Interaction Between Mouse Hepatitis Viruses and Primary Cultures of Kupffer and Endothelial Liver Cells from Resistant and Susceptible Inbred Mouse Strains

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SUMMARY

Infection with mouse hepatitis virus type 3 (MHV 3) of primary cultures of Kupffer and endothelial cells from the livers of resistant (A/J) and susceptible (BALB/c) mice was followed by the appearance of typical syncytia and comparable yields of virus. Using cells from A/J mice there was a delay of about 24 to 36 h in the appearance of the first particles detected by electron microscopy, the maximum viral titre and the number and size of syncytia. The partial resistance to MHV 3 multiplication expressed by cells from A/J mice was also observed when they were infected with a strain of low virulence (JHM strain). The delay in MHV multiplication found in the sinusoidal liver cells, mainly in the endothelial cells, may be an important factor in their resistance, by allowing time for the local and systemic responses to clear the infective particles as well as in determining their degree of hepatotropism.

Mouse hepatitis virus type 3 (MHV 3) has the property of producing focal hepatic necrosis, the severity of which varies according to the mouse strain. After injection with MHV 3, A/J mice develop a small number of very well-defined necrotic foci in the liver which disappear 8 to 12 days later. On the other hand, most mouse strains, including BALB/c, show a large number of necrotic foci 2 to 3 days after the infection and the mice die of acute hepatitis 3 to 4 days later.

Although the hepatocytes constitute the main site of MHV 3 replication, the virus particles carried by the blood have to cross the sinusoidal barrier, made up of Kupffer and endothelial cells, in order to infect the parenchymal cells (Kirn et al., 1982; Steffan & Kirn, 1979). A direct relationship has been demonstrated between the resistance of A/J mice and the ability of their peritoneal macrophages or hepatocytes to resist partially to MHV 3 replication (Virelizier & Allison, 1976; Arnheiter et al., 1982). However, the interaction of MHVs with isolated Kupffer and endothelial liver cells has been rarely studied.

MHV 3, MHV strain JHM (JHMV) and vesicular stomatitis virus (VSV) were propagated in the mouse fibroblast cell line L-929. MHV 3 and JHMV stocks had a titre of $5 \times 10^5$ p.f.u./ml. The VSV stock had a titre of $10^{8.3}$ tissue culture infective doses per ml (TCID$_{50}$/ml). The technique used for isolating and cultivating Kupffer and endothelial liver cells has already been described (Kirn et al., 1980; Steffan et al., 1981). Briefly, mouse livers were perfused in situ with 0.05 ~ collagenase and incubated again in collagenase in a gyratory water-bath for 30 min. The cellular suspension was filtered on nylon, centrifuged on metrizamide and the Kupffer and endothelial cells were separated by centrifugal elutriation. The cells were cultured in 96-well microtitre plates at a concentration of $3 \times 10^5$ cells/well in Dulbecco's HEPES medium supplemented with 20% foetal calf serum. Cultures (24 h) were infected with MHV 3, JHMV and VSV at a multiplicity of 0.1 and unadsorbed virus was removed after 1 h at 37 °C before fresh medium was added. At different time intervals supernatant samples were taken for virus titration.
Short communication

Fig. 1. Kinetics of MHV 3 (a, b) and JHMV (c, d) multiplication in primary cultures of Kupffer cells (a, c) or endothelial liver cells (b, d) from A/J ( ) or BALB/c (——) mice. Cells (3 × 10^5/well) were infected with either virus at an m.o.i. of 0.1.

Although the final MHV 3 yield was similar in cell cultures from resistant (A/J) and susceptible (BALB/c) mice, maximum titres were always observed 24 to 36 h later in the cells from the resistant mice (Fig. 1). As is typical of MHV 3 replication, syncytia (Fig. 2) and virus-containing cytoplasmic vacuoles could be found in all cell cultures. However, all these events were observed with a delay of about 24 to 36 h in cell cultures from resistant mice. In cell cultures from susceptible mice, cell fusion spread rapidly over the entire monolayer, whereas in those from resistant mice reduced numbers of cells and only limited spreading were involved. The longer time lapse needed for MHV 3 replication in Kupffer and endothelial liver cells from resistant mice, together with that observed for hepatocytes (Arnheiter et al., 1982), may be of crucial importance in their resistance to the infection. When viruses present in the blood reach the sinusoids they have first to interact with the Kupffer and/or the endothelial cells and only afterwards with the hepatocytes (Kirn et al., 1982; Steffan & Kirn, 1979). It may be speculated that in vivo the prolongation of the lag phase in these cells allows the specific and non-specific mechanisms to clear a large amount of virus particles thus suppressing virus infection in large numbers of hepatocytes. On the other hand, in susceptible mice where virus is produced very rapidly, the immune system as well as non-specific defences (Le Prevost et al., 1975; Pereira et al., 1984; Virelizier & Gresser, 1978), if stimulated, would not be able to clear the organism of infectious particles.

A delay was likewise observed for JHMV replication in Kupffer and endothelial cells from both mouse strains. However, these cultures always shed less virus than those infected with
Fig. 2. Cytopathic effect in primary cultures of Kupffer cells (a, b) or endothelial liver cells (c, d) from BALB/c mice uninfected (b, c) or infected with MHV 3 (m.o.i. 0·01) (a, d) and fixed 18 h later for scanning electron microscopy. S, Syncytium; N, nucleus.

MHV 3 (Fig. 1). Maximum titres of JHMV replication were $3 \times 10^3$ to $5 \times 10^3$ p.f.u./ml for Kupffer cells and $0·9 \times 10^3$ to $2 \times 10^3$ for endothelial cells, in contrast with those of MHV 3 replication which were nearly $2 \log_{10}$ units higher. JHMV multiplication in endothelial cells was
markedly delayed when compared with MHV 3 multiplication in these cells (Fig. 1). The relatively high resistance of these cells to JHMV replication corresponds with the weak hepatotropism of this strain of MHV. On the other hand, MHV 3, which replicates quite well in endothelial cells, is considered to be a high virulence hepatotropic strain of MHV. Our finding shows a correlation between the degree of hepatotropism of these two strains of MHV and their ability to replicate in cultured sinusoidal liver cells. Although it has been shown recently that cultured hepatocytes reflect susceptibility when the whole animal is concerned (Arnheiter et al., 1982), our observations correspond closely to those of Taguchi et al. (1983) showing that the hepatotropism of a given MHV may be determined by its potential for replication in non-parenchymal cells. Our findings, based on methodology allowing us to use separated Kupffer and endothelial cell populations, suggest that among non-parenchymal liver cells the endothelial ones play a key role, since JHMV (low virulence) replication in these cells was markedly delayed when compared with that of MHV 3 (high virulence).

Last, when Kupffer and endothelial cells were infected with VSV, both cell populations always shed equal amounts of virus with no visible differences in the kinetics of virus production (data not shown). This indicates that the partial resistance of Kupffer and endothelial cells to infection in vitro by MHV is a specific phenomenon.

In conclusion, our results show that both Kupffer cells and endothelial liver cells support MHV replication, giving rise to the appearance of typical syncytia and virus-containing cytoplasmic vacuoles. MHV 3 replication in cultured Kupffer and endothelial cells from A/J and BALB/c mice correlates with the resistance and susceptibility in vivo shown by these strains of mice to MHV 3 infection. A correlation was also observed between the degree of hepatotropism shown by MHV 3 and JHMV and their ability to grow in those cells. MHV replication in endothelial cells, which represent about 70% of the total number of sinusoidal liver cells and which are one of the first cell populations to interact with infectious particles in the liver, may be a crucial step in the resistance exhibited by various strains of mice.

REFERENCES


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