Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Cell Host & Microbe

CellPress

Commentary

Interferons in coronavirus pathogenesis: The good, the bad, and the ugly

Rudragouda Channappanavar[1,*]
[1]Department of Veterinary Pathobiology, College of Veterinary Medicine, Oklahoma State University, Stillwater, OK 74078, USA
*Correspondence: rchanna@okstate.edu
https://doi.org/10.1016/j.chom.2022.03.022

Interferons (IFNs) are a key component of the innate antiviral immunity and are generally implicated in protective host immune responses. Here, I discuss the central role of IFNs during different coronavirus (CoV) infections, the importance of timing of the IFN response, and how emerging human coronaviruses subvert antiviral IFN response to cause severe disease.

Since the time I started my postdoctoral work with Dr. Stanley Perlman in 2012, I have believed that exuberant host innate immune response is one of the critical factors facilitating severe disease observed in humans infected with emerging pathogenic human coronaviruses (hCoVs). My contention, without much coronavirus (CoV) background knowledge at the time, was quite simple. As naive hosts, humans have not seen emerging hCoVs and do not know how to fight and defend against the new enemy, and their immune system is blindly overreacting, causing inflammation and tissue damage in the process. With 10 years of research experience in the field of coronavirus immunology and pathology, a little more understanding of the hCoV biology, and having worked with several animal models, I believe that a virus-induced suboptimal antiviral but a simultaneously excessive inflammatory response is one of the major causes of fatal pneumonia observed following hCoV infections. But I now recognize that such a response, which Stanley and I quoted as "dysregulated immunity," is induced by the hCoVs that replicate to high titers very early and possess multiple highly sophisticated interferon (IFN) and interferon-stimulated gene (ISG) antagonizing proteins in their arsenal.

The good

For several years before the emergence of severe acute respiratory syndrome CoV-2 (SARS-CoV-2), long-time coronavirologists had identified numerous structural and non-structural proteins within several members of the coronavirinae sub-family that efficiently antagonized type I and type III interferon (IFN-I and IFN-IIIs) and ISG responses. The majority of these studies used various strains of mouse hepatitis virus (MHV, a mouse coronavirus) that caused strain-specific illness in mice and were used extensively to study CoV replication, host-virus interactions, and antiviral response. Results from in-vitro studies showed that MHV reduced levels of IFNs in mouse fibroblast L2 cell lines compared to other viruses such as Sendai virus and Newcastle disease virus, and suppression of MHV required high concentrations of recombinant IFNs, suggesting a robust anti-IFN antiviral mechanism elicited by CoVs (Roth-Cross et al., 2007). In contrast, lack of type I IFN receptor (IFNAR) signaling resulted in dramatically high virus replication *in vitro*, and particularly *in vivo*, such that MHV spread to multiple organs and the majority of the IFNAR mice died within 3 to 4 days post-MHV infection (Cervantes-Barragan et al., 2007). These results showed that IFNAR signaling is critical to suppress initial virus replication and prevent the systemic spread of MHV. Our unpublished studies using MHV-1 (a pneumotropic strain of MHV) also confirmed these findings (R.C., unpublished data). Collectively, these results demonstrate "the good" side of IFNs during CoV infections.

The bad

During my graduate studies, I studied the role of programmed death 1 signaling in impaired T cell responses to herpes virus infections in the aged host. Therefore, my first project in Stanley's lab was to examine the role of memory CD8 T cells in protecting highly susceptible middle-aged mice from lethal SARS-CoV infection. While working with SARS-CoV on this project and from the results of other lab members, I had observed that mice infected with a lethal dose of SARS-CoV succumbed to infection beginning on day 3 or 4 post-infection, suggesting a pivotal role for innate immunity in protective versus pathogenic immunity. My veterinary medicine background, a master's degree in veterinary pathology, and immunology training as a PhD student helped me to better understand CoV immunology and pathobiology. Studies following the SARS epidemic described a delayed IFN but robust inflammatory genes expression in SARS-CoV-infected macrophages (Cheung et al., 2005; Law et al., 2005). Similarly, human airway and lung epithelial cells showed robust induction of IFNs upon influenza virus infection, while this response was significantly delayed in cells infected with SARS-CoV and Middle East respiratory syndrome-CoV (MERS-CoV) (Menachery et al., 2014). A further search in literature for correlates of severe disease showed elevated IFN signatures in patients with severe SARS, MERS, and influenza. Based on these results, I hypothesized that a delayed IFN response fails to suppress hCoV replication, thus causing severe disease. Although not convinced initially, Stanley supported me to test the hypothesis and provided enough freedom with key inputs (one of the great traits of Stanley as a mentor) to test other related ideas as well. However, we knew from earlier mouse-adapted SARS-CoV studies that young 6–10-week-old wild-type (WT) and IFNAR mice on C57BL/6 and 129S backgrounds were completely resistant to developing the severe disease (Frieman et al., 2010), whereas age-matched WT BALB/c mice

showed severe respiratory illness. Fortunately, Dr. Wendy Maury in the Department of Microbiology at the University of Iowa had IFNAR mice on a BALB/c background, developed by Dr. Joan Durbin at the Rutgers New Jersey Medical School. Since young BALB/c mice developed severe SARS, we used these mice to evaluate the role of IFNAR signaling in SARS-CoV pathogenesis. To our surprise, but in agreement with our hypothesis, we found that IFNAR mice on BALB/c background were resistant to developing severe SARS compared to WT BALB/c mice. A detailed kinetic analysis of IFN response and virus replication showed that a delayed type I IFN signaling relative to peak virus titers correlated with lung pathology, which was in part mediated by $CCR2^{hi}$ inflammatory monocytes (Channappanavar et al., 2016). Further, the exogenous rIFN-β administration early (6 h) after infection completely protected mice, while a delayed intranasal rIFN-β instillation failed to protect mice from lethal SARS. Additional follow-up studies using a mouse-adapted MERS-CoV showed that, in contrast to SARS-CoV infection, IFNAR signaling protected mice from lethal MERS. Interestingly, however, IFNAR signaling was not required for suppressing early MERS-CoV replication, but it was critical for virus clearance (Channappanavar et al., 2019). Therefore, the pathogenic role of endogenous IFNs and the inability of endogenous IFN-Is to suppress early hCoV replication is depicted as "the bad" host IFN response.

And the ugly

In our manuscript submitted to the *Cell Host & Microbe*, we had termed pathogenic IFN-I activity as the "dysregulated interferon" response. However, when we received the first round of comments, reviewer 1 asked us what we meant by "dysregulated immunity" and whether we were referring to delayed IFN response. We were planning to examine the timing of the IFN response, and the comments from reviewer 1 reinforced the importance of investigating the timing of IFN-I response in hCoV outcome. By this time, we had learned that unlike during SARS-CoV infection, IFN-I signaling protected mice from MERS-CoV infection. We thought it would be interesting to examine the effect of IFN-I on disease outcome when given early and at or after the peak MERS-CoV replication. In a series of experiments conducted and published later in the *Journal of Clinical Investigation*, we showed that timing of IFN-I response relative to virus infection is a critical determinant of the disease outcome. While early IFN-I response or therapy was protective, delayed IFN-I response/therapy was detrimental in mice infected with a sub-lethal dose of MERS-CoV. We also showed that the detrimental effect of delayed recombinant IFN-β therapy was associated with robust recruitment and inflammatory activity of monocyte-macrophages (Channappanavar et al., 2019). Since monocyte-macrophages do not express the IFN-lambda receptor (IFNλR), we then in a series of another set of experiments examined whether delayed recombinant IFN-λ treatment protects mice from lethal disease. To our surprise, it didn't, and we later learned from recent elegant studies that the delayed IFN-λ activity induced lung epithelial death and impaired tissue repair mechanisms (Broggi et al., 2020; Major et al., 2020), likely causing fatal disease. Our unpublished studies using mouse-adapted SARS-CoV-2 also suggested IFN timing-dependent disease outcomes in mouse models (R.C., unpublished data). Several independent studies, including human clinical trials, support our results that showed the protective role of early IFN stimulation, while delayed IFN activity is detrimental. I believe the detrimental effects of late IFN-I/IFN-III treatment in an otherwise sublethal infection shows "the ugly" side of IFNs.

Thus far, we collectively learned that:(1) hCoV replication to high titers very early after infection and robust hCoV-mediated IFN/ISG antagonism lead to a delayed anti-viral IFN/ISG response causing fatal pneumonia; (2) the timing, duration, and level of IFN responses play key roles in determining the disease outcome; and (3) CoVs are differentially susceptible to IFNs, and the role of IFNs in CoV pathogenesis is virus, cell tropism, and to some degree animal model specific.

After proposing the concept of "timing IFN-I response in the outcome of hCoV infection," several recent studies identified an association of elevated IFN and ISG signature in patients with severe COVID-19 (Sposito et al., 2021). In agreement with our studies, clinical trials in humans also showed that late recombinant IFN-I administration caused severe disease while an early treatment provided significant protection (Kalil et al., 2021).

Additional key determinants of the differential effect of IFN activity in CoV pathogenesis

Rapid replication of pathogenic hCoVs to high titers and their relative resistance to IFNs/ISGs

Several elegant studies and reviews before and after the COVID-19 pandemic showed an incredible ability of multiple structural and non-structural coronavirus proteins to antagonize IFN/ISG responses (Frieman et al., 2008; Xia et al., 2020). In animal models, pathogenic hCoVs replicate high titers very early (24–48 h post-infection) after infection compared to other viruses such as influenza A virus (IAV) and respiratory syncytial virus (RSV) that show peak titers 3 to 5 days post-infection. In the latter studies, IFNs and ISGs induced early relative to virus replication (Goritzka et al., 2015). Although it is unclear whether pathogenic and seasonal CoVs differentially replicate in the airway and alveolar epithelial cells, some studies suggest that seasonal hCoVs induce robust interferon response in macrophages (Cheung et al., 2005) and are likely more sensitive to IFN/ISG mediated antiviral immunity compared to pathogenic hCoVs such as MERS-CoV (Dijkman et al., 2021).

Pathogenic hCoVs suppress IFN/ISG activity while inducing a robust inflammatory response

Pathogenic hCoVs efficiently suppress antiviral IFN/ISG responses in the airway and alveolar epithelial cells, whereas they induce poor IFN/ISG response in myeloid cells such as macrophages. Conversely, hCoVs induce robust inflammatory cytokine and chemokine responses in epithelial cells, monocytes, and macrophages (Blanco-Melo et al., 2020; Cheung et al., 2005; Law et al., 2005). These results demonstrate a biased host response that is likely instrumental in causing excessive inflammation and lung pathology during pathogenic hCoV infections.

Differential CoV cell tropism in host response to IFN/ISG mediated antiviral immunity

A careful analysis of existing literature shows unique IFN/ISG responses during

CoV and other RNA virus infections. As mentioned above, IFN-I is extremely critical to suppress early MHV replication and protect the host. Mice that lack IFNAR signaling succumb to MHV infection within 2 to 4 days post-infection, which correlates with a several log increases in virus titers and systemic viral spread compared to WT mice. Such a host response is likely due to the ability of myeloid cells, which are efficiently infected with MHV, to restrict infection and prevent systemic spread. Similarly, in mice where RNA viruses efficiently infect myeloid cells (such as vesicular stomatitis virus, flaviviruses, and alphaviruses), IFNAR is critical to inhibit early virus replication and prevent mortality. Interestingly, in these models, the lack of IFNAR signaling also leads to exaggerated inflammatory cytokine and chemokine activity, suggesting that IFNAR is also critical to moderate virus-induced inflammation. In contrast, hCoVs (SARS-CoV, MERS-CoV, and SARS-CoV-2) efficiently replicate in epithelial cells but only abortively so in hematopoietic cells. In mice infected with the mouse-adapted versions of pathogenic hCoVs, IFNAR signaling is either not required, or if required, it has a marginal effect on suppressing initial virus replication and a rather critical role in virus clearance. Similar observations are also made following infection with other viruses such as IAV and RSV. Collectively, these studies highlight that the role of IFN-I/III signaling in either suppressing initial virus replication and/or in virus clearance, depends on cell tropism of the virus.

The instrumental role of *Cell Host & Microbe* paper in shaping my research career

Our *Cell Host & Microbe* paper (Channappanavar et al., 2016) was a breakthrough paper that gave me identity among the coronavirus researchers and other viral immunologists. This paper, to my knowledge, was also the first to highlight the importance of the timing of IFN response in the outcome of acute respiratory virus infections. This work was also instrumental in providing me opportunities to interview for a faculty position and secure a position. Comments from the reviewers and the editor of the submitted manuscript, Dr. Ella Hinson, were critical to strengthen our conclusions and build on the story. So far, this manuscript has garnered >1,200 citations and continues to be one of the highly cited papers published in *Cell Host & Microbe.*

Key questions and future research direction

As an independent investigator, my laboratory—independently and in collaboration with Drs. Stanley Perlman (University of Iowa), Anthony Fehr (University of Kansas), and Xufang Deng (Oklahoma State University) laboratories—continues to better understand the role and the mechanism by which IFNs induce protective and pathogenic immunity. My laboratory is particularly interested in identifying a specific set of ISGs that provides protection during early IFN therapy and that facilitate lung pathology when administered at the later stages of infection. We are also working to identify critical determinants of hCoVs and the key cell sensors and downstream signaling molecules that facilitate hCoV-induced lethal lung inflammation and pathology. One of the main reasons for focusing on hCoV induced inflammation is that, despite phenomenal progress made toward identification, development, and evaluation of several antiviral agents, the antivirals provide incomplete to minimal protection when given during later stages of infection, suggesting that virus-induced inflammation and tissue damage are the key factors that drive lung pathology and fatal pneumonia. However, the molecular basis for the exaggerated inflammation and severe disease caused by hCoVs and other emerging respiratory viruses is incompletely understood. I believe that identifying key pathways facilitating excessive inflammation is critical to developing novel virus-specific anti-inflammatory therapies to protect patients with severe illness.

ACKNOWLEDGMENTS

I would like to thank Dr. Stanley Perlman for the opportunity to work in his lab as a postdoctoral scholar and then as research scientist. Thanks to Dr. Anthony Fehr for assistance with the *Cell Host & Microbe* study and for continuous collaboration. I would also like to recognize the contributions of several long-time coronavirologists and other virologists and viral immunologists whose work I couldn't cite because of space and citation limitation. Research in my lab is funded by OSU-College of Veterinary Medicine start-up fund and Oklahoma Center for Respiratory and Infectious Disease (OCRID) Centers of Biomedical Research Excellence (COBRE) grant NIH P20GM103648.

DECLARATION OF INTERESTS

The author declares no competing interests.

REFERENCES

Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.C., Uhl, S., Hoagland, D., Moller, R., Jordan, T.X., Oishi, K., Panis, M., Sachs, D., et al. (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell *181*, 1036–1045.e9.

Broggi, A., Ghosh, S., Sposito, B., Spreafico, R., Balzarini, F., Lo Cascio, A., Clementi, N., De Santis, M., Mancini, N., Granucci, F., and Zanoni, I. (2020). Type III interferons disrupt the lung epithelial barrier upon viral recognition. Science *369*, 706–712.

Cervantes-Barragan, L., Züst, R., Weber, F., Spiegel, M., Lang, K.S., Akira, S., Thiel, V., and Ludewig, B. (2007). Control of coronavirus infection through plasmacytoid dendritic-cell-derived type I interferon. Blood *109*, 1131–1137. https://doi.org/10.1182/blood-2006-05-023770.

Channappanavar, R., Fehr, A.R., Vijay, R., Mack, M., Zhao, J., Meyerholz, D.K., and Perlman, S. (2016). Dysregulated Type I Interferon and Inflammatory Monocyte-Macrophage Responses Cause Lethal Pneumonia in SARS-CoV-Infected Mice. Cell Host Microbe *19*, 181–193.

Channappanavar, R., Fehr, A.R., Zheng, J., Wohlford-Lenane, C., Abrahante, J.E., Mack, M., Sompallae, R., McCray, P.B., Jr., Meyerholz, D.K., and Perlman, S. (2019). IFN-I response timing relative to virus replication determines MERS coronavirus infection outcomes. J. Clin. Invest. *129*, 3625–3639.

Cheung, C.Y., Poon, L.L., Ng, I.H., Luk, W., Sia, S.F., Wu, M.H., Chan, K.H., Yuen, K.Y., Gordon, S., Guan, Y., and Peiris, J.S. (2005). Cytokine responses in severe acute respiratory syndrome coronavirus-infected macrophages in vitro: possible relevance to pathogenesis. J. Virol. *79*, 7819–7826.

Dijkman, R., Selvaraj, M., Gad, H.H., Hartmann, R., More, S., Perlman, S., Thiel, V., and Channappanavar, R. (2021). Effective interferon (IFN)-λ treatment regimen to control lethal MERS-CoV infection in mice. Preprint at bioRxiv. https://doi.org/10.1101/2021.05.26.445685.

Frieman, M., Heise, M., and Baric, R. (2008). SARS coronavirus and innate immunity. Virus Res. *133*, 101–112.

Frieman, M.B., Chen, J., Morrison, T.E., Whitmore, A., Funkhouser, W., Ward, J.M., Lamirande, E.W., Roberts, A., Heise, M., Subbarao, K., and Baric, R.S. (2010). SARS-CoV pathogenesis is regulated by a STAT1 dependent but a type I, II and III interferon receptor independent mechanism. PLoS Pathog. *6*, e1000849.

Goritzka, M., Makris, S., Kausar, F., Durant, L.R., Pereira, C., Kumagai, Y., Culley, F.J., Mack, M., Akira, S., and Johansson, C. (2015). Alveolar macrophage-derived type I interferons orchestrate innate immunity to RSV through recruitment

of antiviral monocytes. J. Exp. Med. *212*, 699–714.

Kalil, A.C., Mehta, A.K., Patterson, T.F., Erdmann, N., Gomez, C.A., Jain, M.K., Wolfe, C.R., Ruiz-Palacios, G.M., Kline, S., Regalado Pineda, J., et al.; ACTT-3 study group members (2021). Efficacy of interferon beta-1a plus remdesivir compared with remdesivir alone in hospitalised adults with COVID-19: a double-bind, randomised, placebo-controlled, phase 3 trial. Lancet Respir. Med. *9*, 1365–1376.

Law, H.K., Cheung, C.Y., Ng, H.Y., Sia, S.F., Chan, Y.O., Luk, W., Nicholls, J.M., Peiris, J.S., and Lau, Y.L. (2005). Chemokine up-regulation in SARS-coronavirus-infected, monocyte-derived human dendritic cells. Blood *106*, 2366–2374.

Major, J., Crotta, S., Llorian, M., McCabe, T.M., Gad, H.H., Priestnall, S.L., Hartmann, R., and Wack, A. (2020). Type I and III interferons disrupt lung epithelial repair during recovery from viral infection. Science *369*, 712–717.

Menachery, V.D., Eisfeld, A.J., Schäfer, A., Josset, L., Sims, A.C., Proll, S., Fan, S., Li, C., Neumann, G., Tilton, S.C., et al. (2014). Pathogenic influenza viruses and coronaviruses utilize similar and contrasting approaches to control interferon-stimulated gene responses. MBio *5*, e01174–14.

Roth-Cross, J.K., Martínez-Sobrido, L., Scott, E.P., García-Sastre, A., and Weiss, S.R. (2007). Inhibition of the alpha/beta interferon response by mouse hepatitis virus at multiple levels. J. Virol. *81*, 7189–7199.

Sposito, B., Broggi, A., Pandolfi, L., Crotta, S., Ferrarese, R., Sisti, S., Clementi, N., Ambrosi, A., Liu, E., Frangipane, V., et al. (2021). Severity of SARS-CoV-2 infection as a function of the interferon landscape across the respiratory tract of COVID-19 patients. Preprint at bioRxiv. https://doi.org/10.1101/2021.03.30.437173.

Xia, H., Cao, Z., Xie, X., Zhang, X., Chen, J.Y., Wang, H., Menachery, V.D., Rajsbaum, R., and Shi, P.Y. (2020). Evasion of Type I Interferon by SARS-CoV-2. Cell reports *33*, 108234. https://doi.org/10.1016/j.celrep.2020.108234.