LOCALIZATION OF VIRUS AND ANTIBODY RESPONSE IN MICE INFECTED PERSISTENTLY WITH MHV-JHM

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ABSTRACT

Suckling mice infected intranasally with MHV-JHM and nursed by immunized dams develop a late onset demyelinating encephalomyelitis. Analysis by in situ hybridization revealed that MHV-JHM entered the central nervous system (CNS) via the olfactory and trigeminal nerves and spread over the next two weeks to the spinal cord, prior to amplification at this site. Serial measurements of neutralizing antibody titers showed that the late onset disease developed in some mice at levels of antibody which protected mice from the fatal, acute encephalitis, supporting the notion that cell-mediated and not humoral immunity is important in protecting mice from MHV-JHM persistence.

INTRODUCTION

Intranasal or intracerebral inoculation of weanling or suckling mice with the JHM strain of mouse hepatitis virus (MHV-JHM) causes an invariably fatal encephalitis. In previous reports, we have characterized a model in which suckling C57BL/6 mice are protected from the acute encephalitis if they are nursed by immunized dams (1-3). We have shown that:

1) After inoculation with MHV-JHM intranasally, all of the mice were protected from the fatal encephalomyelitis at 7 d p.i. However, histological evidence of encephalitis was present even though the mice remained asymptomatic. 40% of the maternal antibody-protected mice developed neurological disease expressed clinically by hindlimb paralysis and histologically by a demyelinating encephalomyelitis. Whereas viral antigen could be detected in all mice whether symptomatic or asymptomatic, virus could only be isolated from symptomatic mice. The serum antibody response in both asymptomatic and symptomatic mice was minimal.

2) The astrocyte was an important target cell in the CNS in both symptomatic and asymptomatic mice and accounted for 20-40% of the infected cells in nearly all mice.

3) Using in situ hybridization, we determined that MHV-JHM was present throughout the brain in mice dying from the acute encephalitis. On the other hand, virus could always be detected in the spinal cord and brainstem of mice with hindlimb paralysis. Additionally, it was also present in other

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parts of the brain, including the hippocampus, the thalamus, the cerebral cortex and the optic chiasm, in some mice with the late onset disease.

In this report, we describe the localization of virus in asymptomatic mice at early and late times p.i. We also present experiments which show that high levels of anti-MHV-JHM antibody do not protect against the development of the late onset neurological disease.

MATERIALS AND METHODS

Animals, viruses, sera. MHV-JHM, MHV-A59 and C57BL/6 mice were obtained as previously described (1). In some experiments, mice were immunized with UV-inactivated MHV-JHM, prepared by treatment of MHV-JHM with 18,000 erg/mm^2. No residual virus could be detected by plaque assay. Sera were titered by either a neutralization assay (1) or by ELISA (manuscript in preparation).

In situ hybridization. This was performed as described previously (3).

RESULTS

Clinical studies: In our first study, we showed that suckling mice inoculated at ten days of age and suckled by unimmunized dams all died of an encephalitis, whereas mice suckled by immunized dams all survived. Forty percent of the latter developed hindlimb paralysis at 35 d p. i., with a range of 23 to 60 d (1).

In the present study, 103/114 (90%) of maternal antibody-protected offspring developed hindlimb paralysis. Of the 103 which developed hindlimb paralysis, 15 (15%) recovered with a mild residual paresis; some of these mice also had several additional relapses. Mean maternal antibody titers, as measured by plaque-reduction neutralization assay, were 1:550 in this study, as opposed to 1:2700 in the first one.

Hindlimb paralysis was noted at 25 d (S.D. 11 d) with a range of 9-83 d; only two mice were noted to develop hindlimb paralysis at an age greater than 60 d p.i. Most of the mice had only hindlimb paralysis, with no signs of encephalitis (hunching, ruffled fur, irritability and lethargy). However, some of the mice that developed clinical disease at earlier times after inoculation (less than 18 d p.i.) were observed to have mild encephalitic symptoms as well.

Since mice greater than 60 days of age generally did not develop clinical disease, this suggested that some type of maturation, whether in the immune system or otherwise, had occurred to prevent viral reactivation. To identify a possible active role of the immune system in this process, a group of older mice were immunosuppressed with cyclophosphamide. For this purpose, 12 mice greater than 100 days p.i. were divided into two groups. Six were treated with cyclophosphamide at a dosage shown previously to be immunosuppressive (200 mg/kg/d administered intraperitoneally every other day for three doses (4)). No neurological disease developed in any mouse from either group.

Regional localization of MHV-JHM in mice at early times p.i. In the first experiments, brains isolated from mice inoculated intranasally with MHV-JHM and nursed by unimmunized dams were analyzed by in situ hybridization with [^{35}S] labelled antisense RNA probes (3). At 3 d p.i., viral RNA was readily detected in the olfactory lobes of all mice. By 4 d p.i., viral RNA could be detected in nearby areas of the olfactory system, and could also be detected in the brainstem at a site corresponding to the mesencephalic nucleus of the trigeminal nerve. By 5 d p.i., when the mice were nearly

dead, viral RNA could be detected throughout the brain, with prominent labelling occurring in the hypothalamus, hippocampus, basal ganglia and thalamus. In the computerized version of these data shown in Figure 1, representative sagittal sections from each mouse were digitalized. Viral RNA was clearly present initially in the olfactory lobes and mesencephalic nucleus at 3 and 4 d p.i., with rapid spread to central portions of the brain at 5 d p.i.

Suckling mice nursed by immunized dams are completely protected from the acute encephalitis, but may develop hindlimb paralysis several weeks p.i.(1). When these mice were analyzed at early times p.i., MHV-JHM RNA could be detected in the olfactory lobes and mesencephalic nucleus of the trigeminal nerve, in the same distribution as observed in mice nursed by unimmunized dams. Similarly, the same distribution of viral RNA was observed at 3-4 d p.i. in mice infected with the attenuated A59 strain of MHV. These results suggested that MHV, whether the virulent JHM or avirulent A59 strain, entered the CNS via the olfactory and trigeminal nerves, both of which innervate the nose.

Regional localization in asymptomatic mice at 15 d p.i. Maternal antibody-protected mice remain asymptomatic at 15 d p.i., and MHV-JHM RNA could not be detected in the brains of these mice by in situ hybridization and film autoradiography. However, viral RNA could be detected in the cephalic part of the spinal cord in all mice that were examined. This result suggested that virus was transported from the olfactory/limbic or trigeminal systems to the spinal cord prior to the increase in viral replication which in turn led to clinical disease.

Relationship of serum antibody titer and the development of clinical disease. In the next set of experiments, we determined the level of neutralizing antibody protective against the acute disease, and also determined whether infected mice with low levels of antibody were capable of mounting any antibody response against MHV-JHM. To interpret experiments involving the decay of maternal anti MHV-JHM antibody, we first determined its rate of decay in uninfected suckling mice. Maternal anti-MHV-JHM

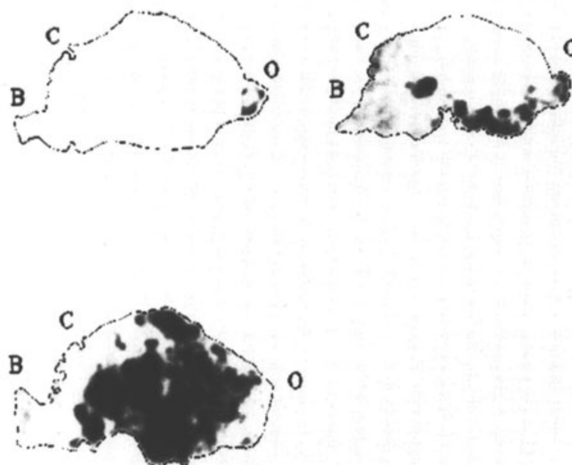

Figure 1-Computer digitalization of mice with acute encephalitis analyzed by in situ hybridization. Digitalization was performed using the LONISP program (Washington University, St. Louis). B-brainstem, O-olfactory lobe, C-cerebellum, arrow-mesencephalic nucleus of the trigeminal nerve. Upper left-3 dp.i.; upper right-4 d p.i.; lower left-5 d p.i.

antibody decayed with a half-life of 5.75 d (S.D. .88 d, range 4.76-6.88 d), in agreement with previous measurements of decay of total maternal antibody (5).

To determine the minimal level of neutralizing antibody protective against the acute encephalitis, antibody titers were measured at 3 and 7 d p.i. in 82 infected mice suckled by immunized dams. Fifty one (62%) had a titer >1:400, 22 (27%) had titers between 1:200 and 1:400 and 9 (11%) had titers between 1:100 and 1:200. These results suggested that titers greater than 1:100 were protective against the acute disease.

Serial titers were measured in 62 of these mice. Thirty six (69%) had neutralizing titers <1:100 when they developed hindlimb paralysis. The remainder had titers >1:100, with 4 mice (6%) developing hindlimb paralysis with serum titers >1:400 (Figure 2A). This result suggested that neutralizing titers effective against the acute disease did not protect against the development of hindlimb paralysis. For greater sensitivity, serial titers were also assayed by ELISA. Antibody titer decayed at the same approximate rate when measured by either neutralization assay or ELISA. However, measurement by ELISA showed that in most mice, the titer either remained constant or rose slightly at 20-30 days p.i. (Figure 2B). Thus, most mice were able to mount a minimal antibody response, which could only be detected by ELISA.

To determine whether the mice which exhibited a low antibody response were capable of mounting a greater response, a group of 18 mice greater than 40 d p.i. were immunized at weekly intervals with either live virus (two times) or UV-inactivated virus (three times) in Freund's adjuvant. 89% (16/18) of the mice so immunized developed an elevated antibody response with a titer (mean 1:692) similar to that observed in uninfected mice immunized as described previously (1).

Antibody response in young mice. The results presented thus far showed that maternal antibody-protected mice did not develop a significant antibody response to MHV-JHM, although they were capable of doing so if immunized in the presence of a strong adjuvant. To determine if younger mice were

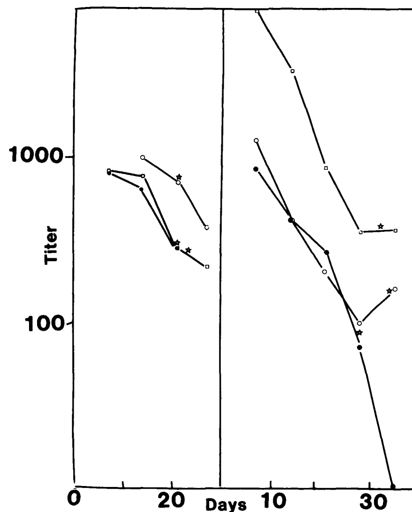

Figure 2-Serial neutralization and ELISA antibody titers in maternal antibody-protected mice. A) Neutralizing titers in 3 mice that developed hindlimb paralysis in the presence of elevated levels of serum antibody. B) Representative serial ELISA titers for three mice that developed hindlimb paralysis. *-time when hindlimb paresis was first noted.

capable of responding to MHV-JHM, uninfected suckling and young weanling mice, suckled by unimmunized dams, were immunized with live MHV-JHM in Freund's adjuvant. Five mice were immunized with MHV-JHM at weekly intervals for three weeks beginning at 14 d after birth. Four weeks later, neutralizing antibody titers ranged from 1:50 to 1:377, with a mean of 1:191. In comparison, mice immunized at 10-12 weeks of age developed mean titers of 1:692 (range 1:200 to 1:1500) as described in the previous section. Thus these mice were capable of mounting an antibody response, although the kinetics of response and maximal titer attained were less than that observed in older mice. Attempts made to immunize young infected mice with MHV-JHM were unsuccessful, probably because the presence of maternal antibody prevented any antibody response.

DISCUSSION

After intranasal inoculation, MHV enters the CNS via the olfactory and trigeminal nerves. If mice are susceptible to the acute encephalitis, virus spreads rapidly from these portals of entry to more central locations, causing death. On the other hand, if the fatal disease is prevented either by using an attenuated virus such as the A59 strain or by inoculating mice that are nursed by immunized dams, virus remains confined to the olfactory and trigeminal systems and their direct connections, such as the limbic system. Viral RNA disappears from the brain over the next few days, but can be detected in the spinal cord at 15 d p.i., although the mice remain asymptomatic.

In mice that develop hindlimb paralysis, viral RNA can always be detected in the spinal cord, suggesting that virus which has been previously transported to this structure is able to amplify and eventually cause clinical disease. At the same time, virus appears to amplify in some mice in other parts of the CNS which are either immediate or distant connections of the olfactory and trigeminal nerves and from there spread to other structures in the CNS.

The factors that are important for suppression of viral replication in asymptomatic mice, and those which facilitate viral amplification and resultant clinical disease have not been determined. One explanation would be that the virus has been modified during the period of clinical latency in mice, as has been shown to occur under certain conditions in rats infected with MHV-JHM (6-8). We have found that virus isolated from mice with hindlimb paralysis is identical to the initial stock of MHV-JHM by protein and RNA blot analysis, by radioimmunoprecipitation and polyacrylamide gel electrophoresis of infected cell proteins and by ELISA with a panel of monoclonal antibodies directed against 5 different epitopes of the E2 glycoprotein of MHV-JHM (antibodies provided by Dr. Michael Buchmeier).

A second explantion is that the immune system is not able to respond appropriately to MHV, thus allowing viral replication. Sufficient levels of maternal antibody protect mice from the acute encephalitis (1,9), but do not prevent viral persistence in the CNS. In this report, we show that after passively-acquired maternal antibody levels have decayed to low levels, antibody response in infected offspring is minimal, whether the mice have developed hindlimb paralysis or remain asymptomatic. Additionally, some of the mice become paralyzed at a time when their serum antibody titers are at a level which would protect against the acute encephalitis, suggesting that even if mice could mount a high antibody response, this would not protect against viral amplification and the development of clinical disease. One caveat is that these measurements were performed on serum, and may not reflect levels present in the CNS.

Neutralizing antibody may not be protective for one of several reasons.

Even though the envelope glycoprotein E2 is usually found on the surface of infected cells, less may be present in cells of the CNS (10). Another possibility is that MHV-JHM spreads primarily via cell to cell spread and is therefore not accessible to antibody.

Maternal antibody-protected C57BL/6 mice for the most part develop only a low level of neutralizing or total antibody, although they are capable of mounting a response if immunized with a strong adjuvant. This may reflect inadequate antigen presentation since the virus is present in the CNS, considered an immunologically protected site. The same difficulty may cause an inadequate cell-mediated immune response, which in turn might account for the inability of the host to eliminate MHV-JHM. An age-dependence exists as well, since antibody response is less in young mice and almost all mice which survive to ten weeks (60 d p.i.) do not develop hindlimb paralysis.

In previous studies, antibody response also has not correlated well with the development of clinical disease or demyelinating lesions in rodents infected with MHV-JHM (4,11). Our results suggest, as do other studies (12), that cellular immunity may be most important in protection of mice against MHV-JHM.

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REFERENCES

1. Perlman, S., Schelper, R., Bolger, E., Ries, D., 1987, Late onset, symptomatic, demyelinating encephalomyelitis in mice infected with MHV-JHM in the presence of maternal antibody. Microbial Pathog. 2:185.
2. Perlman, S., Ries, D., 1987, The astrocyte is a target cell in mice persistently infected with mouse hepatitis virus, strain JHM. Microbial Pathog. 3:309.
3. Perlman, S., Jacobsen, G., Moore, S., 1988, Regional localization of virus in the central nervous system of mice persistently infected with murine coronavirus JHM. Virology 166:328.
4. Stohlman, S., Weiner, L., 1981, Chronic central nervous system demyelination in mice after JHM virus infection. Neurology 31:38.
5. Pace, G., Dresser, D., 1961, The elimination of mouse and bovine globulins from the circulation of newborn and adult mice. Quart. J. Exp. Physiol. 46:369.
6. Taguchi, F. Siddell, S., Wege, H., ter Meulen, V.,1985, Characterization of a variant virus selected in rat brain after infection of mouse coronavirus JHM. J. Virol. 54:429.
7. Taguchi, F., Fleming, J., 1989, Comparison of six different murine coronavirus JHM variants by monoclonal antibodies against the E2 glycoprotein. Virology 169:233.
8. Morris, V., Tieszer, C., MacKinnon, J., Percy, D., 1989, Characterization of coronavirus JHM variants isolated from Wistar Furth rats with a viral-induced demyelinating disease. Virology 169:127.
9. Pickel, K., Muller, M., ter Meulen, V., 1985, Influence of maternal immunity on the outcome of murine coronavirus JHM infection in suckling mice. Med. Micro. Immunol. 174:15.
10. Parham, D., Tereba, A., Talbot, P., Jackson, D., Morris., V., 1986, Analysis of JHM central nervous system infections in rats. Arch. Neurol. 43:702
11. Watanabe, R., Wege, H., ter Meulen, V., 1987, Comparative analysis of coronavirus JHM-induced demyelinating encephalomyelitis in Lewis and Brown Norway rats. Lab. Invest. 57:375.
12. Sussman, M., Shubin, R., Kyuwa, S., Stohlman, S., 1989, T-Cell-Mediated clearance of mouse hepatitis virus strain JHM from the central nervous system. J. Virol. 63:3051