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Characterization of Low-virulent Mouse Coronavirus Isolated from Faeces in a Mouse Colony

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With 5 figures and 2 tables

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Summary

A virus which produced cytopathic effect with syncytium formation was isolated from the faeces of apparently healthy mice by mouse brain tumour cell culture, DBT cells. The isolate was virologically and serologically identified as mouse coronavirus. It grew well in DBT cells, and formed clear plaques much smaller in size than those of other low-virulent strains. In experimental infections, the isolate could produce only a mild hepatitis in cortisone-treated weanling mice. Fatal hepatitis was not observed even in mice treated with 10 mg cortisone; whereas many mice were killed by other low-virulent strains.

Key words: Coronavirus, mouse, plaque formation, experimental infection

Introduction

Mouse coronavirus (murine hepatitis virus; MHV), a member of the family Coronaviridae (23), is one of the most common pathogens of laboratory mice. The virus has many antigenically related strains or isolates and can cause significant diseases such as acute hepatitis, encephalomyelitis, infant diarrhoea and wasting syndrome in athymic nude mice. However, MHV strains vary greatly in their pathogenicity, and the clinical course of the infection depends on the virus strain, route of infection and many host factors (1, 2). Under natural conditions, MHV infection seems to be subclinical in most cases. The virus is highly contagious and probably transmitted by respiratory or oro-faecal routes (1, 2, 13, 24). Concerning the latter route, enteric infection of MHV strains (4–6, 9, 18, 22) and virus excretion in faeces (13, 22) have been reported.

Recently, we have encountered an MHV infection of possible low-virulent strain (20). Since faeces is one of the suitable specimens for the virus isolation from apparently healthy mice (13), we further examined faecal samples and characterized the isolate.

Material and Methods

Viruses and cells: SR-CDF$_1$-DBT (DBT) cells (19) were used throughout the examination. Virus isolation from mouse faeces was performed as described elsewhere (13). Faecal samples were obtained from a mouse colony with the incidence of MHV infection (20). Prototype MHV strains MHV-1 (8), JHM (7), MHV-S (22), recent isolates of low-virulent strains MHV-NuU (16), MHV-D (18), MHV-N (13), and the present isolate were passaged on DBT cells and assayed for infectivity by the plaque assay method (10, 11, 14).

Properties of the isolate: Effect of 5-iodo-2-deoxyuridine (IUDR), ether and chloroform sensitivity, acid (pH 3.0) stability, temperature sensitivity (56 °C · 30 min) and filtration by membrane filters were tested as previously described (12). Growth characteristics on DBT cells was examined as reported elsewhere (13, 14, 17). The virus was inoculated onto the cells at multiplicity of infection of 0.5—1 plaque-forming units (PFU) per cell.

Serology: Serum neutralization test was carried out by the plaque reduction method (13, 21), and immunofluorescence was performed by the indirect method (17). Antisera for the serum neutralization test were prepared in rabbits (13) or mice (21). Anti-JHM rabbit serum and FITC-conjugated anti-rabbit IgG goat serum (Cappel Lab.) were used for the immunofluorescence.

Experimental infection: Specific pathogen-free, 4-week-old female Crj : CD-1 (ICR) mice were obtained from a commercial breeder (Charles River Japan, Inc.). They were fed sterile acid water and γ-ray irradiated commercial diet (NMF; Oriental Yeast Co.) *ad libitum*. Hydrocortisone acetate (HCA) was subcutaneously administered shortly before the virus inoculation. Mice were infected by intraperitoneal (i. p.) and intranasal (i. n.) routes with 0.2 ml (10^5 PFU) and 0.02 ml (10^4 PFU) amounts of viruses (MHV-NuU, MHV-N or the isolate), respectively. As controls, 10 mice each were inoculated with HCA or virus alone, and 10—20 mice each were inoculated with both HCA and virus. After inoculation, they were kept in a class IIA biological contaminant cabinet (Hitachi Ltd.). Surviving mice were killed by ether anesthesia and necropsied 14 days post-inoculation (p. i.).

Results

Virus isolation: Faeces of apparently healthy mice 2 to 6 weeks old were obtained for virological examination. Cytopathic effect (CPE) with syncytium formation (Fig. 1) appeared within 24 h p. i. in DBT cell cultures inoculated with faecal samples of 3- and 4-week-old mice. CPE was more prominent in the former samples, so the isolates from 3-week-old mice were passaged in DBT cells for further studies. The maximal virus titre (10^5—10^6 PFU / 0.2 ml) was obtained at the fifth passage.

Properties of the isolate: Physicochemical properties of the isolate are shown in Table 1. Virus replication was not inhibited by the presence of IUDR. The isolate was passed through a membrane filter of 100 nm pore size, but not through a filter of 50 nm pore size; it was sensitive to ether and chloroform, rather stable at pH 3.0, and showed a 3-log reduction in titre after heating at 56 °C for 30 min.

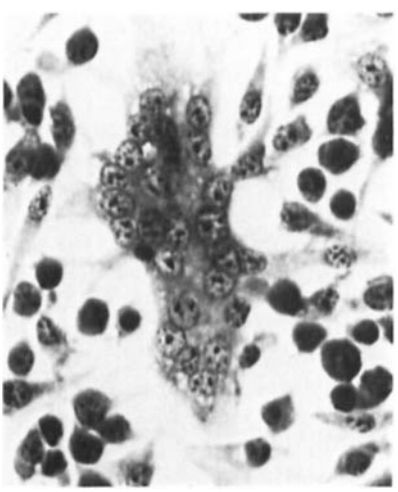

Fig. 1. Syncytium formation of the isolate in DBT cells; 7 h p. i., May-Grünwald Giemsa staining, × 1,600

Table 1. Physico-chemical properties of the isolate

Treatment	Virus titre (PFU / 0.2 ml)
IUDR 10^{-4}M	8.7×10^5
Control	1.2×10^6
Filtration, 450 nm	5.0×10^5
200 nm	3.8×10^5
100 nm	6.5×10^4
50 nm	< 10
Ether, 4 °C · 18 h	< 10
Control, 4 °C · 18 h	2.1×10^5
Chloroform, 22 °C · 15 min	< 10
Control, 22 °C · 15 min	6.5×10^5
pH 3, 22 °C · 3 h	1.0×10^4
pH 7, 22 °C · 3 h	2.3×10^5
56 °C · 30 min	1.2×10^2
4 °C · 30 min	7.7×10^5

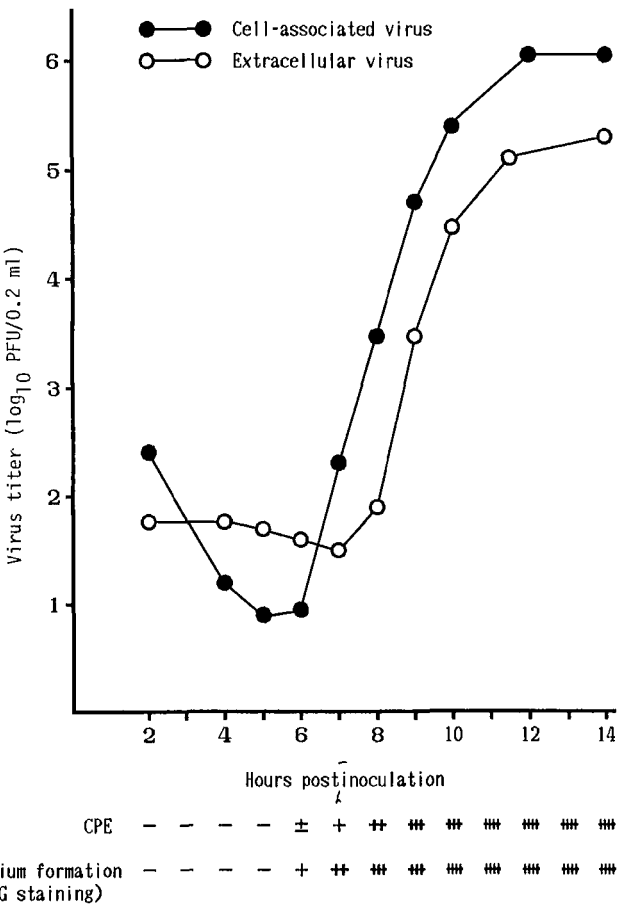

Fig. 2. Growth curve of the isolate in DBT cell cultures

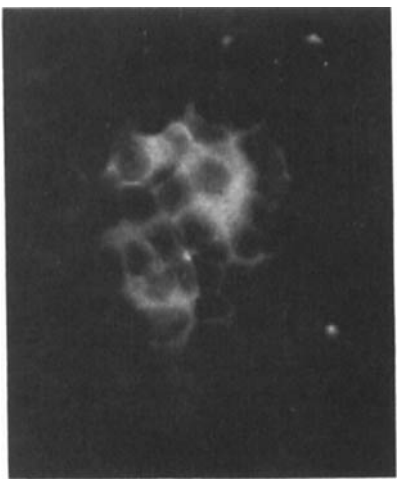

Fig. 3. Virus specific immunofluorescence in the cytoplasm; 7 h p. i., ×1,600

Growth features of the isolate in DBT cell culture are shown in Fig. 2. When weak CPE appeared 6 h p. i., small syncytia were observed in stained cells. CPE spread throughout the culture 10 h p. i.; then cell detachment followed. The virus titre began to increase 7 h p. i. in the cellular phase, 8 h p. i. in the fluid phase, and reached a plateau 12—14 h p. i. Cell-associated virus was about one-log higher in titre than extracellular virus from 7 to 14 h p. i.

The isolate, as well as other MHV strains, formed clear plaques on DBT cells. Without staining, small plaques became visible within 24 h p. i., and a second overlay was added two days p. i. In strains MHV-1, JHM, MHV-S, MHV-D, MHV-N and MHV-NuU, plaques were countable on the day of staining. On the contrary, those of the isolate first became countable one day after staining, with a size of 0.5—1 mm in diameter. Their size was much smaller than that of other strains (Fig. 3), and was stable after 10th passage.

Serology: Virus antigen was detected in the infected cells by the indirect immunofluorescence technique. Specific fluorescence was observed in the cytoplasm, particularly in the fused cytoplasm (Fig. 4). Five virus strains (MHV-D, JHM, MHV-N, MHV-S and the isolate) and respective antisera were prepared for the serum neutralization test. JHM was readily distinguished from other strains. The other four strains showed cross-reaction each other, except the pair of MHV-D and the isolate, in which antiserum against the isolate neutralized only the homologous virus (Table 2).

Experimental infection: Results are summarized in Fig. 5. All the control mice inoculated with HCA (2.5, 5.0, 7.5 or 10 mg) or viruses alone survived for 14 days, and their livers were apparently normal at necropsy. MHV-NuU and MHV-N could produce fatal hepatitis in HCA-treated mice by both i. p. and i. n. inoculation. Mortality increased

Table 2. Relationship of the isolate to MHV strains by serum neutralization test

Virus	Antiserum				
	MHV-D[a]	JHM[b]	MHV-N[b]	MHV-S[b]	Isolate[a]
MHV-D	640[c]	160	640	160	<20
JHM	<20	≧10,240	<20	80	<20
MHV-N	80	160	1,280	1,280	320
MHV-S	160	160	640	2,560	160
Isolate	160	320	320	320	640

[a]: Prepared in mice. [b]: Prepared in rabbits. [c]: Antibody titres are expressed as the reciprocal of the highest serum dilution that showed ≧50 % plaque reduction. Homologous titres are underlined.

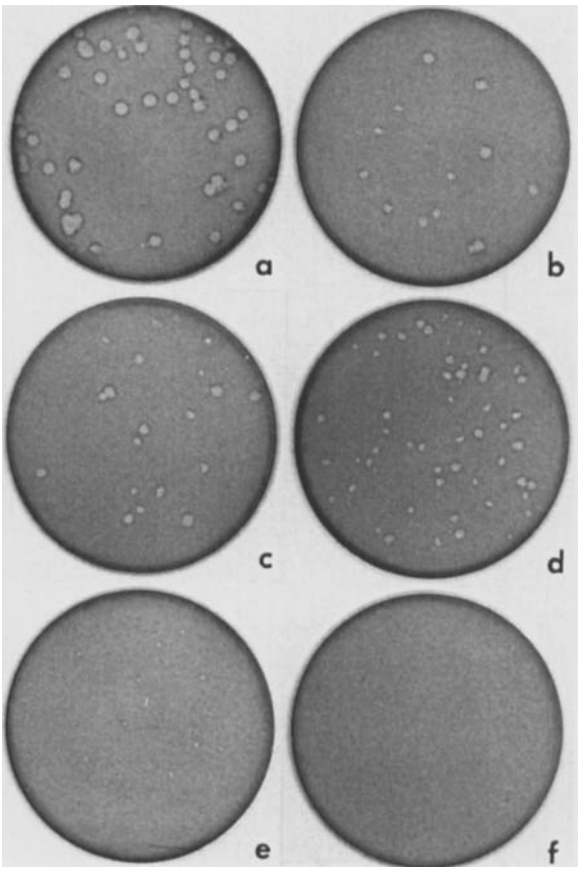

Fig. 4. Plaque formation in DBT cell cultures one day after staining. a: MHV-S, b: MHV-1, c: MHV-N, d: MHV-D, e: isolate and f: control. Six strains (MHV-1, JHM, MHV-S, MHV-D, MHV-N, MHV-NuU and the isolate) were compared for their plaque size. Of the remaining two strains, JHM produced the largest plaques, and MHV-NuU was similar to MHV-1 in size

according to the dose of HCA. In contrast, no mice inoculated with the isolate died regardless of the infection route and the dose of HCA. The occurrence of hepatitis is shown in Fig. 5 b. The rate is expressed as the accumulation of fatal cases and the mice with macroscopic lesions at necropsy, because all the fatal mice showed severe necrotic hepatitis. The isolate, as well as MHV-NuU and MHV-N, could produce hepatitis in HCA-treated mice, and the rate increased according to the dose of HCA. In general, however, the degree of hepatitis was slightest in mice inoculated with the isolate. It could produce sporadic necrosis at the most even in mice treated with 10 mg HCA; whereas many mice were killed by the other two strains.

Discussion

The present isolate was virologically and serologically identified as mouse coronavirus. Its physico-chemical properties were similar to those of previous reports (12, 13): sensitive to ether and chloroform, rather stable at pH 3.0, and not completely inactivated after heating at 56 °C for 30 min. The isolate, as well as other MHV strains (10, 11, 13—16, 18), grew well in DBT cell cultures and produced clear plaques. However, there were some differences in plaque formation between the isolate and other strains (MHV-1, JHM, MHV-S, MHV-D, MHV-N and MHV-NuU were examined), i. e., plaques of the isolate first became countable one day after staining, and they were much smaller in size than those of other strains. About plaque size, virus release from host cells may be concerned, because the isolate was rather cell-associated in the growth curve. Cell-associated virus was about one-log higher in titre than extracellular virus during its

Fig. 5—a

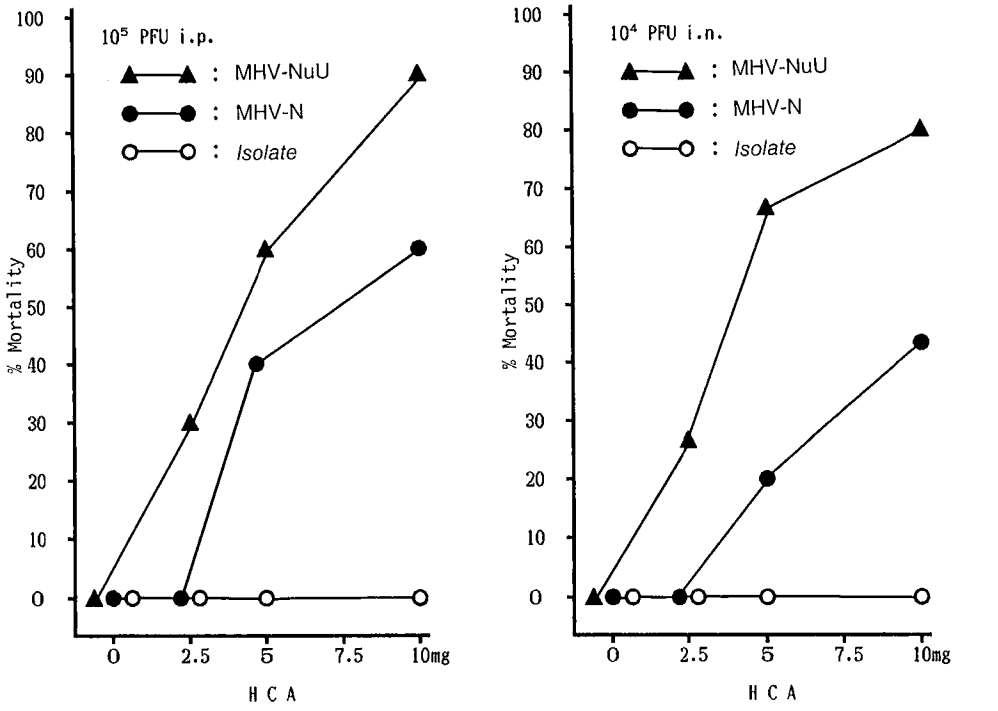

Fig. 5—b

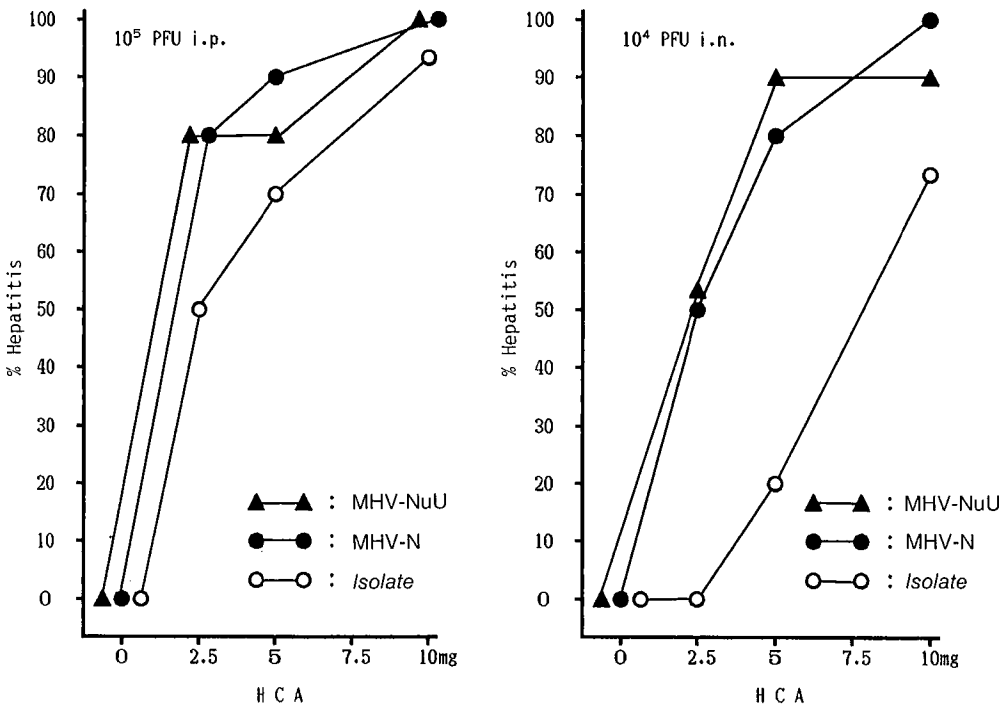

Fig. 5. Experimental infection in 4-week-old female ICR mice. a: mortality after i.p. (left) or i.n. (right) inoculation. b: cumulative rate of hepatitis after i.p. (left) or i.n. (right) inoculation

exponential growth phase (Fig. 2); whereas, in other strains, both virus titres were equal or extracellular virus showed a higher titre during the same phase (11, 13, 14).

In serological studies, the isolate was related to other MHV strains. Indirect immunofluorescence showed a common antigen between the isolate and JHM. Among the five strains in the serum neutralization test (Table 2), the isolate was antigenically most close to MHV-N, which was also isolated from faeces of apparently healthy mice (13).

Recent work (4) has suggested that MHV strains are divided into two groups according to their infection patterns, namely, respiratory MHV strains and enterotropic MHV strains. Since prototype strains and most well known isolates belong to the former group, the pathogenicity of respiratory strains has been studied from many aspects. On the other hand, the category of enterotropic MHV has been proposed only recently and most enterotropic strains are recent isolates. Thus, little is known about their pathogenicity as well as host resistant factors to these strains (1, 2).

The present isolate is probably an enterotropic strain, because it was isolated from faeces of 3-week-old mice and grows well in the intestine of weanling mice (unpublished data). In addition, MHV-N may be also an enterotropic MHV on the following evidence: isolation from faeces, and close antigenical relationship to MHV-D (an enterotropic strain) and the isolate. Since many MHV strains passaged in DBT cells have been studied for their pathogenicity in 4-week-old ICR mice (10, 13, 15, 16, 24), we also examined the pathogenicity of these two possible enterotropic strains in the same mouse strain. As a control, a well characterized low-virulent strain, MHV-NuU (16, 21, 25, 26), was included in the experimental infection. All the mice inoculated with HCA or each virus strain alone survived for 14 days, and the liver was apparently normal at necropsy. The isolate was also of low-virulence to weanling ICR mice. In HCA-treated mice, MHV-NuU and MHV-N could produce fatal hepatitis by i. p. or i. n. inoculation as reported previously (13, 15, 16). MHV-NuU was most virulent at each HCA dose, and, surprisingly, the isolate could not produce fatal hepatitis even in mice treated with 10 mg HCA, in which only at most a sporadic necrosis was observed in the liver. The isolate seems to be much more avirulent to the cortisone-treated ICR mice than other known low-virulent strains, because, after i. p. or i. n. inoculation, low-virulent strains always produced fatal hepatitis in weanling ICR mice treated with cortisone in the previous studies (13, 15, 16, 24).

Since the course of MHV infection is known to depend on the host factors, such as age, genotype and lymphoreticular functions (1, 2), the pathogenicity of the present low-virulent isolate in the host under various conditions is now being studied.

Zusammenfassung

Charakterisierung eines aus Kotproben isolierten, schwach virulenten Mäusehepatitisvirus (MHV)

Aus dem Kot klinisch gesunder Mäuse konnte in einer murinen Hirntumorzellinie (DBT-Zellen) ein cytopathogenes Virus isoliert und aufgrund seiner virologischen und serologischen Eigenschaften als Mäusehepatitisvirus identifiziert werden. Deutlich kleinere Plaques in DBT-Zellkulturen sowie lediglich milde Verlaufsformen einer Hepatitis nach experimenteller Infektion immunsupprimierter Mäuse unterscheiden dieses Isolat von anderen schwach virulenten Stämmen. Auch nach Vorbehandlung der Mäuse mit 10 mg Cortison traten keine schweren Verlaufsformen einer Hepatitis auf, wohingegen die meisten der mit anderen schwach virulenten Stämmen infizierten Mäuse starben.

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