The Relationships Between the Intracellular Steps in the LPS-stimulated Release of Nitric Oxide from Microglia

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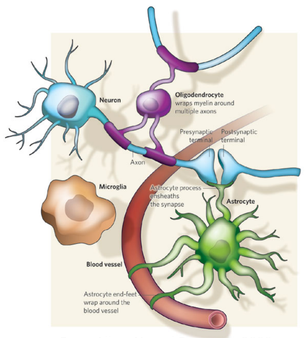
ABSTRACT

Inflammation of the microglia, a type of cell found in the brain, is noted as a symptom in most neurological pathologies of the central nervous system (CNS). For example, microglia play a role in unnecessary neuronal apoptosis in amyotrophic lateral sclerosis (ALS). In a healthy brain, the microglial production of the reactive oxidative species (ROS) nitric oxide (NO) and superoxide is a highly regulated process; however, when inflamed, these ROS are released without regulation. Presently, the mechanism surrounding these reactions is not well-defined, and this project seeks to provide a better understanding.



From Allen, N. and Barres, B. 2009.

INTRODUCTION

Lipospolysaccarides (LPS), which are found in bacterial cell walls, are used by immune response cells to identify "problem" bacteria.[2] Before being eliminated from the body, LPS from these bacteria can stimulate immune response cells to release pro-inflammatory molecules such as NO, promoting in microglia an inflammatory response that causes damage to the neurons:

- NO released from microglia combines with superoxide extracellularly, forming peroxynitrite.[3]
- Peroxynitrite acts on neuronal AMPA/Kainate receptors, causing influx of Ca^{2+} and apoptosis of the neuron.[3]

On the microglia, LPS acts on TLR4, a membrane receptor, to start a cascade of reactions that lead to the release of NO. There are two pathways for NO release, and our study focuses the My-D88 dependent pathway illustrated in figure 1.[4]

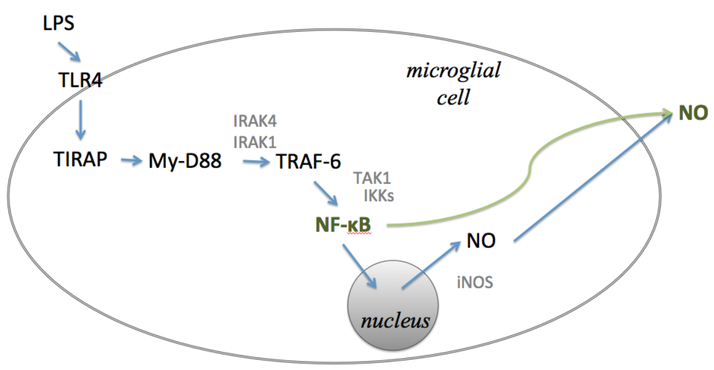


Figure 1. The My-D88 dependent response pathway that leads to nitric oxide release from microglia when activated by LPS. The green arrow represents the results obtained thus far.

- LPS - *lipopolysaccharides*
- TLR4 – *toll-like receptor 4*
- TIRAP – *toll-interleukin-1 receptor domain-containing adaptor protein*
- My-D88 – *myeloid differentiation primary response gene 88*
- IRAK4 – *IL-1 receptor-associated kinase-4*
- IRAK1 – *IL-1 receptor associated kinase-1*
- TRAF6 – *TNF receptor-associated factor-6*
- TAK1 – *transforming growth factor-β-activated kinase-1*
- IKKs – *IκB Kinase*
- NF-κB – *nuclear factor kappa-light-chain-enhancer of activated B cells*
- iNOS – *inducible nitric oxide synthase*
- NO – *nitric oxide*

METHODS & RESULTS

NO release from Microglia:

A Hill equation was used to model the rate of NO release over time when different concentrations of LPS were used to stimulate microglia. This equation was fit to experimental data[5] to allow for determination of the Hill coefficient, constant and scale factor.

$$\frac{\Delta\, NO}{\Delta\, t} = 1.5 * \frac{[LPS]^1}{(0.28)^1 + [LPS]^1}$$

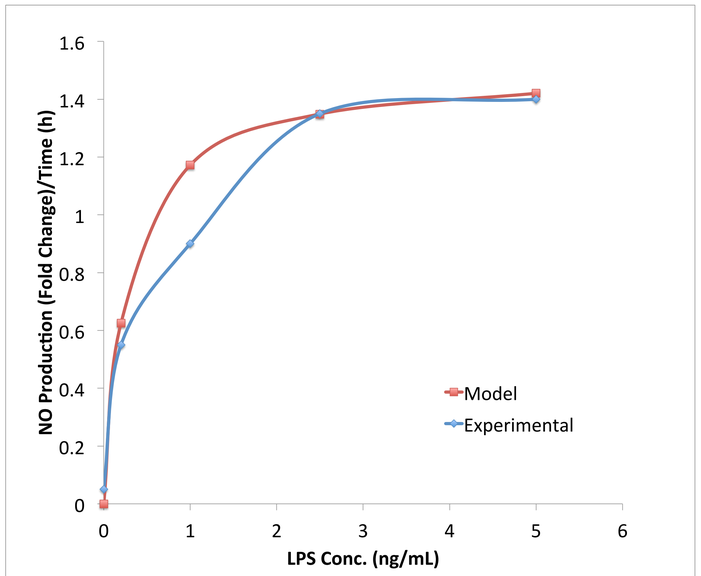


Figure 2. Depiction of the model for NO release rate in microglia activated by LPS at varying concentrations (ηg/mL).

NF-κB activation:

The exponential function was used to model the rate of NF-κB activation over time when varying concentrations of LPS are used to stimulate microglia. This equation was fit to experimental data[6] to allow for determination of the exponential constant and the scale factor.

$$\frac{\Delta\,(NF-\kappa B)}{\Delta\, t} = 3.35 * e^{0.5*\left(\frac{-1}{[LPS]}\right)}$$

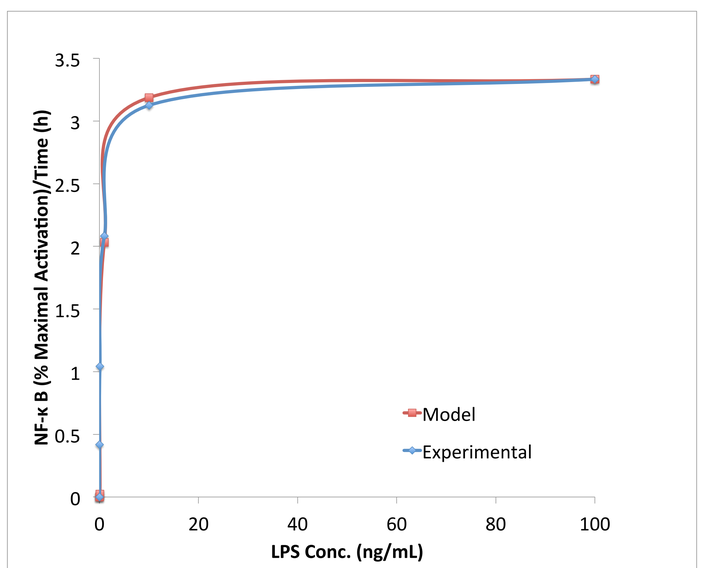


Figure 3. Depiction of the model for NF-κB activation rate in microglia activated by LPS at varying concentrations (ηg/mL).

Relationship between NF-κB activation and NO release :

Comparing the rate of NO production to NF-κB activation, the rate of NO release is approximately half the rate of NF-κB activation.

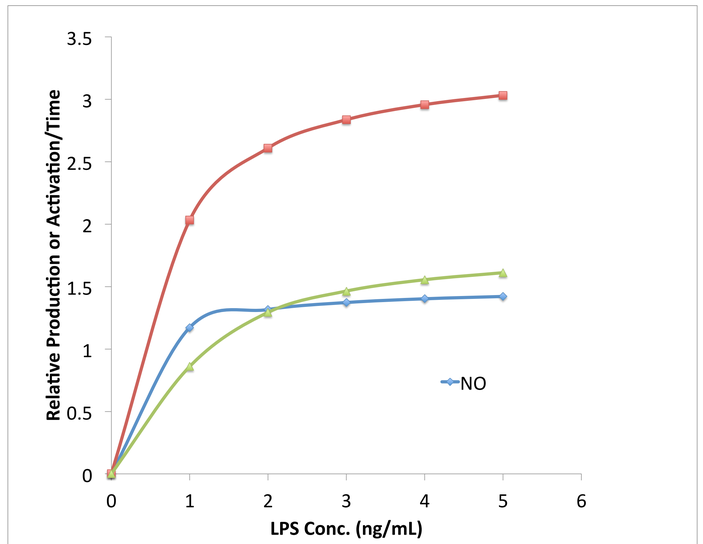


Figure 4. The difference between the rates of NF-κB activation and NO release when activated by LPS at varying concentrations (ηg/mL).

DISCUSSION

Our data suggests that the NO is produced/released with deamplification from the previous step. Research continues on this project with the intent of elucidating the relationships between the rates at the other steps. With all steps understood, we could build a model simulating the affect NO release has on a neuron, providing even greater understanding of the complex systems at play. It is expected that such information would be useful in helping to better direct treatments of inflamed microglia when examining the My-D88 pathway.

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