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Application Note

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Multiplexed Quantification of Secreted Protein Release in Inflammatory and Neuroinflammatory Models using HTS by Cytometry

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Abstract

Cell signaling pathways involve a variety of secreted proteins and cytokines and accurately measuring these molecules is vital for basic research and drug development. The importance of the interface between the nervous and immune systems during inflammation and neurodegenerative disease is increasingly being recognized. Research is focusing on deepening our understanding of cytokines involved in disease development and progression, with drug targets beginning to emerge for neuroinflammatory and neurodegenerative conditions. The data shown highlight how iQue Qbeads® and High-Throughput Screening (HTS) by Cytometry can be utilized to simultaneously investigate a broad range of secreted proteins in physiologically relevant immune cell or iPSC-derived neuroinflammatory models.

Introduction

Cellular signaling pathways regulating cell-to-cell communication involve a diverse array of secreted proteins and cytokines. Accurately quantifying secretion, or abnormal production, of these molecules is essential for basic research and drug development to enhance our understanding of physiological processes, such as immune cell activation, and dysfunctional cellular signaling implicated in nearly all diseases.[1] The importance of the interface between the nervous and immune systems during inflammation and neurodegenerative diseases is increasingly being recognized. Recent studies have shown that inflammation not only plays a crucial role in advancing neurodegeneration but can also act as initiator, with inflammatory signaling exhibiting beneficial and detrimental roles depending on the stage of a disease.[2,3] A more comprehensive understanding of the timing, cell specificity, and molecular mechanisms, alongside the use of more translational cellular models, such as iPSC-derived, will be crucial for developing therapeutic strategies that block or enhance inflammatory signaling pathways involved in neurodegenerative diseases.

Over the past decade, there has been a significant increase in research emphasizing the measurement and analysis of cytokines. These studies focus on the ability to detect, quantify, and differentiate specific multiple secreted factors from a complex mixture of biomolecules in any given sample.[4] A range of established immunoassay methods, such as enzyme-linked immunosorbent assays (ELISA) and flow cytometry-based assays are currently used. However, they have several limitations including being low-throughput, laborious, and time-consuming, often requiring multiple steps of optimization, labeling, long incubations, and repeated washes.[5] iQue Qbeads® enable the capture of specific proteins on distinct bead types and facilitate the multiplexed quantification of a broad range of cytokines, adhesion molecules, enzymes, and growth receptors. These ready-to-run kits have been developed for use on the iQue® High-Throughput Screening (HTS) Cytometry Platform and provide an integrated solution with unique benefits of simple no-wash protocols, a high degree of assay miniaturization, rapid sampling speeds, and lower costs.

This application note demonstrates how a combination of pre-built or custom Qbead® kits and HTS by Cytometry can be utilized to simultaneously quantify multiple secreted proteins in inflammatory and neuroinflammatory models. We also describe how this flexible approach can be used to investigate iPSC-derived neuronal models, where non-perturbing methods and low sample volumes are essential to temporally monitor inflammatory protein release in sensitive and precious cell types.

Materials and Methods

RAW264.7 Stimulation Assay

Murine RAW264.7 macrophages were plated into a 96-well plate at 12,000 cells/well in DMEM media and left to adhere overnight at 37 °C. Cells were serum-starved for 3 hours to reduce basal Akt activation and then treated in serum-free media with a concentration range of the inflammatory agent lipopolysaccharide (LPS; 0.01 – 100 ng/mL), or a single concentration of LPS (100 ng/mL) in the presence of a direct Akt inhibitor MK2206. Supernatant samples were taken at 4 hours post-stimulation, transferred to a v-bottomed 96-well plate, and stored at -20 °C. Materials required are described below (Table 1).

Materials	Supplier	Cat. No.	Final Concentration
DMEM	Gibco	41965039	-
Characterized Foetal Bovine (FBS) serum	Cytiva HyClone™	SH30071.03	10%
Penicillin-Streptomycin	Gibco	15140122	1%
Costar™ 96-well flat-bottom plate	Corning™	3595	-
Costar™ 96-well V-bottomed plate	Corning™	3363	-
LPS	Sigma	L4391	0.01 – 100 ng/mL
MK2206	LKT Labs	M4000	5 µM

Table 1: *Materials required for murine RAW264.7 stimulation assay.*

Monocyte Differentiation

Human primary monocytes were plated into PLO coated 6-well plates at 300,000 cells/well in RPMI media and differentiated into macrophages using granulocyte-macrophage colony-stimulating factor (GM-CSF) or macrophage colony-stimulating factor (M-CSF) for 6 days with a media refresh performed after 3 days. Macrophages were then unstimulated (M0) or polarized for 24 hours to induce an M1 (LPS and interferon gamma (IFNγ)) or M2a (IL-4) macrophage phenotype. Morphological changes accompanying differentiation were monitored using the Incucyte® Live-Cell Analysis System. Supernatant samples (10 µL/well) were taken throughout differentiation (Day 3 and 6) and at 24 hours post-polarization (Day 7) and transferred to v-bottomed 96-well plates and stored at -20 °C. Media formulations and materials used are described below (Table 2).

Media Formulation	Base Medium	FBS	Supplements
Human M0 macrophages	RPMI	10%	50 ng/mL M-CSF
Human M1 macrophages	RPMI	10%	1 µg/mL GM-CSF, 50 ng/mL LPS, 20 ng/mL IFNγ
Human M2a macrophages	RPMI	10%	1 µg/mL M-CSF, 1 µg/mL IL-4
Media Formulation	**Supplier**	**Cat. No.**	**Final Concentration**
Human Peripheral Blood Monocytes	StemCell Technologies	70034	-
RPMI 1640	Gibco	21875091	-
FBS	Cytiva HyClone™	SH30071.03	10%
Poly-L-Ornithine (PLO)	Merck	P4957	0.01%
Costar™ 6-well flat-bottom plate	Corning™	3516	-
Costar™ 96-well V-bottomed plate	Corning™	3363	-
LPS	Sigma	L4391	50 ng/mL
M-CSF	Sartorius	CYK-0100-0031	50 ng/mL – 1 µg/mL
GM-CSF	Sartorius	CYK-0100-1019	1 µg/mL
IFNγ	Sartorius	CYK-0100-1017	20 ng/mL
IL-4	Sartorius	CYK-0100-1006	1 µg/mL

Table 2: *Media formulations and materials used for human primary monocyte to macrophage differentiation.*

Microglia Stimulation Assay

Human iPSC-derived microglia were stabilized and matured in advanced DMEM/F12 using the manufacturer's protocol.[6] Microglia were plated at 10,000 cells/well in a PLL coated 96-well plate and cultured for 14 days. Cells were treated with concentration ranges of IFNγ or LPS, or a single concentration of each stimulus (100 ng/mL) in the presence of the anti-inflammatory compound Dexamethasone (DEX). Treatment with a combination of LPS (100 ng/mL) and IFNγ (20 ng/mL) was used as a positive control for microglia activation into a pro-inflammatory M1 phenotype. Supernatant samples (10 µL/well) were taken at 4, 24, and 48 hours post-stimulation, transferred to v-bottomed 96-well plates and stored at -20 °C. Media formulations and materials used are described below (Table 3).

Media Formulation	Base Medium	Supplements	
Microglia stabilization	Advanced DMEM F12	1X GlutaMAX, 50 µM 2-Mercaptoethanol, 1X N2, 0.1 µg/mL Doxycycline, 10 µM ROCKi, 50 ng/mL M-CSF	
Microglia maturation	Advanced DMEM F12	1X GlutaMAX, 50 µM 2-Mercaptoethanol, 1X N2, 100 ng/m IL-34, 10 ng/mL M-CSF	
Materials	**Supplier**	**Cat. No.**	**Final Concentration**
ioMicroglia™	Bit.bio	io1021	-
Poly-L-Lysine (PLL)	Sigma	P4707	0.01%
Advanced DMEM/F-12	Gibco	12634010	-
Costar™ 96-well flat-bottom plate	Corning™	3595	-
Costar™ 96-well V-bottomed plate	Corning™	3363	-
GlutaMAX	Gibco	35050061	1X
2-Mercaptoethanol	Gibco	31350010	50 µM
N2	Gibco	17502001	1X
ROCKi (Y-27631)	Abcam	Ab144494	10 µM
IL-34	Peprotech	200-34	100 ng/mL
M-CSF	Sartorius	CYK-0100-0031	10 – 50 ng/mL
IFNγ	Sartorius	CYK-0100-1017	0.14 - 100 ng/mL
LPS	Sigma	L4391	0.14 – 100 ng/mL
Dexamethasone	Sigma	D4902	500 nM

Table 3: *Media formulations and materials used for human iPSC-derived microglia.*

Healthy and AD iPSC-derived Co-culture

Healthy and Alzheimer's Disease (AD; PSEN1 mutation) derived iPSCs were seeded in SureBond+ReadySet coated 96-well TPP plates at 25,000 cells/well in plating medium. Media was changed after 24 hours to complete medium and neuronal differentiation was induced as per supplier's protocol on Day 4.[7,8] Healthy or AD neurons were co-cultured on Day 19 with iPSC-derived microglia precursors. On Day 30 supernatants were removed, transferred to a v-bottomed 96-well plate and stored at -20 °C. Media formulations and materials used are described below (Table 4).

Media Formulation	Base Medium	Supplements	
Neuronal plating	NeurONE plating medium	-	
Neuronal culture	NeurONE base medium	NeurONE base supplement (1X)	
Neuronal differentiation	NeurONE differentiation medium	NeurONE differentiation supplements A or B (1X)	
Microglia maintenance and co-culture	Microglia maintenance medium	Supplements A (0.1X), B (0.1X), and C (1X)	
Materials	**Supplier**	**Cat. No.**	**Final Concentration**
hNPC control iPSC-derived neurons	Axol Bioscience	Ax0016	-
hNPC AD iPSC-derived neurons	Axol Bioscience	Ax0012	-
hiPSC microglia precursors	Axol Bioscience	Ax1666-β	-
NeurONE plating medium	Axol Bioscience	Ax0061	-
NeurONE base medium	Axol Bioscience	Ax0062	-
NeurONE base supplement	Axol Bioscience	Ax0063	1X
NeurONE differentiation medium	Axol Bioscience	Ax0064	-
NeuONE differentiation supplement A	Axol Bioscience	Ax0065	1X
NeuONE differentiation supplement B	Axol Bioscience	Ax0065	1X
Microglia Maintenance Kit	Axol Bioscience	Ax0600	-
Readyset	Axol Bioscience	Ax0041+rs	1X
Surebond	Axol Bioscience	Ax0041+rs	20 µg/mL
TPP™ 96-well flat-bottomed plate	TPP™	92096	-
Costar™ 96-well V-bottomed plate	Corning™	3363	-

Table 4: *Media formulations and materials used for human healthy and AD iPSC-derived neuron and microglia co-culture.*

Secreted Protein Release Assay

Supernatant samples were thawed and analyzed using the iQue®, a High-Throughput Screening (HTS) Cytometry Platform, and iQue Qbeads® kits designed to simultaneously quantify the concentration of up to 30 human, mouse, or rat secreted proteins in a 96-well or 384-well format. The pre-built or custom-built panels are supplied as ready-to-use kits complete with buffers, detection reagents, standard analytes, and a pre-gated template, which are analyzed using integrated iQue Forecyt® Software. The workflow involves a no-wash protocol and low sample volumes (Figure 1). Briefly 10 µL of sample and 10 µL of Qbeads® are combined and incubated for 1 hour. After incubation, 10 µL of the detection antibody is added, incubated for 2 hours for human or 1 hour for mouse analytes, and then read on the iQue® HTS Platform. Standard curves were generated using serial titrations of the analytes of interest. Purified analytes (supplied in kit) were pooled, reconstituted with assay dilutent and an 8-point 1:4 serial titration was performed (5,000 to 0 pg/mL). The specific kits used for each assay and the secreted proteins measured are described below (Table 5).

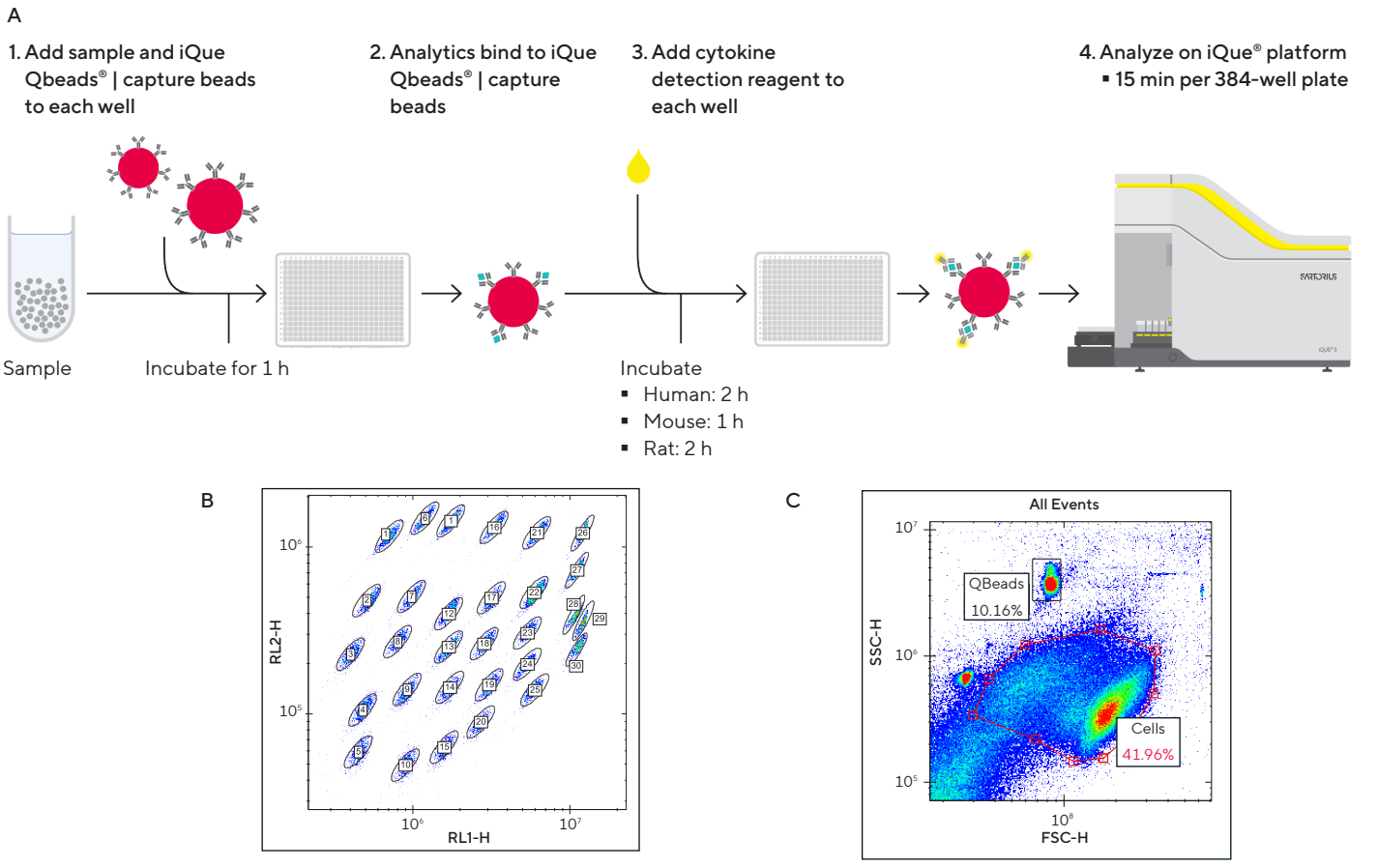

Figure 1: *Schematic of iQue Qbeads® Workflow on the iQue® HTS Platform.*
A) No-wash workflow shown for iQue Qbeads® analyzed on the iQue® HTS Platform. B) The iQue Qbeads® Assay Builder provides a flexible assay format that facilitates up to 30 secreted protein concentrations in a single well. C) Distinct identification of iQue Qbeads® and cells characterized by iQue Forecyt® Software population gating.

Assay	Materials	Supplier	Cat. No.	Secreted Proteins Measured
RAW264.7 Stimulation	iQue Qbeads® Mouse PlexScreen Kit	Sartorius	Qbeads-M (custom)	IL-1α, IL-1β, IL-2, IL-4, IL-6, IL-10 IFNγ, TNFα, CCL2 (MCP-1), CCL3 (MIP-1α), CXCL9 (MIG)
Monocyte differentiation & Microglia Stimulation	iQue Qbeads® Human Inflammation Panel Kit	Sartorius	97097	IL-2, IL-6, IFNγ, CCL2 (MCP-1), CCL3 (MIP-1α), CXCL9 (MIG), CXCL10 (IP-10)
Healthy & AD iPSC-derived co-culture	iQue Qbeads® Human PlexScreen Kit	Sartorius	Qbeads-H (custom)	IL-1β, IL-2, IL-6, IL-10, IFNγ, TNFα, CCL2 (MCP-1), CSF2 (GM-CSF)

Table 5: *iQue Qbeads® Kits used for quantifying secreted protein release.*

Results

Secreted Protein Release in Inflammatory Models of Akt Activity

Stimulation of immune cells by LPS results in cell activation through the release of pro-inflammatory cytokines, a process regulated by the Akt pathway.[9,10] Inhibition of this pathway, either by directly targeting Akt or upstream PI3K, has been demonstrated to reduce Akt phosphorylation and decrease the release of inflammatory cytokines.[11] Dysregulation of the Akt pathway is associated with various diseases, including cancer, inflammatory conditions, and neurodegenerative disorders.[12,13] Therefore, targeting the Akt pathway presents potential therapeutic opportunities for these conditions.

To investigate immune cell activation, a custom iQue Qbeads® kit was designed using the assay builder to measure the concentration of 11 mouse inflammatory cytokines and chemokines of interest (Figure 2). Murine RAW264.7 monocytes were serum starved for 3 hours to inactivate Akt and then treated with a concentration-range of LPS (1.56 - 100 ng/mL) in the absence or presence of an allosteric Akt inhibitor, MK2206. Supernatant samples were taken at 4 hours post-stimulation and analyzed. Each secreted protein was identified using a pre-gated template provided as part of the kit (Figure 2A). Protein concentrations in the samples were then derived from standard curves, with an example curve shown for CCL3 (MIP-1α) (Figure 2B). The heatmap feature provides a quick visualization of secreted protein release (Figure 2C). The results revealed that LPS induced a concentration-dependent increase compared to vehicle in the release of inflammatory proteins CCL3 (Figure 2D) and TNFα (data not shown), with little-to-no release observed for the other cytokines and chemokines of interest. LPS stimulation in the presence of MK2206 showed a reduction in the release of CCL3 compared to LPS alone, with values of 2,174 ± 330 and 3,026 ± 209 pg/mL, respectively (Figure 2E). We also observed that basal release of CCL3 was diminished in the presence of MK2206 alone compared to vehicle; together the results indicate a role of the Akt pathway in the release of inflammatory proteins.

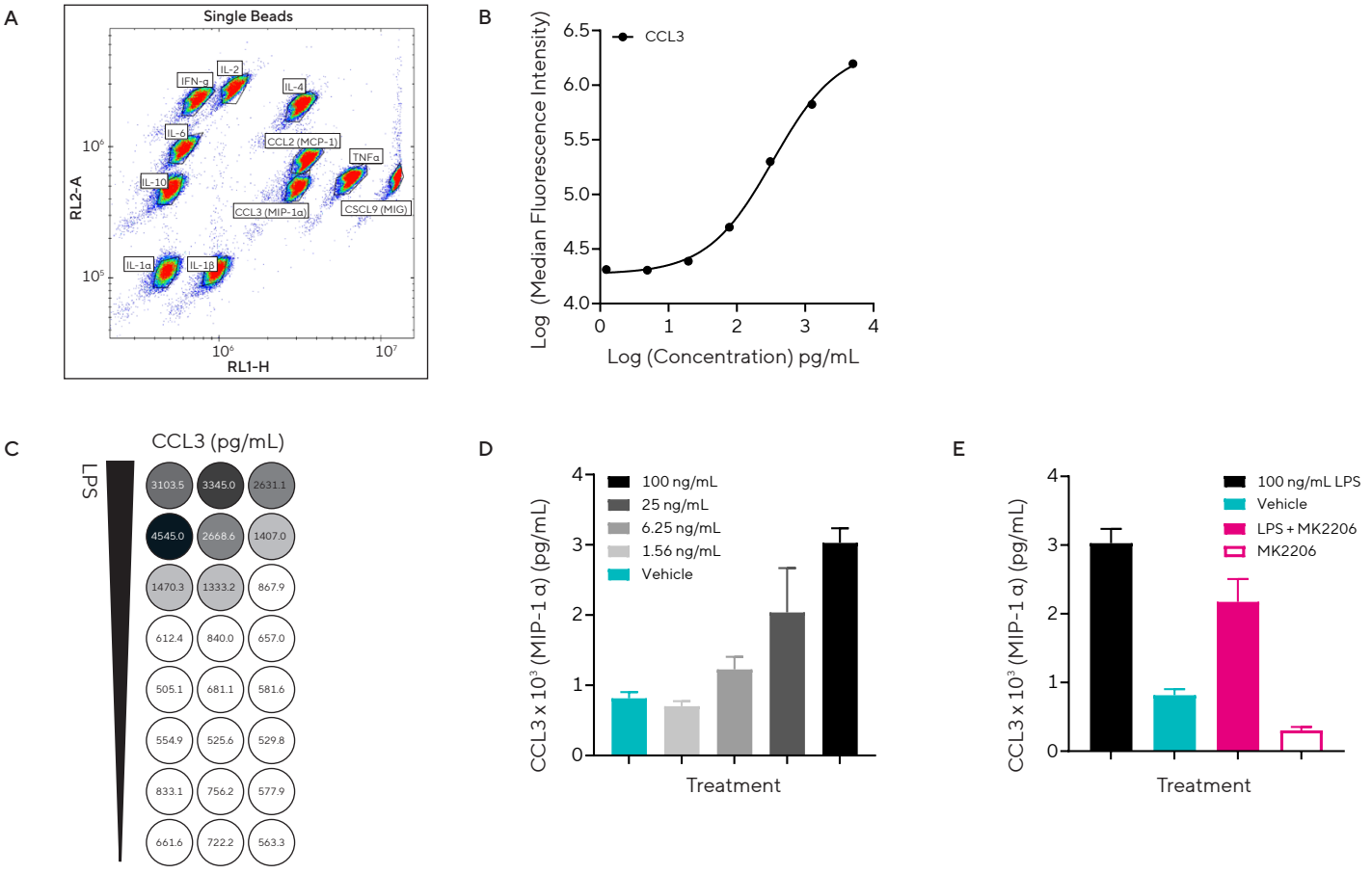

Figure 2. *Pro-inflammatory Stimulation Induces Concentration-Dependent Release of Inflammatory Cytokines.*
A) Dot plot showing single beads and Forecyt® gating strategy for the custom murine Qbead panel designed to assess 11 inflammatory secreted proteins of interest. B) Standard curve from which CCL3 (MIP-1α) protein concentrations were derived. C) Heatmap for CCL3 concentration. D) CCL3 concentration in response to a concentration range of LPS or vehicle. E) CCL3 concentration in response to a single concentration of LPS (100 ng/mL) in the presence of direct Akt inhibitor MK2206 (5 µM). Data presented as mean + SEM at 4 hours post-stimulation, n = 3 replicates.

Utilizing HTS Cytometry to Assess Phenotypic Differences Following Macrophage Polarization

Macrophages are key components of the immune system and under the influence of environmental stimuli can be activated into M1 or M2 phenotypes. M1 macrophages, classically activated by LPS and IFNγ, display a pro-inflammatory phenotype with amoeboid morphology. In contrast, M2 macrophages are alternatively activated and can be divided into at least four types based on the applied stimuli. M2a macrophages are induced by IL-4 and exhibit an anti-inflammatory phenotype with a mixture of ramified and amoeboid morphologies.[14,15] Macrophages can exist as heterogeneous populations and multiparametric approaches are required for their characterization including cell morphology, receptor expression profiles, and their secretion of pro-inflammatory factors.[16]

Here we investigated secreted protein release in human primary monocytes differentiated to M0, M1, or M2a macrophages over 7 days (Figure 3). Morphological changes accompanying differentiation were monitored using live-cell analysis. Supernatant samples taken on Day 3, 6, and 7 of differentiation were assessed using HTS by Cytometry and the pre-built Human Inflammation Panel Kit, which allows for the measurement of 7 human cytokines and chemokines implicated in inflammatory responses to disease states. Representative phase-contrast images of morphological changes indicative of M1 and M2a phenotypes are shown, with expected amoeboid and ramified morphologies observed, respectively (Figure 3A). On Day 7, we observed an increase in the release of five secreted proteins for M1 compared to M0 and M2a macrophages (Figure 3B), with little-to-no IL-2 release shown for any phenotype. M2a macrophages exhibited a slight decrease in CCL2 on Day 7 compared to M0 macrophages (Figure 3C). These findings are consistent with the pro-inflammatory phenotype of M1 macrophages.

Overall, the data indicate successful polarization of macrophages into pro-inflammatory M1 and anti-inflammatory M2a phenotypes. These can be distinguished by their distinct morphologies and profiles of pro-inflammatory secreted protein release.

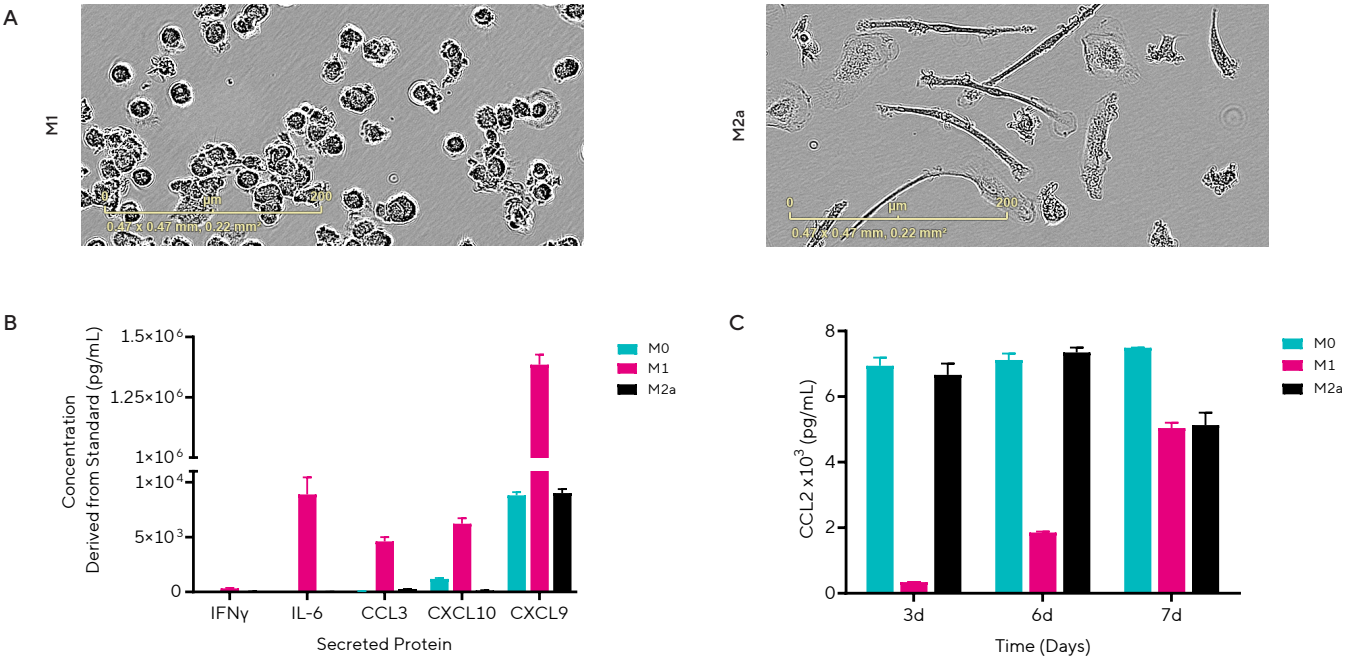

Figure 3: *M1 Macrophage Polarization Induces Inflammatory Secreted Protein Release.*
Representative images on Day 7 of macrophage morphology following polarization to an M1 or M2a phenotype. B) Concentrations of 5 out of the 7 secreted proteins shown for each macrophage phenotype on Day 7. C) Temporal profile of CCL2 (MCP-1) concentrations for each macrophage phenotype. Data presented as mean + SEM, n = 3 replicates.

Neuroinflammatory Protein Release in Healthy and Diseased iPSC-derived Models

Human iPSC-derived models are increasingly being used for *in vitro* studies of the human central nervous system and its interplay with immune systems. These models enable the generation of healthy and disease specific neurons and support cells, such as microglia, providing a more translational system for investigating human development and disease and enabling the study of emerging therapeutics for neuroinflammatory and neurodegenerative conditions.[17] Due to inherent cell and model sensitivity, methods that are non-invasive and cell sparing are essential to reliably monitor and characterize these complex models.

Microglia, the brain's resident macrophages, play a key role in neuroinflammatory responses, existing in either pro-inflammatory (M1) or anti-inflammatory phenotypic states.[18] They have a crucial function in both the development and progression of neurodegenerative diseases.[19,20] In this study, we explored the effects of pro-inflammatory stimuli on iPSC-derived microglia. We examined two different stimuli, IFNγ and LPS, with a combination of both serving as a positive control for inducing an M1 pro-inflammatory microglial phenotype (Figure 4).

Microglia were treated with concentration ranges of these stimuli or a single concentration (100 ng/mL) in the presence of the anti-inflammatory compound DEX (500 nM) and supernatant samples taken at 4, 24, or 48 hours post-treatment were assessed using HTS by Cytometry and the Human Inflammation Panel Kit. The gating strategy used to identify the various secreted proteins (Figure 4A) and example curves for standards used to derive protein concentrations of samples are shown (Figure 4B). IFNγ was only analyzed for conditions where it was not exogenously used as a stimulatory agent. In response to the two stimulants, we observed the release of several secreted proteins. For instance, following stimulation with IFNγ, CXCL10 (IP-10) was released in a temporal and concentration-dependent manner with levels marginally lower than the positive control and this increase was not inhibited in the presence of DEX (Figure 4C). For CCL3 (MIP-1α), we observed similar temporal release with a small concentration-dependent effect which was diminished in the presence of DEX (Figure 4D).

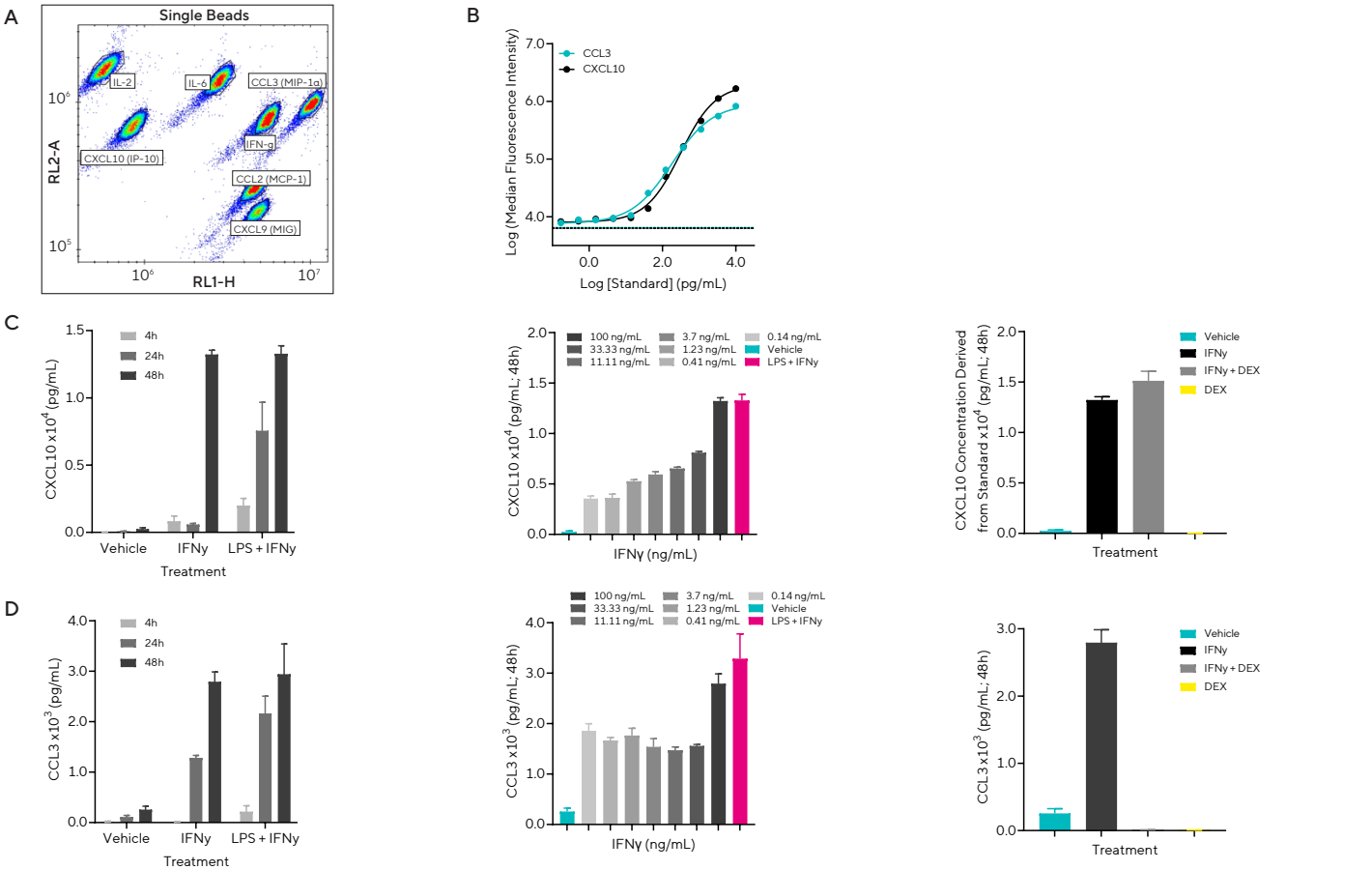

Figure 4: *Pro-inflammatory stimulation of microglia induces inflammatory secreted protein release*
Human iPSC-derived microglia were treated with LPS and IFNγ, in the absence or presence of DEX. A) Forecyt® gating strategy for each secreted protein. B) Cytokine standards from which concentrations were derived shown for two of the seven secreted proteins, CCL3 and CXCL10. C) CXCL10 concentration in response to 100 ng/mL IFNγ or positive control across 4 – 48 hours (left), a concentration range of IFNγ at 48 hours (middle), and 100 ng/mL IFNγ in the presence or absence of DEX at 48 hours (right). D) CCL3 (MIP-1α) concentration in response to 100 ng/mL IFNγ or positive control across 4 – 48 hours (left), a concentration range of IFNγ at 48 hours (middle), and 100 ng/mL IFNγ in the presence or absence of DEX at 48 hours (right). Data presented as mean + SEM, n = 3 replicates.

In a second study of neuroinflammation and neurodegeneration, we assessed secreted protein release in healthy or AD iPSC-derived models and examined neurons in mono- or co-culture with healthy iPSC-derived microglia. Supernatant samples taken on Day 30 were analyzed using HTS by Cytometry and a custom-built kit designed to detect the release of 8 human secreted proteins of interest (Figure 5). Overall, we observed an increase in the levels of GM-CSF and CCL2 proteins for neurons in co-culture compared to mono-culture, suggesting differences in basal secretory profiles of cultures in the presence of microglia. Additionally, the results revealed that the levels of 3 inflammatory cytokines, IL-6, CSF-2, and CCL2, were consistently higher in diseased compared to healthy phenotypes in co-culture conditions. For example, CCL2, a chemokine that has been implicated in mediating microglia accumulation at neuroinflammatory sites and in altered metabolism of amyloid-beta in AD[21], was increased from 3,197 ± 297 to 6,235 ± 628 pg/mL for healthy compared to diseased co-cultures, respectively.

This approach allows for high-throughput multiplexed cytometry to be performed, with the miniaturization of the assay enabling temporal evaluation of secreted protein release in a non-perturbing manner. Ultimately, allowing us to monitor sensitive translational cellular models and probe deeper into neuroinflammatory mechanisms and potential therapeutic effects.

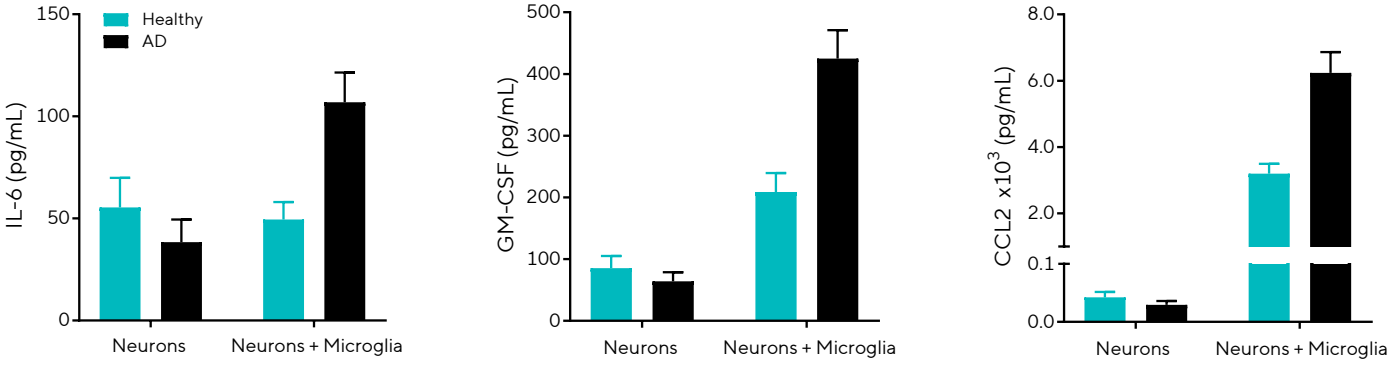

Figure 5: *Elevated pro-inflammatory secreted proteins observed in AD iPSC-derived neuron and microglia co-cultures.*
Healthy or AD (PSEN1 mutation) neurons co-cultured on Day 19 with iPSC-derived monocytes. IL-6, GM-CSF, and CCL2 concentrations derived from standards shown for healthy or AD iPSC-derived mono-cultures or co-cultures. Data presented as mean + SEM, n = 3 replicates.

Summary and Outlook

Quantification of biologically relevant secreted chemokines and cytokines is crucial in basic research and drug development, facilitating understanding of immune cell activation and complex cellular signaling cascades involved in neuroinflammation and neurodegeneration. Existing technologies for the detection of proteins in solution often have limitations and trade-offs between ease, speed, the ability to multiplex and cost. To effectively screen secreted proteins, approaches must have the speed and sensitivity to support high-throughput, whilst also being cost effective and provide meaningful data.

The data shown highlights how iQue Qbeads® and iQue® HTS by Cytometry can be utilized to simultaneously investigate a broad range of secreted proteins in physiologically relevant immune cell or iPSC-derived neuroinflammatory models. These flexible assays enable target proteins of interest to be studied in a miniaturized format, permitting the assessment of multiple treatments at several timepoints, and allowing for a spectrum of biological responses to be observed. We have demonstrated how they can be performed for deeper insights into temporal secreted protein release in response to pro-inflammatory stimuli and examine phenotypic differences in healthy and disease states.

References

1. Liu, C. *et al.* Cytokines: From Clinical Significance to Quantification. *Advanced Science* 8, (2021).
2. DiSabato, D. J., Quan, N. & Godbout, J. P. Neuroinflammation: the devil is in the details. *J Neurochem* 139, 136–153 (2016).
3. Zhang, W., Xiao, D., Mao, Q. & Xia, H. Role of neuroinflammation in neurodegeneration development. *Signal Transduct Target Ther* 8, 267 (2023).
4. Bucheli, O. T. M., Sigvaldadóttir, I. & Eyer, K. Measuring single-cell protein secretion in immunology: Technologies, advances, and applications. *Eur J Immunol* 51, 1334–1347 (2021).
5. Leng, S. X. et al. ELISA and Multiplex Technologies for Cytokine Measurement in Inflammation and Aging Research. J Gerontol A Biol Sci Med Sci 63, 879–884 (2008).
6. bit.bio. ioMicrogliaTM User Manual. https://www.bit.bio/resources/iomicroglia-user-manual
7. Axol Bioscience. Cortical Excitatory Neurons User Guide. https://axolbio.com/publications/https-axolbio-com-wp-content-uploads-2024-06-axol-user-guide-monoculture-axocells-cortical-excitatory-neurons-version-2-june-2024-pdf
8. Axol Bioscience. Microglia User Guide. https://axolbio.com/publications/https-axolbio-com-wp-content-uploads-2024-03-axol-user-guide-monoculture-axocells-microglia-march-2024-pdf.
9. Nam, H. Y. et al. Ibrutinib suppresses LPS-induced neuroinflammatory responses in BV2 microglial cells and wild-type mice. *J Neuroinflammation* 15, 271 (2018).
10. Ngabire, D. et al. Anti-Inflammatory Effects of Aster incisus through the Inhibition of NF-κB, MAPK, and Akt Pathways in LPS-Stimulated RAW 264.7 Macrophages. *Mediators Inflamm* 2018, 1–10 (2018).
11. Xie, S. *et al.* Identification of a Role for the PI3K/AKT/mTOR Signaling Pathway in Innate Immune Cells. *PLoS One* 9, e94496 (2014).
12. Chu, E., Mychasiuk, R., Hibbs, M. L. & Semple, B. D. Dysregulated phosphoinositide 3-kinase signaling in microglia: shaping chronic neuroinflammation. *J Neuroinflammation* 18, 276 (2021).
13. Manning, B. D. & Toker, A. AKT/PKB Signaling: Navigating the Network. *Cell* 169, 381–405 (2017).
14. Genin, M., Clement, F., Fattaccioli, A., Raes, M. & Michiels, C. M1 and M2 macrophages derived from THP-1 cells differentially modulate the response of cancer cells to etoposide. *BMC Cancer* 15, 577 (2015).
15. Mantovani, A., Sozzani, S., Locati, M., Allavena, P. & Sica, A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. T*rends Immunol* 23, 549–555 (2002).
16. Gordon, S. & Taylor, P. R. Monocyte and macrophage heterogeneity. *Nat Rev Immunol* 5, 953–964 (2005).
17. Zhu, Z. & Huangfu, D. Human pluripotent stem cells: an emerging model in developmental biology. *Development* 140, 705–717 (2013).
18. Li, S., Wernersbach, I., Harms, G. S. & Schäfer, M. K. E. Microglia subtypes show substrate- and time-dependent phagocytosis preferences and phenotype plasticity. *Front Immunol* 13, (2022).
19. Woodburn, S. C., Bollinger, J. L. & Wohleb, E. S. The semantics of microglia activation: neuroinflammation, homeostasis, and stress. *J Neuroinflammation* 18, 258 (2021).
20. Kwon, H. S. & Koh, S.-H. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener* 9, 42 (2020).
21. Westin, K. et al. CCL2 Is Associated with a Faster Rate of Cognitive Decline during Early Stages of Alzheimer's Disease. *PLoS One* 7, e30525 (2012).

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