ApplicationForum

Profiling Modulation of Interleukin-10 Immunosuppression Using Antibody Arrays

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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 Jak1/Tyk2-STAT3 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 inflammation and have been reported to respond to IL-10 immunosuppression in a manner similar to primary mouse microglial cells. 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

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% CO₂ 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 0127:B8 (Sigma, Catalog # L4516) for different durations. To investigate the effects of small molecule inhibitors, BV-2 cells were treated for one hour with DMSO carrier, 0.2 µM Tofacitinib (Tocris, Catalog # 4556), or 0.2 µM MLN4924 (R&D Systems, Catalog # I-502) prior to treatment with IL-10 and LPS + IFN-γ.

Proteome Profiler™ Antibody Array Analysis

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

Summary

Proinflammatory cytokines are secreted by mouse microglia BV-2 cells following activation of TLR4 by its agonist LPS. Pretreatment with IL-10, a potent anti-inflammatory cytokine, attenuates IFN-γ and LPS-induced inflammatory cytokine secretion by BV-2 cells. The expression levels of CD40/TNFRSF5, CXCL9/MIG, G-CSF, ICAM-1/CD54, IL-1α/IL-1F1, IL-6, IL-27, and TNF-α appeared to be attenuated. Tofacitinib, a selective Jak inhibitor, modulates microglia cytokine production induced by LPS and IFN-γ.

To learn more, please visit: rndsystems.com/Immunoassays.

BioTechniques 60:205 (April 2016) doi 10.2144/000114406