MOUSE MODELS AND SARS-COV-2

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Abstract
Since December 2019, a novel coronavirus SARS-CoV-2 has emerged and rapidly spread throughout the world, resulting in a global public health emergency. COVID-19 is causing a major once-in-a century global pandemic. The emergences of coronaviruses have caused a serious global public health problem because their infection in humans caused the severe acute respiratory disease and deaths. Much more serious than SARS-CoV in 2002, the current SARS-CoV-2 infection has been spreading to more than 213 countries, areas or territories and causing more than 31 million cases and 962,518 deaths (update september 2020). Intensive research efforts have focused on increasing our understanding of viral biology of SARS-CoV-2, improving antiviral therapy and vaccination strategies. The lack of vaccine and antivirals has brought an urgent need for an animal model. The animal models are important for both the fundamental research and drug discovery of coronavirus.
Keywords: *coronavirus, respiratory syndrome, animal model, vaccine, drug*

1. Introduction

Animal models are critical for us to understand the viral infection and pathogenesis. Moreover, animal models are essential for development and preclinical evaluation of a vaccine or an antiviral agent. An ideal animal model is the one that mimics viral infection and diseases in humans in multiple aspects including morbidity, viral load, typical clinical symptoms, host immune responses and mortality. Therefore, the urgent need of preventing and controlling coronavirus infection necessitates the search for an optimal SARS- CoV-2 animal model. Based on the published studies, animal models of SARS-CoV and MERS-CoV include civet cats, camelidaes, monkeys, mice, hamsters, ferrets, rabbits and other potential hosts. Humanized animal models available to support coronavirus infection and pathogenesis might provide new options to overcome the limitations of the traditional coronavirus animal models. Additionally, animal models for pseudovirus are also prospected to avoid the concern of biosafety.

2. Emerging coronaviruses infections

Clinical symptoms of SARS-CoV- and MERS-CoV- infected patients at early time include fever, chills, coughing, malaise, myalgia, headache, diarrhea, vomiting and nausea. Furthermore, immunohistochemistry (IHC) detection demonstrated the presence of viral antigens in lung tissues. The COVID-19 patients present similar symptoms to those of SARS- CoV- or MERS-CoV-infected patients, while some patients may show no typical clinical symptom in the early stage of infection. The typical pathological features of severe cases include prolonged inflammation with destruction and desquamation of alveolar pneumocytes, hyaline-membrane formation, interstitial inflammatory infiltration and interalveolar hemorrhage. Multinucleated giant cells were also observed in the tissues of COVID-19 patients. Over 70% of COVID-19 patients were diagnosed as pneumonia by chest computed tomography (CT) to be admitted to hospital. CT images showed the typical features of ground-glass opacity and bilateral patchy shadowing in lungs. Recent clinical and experimental studies have demonstrated SARS-COV-2 caused the nosocomial infection and fecal-oral transmission, its infection results in immune abnormality and multiple organ failures of the COVID-19 patients. Moreover, a long incubation period (>29 days) was observed in some

COVID-19 cases, suggesting a risk of occult and chronic infection. Although SARS-COV-2 displays a lower rate of severe cases and case fatality than SARS-CoV and MERS-CoV, its high infectivity, widely spreading and huge infection population have become a catastrophic medical burden and critical social problem (1, 2, 3).

3. Natural Infectious animal models

Viral entry and intracellular replication are two essential steps for a virus to establish a successful infection. The specific receptor proteins anchored on cell mem- brane mediate viral entry and intranuclear transcriptional factors regulate viral replication. Therefore, host-range of virus depends on these two steps. Non-human primates (NHPs), ferrets and hamsters have been demonstrated to support SARS-CoV infection and pathogenesis, but only the NHPs are fully permissive for MERS-CoV infection. The civet cats were demonstrated permissive for SARS-CoV infection by serological antibody detection. The camelidaes have been demonstrated permissive for MERS-CoV infection because the specific antibodies against MERS-CoV S protein were detected at a high rate, and respiratory infections after the administration of MERS-CoV. Immunocompetent young inbred mice support transient SARS-CoV infection without clinical signs of disease, but were not permissive to MERS-CoV due to the lack of functional receptor. After a careful review of their illness, virological, histological and immunological characteristics post infection, NHPs, ferrets and hamsters have been presumed to be potential natural hosts of SARS-CoV- 2. Meanwhile, other experimental animal models such as tree shrew, woodchuck, pangolin, rat, guinea pig and cotton mouse, as well as domesticated animals such as cats and dogs might be potential hosts to support SARS-CoV-2 infection (1, 2, 3, 4, 5, 6, 7, 8).

4. Mouse models

Mouse model has been widely used for many different viral investigations. It has been considered as the best small animal model for hepatitis B virus (HBV), hepatitis C virus (HCV), cytomegalovirus (CMV), Zika virus, and among others. Due to its low cost, small size, easy operation and high reproducibility, mouse model is suitable for large scale studies of viruses not only for the pathogenesis but also for antivirals. In contrast of the NHPs, mice are more convenient to be handled in a higher level of biosafety laboratory due to its relatively small size and less operation difficulties. Importantly, mouse can be easily manipulated at the genetic level for precision research. For instance, a lot of genetically mutated mice are available for studies in anti-viral immunity, viral pathogenesis and viral infection and transmission restriction. Furthermore, current murine immunological reagents are available to study viral pathogenesis and host immune responses. Several strains of mice have been tried to be intranasally infected with SARS-CoV (Urbani strain), it was found that the virus poorly replicated in several young and adult inbred strains of mice (BALB/c, C57BL6 and 129S) to only a low viral yield. Clinical illness was not observed, and the virus was cleared within nine days. Meanwhile, mice are not naturally susceptible to MERS-CoV infection. It was later revealed that the mouse DPP4 receptor differs from human counterpart in crucial domains that is critical to bind the S protein. To overcome the species-specificity barrier for SARS-CoV or MERS-CoV, human receptor protein (hACE2 or hDPP4) was expressed in mice by knocking in or transfecting a human receptor gene in mouse. The studies of hACE2-transgenic mice were performed at almost the same time in 2007 independently in three groups. They reported that hACE2- transgenic mice supported SARS-CoV infection and pathogenesis. McCray et al. demonstrated that systemic expression of hACE2 under the control of an epithelial cell-specific promoter K18 resulted in lethal SARS-CoV infection within five days. Tseng et al. developed two lineages of transgenic mice expressing hACE2 under the CAG promoter. After SARS-CoV infection, the two transgene-positive mice, AC70 and AC63 showed

high viral load, clinical illness and tissue pathology. The lethal lineage of mice (AC70) displayed higher hACE2 expressions in several organs, wider spectrum of clinical illness, including wasting symptom, severe pneumonia and death, rather than the non-lethal lineage mice (AC63) with lower hACE2 expressions. Yang et al. generated another non-lethal lineage mouse that expresses hACE2 under mouse ACE2 promoter. Another study further revealed the differential virological and immunological outcomes among different hACE2 transgenic lineages.

To rapidly generate a MERS-CoV infecting mouse model, Zhao et al. transduced common adult BALB/c and C57BL6 mice with an adenoviral vector expressing hDPP4 (Ad5-hDPP4). After MERS-CoV infection, these mice showed interstitial pneumonia, expressed viral antigen in the lungs and lost weight, but deaths were not observed. Later, hDPP4-transgenic mice were generated to mimic severe MERS-CoV infection and lethal pathogenesis. Agrawal et al. developed a transgenic mouse that expresses hDPP4 under the control of the CAG promoter. This hDPP4-transgenic mouse was fully permissive for MERS-CoV infection to young adult BALB/c and C57BL6 mice might provide an avenue for rapid and robust generation of SARS- CoV-2 infectious mouse model.

Several studies have attempted to use the immunecompromised mice to determine the role of immune effectors in the coronavirus infection. First, the following knock-out mice have been applied for the infection of SARS-CoV: beige lacking functional NK cells, $CD1^{-/-}$ lacking NK-T cells, $Rag^{-/-}$ lacking T and B cells. However, all the immune-compromised mice failed to allow the infection of SARS-CoV. Viral kinetics were not significantly different among the 57BL6 (wild type), beige, $CD1^{-/-}$ and $Rag1^{-/-}$ mice. Histopathological detection showed similar self-limiting bronchiolitis and mild pneumonia among these mice. Interestingly, a prolonged viral replication and illness were observed in $STAT1^{-/-}$ mice with 129S back- ground. In this model, viral replication is detectable until day 22 post infection indicating that a STAT1 mediated type I interferon response is required to control SARS-CoV infection. The $STAT1^{-/-}$ mice were also challenged with MERS-CoV (EMC- 2012 strain). Because the lack of receptor hDPP4, clinical illness were not observed and viral replication was undetectable. However, the hDPP4-transfected mice additional deficiency of interferon-α receptor, MyD88 and MVAS still cannot prolong the viral replication or cause severe cause throughout a MERS-CoV infection course. Generally, mice with targeted immune deficiency are useful tools to investigate interaction between host immunity and coronavirus. Besides the relatively low viral load and mild illness, the immunodeficient mice are of limited value in the studies of vaccine and immunotherapy (9, 10, 11, 12, 13, 14, 15).

5. Humanized animal models for studying coronavirus infection.

Prevention of and recovery from the coronavirus infection are usually associated with adaptive and innate immunity. The outcomes of the coronavirus infection depend on humoral immune responses such as the subtype and titre of antibody. Although NHPs, mice and other experimental animals can effectively mimic coronavirus infection, the genetic diversity might disturb the interpretation of the possibly different results between human and non-human species. To directly study coronavirus infection in human organ or tissue, humanized animal models are the method of choice, which can also be used for evaluation of coronavirus vaccines and host target agents. In the past decades, mice with one or several human tissues or cells engraftment have been widely used to investigate the pathogenesis of HIV, HBV, HCV, CMV, varicella-zoster virus (VZV) and other important pathogens. Lung is the main target organ of coronavirus infection. In 2012, Maidji et al. grafted human fetal lung tissues under the kidney capsule of immunodeficient SCID mice. The lung tissues rapidly grew and developed mature structures resembling the normal human lung and were demonstrated to support CMV infection and pathogenesis. In 2017, Wang et

al. established a human lung-xenografted mouse model for VZV infection. After VZV infection, viral replication, lung pathogenies and pro-inflammatory cytokine responses were detected in the human lung xenografts. More recently, Wahl et al. reported a humanized mouse with mono human lung engraftment (LoM) or incorporated with human bone marrow, liver and thymus (BLT-L). The LoM or BLT-L mice supports infection and replication of human pathogens such as MERS-CoV, RSV, CMV and Zika virus. Antigen-specific humoral and T-cell responses were observed in BLT-L mice, suggesting that human lung and immune cell dual chimeric mice might be an ideal humanized animal model for the pathogenesis and immune study of SARS-CoV-2. Due to the abundant expression of hACE2 in multiple organs of human, multiple organs and tissues including liver, heart, intestine, kidney, bladder and immune cells are susceptible to the infection of SARS-CoV-2, suggesting a reason that causes multi-organ failures. Therefore, mice with engraftment of human somatic, progenitor and stem cells, as well as varied human tissues and organoids might be useful to investigate the tropism of SARS-CoV-2.

Comparing to the traditional animal models, the humanized mice provide new options for investigators to directly study the viral infection in human tissues, which is adequate to delineate the tissue tropism and the host-virus interactions. The development of humanized mice is important to improve our fundamental understanding for mechanisms of coronavirus infection and immunopathophysiology. In the future, humanized mice might become a unique tool to obtain the insights into our strategies for developing coronavirus vaccine, early intervention and antiviral therapy.

6. Conclusions

The coronaviruses are one of the most important pathogens causing robust respiratory infection, severe cases and deaths in humans. A rapid animal test of SARS-CoV-2 is important to assess the efficiencies of the vaccines, antivirals and the sensitivity of the diagnostic tests. We also debated that a rapid generation of MA viral strains or mice carrying human receptor is a good option for urgent and effective animal studies. In addition, development of humanized animal model might provide a direct infection of coronavirus to human tissue. Taken together, animal models are the fundamental tolls to investigate the viral pathogenesis, to develop vaccines and antiviral drugs.

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