

Mouse Hepatitis Virus Strain 3 Infection of C57, A/Sn and A/J Strain Mice and Their Macrophages

Brief Report

By

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With 1 Figure

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Summary

Mouse hepatitis virus strain 3 replicated in C57, A/Sn and A/J strain mouse macrophages with the production of a clear cytopathic effect, although only C57 and A/Sn strains of mice were killed with similar MHV3 dilutions. We could not confirm a previous report showing that *in vitro* cultured macrophages from A/J strain mice were resistant to MHV3 infection.

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Mouse hepatitis virus (MHV) exists in several closely related serotypes with varying degrees of pathogenicity in mice (8). A number of studies have shown a correlation between the pathogenicity of a certain type of virus and its ability to grow in cultured macrophages (3, 9). Thus, the non-pathogenic MHV1 does not replicate in mouse macrophages (1), whereas the pathogenic types MHV2 and MHV3 readily infect mouse peritoneal macrophages (2, 7, 11, 12). Most strains of mice can be infected with MHV3 with the development of fulminant hepatitis. Previous studies using A/J strain mice showed that these mice were resistant to MHV3 infection, with only a mild disease occurring with large doses of virus (4, 12). During the first four days of infection virus was recovered from infected mice although there were only minimal changes to their livers (4). However, young A/J strain mice were susceptible up to 3 weeks of age (4). Other mouse strains, including the C57 strain (4, 5, 12) and the A/Sn strain (5) were sensitive to MHV3 infection. Furthermore, VIRELIZIER and ALLISON (12) have reported that there was no detectable growth of MHV3 in macrophages from A/J mice; whilst MHV3 replicated in macrophages from C57 strain mice. We were interested in analysing this reported resistance of A/J strain mouse macrophages to MHV3 infection and attempted to repeat this observation by comparing the effect of MHV3 infection on C57, A/Sn and A/J strain mice and their macrophages.

5 and 10 week old C57 BL/10 Cre and A/Sn Cre strain mice were obtained from the specific-pathogen-free (SPF) unit of the Clinical Research Centre, Harrow, and A/J strain mice were supplied from the SPF unit of the National Institute for Medical Research, Mill Hill. Extreme care was taken to ensure that these mice had no previous exposure to MHV3 and other pathogens, as certain batches of SPF mice from other sources were found to have antibodies to MHV, and were resistant to infection. MHV3 was obtained from Dr. Tapani Hovi, Clinical Research Centre, Harrow, and was grown in confluent secondary mouse embryonic fibroblasts. Monolayers were infected at an input multiplicity of 0.1 infectious particles per cell and, following an adsorption period of 1.5 hours at 37° C, were incubated for 72 hours at 37° C in Eagle's MEM with 2 per cent foetal calf serum (6). Aliquots of this virus suspension were used after low speed clarification. Unstimulated peritoneal macrophages were washed from the peritoneal cavities of mice and were cultured in 30 mm petri dishes in 2 ml of 199 medium, supplemented with 10 per cent heat inactivated foetal calf serum, at a concentration of 5×10^5 cells per ml (12). Cultures of adherent macrophages were infected 24 hours after seeding by removing the medium and replacing it with appropriate dilutions of virus in culture medium. The appearance of giant cells was checked after 24, 48 and 72 hours by examining unfixed cultures with an inverted microscope (12). At 24 hours after infection some cultures were fixed for 1 hour at room temperature in 3 per cent glutaraldehyde buffered with 0.1 M cacodylate buffer (pH 7.2) containing 5 per cent sucrose. Fixed cells were subsequently processed for electron microscopy as previously described (10). For *in vivo* infection, groups of 10 ten-week-old mice were injected intraperitoneally with 0.1 ml of appropriate dilutions of MHV3 in PBSA. The mortality and clinical symptoms were recorded during several weeks after infection.

In order that our results would be comparable with those of VIRELIZIER and ALLISON (12), similar conditions of MHV3 infection and macrophage culture were used. As the titre of virus we used could not be exactly correlated with that used previously, a number of virus dilutions were used. Furthermore, the macrophages used in the previous report were from mice ranging in age from 5 to 10 weeks: we used macrophages from mice aged 5 and 10 weeks in this report.

The result of infecting 10 week old C57, A/Sn and A/J strain mice with various dilutions of MHV3 is shown in Table 1. Both C57 and A/Sn strain mice were sensitive to similar dilutions of virus and infected mice of both strains showed similar clinical symptoms, developing a fulminant hepatitis and died within 8 days. Mice that did not die after infection showed no symptoms of the disease at any time. However, A/J strain mice were not killed by any of the virus dilutions tested and none of the mice showed any evidence of disease. Similar results were obtained on infecting 5 week old mice with the same MHV3 dilutions.

Table 2 shows the results of a typical experiment in which macrophages from 10 week old mice were infected with various MHV3 dilutions. There was a difference in the appearance of CPE in macrophages from different strains of mice on infection with MHV3, as measured by giant cell formation. Although CPE appeared sooner in infected C57 strain macrophages than A/Sn and A/J strain macrophages, the CPE was qualitatively similar for all macrophage strains with a gradual rounding up of the macrophages followed by giant cell formation.

Supernatants from infected macrophage cultures were titrated in macrophages from T0 strain mice. In all infected samples where CPE was observed, titres of at least 10^4 ID₅₀ were obtained; and no virus was obtained from infected macrophages showing no CPE (results not shown). These results indicate that giant cell formation was an adequate marker for virus replication. Thus, C57 strain macrophages from 10 week old mice were infected with MHV3 more readily than A/Sn or A/J strain macrophages. Similar, but less pronounced differences in sensitivity to MHV3 replication were observed in MHV3 infected macrophages from 5 week old mice.

Table 1. In vivo susceptibility to MHV 3 of 10 week old C57, A/Sn and A/J strain mice

Mouse strain	Virus titre (ID ₅₀) per ml ^b	Number of mice surviving after infection (days) ^a			
		5	6	7	14
C57	No virus	10	10	10	10
	10 ¹	10	9	8	8
	10 ²	10	7	4	3
	10 ³	9	1	0	0
A/Sn	No virus	10	10	10	10
	10 ¹	10	10	10	9
	10 ²	10	6	0	0
	10 ³	10	4	0	0
A/J	No virus	10	10	10	10
	10 ¹	10	10	10	10
	10 ²	10	10	10	10
	10 ³	10	10	10	10

^a 10 mice of each strain were used for each virus dilution

^b 0.1 ml inoculum of virus in PBSA was given intraperitoneally. The mice given no virus were injected with 0.1 ml PBSA

Table 2. Cytopathic effect induced by MHV 3 in macrophages from 10 week old C57, A/Sn and A/J strain mice

Mouse strain	Time after infection (hours)	Virus titre (ID ₅₀) per ml ^a			
		10 ⁴	10 ³	10 ²	10 ¹
C57	24	+++	+	—	—
	48	++++	++++	++++	++
	72	++++	++++	++++	++++
A/Sn	24	+	—	—	—
	48	++	+	—	—
	72	+++	++	+	—
A/J	24	+	+	—	—
	48	+++	++	—	—
	72	+++	++	+	—

^a Virus titre determined from titration of virus in T0 strain mouse macrophages. The number of crosses represents the percentage of giant cells. — no CPE, + ≤25, ++ ≤50, +++ ≤75, ++++ ≤100

Virus replication in the *in vitro* cultured macrophages was also confirmed by electron microscopy. Fig. 1 shows virus particles present in macrophages from A/J strain mice which had been infected with a virus titre of 10^2 ID₅₀ and fixed after 24 hours. A/J strain mouse macrophages infected with 10^2 ID₅₀ of virus did not show a clear cytopathic effect until 72 hours after infection. However, after 24 hours approximately 10 per cent of these cells were found, by electron microscopy, to contain virus particles. Virus particles were not observed in A/J strain mouse macrophages infected with 10^1 ID₅₀ of virus.

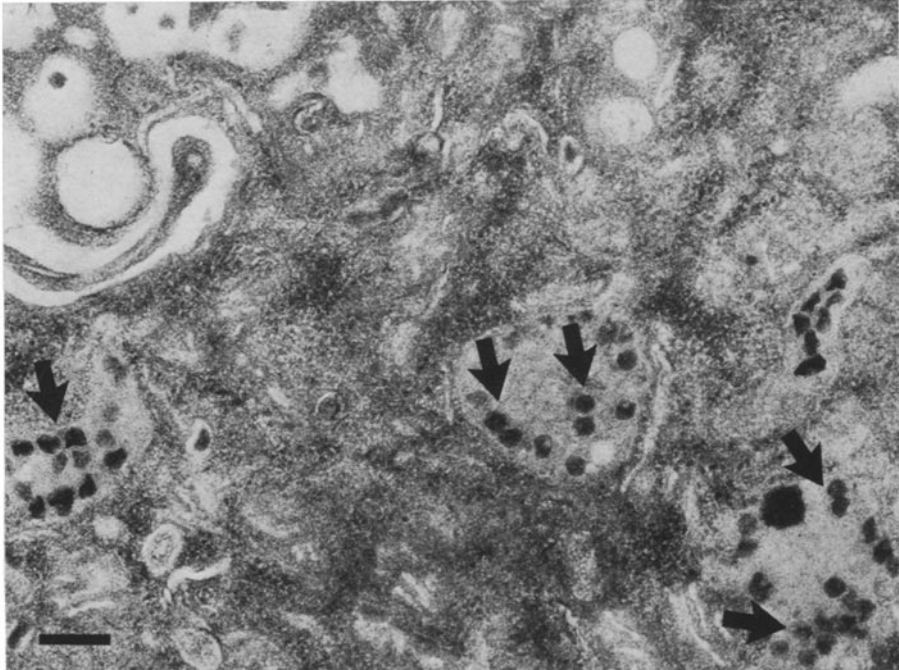


Fig. 1. Virus particles in macrophages from A/J strain mice, which had been infected for 24 hours with a virus titre of 10^2 ID₅₀. Arrows indicate virus particles. Bar represents 200 nm

These results appear to be at variance with those of VIRELIZIER and ALLISON (12), which suggested that macrophages from A/J mice were resistant to MHV3 infection. However, they showed results using only a low virus dilution, probably corresponding to our inoculum with a virus titre of 10^2 or 10^1 ID₅₀ per ml. At this titre, a significantly reduced CPE was observed in infected macrophages from 10 week old A/J strain mice (Table 2). However, they also showed that "very heavy inocula" of MHV3 produced giant cells in these macrophages cultures.

We did not observe a simple correlation between the pathogenicity of MHV3 and its ability to replicate in cultured macrophages. However, this was not entirely unexpected as macrophages are only one constituent of the whole mouse immune defence system and multiple factors, such as immune status, genetic composition and age are all important in the determination of susceptibility to infection.

In conclusion, we have confirmed that C57 and A/Sn strain mice are susceptible to MHV3, and shown that MHV3 replicated in macrophages from these mice with the formation of giant cells. Furthermore, we have shown that macrophages from A/J strain mice were susceptible to MHV3 infection, even though the whole animals were resistant to similar dilutions of MHV3. Although these results appear to partially differ from a previous report (12), we have suggested how they may be compatible. However, it is always difficult to compare results from different laboratories using similar, although not necessarily identical SPF animals and viruses and experimental procedures.

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