PATHOLOGICAL AND VIROLOGICAL ANALYSES OF SEVERE ACUTE RESPIRATORY SYNDROME–ASSOCIATED CORONAVIRUS INFECTIONS IN EXPERIMENTAL ANIMALS

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1. INTRODUCTION

Severe acute respiratory syndrome (SARS) is a recently identified emerging infectious disease caused by SARS-associated coronavirus (SARS-CoV). To determine the pathological features of SARS-CoV infection in experimental animals, its clinical, pathological, and virological features were investigated in cynomolgus monkeys, BALB/c mice, and F344 rats. The susceptibility of these animals to SARS-CoV infection was evaluated to identify suitable animal models for studies of the pathogenesis and treatment of SARS.

2. MATERIALS AND METHODS

The SARS-CoV, HKU39849 isolate was used in the present study.[1] The virus was propagated three times in Vero E6 cells, and the infectious doses of the virus stock were expressed as the 50% tissue culture infective dose ($TCID_{50}$) on these cells. Three-year-old male cynomolgus monkeys (*Macaca fascicularis*), 4-week-old female BALB/c mice, and 4-week-old F344 rats were used. Monkeys were inoculated intranasally with 10^3 or 10^6 $TCID_{50}$ of SARS-CoV in 3.5 ml of medium, or intratracheally with 10^8 $TCID_{50}$ in 5 ml of medium. BALB/c mice and F344 rats were inoculated intranasally with $2x10^6$ $TCID_{50}$ of SARS-CoV in 20 µl of medium and 10^7 $TCID_{50}$ in 100 µl of medium, respectively. After inoculation, these animals were observed for clinical symptoms and sacrificed for pathological examination. Virus isolation and viral infectivity titers were investigated in

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Vero E6 cell cultures. The SARS-CoV genome quantified by one-step reverse transcription and quantitative PCR assay using a LightCycler SARS-CoV quantification kit (Roche Diagnostics, Indianapolis, IN). All procedures in which infectious SARS-CoV was manipulated were carried out under biosafety level 3 conditions. The National Institute of Infectious Diseases Animal Care and Use Committee approved the animal studies to be carried out in an animal biosafety level 3 facility.

3. RESULTS

In monkeys, following intranasal inoculation with 10^6 $TCID_{50}$ of SARS-CoV, the virus was isolated from throat and nasal swabs, and the viral genome was detected in rectal swabs collected between 2 and 7 days postinoculation (p.i.). In one of the two monkeys inoculated intratracheally with 10^8 $TCID_{50}$ of SARS-CoV, virus and viral genome were detected in throat swabs collected on day 2 p.i. and in rectal swabs collected from days 4 to 7 p.i., respectively. Virus antigen-positive alveolar cells and macrophages were detected in the lower lobes of the lungs on day 7 p.i. in monkeys after intratracheal inoculation (Figure 1A). Angiotensin-converting enzyme 2 (ACE2, a receptor for SARS-CoV[2] antigen-positive cells were observed in the virus-infected area and were repairing swelled type II alveolar epithelium in the lung of monkeys (Figure 1A, B, and C). In BALB/c mice, the virus was detected in nasal and lung washes on days 3 and 5 after intranasal inoculation with 10^6 $TCID_{50}$ of SARS-CoV. Virus antigen was found in the epithelial cells in the lung alveolar and nasal cavities on day 3 as well as slight inflammatory reaction. In contrast, virus antigen was observed in the epithelial cells of intrapulmonary bronchi with inflammatory cells, including macrophages, in F344 rats after intranasal inoculation with 10^7 $TCID_{50}$ of SARS-CoV (Figure 1D). ACE2 antigen was observed in the epithelial cells of the respiratory tract in mock-infected F344 rats (Figure 1E). In BALB/c mice, ACE2 antigen-positive cells were not detected in non-infected areas (Figure 1F). In these experimental animals, ACE2 antigen-positive cells were observed in the virus-infected area and were repairing swelled type II alveolar epithelium (Table 1). In BALB/c mice and F344 rats, the virus was eliminated by day 7 p.i. None of the three experimental animals developed any clinical symptoms similar to SARS-like disease.

4. DISCUSSION

It was suggested that the infection and replication of SARS-CoV occurred in the respiratory tract of these experimental animals. However, there were differences in pathological findings, such as distribution of SARS-CoV and ACE2 antigen in the lungs between monkey, mouse, and rat (Table 1). The localization of infection with SARS-CoV was associated with the distribution of ACE2 antigen. These results indicated that ACE2 was used as a receptor for SARS-CoV infection in these animals. Furthermore, pathological dissimilarities in infectious and inflammatory reactions were observed between BALB/c mice and F334 rats. It was reported previously that monkeys were not suitable for studies of SARS.[3,4] Although none of the three types of experimental animal examined here developed any SARS-like symptoms, SARS-CoV could infect and replicate in these animals after intranasal or intratracheal inoculation. Therefore, these

animal models are thought to be useful not only for studies on SARS-CoV infection and pathogenesis, but also for evaluation of novel vaccine and antiviral drugs against this virus.

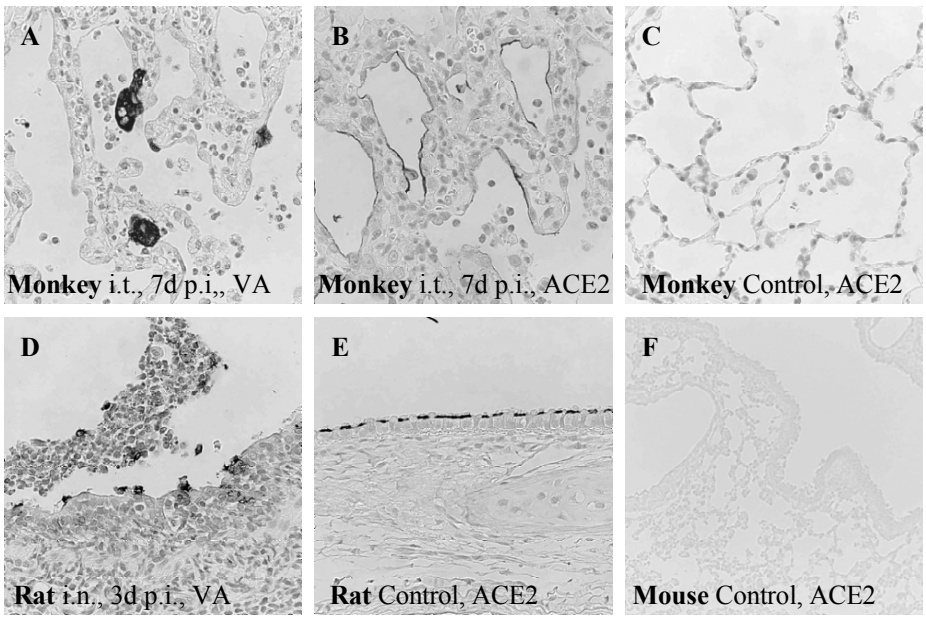

Figure 1. Distribution of SARS-CoV and ACE2 antigens in lung tissue determined using the immunoperoxidase method. VA, virus antigen. Original magnification: A–E, x400; F, x100.

Table 1. Distribution of SARS-CoV antigen and expression of ACE2 in experimental animals.

		Virus antigen			Expression of ACE2		
		Monkey	Mouse	Rat	Monkey	Mouse	Rat
Nasal cavity	Respiratory area	-	-	+	-	-	+
	Olfactory area	+	+	-	-	-	-
Trachea	Trachea	-	-	+	-	-	+
Lung	Intrapulmonary bronchi	-	-	+	-	-	+
	Bronchioles	-	-	+	-	-	+
	Alveoli	+	+	+	(+)*	(+)*	(+)*

* ACE2 antigen-positive cells were observed in the virus-infected area and were repairing swelled type II alveolar epithelium.

5. ACKNOWLEDGMENTS

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