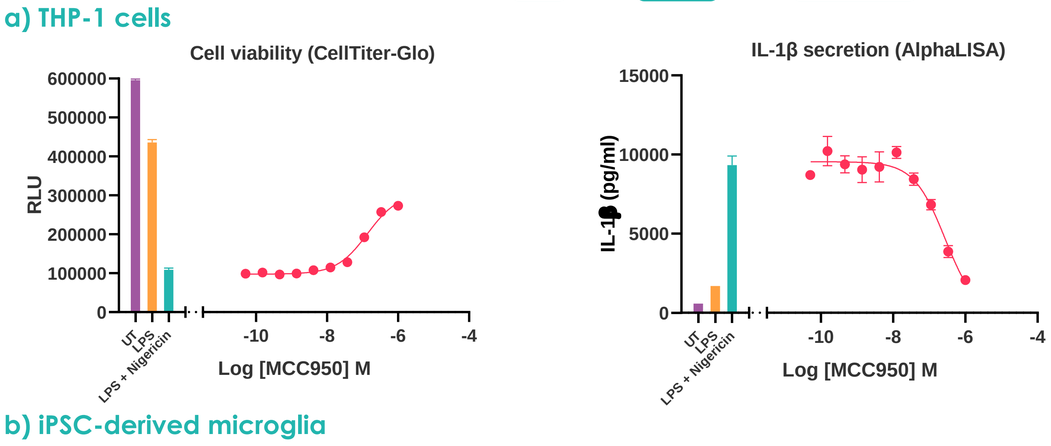


Chemotaxis was measured in real-time using the Incucyte S3 for both a) M0 differentiated THP-1 cells and b) microglia. Compound concentration-response curves for cell migration was measured at 48 hours (THP-1 cells) and 12 hours (microglia).

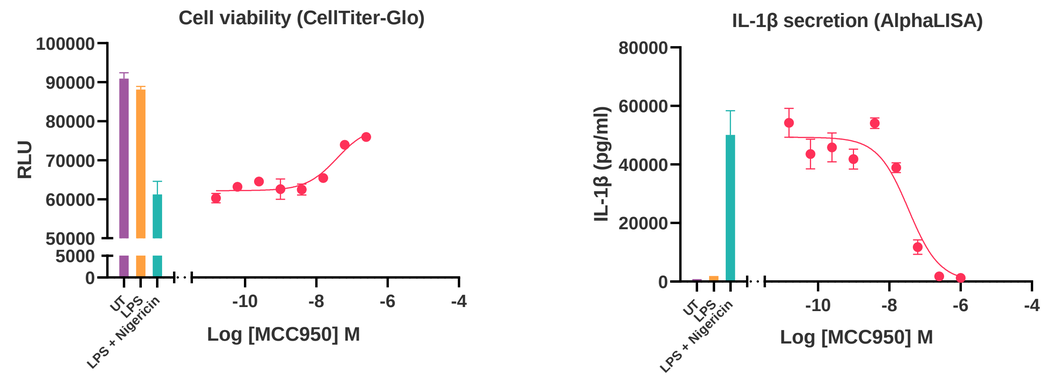
Inflammasome

- NLR family pyrin domain containing 3 (NLRP3) is a key regulator of inflammation
- Hallmarks of NLRP3 inflammasome activation include the release of pro-inflammatory cytokines such as of IL-1β, and cell death (pyroptosis)
- Domainex have developed robust screening assays measuring inflammasome activation in THP-1 cells differentiated into M0 macrophages, whole blood and iPSC-derived microglia:

a) THP-1 cells



b) iPSC-derived microglia

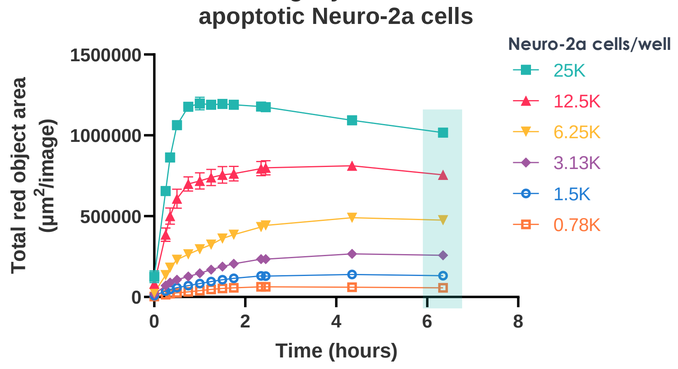


To stimulate the NLRP3 inflammasome (a) M0 differentiated THP-1 cells and (b) iPSC microglia have been treated with LPS +/- Nigericin. Concentration-response curves are observed with the NLRP3 inhibitor MCC950.

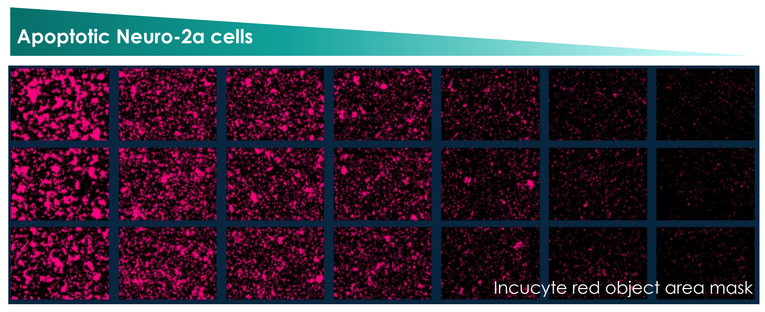
Phagocytosis

- A key process in an inflammatory-response is clearing of pathogens, cell debris and apoptotic cells via phagocytosis
- Domainex have developed assays to quantify THP-1-mediated phagocytosis of apoptotic Neuro-2a cells labelled with pHrodo™ Red dye
- pHrodo™ fluorescence increases once inside the acidic environment of the phagosome and thus acts as a marker of phagocytosis
- We are now working to optimize this assay to measure iPSC-derived microglia phagocytosis

a)



b)



Apoptotic Neuro2a-cells (pre-treated with Staurosporine and labelled with pHrodo™ Red) were co-cultured with M0 differentiated THP-1 cells. Phagocytosis was quantified over time using the Incucyte S3 (a). Representative images of phagocytosed Neuro-2a cells at 6.5 hours are shown in b).

Summary

- Domainex has developed a comprehensive suite of assays to enable the profiling and development of novel compounds targeting inflammatory and neuro-inflammatory targets
- These robust assays can be performed routinely, allowing progression of hit-to-lead and lead optimisation programmes, ultimately assisting in the nomination of a pre-clinical candidate
- Furthermore, these assays can be performed in a variety of relevant cellular models, and further developed as required for each project's specific needs

Services/Contact

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