The Location of NF-κB and AKAP-95 in Lipopolysaccharide Treated RT4-D6P2T Schwannoma Cells

Mackenzie Wilcox and Angela L. Asirvatham, Ph.D.
Department of Biology, Misericordia University, Dallas, PA

Introduction

Schwann cells (SCs) are the primary supportive cell of the peripheral nervous system (PNS), which form the myelin sheath around the axon. During nerve injury, SCs secrete pro-inflammatory cytokines, such as TNF-α, through pathways, such as the NF-κB pathway,[3] to combat the injury.[2] Then, SCs alter their phenotype to begin dividing.[5] SC growth is regulated by forskolin, a plant extract, which activates the cAMP pathway required for SC division.[4] Lipopolysaccharide (LPS) triggers an inflammatory condition within SCs and therefore has been used *in vitro* to simulate an inflammatory model of SCs.[1] Preliminary observations have indicated that LPS treatment upregulates TNF-α expression, but downregulates NF-κB expression. In cAMP stimulated cells, AKAP-95, an important anchoring protein of the cAMP/protein kinase A pathway, also expresses an altered expression when treated with LPS. The primary goal of this study was to investigate the location, translocation, and interaction of NF-κB, TNF-α, and AKAP-95 after LPS treatment and cAMP stimulation. It was hypothesized that LPS treatment of cAMP-stimulated Schwann cells will increase cell viability at low dose and cause NF-κB to remain in the cytoplasm and AKAP-95 in the nucleus.

Methods

Cell Culture:

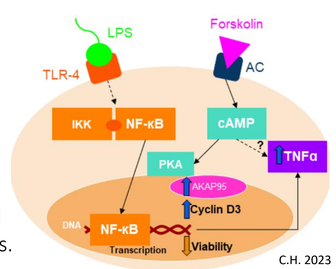
- RT4-D6P2T Schwannoma Cell Line (ATCC Cat# CRL-2768) was grown on poly-L-lysine coated culture dishes in DMEM at 37°C and 5% CO_2.

Cell Viability:

- The RT4-D6P2T Schwannoma Cell line was cultured in 96-well dishes and treated with various doses of LPS with or without 2mM forskolin for 1-, 3-, 12-, or 24-hours .
- Using the MTT cell viability assay, the optical density of each treatment was read at 570nm as an indicator of cell viability.

Immunofluorescence:

- RT4-D6P2T Schwannoma Cell line was cultured in 8-well chamber slides and incubated in primary antibodies against NF-κB and AKAP-95.
- Proteins were visualized using fluorescently-tagged secondary antibodies.



The Effects of LPS on RT4-D6P2T Schwannoma Cells

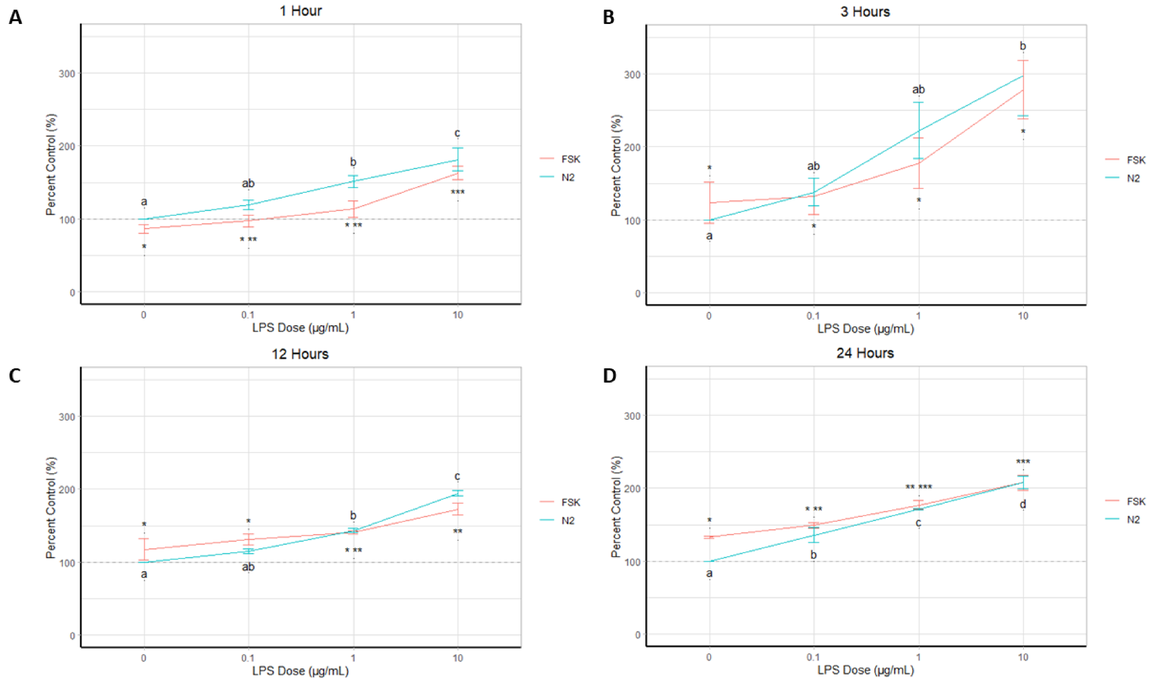


Figure 1. Effect of Lipopolysaccharide (LPS) on RT4-D6P2T Schwannoma cells with and without forskolin. Using the MTT viability assay, the RT4 Schwannoma cell line was treated for (**A)** 1-, (**B)** 3-, (C) 12-, and (**D**) 24-hours with no growth factors (control media, N2) or 2mM forskolin (F) and 0µg/mL LPS, 0.5µg/mL LPS, 1µg/mL LPS, or 10µg/mL LPS in 96-well plates (n=3). The optical density of each treatment was read at 570nm as an indicator of cell viability and was analyzed and displayed as percent control (%).

The Effects of LPS on RT4-D6P2T Schwannoma Cells

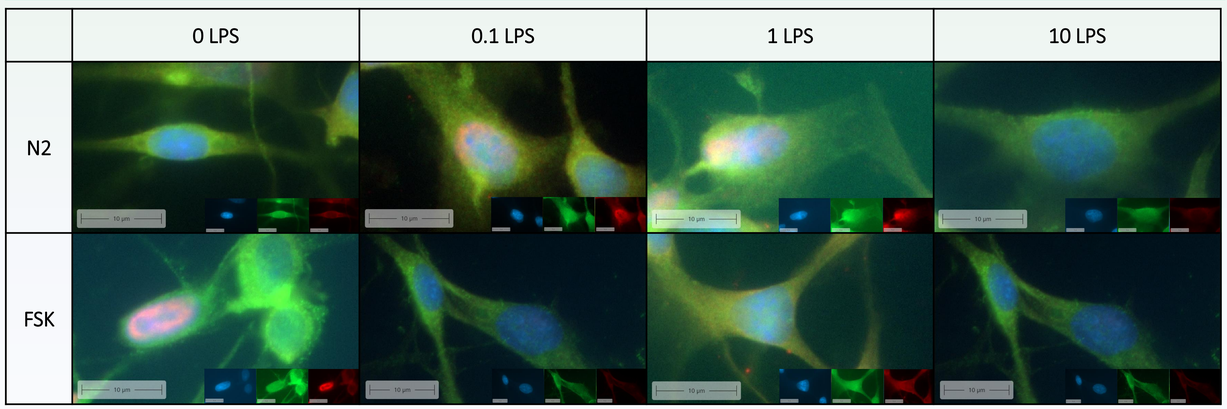


Figure 2. Effect of Lipopolysaccharide (LPS) on RT4-D6P2T Schwannoma cells NF-κB and AKAP-95 location with and without forskolin. RT4-D6P2T Schwannoma cells were treated for 1-hour with no growth factors (control media, N2) or 2µM forskolin (F) and 0µg/mL LPS, 0.5µg/mL LPS, 1µg/mL LPS, or 10µg/mL LPS in 8-well chamber slides, then incubated with primary antibodies against NF- κB and AKAP-95. Proteins were identified using secondary antibodies tagged with fluorescein isothiocyanate (FITC) or Texas Rhodamine red (TXR) (n=3). Images were visualized using a Zeiss Axio Observer Z1 inverted fluorescent microscope.
Video of colocalization available upon request.

Conclusion

- Overall, 10µg/mL LPS increased proliferation significantly regardless of cAMP stimulation.
- Cell viability based on cAMP-stimulated with LPS-treatment is time-dependent and most effective after 12-hours, with the exception of 10µg/mL LPS-treated cells.
- Overlapping staining patterns of DAPI and NF-κB and NF-κB and AKAP-95 did not appear to be present.
- From qualitative image analysis, there appears to be no translocation of NF-κB from the cytoplasm into the nucleus.

References

1. Cheng C, Qin Y, Shao X, Wang H, Gao Y, Cheng M, Shen A. 2007. Induction of TNF-α by LPS in Schwann cell is regulated by MAPK activation signals. Cell Mol Neurobiol 27:909-921.
2. Orr M, Gensel J. 2018. Spinal cord injury scarring and inflammation: therapies targeting glial and inflammatory responses. Neurotherapeutics. 15:541-553.
3. Qin Y, Hua M, Duan Y, Gao Y, Shao X, Wang H, Tao T, Shen A, Cheng C. 2012. TNFα expression in Schwann cells is induced by LPS and NF-kB-dependent pathways. Neurochem Res. 37:722-731.
4. Rahmatullah, M, Schroering, A, Rothblum, K, Stahl, R.C, Urban, B, Carey, D.J. 1998. Synergistic regulation of Schwann cell proliferation by heregulin and forskolin. Molecular and Cellular Biology 18(11): 6245-6252.
5. Soto J, Monje P. 2017. Axon contact-driven Schwann cell dedifferentiation. Glia. 65(6):864-882.
6. Wall EA, Zavzavadjian JR, Chang MS, Randhawa B, Zhu X, Hsueh RC, Liu J, Driver A, Bao XR, Sternweis PC, Simon MI, Fraser IDC. 2009. Supression of LPS-induced TNFalpha production in macrophages by cAMP is mediated by PKA-AKAP95-p105. Sci Signal 2(75):ra28

Acknowledgements

The researcher would like to thank the Misericordia University Summer Undergraduate Research Fellowship committee, the Pennsylvania Academy of Sciences, the Beta Beta Beta Biological Honor Society, and the Misericordia University Student Research Grant committee for financial assistance. The researcher would also like to acknowledge Leo Carr, Jill Dillon, Helen Bogdon, Caitlyn Henry, and the Misericordia University Biology Department for their assistance with the research.

Misericordia University Printing Services