

Role of Antimicrobial Peptide, SAA3, in inducible protection by Pam2-ODN in mouse lung epithelial cells

Lijo Andrews, Shreya Bhattacharya, PhD, Scott E. Evans, MD

The University of Texas MD Anderson Cancer Center, Department of Pulmonary Medicine

Abstract

It has been shown that lung epithelial cells have displayed to be active effectors of microbial defense. Stimulation of these cells by a treatment consisting of the combination of Toll-like Receptor (TLR) agonists have shown to increase defenses towards commonly lethal pneumonias. This study examined the synergistic interactions between TLR2/6 and TLR9, more commonly known as Pam2-ODN, and the resulting immune response of MLE15, a mouse lung epithelial cell line. Results were collected by measuring the increased production of known antimicrobial peptide (AMP) receptors, indicating an increased immune response. The AMP of interest in this study is Serum amyloid A-3 (SAA3). SAA3 levels were measured after the addition of treatment at various time frames between 1-6 hours. Results showed the most significant level of SAA3 production at 2 hours and 4 hours, suggesting these time frames as optimal following the addition of treatment.

Introduction

Pneumonia is an invasive disease that results in the inflammation of the alveolar sacs in the lungs, commonly causing fluid retention in these sacs. The retention of fluid thus results in the impairment of breathing and can even cause sepsis, often leading to death. The disease is most commonly seen in those with illness or a weakened immune system, such as persons undergoing chemotherapy, and affects close to one hundred million people annually¹.

Previous studies have indicated that the epithelial cells of the lungs can be stimulated to provide protection against these pneumonias². This stimulation was seen to be induced by treatment consisting of the combination of two TLR agonists. The first agonist, TLR2/6 is a heterodimer that recognizes diacylated lipopeptides such as Pam2CSK4 (Pam2), an immune modulator that contributes to the early immune response after infection by a pathogen³. The second receptor that is used in conjunction with TLR 2/6 is TLR9. TLR9 works by binding un-methylated cytosine-guanine dinucleotide (CpG) motifs in both bacteria and viruses, which triggers signaling cascades, also resulting in the

regulation of pro-inflammatory immune response. The CpG motif that is responsible for this cascade in TLR9 is oligonucleotide (ODN) M362. Combined, the two ligands: known as Pam2-ODN, displays an increased immune response to pneumonia infection⁴.

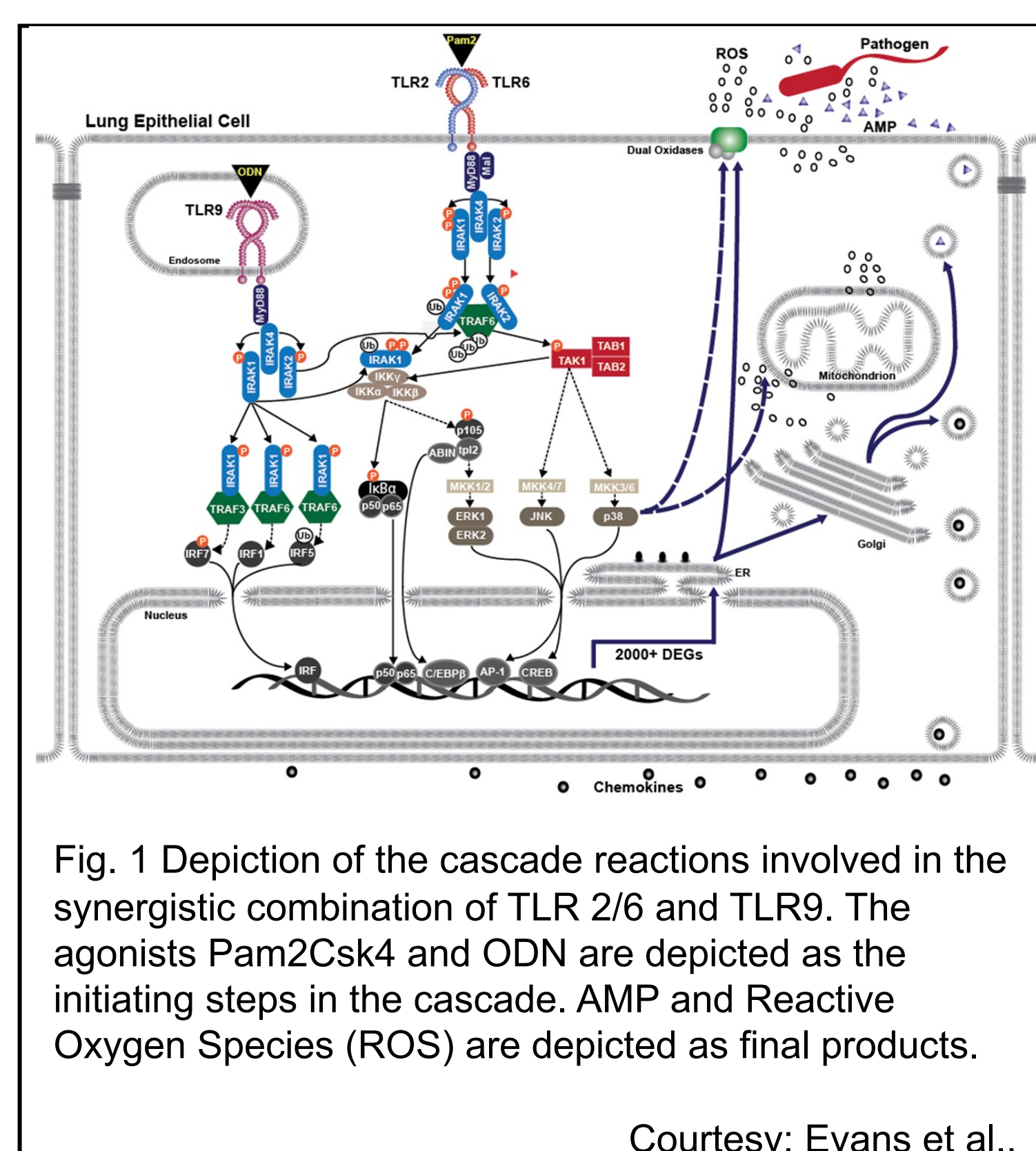


Fig. 1 Depiction of the cascade reactions involved in the synergistic combination of TLR 2/6 and TLR9. The agonists Pam2Csk4 and ODN are depicted as the initiating steps in the cascade. AMP and Reactive Oxygen Species (ROS) are depicted as final products.

Courtesy: Evans et al.,

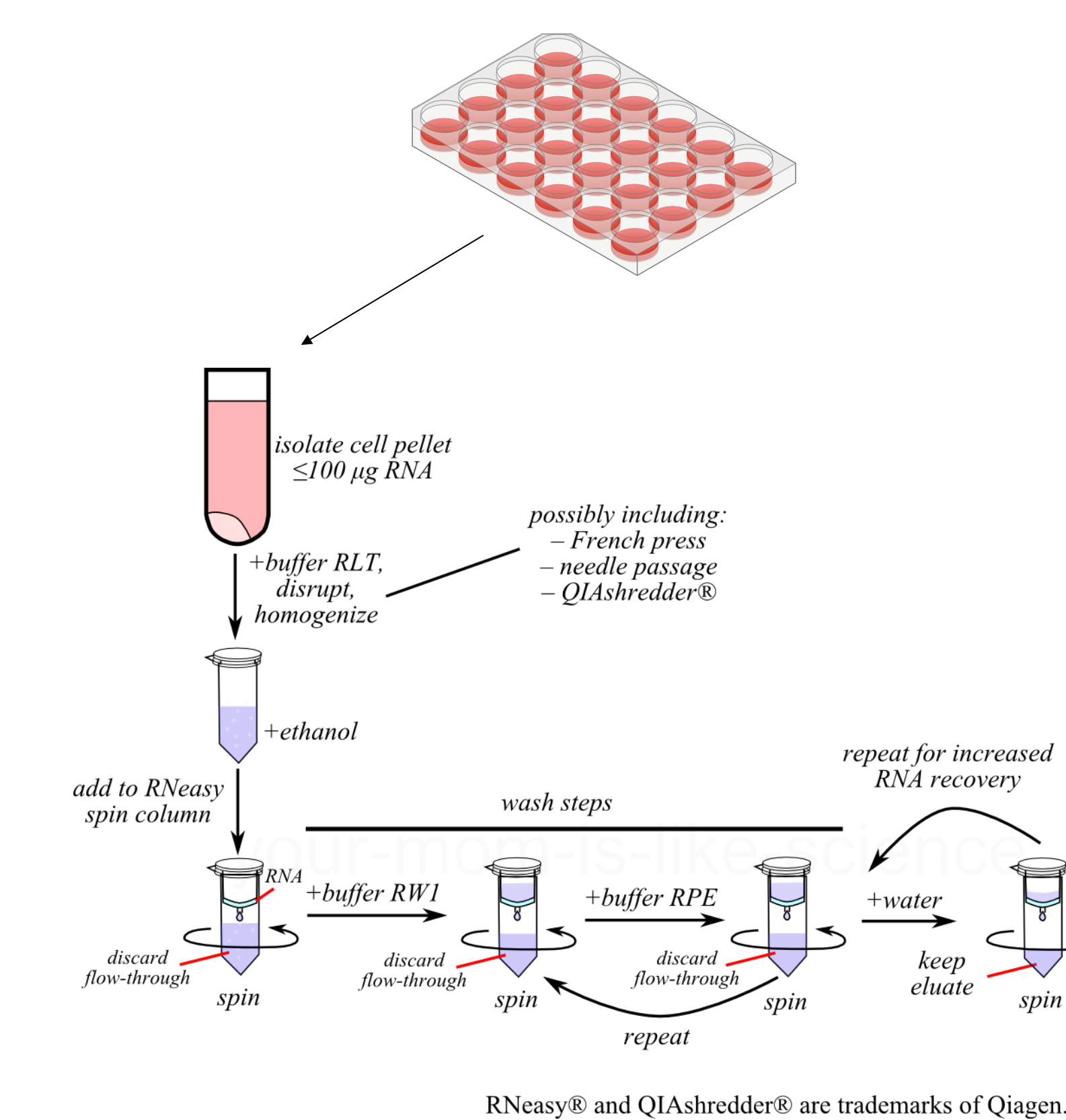
Aim

Previous microarray analyses have indicated upregulated genes coding for antimicrobial peptides (AMP's) after the addition of PAM2-ODN treatment to lung epithelial cells. One of the AMP's that were found to be upregulated is Serum amyloid A-3. Serum amyloid A (SAA) is a 104 amino acid long AMP that is known to play roles in cell-to-cell communication as well as in modulating inflammatory feedback responses⁵. In humans, SAA1 and SAA2 are most commonly seen to increase in level by more than 1000-fold during an acute-phase immune response⁶. A third isoform of the protein, known as SAA3 is seen to exist in mice, but not humans, and it produces a similar outcome. This response renders the protein as a biomarker for infection, allowing us to use it as a measurement tool following the administration of treatment. As such, this study aimed to validate the microarray analysis by performing qRT-PCR ran at various time points to better understand the level of the gene expression over time.

Methodology

This study consisted of various timed trials ran in five phases: cell culture, addition of treatment, RNA Extraction, cDNA conversion, and qRT-PCR analysis. Once

the cells reached full confluence, treatments were added according to the time frame indicated (e.g. 2 hr. group was treated for 2 hours). Once the time frame was complete, cells were then lysed, and RNA was extracted using a spin column. Gene expression of SAA3 was measured by qRT-PCR. The data were then analyzed using the GraphPad software.



Results

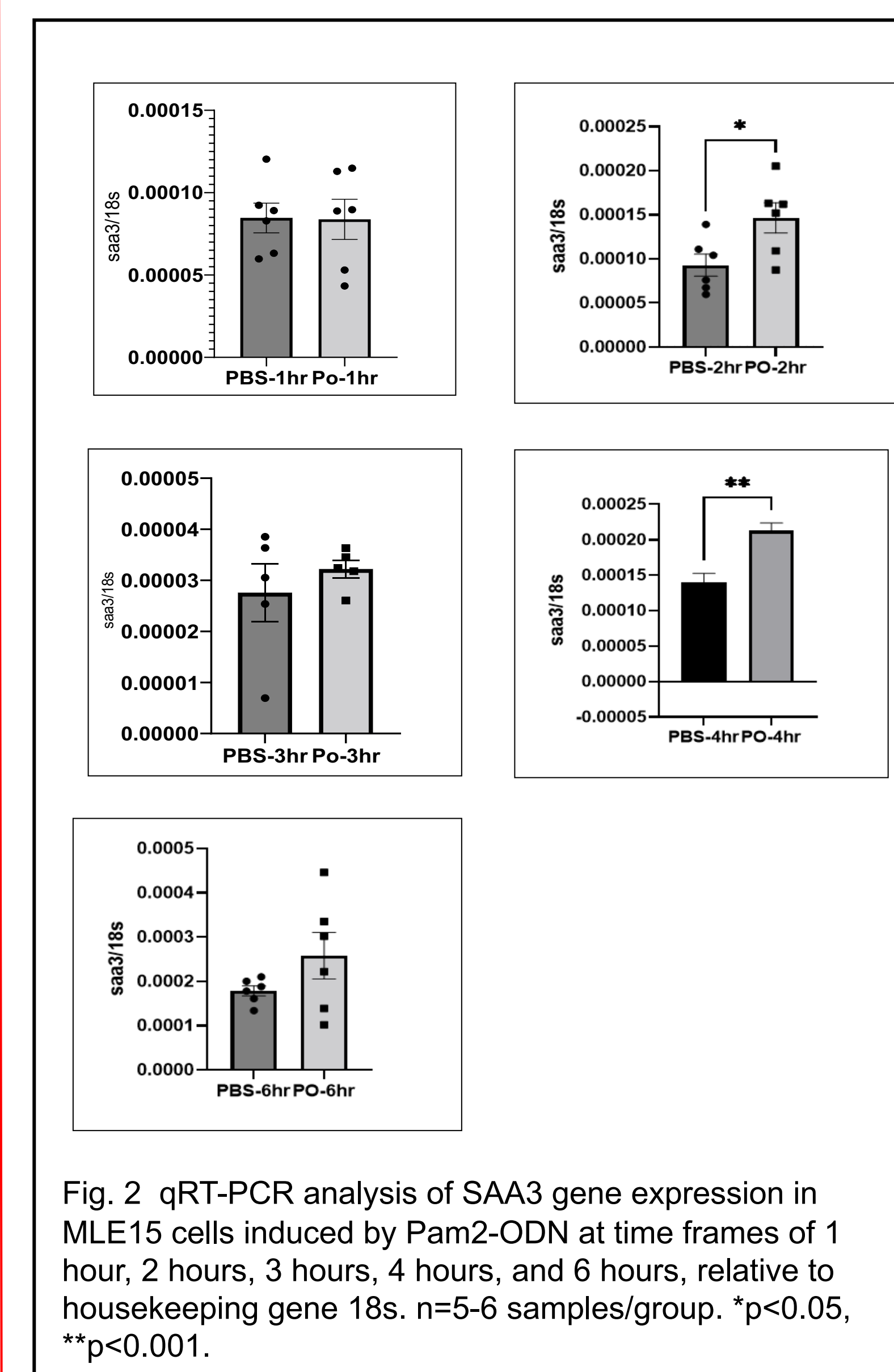


Fig. 2 qRT-PCR analysis of SAA3 gene expression in MLE15 cells induced by Pam2-ODN at time frames of 1 hour, 2 hours, 3 hours, 4 hours, and 6 hours, relative to housekeeping gene 18s. n=5-6 samples/group. *p<0.05, **p<0.001.

Conclusions

The results show SAA3 is expressed significantly at 2 hours and 4 hours due to Pam2-ODN treatment. Pam2-ODN induces a protective response through AMPs such as SAA3, especially at these frames in the MLE cell line.

Future Direction

Further studies involving *in-vitro* infection models need to be performed to assess the protectiveness of SAA3 and other antimicrobial peptides induced by Pam2-ODN. Results will allow for increased insights into the mechanism of action behind this induced protection.

References

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