In cells defective for the dominant antiviral pathway, the normally redundant piRNA pathway becomes an effective response against the Venezuelan Equine Encephalitis Virus vaccine strain.

The importance of this work with Venezuelan Equine Encephalitis Virus

Venezuelan Equine Encephalitis Virus (VEEV) is an **important human pathogen** that causes fatal infection of the brain and spinal cord[1]. VEEV circulates between a mosquito vector and mammalian host, periodically emerging to infect humans and equids[1]. The VEEV vaccine strain (TC-83) produces more **mutations** than wild-type VEEV and was unable to efficiently infect mosquito cells[2]. We hypothesized this was due to a key mosquito immune response, RNAi (a collection of three pathways including the piRNA pathway that recognize, produce and degrade viral RNA during infection). As mutations are **key to viral infection and transmission,** it important we understand how altering the mutation rate impacts the virus' interactions with host immune systems.

What are the characteristics of piRNAs?

- **Viral 24-31nt length sequences** (these are recognised, produced and degraded by the piRNA pathway)[3].
- **A10** positive strand bias and **U/T1** negative strand bias[3].
- **Uneven distribution** of these sequences along the virus genome[3].
- **Sequence bias** towards the positive strand[3].
- **Why does this work matter?** Our previous work showed the siRNA response was dominant against the VEEV vaccine strain. Viruses able to evade the dominant siRNA response may instead be targeted by the piRNA response, providing a second barrier.

Methods

C7/10 cells (siRNA deficient) were infected with the vaccine strain at a controlled volume

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Cellular RNA was extracted and sent for sequencing

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We identified sequences mapping to the VEEV vaccine strain

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R, Excel and GraphPad Prism were used to analyze these sequences

We Identified Sequence Lengths of 25 and 26nts

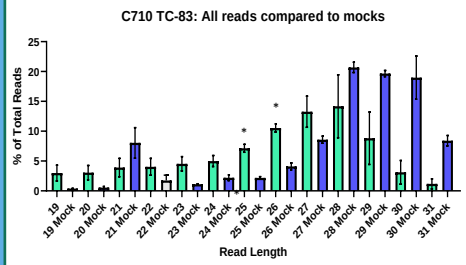

Figure 1. In C7/10 cells percentage counts of 25nt and 26nt length sequences were significant compared to mocks. Significance was determined using an unpaied T-test on Graphpad Prism.

Positive strand sequences showed characteristic A10 piRNA bias

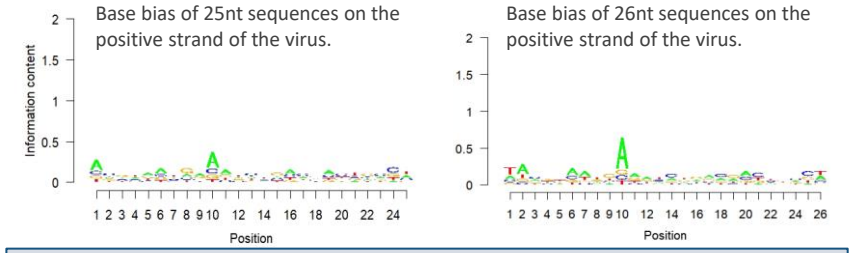

Figure 2. The positive strands of the vaccine strain show strong bias for A10. There is also bias for A1 on 25nt sequences and T1 and A2 on 26nt sequences.

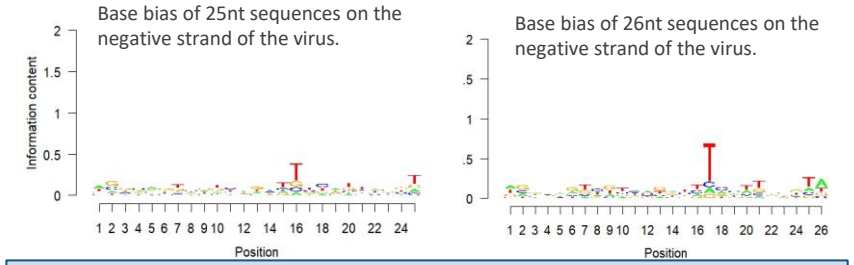

Figure 3. There is T bias at nucleotide positions 16 and 25 for 25nt sequences and 17, 21 and 25 and A bias at 26 for 26nt sequences on the negative strand of the virus.

1. Aguilar, P. V., Estrada-Franco, J. G., Navarro-Lopez, R., Ferro, C., Haddow, A. D., & Weaver, S. C. (2011). Endemic Venezuelan equine encephalitis in the Americas: hidden under the dengue umbrella. Future Virol, 6(6), 721-740. doi:10.2217/FVL.11.5
2. Kautz, T. F., Guerbois, M., Khanipov, K., Patterson, E. I., Langsjoen, R. M., Yun, R., Warmbrod, K. L., Fofanov, Y., Weaver, S. C., Forrester, N. L. 2018. Low-fidelity Venezuelan equine encephalitis virus polymerase mutants to improve live-attenuated vaccine safety and efficacy. Virus Evolution, 6:4(1). doi: 10.1093/ve/vey004. eCollection 2018 Jan.
3. Ruckert, C., Prasad, A. N., Garcia-Luna, S. M., Robison, A., Grubaugh, N. D., Weger-Lucarelli, J., Ebel, G. D. (2019). Small RNA responses of culex mosquitoes and cell lines during acute and persistent virus infection. Insect Biochem Mol Biol. 109:13-23. doi: 10.1016/j.ibmb.2019.04.008.
4. Miesen P, Ivens A, Buck AH, and van Rij RP (2016). Small RNA Profiling in Dengue Virus 2-Infected Aedes Mosquito Cells Reveals Viral piRNAs and Novel Host miRNAs. PLoS neglected tropical diseases 10, e0004452.
5. Varjak M, Donald CL, Mottram TJ, Sreenu VB, Merits A, Maringer K, Schnettler E, and Kohl A (2017a). Characterization of the Zika virus induced small RNA response in Aedes aegypti cells. PLoS neglected tropical diseases 11, e0006010.

Sequences Showed Uneven Distribution

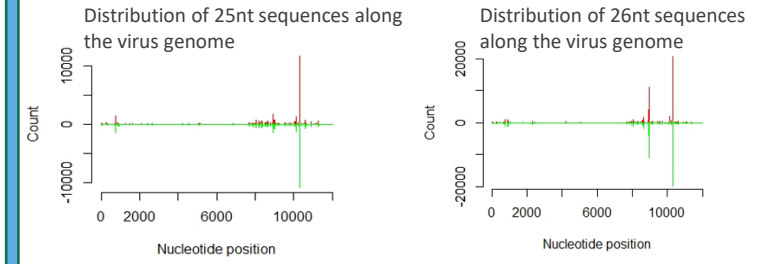

Figure 4. Both 25nt and 26nt sequences map to specific sections of the viral genome. These include clusters at 8000 to 11,000 base pairs.

Discussion

C7/10 cells defective for the siRNA response show characteristics of the piRNA response. Sequences of 25nt and 26nt in length are identified that have A10 bias and are mapped to specific points of the genome. These are common characteristics of piRNAs[3]. Whilst there is not the characteristic positive strand bias (graphs not shown) or U/T1 bias on the negative strand of either sequences, several other studies have identified piRNAs against Zika Virus, Dengue Virus and West Nile Virus that showed no classic nucleotide bias[3,4,5]. More analysis is needed to confirm the lack of bais still represents piRNAs. Our previous work has shown that the siRNA response is dominant against the vaccine strain in siRNA competent cells. This work indicates the **piRNA response is a secondary response** when the siRNA is unavailable. **This is important as mutant viruses able to evade the siRNA response may instead be targeted by the piRNA response.** In the case of the vaccine strain, it appears increasing the mutation frequency does not enable the virus to evade either response and this may be the reason the vaccine strain is unable to efficiently infect mosquito cells.

Conclusion

- The piRNA response is likely redundant to the siRNA response.
- Mutant viruses able to evade the siRNA response are likely targeted by a second response: the piRNA.
- Increasing the mutation frequency of the vaccine strain does not enable VEEV to evade these pathways.

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