

The Role of Basigin as an Immune Mediator in the CNS

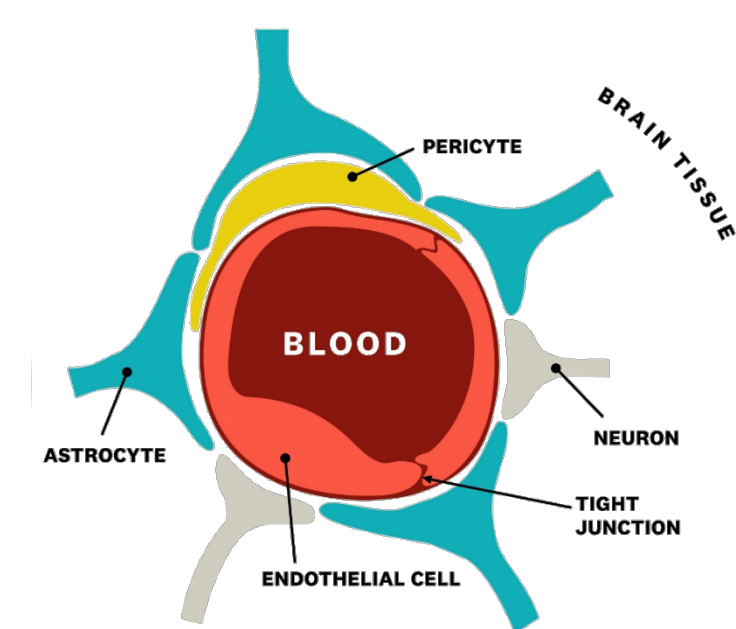
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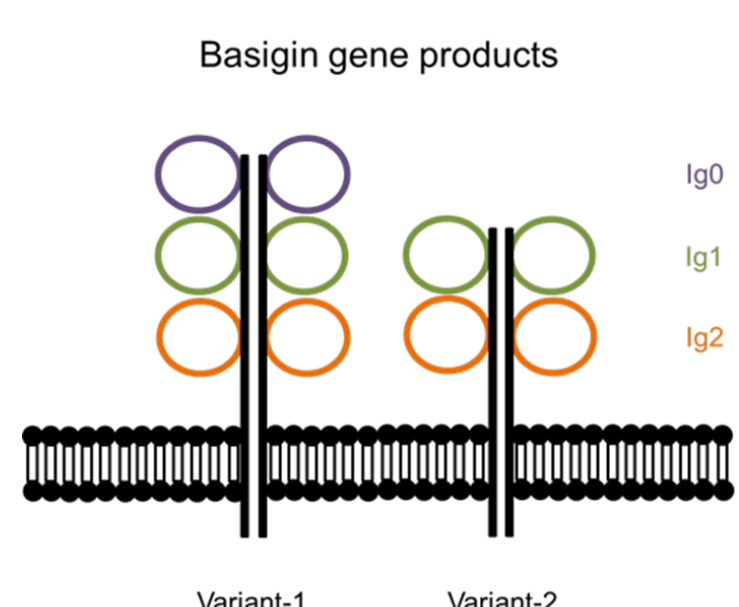
Introduction

The Blood Brain Barrier (BBB) is comprised of nonfenestrated endothelia, astrocytes, and pericytes, which serve to limit the entrance of molecules into the central nervous system (CNS; Serlin *et al.*, 2016).

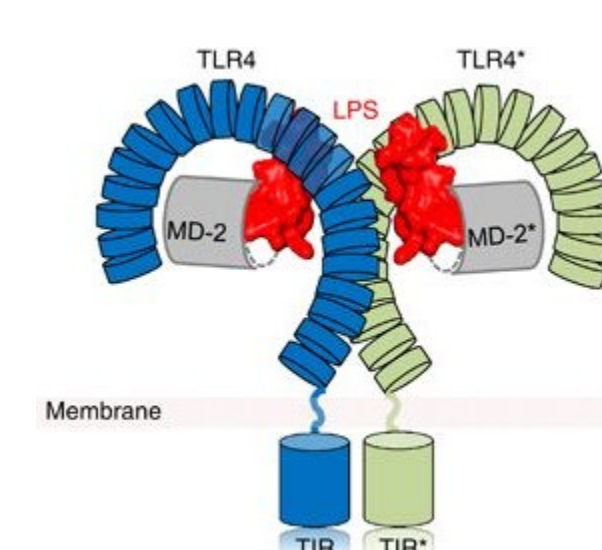


Molleda D, 2019

Basigin gene products are Immunoglobulin-like (Ig-like) cell adhesion molecules with extracellular Ig domains, a single-pass transmembrane domain, and a short cytoplasmic tail (Muramatsu, 2016). Basigin is expressed on endothelial cells and is thought to interact with Toll-like receptor 4 (TLR4; Brown, 2016, UNF Graduate Thesis).

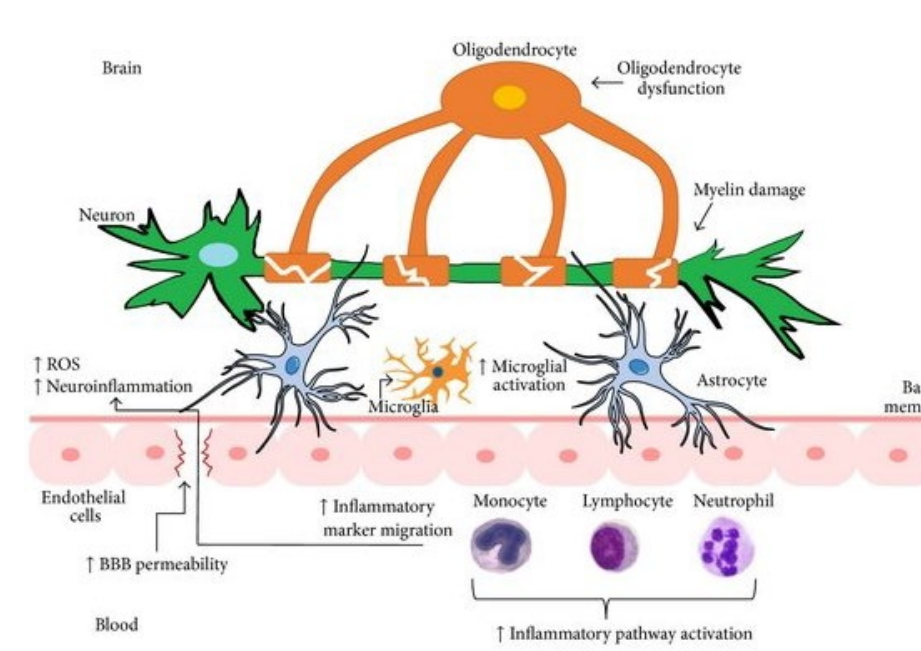


Toll-like receptor 4 (TLR4) is a single-pass transmembrane protein expressed on T-cells and macrophages (Krutzik *et al.*, 2005). When presented with lipopolysaccharide (LPS), TLR4 initiates transcription of pro-inflammatory cytokines, like interleukin 6 (IL-6). An increase in peripheral IL-6 can disrupt the BBB, causing an infiltration of peripheral IL-6 and T-cells into the brain (Rothaug *et al.*, 2016).



Parks and Lee, 2013

Infiltration of IL-6 and T-cells into the CNS activates astrocytes and microglia. Activation of these cells produce reactive oxygen species (ROS) and promote neuroinflammation by increasing pro-inflammatory cytokine production (Perry *et al.*, 2010).



The ROS released by microglia and astrocytes attack the myelin sheath around neurons. The breakdown of myelin promotes phagocytosis by microglia, which respond by producing more ROS. This positive feedback loop ultimately leads to neurodegenerative disorders, such as Parkinson's disease and Multiple Sclerosis (Van der Goes *et al.*, 1998).

Purpose / Hypothesis

The purpose of the present study was to quantify the expression of Basigin and TLR4 in neonatal and adolescent mice in the central nervous system (CNS) after 3, 6, 12, and 24 hours of exposure to lipopolysaccharide (LPS) to mirror acute and chronic inflammation.

Inflammation, an immune response to harmful stimuli, occurs in two stages: acute and chronic. The acute inflammatory response is activated immediately after the harmful stimulus is presented. Chronic inflammation is a consequence of an acute response, as it is marked by prolonged inflammation. It was expected that an increase in Basigin transcript levels would be observed after 3 and 24 hours. Due to their lack of immunological memory, it was expected that less Basigin transcript expression would be observed in neonates as compared to the adolescent mice.

Methodology

Animal care and use

All animal procedures were conducted under the approval of the UNF Institutional Animal Care and Use Committee (IACUC). Animals were maintained under standard mouse husbandry conditions with a 12h:12h light/dark cycle and received *ad libitum* water and standard rodent chow. Euthanasia was carried out by decapitation for animals less than 2 weeks of age and by asphyxiation using CO₂ for animals greater than 2 weeks of age.

Treatment of tissue

Brains were dissected from mice at postnatal day (PD) 7 and 30. Brains from PD 7 mice were further sectioned down their midsagittal plane, while PD 30 brains were sectioned into 4 equal parts. Each section was incubated in DMEM ± LPS (10 µg/mL, InvivoGen) at 37°C with 5% CO₂ for 3, 6, 12, and 24 hours. Incubation periods for each treatment were performed in triplicate for both age groups.

q-RT-PCR

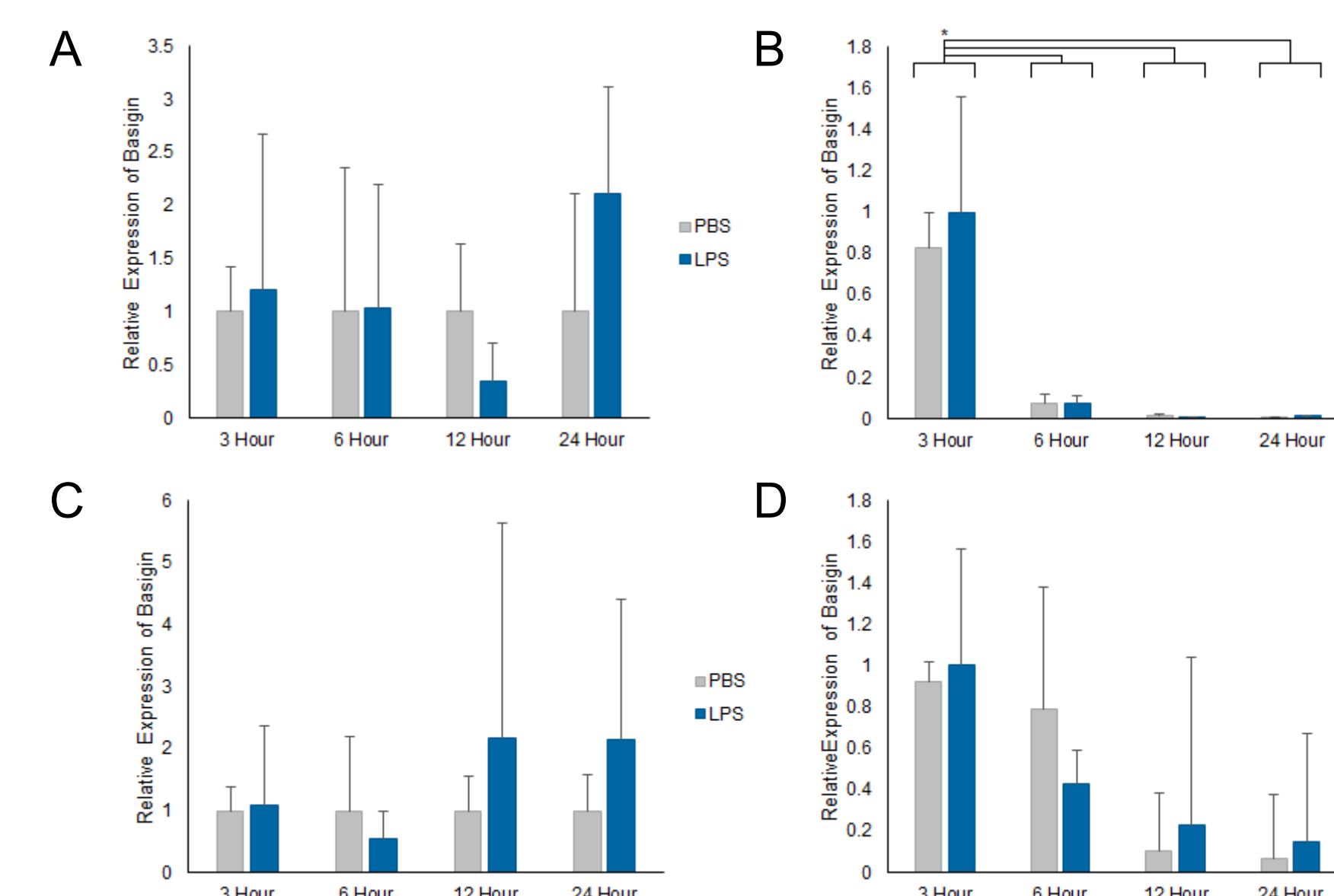
Following incubation, RNA was isolated from tissue samples using the TRI Reagent protocol (Molecular Research Center, Inc.). Quantitative reverse transcription polymerase chain reaction (q-RT-PCR) was performed, in triplicate, utilizing 100 ng of isolated RNA, iTaq™ Universal SYBR® Green Supermix (Bio-Rad Laboratories, Inc.) and primers specific to either Basigin-variant-2, or TLR4. The quantity of each transcript was determined using a standard curve and normalized to 18S rRNA.

Protein Expression

Basigin and TLR4 protein expression was determined using an ELISA using antibodies specific for Basigin (Ochrietor *et al.*, 2003) and TLR4 (ThermoFisher Scientific). Total protein (100 µg/mL) isolated from mouse tissue via the TRI Reagent protocol was analyzed in triplicate. An alkaline phosphatase detection system was used, and the absorbance was measured at 405 nm using a spectrophotometer. The data were plotted as absorbance, which directly correlates to the respective protein expression.

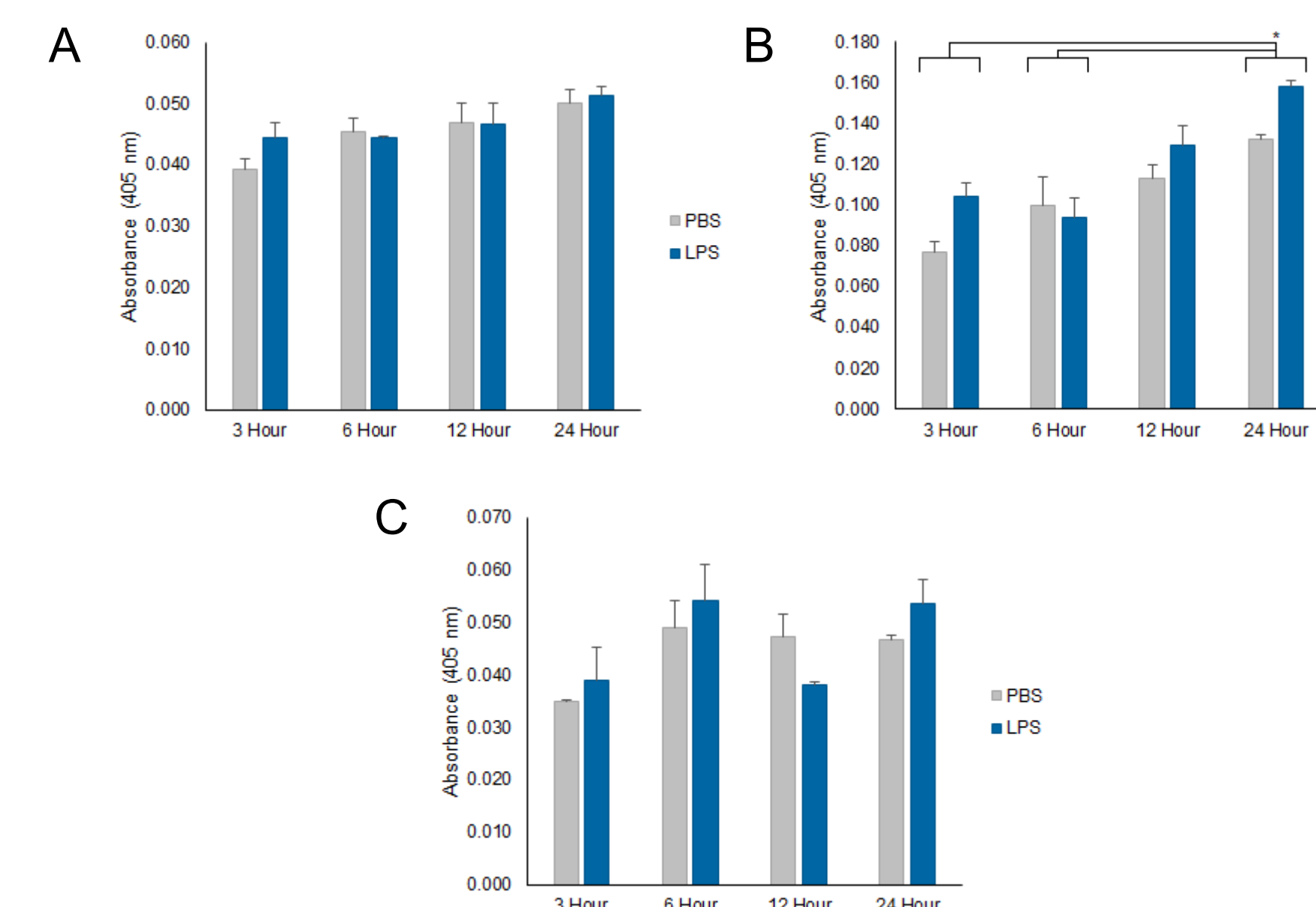
Results

Transcript expression of Basigin at PD 7 and PD 30



qRT-PCR was performed using isolated RNA from mouse brains at PD 7 (A, B) and PD 30 (C, D). Expression of Basigin transcripts after incubation with LPS for 3, 6, 12, and 24 hours are shown relative to PBS control (A, C) or relative to the 3-hour control (B, D). The error bars represent the coefficient of variation. A two-way ANOVA, followed by Tukey's HSD test determined no statistical significance between treated (LPS) and untreated (PBS) groups at both PD 7 and 30. However, Basigin expression at the 3-hour time point in PD 7 mice (B) was significantly different ($P < 0.05$) from other time points. This was not observed in PD 30 mice (D). Though no statistical significance was found, Cohen's D determined a moderate and small effect size between treatment and control groups at 12 and 24 hours, respectively, for PD 7 mice. A small effect size was determined between treatment and control groups at 24 hours for PD 30 mice.

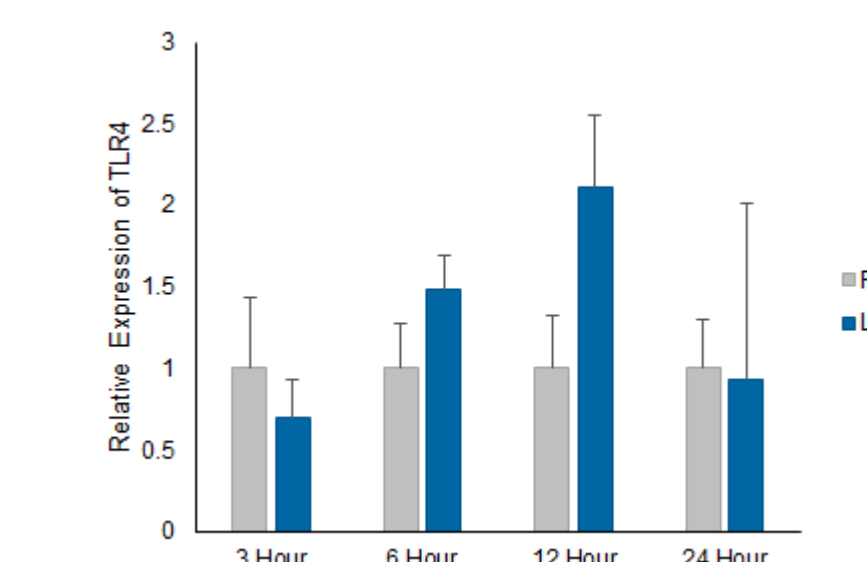
Protein expression of Basigin increases at PD 30



ELISA analyses using total isolated protein from mouse brains at PD 7 (A) and PD 30 (B, C) were performed for Basigin (A, B) and TLR4 (C). The average of 3 runs were plotted for the treated (LPS) and untreated (PBS) tissues. The error bars represent the standard error. A two-way ANOVA, followed by a Tukey's HSD test determined the 24-hour time point was significantly different ($p < 0.05$) than the 3- and 6-hour time points at PD 30. Cohen's D determined a moderate and large effect size between treated and control groups at 3 and 24 hours, respectively, for PD 30.

Results

Transcript expression of TLR4 at PD 7



qRT-PCR was performed using isolated RNA from mouse brains at PD 7. Expression of TLR4 transcripts after incubation with LPS for 3, 6, 12, and 24 hours are shown relative to the PBS control. The bars represent the triplicate runs and the error bars represent the coefficients of variation. A two-way ANOVA, followed by Tukey's HSD test determined no statistical significance between treated (LPS) and untreated (PBS) groups. Though no statistical significance was found, Cohen's D determined a moderate effect size between treated and control groups at 6 and 12 hours.

Conclusions and Comments

Regulation of Basigin in mouse neonate and adolescent brains does not significantly change when exposed to LPS under either acute or chronic conditions. Although no statistical significance was detected, a practical significance was determined at 24 hours for both age groups, suggesting chronic exposure to LPS increases Basigin transcripts. The lack of statistical significance, and high error, can be attributed to low sample size. Further, sex differences in baseline expression of Basigin were not measured, though they may exist.

TLR4 transcript levels do not change significantly, but practical significance was determined for the late stage of acute exposure to LPS. During the late stage of acute exposure, Basigin mRNA expression is inversely related to TLR4 expression in neonate mice.

Protein expression of Basigin was determined to be neither statistically nor practically significant in neonate mice, but the increase of Basigin protein in adolescent mice at 3 and 24 hours had a large effect size. Acute exposure may spur an onset of Basigin protein production, followed by another onset after prolonged exposure.

Acknowledgements

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