Studying the Impact of NS1 on Host Tropism of Influenza A Virus

Sara S. El Zahed[1], Soner Yildiz[1], Ignacio Mena[1], Arturo Marin[1], and Adolfo García-Sastre[1,2]

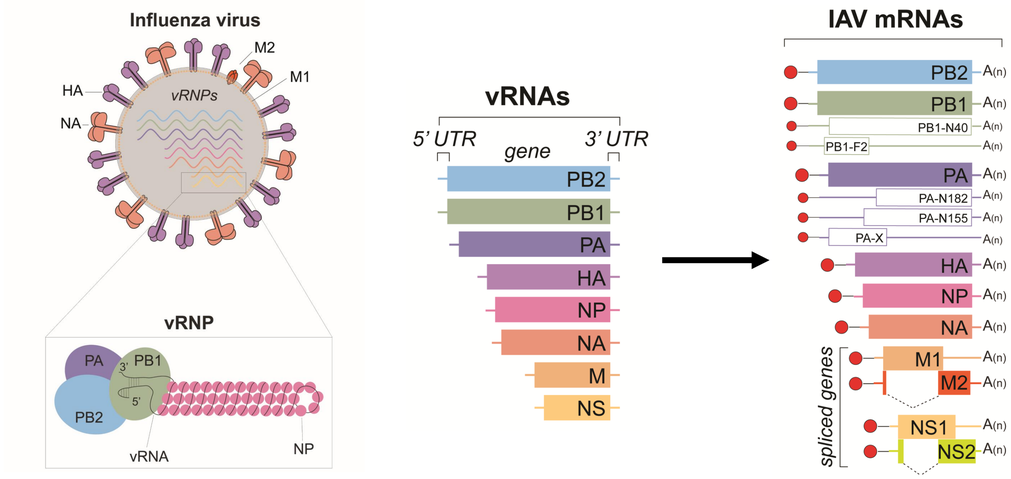
[1]Department of Microbiology, Icahn School of Medicine at Mount Sinai, New York, New York

[2]Institute of Global Health and Emerging Pathogens, Icahn School of Medicine at Mount Sinai, New York, New York

Summary

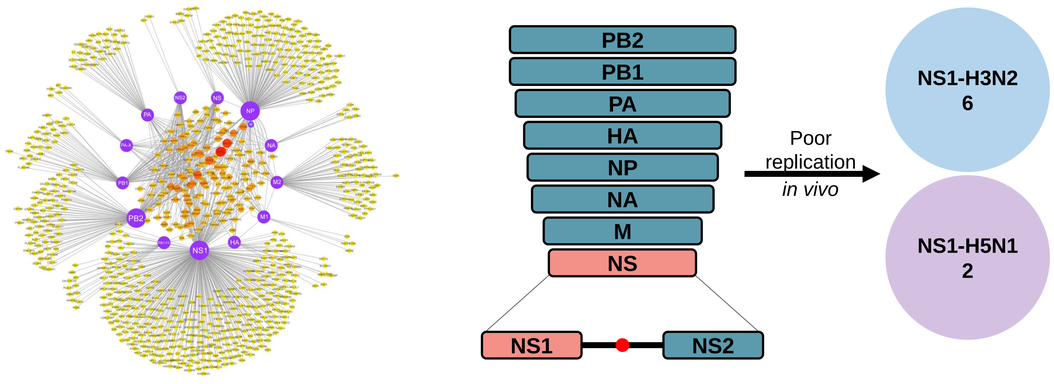
Influenza A virus (IAV) has been recognized for its ability to infect diverse hosts. While this characteristic has been largely attributed to hemagglutinin, neuraminidase, and polymerase proteins of IAV, host-pathogen interaction networks suggest that the NS1 virulence factor of influenza viruses could also contribute to host tropism. To further understand the impact of NS1 on tropism, we assessed *in vivo* adaptation of eight recombinant IAVs replicating poorly in mice. Except for the NS segment, these viruses share a common H1N1 backbone. Particularly, the NS segment has been modified to express different NS1 proteins. Indeed, following some rounds of passaging in mice, all viruses showed increased viral lung titers and reduced percent survival, suggesting *in vivo* adaptation. To identify mutations acquired by each virus and assess their impact on adaptation, we isolated viral RNA from mouse lung samples for whole genome sequencing. Indeed, some viral genomes revealed single nucleotide polymorphisms in NS1. As such, we will further study such mutations and determine their impact on adaptation. In all, this work could further support a potential role for NS1 in defining host tropism.

[A]Influenza A Virus



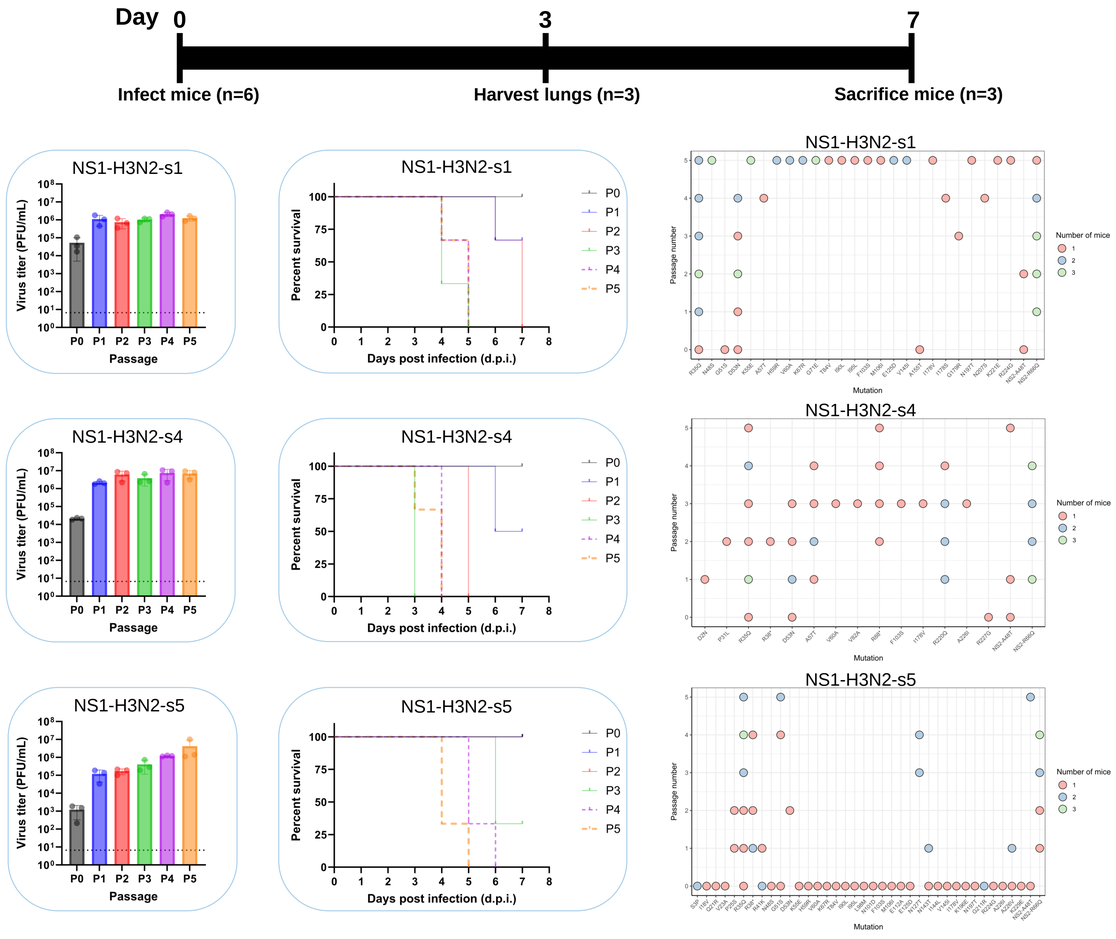
A. Influenza A is an enveloped virus that contains eight negative-sense single stranded RNA segments in the form of viral ribonucleoproteins (vRNPs). Following infection, these viral RNAs (vRNAs) are transcribed into their respective viral mRNAs. While the M and NS transcripts largely encode M1 and NS1, respectively, some of these mRNAs could undergo splicing, thus coding for M2 and NS2, respectively. Figure is adapted from Dou *et al.*, 2018, *Front. Immunol.*, **9**:1581.

[B]NS1 and Host Tropism



B. Protein-protein interaction network of influenza A virus (IAV) and *Homo sapiens* (left figure). To understand the impact of NS1 on host tropism *in vivo*, six poorly replicating recombinant IAVs expressing different NS1 proteins were used to infect mice and promote adaptation (right figure). Filled red circle in the left figure refers to a barcode. Left figure adapted from Farooq *et al.*, 2020, *BMC Infect. Dis.*, **20**:480.

[C]Lung Viral Titers, Survival Curves, and Missense Mutation Profiles



C. Timeline of passaging experiments *in vivo* to promote adaption of eight recombinant IAVs expressing different NS1 proteins. Experimental protocols were approved by the Institutional Animal Care and Use Committee at Icahn School of Medicine at Mount Sinai. For each passage, six mice were infected with one of the eight strains of interest and monitored for morbidity and mortality. Three days post-infection, three mice were sacrificed, and their lungs were harvested to determine lung viral titers (left figures). The remaining mice continued to be monitored for morbidity and mortality up to day seven post-infection (middle figures). Three recombinant IAVs with NS1 fragments from viruses belonging to the H3N2 subtype revealed some mutations in NS1 that were acquired and maintained over passages.

Significance

Understanding how NS1 contributes to host tropism of influenza A viruses could validate some of the mapped interactions between NS1 and host factors. Indeed, defining key amino acid residues of NS1 that drive host tropism could highlight their role in virulence in different hosts. Further, resistance to clinically approved antiviral drugs has limited our reservoir of available therapeutics. As such, validated interactions between NS1 and some host factors could identify potential targets for therapeutic intervention.

References

1. Dou, D. *et al*. (2018). Influenza A virus cell entry, replication, virion assembly, and movement. *Front. Immunol.*, **9**:1581.
2. Farooq, Q.u.A. *et al.* (2020). A systems biology-driven approach to construct a comprehensive protein interaction network of influenza A virus with its host. *BMC Infect. Dis.*, **20**:480.