Evaluation of a lepidopteran *in vitro* model of interactions between extracellular vesicles and viruses

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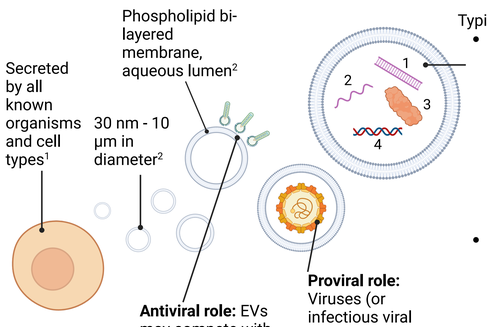
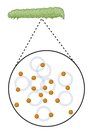
INTRODUCTION

Extracellular vesicles

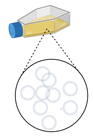
and their potential link to antiviral immunity in insects and other animals



Why an *in vitro* model?



Live insects: lipoproteins contaminate EV preps



Cell cultures: no contaminating lipoproteins; more amenable to transfections, infections , and gene editing

Fig. 1: introduction to extracellular vesicles (EVs). Created with BioRender.com.

Aim 1: confirm **successful isolation** of EVs from a cell line (**Sf9**; from *Spodoptera frugiperda* [Noctuidae]) by comparing with EV preparations from cotton bollworm **larvae** (*Helicoverpa armigera* [Noctuidae]).

Aim 2: determine if EVs **modulate permissiveness of Sf9 cells to infection** by *Autographa californica* multiple nucleopolyhedrovirus (**AcMNPV**; a lepidopteran-specific baculovirus), and if so, whether pre-infection of the EV-producing cells has any effect.

METHODS

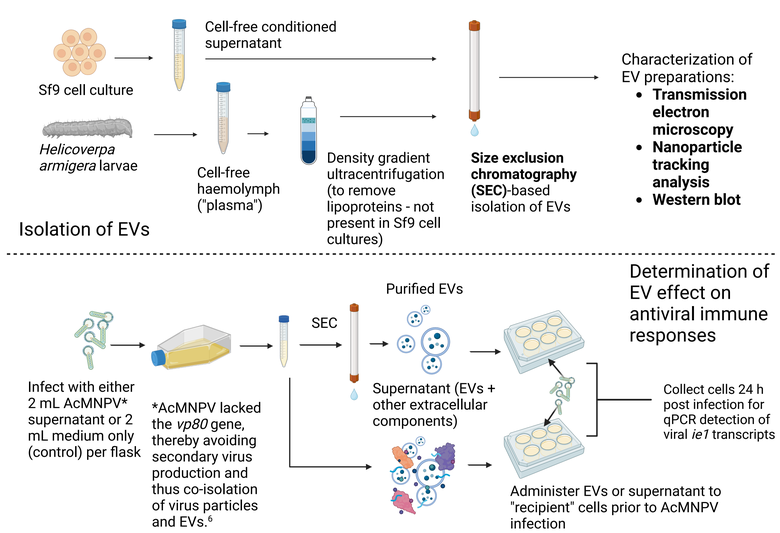
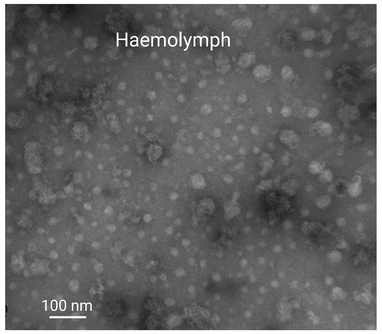


Fig. 2: Isolation procedure for extracellular vesicles (top). Experimental procedure to determine if EVs from infected cells modulate immune responses of naïve cells (bottom). Created with BioRender.com.

RESULTS & DISCUSSION



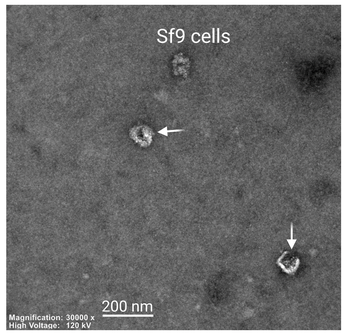


Fig. 3: **Transmission electron microscopy** (TEM) images of EV-like structures from *Helicoverpa armigera* larval haemolymph (left) and Sf9 cell culture supernatant (right).TEM shows **particles within the size range of EVs**, some of which (especially in the Sf9-derived samples) show a **cup shape** (defining characteristic for EVs in TEM preparation[2]; white arrows). Created with BioRender.com.

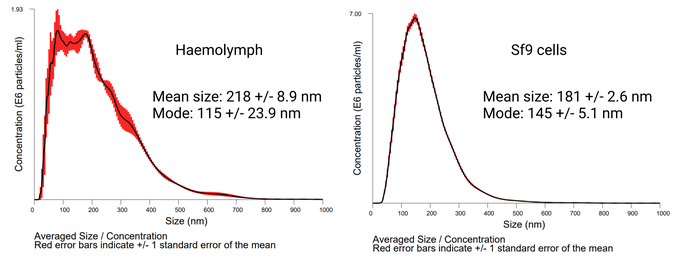


Fig. 4: **Nanoparticle tracking analysis** (NTA)-based size distribution graphs of EV preparations from *Helicoverpa armigera* haemolymph (left) and Sf9 cell culture supernatant (right). NTA indicates **typical EV size distribution** (right-skewed , with peak at ca. 200 nm) for both Sf9- and *H. armigera*-derived EVs. Created with BioRender.com.

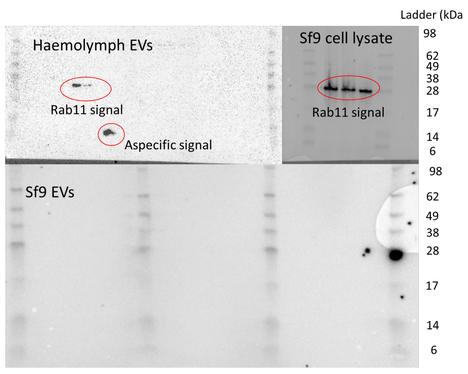


Fig. 5: **Western blot** against the **EV marker Rab11** (expected molecular mass: 24 kDa) for EV preparations from *H. armigera* haemolymph (top left), Sf9 cell lysate (top right), and Sf9 cell EV preparations (bottom).Western blotting confirms the presence of the EV marker Rab11 in *H. armigera*-derived EV preparations and in Sf9 cell lysate, but not in Sf9 EV preparations: **Rab11 is not a suitable EV marker for Sf9 cells** (note that the antibody was raised against vertebrate Rab11). Hence, other EV markers for Sf9-derived EVs may need to be searched out and tested.

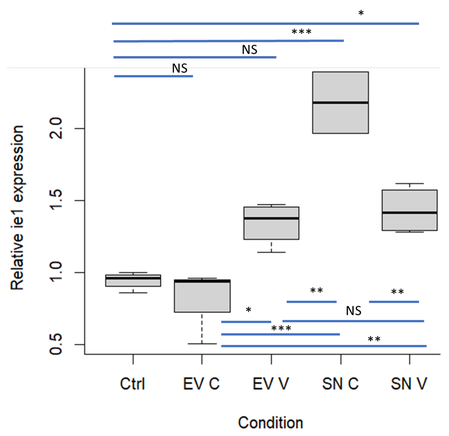


Fig. 6: **Effect of EV or supernatant pre-treatment on expression of viral (*ie1*) transcripts.** Prior to viral infection, Sf9 "recipient" cells were incubated overnight with fresh cell medium (Ctrl), EVs from control (EV C) or infected (EV V) cells, or supernatants from control (SN C) or infected (SN V) cells. P-value cutoff was 0.05. (p = 0 < *** < 0.001 < ** < 0.01 < * < 0.05; NS = p > 0.05).

Comparison of viral transcript levels suggests that secreted components in the **cell culture medium increase viral replication**, and that this effect is **reduced** if the cells were pre-treated with virus. However, **EVs** within the medium seem to **play little role** in modulating viral transcript levels.

CONCLUSION

- Sf9 culture supernatant exhibits particles which show characteristics of EVs, but optimization of EV marker proteins is needed. Hence, Sf9 cells can be considered a **suitable *in vitro* model** for some EV research in Lepidoptera (especially noctuids).
- Although an **extracellular component** appears to play a role in baculoviral infection, this **effect is probably not attributable to EVs.**

ACKNOWLEDGEMENTS

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REFERENCES

1. Gill, S. et al. (2019). *FEMS Microbiology Reviews, 43*(3), 273-303.
2. van Niel, G. et al. (2018). *Nature Reviews Molecular Cell Biology, 19*, 213-228.
3. Bou, J-V. et al. (2023). *Vaccines, 11*(10), 1532.
4. Kerviel, A. et al. (2021). *Annual Review of Cell and Developmental Biology, 37*, 171-197.
5. Santos, D. et al. (2021). *Plants, 10*(3), 484.
6. Marek, M. et al. (2011). *Biotechnology and Bioengineering, 108*(5), 1056-1067.