



Differential Responses of Human and Mouse Lung Epithelial Cells in Inducible Resistance to Viral Infection

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Introduction

Respiratory infections are the leading cause of mortality globally. The advent of the SARS-CoV-2 pandemic has claimed over 4 million lives and many more are suffering sequelae post-infection. The past year has shown us that there is a pressing need for improved treatments and symptomatic management of viral respiratory infections.

Preliminary studies in HBEC3-KT cells have demonstrated that Pam2/ODN (Pam2CSK4 and ODN M362) results in overall increased reactive oxygen species (ROS) activity.

We used Influenza A Virus (IAV), an orthomyxovirus, and Mouse Hepatitis Virus (MHV), a coronavirus, in this study because of the differential responses seen in infected cells. For example, Coronaviruses are known to inhibit the RIG-I pathway, thereby suppressing maximal interferon response. Observing the differences in responses could help us understand the mechanism of the protective phenotype post-administration of Pam2/ODN.

Results

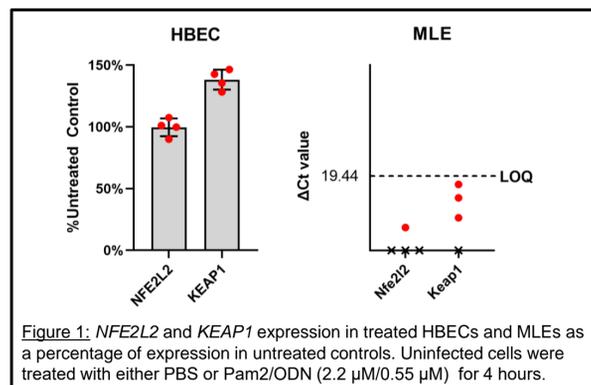


Figure 1: *NFE2L2* and *KEAP1* expression in treated HBECs and MLEs as a percentage of expression in untreated controls. Uninfected cells were treated with either PBS or Pam2/ODN (2.2 μM/0.55 μM) for 4 hours.

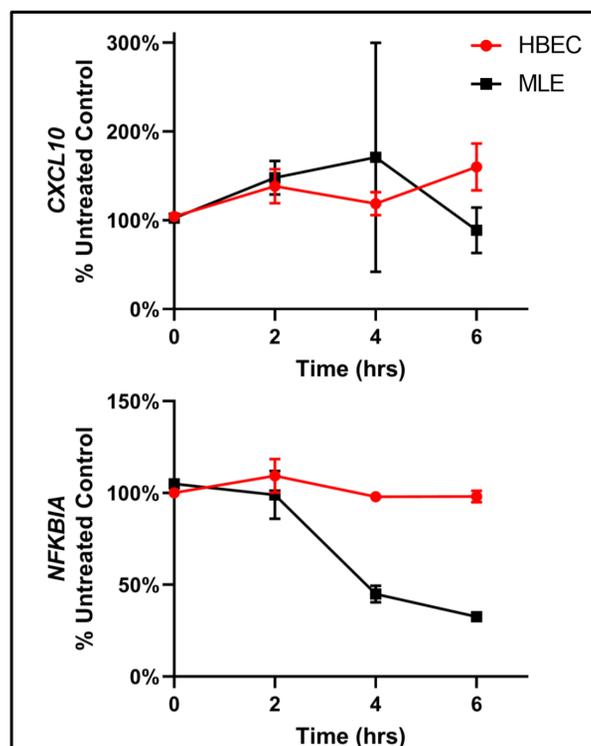


Figure 2: Uninfected cells treated for 2, 4, and 6 hours with Pam2/ODN as a percentage of expression in untreated controls

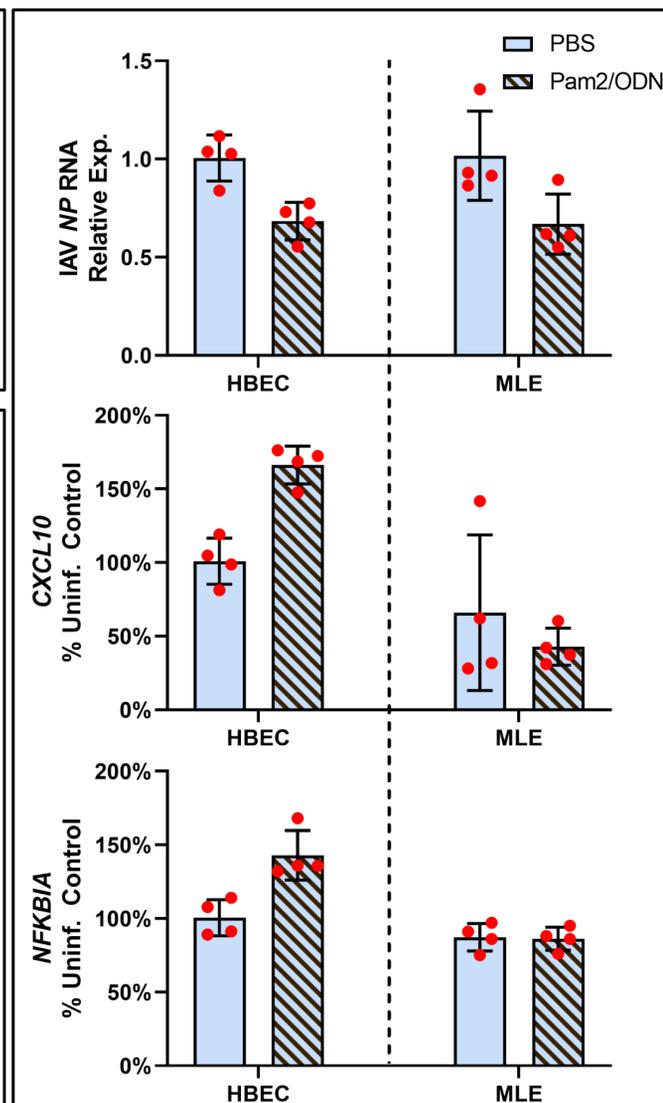


Figure 3: MLE-15 and HBEC3-KT cells treated with or without Pam2/ODN (2.2 μM/0.55 μM) as a percentage of expression in uninfected controls. Cells were treated for 4 hours prior to inoculation with IAV-H3N2 at a multiplicity of infection (MOI) of 0.1 for 24 hours.

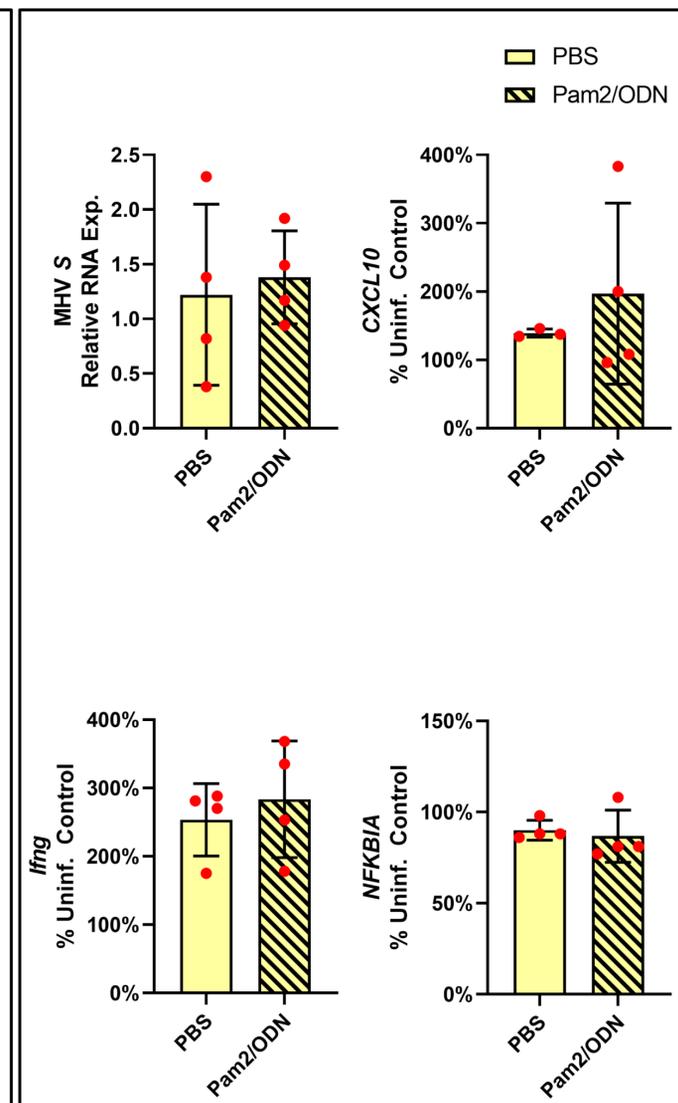


Figure 4: MLE-15 cells treated with or without Pam2/ODN as a percentage of expression in uninfected controls. Cells were treated for 4 hours prior to inoculation with MHV-A59 at a multiplicity of infection (MOI) of 0.1 for 24 hours.

Discussion

In HBECs, no change in the expression of genes that promote antioxidant pathways (*NFE2L2*) was observed while the expression of inhibitors of those same pathways (*KEAP1*) was elevated. This leads to an overall increase in the activity of ROS, bolstering defense against viral and other intracellular pathogens. Interestingly enough, we do not see any expression of these genes in MLEs at baseline, regardless of treatment (Figure 1).

There is a slight upward trend in the expression of *Cxcl10* (CXCL10) in HBECs 6 hours post Pam2/ODN treatment, we don't see any significant trends in MLEs.

In MLEs, we have shown that acutely, Pam2/ODN decreases the expression of *Nfkb1a* ($I\kappa B\alpha$), an inhibitor of NF- κ B. We

hypothesize this could be partially responsible for the protective phenotype described in previous studies. Importantly, we do not see this result replicated in HBECs (Figure 2).

We see significant decrease in IAV NP RNA in IAV-infected HBECs treated with Pam2/ODN. Conversely, we see an increase in the expression of *CXCL10*, which falls in line with the anticipated enhanced immune response. Finally, we don't observe a difference in the expression of *NFKBIA*.

We see a slight downward trend in IAV NP RNA expression in IAV-infected MLEs treated with Pam2/ODN, although the difference is not significant. A similar trend can be seen in *Cxcl10*, which may be an

incidental finding (Figure 3).

In MHV-infected MLEs, we see no significant changes in the expression of *Irfng* (IFN- γ), *Nfkb1a*, *Cxcl10*, or MHV S protein after treatment with Pam2/ODN compared to PBS. This is most likely attributed to the limited inoculation time of 24 hours. Previous studies have shown significant changes in expression at 72-hour timepoints post-infection (Figure 4).

We anticipated a differential response in gene expression between different cell types after treatment with Pam2/ODN; HBECs are bronchiolar cells while MLEs are more akin to alveolar cells, which could suggest there is a differential response dependent on cell type.

Conclusion

Pam2/ODN presents a novel means to combat viral pneumonia. Prophylactic administration preferentially promotes CXCL10 activity in IAV-infected HBECs while downregulating inhibitors of NF- κ B in uninfected MLEs. Overall, this study validates the protective phenotype observed in MLE-15 and HBEC3-KT cells while also pointing towards a differential mechanism for the observed phenotype.

References and Acknowledgements

Ware, H. H., Kulkarni, V. V., Wang, Y., Pantaleón García, J., Leiva Juárez, M., Kirkpatrick, C. T., Wali, S., Syed, S., Kontoyiannis, A. D., Sikkema, W., Tour, J. M., & Evans, S. E. (2019). Inducible lung epithelial resistance requires multisource reactive oxygen species generation to protect against bacterial infections. *PLoS one*, 14(2), e0208216. <https://doi.org/10.1371/journal.pone.0208216>