

Isolation of Lymphocytic Choriomeningitis Virus from Wild House Mice (*Mus musculus*) in Osaka Port, Japan

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ABSTRACT. Lymphocytic choriomeningitis virus (LCMV) was isolated from 14 out of 35 wild house mouse samples captured on two piers of Osaka port in Japan. Four of them were isolated from 18 antibody positive mice while 10 were from 17 antibody negative mice. This is the first report of the isolation of LCMV from wild mice in Japan.—**KEY WORDS:** lymphocytic choriomeningitis virus, *Mus musculus*, Osaka, wild house mouse.

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Lymphocytic choriomeningitis virus (LCMV) is a member of family *Arenaviridae* and is well known as one of viral zoonoses. It is often said that LCMV exists in all parts of the world. Although most of isolations of LCMV from wild rodents were reported from Europe and America, well-documented proof is not available for Asia and Africa.

In Japan, the isolation of LCMV was first reported by Kasahara *et al.* in 1937 [1, 2]. They isolated five strains of LCMV from experimental animals in the course of a study of Japanese B encephalitis. Two of the strains were assumed to be from mice and another two were from guinea pigs. The origin of the experimental mice they used in the study was unknown, although experimental mouse colonies had been introduced to Japan from Germany (Dr. S. Kasahara personal communication).

Although the ultimate reservoir of the virus seems to be the house mouse (*Mus musculus*), the presence of LCMV in wild house mice had not been investigated in Japan prior to this. Recently we reported occurrence of LCMV antibody in wild house mice in Yokohama port [4]. Therefore, further investigation was conducted to reveal presence of LCMV. This paper is the first report of the isolation of LCMV from wild house mice in Japan.

MATERIALS AND METHODS

The indirect fluorescent antibody (IFA) test to detection of antibody or viral antigen and estimating the ages of wild house mice were as described previously [4]. Antibody titers of more than 16 were considered as positive.

Laboratory mice: BALB/c mice at 3–4 weeks of age were obtained from a LCMV-free colony of our institute (National Institute of Health). The spleens of wild house mice were homogenized with Eagle's minimum essential medium (MEM, Nissui Seiyaku). The homogenates were allowed to stand for 5 minutes without centrifuging. Then the supernatants were inoculated intracerebrally into mice. From day 7 to day 10 after inoculation, the brains of mice which showed nervous symptoms were harvested for passage to the next series of mice and cell cultures. Serum samples from sacrificed and surviving mice were also tested for anti LCMV antibody by IFA test.

Cell culture: Vero-E6 cells were used throughout the experiments. Ten to 20% brain homogenate in MEM was inoculated onto the cell culture. On day 7 after the incubation, a portion of the cells was subjected to IFA test with an immune hamster serum against WE strain of LCMV as a positive control as described previously [4].

RESULTS

Prevalence of the LCMV antibody in wild house mice in Osaka port: From January to March 1990, seven out of 27 mice (25.9%) captured from 3 piers in Osaka port were antibody positive to WE strain. Although 10 mice captured from the third pier were negative, two out of six mice (33.3%) from the central pier and five out of 11 mice (45.4%) from the first pier were positive.

In April 1990, 18 out of 35 mice (51.4%) captured from the central and the first piers were positive

Table 1. Individual data of mouse samples captured in Osaka port

Mouse sample	Pier	Sex	Body weight (g)	Age in months	Antibody titer	Isolation of virus		
						Mouse		Cell
						Mortality	Antibody	Antigen
OQ-27	Central	M	12	8.3	— ^{a)}	4/4	+	+
OQ-28	First	M	12	5.0	—	0/4 ^{b)} (0/5) ^{d)}	+	+
OQ-29	First	M	14	7.0	—	4/4	+	+
OQ-30	Central	F	12	9.2	—	0/4 (5/5)	+	+
OQ-31	First	F	15	7.0	—	2/4	+	+
OQ-32	First	M	8	2.0	—	2/4	+	+
OQ-33	First	F	21	21.5	512	— ^{c)}		
OQ-35	First	M	18	17.4	512	—		
OQ-36	First	M	14	11.8	128	—		
OQ-37	First	M	18	4.3	—	—		
OQ-38	First	F	10	1.8	—	0/4 (0/5)	+	+
OQ-39	First	F	13	3.3	1024	—		
OQ-40	First	F	20	3.7	—	—		
OQ-41	First	F	15	26.9	512	—		
OQ-42	First	M	10	1.3	128	1/4	+	+
OQ-43	First	F	18	13.8	—	—		
OQ-44	First	F	20	9.2	512	—		
OQ-45	Central	M	12	8.9	—	—		
OQ-46	Central	M	13	3.6	1024	—		
OQ-47	Central	M	12	7.8	—	4/4	— ^{e)}	+
OQ-48	Central	F	12	11.3	32	—		
OQ-49	First	F	8	1.4	512	4/4	ND ^{f)}	+
OQ-50	First	F	8	1.1	—	—		
OQ-51	First	M	16	9.9	1024	—		
OQ-52	First	M	5	1.4	—	0/4 (5/5)	+	+
OQ-53	First	M	10	1.9	512	0/4 (1/5)	+	+
OQ-54	First	F	14	6.2	64	—		
OQ-55	First	M	8	0.9	—	—		
OQ-56	First	F	19	6.1	64	—		
OQ-57	First	M	10	2.0	128	0/4 (4/5)	+	+
OQ-58	Central	F	13	5.1	1024	—		
OQ-59	Central	M	12	30.4	64	—		
OQ-60	First	M	13	10.9	128	—		
OQ-62	First	F	18	3.3	—	—		
OQ-63	First	M	22	18.4	—	0/4 (2/5)	+	+

a) 16>.

b) With symptoms.

c) Without symptoms.

d) Mortality at 2nd passage.

e) Collected from a moribund mouse on day 6.

f) Not done.

(Table 1). Between January and April, antibody positive ratio of mice from the central pier was 6/14 or 42.8%, while that of mice from the first pier was 19/38 or 50.0%. The difference was not significant. There was no significant difference between antibody positive ratio of female mice (12/21, 57.1%) and that of male mice (13/31, 41.9%).

Virus isolation: The spleen homogenates of 35 mice captured in April 1990 were inoculated into mice. Symptoms appeared on day 6 to day 8 after the inoculation. In typical cases, the mice showed ruffled fur, half-closed eyes and hunched backs. When the mice were suspended by the tail, the hind

limbs of the mice experienced tremors. Death occurred from 1 to 4 days after onset of the clinical disease.

LCMV was isolated from 14 mice (Table 1). Four of them were isolated from 18 antibody positive mice and 10 of them from 17 antibody negative mice. Although the isolation ratio of the virus from the central pier (3/8, 37.5%) was not differ from that of the first pier (11/27, 40.7%) and also that from female mice (4/16, 25.0%) was not differ from that of male mice (10/19, 52.6%), the isolation ratio of antibody positive mice (4/18, 22.2%) was significantly lower than that of antibody negative mice (10/17,

Table 2. Age distribution of captured mice in Osaka port

	Age (in months)					Total
	2>	2.0-3.9	4.0-7.9	8.0-12.0	12<	
Antibody positive	3 ^{a)} (3) ^{b)}	3(1)	3(0)	5(0)	4(0)	18(4)
Antibody negative	4(2)	4(1)	5(3)	3(2)	2(1)	17(10)
Total	7(5)	7(2)	8(3)	8(2)	6(1)	35(14)

a) Number of mice.

b) Number of mice from which LCMV was isolated.

Table 3. Antibody titers of inoculated mice against isolates

Sample inoculated	Days after inoculation	Antigen			
		WE	OQ-30	OQ-38	OQ-49
OQ-47	6 ^{a)}	16>	16>	16>	16>
OQ-27	7	512	512	512	512
OQ-28	7	512	512	512	512
OQ-29	7	128	128	128	128
OQ-30	7	256	256	256	256
OQ-31	7	256	256	256	256
OQ-32	7	128	128	128	128
OQ-63	8	256	256	512	256
OQ-38	9	1024	1024	1024	1024
OQ-52	9	1024	1024	1024	1024
OQ-53	9	1024	1024	1024	1024
OQ-57	9	2048	2048	1024	1024
OQ-42	15	4096	4096	4096	4096
Immune hamster ^{b)}		512	512	512	512

a) Moribund mouse.

b) Anti WE strain.

58.8%, $p < 0.05$).

Age of mice: In antibody positive mice, the virus was isolated only from those under 4 months of age (Table 2). However, virus was isolated from antibody negative mice of various age groups. Antibody-positive, virus-negative mice ($N=14$, 12.40 ± 8.56) were significantly older than those of antibody-negative, virus-positive mice ($N=9$, 5.50 ± 3.04 , excluding sample OQ-63 by using Smirnov's calculation, $p < 0.05$) and also older than antibody-positive, virus-positive mice ($N=$, 1.65 ± 0.35 , $p < 0.05$).

Pathogenicity of the isolates in mice: Although mice inoculated with 7 (OQ-28, OQ-30, OQ-38, OQ-52, OQ-53, OQ-57 and OQ-63) out of 14 samples from which the virus was isolated showed typical symptoms from day 7 after inoculation, their nervous symptoms were disappeared on the 12th

day, and the mice survived. The brain homogenates of these isolates were reinoculated into mice (Table 1). Five out of 7 isolates killed some of the inoculated mice. However, even after three serial passages in mice, the isolates, OQ-28 and OQ-38, did not kill any mice by intracerebral inoculation.

Antigenicity of isolates: Eighteen surviving mice (OQ-28:5, OQ-38:5, OQ-53:4, OQ-57:1 and OQ-63:3) after inoculation of the isolate at the 2nd mouse-passage mentioned above (Table 1) were challenged intracerebrally with WE strain of LCMV which killed the untreated mice within 8 days of inoculation. All surviving mice were resistant to the challenge with the WE stain. The serum titers of the mice at 3 weeks after the challenge inoculation became 16384 or more.

Antigens in the IFA test made from Vero-E6 cells infected with WE strain and three representative

isolates (OQ-38, OQ-57, and OQ-63) were compared by using sera from inoculated mice and an immune hamster serum against WE strain. There was no significant difference among antibody titers against the four antigens and antibody titers increased concomitantly with the time elapsed after the inoculation (Table 3).

DISCUSSION

Several viruses identical to LCMV were isolated from wild house mice in certain piers in Osaka port. These viruses were highly prevalent in the population of wild house mice in these areas. The origin of the virus is unknown, although mice obtained in this survey were identified as a mixed breed among the subspecies *M. m. mollossinus*, *M. m. domesticus* and *M. m. castaneus*. (Suzuki H. and Morita C. unpublished data). Since *M. m. domesticus* and *M. m. castaneus* are not indigenous to Japan, there is a strong possibility that virus positive mice could have come from outside Japan.

As a rule, neonatally or congenitally infected mice remained carriers for the rest of their life. Most antibody-negative, virus-positive mice were assumed to be carriers of the virus. From these mice, the uninfected mice could be infected within the first 4 months after birth because the virus had not been isolated from antibody positive mice older than 4 months. The experimentally induced carrier mice often show glomerulonephritis and other immune complex diseases within 12 months after the birth. The carrier in wild house mice would be short-lived than antibody-positive and virus negative mice.

Isolates OQ-28 and OQ-38 did not kill mice even

after 3 serial passages in mice. Both isolates were isolated from the same pier. The LCMV which resulted in low mortality amongst mice as reported by Wiktor *et al.* [5], was isolated from a cell culture system infected with a rabies virus. There is no record of the isolation of LCMV with low mortality from animals [3]. It is interesting that the isolates from the small endemic focus showed different pathogenicities against mice.

LCMV was successfully isolated from wild house mice in Osaka port. Endemic areas in the port are limited and no human cases were reported. However, more intensive studies on human populations should be pursued.

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