PERSISTENT INFECTION WITH MOUSE HEPATITIS VIRUS, JHM STRAIN IN DBT CELL CULTURE

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SUMMARY

After inoculation with JHM strain into DBT cell monolayers, a persistently infected DBT cell culture was established without producing typical cytopathic changes after about 15th passages. By immunofluorescence virus specific antigen was demonstrated in 10 to 15% DBT cells. This persistently infected culture (JHM-CC) was resistant to superinfection with parental JHM, but such resistance was not shown against vesicular stomatitis virus. JHM-CC virus produced small plaques on DBT cell monolayers. Temperature sensitive (TS) mutant, defective interfering (DI) particle or interferon was not detected in the JHM-CC. To intracerebral inoculation with JHM-CC virus, cortisone treated ICR mice survived without showing clinical signs, however, demyelinating lesions were produced in the brain and spinal cord of them.

INTRODUCTION

Mouse hepatitis virus (MHV), a member of coronavirus group, is known to cause hepatitis, encephalitis or enteritis in mice (Robb and Bond, 1978). Among MHV strains, JHM has strong neruotropism characterized by acute and chronic demyelinating encephalitis in mice. Recently, Stohlman and Weiner (1978) reported a persistent infection of JHM in neuroblastoma cells, suggesting that it may provide useful model for studying the mechanism of chronic infection in animal and man.

This study deals with the establishment of carrier culture and some properties of viruses originating from the culture.

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MATERIALS AND METHODS

Virus and cell culture : Plaque-purified JHM was propagated in DBT cells and infectivity was assayed by the method as reported previously (Hirano et al, 1974, 1978). DBT cells were grown in Eagle's minimum essential medium (MEM) containing 10% calf serum and 10% tryptose phosphate broth. For virus harvesting and cell maintenance, serum content was reduced to 5%.

Immunofluorescence : Direct immunofluorescence was performed using fluorescein isotyiocyanate-conjugated rabbit IgG against MHV-2 (Hirano et al., 1978). The samples were treated with conjugated antiserum at 37 C for 1 hr.

Interferon assay : Interferon assay was performed by a plaque reduction test using vesicular stomatitis virus (VSV, New Jersy strain) and L cell system as reported previously (Taguchi et al, 1976).

Animal inoculation : Four-week-old male ICR mice were obtained from a breeder colony which was serologically checked for the absence of MHV infection (Fujiwara, 1969). Mice were inoculated intracerebrally (i.c.) with 0.02 ml of virus material, and some mice were injected subcutaneously with 2.5 mg cortisone acetate shortly after virus inoculation.

RESULTS

Establishment and some characterization of a persistently infected DBT cell line : Within 12 hr postinoculation (p.i.) with original JHM, cytopathic effect (CPE) with syncytium formation was evident on DBT cell monolayers and most cells became detached from the glass. However, a few cells still remained and appeared normal in morphology. These remaining cells grew up after changing culture medium and formed a new monolayer. When about 50 to 70% of cell monolayer was established on the glass, passage was done, and syncytium formation was observed in a part of newly established monolayers. After 16th or more passages, syncytium was never formed, and this cell culture was designated as JHM-CC.

By direct immunofluorescence about 10 to 15% cells of the JHM-CC monolayer were found to have virus specific antigen. Especially, strong fluorescence was observed in cells characterized by rounding appearance.

When the culture fluid of JHM-CC was inoculated into a fresh DBT cell monolayer, smaller plaques than those produced by parental JHM were produced in DBT cells. The sizes of plaques formed by JHM-CC virus and JHM were 0.5 to 0.8 and 2 to 4 mm in diameter, respectively (Figure 1). Virus yield was not changed during 80 to 110th passage levels. Figure 2 showed yield of small plaque forming viruses from JHM-CC at the 110 to 130th passage levels.

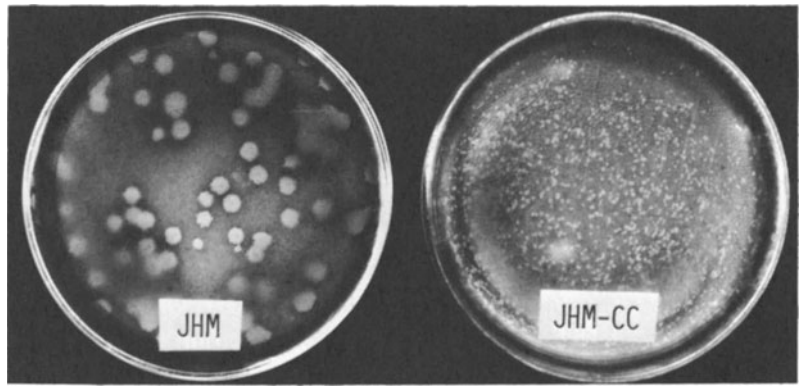


Figure 1. Plaques produced on DBT cell monolayer by JHM and JHM-CC virus at 48 hr p.i.

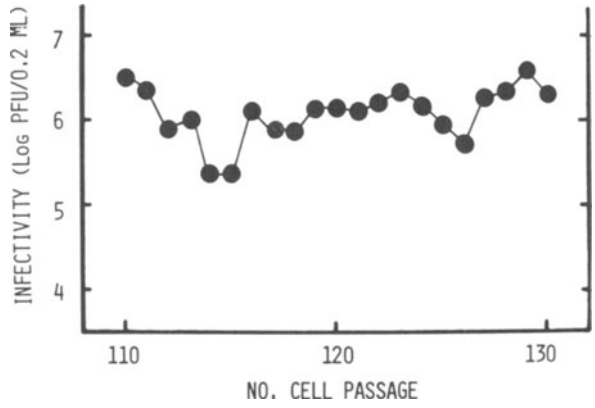


Figure 2. Yield of small plaque forming viruses from the JHM-CC at 110 to 130th passage levels.

JHM-CC monolayers as well as DBT cell monolayers which had been inoculated with JHM (3 PFU/cell) were incubated at different tempertures, 31, 37 and 39 C, and the infectivity of culture fluid was examined at the same temperatures as test incubation at 24 and 72 hr p.i. on DBT cells. As shown in Table 1, there was no difference in virus yield between given temperatures in JHM-CC as well as JHM-infected DBT cells, suggesting that JHM-CC virus is not a temperature sensitive mutant.

From the results obtained, it was evident that JHM-CC virus was less virulent to DBT cells than parent JHM.

To see neurovirulence of JHM-CC virus for mice, 4-week-old ICR

Table 1. Effect of temperatures on virus growth

Virus		Temperature 31	37	39
JHM*	exp.1	6.5**	6.8	6.7
	exp.2	6.5	6.4	6.5
JHM-CC***	exp.1	5.6	5.3	5.5
	exp.2	5.4	5.5	5.3

* JHM harvested at 24 hr p.i. ** Log PFU/0.2 ml
*** JHM-CC harvested at 72 hr p.i.

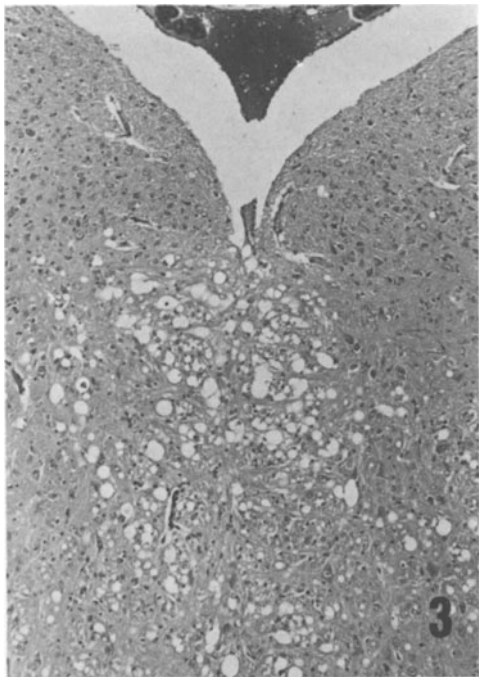
mice were inoculated i.c. with 10^3 or 10^4 PFU of JHM-CC virus as well as JHM virus. As shown in Table 2, within 3 to 5 days p.i., most of JHM-inoculated mice developed typical central nervous system disorders and died of encephalitis within 10 days. After inoculation with 10^3 to 10^4 PFU of JHM-CC virus, all mice survived for 14 days without any clinical signs even with cortisone treatment. No virus was recovered from the brains of JHM-CC inoculated mice which were sacrificed at 14 days while histopathologically some demyelinating lesions were found in the brain and spinal cord of the survived mice as shown in Figure 3. After inoculation with 10^5 PFU of JHM-CC virus, extensive demyelination was found in the brain stem and cerebellum of the inoculated mice killed at 4 weeks p.i. as shown in Figure 4.

Table 2. Neurovirulence of JHM and JHM-CC virus for 4-week-old mice

Virus	Dose (PFU)	Mortality	Time to death
JHM	10	3/5*	8.3 (7-9)**
	10^2	5/5	8.4 (6-9)
JHM-CC	10^3	0/5	-
	10^4	0/5	-
	10^4 (C)***	0/5	-

* No. dead/tested, 14 days p.i
** Mean time to death in days with range in parenthesis
*** 2.5 mg cortisone administration shortly after virus inoculation.

Resistance of JHM-CC to superinfection : The monolayers of JHM-CC and uninfected DBT cells were inoculated with JHM or other MHV strains (3 PFU/cell). As shown in Table 3, although typical CPE was produced in control DBT cells within 12 hr p.i., no change was detected for 48 hr in the superinfected JHM-CC with JHM or other



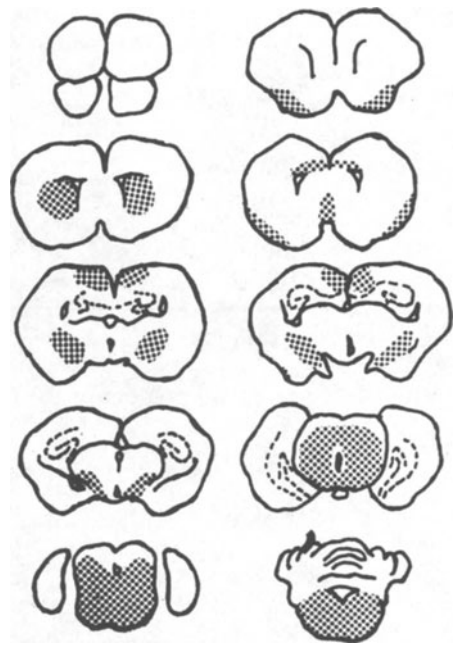


Figure 3. Demyelinating lesions in midbrain of the survived mice after inoculation with 10^4 PFU of JHM-CC virus. Killed at 14 days p.i.

Figure 4. Distribution of demyelinated lesions in the mice inoculated with 10^5 PFU of JHM-CC virus. Killed at 4 weeks p.i.

MHV strains. No progeny of the challenged MHV was recovered from the superinfected JHM-CC, whereas the control DBT cell culture yielded more than 10^6 PFU of MHV after challenge. These findings suggest that JHM-CC interfers with the growth of challeged MHV.

Next, JHM-CC was tested for susceptibility to VSV challenge. JHM-CC and DBT cell monolayers were inoculated with VSV at different doses. After virus inoculation, complete CPE was observed in both JHM-CC and DBT cells, and about 10^7 PFU of VSV were recovered from the both inoculated cultures irrespective of inoculum doses at 24 hr p.i. (Table 4).

Interferon activity was not detected in JHM-CC by assay using VSV and L cell system.

To see the existence of DI particles, DBT cell monolayers were inoculated simultaneously with both JHM virus (3 PFU/cell) and virus from JHM-CC (2 PFU/cell). As control, another group of DBT cell monolayers received a single inoculation with the same dose of JHM. After virus inoculation at 37 C for 1 hr, the inoculated cultures were washed with MEM, given maintenance medium and then incubated

Table 3. Resistance of JHM-CC to superinfection with MHV

Challenge with	CPE		Yield	
	DBT	JHM-CC	DBT	JHM-CC
MHV-1	+	-	6.8*	<1.0
MHV-2	+	-	7.3	<1.0
MHV-3	+	-	6.8	<1.0
JHM	+	-	6.6	<1.0
MHV-A59	+	-	6.8	<1.0

* log PFU/0.2 ml

Table 4. Susceptibility of JHM-CC to superinfection with VSV

VSV (PFU/cell)	Yield	
	DBT	JHM-CC
100	7.5*	6.9
10	7.7	7.0
1	7.7	7.2

* log PFU/0.2 ml

at 37 C for 12 or 24 hr. Within 12 hr p.i. typical syncytium formation was found in the cultures inoculated with two viruses and no difference was seen in yield of JHM virus between single or double infected cultures as shown in Table 5. This suggests that there exists no DI particles in the JHM-CC virus stock.

Table 5. Co-infection of JHM and JHM-CC viruses on DBT cells

Virus (PFU/cell)	Yield	
	12 hr	24 hr p.i
JHM (3)	5.7*	4.1
JHM (3) + JHM-CC (2)	5.8	4.4

* log PFU/0.2 ml

DISCUSSION

The presistent infection seems to result from multiple factors involved in the virus-cell interaction such as the generation of TS mutants (Preble and Younger, 1975), formation of DI particles (Huang and Baltimore, 1970), presence of some virus inhibiting factots (ter Meulen and Martin, 1976) or induction of interferon. However,

the persistent infection established with JHM in JHM-CC seems not due to the presence of DI particles, TS mutants or interferon.

Virus specific antigen was found in only 10 to 15% cells of JHM-CC through the experiments. The persistently infected "carrier culture" was divided into three types by Walker (1964), and the persistent case of JHM-CC seems to correspond to that in which the infection is perpetuated in a minority of cells while the most cells are resistant.

JHM-CC virus produced smaller plaques than parent JHM virus in DBT cells and it was less virulent for mice. The correlation between virus plaque size and virulence is well documented with pair of plaque size mutants from a patental stock (Takemoto, 1966). In this case, smaller plaque mutants tend to be less virulent, as shown in this study.

Recently, Stohlman and Weiner (1978) established a persistent infection of JHM in murine neuroblastoma cells and stated that the carried viruses capable causing acute encephalitis were not TS nor plaque mutants. Weiner (1973) described that fatality of JHM infection in mice was dependent upon host age and inoculum dose and that demyelination frequently occurred in non-fatal infection. JHM-CC virus never produced acute encephalitis which is common in parental JHM infection and demyelination occurs in chronic stage of mice that have shown no clinical signs of acute phase.

The persistent infection in JHM-CC in vitro and in vivo system would be helpful in studying the mechanism of chronic and persistent infection with human and animal coronaviruses.

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