Different Transmissibility of 2 Isolates of Seoul Virus from the Same Wild Brown Rat (*Rattus norvegicus*)

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ABSTRACT. Two Seoul virus strains were isolated from the same wild brown rat (*Rattus norvegicus*) by 2 different procedures. While one isolate (TR-352) by means of a cell culture system had no horizontal transmissibility in 3-week-old rats, another isolate (TR-352R) by means of inoculation to newborn rats had transmissibility in rats.—**KEY WORDS:** horizontal transmission, rat, Seoul virus.

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The mechanism of transmission of Seoul virus in rats is still obscure. Previously, we reported that the transmission of SR-11 strain of the virus is strictly age-dependent [3]. Horizontal transmission of the strain to cagemates occurred from rats inoculated with the virus within 48 hr after birth, but not from those inoculated at 3 weeks of age. In addition, passive administration of antibody protected from infection by the virus [4], and Zhang *et al.* also reported that transmission of the virus from infected mother rats to their offspring was prevented by maternal antibody [7]. This means that the SR-11 strain of Seoul virus is rarely transmitted to other rats and the chain of infection of the virus might reach a deadend. On the other hand, from the epidemiologic observation of wild rats in the port area of Tokyo Bay, the transmission of the virus seems to occur among adults rats [5]. To clarify the transmission mechanism of the virus in rats, it is necessary to determine the difference between the transmissible virus in adult rats and the non-transmissible one. In an attempt to obtain horizontally transmissible strain(s) in adults rats, we utilized 2 isolation methods, by means of inoculation onto Vero-E6 cells and newborn rats, for the specimen from a rat captured in port area of Tokyo Bay [4]. Those isolated with using Vero-E6 cells and those with newborn rats were designated TR-352 and TR-352R, respectively. Wistar rats were obtained from a Seoul virus free colony (Nippon SLC, Japan) and used for the virus inoculation and passage. For the virus passage, newborn rats were injected subcutaneously with 10% lung suspension of inoculated rats within 48 hr after birth. After 3 weeks, the serum antibody was assayed and the lungs of seropositive rats were used for the next passage. TR-352 strain passaged 8 times in Vero-E6 cells and TR-352R strain passaged 3 times in newborn rats were used in this experiment. The viral titer was expressed as 50% tissue culture infectious dose (TCID₅₀) after 14 days incubation [2]. For antibody detection, indirect immunofluorescence test (IFA) employing by the methods described previously was used [1]. The antigen for IFA was prepared from TR-352 infected Vero-E6 cells. Viral antigen in lung tissue was also examined by IFA staining as described previously [3].

The experimental design of the present experiment was as follows; Two groups of 3-week-old rats were inoculated subcutaneously with TR-352 (1×10^4 TCID₅₀/0.2 ml/rat) or TR-352R strains (1×10^3 TCID₅₀/0.2 ml/rat) of Seoul virus. Three, 6 and 9 weeks after inoculation, 3 or 4 rats

from each group were sacrificed to be examined for the presence of antibody and the viral antigen in the lung tissue. Normal 3-week-old rats were placed in the same cage as cagemates. At six weeks after inoculation, the cagemates were separated from the inoculated rats and kept for a further 3 weeks to be checked for conversion of the antibody and the viral antigen in the lung. All experiments except IFA were performed in the containment laboratories on P3 level of the National Institute of Health.

As shown in Table 1, the rats inoculated with TR-352 or TR-352R had the antibody to the Seoul virus at 3, 6 and 9 weeks after inoculation. Although the rats inoculated with TR-352 were inoculated with a 10 times higher titer of the virus than those with TR-352R, 3 of 4 rats had the viral antigen in their lungs at 3 weeks after inoculation and none of rats had it at 6 or 9 weeks after inoculation. On the other hand, the viral antigens were detected in the lungs of rats infected with TR-352R strain until 9 weeks after inoculation. Four of 6 cagemates with TR-352R inoculated rats had the antibody and the viral antigen was also detected in the lungs of the antibody positive rats. The rats could be infected with TR-352, but did not transmit infection to their cagemate.

The data mentioned above might be interpreted as indicating that the horizontally transmissible strain (TR-352R) lost its transmissibility during the adaptation to the cell culture. This characteristic of the TR-352 strain seems

Table 1. Antibody and antigen of Seoul virus in experimental rats

Virus inoculated	Weeks after inoculation	No. tested	IFA ^{a)} antibody		Viral antigen in lung No. positive
			No. positive	titer (GM) ^{b)}	
TR-352	3	4	4	8192	3
	6	3	3	8192	0
	9	3	3	8192	0
	(9)	6	0	0	0
TR-352R	3	4	4	8192	4
	6	3	3	8192	3
	9	3	3	8192	3
	(9)	6	4	6888	4

a) Indirect immunofluorescence test.

b) Geometric mean of antibody titer of positive rats.

to resemble that of the SR-11 strain. As described in the previous paper, the SR-11 strain was transmissible when the rats were inoculated in the neonatal period but not at 3 weeks old. The nature of SR-11 was not due to the adaptation to the cell culture but originated in the characteristics of the virus contained in the lung sample from the rat from which the SR-11 strain had been isolated [4]. It is also possible that the TR-352 strain was selected from a mixed population of a horizontally transmissible virus and non-transmissible ones through the procedure of isolation by means of the cell culture system. Sugiyama *et al.* found that a Seoul virus strain was isolated from a lung sample of a wild brown rat by means of Vero-E6 cells but failed to isolate a virus from the same sample by means of inoculation into newborn rats [5]. This may mean that the isolate was not able to be transmitted among rats and could only propagate in a cell culture system. Hence Seoul viruses with various degree of transmissibility might exist among rat populations in nature.

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