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INSIGHTS

An ace model for SARS-CoV-2 infection

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Developing effective in vivo models for SARS-CoV-2 infection is crucial for mechanistic studies of COVID-19 disease progression. In this issue of *JEM*, Israelow et al. (https://doi.org/10.1084/jem.20201241) generate a model that supports SARS-CoV-2 infection in mice, which they use to characterize type I IFN–driven pulmonary inflammation.

The ongoing COVID-19 pandemic has spurred research at an unprecedented speed. In vivo infection models are indispensable to fully understand the complex interactions of SARS-CoV-2 with different lung cell types, the immune system, and other affected organs. However, evidence emerging from initial studies in animals has presented significant problems. Ferret infection with SARS-CoV-2 causes mild disease (Blanco-Melo et al., 2020); nonhuman primates can be infected, but there are important ethical considerations and logistical limitations; hamsters appear to develop lung disease similar to humans, but the available tools are limited (Boudewijns et al., 2020); and the mouse orthologue of human angiotensin-converting enzyme 2 (hACE2) does not support SARS-CoV-2 infection (Letko et al., 2020; Hoffmann et al., 2020). Therefore, hACE2 needs to be introduced into mice, unless a mouse-adapted virus is used. Mice transgenic for hACE2 (expressed under a keratin 18 promoter; K18-hACE2 Tg mice) exist, but they are scarcely available and are limited so far to one genetic background.

In this issue of *JEM*, Israelow et al. (2020) generated a model for SARS-CoV-2 infection in mice by delivery of an hACE2 transgene by adeno-associated virus (AAV) infection. Adenovirus-based technology has previously been used for vaccination and gene therapy, as well as the generation of mouse models to study human disease, including Middle East respiratory syndrome coronavirus infection (Zhao et al., 2014). The vector used in this study, AAV9, efficiently transduces alveolar epithelial cells owing to its tropism for β-galactose links to terminal sialic acids (Bell et al., 2011), which are dispersed widely on the surface of the respiratory epithelium. Mice were transduced intratracheally with AAV9-encoding hACE2 and infected 2 wk later with SARS-CoV-2. AAV9-hACE2–transfected mice support SARS-CoV-2 replication, with viral titers peaking, at the latest, on day 2 after infection. While infection induced no weight loss or mortality, it triggered an inflammatory cell infiltrate with increases in lung monocytes and activated lymphocytes, a lung cytokine and IFN signature, and humoral immunity at later stages.

There are several biological caveats to consider when using such a model: 1) baseline hACE2 expression may differ mouse-to-mouse, owing to potentially varying degrees of transduction efficiency; 2) the adenovirus vector may itself induce inflammation or an IFN response; 3) transduced hACE2 lacks physiological regulation, for instance, in response to infection or cellular stress. However, the latter disadvantage also applies to K18-hACE2 Tg mice. A technical caveat involves the use of tracheotomy for intratracheal administration of a large vector volume (50 µl). In addition to being a highly invasive procedure, direct administration to the alveoli potentially means transduction of the upper respiratory epithelium is bypassed, and therefore this model would lack the capacity for SARS-CoV-2 infection in multiciliated airway cells, as is the case in human infection (Qi et al., 2020).



Insights from Jack Major and Andreas Wack.

The biggest advantage of this approach is its versatility. Mice of any genetic background can be transduced by this vector, thus accelerating research into COVID-19 disease mechanisms. The Iwasaki team takes advantage of this, using IFNAR1 KO and IRF3/7 double KO (DKO) strains to explore IFN biology following SARS-CoV-2 infection. Type I (IFN-α/β) and III (IFN-λ) IFNs are important antiviral cytokines, yet are also associated with pathogenic effects. Israelow et al. (2020) show that SARS-CoV-2–infected IFNAR1 KO mice, lacking IFN-α/β signaling, have reduced inflammation and lymphocyte activation, which would support the notion that IFN-α/β can drive pathogenic inflammation in respiratory viral infections (Davidson et al., 2014; Channappanavar et al., 2016). The observed improvement in IFNAR1 KO mice is in line

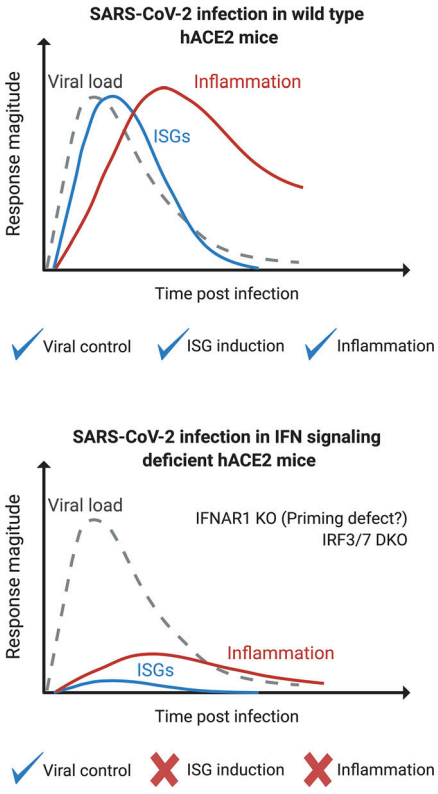
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IFN-independent viral control in hACE2-transduced mice. Viral titers peak on day 2 after SARS-CoV-2 infection in hACE2-expressing wild type mice, triggering IFNAR1-dependent expression of ISGs and pulmonary inflammation characterized by immune cell recruitment and activation. Mice deficient for IFN signaling (either by IRF3 and IRF7 deficiency, or a potential IFN-priming defect in IFNAR1 KO mice), therefore lacking ISG induction, have reduced inflammation, and surprisingly, a comparable viral burden compared to wild type mice. This may occur for a number of reasons, including a possible type I and III IFN–independent mechanism of viral control or efficient viral mechanisms of IFN antagonism. Created with BioRender.com.

with Sun et al. (2020), who also find a trend toward improved recovery and reduced pulmonary inflammation in absence of IFN-α/β–induced signaling.

The assumption would be that in the absence of type I IFN signaling, viral replication would be controlled by IFN-λ. This would suggest higher virus levels in IRF3/7 DKO (Israelow et al., 2020) and STAT1 KO (Sun et al., 2020) mice, which lack the pathways required for type I and III IFNs production and signaling, respectively. However, these mice display similar levels of viral control compared with wild type and IFNAR KO mice. These findings are surprising, suggesting that SARS-CoV-2 antiviral immunity operates independently to type I and III IFN induction of IFN-stimulated genes (ISGs). It may also indicate potent IFN pathway antagonism by virally encoded proteins (Blanco-Melo et al., 2020). These data are also in contrast to observations following SARS-CoV-2 infection in hamsters. STAT2-deficient hamsters, which presumably lack both type I and III IFN signaling, had increased viral titers in the lung and in distal tissue sites (Boudewijns et al., 2020). The mouse data potentially indicate a role for IRF3/7-independent type II IFN (IFN-γ)–mediated antiviral protection (Carlin et al., 2017); however, Sun et al. (2020) show comparable viral control in WT and IFN-γ KO mice, and STAT1 KO mice also have impaired IFN-γ signaling.

How important IFN-λ signals are in substitute of IFN-α/β in the IFNAR1 KO mice is an open question, as ISG induction in IFNAR1 KOs appears completely ablated, arguing against an important role for IFN-λ (Israelow et al., 2020). One possibility is that expression of ISGs induced by IFN-λ is diluted in whole organ analysis, as the effects of IFN-λ are largely restricted to the lung epithelium. It would also be interesting to quantify IFN-λ levels in IFNAR1 KO mice, or measure the effects of temporary αIFNAR antibody blockade on ISG induction, as priming defects in IFNAR1 KO mice significantly dampen production of IFN-λ following respiratory virus infection (Major et al., 2020). Hassan et al. (2020) attempted αIFNAR treatment in their hACE2 adenovirus model, but the situation is complex, as they also block IFNAR signaling to improve adenovector transduction before SARS-CoV-2 infection. However, Hassan et al. (2020) show virus load is not affected by αIFNAR treatment, suggesting a potential role of IFN-λ, or alternatively the complete independence of virus control from IFNs. The latter option would be in contradiction with findings in primary human airway epithelial cell cultures, which show that SARS-CoV-2 is sensitive to IFN treatment (Lokugamage et al., 2020), unless the virus is able to suppress ISG expression in all infected cells.

Given the prolonged expression of hACE2 following AAV9 transduction by Israelow et al. (2020), the time delay between transduction and infection is 2 wk, rather than the 5 d reported in other models (Sun et al., 2020; Hassan et al., 2020). This is advantageous in the avoidance of residual activity of antiviral and inflammatory programs induced by vector transduction, which may explain contradicting results between the studies. Could there be a residual IFN signature after adenovirus transduction in the study by Sun et al. (2020) that contributes to virus control? This would be absent in IFNAR1 and STAT1 KO mice, potentially explaining the early differences in viral load. In contrast, any potential ISG response to AAV transduction in Israelow et al. (2020) would likely dissipate in the 2 wk before SARS-CoV-2 infection, meaning there would be no baseline protection in WT over KO mice, and indeed, there was no observed difference in viral control.

As Israelow et al. (2020) show, the generation of in vivo SARS-CoV-2 infection mouse models provides us with an invaluable tool for characterizing aspects of COVID-19 immunity, which has a significant clinical impact. Collective evidence emerging from patient cohort sample analyses reveals a complex picture regarding the role of IFNs in COVID-19 disease progression, with significant questions remaining. A comprehensive integrated immune analysis of whole blood from 50 COVID-19 patients by Hadjadj et al. (2020) identified a significantly dampened type I IFN and ISG signature in individuals suffering severe disease, as compared with mild cases. This is in contrast to an additional recent publication by the Iwasaki team, which studied COVID-19 immunological correlates in patient blood over time (Lucas et al., 2020). In that study, they found a correlation of increased proinflammatory cytokines and type I and III IFN levels in cases of severe disease. They identified a common core cytokine and IFN signature in both moderate and severe disease groups early during infection. It is important to note that this was observed in the first 10 d after disease onset, similar to the timings of sample collection in the Hadjadj et al. (2020) study, which had a median sample collection time of 10 d after disease onset. Interesting and important observations additionally came from comparisons in later disease phases. While cytokine and IFN markers declined in moderate patients, they persisted in severe cases, including type I and III IFN (Lucas et al., 2020). Collectively, these studies

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suggest that impaired early antiviral immunity may result in viral persistence and subsequent prolonged IFN production in severe COVID-19 cases. This is supported by a recent analysis of COVID-19 IFN responses, which revealed delayed type I and III IFN induction in a subset of COVID-19 patients compared with those of influenza patients (Galani et al., 2020). Thus, in COVID-19, a delayed IFN presence may skew a potentially protective antiviral response toward a pathogenic proinflammatory response. These clinical studies also reveal a heterogeneity in the manifestation of individual COVID-19 responses, highlighting the need for mechanistic immune studies using in vivo models.

The important study by Israelow et al. (2020) falls into a lively debate about the benefits and potential harmful effects of IFN-based host-directed COVID-19 therapeutics. A problem for clinicians is pinpointing a time window, or identifying biomarkers for the disease stage in which IFN administration is beneficial. How important timing is for IFN therapeutic effects was evidenced in a retrospective study of 446 patients with COVID-19, which reported reduced mortality following early IFN-α administration, whereas late use was associated with delayed recovery and increased mortality (Wang et al., 2020). Combined evidence from further clinical and in vivo studies will be invaluable to determine the effective application of antiviral immune therapies.

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