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ELISA AND INDIRECT IMMUNOFLUORESCENCE IN THE DIAGNOSIS OF LCM VIRUS INFECTIONS

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Summary. – Thirty-seven matched samples of patient sera with the clinical diagnosis of a lymphocytic choriomeningitis (LCM) infection, as well as 56 matched samples of patient sera with the clinical diagnosis of a CNS infection of vague etiology were examined. Two serological techniques, indirect immunofluorescence (IF) and ELISA were used. They revealed 16.2 % of positive sera confirming the clinical diagnosis of the disease; in the cases of clinical diagnosis of CNS infection of vague etiology 8.9 % of positive sera were found, which points to an LCM virus-caused infection.

Key words: immunofluorescence; ELISA; lymphocytic choriomeningitis

Introduction

The disease caused by the LCM virus is one of the zoonoses. Domestic mice (*Mus musculus*) which excrete the virus through urine and faeces, thus polluting the environment, are the most common source of human LCM infection. The route of infection in humans is via the respiratory tract. Since the infection in mice is permanent, with females transmitting the virus to their progeny, there is a constant health hazard to humans of becoming infected with the virus. Other rodents may also be the source of human infection. There have been reports of epidemics in the U. S. A. with the Syrian hamsters, which served as laboratory animals or were kept as pets (Biggar *et al.*, 1975; Deibel *et al.*, 1975; Gregg, 1975; Hinman *et al.*, 1975; Hotchin *et al.*, 1975*a*), as the source of infection. In man the infection is manifested as a mild form of encephalitis. Although some 50 % of the infections are unaccompanied by symptoms, in 15 % of the cases symptoms of CNS infection do occur (Lehmann-Grube, 1971).

Until now, complement fixation (CF) reaction has been used in our serological diagnosis of LCM. However, CF is known not to be invariably reliable in the serological diagnosis of this disease. In LCM the antibodies fixing the complement take as long as 3–4 weeks to occur after the infection and are only present in the serum for a relatively short time (Hotchin *et al.* 1975*b*; Lewis *et al.*, 1975). Cohen *et al.* (1966) have isolated LCM virus from the cerebrospinal liquor of

some patients, but the sera of these patients were CF-negative. Thus the present study attempts to improve the quality and rapidity of laboratory diagnosis of LCM by means of new serological methods (ELISA, IF).

Materials and Methods

Sera originated from the patients admitted at Dr. F. Mihaljevic, Infectious Disease Teaching Hospital, Zagreb. Of a total of 93 matched serum samples, 37 were from the clinically diagnosed cases of LCM virus infection, 56 deriving from the cases of CNS with vague etiology.

Virus. The WE 123 LCM virus strain, which originated from the Virus Reference Laboratory of Central Public Health Laboratory, Colindale was used in preparing the antigen. The strain was obtained as a lyophilized 10 % suspension of brains of mice from a new litter.

Cell culture. Antigens were prepared in BHK-21 cell line cultures. The cell were cultivated at 37 °C in MEM 99 medium with 10 % of inactivated foetal serum.

Antigen for IF test. A suspension of infected mouse brains was adsorbed onto a grown layer of cells. The cells were infected with 10^3-10^5 LD_{50} in 1 ml per Demeter's bottle. The virus adsorption lasted 60 min at 37 °C, then Eagle's MEM medium with 5 % foetal serum was added and cultures were incubated till the occurrence of cytopathic effect (CPE), which was usually 3–4 days after the infection. The cells were then removed, resuspended in phosphate borate buffer (PBS) pH 7.2 and re-centrifuged. Cell elution was repeated thrice. After the last centrifugation, buffer was poured off and cells were resuspended in the medium without foetal serum. 20 μl aliquots thus resuspended cells were placed on microscopic slides at concentration 1–2x10^6/ml. Slides were dried at room temperature and fixed for 10 min in acetone cooled to –20 °C. Cells fixed in this way were used as the antigen for the indirect IF test.

Antigen for ELISA test. After the infected monolayers of BHK-21 cells showed the CPE medium was decanted and inactivated with gamma irradiation (2x10^7 rad, 10 sec). Virus was then concentrated and partially purified by sucrose density gradient centrifugation (Gschwender *et al.*, 1975).

IF test (indirect method). 20 μl of twofold serial dilutions of sera (1:8 – 1:256) were placed onto microscopic slides with fixed antigen and incubated at 37 °C for 30 min. The slides were then eluted 5 times with PBS over a 10 min periods. After the elution, antihuman IgG and fluoresceinisothiocyanate-labelled IgM in a 1:10 dilution (Torlak, Belgrade), were placed into the antigen-antibody complex and incubated anew for 30 min. After two elutions with PBS and contrast-staining with Evans blue for 10 min the preparations were viewed under a fluorescent microscope at 10x40 magnification.

ELISA test. Antigen designed for the test was diluted to 1:200 in a coating buffer (0.05 mol/l sodium carbonate, pH 9.5), transferred into microtiter plates and incubated at 37 °C for 1 hr. The plates were then eluted 4 times in PBS with 0.05 % Tween-20. After the elution 100 μl of tested serum was added into the wells and incubated for 1 hr at 37 °C. The incubation was followed by eluting the plates 4 times with PBS and by adding the antihuman IgG or IgM labelled with alkaline phosphatase (Orion Diagnostica, Helsinki), which was diluted in ELISA buffer to 1:200. After incubation at 37 °C for 1 hr and another elution, 100 μl of p-nitrophenylphosphate (Sigma) diluted in 1 mol/l pH 9.8 diethanolamine buffer with 0.5 mmol/l $MgCl_2$ was added to each well. The reaction developed for 20 min at room temperature, stopped by adding 1 μl 3 mol/l NaOH and the A_{405} read in automatic Kontron SE 100 reader (Wolley *et al.*, 1976; Niklasson *et al.*, 1983; Niklasson *et al.*, 1984).

Results

The results of serological tests are shown in Tables 1 and 2

Six (16.2 %) of the 37 cases of clinically diagnosed LCM virus infection were

Table 1. The results of serological tests on matched sera of patients with the clinical diagnosis of LCM virus infection

Patient	Serum sample	IgG antibody titer		IgM antibody titer	
		IF	ELISA	IF	ELISA
P.S.	1st	16	200	8	100
	2nd	32	1600	-	-
T.M.	1st	16	400	16	200
	2nd	16	400	-	-
V.S.	1st	16	200	8	200
	2nd	32	1600	-	-
M.K.	1st	16	400	32	1600
	2nd	64	1600	-	-
S.A.	1st	8	200	16	800
	2nd	16	400	-	-
B.D.	1st	16	200	16	400
	2nd	16	400	-	-

Table 2. The results of serological tests on matched sera of patients with the clinical diagnosis of CNS infection of vague etiology

Patient	Serum sample	IgG antibody titer		IgM antibody titer	
		IF	ELISA	IF	ELISA
A.Z.	1st	16	200	8	100
	2nd	32	400	-	-
F.N.	1st	32	1600	16	400
	2nd	32	1600	-	-
S.D.	1st	16	200	neg.	200
	2nd	32	1600	-	-
T.V.	1st	16	400	8	200
	2nd	64	3200	-	-
M.K.	1st	32	800	32	3200
	2nd	32	1600	-	-

positive in both tests. IgG antibody titer levels in the IF test ranged from 1:8 to 1:16 in the first and from 1:16 to 1:64 in the second serum samples. In ELISA test, these antibody titers ranged from 1:200 to 1:400 and from 1:400 to 1:1600 in the first and second serum samples, respectively. In the positive first serum samples both tests demonstrated the presence of IgM antibodies as well.

Five (8.9 %) of the 56 cases with the clinical diagnosis of CNS infection of vague etiology were serologically positive in both tests. In IF, IgG antibody titer levels ranged from 1:8 to 1:32 in the first and from 1:32 to 1:64 in the second serum samples. The presence of this class of Ig was also demonstarted by the ELISA test, where titer levels ranged from 1:200 to 1:1600 in the first and from 1:400 to 1:3200 in the second serum samples. These patient's first serum samples were also examined for the presence of IgM class antibodies. While the ELISA test demonstrated their presence in all 5 sera, IF did so in 4 sera only.

Discussion

The present investigation shows that the IF and ELISA tests are almost equally sensitive in detecting specific antibodies in LCM. All sera except one (patient S. D., Table 2) that were positive in IF were also positive in ELISA test. In S. D. serum IgM antibodies could be demonstrated with ELISA only, which seems to support the argument that ELISA is the more sensitive of the two assays. Nevertheless, one cause of the conflicting results of these two methods might be the variability of reading the reactions in these tests.

With regard to IF sensitivity our findings are in agreement with the literature data. Lewis *et al.* (1975) and, independently, Cohen *et al.* (1966) have compared the IF, CF and neutralization test (NT) in respect of the methods sensitivity in detecting LCM-specific antibodies. Their reports show that IF permits the earliest demonstration of antibodies in this disease, i.e. already in the first week of illness or 3 days after the appearance of symptoms. Complemet-fixing and neutralizing antibodies occur later.

Lewis *et al.* (1975) examined serologically a group of cases in which it was possible to determine the onset of disease with some degree of precision. Of the 39 serum samples (acute and convalescent) which were IF-positive, 6 were CF-negative and 31 NT-negative. In all the cases serologically positive in the IF test, neutralizing antibodies were also demonstrated in the subsequent course of the disease. In two patients they monitored serological response 10 months after the appearance of symptoms and, throughout the monitoring, they were able to prove the presence of specific antibodies in the serum with both IF and ELISA.

In the serological diagnosis of LCM, some researches (Hotchin *et al.*, 1987*5c*; Lehmann-Grube and Ambrassat, 1977) have used the plaque reduction methods (RP) which is as sensitive and specific as NT, but much faster. RP takes 4 days to do, a big advantage over NT which takes 3 weeks.

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