Simulation of an Inflammatory Model Using Schwann Cells

Caitlyn Henry, Peyton Kimmel, Mackenzie Wilcox & Angela Asirvatham, Ph.D.
Department of Biology, Misericordia University, Dallas, PA

MISERICORDIA UNIVERSITY

Introduction

Schwann cells are a type of glial cell in the peripheral nervous system that produce the myelin sheath surrounding neuronal axons. This myelin insulates the neurons and promotes the rapid conduction of electrical impulses throughout the body. Schwann cells have also been found to play a critical role in neuron repair following nerve injury. During nerve injury, the myelin sheath is damaged, stimulating Schwann cells to release cytokines, or inflammatory mediators, that recruit immune cells to the site of injury so that the myelin debris can be cleared, and repair can take place.[1] Then neuronal growth is facilitated by heregulin and an unknown growth factor that stimulates the cyclic adenosine monophosphate (cAMP) pathway.[2,3] There is still yet to be known regarding the exact mechanisms by which Schwann cells mediate nerve repair. Two pathways of interest are the nuclear factor kappa B (NK-κB) and cAMP pathways. The NF-κB pathway plays a major role in inflammation through the production of cytokines like tumor necrosis factor alpha (TNF-α) and can be stimulated *in vitro* by treating cells with lipopolysaccharide (LPS), a cell wall immunostimulatory component of Gram-negative bacteria.[1] The cAMP pathway is a key regulator of cell division[2,4] and can be stimulated by treating cells with an artificial plant extract called forskolin.[2] This study aims to examine proteins of the NF-κB pathway when stimulated with cAMP-activating growth factors. It was hypothesized that cells treated with LPS and growth factors express less NF-κB and TNF-α than cells treated with LPS only. A better understanding of the mechanisms underlying nerve injury and Schwann cell-mediated repair will hopefully shed light on a potential therapeutic target in the treatment of nerve injury and inflammation.

Methods

1. Cell Culture: The S16 immortalized rat Schwann cell line (ATCC) was aseptically cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% 1:1 penicillin:streptomycin at 37°C and 5% CO_2.
2. Lysate Preparation: Cells were treated with 1 µg/mL of LPS with N_2 (control media), heregulin (H), forskolin (F), or heregulin plus forskolin, for 3 hours. Cell lysates were prepared using lysis buffer.
3. Immunoblotting: Cell lysates were separated using SDS-PAGE gel electrophoresis, followed by Western blot using antibodies against NF-κB and TNF-α. β-actin was used as a loading control. NF-κB and TNF-α expression was quantified using densitometry analysis.
4. Immunofluorescence: Cells were cultured in 8-well chamber slides and incubated in antibodies against NF-κB and TNF-α. Proteins were visualized using fluorescently-tagged secondary antibodies.

Results

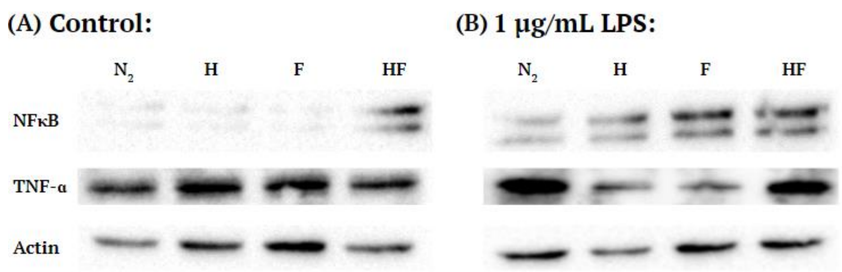

Figure 1. NF-κB and TNF-α expression in (A) unstimulated SC-2941 cells and (B) SC-2941 cells stimulated with 1 µg/mL LPS for 3 hours with different growth factor treatments (N_2 = control media; H = heregulin; F = forskolin; HF = heregulin + forskolin). Actin is shown as a loading control. n=1.

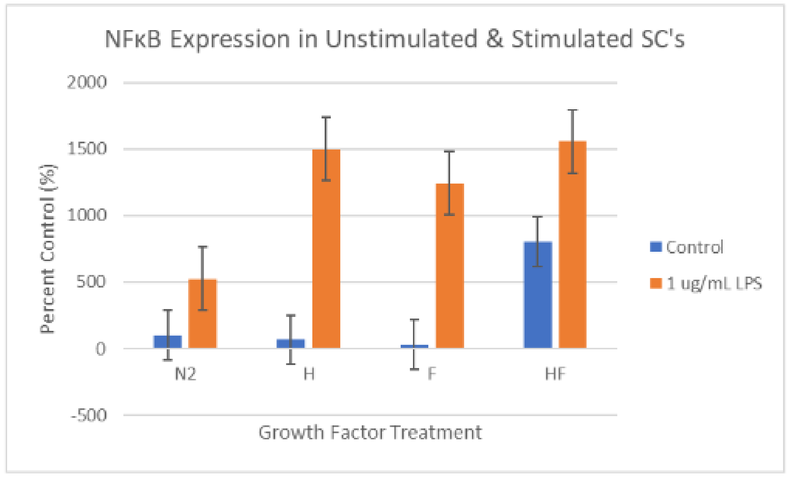

Figure 2. Relative NF-κB expression in unstimulated SC-2941 cells and SC-2941 cells stimulated with 1 µg/mL LPS for 3 hours with different growth factor treatments. Band densities expressed as percent control ± SEM (n=1).

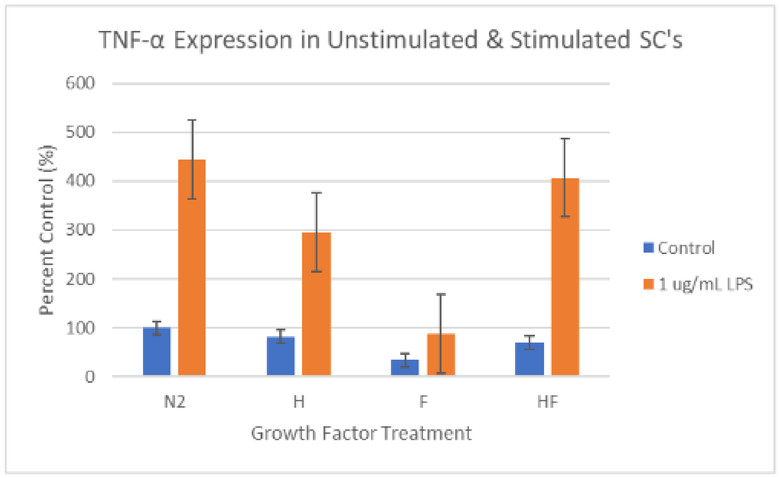

Figure 3. Relative TNF-α expression in unstimulated SC-2941 cells and SC-2941 cells stimulated with 1 µg/mL LPS for 3 hours with different growth factor treatments. Band densities expressed as percent control ± SEM (n=1).

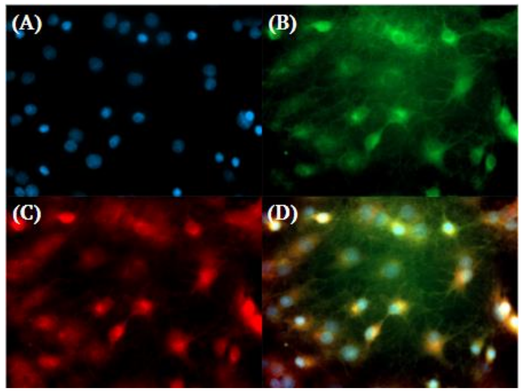

Figure 4. Representative set of images depicting the locations of NF-κB and TNF-α in the cell regardless of LPS-stimulation. Images taken at 63x magnification using a Zeiss Observer Z1 with AxioCam MRm (A = DAPI; B = NF-κB; C = TNF-α; D = Merged).

Conclusions

- Schwann cells appear to produce TNF-α with or without incubation with LPS.
- 1 µg/mL LPS and growth factors may act synergistically to upregulate NF-κB expression while downregulating TNF-α expression.
- Potential crosstalk between NF-κB and cAMP pathways during LPS-stimulated inflammation

Future Plans

- Use a different Schwann cell line: RT4-D6P2T
- Try higher doses of LPS to stimulate inflammation
- Incubate Schwann cells in LPS for various time points to determine time-dependent effects
- Measure expression of other pro-/anti-inflammatory mediators downstream of the NF-κB pathway.

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