— Note —

Detection of Lymphocytic Choriomeningitis Virus Antibody in Colonies of Laboratory Animals in Japan

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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, 2.2%) 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 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 murinus*, 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.

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 meningoencephalomyelitis [1]. The virus was first isolated from a patient with acute aseptic meningitis by Armstrong and Lillie in 1934 [2]. 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 hamsters (*Mesocricetus auratus*) in biomedical laboratory colonies [3, 4]. Recently a case of human infection possibly caused by an exposure to house mice was reported in the United States [5].

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 [6]. 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 [7]. 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 animals in Japan, indicating the contamination with Arena virus group similar to Europe and North America [5].

A monitoring the antibody level to LCMV in laboratory and field rodents is a crucial key-point for preventing the human cases in the

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 cytoplasm.

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 [8, 9].

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 LD₅₀/0.02 ml when inoculated intracerebrally (i.c.) into 3 week-old mice. 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% CO₂ 95% air. The virus-infected and normal L cells were trypsinized, and 5×10^5 cells/ml of the infected cells and 2×10^5 cells/ml of the normal cells were mixed in 0.01 M phosphate buffered saline (PBS). Ten microliter 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 (0.01 M, 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 agarius*. 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% glycerine 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.

Typical patterns of virus antigen localization demonstrated by the virus-infected hamster serum are shown Fig. 1; granular antigens

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

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

* Laboratory-bred animal sera were obtained from conventional animals except parts of mice and rats.

** Reciprocal highest dilution of serum.

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 serum, because of the nature of anti-LCMV antibody reported as low affinity and avidity [10].

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 2.2% 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. These results were clearly indicate that LCMV infection is present in laboratory animals in Japan. More comprehensive analysis of the mode of LCMV infection in breeding colonies is required.

The presence of the antibody in SPF mice points out the importance of microbiological monitoring for LCMV. Furthermore, special attention has to be paid for possible human infection with LCMV similar to that in the Western countries [7].

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リンパ球性脈絡髄膜炎 (LCM) ウイルス抗体の検出

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我国の実験小動物コロニーにリンパ球性脈絡髄膜炎ウイルス (LCMV) の感染が存在するか否かを蛍光抗体間接法を使用して検索した。その結果, SPF マウスで 3/152 (2.2%), コンベンショナルマウスで 30/539 (5.6%) の抗体陽性を確認し, 抗体価は 1:10-1:160 の分布を示した。またシリアンハムスターならびに捕獲後飼育室で繁殖させたセズネズミ, カヤネズミの抗体保有率

もそれぞれ 2/89 (2.2%), 2/68 (2.9%), 3/14 (21.4%) を示した。一方, スナネズミ, スンクス, モルモット, ラットにおいては今回の調査範囲では抗体陰性であった。以上の結果より, 我国の実験小動物の一部に LCMV の感染があることが示唆され, 今後, 小動物の微生物学的モニタリングやヒト髄膜炎患者に対する検査体制の確立が望まれる。