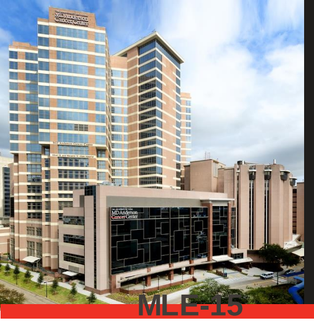

The Effects of the Transcription Factor IRF-3 in Pam2ODN Microbial Resistance

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Introduction

Pneumonia is a common and dangerous clinical condition affecting many people worldwide. It is most lethal in immunosuppressed and immunocompromised patients; thus, major effort has been undertaken to discover novel preventative measures and treatments to ameliorate the effects from acquiring this condition. One treatment, the use of the TLR2 agonist Pam2CSK (Pam2) with the Class C oligonucleotide TLR9 agonist ODN M362 (ODN), has been shown to resist microbial infection. However, the complete mechanism of Pam2/ODN's action is not fully understood(1). Previous research has shown that Pam2ODN has activates the transcription factor NF-kB, but only little is known about the combined drug's effects on the transcription factor interferon regulatory factor 3 (IRF-3) and how IRF-3 contributes to Pam2/ODN's resistance to infections. IRF-3 is an interferon regulatory factor that when activated upregulates the transcription of antimicrobial factors, such as Type 1 Interferons.

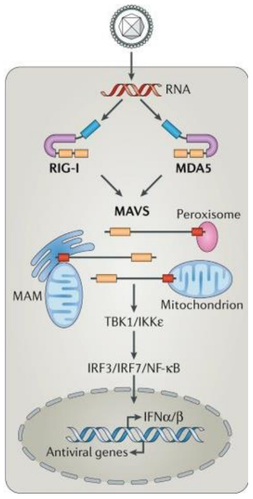

Fig 1. RNA activation pathway of IRF-3 (2).

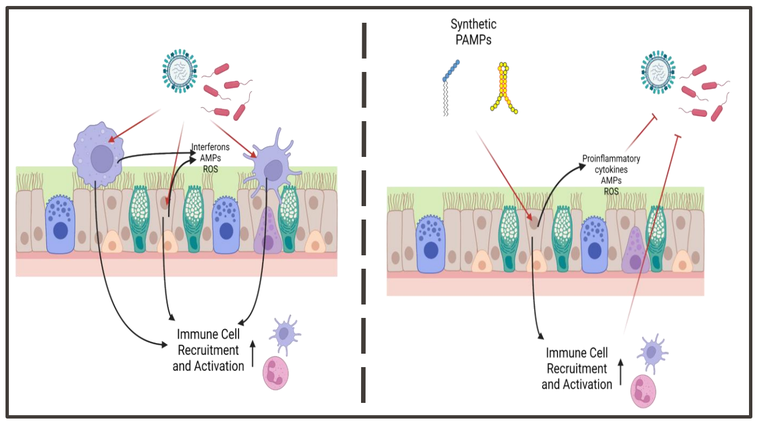

Fig 2. Pathogen activation of the innate immune system compared to Pam2/ODN activation.

Methods

To determine the effects of IRF-3 on Pam2/ODN's resistance against microbial infections a series of experiments were executed:

1. MLE-15 cells were cultured either alone or treated with Pam2/ODN to determine the level of IRF-3 activation.
2. MLE-15 cells were treated separately by two TBK1/IKKe inhibitors, Amlexanox and MRT67307, to prove that they could inhibit the phosphorylation of IRF-3 when compared to negative controls.
3. MLE-15 were treated with one of the TBK1/Ikke inhibitors and Pam2/ODN and infected influenza virus to measure viral burden when compared to negative controls.

Results

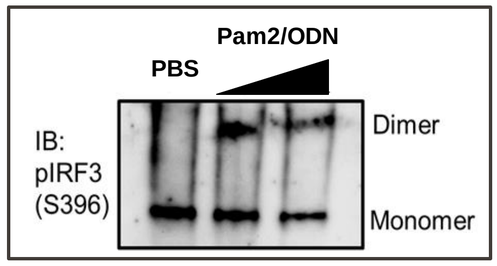

Fig 3. MLE-15 cells treated for either PBS or two different Pam2/ODN concentrations. Separation of phosphorylated IRF-3 by dimerized status.

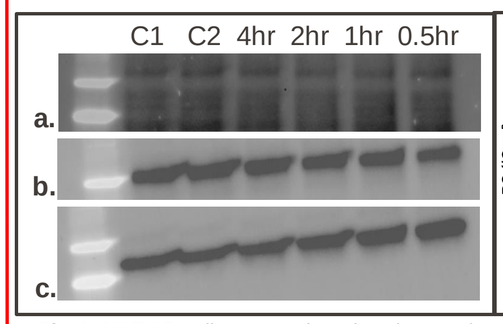

Fig 4. MLE-15 cells were cultured and treated with Pam2/ODN for 4hr, 2hr, 1hr, and 0.5hrs. **a.** Western blot for phosphorylated IRF-3. **b.** Western blot for polyclonal IRF-3. **c.** Western blot of beta-actin.

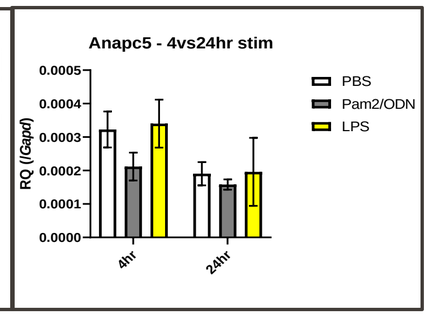

Fig 5. MLE-15 cells were treated with PBS, Pam2/ODN, or LPS for either 4 hours or 24 hours. The relative gene expression of Anapc5 compared to Gapd is shown.

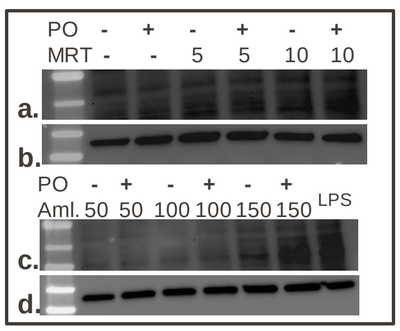

Fig 6. MLE-15 cells were cultured in Pam2/ODN for four hours after beginning treatment of one of two inhibitors, Amlexanox and MRT67307, for one hour at various micromolar concentrations with appropriate negative controls. **a**. Western blot of MRT67307 treated cells for phosphorylated IRF-3. **b.** Western blot of MRT67307 treated cells for beta-actin. **c.** Western blot of Amlexanox treated cells for phosphorylated IRF-3. **d.** Western blot of Amlexanox treated cells for beta-actin.

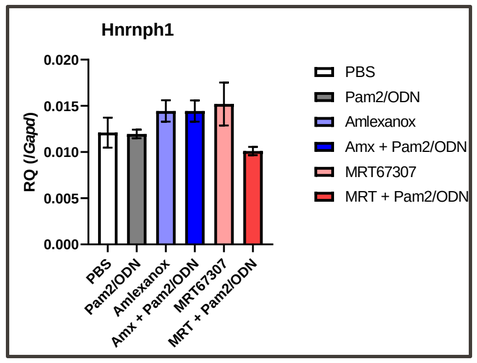

Fig 7. MLE-15 cells were treated with either Amlexanox or MRT67307 for one hour then treated with Pam2/ODN for four hours. The relative gene expression of Hnrnph1 compared to Gapd is shown.

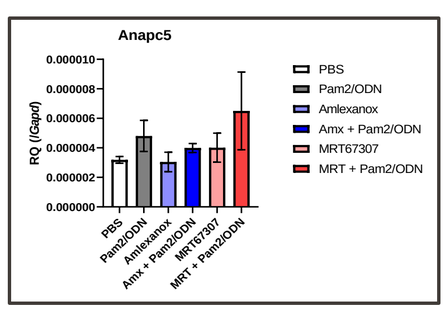

Fig 8. MLE-15 cells were treated with either Amlexanox or MRT67307 for one hour then treated with Pam2/ODN for four hours. The relative gene expression of Hnrnph1 compared to Gapd is shown.

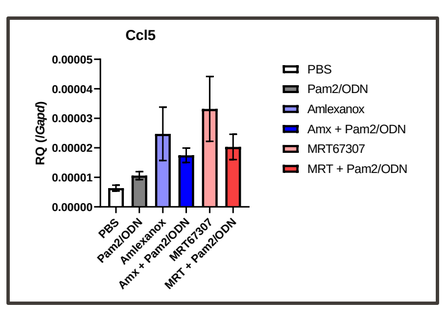

Fig 9. MLE-15 cells were treated with either Amlexanox or MRT67307 for one hour then treated with Pam2/ODN for four hours. The relative gene expression of Ccl5 compared to Gapd is shown.

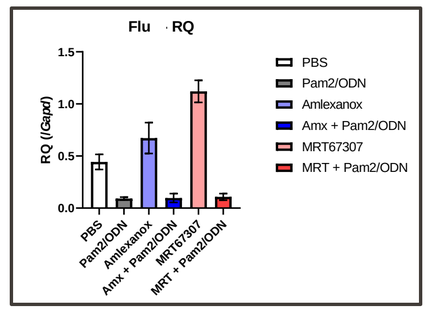

Fig 10. MLE-15 cells were treated with either Amlexanox or MRT67307 for one hour, then Pam2/ODN for four hours and influenza virus for 24 hours. Viral burden was measured by comparing NP to Gapd.

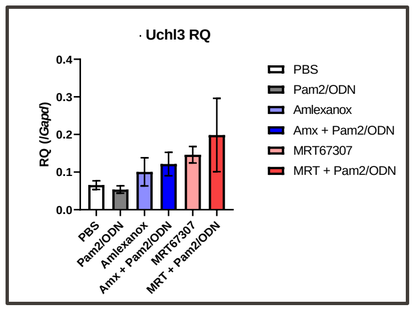

Fig 11. MLE-15 cells were treated with either Amlexanox or MRT67307 for one hour, then Pam2/ODN for four hours and influenza virus for 24 hours. Viral burden was measured by comparing Uchl3 to Gapd.

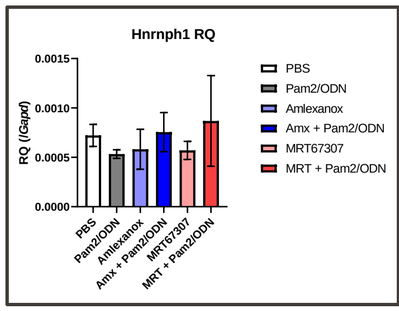

Fig 12. MLE-15 cells were treated with either Amlexanox or MRT67307 for one hour, then Pam2/ODN for four hours and influenza virus for 24 hours. Viral burden was measured by comparing Hnrnph1 to Gapd.

Future Directions

In future studies, the same experiments should be repeated using alternate cell lines to achieve more conclusive results. The immortalized human bronchial epithelial cell line, HBEC3-KT, should be tested to give more representative results to the human response. In addition, mouse tracheal epithelial cells (mTECs) can give valuable data that can be used as the foundation for in-vivo experiments.

Second, in-vivo studies on IRF-3 knockout mice that have been treated with Pam2/ODN should be conducted to determine how IRF-3 plays a role in survivability, when mice are infected with a pathogen.

Acknowledgements

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1) Wali, Shradha, et al. "Immune Modulation to Improve Survival of Viral Pneumonia in Mice." *American Journal of Respiratory Cell and Molecular Biology*, vol. 63, no. 6, 6 Aug. 2020, pp. 758–766, https://doi.org/10.1165/rcmb.2020-0241oc.

2)Rehwinkel J, Gack MU. RIG-I-like receptors: their regulation and roles in RNA sensing. Nat Rev Immunol. 2020 Sep;20(9):537-551. doi: 10.1038/s41577-020-0288-3. Epub 2020 Mar 13. PMID: 32203325; PMCID: PMC7094958.