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# Simulation of an Inflammatory Model Using Schwann Cells

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# Simulation of an inflammatory model using Schwann Cells

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## Abstract

During peripheral nerve injury, the myelin surrounding the neuronal axons is damaged, initiating an inflammatory response to remove myelin debris. Once myelin debris is cleared, Schwann cells acquire a proliferating phenotype which allows them to grow and divide so that remyelination can occur. The neuron stimulates Schwann cell division by secreting growth factors, like heregulin, and an unknown growth factor that activates the cAMP pathway. Although the role of cAMP in axonal regeneration is well-known, not much has been explored about its function in Schwann cells during nerve injury and inflammation. To simulate an inflammatory environment, the S16 Schwann cell line (SC-2941) was activated with lipopolysaccharide (LPS), a cell-wall immunostimulatory component of Gram-negative bacteria. It was hypothesized that Schwann cells stimulated with LPS and growth factors will have higher proliferation in comparison to LPS treatment only. Schwann cells were treated for 1, 3, 12 or 24 hours with no growth factors (control media, N2), 12.5 ng/mL heregulin (H), 2mM forskolin (F) or H+F and various doses of LPS at 5, 50 or 500 ng/mL. Using the MTT proliferation assay, preliminary studies in 24-hour cultures reveal that cell proliferation, as measured by optical density, was significantly higher in cells treated with 5 ng/mL of LPS+F (0.846 ± 0.054), and H+F (1.023 ± 0.189) in comparison to cells grown with H (0.699 ± 0.057) or $N_2$ only (0.765 ± 0.016). In contrast, cells treated for 1, 3 and 12 hours, with various concentrations of LPS revealed an overall decrease in proliferation when compared to 24-hour cultures. However, cultures treated with LPS and F or H+F, for all time points, showed an increase in cell growth when compared to $N_2$ and H. In summary, it appears as though a combination of LPS and forskolin, with or without heregulin, may promote more Schwann cell proliferation than LPS alone. These findings also suggest that, when LPS-activated cells are treated with heregulin or forskolin, alone, they may activate two very distinct pathways to initiate opposite responses, with heregulin hindering cell division and forskolin promoting cell division. However, when heregulin and forskolin are combined, the forskolin-activated cAMP pathway appears to promote higher proliferation to offset the decrease in proliferation initiated by the heregulin pathway. Considering these results, it appears that the cAMP pathway in Schwann cells may play a major role in the inflammatory environment during nerve injury.

## Introduction

Schwann cells are the main supporting cell in the PNS that secrete myelin to insulate neurons and promote the rapid conduction of electrical signals throughout the body. In addition to their neurological functions, Schwann cells play a critical role in the immune system, especially during nerve injury when the myelin surrounding the axon is damaged. In response to this, Schwann cells secrete cytokines, or inflammatory mediators, that attract other immune cells to the site of injury so that myelin debris can be cleared, and repair can take place[1]. Once the debris is cleared, the neuron stimulates Schwann cell growth, or proliferation, by secreting growth factors, like heregulin, and an unknown growth factor that activates the cyclic adenosine monophosphate (cAMP) pathway, which is an important regulator of cell division[2,3]. In vitro, an artificial plant extract called forskolin can be used to stimulate the cAMP pathway[2]. cAMP is well-known as an anti-inflammatory signal in injury[4], but its role in neuronal injury is unclear as it normally promotes cell proliferation. The cAMP pathway in Schwann cells was explored by simulating inflammation using lipopolysaccharide (LPS), a bacterial endotoxin found in the membranes of Gram-negative bacteria. The objective of this research project was to identify the optimal dose (5, 50, or 500 ng/mL of LPS) and time (1, 3, 12, or 24 hours) at which LPS, with or without growth factors, promotes maximum Schwann cell proliferation. It was hypothesized that Schwann cells stimulated with LPS and growth factors for shorter periods of time will have higher proliferation in comparison to LPS treatment only.

## LPS Dose Response

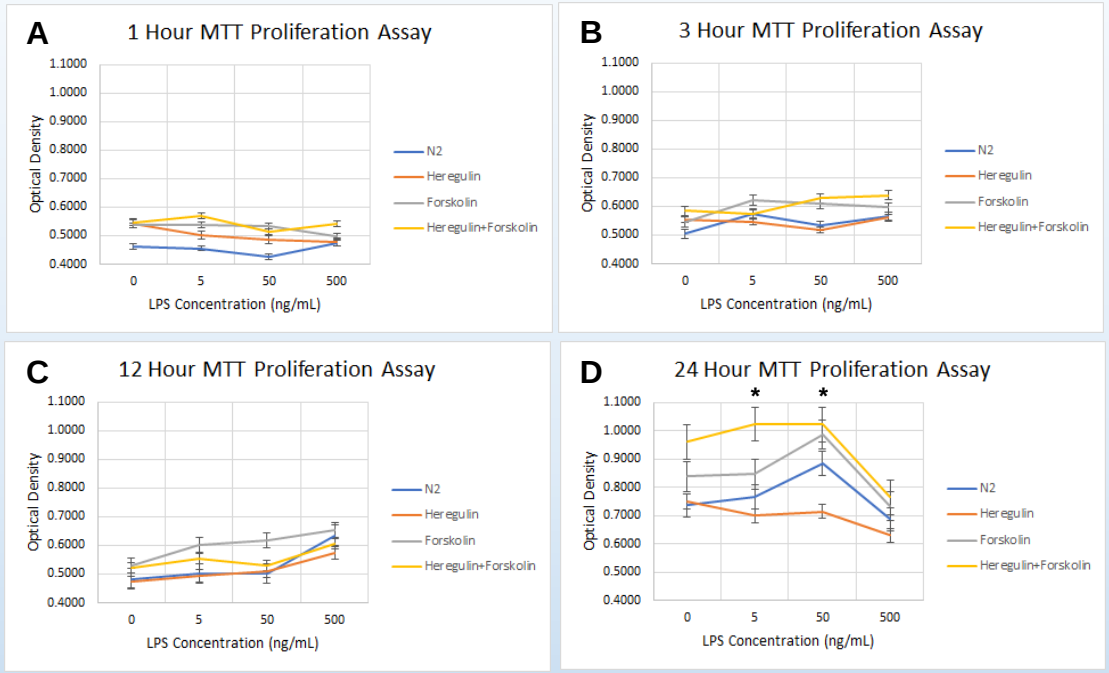


**Figure 1.** Effects of LPS Treatment With or Without Growth Factors on SC Proliferation. Using an MTT proliferation assay, the S16 SC line (SC-2941) was treated for 1 (A), 3 (B), 12 (C), or 24 hours (D) with no growth factors (control media, $N_2$), 12.5 ng/mL heregulin (H), 2 mM forskolin (F), or H+F, and various doses of LPS at 0, 5, 50, or 500 ng/mL, in 96-well plates. After incubation with LPS, each SC treatment was incubated in 12 mM MTT for 2 hours and SDS-HCl for another hour. The optical density of each treatment was read at 570 nm as an indicator of cell proliferation. Data plotted as mean ± SEM. *p < 0.05, significantly different from other treatments within the same time point [24-hour time point only]. (n = 2 for 1, 3, and 12 hours; n = 3 for 24 hours and 0, 5, and 50 ng/mL treatments; n = 1 for 24 hours and 500 ng/mL treatment).

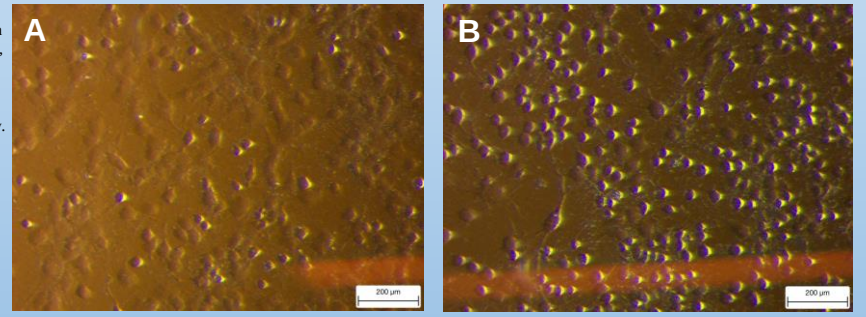


**Figure 2.** S16 SCs were cultured with no growth factors (control media, $N_2$), 12.5 ng/mL H, 2 mM F, or H+F, and incubated for various times with various dosages of LPS as specified above for the MTT proliferation assay. SCs were imaged using a Zeiss Primovert iLED equipped with Zeiss Axiocam ERc 5s microscope camera 5mp. The images on the right are representative of SCs treated with H+F alone (A) and after a 12-hour incubation with 500 ng/mL LPS (B). Both images are displayed at a magnification of 10x.

## Conclusions

- Between the treatments used, it appears as though either 5 or 50 ng/mL of LPS combined with both heregulin and forskolin for 24 hours results in the most Schwann cell proliferation.
- Considering Schwann cells treated with LPS for 24 hours had generally higher optical densities than cells treated with LPS for 1, 3, and 12 hours, there may be some period of recovery between 12 and 24 hours.
- These findings suggest that, when LPS-activated cells are treated with heregulin or forskolin, alone, they may activate two very distinct pathways to initiate opposite responses, with heregulin hindering cell division and forskolin promoting cell division. However, when heregulin and forskolin are combined, the forskolin-activated cAMP pathway appears to promote higher proliferation to offset the decrease in proliferation initiated by the heregulin pathway.

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