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Correlation Between Growth Potential of Mouse Hepatitis Viruses in Macrophages and Their Virulence for Mice

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Correlation between the virulence of mouse hepatitis virus (MHV) for mice and the growth potential of the virus in peritoneal adherent cells was observed for highly virulent MHV-2 and avirulent MHV-1, JHM, and MHV-S strains. However, this phenomenon was not observed in strain MHV-3, which multiplied to almost the same degree in peritoneal adherent cells from susceptible and resistant mouse strains.

It is well known that the many strains of mouse hepatitis virus (MHV) vary in multiplication site and virulence in mice (6, 7). MHV is prevalent in mouse colonies, causing serious problems in maintenance and breeding (2). It is also widely recognized that the resistance of some mouse strains to highly virulent MHV is dependent upon protective mechanisms involving macrophages (1, 8). It is of interest to us whether such variation in virulence observed among MHV strains is connected with the interaction of macrophages with each MHV strain. In this study we examined the multiplication in macrophages of five MHV strains showing different virulences for mice to learn whether a correlation exists between virulence and multiplication capability in macrophages of MHV.

We selected MHV strains MHV-1, MHV-2, MHV-3, JHM, and MHV-S because they were reported to be extremely different in virulence (6). These virus strains were propagated and assayed in DBT cells as previously reported (3, 8). First, to ascertain the virulence of each strain of MHV, 10 to 10^5 plaque-forming units (PFU) of virus in 0.2 ml was inoculated intraperitoneally into 4-week-old CDF1 (BALB/c × DBA) mice maintained in our Institute, and mortality was followed for 2 weeks. All mice died after inoculation with 10 PFU of MHV-2, and all mice survived when 10^5 PFU of MHV-1, JHM, or MHV-S was inoculated (Table 1). The 50% lethal dose of MHV-2 and that of MHV-1, JHM, or MHV-S were calculated as less than 1 and more than 5 (in $\log_{10}$), respectively. MHV-3 caused death when more than 10^5 PFU of virus was injected, but mice survived when inoculated with less than 10^4 PFU of MHV-3, indicating that its 50% lethal dose is 4.5 $\log_{10}$. From these results, MHV-2 was designated as highly virulent, MHV-1, JHM, and MHV-S were termed avirulent, and MHV-3 was a low-virulence strain for CDF1 mice.

CDF1 mice were then infected intraperitoneally with 5×10^4 PFU of each strain of MHV, and the virus titer in the liver or spleen was assayed as previously reported (8). Highly virulent MHV-2 multiplied well both in the liver and spleen, and this resulted in the death of all mice within 48 to 60 h postinoculation (Fig. 1). Avirulent MHV-S did not multiply in either of these organs. Unexpectedly, the other avirulent strains, MHV-1 and JHM, multiplied fairly well to titers of 10^3 to 10^5 PFU per 0.2 g, but the growth of these viruses subsided on day 4, and only a slight amount of virus was detected on day 6. Low-virulence MHV-3 multiplied, reaching a titer of 10^5 to 10^6 PFU per 0.2 g. The growth of MHV-3 also decreased from day 4 on.

Next, the growth of each MHV strain in cultured peritoneal adherent cells (PAC) of CDF1 mice was compared with virus growth in highly permissive DBT cells. The preparation of resident PAC has been described elsewhere (9). Cultured PAC at a concentration of 10^6 cells per ml in Eagle minimal essential medium (Nissui, Tokyo) containing 10% fetal calf serum in a Multiwell tissue culture plate (15-mm diameter; Falcon, Oxnard, Calif.) were infected with 10^5 PFU of each MHV strain, and virus titer in the supernatant was assayed. There was little difference in virus growth in DBT cells among the five strains: they all multiplied well, attaining titers of 1×10^6 to 5×10^6 PFU per 0.2 ml at 24 h after inoculation. There were striking differences among the strains, however, when grown on PAC (Fig. 2). Highly virulent strain MHV-2 multiplied in PAC to a titer almost equal to that in DBT cells, with cytopathic effect (CPE) evident as cell rounding. Avirulent MHV-S multiplied poorly in PAC, reaching ca. 10^2 PFU per

TABLE 1. *Virulence of five MHV strains for CDF1 mice*[a]

PFU inoculated	No. of dead mice/no. tested				
	MHV-1	MHV-2	MHV-3	JHM	MHV-S
10^5	0/4	ND[b]	4/4	0/4	0/4
10^4	0/4	ND	0/4	0/4	0/4
10^3	0/4	4/4	0/4	0/4	0/4
10^2	ND	4/4	ND	ND	ND
10	ND	4/4	ND	ND	ND
LD_{50}[c]	>5.0	<1.0	4.5	>5.0	>5.0

[a] Four-week-old CDF1 mice were inoculated intraperitoneally with various doses of each MHV strain, and mortality was checked for 2 weeks.

[b] ND, Not done.

[c] One 50% lethal dose (LD_{50}; Reed-Muench method) shown in $\log_{10}$ PFU.

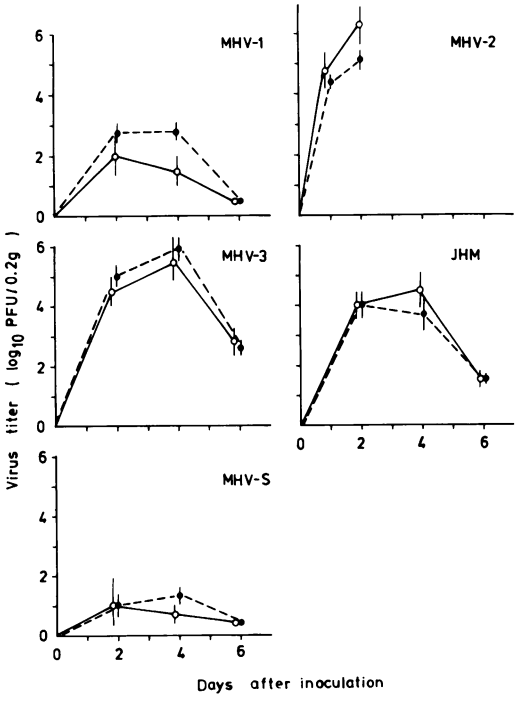

FIG. 1. *Growth of five MHV strains in the liver* (○) *and spleen* (●) *of CDF1 mice infected intraperitoneally with* 5×10^4 *PFU of each MHV strain. Each point represents the mean value, and the vertical bars indicate the range of three samples.*

0.2 ml, which is only 10^{-4} of its growth in DBT cells, and CPE was rarely observed. The growth of JHM was slightly higher than that of MHV-S, but minimal CPE was observed as the formation of polykaryocytes. The growth of MHV-1 and MHV-3 was intermediate between those of MHV-2 and JHM, but there was an apparent

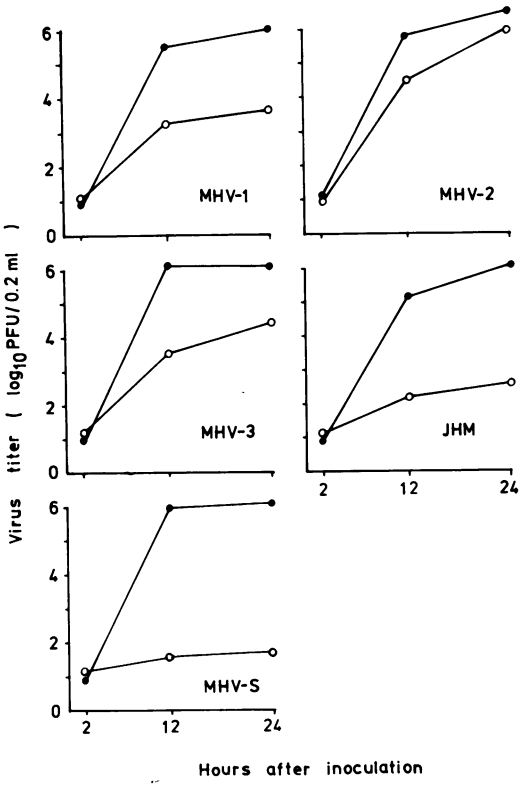

FIG. 2. *Growth of five MHV strains in PAC* (○) *and DBT cells* (●). *PAC and DBT cells were infected with each MHV strain at a multiplicity of infection of 1 and 0.1, respectively, and virus titers in the supernatant were plaque assayed.*

difference between these two strains in terms of CPE: MHV-3 produced prominent polykaryocytes as early as 8 to 10 h after inoculation, whereas the severity of MHV-1 CPE was not as extreme.

For MHV strains other than MHV-3 there seemed to be a correlation between virulence for CDF1 mice and growth potential in PAC from the same mice. To see whether such a correlation existed in other strains of mice, these two characteristics were examined using C3H/HeJms, C57BL/6J, and BALB/cCr mouse strains, all of which are maintained in our Institute, in comparison to CDF1 mice. The virulence of MHV-1, MHV-2, JHM, or MHV-S for these four strains of mice was not variable: MHV-2 showed high virulence, and MHV-1, JHM, and MHV-S were avirulent for all four strains. MHV-3, however, was virulent for all mice except for CDF1 mice, with a 50% lethal dose of less than 1 ($\log_{10}$) (data not shown). We examined virus growth in PAC prepared from these mice. No striking difference was observed in the growth

of a given MHV strain in PAC from four separate mouse strains: MHV-2 multiplied to the highest titer, attaining 10^5 to 10^6 PFU; MHV-1 and MHV-3 reached 10^3 to 10^4 PFU; JHM and MHV-S reached $10^{1.5}$ to 10^3 PFU. The CPE of these five strains of MHV in PAC from C3H, C57BL, or BALB/c was similar to that in PAC from CDF1 mice. These results indicate that there appears to be a correlation in MHV-1, MHV-2, JHM, and MHV-S between virulence and growth potential in PAC in all mouse strains examined, but MHV-3 did not show such a correlation.

A correlation between the virulence of MHV-3 and its multiplication in macrophages has been reported for three mouse strains with different virus susceptibilities (10). Such a correlation was not observed in another laboratory, however (5). Other evidence to support the involvement of macrophages in resistance is found in a report by Levy-Leblond and Dupuy (4), who showed that both spleen macrophages and T lymphocytes are necessary for complete resistance. We established that the susceptibility of mice depends upon the permissiveness of T lymphocytes to MHV-3 replication, and we observed no difference in MHV-3 multiplication in macrophages from resistant and susceptible mouse strains (11). This inconsistency observed among several laboratories could be explained by differences in the inbred mice used, or a small variation within a given inbred mouse strain, i.e., a difference at the subline level. In MHV-3 infection, it is postulated that some factors other than macrophages are deeply involved in the resistance mechanisms. In the present case, we consider that all of our mouse strains showed resistance at the macrophage level, because the permissiveness of macrophages for MHV-3 replication was almost equal to that for avirulent MHV-1. In reference to the other resistance factor(s), however, CDF1 mice showed resistance and the rest of the mouse strains showed susceptibility. This resulted in a difference in susceptibility of the whole animal.

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