West-Nile virus replicon particles infect 293T cells expressing DC-SIGNR

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Abstract

West-Nile virus (WNV) is an arbovirus usually transmitted to humans via a mosquito vector. Infections commonly result in febrile symptoms while rare severe neuroinvasive cases may result in encephalitis or meningitis. Studies have shown that WNV infection efficiency is enhanced by expression of DC-SIGNR on target cells, which normally do not express DC-SIGNR. To investigate WNV tropism, we established 293T kidney epithelial cell lines that stably express vector, DC-SIGNR and mutants of DC-SIGNR that lack the entire carbohydrate-recognition domain (CRD) or lack the C-terminal half of the CRD. We demonstrate successful surface expression of DC-SIGNR and its mutants from stably-transfected 293T cells, but not vector-transfected 293T cells. Further, we show that monoclonal antibody 120604 which binds specifically to the DC-SIGNR CRD binds to DC-SIGNR expressing 293T cells, but not to vector nor any of the DC-SIGNR mutants expressing cells. Virus replicon particles (VRPs), replication-incompetent viral particles containing necessary structural proteins for infection and a viral plasmid including a GFP reporter are used to safely and conveniently study viral entry. Entry assays using WNV (NY99) VRPs as well as a variant of WNV (NY99) which contains the beta-lactamase enzyme show significant entry into DC-SIGNR expressing cell lines, but not in controls that do not express DC-SIGNR. Additionally, we show that WNV VRPs do not enter DC-SIGNR expressing cells that lack the CRD or the C-terminal half of the CRD suggesting that the C-terminal half of the CRD is required for successful entry of WNV via DC-SIGNR. Future experiments may be able to shed light on which amino acids are required for entry.

Introduction

Since its migration to New York in 1999, a strain of West-Nile Virus (WNV) that primarily used a bird host and mosquito vector transmission cycle has swept across the western hemisphere into Central and South America (1). Human WNV infection may lead to West Nile fever, associated with symptoms that persist past 30 days in >60% of cases (1,2). In addition, it has been estimated that ≈ 1 in 50 infected patients ≥65 years develop encephalitis, meningitis, and paralysis (1, 3). Since, presently, there exist no accepted human vaccine, vector preventive measures serve as the primary avenue for combating primary infection. Current treatments are mostly supportive (1).

Dendritic Cell-Specific Intercellular adhesion molecule-3-Grabbing Non-integrin Related protein (DC-SIGNR) on lung, lymph, and liver endothelia when expressed on a cell line, enhanced WNV infection (4) as well as related Flaviviruses Japanese encephalitis virus(5) and dengue virus(6). Davis (2006) proposed that N-linked high-mannose oligosaccharides primarily on WNV transmembrane protein E have multivalent interactions with the carbohydrate recognition domain (CRD) of DC-SIGNR as both anti-CRD antibodies and E protein gene knockout reduced infection to levels comparable to control cell lines.

Further research will identify which amino acids are important for DC-SIGNR-dependent WNV infection. DC-SIGN, a related molecule of DC-SIGNR, enhances Flavivirus entry and the C-Terminus half of its CRD is primarily responsible for this enhancement (5, 6, 7). Although similar, the CRD of DC-SIGN and DC-SIGNR have different ligand molecular interactions (7). The purpose of this study is to test whether WNV entry requires the C-terminal half of the CRD of DC-SIGNR.

WNV Structure and Tropism

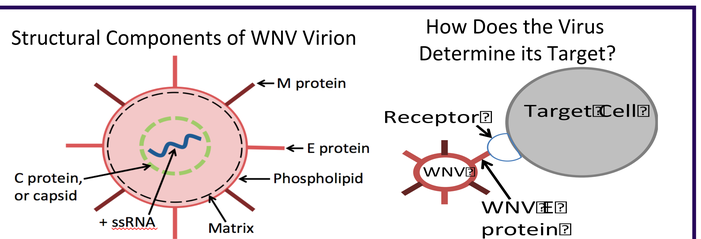
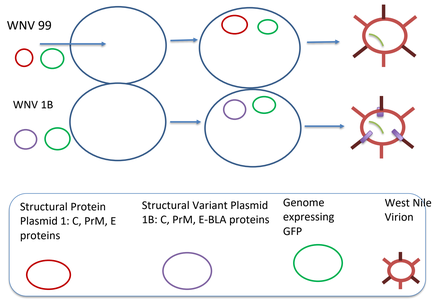


Figure 1. The WNV virion attachment E protein in part determines the tropism of the virus by attaching to target cell surface receptors.

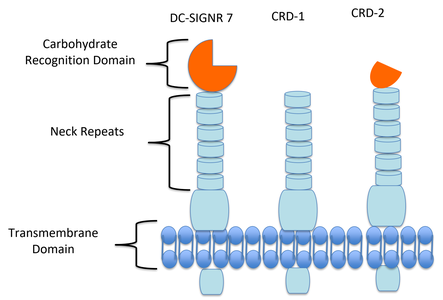
Virus Replicon Particle (VRP) System

Figure 2. Lab produced VRPs are used to safely test viral infection. Transfection of two plasmids into HEK-293T cells leads to the production of WNV VRPs. One plasmid expresses the structural membrane, capsid, and attachment proteins; PrM, C, and E, respectively. While the other plasmid contains a green fluorescent protein (GFP) expressed from within a mutated viral genome. The structural proteins and GFP containing genome assemble to create a non-replicating infectious WNV virions.



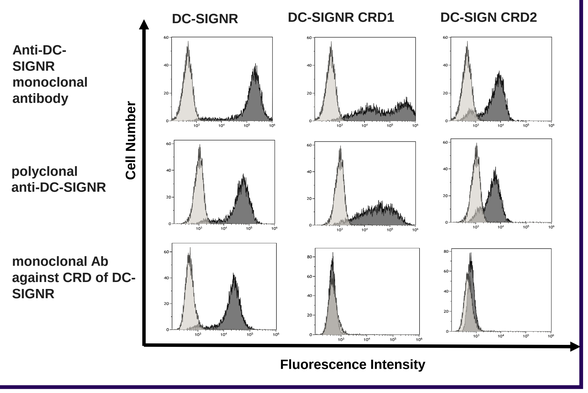
Generating DC-SIGNR expressing cells with mutations

Figure 3. To generate stable expressing DC-SIGNR from normally non-expressing HEK-293T cells, the open reading frames of DC-SIGNR7 was subcloned into SBP-mini vector. The SBP-mini vector co-expresses a streptavidin-binding mini protein on the cell surface as well as hygromycin resistance which was used to select out stable cells. Mutations were made to DC-SIGNR7. The CRD-1 mutation removed the carbohydrate recognition domain and the CRD-2 mutation removed half of the domain. These mutants were also used to generate CRD-1 and CRD-2 stably expressing 293T cells.



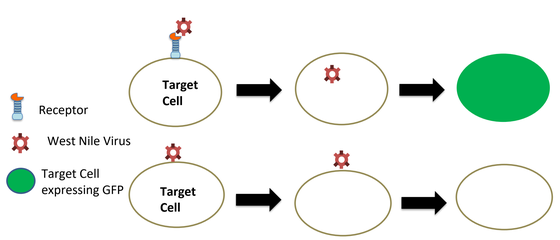
Assaying for WNV PRP Infection

Figure 4. DC-SIGNR expression on 293T cells. All cells (293T expressing nothing, DC-SIGNR, 293T cells expressing DC-SIGNR CRD1 and 293T cells expressing DC-SIGNR CRD2) were stained with anti-DC-SIGNR monoclonal antibody, polyclonal anti-DC-SIGNR antibody and finally a monoclonal antibody that stains only the CRD in DC-SIGNR. The light colored histogram represents stained 293T cells expressing nothing.



Surface expression of DC-SIGNR and its mutants

Figure 5. WNV uses the E attachment protein to bind to a target cell. If the virus gains entry into the cell, it releases its viral genome into the cytoplasm of the target cell. GFP is expressed from the modified genome and the cell fluoresces green.



WNV Infection of DC-SIGNR expressing cells

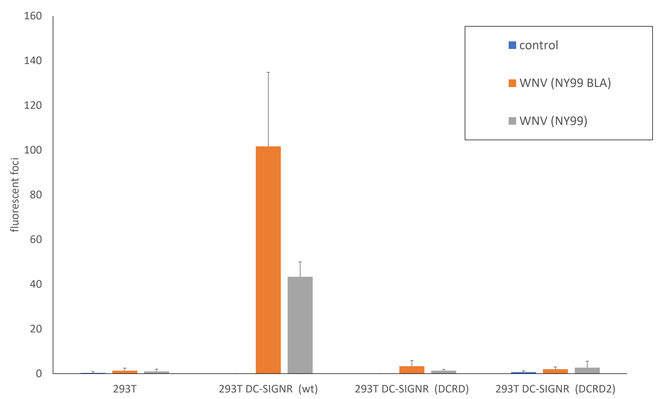


Figure 6. WNV entry requires the C-terminal half of the CRD of DC-SIGNR. 293T, 293T expressing DC-SIGNR, DC-SIGNR CRD1 (DCRD) and DC-SIGNR CRD (DCRD2) were plated in 96 well plates and infected in triplicate with control, WNV (NY99) containing beta-lactamase and WNV (NY99) for three days. Green fluorescent foci were manually counted from each of the three infection wells and graphed above.

Discussion

These results show that WNV VRPs infect 293T DC-SIGNR expressing cells. Eliminating the CRD or the C-terminal portion of the CRD ablates efficient entry into the 293T cells. We also show that eliminating the CRD from DC-SIGNR does not prevent the mutant's cell surface expression (Figure 4) and therefore the lack of entry by the WNV VRPs is not due to lack of cell surface expression of the protein. Altogether, these results demonstrate that the CRD is critical for viral entry and suggest that the virus may specifically bind to DC-SIGNR via the C-terminal portion of the CRD. Further experiments will be performed to identify those amino acids in DC-SIGNR responsible for mediating entry of the WNV.

Acknowledgements

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