COVID-19

SARS-CoV-2 targets MAVS for immune evasion

A new study shows that the SARS-CoV-2 nucleocapsid protein represses the antiviral type I interferon response through direct interaction with the signalling adaptor protein MAVS. Targeting this process might be a useful therapeutic strategy to boost immunity against COVID-19.

Alessia Zotta, Alexander Hooftman and Luke A. J. O'Neill

COVID-19 is a respiratory disease caused by SARS-CoV-2 and remains a major global public health issue despite recent therapeutic advances and the approval of several vaccines for use[1]. Many studies have focused on how the immune system responds to infection with SARS-CoV-2 (ref. [2]). The induction of antiviral type I interferons (IFNs) is a critical response and is driven by the sensing of viral single-stranded RNA, most likely by RNA receptors such as RIG-I[3]. SARS-CoV-2 RNA is contained within the viral particle that is composed of four structural proteins: the spike glycoprotein (S), the membrane protein (M), the ion channel envelope protein (E) and the nucleocapsid protein (NP), which are involved in viral invasion and replication[4]. The S protein binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the respiratory epithelium[5] and is responsible for the entry of the virus into host cells. The NP is involved in the packaging of RNA into new virions. Whether NP has other roles is not known. In this issue of *Nature Cell Biology*, Wang et al.[6] report that NP can inhibit the key signalling adaptor mitochondrial antiviral-signalling protein (MAVS), which is activated by RIG-I, limiting the host defence response to the virus. They demonstrate that this process can be blocked with a peptide, raising the prospect of a novel therapy to boost immunity and limit pathology in COVID-19 (Fig. 1).

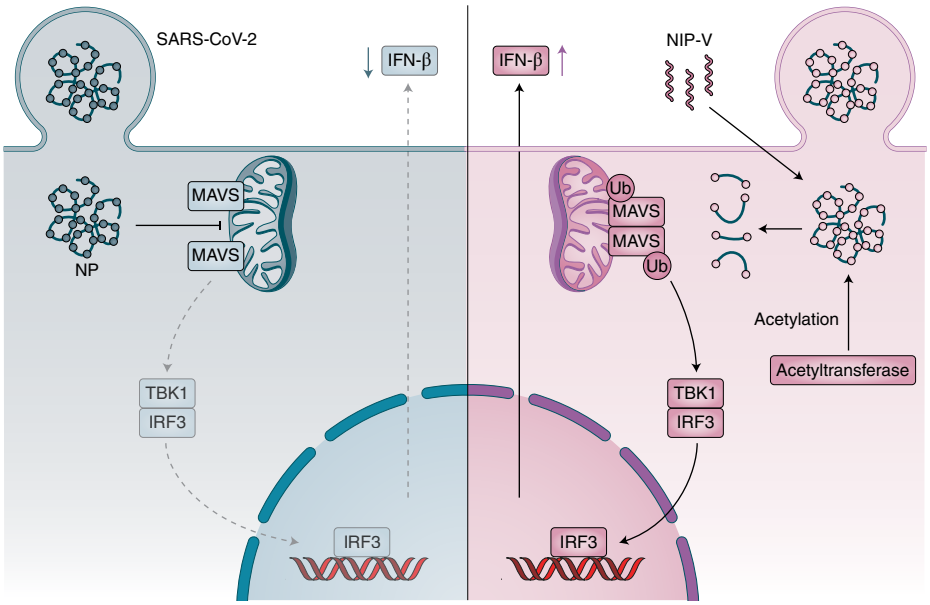

Fig. 1 | SARS-CoV-2 nucleocapsid protein (NP) represses MAVS signalling and IFN-β release. SARS-CoV-2 NP inhibits polyubiquitination and aggregation of MAVS, repressing the downstream TBK1–IRF3-mediated upregulation and release of IFN-β (left panel). Targeting of NP by host acetyltransferases or interfering peptides such as NIP-V disrupts NP droplet formation, preventing NP-mediated repression of MAVS. This facilitates release of IFN-β, thereby promoting antiviral immunity and viral clearance (right panel).

The study began with the demonstration that NP can undergo phase separation and form micro-sized droplets that can co-localise with viral RNA in infected HeLa cells. The dimerisation domain (DD) in NP was found to be essential for droplet formation and RNA binding.

The effect of NP on the antiviral response was examined next. The authors studied IFNs, as well as RIG-I and MAVS[7]. Transfection of HEK293 cells with NP completely blocked the expression of IFN-β in response to Sendai virus (SeV) and vesicular stomatitis virus (VSV) infection, an effect requiring the DD. NP expression also inhibited the induction of IFN-β by 5′-pppRNA (which acts via RIG-I) and Poly (I:C) (which like double-stranded RNA is detected by TLR3). Mice infected with genetically engineered recombinant VSV containing NP lacking the DD exhibited lower viral load in lungs, liver and spleen compared to VSV containing intact NP (NP-VSV). This was likely due to NP limiting the induction of IFN-β via its DD domain, promoting viral replication.

The authors then examined the signalling pathway driven by RIG-I, which involves the protein kinases TBK1 and IKKε and the transcription factor they activate, IRF3, which regulates IFN transcription. Each of the kinases becomes phosphorylated and then activated; they phosphorylate IRF3, which then dimerises and translocates to the nucleus[7,8]. NP transfection was shown to reduce phosphorylation of TBK1, IKKε and IRF3, as well as IRF3 dimerisation and nuclear translocation induced by SeV. These inhibitory effects also required the DD.

MAVS is the key signal for activation of TBK1 and IKKε, and this was examined next. MAVS is known to undergo K63-linked polyubiquitination by the E3 ligase TRIM31, leading to its aggregation and recruitment of TRAF3, TRADD and TRAF6 (ref. [9]). Co-immunoprecipitation experiments showed a direct interaction between NP and MAVS. The CARD and proline-rich region of MAVS were required for the interaction. NP was shown to disrupt the interaction between MAVS and RIG-I, as well as the interaction between RIG-I and TRIM31. MAVS was also found to undergo phase separation and form micro-sized droplets, a process which was disrupted by NP. NP also blocked K63-linked

polyubiquitination of MAVS by TRIM31, impairing TBK1 and IKKε recruitment to MAVS.

These findings led the authors to examine whether the infected cells might somehow target NP. They demonstrated that NP undergoes acetylation at K375, a residue next to the DD that is conserved in the NP of the related viruses SARS-CoV and MERS-CoV. Acetylation of this amino acid by the host acetyltransferase CREB-binding protein inhibited NP phase transition and prevented NP from blocking MAVS signalling and the IFN-β response. Infected cells therefore appear to possess a mechanism to limit the inhibitory effect of NP, allowing them to counter the infection.

RNA viruses like SARS-CoV-2 have a high mutation rate generating viral variants[10]. The authors next characterised some of the reported SARS-CoV-2 variants that exhibited mutations in the NP, including K375Q, K375N and K375E. These changes mimic acetylation of K375 and attenuate phase separation and dimerisation of the NP, impeding the ability of these variant NPs to suppress MAVS aggregation and limit IFN-β production. Mice infected with recombinant VSV expressing these NP variants exhibited lower viral loads compared with those infected with NP-VSV, implying that these variants are less virulent because of the alteration in NP.

Finally, the authors designed interfering peptides targeting the regions of the NP DD involved in the interaction with MAVS. One particular peptide, termed NIP-V, was shown to suppress droplet formation of NP and viral load in vitro and in vivo and boosted IFN-β signalling in response to NP-VSV infection. Humanised ACE2 transgenic mice were injected with NIP-V and infected with SARS-CoV-2. The peptide led to an elevated antiviral response, as characterised by increased *Ifnb1* and *Isg56* expression in lungs and lower SARS-CoV-2 genomic RNA levels in spleen and liver. The severe lung damage associated with SARS-CoV-2 infection was also reduced by administration of NIP-V in this model.

This work therefore provides a mechanism for immune evasion by SARS-CoV-2. This could be a major mechanism whereby SARS-CoV-2 limits IFN production, as the virus is known to induce low levels of IFNs both in vitro and in vivo[10]. The DD in the NP protein promotes liquid phase interaction with MAVS, repressing the IFN response in infected cells. Acetylation of NP by host acetyltransferases or its targeting by interfering peptides boosts anti-viral immunity.

There is complex biochemistry at play here, with the prion-like aggregation of MAVS being controlled by the liquid-liquid phase separation of the SARS-CoV-2 NP. A key question therefore is whether this process would be amenable to therapeutic targeting, most likely with small molecules that will mimic the peptide approach, or perhaps with modified peptides. Information on the control of acetylation of NP might provide additional insights which could lend themselves to other therapeutic approaches. Further investigation is required to study whether this effect of NP is a major mechanism of immune evasion by SARS-CoV-2 relative to other proteins in SARS-CoV-2. The work might inform studies into other viruses and how they might target MAVS as an immune evasion strategy. There is also interesting information on some of the variants in SARS-CoV-2, in this case variants that are less virulent, which might be useful in the effort to understand the evolutionary trajectory of SARS-CoV-2. This study reveals an elegant mechanism of immune targeting by SARS-CoV-2 which could well lead to a whole new approach to boosting anti-viral immunity against COVID-19. ❐

Alessia Zotta, Alexander Hooftman and Luke A. J. O'Neill ✉

School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Trinity College, Dublin, Ireland.

✉*e-mail: laoneill@tcd.ie*

Published online: 8 July 2021
https://doi.org/10.1038/s41556-021-00712-y

References

1. Huang, C. et al. *Lancet* **395**, 497–506 (2020).
2. Paces, J., Strizova, Z., Smrz, D. & Cerny, J. *Physiol. Res.* **69**, 379–388 (2020).
3. Kadowaki, N., Antonenko, S., Lau, J. Y. & Liu, Y. J. *J. Exp. Med.* **192**, 219–226 (2000).
4. Wu, A. et al. *Cell Host Microbe* **27**, 325–328 (2020).
5. Jia, H. P. et al. *J. Virol.* **79**, 14614–14621 (2005).
6. Wang, S. et al. *Nat Cell Biol.* https://doi.org/10.1038/s41556-021-00710-0 (2021).
7. Dutta, S., Das, N. & Mukherjee, P. *Front. Microbiol.* **11**, 1990 (2020).
8. Liu, B. et al. *Nat. Immunol.* **18**, 214–224 (2017).
9. Blanco-Melo, D. et al. *Cell* **181**, 1036–1045.e9 (2020).
10. Majumdar, P. & Niyogi, S. *Epidemiol. Infect.* **149**, e110 (2021).

Competing interests

The authors declare no competing interests.