SPREAD OF MHV-JHM FROM NASAL CAVITY TO WHITE MATTER OF SPINAL CORD

Transneuronal Movement and Involvement of Astrocytes

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ABSTRACT

C57Bl/6 mice infected intranasally with mouse hepatitis virus, strain JHM (MHV-JHM) develop hindlimb paralysis with histological evidence of demyelination several weeks after inoculation. Virus must spread from the site of inoculation, the nasal cavity, to the site of disease, the white matter of the spinal cord. It has been shown previously that after intranasal inoculation, virus enters the brain via the olfactory nerve and spreads to infect many of its neuroanatomic connections within the central nervous system (CNS). In this report, it is shown that virus infecting the spinal cord is first detected in the gray matter, with spread occurring to the white matter soon thereafter. Astrocytes are heavily infected during the process of spread from the gray to the white matter of the spinal cord. Since astrocytes are in intimate contact with neuronal synapses and are themselves connected via gap junctions, these results suggest that astrocytes may be a conduit for the spread of virus in these mice. Astrocytes provide factors for the proliferation and survival of oligodendrocytes, and widespread infection of these cells might contribute to the demyelinating process eventually observed in these mice. Additionally, since virus first appears at specific locations in the spinal cord, it should be possible to determine the source of the virus infecting the cord. While the results are not definitive, the data are most consistent with virus spreading from the ventral reticular formation to the gray matter of the cervical spinal cord.

INTRODUCTION

Mouse hepatitis virus (MHV), a member of the coronavirus family, causes hepatitis, enteritis and encephalitis in susceptible rodents[1]. MHV strain JHM (MHV-JHM) is highly neurotropic and causes acute and chronic encephalomyelitis[2]. The most virulent strains,

Corona- and Related Viruses, Edited by P. J. Talbot and G. A. Levy
Plenum Press, New York, 1995

characterized in part by encoding a full length (4139 nucleotides) surface glycoprotein (S)[3], cause an acute encephalitis in nearly all strains of mice, with death occurring in 5-7 days. This acute infection can be modified so that most mice survive, but instead develop histological evidence of demyelination. Modifications that result in this scenario include use of attenuated strains of MHV-JHM[4] or passive administration of either protective antibody or T cells[5,6]. Virus or viral products can be detected in the white matter of these mice, although mice are asymptomatic in most cases.

In the model developed in this laboratory, suckling mice are inoculated intranasally with MHV-JHM and are protected from the acute, fatal infection by nursing with dams previously immunized against the virus[7]. Several weeks later, a variable percentage (40-90%) develop hindlimb paralysis with histological evidence of a demyelinating encephalomyelitis. Both the clinical and histological manifestations of this disease are primarily in the spinal cord suggesting that virus spreads to the spinal cord from the original site of inoculation (the nasal cavity). In several recent publications, the possible pathways used by the virus to spread within the brain have been described. First, the results indicate that virus spreads transneuronally, and presumably, trans-synaptically from the olfactory bulb. Surgical ablation of both olfactory bulbs or chemical destruction of the olfactory epithelium prevents virus entry into the brain, suggesting that virus spreads via infection of olfactory receptor neurons[8]. In contrast to what is observed with pseudorabies virus or herpes simplex virus[9,10], MHV-JHM does not enter the brain via the trigeminal nerve or via the sympathetic and parasympathetic nerves which also innervate the nasal cavity. Consistent with these observations, direct inoculation into the olfactory bulb labels precisely the same structures as does inoculation into the nasal cavity. The tropism for the olfactory bulb is so great that virus appears to enter the brain solely via the olfactory nerve even after inoculation into peripheral sites such as the tooth pulp or peritoneum (unpublished observations). Second, virus appears to spread primarily in a retrograde direction (from the axon to the cell body), although this is difficult to prove definitively since the olfactory bulb is reciprocally connected to its primary connections in most cases. Consistent with this, the olfactory tubercle, which receives projections from the bulb, but does not itself project to the bulb, only is rarely infected by MHV-JHM[10]. A recent publication suggests that MHV-JHM is released from both the dendritic and axonal surfaces of cultured neurons[11]. Release from the apical surface would make anterograde spread possible, but this direction of spread does not appear to occur in the infected animal to a significant extent.

Third, many, but not all of the primary connections of the main olfactory bulb are infected by MHV-JHM. Of particular note, the locus coeruleus is not infected by MHV-JHM and the hippocampus shows only minimal evidence of infection[10]. Both of these structures send prominent projections to the olfactory bulb. This distribution of infection is not shared by all viruses. Thus, after intranasal or intrabulbar infection, herpes simplex virus type I infects both of these structures, but does not infect other structures which are infected by MHV-JHM[10]. In a recent publication, rabies virus was shown to infect nearly the same structures as MHV-JHM, with sparing of the locus coeruleus[12]. The explanation for this sparing of the locus coeruleus by these two neurotropic RNA viruses, but not by a neurotropic herpesvirus remains to be determined.

RESULTS AND DISCUSSION

These previous experiments were all performed with young adult mice (6 weeks old) in which MHV-JHM was inoculated into the nasal cavity or olfactory bulb. These mice die before there is sufficient time for virus to reach the spinal cord. In previous reports, passive administration of neutralizing anti-MHV monoclonal antibody directed against the S glyco-

protein fully protected mice from the acute encephalitis, but did not prevent demyelination[5]. Mice remained asymptomatic in that study. In preliminary studies, we delivered varying amounts of antibody at different times relative to the intranasal inoculation of virus. Administration of antibody prior to virus resulted in complete protection from the acute disease, but protection was so complete that we could not detect virus in the brain by in situ hybridization. We determined that 7 μL of antibody (1:1 mixture of two anti-S monoclonal antibodies-5A13.5 and 5B19.2, kindly provided by Dr. M. Buchmeier, The Scripps Research Institute) administered intraperitoneally 72 hours after infection fully protected mice from acute encephalitis, but virus could still be detected in the brain by in situ hybridization. By 72 hours, MHV-JHM has caused a significant infection of the olfactory bulb which would spread in the absence of antibody throughout the entire brain over the next 48-72 hours. The presence of relatively small amounts of protective antibody administered even after infection was well established in the olfactory bulb thus prevents an acute, fatal infection but not the transneuronal spread of virus to distal connections of the bulb. In our initial experiments we showed that the same brain structures were infected in the presence of antibody as we observed previously in the absence of antibody. We also showed that histological evidence of demyelination could be detected in the spinal cord 2 months after inoculation even though the mice remained asymptomatic, suggesting that virus had spread to the cord and persisted in this structure.

To determine the initial site of infection within the spinal cord, three week old C57Bl/6 mice were inoculated intranasally with virus and protected from the acute, fatal infection by the intraperitoneal administration of monoclonal antibody. At 6-7 days post infection, virus was readily detected in the spinal cord by both in situ hybridization and immunohistochemical techniques (Figure 1). Virus was first noted in the gray matter of the cervical spinal cord in laminae V-VII, although spread to the white matter occurred very soon thereafter. The initial site of labeling was always the same and suggested that spread to the spinal cord occurred from a single site or a few sites in the brain. In the next set of analyses, we attempted to determine the source of the virus which infected the cord. For these analyses, we assumed that virus spread solely in the retrograde direction, as this is consistent with previous studies.

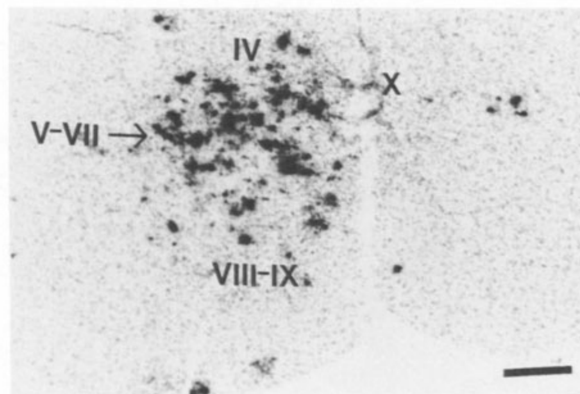

Figure 1. Virus appearance in the gray matter of the spinal cord. Brains and spinal cords were harvested at 6-7 days after intranasal inoculation with MHV-JHM and frozen in OCT. Sections were prepared and analyzed by in situ hybridization as described previously[10]. Virus was initially detected in laminae V-VII and then as shown in the figure, rapidly spread to involve other parts of the gray matter. A small amount of virus can also be detected in the white matter in this section. The approximate position of each lamina is shown in the figure. Magnification bar-200 μm.

The spinal cord has major projections to the reticular formation, the thalamus, the hypothalamus, the midbrain and the cerebellum, as well as less prominent connections to other CNS areas such as the ventral forebrain[13 17] Specific sites in the spinal cord project to each location For example, the spinothalamic tract originates primarily in the cervical cord, with a smaller projection from the lumbar spinal cord Within the rostral spinal cord, this tract originates from both dorsal and ventral sites Analysis of each of the above sites did not establish any single one as the definitive source for the virus infecting the spinal cord However, the cerebellum was not likely to be the source for virus, since the spinal cord projects to the cerebellar cortex and medial and interposed nuclei, none of which is infected by MHV-JHM to a significant extent On the other hand, the ventral reticular formation is likely to be the source for virus infecting the cord First, the ventral reticular formation receives many projections from the spinal cord, with a very high representation of fibers originating in the appropriate laminae (V-VII) of the cervical region Second, the ventral reticular formation is heavily infected by MHV-JHM in all cases, and this structure is labeled just prior to infection of the spinal cord Other structures which receive projections from the upper spinal cord, such as the midline thalamic nuclei, are not as heavily or consistently infected by MHV-JHM as is the reticular formation Thus, the specificity of labeling in the cervical spinal cord and reticular nuclei and the time course of the MHV-JHM infection all strongly suggest that these nuclei are the source for the spinal cord infection

A summary of the data is shown in Figure 2 In this figure, we assume that virus spreading to the cord originates from one of the four structures (ventral reticular formation, midline thalamic nuclei, lateral hypothalamus, central gray) which receives projections from the cord and is infected by MHV-JHM Likely pathways from the nasal cavity to these structures are shown, although connections to the pontine and medullary reticular formation are sufficiently ill-defined so that this approach is not possible for these structures

Once virus has spread to the gray matter of the spinal cord, it can be detected soon thereafter in the white matter In the next set of experiments, we defined more precisely the basis for this spread The presence of neutralizing antibody made it likely that virus moved to the white matter via cell-to-cell spread and not via the extracellular fluid In theory, virus could spread from the gray to the white matter without exposure to the extracellular fluid via either axons or astrocyte-astrocyte connections Astrocytes are extensively connected via gap junctions and have been postulated to form a giant functional syncytium[18] Astrocytes can be specifically identified using antibody to glial fibrillary acidic protein (GFAP) In the next set of experiments, infected spinal cords were simultaneously assayed for MHV-JHM

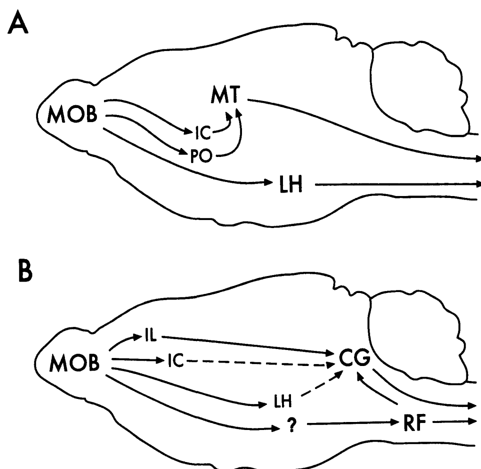

Figure 2. Pathways of virus spread from the site of inoculation, the nasal cavity, to the site of disease, the spinal cord Virus could spread from the brain to the spinal cord via the thalamus (A), the lateral hypothalamus (A), the reticular formation (B) or the midbrain (central gray) (B) The probable pathways involving these structures are shown in the figure and are indicated by solid lines Dotted lines show other pathways which are present, but less likely to be used by the virus to spread from the nasal cavity to the spinal cord Virus could spread to the reticular formation via many possible pathways, so for the sake of simplicity, no specific one is indicated Abbreviations-MOB-main olfactory bulb, PO-piriform cortex, MT-midline thalamic nuclei, IC-insular cortex, LH-lateral hypothalamus, IL-infralimbic cortex, CG-central gray, RF-reticular formation

RNA and astrocyte antigens The results showed that astrocytes were heavily infected during the initial stages (days 6-7) of virus spread to the white matter, with infection appearing to radiate throughout the ventral and lateral parts of the spinal cord These results are in general agreement with our previous results, in which we showed that a substantial fraction of infected cells in both symptomatic and asymptomatic mice were astrocytes[19]

Although these mice are asymptomatic, these results have implications for the pathogenesis of the demyelinating disease caused by MHV-JHM First, astrocytes are in close contact with oligodendrocytes, and are connected to them via gap junctions Virus could spread from astrocytes to oligodendrocytes at sites of gap junctions, since the two cellular membranes are juxtaposed at these locations MHV-JHM has a very fusogenic surface glycoprotein which is able to fuse cells lacking cellular receptor for the virus[20] Presumably, this protein would facilitate viral spread, even at sites lacking virus receptor Second, astrocytes are believed to produce factors critical for oligodendrocyte differentiation, proliferation and survival, as well as cytokines, such as a tumor necrosis factor, which are toxic for oligodendrocytes[21] Astrocyte dysfunction might contribute to the demyelinating process, either by lack of production of important trophic factors, or production of toxic agents In conclusion, infection of astrocytes may be the pathway by which virus spreads from neurons to oligodendrocytes and may also indirectly contribute to the demyelinating process

ACKNOWLEDGMENTS

This research was supported by grants from the N I H S P was supported by a Research Career Development Award from the N I H , N S by an N I H Institutional Training Grant and E B by a N I M H predoctoral fellowship

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