Modulation of Interleukin-10 Immunosuppression by Tofacitinib (CP 690550) in the Mouse Microglial Cell Line BV-2


Introduction
The anti-inflammatory cytokine interleukin-10 (IL-10) is known to be necessary for down regulating proinflammatory responses toward pathogens. IL-10 inhibits the production of many cytokines downstream of its receptor via the Jak/STAT signaling pathway. The small molecule Tofacitinib, also called CP 690550, is a selective inhibitor of the Jak family of cytoplasmic tyrosine kinases. It has been shown to potently inhibit both Jak3 and Jak1- dependent STAT activation. Mouse microglial BV-2 cells are an established in vitro model for neuronal and inflammatory disease and have been reported to respond to IL-10 immunosuppression in a manner similar to primary mouse microglia. We used this model to examine the effects of Tofacitinib on the immunosuppression of proinflammatory cytokine secretion and the activation of the Jak/STAT signaling pathway by IL-10.

Materials and Methods

Cell Culture and Treatments
Immunoblotted mouse microglia BV-2 cells were cultured using DMEM (high glucose) supplemented with 5% FCS containing 100 units/ml penicillin and 100 μg/ml streptomycin, and maintained in a 5% CO2 incubator at 37 °C. BV-2 cells remained untreated or were treated for one hour with 10 ng/ml Recombinant Mouse IL-3 (R&D Systems, Catalog # 417-M3) followed by 0.5 ng/mL Recombinant Mouse IFN-γ (R&D Systems, Catalog # 417-γ1) prior to treatment with IL-10 and LPS + IFN-γ. BV-2 cells were treated for one hour with ZM244740 (R&D Systems, Catalog # HAF016). Cell culture supernates from BV-2 cells were analyzed using the Proteome Profiler Mouse XL Cytokine Array (R&D Systems, Catalog # ARY028).

Phospho-STAT1 (Y701) Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF2894) followed by a HRP-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (R&D Systems, Catalog # AF5809) followed by a HRP-Conjugated Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF4809) followed by a HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # AF5120). A goat anti-rabbit IgG HRP conjugate was used for detection. ICAM-1/CD54, IL-1, IL-6, Cystatin C, IP-10/CRG-2, MCP-1, CXCL2/MIP-2, CXCL10/IP-10/CRG-2, CCL3/4 (MIP-1β), CXCL9/MIG, G-CSF, IL-1β, IL-1a, IL-1α, IL-6, Osteopontin (OPN) and TNF-α, were measured using the Mouse Luminex Bead-Based Multianalyte Profiling Screening Assay (R&D Systems, Catalog # BBA00M). The concentrations of the analytes were determined by interpolation using their standard curves.

Figure 1. Tofacitinib Attenuates IL-10 Immunosuppression of LPS + IFN-γ-Induced Cytokine Secretion.

Figure 2. Quantitative Analysis of Cytokine Levels Following Treatment with LPS + IFN-γ, IL-10, and Tofacitinib.

Figure 3. STAT1, STAT3, and Il-6/Il-10 are Phosphorylated with LPS + IFN-γ and/or IL-10.

Figure 4. Tofacitinib Inhibits Jak-Dependent STAT1 and STAT3 Phosphorylation.

Cell Culture supernates from BV-2 cells were diluted 1:2 and 1:50 and then the concentrations of 52 different analytes, including CCL2/E/MCP-1, CCL4/40, CCL5/RANTES, CCL2/JE/MCP-2, CXCL8/IL-8, CXCL10/IP-10/CRG-2, CXCL2/MIP-2, CCL3/4 (MIP-1α), and low (0.2 pg/mL) Tofacitinib (Toolls, Catalog # 45305) or 0.2 μM MLN4924 (R&D Systems, Catalog # AM001) prior to treatment with IL-10 and LPS + IFN-γ.

Proteome Profiler® Antibody Array Analysis
Cell culture supernatants from BV-2 cells were analyzed using the Proteome Profiler® Mouse XL Cytokine Array Assay (R&D Systems, Catalog # ARY028).

Western blot analysis
Cell culture supernatants from BV-2 cells were dialyzed 1:2 and 1:5 and then the concentrations of 52 different analytes, including CCL2/E/MCP-1, CCL4/40, CCL5/RANTES, CCL2/JE/MCP-2, CXCL8/IL-8, CXCL10/IP-10/CRG-2, CXCL2/MIP-2, CCL3/4 (MIP-1α), and low (0.2 pg/mL) Tofacitinib (Toolls, Catalog # 45305) or 0.2 μM MLN4924 (R&D Systems, Catalog # AM001) prior to treatment with IL-10 and LPS + IFN-γ.

Western blot analysis of cell cultures was performed using a Rabbit Anti-Human/Mouse IL-6/IL-27/IL-22/IL-1b/IL-17/IL-10/IL-12 antibody, a Goat Anti-Human/Mouse IL-1β/IL-6/IL-12/IL-13/IL-22/IL-23/IL-27 antibody, a Goat Anti-Human/Mouse IFN-γ/FN-γ/IFN-γR1/IFN-γR2 antibody, a Mouse Anti-Human/Mouse IL-17A/17F/21/23/27/28 antibody, and a Rabbit Anti-Human/Mouse IL-12 antibody (R&D Systems, Catalog # MAB1799) followed by a HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # HAF031), a Goat Anti-Human/Mouse STAT1(S32/36) antibody (R&D Systems, Catalog # AF4607) followed by a HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # HAF031), a Goat Anti-Human/Mouse STAT3(S32/36) antibody (R&D Systems, Catalog # AF4607) followed by a HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # HAF031), and a Mouse Anti-Human/Mouse IFN-κBα antibody (R&D Systems, Catalog # AB4812) followed by a HRP-Conjugated Goat Anti-Mouse IgG Secondary Antibody (R&D Systems, Catalog # AF0494) followed by a HRP-Conjugated Goat Anti-Rabbit IgG Secondary Antibody (R&D Systems, Catalog # HAF031), or a Sheep Anti- Human/Mouse IFN-κBα Affinity-Purified Polyclonal Antibody (R&D Systems, Catalog # AF4299) followed by a HRP-Conjugated Donkey Anti-Sheep IgG Secondary Antibody (R&D Systems, Catalog # AF5120).

Figure 2. Quantitative Analysis of Cytokine Levels Following Treatment with LPS + IFN-γ, IL-10, and Tofacitinib.

Summary
• Pretreatment with IL-10, a potent anti-inflammatory cytokine, attenuates IFN-γ- and LPS-induced inflammatory cytokine secretion by BV-2 cells.
• Tofacitinib, a selective Jak inhibitor, modulates microglial cytokine production induced by LPS and IFN-γ.

References

• Phosphorylation of the Jak substrates STAT1 (701) and STAT3 (705) is inhibited by Tofacitinib.
• These results suggest that Jak inhibition may repress the anti-inflammatory action of IL-10 in vivo and increase the inflammatory responses of microglia that are activated by proinflammatory cytokines.