Modulation of neuroinflammation by interleukin-10 in mouse microglial cells

Kathryn M Nelson, Nichole Schrantz, Isabel O'Brien, Jim Rivard, Callie Sagarsky, Rainer Grant, Makenna Carlson-Habegger, Megan E Larson, Mandy Kubik

Email: kathryn.nelson@bio-techne.com
Bio-techne, Inc., Minneapolis, MN 55413

Microglia are resident macrophages of the central nervous system that are important for maintaining brain homeostasis. Persistent activation of microglia is associated with neuroinflammation in neurodegenerative disorders. Interest in immortalized microglia cell lines as cell culture models to study their biology and role in neuroinflammation has increased in the past decade, with mouse microglial BV-2 cells emerging as an *in vitro* model for neuronal inflammation similar to primary mouse microglial cells. We report here IL-10 pretreatment of BV-2 cells compared to primary microglia from CD-1 mice. A 45-plex Luminex® immunoassay was used to measure IL-10 pretreatment attenuation of TNF-α, IL-6, CCL5/RANTES, and G-CSF secretion from cells treated with IFN-γ and LPS. In addition, we demonstrate the effects of small molecule inhibitors MLN 4924, CP 690550 (tofacitinib), and Cardamonin on IL-10 pretreatment. MLN4924 inhibits the NF-κB pathway and decreased secretion of multiple cytokines and chemokines, including TNF-α, IL-6, CCL2/MCP-1, CCL5, CCL3/MIP-1α, and CCL4/MIP-1β. CP 690550 inhibits the Jak1/Tyk2-STAT3 pathway, decreasing STAT-3 TYR705 phosphorylation, but had little effect on IL-10 cytokine suppression. Interestingly, IL-10 pretreatment increased secretion of IL-1ra, an M2b microglia phenotype marker, and chemokines CCL2 and CCL3. Multiplex immunoassays are a valuable tool for investigating the mechanisms of neuroinflammation in primary and immortalized cell lines.

Materials and Methods

Cell Culture and Treatments

Immortalized mouse microglia BV-2 cells were cultured using DMEM (high glucose) supplemented with 5% FBS containing 100 units/mL penicillin and 100 µg/mL streptomycin, and maintained in a 5% CO_2 incubator at 37 °C. BV-2 cells remained untreated or were treated for one hour with 50 ng/mL Recombinant Mouse IL-10 (R&D Systems, Catalog # 417-ML) followed by 0.5 ng/mL Recombinant Mouse IFN-γ (R&D Systems, Catalog # 485-MI) and 100 ng/mL *E. coli* Lipopolysaccharide type O127:B8 (Sigma, Catalog # L4516) for different time points. To investigate the effects of small molecule inhibitors, BV-2 cells were treated for one hour with DMSO carrier, 0.2 µM CP 690550 (Tocris, Catalog # 4556), 0.2 µM MLN 4924 (Tocris, Catalog # 6499) or 0.5 µM Cardamonin (Tocris, Catalog # 2509) prior to treatment with IL-10 and LPS + IFN-γ for 0 min, 15 min, 2 hours, and 18 hours. Primary mouse microglia isolated from postnatal day 2 CD-1 mouse brain were obtained from ScienCell (Carlsbad, CA, USA) and cultured using the recommended cell culture media. Cell culture supernates from BV-2 cells and primary microglia treated for 18 hours with LPS + IFN-γ were tested using Luminex and Ella immunoassays.

Luminex Analysis

Cell culture supernate samples were analyzed for 45 different analytes using the Mouse XL Cytokine Luminex Performance Panel (Bio-Techne, Catalog # FCSTM20). Reported concentrations are back-calculated for dilution factor.

Ella Immunoassay Analysis

Cell culture supernate samples were analyzed for nine analytes using the Ella™ Microfluidic-based Immunoassay System. Simple Plex cartridges for CCL2/JE/MCP-1 (Bio-techne, Catalog # SPCKB-MP-000584), CCL3/MIP-1α (Bio-techne, Catalog # SPCKB-MP-007987), CCL4/MIP-1β (Bio-techne, Catalog # SPCKB-MP-003557), G-CSF (Bio-techne, Catalog # SPCKB-MP-007990), CCL5/RANTES (Bio-techne, Catalog # SPCKB-MP-007962), IL-1ra/IL-1F3 (Bio-techne, Catalog # SPCKB-MP-004019), TNF-α (Bio-techne, Catalog # SPCKB-MP-000822), and IL-6 (Bio-techne, Catalog # SPCKB-MP-000466).

Immunoblot Analysis

Cell lysates were prepared from BV-2 cells treated for short time points (0 to 2 hours) using Lysis Buffer 17 (Catalog # 895943) and tested on the Simple Western capillary-based immunoassay instrument Jess (Protein Simple). Phospho-STAT1 was detected using Rabbit Anti-Human/Mouse Phospho-STAT1 (Y701) Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF2894) followed by HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # HAF008). Total STAT1 was detected using Goat Anti-Human/Mouse STAT1 p91 Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # PAF-ST1) followed by HRP-Conjugated Donkey Anti-Goat IgG Secondary Antibody (R&D Systems, Catalog # HAF109). Phospho-STAT3 was detected using Rabbit Anti-Human Phospho- STAT3 (Y705) Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF4607) followed by HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # HAF008). Total STAT3 was detected using Mouse Anti-Human/Mouse/Rat/Monkey STAT3 Monoclonal Antibody (Cell Signaling Technology, Danvers, MA, USA, Catalog # 4904T) followed by HRP-Conjugated Goat Anti-Mouse IgG Secondary Antibody (R&D Systems, Catalog # HAF007).

References

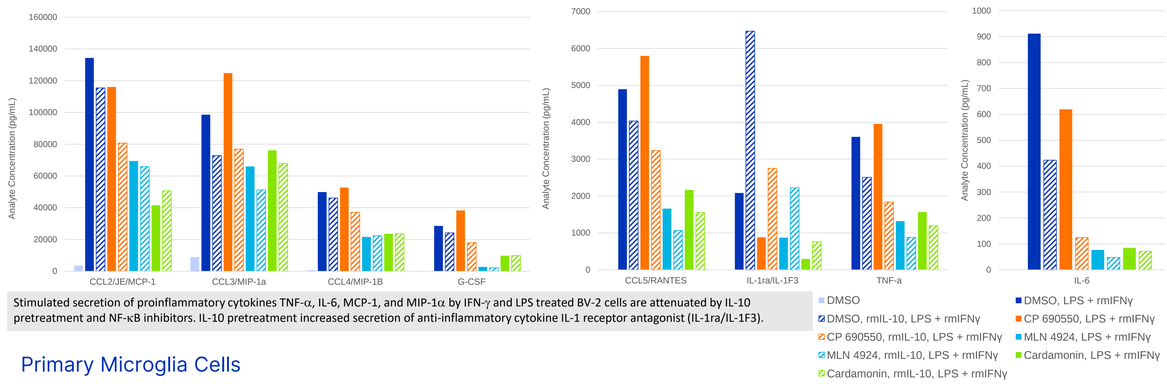
Boyle, D.L. *et al.* (2015) Ann. Rheum. Dis. 74:1311.

Gresa-Arribas, N. *et al.* (2012) PLoS on, ONE 7:e45 227.

Pattis M.J. *et al.* (2012) J. Immunol. 189:2784.

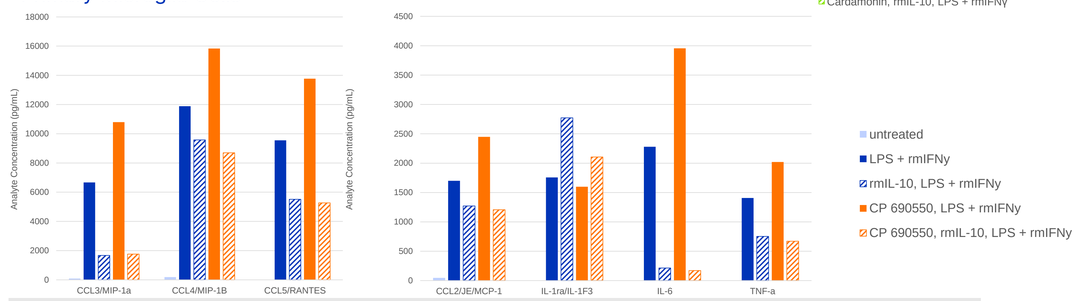
Cytokine Levels Measured in Bio-Techne Luminex Assay

BV-2 Cells



Stimulated secretion of proinflammatory cytokines TNF-α, IL-6, MCP-1, and MIP-1α by IFN-γ and LPS treated BV-2 cells are attenuated by IL-10 pretreatment and NF-κB inhibitors. IL-10 pretreatment increased secretion of anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra/IL-1F3).

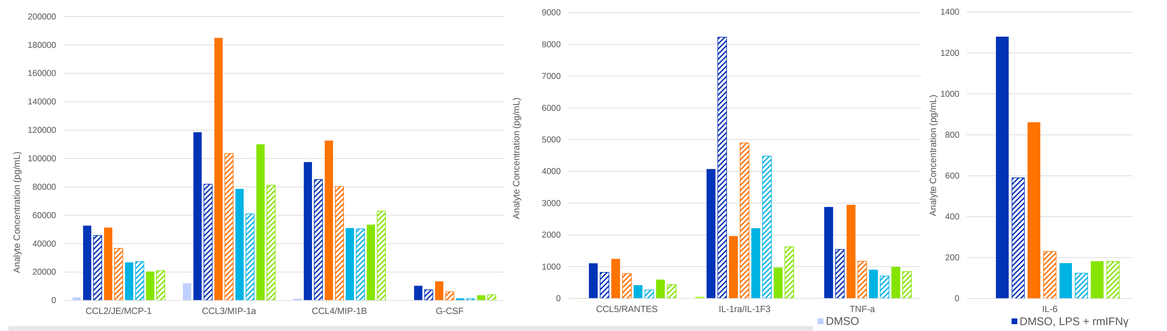
Primary Microglia Cells



TNF-α, IL-6, MCP-1, and MIP-1α secreted by IFN-γ and LPS treated primary microglia are attenuated by IL-10 pretreatment and are attenuated by the Jak kinase inhibitor CP 690550. IL-10 pretreatment increased secretion of anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra/IL-1F3).

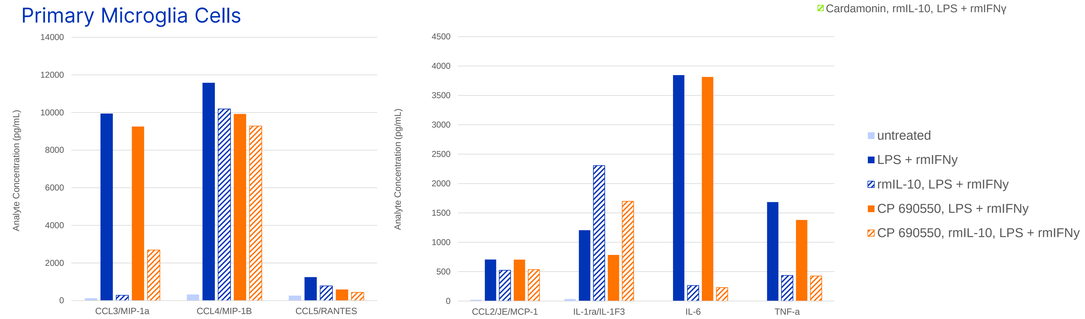
Cytokine Levels Measured by Ella Immunoassay

BV-2 Cells



TNF-α, IL-6, MCP-1, and MIP-1α secreted by IFN-γ and LPS treated BV-2 cells are attenuated by IL-10 pretreatment and NF-κB inhibitors. IL-10 pretreatment increased secretion of anti-inflammatory cytokine IL-1 receptor antagonist (IL-1ra/IL-1F3). These data have a similar pattern to Luminex.

Primary Microglia Cells

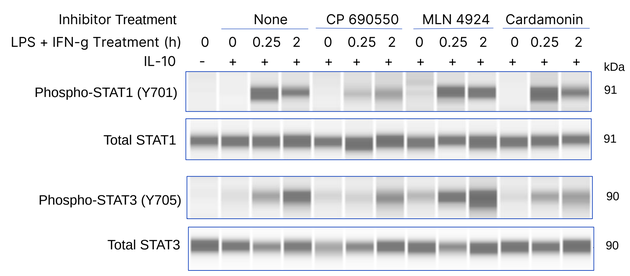


TNF-α, IL-6, MCP-1, and MIP-1α secreted by IFN-γ and LPS treated primary microglia are attenuated by IL-10 pretreatment and are not attenuated by the Jak kinase inhibitor CP 690550. IL-10 pretreatment increased secretion of anti-inflammatory cytokine IL-1 receptor antagonist (IL-1 ra/IL-1F3). These data have a similar pattern to Luminex.

Mouse XL Cytokine Panel, 45-plex

BAFF/BLyS/TNFSF13B	GM-CSF	IL-12 p70
CCL2/JE/MCP-1	ICAM-1/CD54	IL-13
CCL3/MIP-1α	IFN-γ	IL-16
CCL4/MIP-1β	IL-1α/IL-1F1	IL-17/IL-17A
CCL5/RANTES	IL-1β/IL-1F2	IL-18/IL-1F4
CCL11/Eotaxin	IL-1ra/IL-1F3	IL-21
CCL19/MIP-3β	IL-2	IL-27
CXCL1/GROα/KC/CINC-1	IL-3	IL-31
CXCL10/IP-10/CRG-2	IL-4	LDLR
Chitinase 3-like 1/YKL-40	IL-5	LIF
EGF	IL-6	LIX
FGF basic/FGF2/bFGF	IL-7	M-CSF
Flt-3 Ligand/FLT3L	IL-9	TIMP-1
G-CSF	IL-10	TNF-α
GDF-15	IL-11	VEGF

CP 690550 Inhibits Jak-Dependent STAT1 and STAT3 Phosphorylation



CP 690550 (tofacitinib) attenuated Jak kinase-dependent STAT1 and STAT3 phosphorylation, while NF-κ B pathway inhibitors MLN 4924 and Cardamonin did not. Simple Western capillary-based immunoassay instrument Jess (Protein Simple) was used for this testing.

Conclusions

- Neuroinflammatory cytokine secretion by immortalized and primary microglial was modulated by IL-10 pretreatment and small molecule inhibitors of JAK-STAT and NF-κB pathways.
- The Mouse XL Cytokine Luminex Performance Assay (Cat # FCSTM20) simultaneously measured levels of 45 cytokines using only 60 µL. The large number of analytes in this multiplex panel provided a more complete picture of cytokines, particularly IL-1 receptor antagonist (IL-1ra).
- Ella capillary-based automated immunoassay measurement of eight key analytes corroborated results and trends from Luminex Assays.
- IL-10 pretreatment of IFN-γ/LPS treated BV-2 cells and primary microglia decreased secretion of TNF-α, IL-6, CCL5/RANTES, and G-CSF, while increasing secretion of IL-1ra.
- Jess capillary-based immunoassays are an important tool for showing small molecule inhibitor mechanism of action.
- Luminex and Ella immunoassays are powerful workflows for better understanding inflammatory cytokine secretion in neuronal cells.