Dengue Virus Non-Structural Protein 1 Inhibits Pattern Recognition Receptor Signaling
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Introduction
West Nile virus (WNV) and Dengue virus (DENV) are RNA viruses belonging to the Flaviviridae family. Their 5′-3′ UTR genome encodes 3 structural and 7 non-structural proteins. Both viruses are transmitted to humans by mosquito vectors and are mainly prevalent in the subtropical regions of the world where 100 million people are at risk of getting infected annually. These viruses typically cause self-limiting disease. However, in some cases WNV infection can lead to fatal encephalitis and Dengue fever can result in Dengue Hemorrhagic Fever or Dengue Shock Syndrome, both life-threatening conditions. The innate immune pattern recognition receptors (PRRs) such as TLR-like receptors and RNA-Helicases (RIG-I-like) play an important role in recognizing viral pathogens associated molecular patterns (PAMPs) such as single and double stranded RNA, and triggering a cascade of pro-inflammatory cytokines to limit virus spread. However, many viruses including WNV and DENV have evolved mechanisms to evade innate immunity leading to enhanced virus replication and high virulence, hence more severe disease. Nonstructural protein 1 (NS1) in both viruses is a secreted glycoprotein protein that is necessary for virus replication and has been shown to have immunomodulatory function. Our lab has shown that both secreted and intracellularly expressed WNV NS1 inhibit TLR signal transduction leading to inhibition of the overall antiviral state when challenged with viral infection.

The objective of this study was to compare the immunomodulatory role of intracellularly expressed and secreted DENV NS1 to our findings with WNV NS1 in an effort to better understand the virus-host interaction during dengue infection.

Hypothesis
Dengue NS1 inhibits PRR signaling in early dengue infection and plays a major role in increasing viremia and development of severe disease.

Background

Materials and Methods
Creation of the Stable Cell Lines: A recombinant retrovirus expression system was used to transduce Hela and 293T cell lines to create stable NS1 expressing cell lines.

IL-6 and Protein Quantification: Supernatants were collected after the treatments, cells were washed with PBS and lysed using Triton X-100 buffer. Human and Mouse IL-6 ELISA kits (CytoBioscience) were used to quantify IL-6 (pg/ml).

Western Blot: Protein was quantified using Bradford protein assay. Protein was separated on 4-12% SDS-PAGE gel, transferred to nitrocellulose membrane and probed with mouse anti-IL-6 antibody and goat anti-mouse secondary antibody.

Nickel Column Purification: Supernatants were collected for 3 days in DMEM from DENV NS1 or control 293T cell lines. Ni-NTA purification system (Invivogen) was used to purify His-tagged NS1 from the supernatants. The resin was packed through a column and washed a two times before the His tagged NS1 was eluted using high imidazole solution. NS1 presence in the fractions was verified by western blot.

Results

Summary and Conclusions

- Intracellular and secreted Dengue NS1 inhibit TLR3 signaling in Hela cells.
- Secreted Dengue NS1 inhibits TLR3/4 signaling in murine bone marrow derived macrophages.
- Histidine tagged DENV NS1 can be purified using Nickel column purification and is still able to inhibit TLR3 signaling in Hela cells.

Future Directions

- Explore the inhibitory effect of intracellular and secreted dengue NS1 in human monocytes and macrophages.
- Investigate the effect of Dengue NS1 inhibition of PRR signaling on the viral genome copy number in early dengue infection.
- Investigate whether NS1 is secreted from mosquito cells and whether it is inhibitory in the mammalian system.

Citations

Acknowledgments
Krisan Crook, Clayton Morrison, Mindy Miller-Kittrell, Shaikhah Lawson