Silvina Goenaga

Dra. Silvina Goenaga obtained her Bachelor's degree in Biology from Universidad de Buenos Aires in 2006 and her Ph.D. in Biological Chemistry from the same University in 2015 working on the characterization of insect specific flavivirus. She trained in the US working on the potential for co-infection of novel mosquito-specific flaviviruses to block human flaviviral disease agent infection and transmission in mosquitoes at the Centers for Diseases Control and Prevention, Fort Collins, Colorado. Her research interests include arboviruses and the role of mosquitoes as vectors.

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ISID Research Grant Report

Potential for co-infection of novel mosquito-specific flaviviruses to block human flaviviral disease agent infection and/or transmission in mosquitoes

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Introduction

The genus *Flavivirus* comprises over 70 viruses that include several human pathogens such as West Nile virus (WNVV), St. Louis encephalitis virus (SLEV) and Japanese encephalitis virus (JEV) and include a group of "insect-specific" flavivirus (ISFs) that do not infect vertebrates and they will be maintained in nature by vertical transmission. Recently, a new ISF called Nhumirim virus (NHUV) (Pauvolid-Correa et al., 2015) was isolated in *Culex sp.* in Brazil. Co-infection experiments in mosquito cell culture indicated that prior or concurrent infection with NHUV resulted in significant reduction in viral production of WNV, SLEV and JEV (Kenney et al., 2014). These data indicate the potential modulatory effect of flaviviral mosquito co-infections in the field and serve to identify a potential target for blocking mosquito infection as a public health measure. Here, we determine the potential inhibitory role of infection of NHUV on infection with and transmission of WNV in *Cx. quinquefasciatus* and *Cx. pipiens* mosquitoes. Moreover, we assess the efficiency of vertical transmission of NHUV in *Culex quinquefasciatus* mosquitoes.

Materials and Methods

In order to asses NHUV vertical transmission *Cx. pipiens* mosquitoes were intrathoracically inoculated (IT) with NHUV and the offspring were reared to adult stage. We tested for the presence of NHUV RNA and infectious virus by RT-PCR and immunofluorescence assay (IFA) respectively. All F1 emergent adult mosquitoes from those females that were found to be positive by IFA and/or RT-PCR for NHUV were processed.

Dual infection vector competence assay. 3–5 d post-emergence *Cx. quinquefasciatus* mosquitoes and *Cx. pipiens* were intrathoracically co-inoculated with NHUV and WNV or solely with WNV as a control. *Cx. pipiens* mosquitoes were allowed to extrinsically incubate the viruses for 14 days and *Cx. quinquefasciatus* for 3, 5, 7 or 9 days, at which point transmissibility and relative replication of the WNV determined. Bodies and saliva from each mosquito were processed and WNV infection rate (body) and WNV transmission rate (saliva) were analyzed by plaque titration on 12-well plates of Vero cells. The percentage of mosquitoes that were infected, demonstrated disseminated infections, and demonstrated theoretical transmission of WNV were compared by a Fisher exact test.

Results and Discussion

Evidence of vertical transmission of NHUV in *Cx. pipiens* mosquitoes following intrathoracic inoculation was demonstrated. Sixty-eight *Cx. pipiens* females were IT-inoculated with NHUV; however, offspring were reared to adults (n=3) from only one NHUV IT-inoculated female that successfully oviposited. Of these, one mosquito was positive for

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NHUV by RT-PCR and by IFA after homogenization and inoculation on C6/36 cells. Co-infection of NHUV/WNV in *Cx. pipiens* mosquitoes. In order to assess the potential inhibitory effect of NHUV replication on WNV infectivity and transmissibility, *Cx. pipiens* mosquitoes were intrathoracically co-inoculated with NHUV and WNV or solely with WNV as a control. Mosquitoes co-inoculated with NHUV +WNV (n=33) were processed at 14 dpi. Transmission, dissemination and infection rates were analyzed. WNV infection and dissemination rates were 100% for both groups (co-infected and control mosquitoes). The transmission rate in the NHUV+WNV group (45.5%, n=33) was not significantly different from those inoculated with WNV alone (54.6% n=11) (Figure 1).

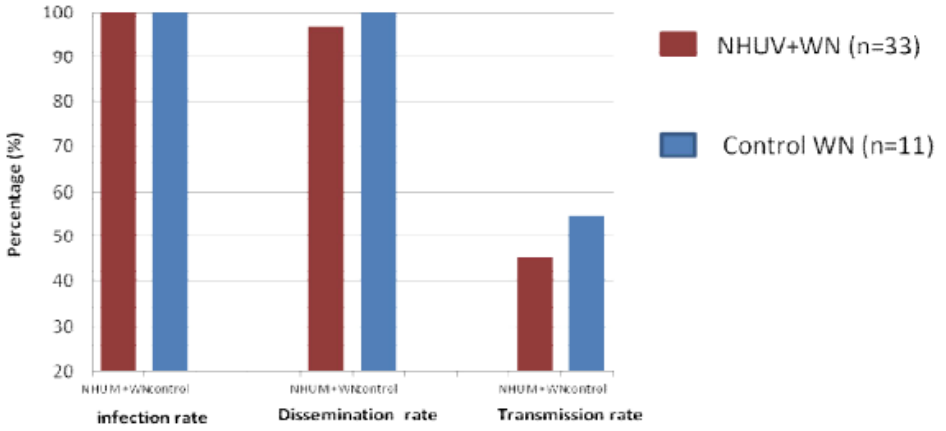

Figure 1. Transmission, dissemination and infection rates in *Cx. pipiens* mosquitoes co-inoculated with NHUV +WNV.

Co-infection of NHUV/WNV in *Cx. quinquefasciatus* mosquitoes. The capacity of *Cx. quinquefasciatus* for being infected and transmitting WNV when co-inoculated simultaneously with NHUV was also assessed. Coinoculated mosquitoes were harvested and processed at 3, 5, 7 and 9 dpi. Results demonstrated 100% infection with WNV of the *Cx. quinquefasciatus* mosquitoes at all four time points in the control groups. Similarly, the NHUV + WNV experimental infection group exhibited 100% infection at dpi 5, 7 and 9, with 90% infection in the co-inoculated group at dpi 3 (Table 2). Nevertheless, differences in the proportion of mosquitoes that were capable of transmitting WNV (≥ 21 mosquitoes per sampling time point) were significantly lower for the NHUV+ WNV group than the WNV control at dpi 7 and 9 (Table 2) [6.3 odds ratio (95% CI 1.4 to 27.8) for WNV vs. NHUV+WNV at dpi 7 and 6.4 odds ratio (95% CI 1.3 to 31.5) for WNV vs. NHUV+WNV at dpi 9].

Sampling	Treatment group	Infection rate (%)	Transmission rate (%)
3 dpi	NHUV+WNVV	19/21 (91)	0/21 (0)
	WNVV	32/32 (100)	7/32 (21.8)
5 dpi	NHUV+WNVV	38/38 (100)	19/38 (50)
	WNVV	32/32 (100)	11/32 (34)
7 dpi	NHUV+WNVV	30/30 (100)	10/30 (33.3)
	WNVV	25/25 (100)	19/25 (76)*
9 dpi	NHUV+WNVV	21/21 (100)	5/21 (23.8)
	WNVV	27/27 (100)	18/27 (66.7)*

Table 2. WNV infection and transmission rates of NHUV co-inoculated *Cx. quinquefasciatus* mosquitoes at 3, 5, 7 and 9 dpi. *Indicates significantly higher transmission rates for WNV infection group versus the dual inoculation group.

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Conclusions

Attempts to grow NHUV in limited vertebrate cell lines have proven unsuccessful (Kenney et al., 2014; Pauvolid-Correaet al., 2015). Despite a small sample size, vertical transmission of NHUV in F1 progeny of IT-inoculated female *Culex* mosquitoes was demonstrated herein. While the assessment of the mechanisms and efficiency of vertical transmission of NHUV should be repeated on a much larger scale, it does suggest that this virus uses a maintenance method demonstrated by many classical ISFs (Bolling et al., 2012; Saiyasombat et al., 2011; Bolling et al., 2011).

Vector competence studies for WNVV in *Cx. quinquefasciatus* mosquitoes co-infected with NHUV were performed. The results demonstrating significant reduction in the transmissibility of WNVV in *Culex* mosquitoes co-inoculated with NHUV at later rather than earlier time points is interesting and portends that prior infection of mosquitoes with NHUV and an establishment of superinfection exclusion mechanisms in salivary acinar cells critical for viral egress prior to exposure to WNV could result in an even more striking inhibition of WNV transmissibility.

References

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