Serological Evidence of Lymphocytic Choriomeningitis
Virus Infection in Japan

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SUMMARY: Indirect fluorescent antibody method was applied for a detection of lymphocytic choriomeningitis virus (LCMV) antibody in colonies of laboratory animals in Japan. The results showed that the antibody exist in SPF mice (3/152, 2.0%) and conventional mice (30/539, 5.6%) with the titers ranging from 1:10 to 1:160. The antibody was also detected in 2.2% (2/89) of Syrian golden hamsters, and 2.9% (2/68) of Apodemus agrarius, 21.4% (3/14) of Japanese harvest mice which have been maintained as laboratory colony for several years. However, the antibody was not demonstrated in Mongolian gerbils, Suncus murius, guinea pigs and rats thus far. These results indicate that LCMV infection is present in laboratory animals in Japan, and pointed out the importance of microbiological monitoring for LCMV.

INTRODUCTION

Lymphocytic choriomeningitis virus (LCMV) is a member of the arenavirus group, and is well-known as one of viral zoonoses associated with three clinical forms, i.e., influenza-like symptoms, meningitis, and meningo-encephalomyelitis. The virus was first isolated from a patient with acute aseptic meningitis by Armstrong and Lillie in 1934. House mice (Mus musculus) is considered as a natural reservoir of the virus. Laboratory mice are easily infected with LCMV from wild mice and the infection persists in colonies of laboratory mice. Hamsters have also high susceptibility to LCMV and become carrier. Human infections from these rodents have been reported. In the United States human infections with LCMV occurred by contact with pet hamsters and Syrian golden hamsters (Mesocricetus auratus) in biomedical laboratory colonies. Recently a case of human infection possibly caused by an exposure to house mice was reported in the United States.

When immunocompetent mice and guinea pigs are experimentally infected with the virus, acute clinical signs including humped posture, chronic convulsive seizures, tonic extension of hind limbs, and lethargy are frequently observed. However, in colonies of laboratory rodents natural infection usually occurs at newborn stage and easily tends to lead to persistence of virus or slow, inefficient clearance, and chronic or life-long shedding in urines without showing acute signs of disease. Thus, control of LCMV is important from both standpoints of zoonosis control and of microbiological monitoring of laboratory rodents.

The contamination of wild and laboratory rodents has been reported in various countries. However, the situation of LCMV contamination in Japanese laboratories has been unknown. In this paper, we report that the presence of LCMV antibody in laboratory and field rodents is a crucial key-point for preventing the human cases in the same way as the virus of hemorrhagic fever with renal syndrome (HFRS) in Japan. Among several serological methods, currently available for the detection of specific LCMV antibodies, i.e., indirect fluorescent
antibody (IFA) test, complement fixation test, fluorescent foci inhibition test, radioimmunoassay, IFA test was employed in the present study because of its safety procedure as well as high specificity and sensitivity.

MATERIALS AND METHODS

Virus: WE strain of LCMV was obtained from Dr. R. Mori, Faculty of Medicine, Kyushu University, Fukuoka, Japan. A stock of the virus was prepared by passing three times in newborn hamster brains, and showed $10^8$ LD$_{50}$ /0.02ml when inoculated intracerebrally (i.c.) into 3 week-old mice.

Indirect fluorescent antibody (IFA) assay: L cells grown in Eagle's minimum essential medium supplemented with 5% heat-inactivated fetal bovine serum were infected with the stock of LCMV at a multiplicity of infection of 1 and incubated for 2 days at 37 C in 5% CO$_2$-95 % air. The virus-infected and normal L cells were trypsinized, and $5 \times 10^5$ cells/ml of the infected cells and $2 \times 10^5$ cells/ml of the normal cells were mixed in 0.01M phosphate buffered saline (PBS). Ten microliters of the cell mixture was dropped into each well of 3 mm diameter in the spot slides (Fuji Glass Co., Fukuoka, Japan) and air-dried for 1 hr at room temperature. After fixation with cold acetone for 20 min., the slides were dried and stored at-80 C until use. In a part of this study, the virus which was plaque-purified four times was employed. Antibody titration was conducted as follows. The slides were washed in deionized water for 3 min., air-dried and covered with two-fold dilutions of test sera in PBS (0.01M, pH 7.2). For a screening of antibody the dilution of 1 : 10 and 1 : 40 were examined. The slides were rinsed three times with PBS for 3 min. each and thereafter the spots were filled with fluorescein conjugated anti-IgG (heavy & light chains) rabbit serum (Cappel Laboratories Co., U. S. A.). An anti-mouse serum was applied for the sera of Suncus murinus, Japanese harvest mouse, and Apodemus agrarius. On the other hand, anti-rat serum was employed to the serum of Mongolian gerbil, considering the cross-reactivity. The dilutions of these antisera were determined by Ouchterlony double diffusion test using 0.35% agarose in PBS before the following experiments. After incubation at 20-25 C in a moisture chamber for 60 min., the slides were washed and mounted with 90% glycerin in carbonate buffer (pH 9.0) and observed under fluorescent microscope (Olympus Optical Co., Tokyo, Japan). An antibody titer more than 1 : 10 was taken as positive one.

RESULTS AND DISCUSSION

Specific patterns of LCMV-IFA: Typical patterns of localization of virus antigen demonstrated by the virus-infected hamster serum are shown in Fig. 1; granular antigens were observed mainly in the cytoplasm of the virus-infected L cells. The specificity of the IFA test was examined by using the antisera against Sendai virus, reovirus type 1 to 3, HFRS (76-118) virus. As a positive control, two convalescent human sera of LCMV infected humans which were obtained from Dr. Lehmann-Grube, Heinrich-Pette Institute for Experimental Virology and Immunology, Hamburg University, West Germany, was employed. None of these antisera showed specific fluorescence except anti-LCMV human sera which detected intracytoplasmic virus antigen at dilutions of 1 : 160, and 1 : 320. The specificity of IFA test was further confirmed by the use of plaque-purified LCMV and a blocking test, although the virus-infected hamster serum was not completely blocked against positive animal.

Fig. 1. Mixtures of LCMV-infected and uninfected L cells were stained with anti-LCMV hamster serum diluted 1 : 256. LCMV-infected cells showed typical fluorescence with the granular antigens in the cytoplasms.
serum, because of the nature of anti-LCMV antibody reported as low affinity and avidity.\(^\text{10}\)

**LCMV antibody in the sera of laboratory animals**: The sera of various species of laboratory animals collected from several colonies were examined for anti LCMV antibody. The results are shown in Table 1. The antibody was detected in 2.0% of SPF mice and 5.6% of conventional mice with titers ranging from 1:10 to 1:160. The antibody was also demonstrated in 2.2% of Syrian golden hamsters, and 2.9% of Apodemus agrarius, 21.4% of Japanese harvest mice which have been maintained as laboratory colony for several years in Japan. It is noteworthy that nine conventional mice, two Apodemus agrarius and one Japanese harvest mouse showed high antibody titer of 1:160. The antibody was not detected in Mongolian gerbils, Suncus, guinea pigs and rats, thus far.

LCMV infection in animal sera and humans in Japan has not been reported. Although more comprehensive analysis of the mode of LCMV infection in breeding colonies is required, these results in this paper were clearly indicate that LCMV infection analyzed with serological method is present in laboratory animals in Japan.

The presence of the antibody in SPF mice points out the importance of microbiological monitoring in our country as well as U.S.A. or Europe countries. Furthermore, special attention has to be paid for possible human infection with LCMV similar to that in the Western countries.\(^\text{3}^\)

### REFERENCES


### Table 1. Prevalence of anti-LCMV antibody in the sera of laboratory animals*

<table>
<thead>
<tr>
<th>Animals</th>
<th>Antibody titer††</th>
<th>Positive rate (%)</th>
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<tbody>
<tr>
<td></td>
<td>&lt;10 10 20 40 80 160</td>
<td></td>
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<tr>
<td>Mouse</td>
<td></td>
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<tr>
<td>SPF</td>
<td>149 1 1 1 3/152 (2.0)</td>
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<tr>
<td>Conv.</td>
<td>510 3 5 6 7 9 30/539 (5.6)</td>
<td></td>
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<tr>
<td>Syrian golden hamster</td>
<td>87 1 1 2/89 (2.2)</td>
<td></td>
</tr>
<tr>
<td>Mongolian gerbil</td>
<td>46</td>
<td>0/46</td>
</tr>
<tr>
<td>Apodemus agrarius</td>
<td>66 2 2/68 (2.9)</td>
<td></td>
</tr>
<tr>
<td>Suncus murinus</td>
<td>17</td>
<td>0/17</td>
</tr>
<tr>
<td>Japanese harvest mouse</td>
<td>11 1 1 3/14 (21.4)</td>
<td></td>
</tr>
<tr>
<td>Guinea pig</td>
<td>31</td>
<td>0/31</td>
</tr>
<tr>
<td>Rat</td>
<td>70</td>
<td>0/70</td>
</tr>
</tbody>
</table>

* Laboratory-bred animal sera were obtained from conventional animal except parts of mice and rats.
** Reciprocal highest dilution of serum.