RESEARCH HIGHLIGHT

SARS-CoV-2 infection in K18-ACE2 transgenic mice replicates human pulmonary disease in COVID-19

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Over the last several months, a global pandemic has developed due to the emergence of the novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is capable of infecting humans. The lack of preexisting immunity to SARS-CoV-2 in humans has prompted the scientific community to strive to understand the mechanisms underlying SARS-CoV-2 pathogenesis and to test new therapeutic agents and vaccines for the clinical management of coronavirus disease 2019 (COVID-19). In these efforts, the development of appropriate animal models of the disease is one of the most valuable strategies.

Unfortunately, although several animal models of COVID-19 have been investigated, only a few (hamsters, ferrets, minks, cats, and nonhuman primates) have been found to be susceptible to the disease.¹ In all cases, the critical issue appears to be the specificity of the viral tropism for human angiotensin-converting enzyme 2 (hACE2), the key factor in the entry of the virus into human cells. Mice are commonly used to study emerging viruses but do not provide a suitable model for SARS-CoV-2 infection because mouse ACE2 (mACE2) is not an appropriate receptor for SARS-CoV-2 and does not facilitate the entry of the virus into the cells. Therefore, the use of mouse models for COVID-19 is limited to genetically modified mice (or, alternatively, engineered viruses that effectively bind mACE2).

To date, several genetically modified mouse models have been developed, in which hACE2 is permanently expressed under the regulation of either global or specific promoters.^{2–4} Winkler et al.⁵ recently reported the use of transgenic mice expressing hACE2 driven by the cytokeratin-18 (K18) gene promoter (K18-hACE2), a model that was originally developed for the severe acute respiratory syndrome (SARS),³ to investigate the effects of SARS-CoV-2 infection (Fig. 1). The study showed the presence of high levels of viral infection in the lungs following intranasal inoculation with 1×10^2 plaque-forming units (p.f.u.) in both male and female mice. These lung infections shared many features with severe COVID-19 in humans, including the presence of an inflammatory response characterized by high levels of proinflammatory cytokines and chemokines; a cellular infiltrate composed mainly of monocytes, neutrophils, and T cells; and perivascular inflammation. Since mild pulmonary disease is the characteristic result of SARS-CoV-2 infection in naturally susceptible animals, the severity of disease observed following SARS-CoV-2 infection in K18-hACE2 mice provides a useful model in which to investigate pulmonary disease and test antiviral countermeasures in this species.

However, there are also some caveats to this model that are mainly related to the fact that, despite the fact that viral spread to other organs such as heart, spleen, and kidney has been detected, extrapulmonary manifestations are usually mild in these animals and, therefore, do not completely mimic the severe clinical symptoms observed in humans. Thus, evidence of thrombosis and vasculitis is present in K18-hACE2 mice with severe pneumonia, but there is no clear proof of widespread thrombosis with microangiopathy and coagulopathy, as have been observed in many severe cases in humans.⁶ Similarly, although some mice develop anosmia soon after infection, neurological involvement is less severe in these mice than in humans.⁷ These differences between human and mouse findings likely reflect the fact that the expression of hACE2 is driven by a nonnative promotor, resulting in a nonphysiological tissue distribution pattern of ACE2 expression. In fact, while hACE2 expression is mainly limited to lung epithelia in K18-hACE2 mice, the protein is expressed in the vascular endothelia, renal and cardiovascular tissue, and epithelia of the small intestine, testes, and lung in humans.⁸ Therefore, the presence of high levels of hACE in the pulmonary epithelia of K18-hACE2 mice may account for the observed severity of pulmonary manifestations, but the lack of expression in other tissues may prevent a direct effect of the virus that may be present in humans with COVID-19.

Second, unlike ACE, which converts angiotensin I (Ang I) to angiotensin II (Ang II), ACE2 promotes the formation of Ang-1-9.⁹ In contrast to Ang I, which induces vasoconstriction and promotes fibrosis and inflammation, Ang-1-9 has vasodilatory, antifibrotic, and anti-inflammatory effects. Interestingly, ACE2 activity is reduced by either downregulation or shedding upon SARS-CoV-2 infection,¹⁰ which likely contributes to the severity of the disease in humans. As there is no evidence of the existence of reduced mACE2 expression in K18-hACE2 mice, it is tempting to speculate that the level of Ang-1-9, and therefore its protective effects, are maintained, thus avoiding the development of some severe symptoms that may be present in humans.

Finally, as already acknowledged by Wilken et al., some of the more relevant risk factors for developing severe COVID-19 in humans, such as female sex, old age, and comorbid cardiovascular disease or diabetes, are not present in K18-hACE2 mice, likely preventing some of the adverse clinical outcomes observed in humans.

In summary, the animal model proposed for COVID-19 mimics many of the clinical features of the disease, making it one of the

¹Molecular Oncology Laboratory (MOL), Departamento de Fisiología, Centro Singular de Investigación en Medicina Molecular e Enfermedades Crónicas (CIMUS), Facultade de Medicina, Universidade de Santiago de Compostela, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), 15782 Santiago de Compostela, Spain
Correspondence: Jose A. Costoya (josea.costoya@usc.es)

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Fig. 1 Overexpression of ACE2 in the lung epithelium facilitates SARS-CoV-2 infection in mice

best models available. A future comprehensive model will include additional aspects, such as the vasculopathy and coagulopathy associated with SARS-CoV-2 infection in humans or the effects of

different chronic diseases, advanced age, or male sex, which put patients at greater risk of adverse outcomes after viral infection.

ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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