Structural biology of flavivirus NS1 protein and its antibody complexes

Bing Liang Alvin Chew, Qi Pan, Hongli Hu, Dahai Luo
Speakers: Caryn Quek, Dylan Cheong, Ryan Lee, Sarah Yip (T22B)

INTRODUCTION

Every year, viruses belonging to the genus Flavivirus such as the Zika Virus (ZIKV), Dengue Viruses (DENV1-4), Yellow Fever Virus (YFV), and West Nile Virus (WNV) cause widespread epidemics, impacting millions. These viruses are mainly spread through insect vectors with WNV causing flu-like symptoms or severe neuroinvasive diseases and Yellow fever, Zika, and Dengue causing mild symptoms to severe outcomes like hemorrhagic fever, microcephaly, and organ failure.

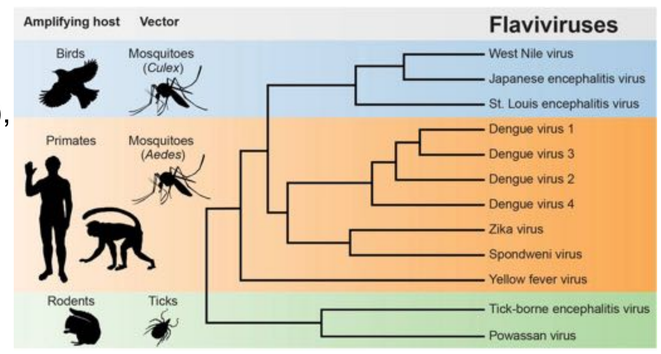

Fig. 1 Phylogenetic tree of *Flavivirus*

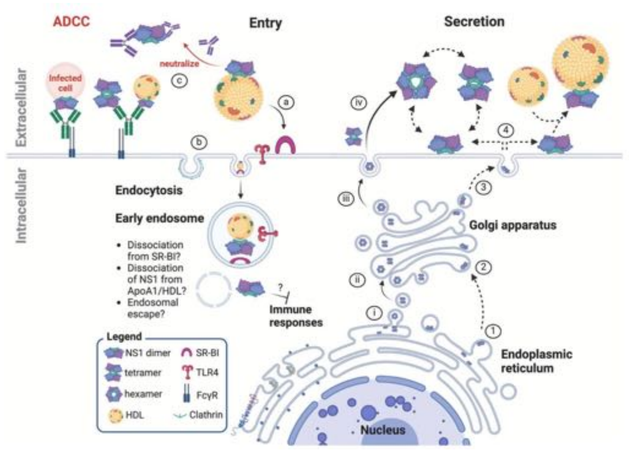

Fig. 2 Current views and unknowns in NS1 secretion, entry and inhibition

Among the 10 viral proteins it produces, the Nonstructural Protein 1 (NS1) is the only secreted protein vital for viral replication, immune response modulation, and viral pathogenesis. Thus, it serves as not only a diagnostic biomarker but also a promising vaccine target. This review mainly explores the rapidly evolving structural diversity of NS1 and its interactions with monoclonal antibodies (mAbs), which highlight their protective roles in the course of an infection. Further research is hence needed to understand the functional relevance of NS1's diverse forms in viral pathogenesis and the development of therapeutic modalities.

METHODS

Cryogenic electron microscopy (Cryo-EM) was used to elucidate the structure of NS1 proteins and interactions with mABs.[1] Cryo-EM is a technique used to study the detailed structures of biological molecules. Samples are flash-frozen, preserving them in their near-native forms. Electron microscopy is then used to capture its 2D structure.

RESULTS

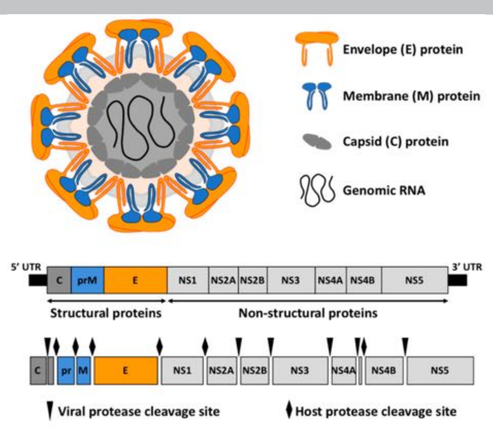

Fig. 3 Viral structure and mRNA showing its encoded proteins

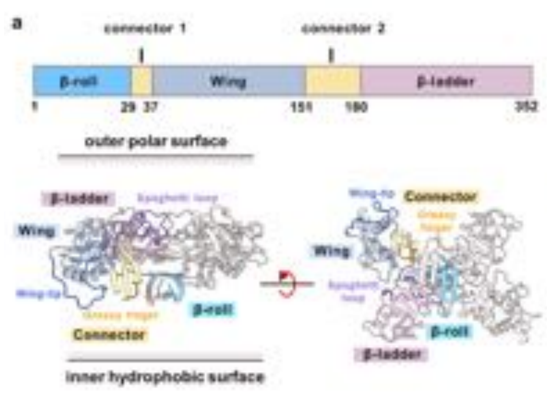

Fig. 4 Sequence of DENV NS1 protomer and cryo-em structure

Flaviviruses have single-stranded, positive-sense RNA genomes encoding polyproteins consisting of 3 structural proteins and 7 nonstructual proteins. Among which, NS1 is the only secreted protein and is thus, the interest of our study.

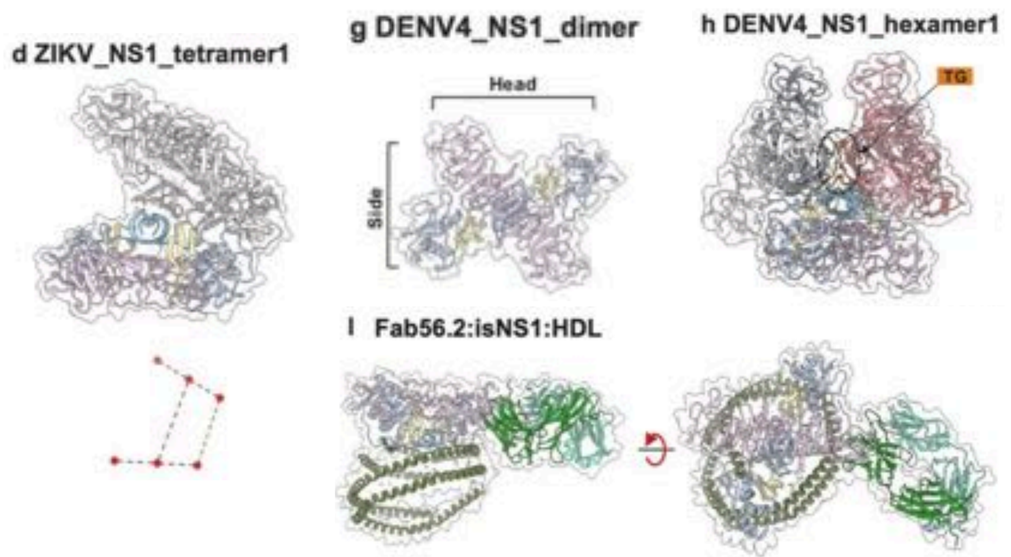

Fig. 5 Structural landscape of NS1 protein. (d) Cryo-em structure of Secretory NS1 (sNS1) tetramer for ZIKV (g) Cryo-EM structure of a NS1 dimer. (h-i) Cryo-em structure for DENV4 sNS1 hexamers. (l) Representation of isNS1 model in complex with Fab56.2 and HDL

Depending on its secretion, association with host factors and recombinant preparation, the flavivirus NS1 can exist in multiple oligomeric states - that of dimeric, tetrameric and hexameric forms.

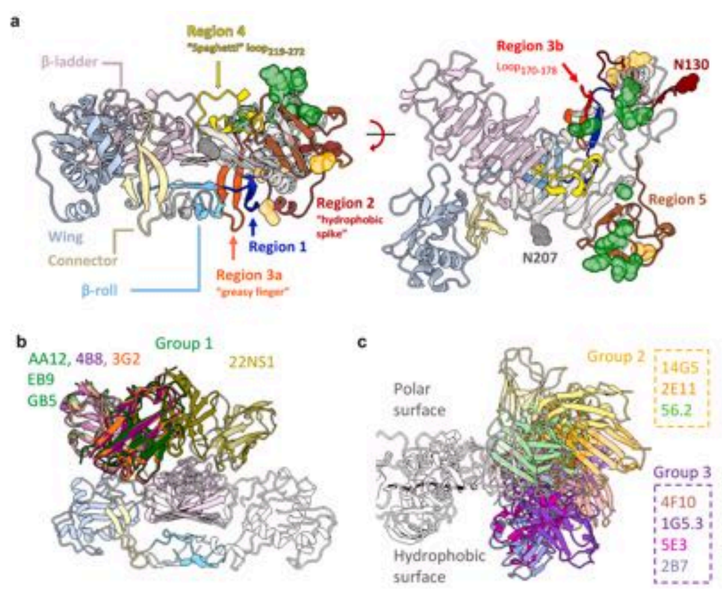

Fig 2. Structural model of NS1 key epitope regions and in complex with available antibody-NS1 complex structures. (a) side and top views of DENV2 NS1 dimer model with left and right monomers coloured and labelled by its domains.[2-4] Critical conformational epitopes were mapped on the right monomer, with glycosylation sites (N130 and N270) labelled as well.[5] (b) Group 1 antibodies bind to NS1 polar surface, aligned to ZIKV NS1 in EB9:NS1 complex (PDB: 8WO4). (c) Groups 2 and 3 antibodies recognise the distal β-ladder end but are differentiated by the antibody-NS1 complex shape.

DISCUSSION

- NS1 exists in various oligomeric states, mainly: dimers, tetramers, hexamers. Recombinant NS1 predominantly forms tetramers and hexamers, while Infection-derived NS1 associates with HDL to become semi-embedded dimers
- NS1-HDL interactions is mediated by the scavenger receptor B1, which facilitates entry into the cell while triggering pro-inflammatory signalling pathways
- NS1's structural diversity is therefore critical for its roles in immune evasion, modulation of vascular permeability and amplification of inflammatory responses
- Gaps still remain in understanding how different oligomeric forms of NS1 precisely contribute to flavivirus pathogenesis
- Anti-NS1 monoclonal antibodies show promise in neutralising NS1 and its effects. Differences in their efficacies demonstrate the importance of targeting specific structural epitopes, particularly within the β-ladder and wing domains
- This emphasises the need to consider NS1's structural diversity when designing vaccines and therapeutic antibodies to better enhance immune efficacy

CONCLUSION

- Understanding NS1's structural complexity and oligomeric diversity is crucial to unravelling its role in flavivirus pathogenesis
- NS1 contributes to immune evasion, vascular leakage and severe disease manifestations, suggesting potential for anti-NS1 antibodies to mitigate its effects
- Anti-NS1 antibodies show promise by targeting specific epitopes, but variations in efficacies across serotypes highlight the need for further structural exploration.
- Key areas for future research include: the precise pathways underlying NS1 secretion, the influence of lipids on NS1 oligomerisation and pathogenesis, and the structural basis for NS1's differing immunogenicities across different serotypes
- Advancing our knowledge in these areas will inform the development of targeted therapies and vaccines with improved cross-serotype efficacy against flavivirus infections

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