00309

Systematic Analysis of Innate Immune Antagonism of SARS-CoV-2 and its Evolution

Lennart Koepke[1], Rayhane Nchioua[1], Manuel Hayn[1], Maximilian Hirschenberger[1], Annika Schundner[2], Fabian Zech[1], Susanne Klute[1], Steffen Stenger[1], Karl-Klaus Conzelmann[3], Jan Münch[1], Dorota Kmiec[1], Manfred Frick[2], Frank Kirchhoff[1], Konstantin Maria Johannes Sparrer[1]

[1]Ulm University Medical Center, Ulm, Germany, [2]Ulm University, Ulm, Germany, [3]Ludwig-Maximilians-Universität München, Munich, Germany

BACKGROUND

The innate immune system is a powerful anti-viral defense mechanism, which includes the interferon (IFN) system and autophagy. Thus, successful pathogens like SARS-CoV-2 need to counteract or evade these defense pathways to establish an infection. However, due to ongoing, worldwide spread in the human population SARS-CoV-2 is evolving and in the meantime five variants with selection advantages (variants of concern, VOC) have emerged.

Key question: Which SARS-CoV-2 proteins antagonize the innate immune system and do these antagonists evolve in VOCs?

METHODS

- Luciferase reporter based assays to evaluate innate immune activation.
- FACS based assay that quantifies changes in autophagosome numbers.
- Western blot analysis of markers related to innate immune pathways.
- Infection assays using SARS-CoV-2 isolates, and immortalized cell lines and primary cells.

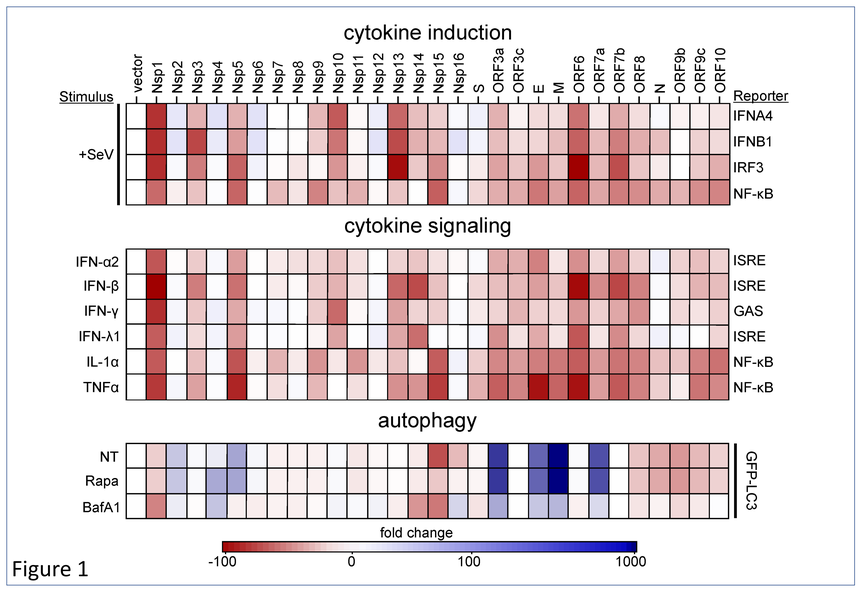


Figure 1

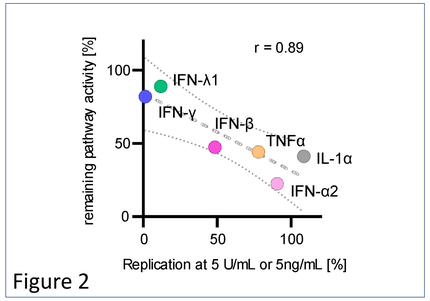


Figure 2

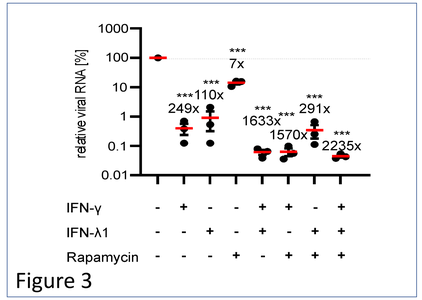
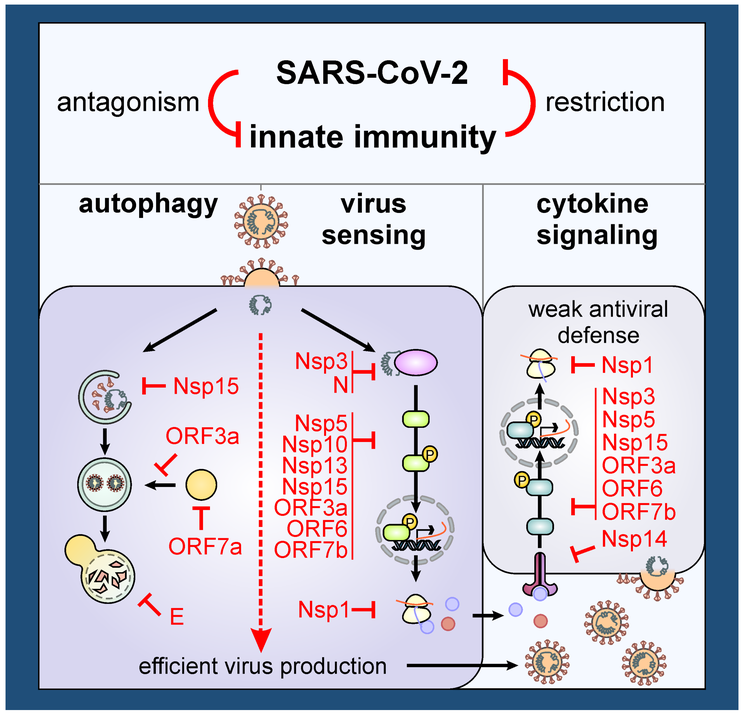


Figure 3



RESULTS

We systematically analyzed the impact of SARS-CoV-2 encoded proteins on induction of IFNs and other pro-inflammatory cytokines, IFN signaling, and autophagy. More than 50% of the proteins encoded by SARS-CoV-2 antagonize innate immunity (Fig. 1). We described the molecular mechanism of Nsp1 (shutdown of host translation) and Nsp14 (degradation of IFNAR1) as potent antagonists of the IFN system, and ORF3a (inhibition of autophagosome-lysosome fusion) and ORF7a (Inhibition of lysosome acidification) as inhibitors of autophagy (Fig. 1). The remaining pathway activity and the sensitivity towards the tested cytokines show an inverse correlation (Fig. 2). Of note, SARS-CoV-2 remains sensitive towards IFN-λ1 and IFN-γ. Combined treatment with both IFNs inhibits SARS-CoV-2 synergistically (Fig. 3). This effect is further enhanced by co-treatment with Rapamycin, which induces higher levels of autophagy. Overall, the IFN/autophagy antagonism of SARS-CoV-2 proteins is shared with its bat relative RaTG13-CoV and SARS-CoV-1 (Fig. 4). Since the end of 2021, 5 VOCs have emerged (Fig. 5). The delta VOC of SARS-CoV-2 remained overall sensitive towards inhibition by IFNs, but the alpha, beta and gamma variants are less sensitive towards IFN-λ1 (Fig. 6).

CONCLUSIONS

- SARS-CoV-2 utilizes synergistic activities to counteract innate immunity.
- SARS-CoV-2 is particularly sensitive to type II and III IFN treatment.
- The functions of main innate immune antagonists are conserved among SARS-CoV-2, SARS-CoV-1 and RaTG13-CoV.
- VOCs with decreased sensitivity towards innate immunity have emerged.
- The Delta VOC is still sensitive to inhibition by IFNs.

ADDITIONAL KEY INFORMATION

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Presenting author contact:

Lennart Koepke
Institute of Molecular Virology,
Ulm University Medical Center
Lennart.Koepke@uni-ulm.de

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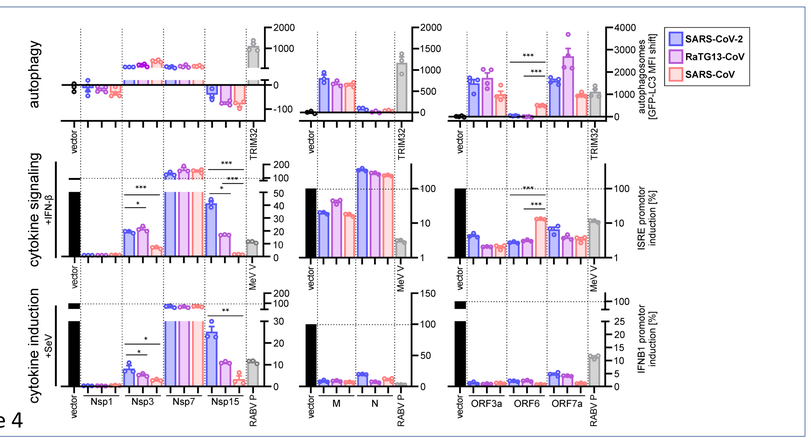


Figure 4

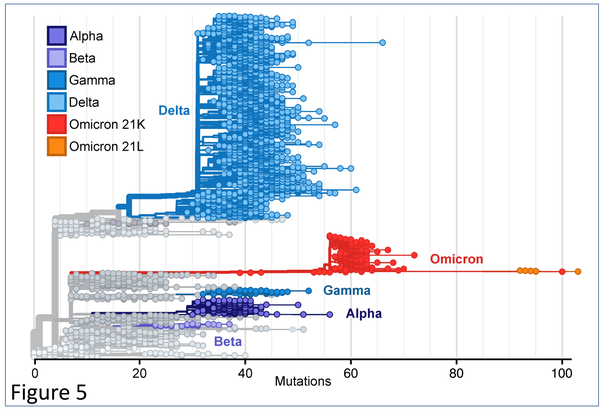


Figure 5

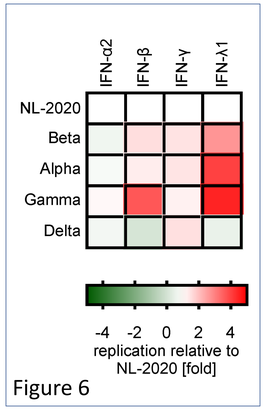


Figure 6