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Asymptomatic Infection of Mouse Hepatitis Virus in the Rat

Brief Report

By

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With 2 Figures

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Summary

After intranasal inoculation of suckling rats mouse hepatitis virus multiplied mostly in the nasal epithelium; though there were no symptoms, antibodies were produced. Antibodies were also demonstrated in adult rats. These findings suggest that the rat may be a natural host for the virus.

*

Mouse hepatitis virus (MHV) is prevalent among mouse colonies (6) and pathogenic for immunodeficient nude (10) or suckling (4) mice, but not for normal adult mice (4, 10). It is well documented that most naturally occurring infections with MHV are transmitted by apparently healthy carrier mice (4), although other species of animals may also be involved in transmission. Antibodies against some strains of MHV have been detected in the sera of other species such as human or rat (8), but these antibodies may be evoked by MHV or other coronaviruses that crossreact with MHV. When inoculated experimentally by intracerebral route, hamsters and rats as well as mice were shown to be sensitive to a strain of MHV, JHM (1, 5), but there was no evidence that MHVs infect these animals when inoculated perorally or intranasally (i.n.) (7, 13) the routes by which natural infection is likely to occur (9, 16). In the present paper, we describe the multiplication of MHV and the production of neutralizing antibody in the rat after i.n. inoculation.

Ten-day-old Wistar rats were purchased from a commercial breeder and inoculated with 10^5 PFU of a low-virulent strain of MHV, namely MHV-S (14). The multiplication of the virus (15), the distribution of viral antigen (15), histopathological changes (16) and the antibody responses (9) were examined as reported elsewhere.

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As shown in Figure 1, MHV-S multiplied in the "anterior part of the head", which includes the nasal bones as well as the nasal mucosa, and reached a maximum titer of about 10^5 PFU/0.2 g on day 2. Virus grew to much lower titres in the brain and low titres (less than 10^2 PFU/0.2 g) were also detected in the lung, liver and spleen in some animals examined 1 to 4 days after virus inoculation. Other tissues, such as salivary glands or blood, contained no infectious virus.

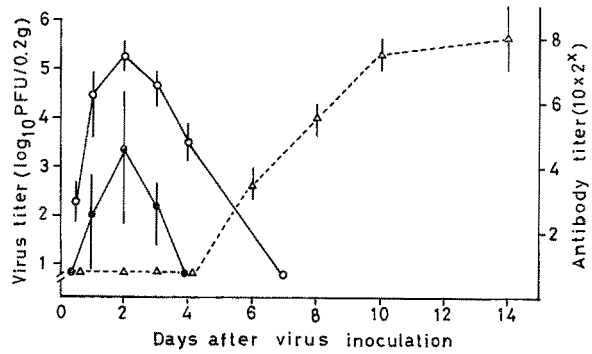


Fig. 1. Effects of inoculating 10-day-old rats i.n. with 10^5 PFU of MHV-S. Titer of virus in the anterior part of the brain (○) and brain (●), and neutralizing antibody titer (△). Each point represents the mean value and the vertical bar the range

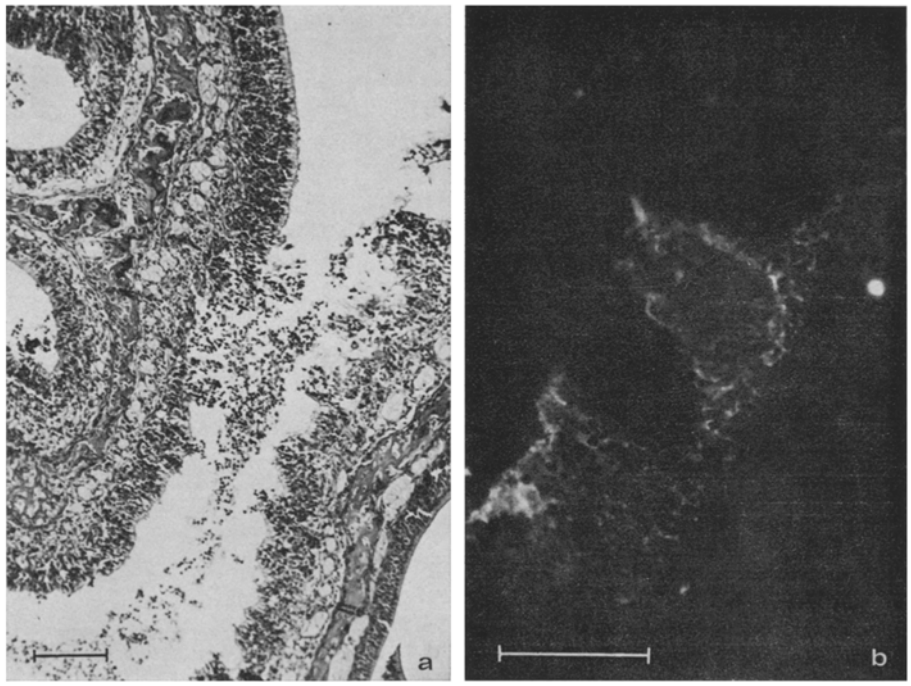
Tissue sections were examined by immunofluorescence as previously reported (15) and viral antigen was detected only in the epithelial cells of the nasal mucosa (Fig. 2b), where histopathological changes such as necrosis and desquamation were found (Fig. 2a). However, no rats showed any clinical signs during 14 days of observation. Neutralizing antibody was detected first on day 6, and thereafter rapidly increased in titer as shown in Figure 1. This mode of infection of MHV-S in rats resembles that found in weanling mice, in which viral multiplication was

Table 1. *Presence of neutralizing antibody in the sera of rats inoculated with MHV-S*[a] *at various ages*

Age at inoculation (weeks)	Sera collected on day[b]	
	0	14
2	0/5[c]	5/5[d]
4	0/5	5/5
6	0/5	3/5
10	0/5	5/5

[a] Various aged rats were inoculated i.n. with 10^5 PFU of MHV-S
[b] Days after inoculation sera were collected
[c] Proportion of sera which were positive when tested at 1:10
[d] Proportion of sera which were positive when tested at 1:100

limited to the nasal mucosa and brain (manuscript in preparation). Two other strains of MHV, namely JHM and MHV-Nu66 (10), were also inoculated into 10-day-old rats and were found to multiply, but to a lesser extent than MHV-S.



Figs. 2a and 2b. Necrotic changes (Fig. 2a) and cytoplasmic immunofluorescence (Fig. 2b) in epithelial cells of the nasal mucosa. Tissue from 10-day-old rats 2 days after i.n. inoculation with 10^5 PFU of MHV-S. Bar represents 0.1 mm

Two-, 4-, 6- and 10-week-old rats were also inoculated i.n. with 10^5 PFU of MHV-S and infectious viruses were assayed in the lung, brain, salivary glands and liver. In less than one-third of the total, were infectious viruses detected in the lung and salivary glands and the titers were less than 10^2 PFU/0.2 g. No infectious virus was demonstrable in the liver. Sera were collected 14 days after inoculation and examined for neutralizing antibody. Table 1 shows that almost all contained neutralizing antibody to a titre of >100. The two rats inoculated at 6 weeks and seronegative when tested at 1:100 were nevertheless positive at 1:50.

So far only mice have been considered to be natural hosts of MHV and rat has been excluded because highly virulent MHV inoculated by various routes did not cause any symptomatic infection like that produced in the mouse (7, 13). However, this does not imply that MHV does not infect the rat. In the present study, we clearly demonstrated that by the intranasal route MHV-S infects rats of various ages from the following criteria: 1. MHV replicated in the "anterior part of the head" and several other organs; 2. viral antigen was detected by immunofluorescence in the epithelial cells of nasal mucosa where histopathological changes were also found; 3. rats of all ages produced neutralizing antibodies

after virus inoculation. In some cases, neither infectious virus nor histopathological changes were demonstrable in infected rats, however, neutralizing antibodies were constantly detected in the sera of inoculated rats, revealing that MHV replication occurs in every cases, but may be below the detection level in some cases.

Two coronaviruses, namely rat coronavirus (RCV) (12) and sialodacryoadenitis virus (SDAV) (11), are known to infect and cause disease in the rat. These viruses multiply first in the epithelial cells of the nasal cavity and thereafter, they manifest their organ tropism i.e., RCV goes to the respiratory system and produces interstitial pneumonia (2, 12) and SDAV affects the salivary and lacrymal glands, and produces adenitis (11). The pathogenesis of MHV-S infection in rats resembles, in part, that of SDAV or RCV, in that these viruses cause the necrotic changes in the epithelial cells of the nasal mucosa at early stage of infection (2). However MHV is confined there, or affects slightly the central nervous system as it does in infections of mice (manuscript in preparation).

Bhatt and his coworkers showed that SDAV multiplied in mice producing some histopathological changes in the respiratory system after i.n. inoculation (3) and we demonstrated in the present paper that MHV infected rats of any ages. These facts indicate that SDAV and MHV are infectious for both mice and rats, so that both may play an important part in the survival of these viruses.

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