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Animal models: important tools for studying SARS-Cov-2 infection

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Abstract

Ever since the appearance of COVID-19, the pathophysiology of SARS-CoV2 infection, the identification of treatments and the development of vaccines have been priorities. This search for preventive and therapeutic strategies has been carried out using animal models adapted to the problem under study.

Keywords: Animal research, models, SARS-CoV-2

SARS-COV-2 INFECTIVITY

SARS-CoV2 (Severe Acute Respiratory Syndrome CoronaVirus type 2), the cause of COVID-19, is a single-stranded RNA virus belonging to the Coronaviridae family, a name derived from the presence of a halo of "spike" viral protein trimers forming a crown of spicules. This family comprises 7 viruses that infect humans, of which SARS-CoV2 is the third after SARS-CoV (or SARS-CoV1) and MERS-CoV (Middle East Respiratory Syndrome-CoV) to cause a fatal epidemic. The genome sequence of SARS-CoV2 is 79.4% similar to that of SARS-CoV1 and 50% similar to that of MERS-CoV. The SARS-CoV2 genome contains 11 genes enabling the production of 29 to 33 viral proteins. Of these, 3 proteins are present in the virus envelope: the membrane protein (M), the envelope protein (E) and the spicule protein, S. The S protein is the viral protein responsible for SARS-CoV2 infectivity in humans. It consists of 2 subunits, S1 and S2. S1 contains an angiotensin-converting enzyme 2 (ACE2)-binding domain. ACE2 is an enzymatically active protein of the renin-angiotensin system, considered to be the most widely recognized SARS-CoV2 receptor by the scientific community. Since 2003, ACE2 has been recognized as a receptor for SARS-CoV1. S2 contains the sequence that enables fusion of the viral envelope with the cell membrane, a fusion that leads to endocytosis of the virus and thus entry of the viral genome into the cell. The pathogenicity of SARS-CoV2 is greater than that of SARS-CoV1, due to its greater affinity for ACE2.

Animals models and their specificity

Mouse. Rodents are the most widely used laboratory animals today, and among them the mouse is the animal of choice in biology. Wild mice are not naturally susceptible to SARS-CoV2, which does not bind effectively to their Ace2 protein. Genetically modified mouse models stably expressing the human ACE2 protein (hACE2) have therefore proved essential. Most of these models were generated during the SARS-CoV1 epidemic in 2003 [2; 3]. These models express hACE2 under the control of different promoters that exhibit tissue- and cell-specific expression [3]. The most widely used model is one in which hACE2 expression is under the control of the cytokeratin K18 promoter (K18-hACE2). In this model, hACE2 mRNA and hACE2 protein were detected in the lungs, encephalon, trachea, digestive tract organs, kidneys and testes, with highest expression in the lungs and encephalon [4]. The encephalic presence of hACE2 is greater in the K18-hACE2 model than in humans. The lungs and brain of these mice are severely affected. K18-hACE2 mice show very high mortality, peaking 6 to 7 days after infection at high infection rates (2x10-3/2x10-4 PFU) and around 10 days after infection at lower

rates [3]. As in humans, SARS-CoV2 infection leads to systemic and local (pulmonary and encephalic) cytokine shock; in the K18-hACE2 model, the pulmonary cytokine response precedes encephalic cytokine shock, the latter being synchronous with the encephalic viral peak and peak mortality, suggesting that encephalic involvement is responsible for the severity of the phenotype developed by these mice [3; 5; 6]. In this model, infection induces a hypoxic environment favoring the expression of HIF1α (Hypoxic Induced Factor), which increases ACE2 addressing to the neuronal membrane, making cells more susceptible to SARS-CoV2 binding. This model, characterized by severe nervous tissue damage (very high neuroinflammation) and a very high mortality rate, is therefore relevant for studying the pathophysiological mechanisms observed in humans developing severe COVID-19. However, this model is not very suitable for studying the long-term effects of infection, or for assessing the value of certain treatments, at least until future studies have determined the infection conditions (viral dose) that will allow better survival of the animals for longer-term study.

The ferret. The respiratory tract of the ferret (Mustela putorius furo), and in particular its long trachea which allows easy compartmentalization of the upper and lower respiratory tracts as in humans, make it a relevant animal model for studying the virulence and spread of respiratory viruses. The ferret is susceptible to infection by SARS-CoV2 (its ACE2 is close to human ACE2), but clinical manifestations are mild (mild respiratory symptoms and fever), with none of the severe symptoms seen in humans and no significant mortality. The ferret coughs and sneezes, and can be infected by indirect contact with conspecifics over a distance of more than one meter; this model is therefore used to simulate the transmission of SARS-CoV2 in humans [1; 7]. On the other hand, the ferret's immune responses to SARS-CoV2 infection are similar to those of humans (similar inflammatory cytokine profile in the airways), which led to its use in the development of antiviral and vaccine treatments prior to the launch of clinical trials [7].

The hamster. Two hamster lines were studied: the golden hamster (Mesocricetus auratus) and the Roborovski hamster (Phodopus roborovskii). These hamsters are naturally susceptible to SARS-CoV2; their ACE2 protein interacts with the virus' spike protein. Hamsters can be infected by both intranasal and ocular routes. Following intranasal infection, hamsters rapidly display many of the symptoms described in humans, with the exception of fever and thermal chills, which are only seen in Roborovski hamsters, where infection is 100% fatal, whereas it is never fatal in golden hamsters [8]. In golden hamsters, the onset of symptoms and their regression are identical in males and females: they present a peak of respiratory distress, a decrease in body mass, lethargy and reduced mobility between 2 and 4 days after infection. Symptoms are less pronounced after ocular infection [9]. An improvement in general condition is observed from 7 days post-infection, and recovery generally occurs 14 days after infection. Virus titration or the presence of viral particles has been demonstrated in the lungs, accompanied by pronounced inflammation leading to severe pneumonia. The presence of viral particles or inflammation in other tissues remains controversial. Golden hamsters were therefore used to study the immunity conferred by a primary infection with SARS CoV2, and also for transmission from one congener to another. This work has shown that a second exposure to SARS-CoV2 does not lead to visible symptoms, indicating that immunity is established after a primary infection. The golden hamster is therefore being used in vaccine trials. Viral transmission from one congener to another is possible, and the course and severity of symptoms are similar to those observed in intranasal infection. Hamsters have also been used to study the anosmia and agueusia often described in patients with COVID-19 [10]. This work demonstrated that infected hamsters showed anosmia and agueusia that persisted as long as the virus was present in the olfactory epithelium.

Macaques. Among non-human primates, rhesus macaques (Macaca mulatta) have been the most widely used [11]. Their infection with SARS-CoV2 causes predominantly respiratory symptoms, with the development of pneumonia present from the day after infection and lasting at least the 1st week post-infection, which then subsides. These respiratory symptoms are accompanied by a lack of appetite, but fever is rarely reported. Several routes of infection have been tested: intratracheal, intranasal and aerosol; the mode of infection has little influence on the course of the disease. In addition to the lungs and respiratory tract, the virus is also found in the central nervous system, liver, heart, kidneys, spleen and intestines. With regard to central nervous system involvement, despite the absence of neurological signs, astrocytic activation has been described in the cortex two weeks after infection, and neuroinflammation accompanied by microglial activation and the presence of alpha-

synuclein aggregates in Lewy body-like structures in the ventral region of the midbrain has been demonstrated 5-6 weeks after infection [12]. These findings suggest that the rhesus macaque is a relevant model for the study of neurological disorders associated with COVID-19, and in particular for the study of phenomena associated with "long COVID". Furthermore, secondary infection with the same variant as the primary infection indicates the development of immunity. Rhesus macaques were therefore used for vaccine testing

CONCLUSIONS

THE K18-hACE2 mice are the most suitable model for studying the neurological changes associated with SARS-CoV2 infection. It should be noted, however, that since the infection conditions required for significant animal survival have not yet been established, this model is not suitable for studying long-term effects.

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