# Investigation of autophagy in activated monocytes

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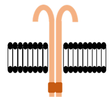
## Introduction

The innate immune response is the first line of defense against pathogens. The response is non-specific in that patterns rather than specific molecules, are detected. White blood cells, including monocytes, express cell-surface proteins that detect pathogens and initiate an immune response.

Toll-like receptors (TLRs) are a major group of pattern recognition receptors (PRRs) expressed on the surface of a variety of cells that function to recognize molecular patterns associated with all classes of pathogenic microorganisms (reviewed by Kawasaki et al., 2014). TLR4 specifically recognizes the lipopolysaccharide (LPS) component of Gram-negative bacterial cell walls.

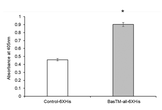


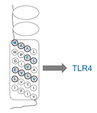
TLR4 has leucine-rich repeats in the extracellular domain, a single-pass transmembrane domain, and a toll-interferon receptor (TIR) domain in the cytoplasmic tail

Basigin (CD147) is a member of the immunoglobulin superfamily (IgSF) that functions as a cell adhesion molecule. It has been implicated in a variety of processes, including reproduction and development, metabolism, and cancer (reviewed by Muramatsu, 2016).

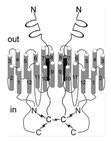
Basigin (CD147) has two extracellular Ig domains, a single-pass transmembrane domain, and a short cytoplasmic tail

A recent study by this laboratory suggests that TLR4 and Basigin interact via their transmembrane domains (Brown, 2016, UNF Graduate thesis).

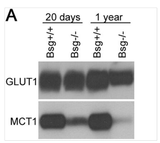


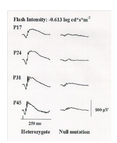

Studies of the neural retina indicate that Basigin gene expression is linked to expression of Monocarboxylate Transporter 1 (MCT1), which moves lactate, pyruvate, and ketone bodies across the plasma membrane via facilitated diffusion (Philp et al., 2003; Halestrap and Price, 1999). Basigin-null mice do not express Basigin or MCT1 on photoreceptors or Müller cells, which impairs delivery of metabolites from the Müller cells to the photoreceptor cells (Philp et al., 2003). The result is blindness in Basigin-null mice from the time of eye opening (Ochrietor et al., 2002).




Wilson et al., 2002



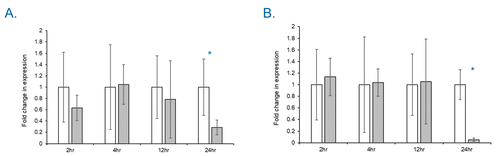

Philp et al., 2003



Ochrietor et al., 2002

## Purpose / Hypothesis

Two recent study by this laboratory showed that the expression of Basigin and MCT1 transcripts is significantly decreased in monocytes treated with LPS for 24 hours. Expression of GLUT1 was also determined to not increase.




Expression of Basigin (A) and MCT1 (B) transcripts in RAW 264.7 cells. The open bars represent D-PBS-treatment and the gray bars represent LPS-treatment. The error bars represent the Coefficient of Variation.
*=p<0.05 via T-test

Because metabolic transporters do not increase in expression in activated monocytes, the purpose of the present study was to determine if those cells rely on substrates already found within the cell by using the autophagy process. It was hypothesized That proteins involved in autophagy would increase in expression in response to exposure to LPS when compared to non-treated conditions.

## Methodology

RAW 264.7 monocytes were grown at 37°C in 5% $CO_2$ in RPMI 1640 medium (Gibco – Life Technologies, Grand Island, NY) containing 10% FBS (HyClone, GE Healthcare-BioSciences, Marlborough, MA).

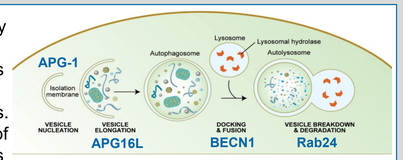
RAW 264.7 cells were plated in serum-free RPMI 1640 medium (Gibco – Life Technologies) and allowed to attach.

The cells were treated with LPS (1 μg/mL; InvivoGen, San Diego, CA) or Dulbecco's phosphate buffered saline (D-PBS; Gibco – Life Technologies) for 24 hours at 37°C in 5% $CO_2$.

Cells were harvested and detergent protein lysates were generated (Philp et al., 2003).

APG-1, APG16L, BECN1, and Rab24 protein expression was determined by a direct ELISA using antibodies specific for each protein (Abgent Antibodies, San Diego, CA). Expression within LPS treated cells and D-PBS-treated cells were analyzed using one-way ANOVA and post one-way T Test.

Autophagy is a tightly regulated process in which a vesicle forms to consume intracellular resources. The regulatory point of each protein tested is shown.






## Results

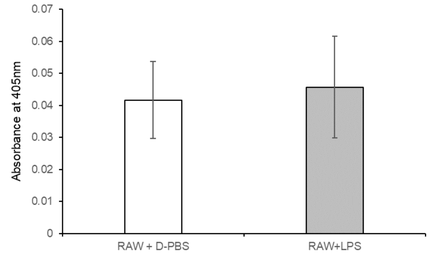



Figure 1. Expression of APG-1 is not increased in monocytes in response to LPS treatment. The absorbance at 405 nm corresponds to the expression of APG-1 in RAW 264.7 monocytes. The values represent the average of two runs and the error bars represent the standard deviation. p=0.369 via a paired, one-tailed T-test.

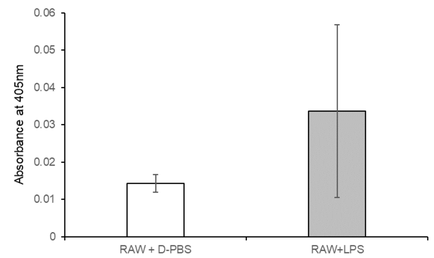



Figure 2. Expression of APG16L is not increased in monocytes in response to LPS treatment. The absorbance at 405 nm corresponds to the expression of APG16L in RAW 264.7 monocytes. The values represent the average of two runs and the error bars represent the standard deviation. p=0.157 via a paired, one-tailed T-test.

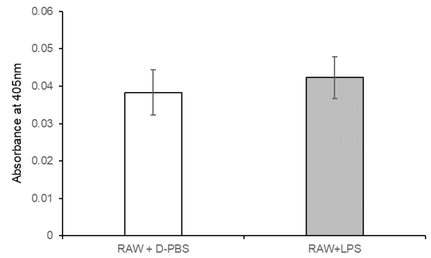



Figure 3. Expression of BECN1 is not increased in monocytes in response to LPS treatment. The absorbance at 405 nm corresponds to the expression of BECN1 in RAW 264.7 monocytes. The values represent the average of two runs and the error bars represent the standard deviation. p=0.238 via a paired, one-tailed T-test.

## Results

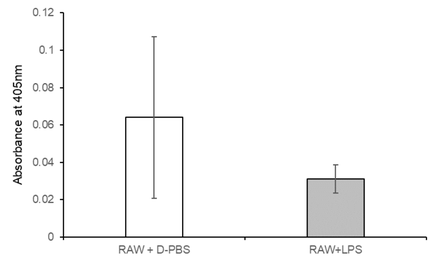



Figure 3. Expression of BECN1 is not increased in monocytes in response to LPS treatment. The absorbance at 405 nm corresponds to the expression of BECN1 in RAW 264.7 monocytes. The values represent the average of two runs and the error bars represent the standard deviation. p=0.152 via a paired, one-tailed T-test.

## Conclusions and Comments

Proteins at different phases of the autophagy pathway were tested so that the full process could be evaluated (Kroemer and Levine, 2008). The protein APG-1 is used to initiate the process. The APG16L protein is used in autophagy vesicle formation. The BECN1 protein regulates endolytic processing in the late stages of autophagy. The Rab24 protein is a G-protein that is expressed during the formation and regulation of autophagic vacuoles.

The expression of the autophagy proteins APG-1, APG16L, BECN1, and Rab24 within RAW 264.7 monocytes were determined to not be upregulated in response to treatment with LPS. This suggests that autophagy is not used as a means of obtaining metabolic substrates during an immune response.

Future studies will continue to assess the shift in metabolism observed in monocytes during an immune response to determine what substrates other than monocarboxylates are used.

## Acknowledgements

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