Contamination of Mouse-adapted Influenza Virus with Sendai Virus

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Abstract: In our laboratory animal facility, Sendai virus (HVJ) contamination occurred in a negative flow rack used for experimental infection with 4 strains of mouse-adapted influenza virus (Inf.V). Anti-HVJ antibody (Ab) was detected in 35/42 mice in the rack. To specify the strain of Inf.V contaminated with HVJ, experimental infection was performed by using A, B and D strains of Inf.V in each vinyl isolator. Anti-HVJ Ab was detected in all mice infected with A strain at day 28 post-infection. As a result of experimental infection with A strain of Inf.V which was treated with anti-HVJ mouse serum, the virus suspension was determined not to contain HVJ and allowed for experimental use in our facility. Since then, HVJ contamination has not occurred in our facility.

Key words: influenza virus, mouse, Sendai virus

Sendai virus (HVJ: hemagglutinating virus of Japan) is a RNA paramyxovirus-parainfluenza type I which causes respiratory disease. In general mice and rats are seriously affected by HVJ [4]. In laboratory animal facilities, HVJ is one of the most important pathogens contamination with which is to be prevented because it is highly contagious and difficult to control. Fortunately, a stable supply of HVJ-free mice has been provided by breeders in recent years. In our facility, the microbiological quality of mice is examined monthly by cultivation of pathogenic bacteria and *Mycoplasma pulmonis* as well as detection of antibodies (Ab) to murine viruses by serological tests, such as enzyme-

linked immunosorbent assay (ELISA) and complement fixation (CF) test. Whenever anti-HVJ Ab is detected by serological tests, the contaminated animal room must be rapidly cleaned by killing the mice in the room and spraying appropriate disinfectants. In addition, it is important to determine the route of HVJ infection in order to prevent future contamination. On the other hand, because materials such as viruses, bacteria, transplantable tumors and cell lines used for experiments are not subjected to an examination of microbiological quality in our facility, it is possible that these materials will cause pathogenic contamination in the laboratory animal facility.

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Recently, mice used as controls for experimental infection with a mouse-adapted influenza virus (Inf.V) suddenly died in a negative flow rack in our facility. As a result of microbiological testing by killing other mice in the same rack, anti-HVJ Ab was detected. To specify the route of HVJ contamination, anti-HVJ Ab in the sera of mice maintained in other racks was examined by ELISA, but no anti-HVJ Ab was detected in the sera of any of the mice examined. In addition, a specific pathogen-free (SPF) environment was maintained at the facilities of breeders which provide mice to our facility. Because routine monitoring in our facility had not found the HVJ contamination by that time, HVJ was considered to be contained in the Inf.V suspension, the suspension having been passaged in mouse lungs and 10-day-old fertile chicken eggs [10]. We report here the results of our research on the cause of HVJ contamination and elimination of HVJ from Inf.V.

We used 42 mice (37 of BALB/c and 5 of C3H strain) divided into 10 groups, and 4 strains of Inf.V, called A, B, C and D strains in this report, in which transmission by respiratory aerosol is almost impossible. Anti-HVJ Ab in mice sera was examined by solid-phase ELISA and CF test. ELISA was carried out by using a commercially available HVJ-antigen (DENKA SEIKEN Co., Ltd., Tokyo). The reaction was visualized with peroxidase-labeled and affinity-pu-

rified Fab' portion of goat anti-mouse IgG (H+L-chain) (MBL, Nagoya), and *o*-phenylenediamine was used as a substrate. The chromogen produced was measured for absorbance at 492 nm. CF test was carried out by using the same HVJ-antigen as above, guinea pig sera as a complement and hemolysin-conjugated sheep erythrocytes (Japan Lyophilization Lab., Tokyo). Subsequently, solid-phase ELISA to detect anti-Inf.V Ab in mouse sera was performed as described previously [8], with purified hemagglutinin [7] and anti-mouse IgG (γ -chain specific) conjugated with alkaline phosphatase (Zymed Laboratories, Inc., San Francisco, CA). The chromogen was produced with *p*-nitrophenyl-phosphate and measured for absorbance at 410 nm, and the IgG Ab concentration was determined as described previously [9]. Table 1 shows the rate of detection of anti-HVJ Ab and anti-Inf.V Ab in each experimental group. Anti-HVJ Ab was detected in sera of mice in all groups by ELISA. In addition, the anti-HVJ Ab was detected in sera of more than 75 percent of mice in groups V, VII, VIII and IX by CF test. The susceptibility of C3H mouse to HVJ [6] may have caused the high rate of detection of anti-HVJ Ab in group VIII and IX by both ELISA and CF test. As mentioned above, anti-HVJ Ab was detected in most of the mice examined by ELISA. It was unknown, however, which strain was contaminated with HVJ. Because there is also the possibility that anti-Inf.V Ab cross-

Table 1. Detection of anti-HVJ antibody and anti-influenza virus antibody in mice in the HVJ contaminated rack

Group				No. of positive/No. of animals examined				
Primary				ELISA				CF
Immunization ^{a)}	Infection ^{b)}	Challenge ^{c)}	Mouse strain	Anti-A	Anti-B	Anti-D	Anti HVJ	Anti-HVJ
I	D vaccine	D	BALB/c	NT ^{d)}	NT	8/8	8/8	5/8
II	A vaccine	D	BALB/c	1/1	0/1	1/1	1/1	0/1
III	B vaccine	D	BALB/c	NT	NT	0/2	2/2	1/2
IV	D	D	BALB/c	NT	NT	NT	4/9	3/9
V	A	D	BALB/c	9/9	NT	NT	9/9	9/9
VI	C	D	BALB/c	NT	1/2	NT	2/2	1/2
VII	B	D	BALB/c	NT	4/4	NT	3/4	3/4
VIII	D		C3H	NT	NT	NT	1/1	1/1
IX	Non treatment		C3H	NT	0/4	NT	4/4	4/4
X	Non treatment		BALB/c	NT	0/1 ^{e)}	NT	1/2	0/2

^{a)}Mice were immunized intranasally with 1 μ l of PBS containing each HA vaccine. ^{b)}Mice were infected intranasally with 1 μ l of each mouse-adapted influenza virus suspension. ^{c)}Three weeks after the immunization or the infection, mice were infected intranasally with 2 μ l of D strain of influenza virus. Eighteen days after the challenge, mice were autopsied. ^{d)}Not tested. ^{e)}One mouse in group X was examined.

reacts with HVJ antigen, experimental infection was performed by using *A* and *B* strains which showed a high CF titer of anti-HVJ Ab, and *D* strain which was used for all experimental groups.

SPF BALB/c and C3H mice were obtained from Japan SLC Inc. (Hamamatsu) for the experimental infection. BALB/c mice were used for the infection of *A* or *B* strain, and BALB/c and C3H mice were used for *D* strain. Mice were infected intranasally with Inf.V suspension. The infected mice were kept together with sentinel mice in a separate cage in each vinyl isolator until autopsy. At day 28 post-infection (PI), the mice infected with *A* or *B* strain were anesthetized with chloroform and then exsanguinated from the heart with a syringe. Sera were collected by centrifugation and subsequently used for Ab determination. Similarly, the mice infected with *D* strain were autopsied at day 25 or 35 PI. Table 2 shows the detection rates and ELISA titers of anti-HVJ Ab and anti-Inf.V Ab in the sera from the mice. In the sera of mice infected with *B* or *D* strain, only anti-Inf.V Ab was detected. On the other

hand, both anti-Inf.V Ab and anti-HVJ Ab were detected in all the mice infected with *A* strain. In addition, anti-HVJ Ab was detected in all the sentinel mice, but anti-Inf.V Ab was detected in only 2 of 5 sentinel mice in the same isolator where mice infected with *A* strain were maintained. These findings indicated that some of the sentinel mice were infected with only HVJ which may be transmitted by respiratory aerosol. Finally, these results made it clear that *A* strain of Inf.V suspension was contaminated with HVJ.

HVJ was eliminated from Inf.V by repeatedly passaging the virus in the presence of anti-HVJ Ab. Briefly, anti-HVJ mouse serum was added to the Inf.V suspension contaminated with HVJ and incubated at 34°C for 60 min. The treated suspension was then inoculated into Madin-Darby canine kidney cells and incubated in Eagle's minimum essential medium supplemented with 5 µg/ml of trypsin at 34°C for 3 days. Finally the supernatant was collected by centrifugation and diluted 1:10 in 0.01 M phosphate-buffered saline (PBS) (pH 7.3). The Inf.V was passaged in this man-

Table 2. Detection of antibody in sera of mice infected with *A*, *B* or *D* strain of influenza virus

Influenza virus strain	Mouse strain	Group (DPI) ^{a)}	No. of positive/No. of animals examined ELISA titer: Means ± S.D. (range)			
			Anti- <i>A</i> (unit/ml)	Anti- <i>B</i> (unit/ml)	Anti- <i>D</i> (unit/ml)	Anti-HVJ (OD)
<i>A</i>	BALB/c	Infection (28)	6/6 1235 ± 321 (732–1612) ^{b)}	NT ^{d)}	NT	6/6 2.27 ± 0.21 (2.04–2.62) ^{e)}
		Sentinel (28)	2/5 273 ± 255 (108–713)	NT	NT	5/5 2.06 ± 0.14 (1.93–2.26)
<i>B</i>	BALB/c	Infection (28)	0/5 93 ± 48 (35–146)	5/5 2796 ± 1130 (797–3585) ^{e)}	NT	0/5 0.33 ± 0.03 (0.29–0.36)
		Sentinel (28)	0/5 79 ± 45 (45–159)	1/5 90 ± 62 (60–200)	NT	0/5 0.28 ± 0.04 (0.23–0.34)
<i>D</i>	C3H	Infection (25)	NT	NT	2/2 1974 ± 171 (1853–2095) ^{d)}	0/2 0.11 ± 0.01 (0.10–0.12)
		Infection (35)	NT	NT	3/3 1340 ± 782 (681–2204)	0/3 0.19 ± 0.03 (0.15–0.21)
		Sentinel (35)	NT	NT	0/2 16 ± 21 (1–30)	0/2 0.15 ± 0.02 (0.13–0.16)
	BALB/c	Infection (25)	NT	NT	2/2 791 ± 699 (296–1285)	0/2 0.08 ± 0.02 (0.06–0.09)
		Infection (35)	NT	NT	3/3 1361 ± 702 (615–2008)	0/3 0.11 ± 0.01 (0.10–0.12)
		Sentinel (35)	NT	NT	0/2 16 ± 21 (1–30)	0/2 0.15 ± 0.02 (0.13–0.16)

^{a)}Days post infection. ^{b)}Value lower than 200 is negative. ^{c)}Value lower than 200 is negative. ^{d)}Value lower than 100 is negative. ^{e)}Value lower than 0.5 is negative. ^{f)}Not tested.

Table 3. Detection of antibody in sera of mice infected with A strain of influenza virus treated with anti-HVJ antibody

Group (28 DPI) ^{a)}	No. of positive/No. of animals examined ELISA titer: Means $\pm$ S.D. (range)	
	Anti-A (unit/ml)	Anti-HVJ (OD)
Infection	4/5 1532 $\pm$ 884 (23–2177) ^{b)}	0/5 0.22 $\pm$ 0.02 (0.19–0.25) ^{c)}
Sentinel	0/4 0	0/4 0.22 $\pm$ 0.02 (0.19–0.26)

^{a)}Days post infection. ^{b)}Value lower than 200 is negative. ^{c)}Value lower than 0.5 is negative.

ner another two times. The resulting virus suspension obtained after the third passage was then diluted 1:10 in 0.01 M PBS (pH 7.3). After incubation with anti-HVJ mouse serum at 34°C for 60 min, 0.2 ml of the suspension was injected into an embryonated egg and incubated at 34°C for 48 hrs.

To confirm the elimination of HVJ from A strain of Inf.V treated with anti-HVJ Ab, 5 BALB/c mice were infected with A strain of Inf.V, and 4 BALB/c mice were maintained as a sentinel. All the mice were autopsied at day 28 PI. Table 3 shows the detection rates and ELISA titers of anti-HVJ Ab and anti-Inf.V Ab in the sera from the above mice. Anti-HVJ Ab was not detected in any mice, either infected or not infected with A strain. From this result, A strain of Inf.V suspension was determined not to contain HVJ and allowed for experimental use in our facility. In addition, it was denied that anti-Inf.V Ab cross-reacted with HVJ antigen. Since then HVJ contamination has not occurred in our facility.

In this study, we reported that mouse-adapted virus suspension was contaminated with HVJ. Fukumi *et al.*

have reported that HVJ was isolated during the passage of Inf.V through mice [3]. Similarly, it was reported that a transplantable tumor was contaminated with murine pathogens, such as mouse hepatitis virus, lymphocytic choriomeningitis virus and *M. pulmonis* [1, 2, 5]. The HVJ contamination in this report may have derived from the mice used for adapting Inf.V in another institute. It is necessary to use SPF mice when the mouse-adapted virus strains are produced. Furthermore, the products derived from animals should be subjected to a microbiological examination before subsequent experimental use.

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