Seroepidemiological Survey of Lymphocytic Choriomeningitis Virus in Wild House Mouse (Mus musculus) in Yokohama Port, Japan

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ABSTRACT. From 1985 to 1989, a total of 129 mice was captured from 7 piers of Yokohama port. Of these, 9 (7.0%) were positive to lymphocytic choriomeningitis virus (LCMV) antigen in indirect fluorescence antibody test. Six out of 31 mice (19.4%) in 1985 and 3 out of 23 mice (13.0%) captured in 1986 were positive. All the mice (74) captured in 1988 and 1989 were negative. Although 7 out of 17 mice (41.2%) in Osanbashi-Shinko pier and 2 out of 23 (8.7%) in Honmoku pier were positive in 1985 and 1986, all mice captured in other piers were negative. This is the first report detecting LCMV antibody in wild house mice in Japan.——KEY WORDS: lymphocytic choriomeningitis virus, Mus musculus, wild house mouse.


Lymphocytic choriomeningitis virus (LCMV) is a member of family Arenaviridae. Each arenavirus is maintained in nature by one or two rodents species in which the virus persistently infected. In the case of LMCV, transmission to humans and transmission to colonies of laboratory rodents are occurred. LCMV was isolated in Japan from experimental mice and guinea pigs in the course of isolation study of Japanese B encephalitis more than 50 years ago [1, 2]. Recently, prevalence of the antibodies against LCMV in laboratory rodents has been reported [4]. Although house mouse (Mus musculus) are assumed as the natural reservoir of the virus, prevalence of LCMV in wild house mice was not investigated in Japan. In this study, we attempt to test the presence of antibody against LCMV in house mice captured in Yokohama port.

MATERIALS AND METHODS

Sera: House mouse sera were obtained from 7 piers of Yokohama port from February 1985 to December 1986 and from January 1988 to December 1989 (Fig. 1). Most of the serum samples were collected from mice by using blood sampling paper (Toyo-roshi, strip type). One tenth milliliters of blood was absorbed onto a paper. After drying, the blood was eluted into 0.6 ml of phosphate buffered saline (PBS). The eluted sera were assumed to be diluted by a ratio of 1:16.

Indirect fluorescence antibody (IFA) test: WE strain of LCMV and hamster immune serum against LCMV were kindly provided by Dr. H. Sato, Nagasaki University, School of Medicine. WE strain was inoculated onto Vero-E6 cells. After 3 days cultivation, the virus infected cells were trypsinized and washed 3 times with PBS and then 1–2 × 10⁴ cells in 10 µl were dispersed in each well of a microscopic slide (12 wells per slide). The slides were dried in a safety cabinet (class II) under UV light and then treated with cold acetone for 10 min. The slides were kept at −80°C until use. About 70% of the cells showed the antigen of LCMV by using the hamster immune serum in IFA test, the method of which was described elsewhere [3]. Fluorescein-isothiocyanate conjugated anti mouse or hamster Ig G goat serum (Cappel) at the dilution of 4 staining units, were employed in the IFA test. If the sample showed specific granular fluorescence in the cytoplasm of infected cells, the reaction was considered as positive. Antibody titers were expressed as the reciprocal of the dilution of the test serum. Titers of more than 16 were considered as positive.

Western blotting: The method used for Western blotting analysis was as described by Towbin et al. [6]. LCMV in supernatant of virus infected cell culture was initially purified by using polyethylene glycol and then by sucrose density gradient method. Purified virus suspension was mixed with an equal volume of sample buffer (0.05M Tris-HCl, 4.6% SDS, 20% glycerol, bromphenol blue), boiled for 5 min and then used as antigen. The antigen was
electrophoresed on 10% SDS-polyacrylamide gel and transferred to a nitrocellulose filter. The filters were incubated with 1:100 diluted test sera for 1 hr at room temperature, and then reacted with peroxidase conjugated anti-mouse or hamster Ig G goat serum (Cappel) for 1 hr at room temperature. The sera was considered as positive if a serum reacted to viral nucleoprotein (molecular weight: 63 K Dalton). The immune hamster serum was also employed as positive control.

Estimation of age in mice: The age of house mouse was estimated from the weight of the eyelens by using the method of Takada [5]. In brief, flowing fixation with 10% formalin, each pair of eye balls were rinsed in water and the lenses removed. Lenses were dried in an oven at 80°C for 1 day and then weighted. Age (in day) was calculated by following formula:

\[ Y = 4.130 \log X - 2.954 \]

Y: weight of a pair of lenses (mg), X: age in day

RESULTS

Prevalence of the antibody in Yokohama 1985–1986:

1985: Six out of 31 mice captured from 5 piers of Yokohama port were positive (19.4%) (Table 1). Five out of 7 mice captured in Osanbashi-Shinko pier (71.4%) and 1 out of 14 mice in Honmoku pier (7.1%) were positive. Ten mice from other 3 piers were negative. All the 5 positive mice in Osanbashi-Shinko pier were captured in the same warehouse and on the same day. However, the age of mice were ranged from 1.6 months to 19.2 months (Table 2). One mouse captured in the same warehouse (TU) 8 months before obtaining the 5 positive mice there, had been negative.

1986: Three out of 24 mice captured from 5 piers were positive (12.5%). Two out of 10 mice captured in Osanbashi-Shinko pier (20.0%) and 1 out of 9 mice in Honmoku pier (11.1%) were positive. Five mice captured from 5 other piers were negative. No mouse was captured in the warehouse (TU) where the 5 positive mice had been captured in 1985.

Prevalence of the antibody in Yokohama port 1988–1989: Forty nine mice and 25 mice captured from 4 piers (Osanbashi-Shinko, Honmoku, Shinyamashita, Yamashita) in 1988 and 1989 respectively were negative. The mouse captured in the warehouse (TU) was negative.

Confirmation of positive sera by using western blotting: Although visual density of reaction to the viral nucleoprotein was not associated to the antibody titer of each serum, all 9 IFA-positive sera
LCMV IN WILD HOUSE MOUSE IN YOKOHAMA

Table 1. Prevalence of LCMV antibody in mice captured from 6 piers of Yokohama port 1985–1986

<table>
<thead>
<tr>
<th>Year</th>
<th>Pier</th>
<th>Daikoku</th>
<th>Detamachi</th>
<th>Yamanouchi</th>
<th>Osanbashi</th>
<th>Honmoku</th>
<th>Yamashita</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1985</td>
<td></td>
<td>0/7</td>
<td>0/2</td>
<td>5/7</td>
<td>1/14</td>
<td>0</td>
<td>6/31</td>
<td></td>
</tr>
<tr>
<td>1986</td>
<td></td>
<td>0/2</td>
<td>0/9</td>
<td>2/10</td>
<td>1/9</td>
<td>0/2</td>
<td>3/24</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>0/2</td>
<td>0/9</td>
<td>7/17</td>
<td>2/23</td>
<td>0/2</td>
<td>9/55</td>
<td></td>
</tr>
</tbody>
</table>

a) Number of positive.
b) Number of tested.

Table 2. Individual data on nine positive house mice in Yokohama port

<table>
<thead>
<tr>
<th>Sample Mouse</th>
<th>Date</th>
<th>Pier</th>
<th>Warehouse</th>
<th>Sex</th>
<th>Body weight (g)</th>
<th>Age (in month)</th>
<th>Titer (IFA)</th>
<th>Number of Strip in Fig. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>100-85</td>
<td>12/09/85</td>
<td>Hon</td>
<td>CS</td>
<td>M</td>
<td>12</td>
<td>1.2</td>
<td>512</td>
<td>2</td>
</tr>
<tr>
<td>104-85</td>
<td>22/10/85</td>
<td>Os-Shi</td>
<td>TU</td>
<td>F</td>
<td>12</td>
<td>9.3</td>
<td>64</td>
<td>3</td>
</tr>
<tr>
<td>105-85</td>
<td></td>
<td></td>
<td>F</td>
<td>22</td>
<td>8.8</td>
<td>256</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>106-85</td>
<td></td>
<td></td>
<td>M</td>
<td>8</td>
<td>1.5</td>
<td>64</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>107-85</td>
<td></td>
<td></td>
<td>F</td>
<td>10</td>
<td>19.2</td>
<td>256</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>108-85</td>
<td></td>
<td></td>
<td>M</td>
<td>15</td>
<td>3.4</td>
<td>128</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>16-86</td>
<td>04/02/86</td>
<td>Hon</td>
<td>KS</td>
<td>M</td>
<td>20</td>
<td>11.0</td>
<td>512</td>
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<tr>
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<td>MS</td>
<td>F</td>
<td>20</td>
<td>9.4</td>
<td>1024</td>
<td>9</td>
</tr>
<tr>
<td>117-86</td>
<td>09/12/86</td>
<td></td>
<td>MB</td>
<td>M</td>
<td>16</td>
<td>11.3</td>
<td>128</td>
<td>10</td>
</tr>
</tbody>
</table>

a) Honmoku pier.
b) Osanbashi-Shinko pier.

Fig. 2. Confirmation of IFA-positive sera by western blotting.
reacted against the nucleoprotein confirming as positive results (Fig. 2, Table 2).

DISCUSSION

Nine out of 129 mice captured from 7 piers from 1985 to 1989 were positive against LCMV antigen (7.0%). Six out of 31 mice (19.4%) and 3 out of 23 mice (13.0%) were positive in 1985 and 1986, respectively. All the mice (74) captured in 1988 and 1989 were negative. Although 7 out of 17 mice (42.1%) in Osanbashi-Shinko pier and 2 out of 23 (8.7%) in Honmoku pier were positive in 1985 and 1986, the remaining mice captured in other piers were negative. Five out of 9 positive mice were captured in the same warehouse at the same time. These data clearly indicate that the foci of LCMV in Yokohama port was limited to certain area only. Further, endemicity of LCMV in mice in this area did not persist for a long time. Although, the ecology of house mouse in warehouses has not been studied, it can be speculated that the population of house mouse is depend on the kind of stocks in warehouses. The reason why we could not obtained enough samples from the warehouse where we captured 5 positive mice in 1985, may be due to changes of stocks in the warehouse. In 1988–1989, we could only obtain one sample which is negative.

Consequently existence of LCMV was revealed in house mice in Yokohama port. More intensive studies are necessary to clarify prevalence of the virus in Japan.

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REFERENCES