VACUOLAR DEGENERATION INDUCED BY JHM-CC VIRUS IN THE CNS OF CYCLOPHOSPHAMIDE-TREATED MICE

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INTRODUCTION

The infection with JHM-CC virus (1), a small plaque mutant of neuro-virulent JHM virus, is non-fatal in immunocompetent mice but constantly induces vacuolar degeneration in the brain and spinal cord (2). To elucidate the role of host immune response in pathogenesis of JHM-CC induced vacuolar degeneration in the central nervous system (CNS), the kinetics of virus growth and the temporal sequence of viral antigen distribution were correlated with histopathological changes in cyclophosphamide-treated adult ICR mice.

MATERIALS AND METHODS

A total of 204 male ICR mice of 4 to 6 week-old of age were inoculated i.c. with 0.02 ml of undiluted JHM-CC virus preparation. The stock virus of JHM-CC was prepared after 3 serial plaque-purifications in DBT cell monolayer cultures. After centrifugation at 8,000 rpm for 10 min, the supernatant of infected cultures was stored at -80°C until use. This virus preparation had an infectivity of 5×10^6 PFU/ml. Ninety-four out of 204 infected mice were given i.p. 100mg/kg of cyclophosphamide (Cy) 1, 3 and 5 days after virus inoculation. Both Cy-treated and untreated mice were randomly sacrificed between day 1 and day 21 for virus titration, immunohistochemistry and histological examinations.

For virus titration, the tissue homogenates were prepared from the brain and spinal cord separately. The infectivity was assayed by plaqueing in the DBT culture as reported previously (1). For histological and immunohistochemical examinations, mice were perfused with a 10% phosphate buffered formalin solution. Multiple coronal sections of the brain and longitudinal sections of the spinal cord were embedded in paraffin. Serial paraffin sections were stained with H&E and by the Kluver-Barrera and Bodian methods for myelin and axon. The viral antigen was detected in paraffin sections with a hyperimmune rabbit serum raised against the DBT-culture-grown JHM virus by the avidin-biotin peroxidase complex (ABC) method. Some sections were also subjected to immunohistochemistry with polyclonal antibodies against neurofilaments (150k) and GFAP.

RESULTS

Clinical symptoms and virus titer in the CNS

The JHM virus infection was usually dormant in immunocompetent mice, although approximately 20% of them developed mild hindlimb paralysis. On the contrary, the infection in Cy-treated mice was often fatal. Almost half of Cy-treated mice became very inactive between day 7 and day 12, and 40 out of 94 Cy-treated mice died with or without paraplegia.

The kinetics of virus titer in the CNS tissue is shown in Figure 1. Each point represents the average of 3 mice. In immunocompetent mice, the virus titer of the brain (closed circles with a dotted line) reached its peak on day 5 and then rapidly decreased. On the other hand, the virus titer of the spinal cord (open circles with a dotted line) increased slowly and gradually decreased. In Cy-treated mice, the mean virus titer of the brain (closed circles with a solid line) rapidly rose with a peak on day 7. The high titer in the brain was sustained for another week, and then it appeared to decline gradually. The virus titer of the spinal cord in Cy-treated mice (open circles with a solid line) slowly increased reaching its peak on day 10 and then declined. In both groups, the highest titer in the spinal cord was always lower than that in the brain.

Fig.1. Virus titer in the brain and spinal cord.

Viral antigen distribution and histopathological changes in the CNS of Cy-treated mice

Since histopathology of this virus infection in immunocompetent mice is presented in another chapter of this book (T. Tsukamoto et al), only the findings in Cy-treated mice will be described in this chapter. In Cy-treated mice, inflammatory cell infiltration in the CNS was almost completely abrogated during an observation period of 3 weeks. Although a few mononuclear cells were seen in the subarachnoid space of the mice sacrificed on days 3 and 5, and massive cell necrosis was often accompanied by a focal microglial reaction, heavy mononuclear cell infiltration into parenchymal tissues and in perivascular spaces was totally absent in Cy-treated mice.

The brain: In asymptomatic mice sacrificed on days 3 and 5, the viral antigen was readily detected in a large number of neurons in the brain. In addition to sporadic occurrence of viral antigen positive (Ag-positive) cells in the ependymal linings of the lateral, 3rd and 4th ventricles and of the choroid plexus, in the paraventricular tissue and the tissue adjacent to the needle tract, the viral antigen was detected in the majority of mitral cells of the bilateral olfactory bulb (Fig. 2). Some neurons in the anterior olfactory nuclei were also Ag-positive but the glomerular and internal granule layers of the olfactory bulb were virtually free of the viral antigen. Groups of Ag-positive cells were also found in the habenular nuclei, the perirhinal, pyriform and entorhinal and somatosensory cortices. Furthermore, a few Ag-positive neurons were always present in the mesencephalic reticular formation and the gigantocellular reticular nuclei of the pons. The number of Ag-positive cells apparently increased on day 5 but their distribution remained unchanged. In many neurons, not only their perikaryon but also the entire cellular processes were heavily labeled with anti-JHM antibody. No glial cells, however, were convincingly labeled with the antibody.

Fig. 2. JHM virus antigens are strongly expressed in the majority of mitral cells of the bilateral olfactory bulb while the glomerular and internal granule cell layers are free of the antigens. 5th post-inoculation day. ABC method.

At this stage of infection, the majority of Ag-positive cells showed no cytopathic changes. Necrotic cells were only rarely encountered among the largely normal ependymal lining cells, the medial habenular nucleus, the hypothalamic nuclei, the deep layer of the cerebral cortex and the roof of the 4th ventricle. Those cells had a deeply eosinophilic cytoplasm and their nuclei were often fragmented. In the 3rd and 4th ventricles, a few desquamated ependymal cells were found on the ventricular surface. Proliferation of microglial cells was evident at the sites of neuronal degeneration. In some mice, mild subarachnoid mononuclear cell infiltration was seen.

On days 7 and 10, Ag-positive cells were more widely distributed in the brain. In the olfactory system, the viral antigen was not confined to the mitral cell layer. Many cells in the molecular and glomerular layers and in the anterior olfactory nuclei were also Ag-positive. The internal granule cell layer, however, was almost completely spared. In the mid-brain and pons, Ag-positive large neurons were widely distributed (Fig. 3). The perikarya and neuritic processes of the Ag-positive neurons often appeared vesicular, and some had a ghost-like appearance (Fig. 3, arrows). On day 10, Ag-positive cells were more randomly distributed in the brain.

In some mice sacrificed on day 10, a large number of the Purkinje cell perikarya and their apical dendrites were heavily labeled. Furthermore, groups of Ag-positive neurons were randomly distributed in the neocortex, the hippocampus and entorhinal cortex. The thalamus and caudoputamen, however, were usually spared. Histological examinations disclosed that some neurons had already been lost and many of the remaining neurons were either chromatolytic or severely pyknotic in the mitral cell layer. In the mesencephalic and pontine reticular formation, swelling of large neurons was conspicuous. Both their perikarya and neuritic processes were markedly swollen and appeared watery, and the neuropil in these regions was grossly vesicular on day 10 (Fig. 4). Such vacuolar changes often involved the bilateral fasciculus retroflexus, the bilateral olfactory and mammillothalamic tracts. Although the majority of the Ag-positive cells randomly distributed in the brain showed little cytopathic change, massive neuronal degeneration was commonly seen in the bilateral entopeduncular and subthalamic nuclei. In addition, necrotic Purkinje and Golgi cells were sporadically found in the cerebellar cortex.

Fig. 3. Presence of the viral antigens in a large number of neurons in the gigantocellular reticular nuclei of the pons on day 7. Note the viral antigens in severely degenerated cells and their processes (arrows). ABC method.

Fig. 4. Vacuolar degeneration of the pontine reticular formation on day 10. The presence of nuclear remnants in some vacuoles (arrows) could be suggestive of the neuronal cell origin of these vacuoles. H&E stain.

On day 14 and thereafter, the number of Ag-positive cells in the brain gradually decreased while vacuolar degeneration was steadily progressive. On day 21, the viral antigen was virtually absent in the olfactory bulb, and was sparsely distributed in the severely vacuolated midbrain and pons and rarely in the cerebral cortex. In some mice, however, a large number of Purkinje cells remained strongly Ag-positive while the antigen was hardly detectable in other parts of the brain. Histological examinations on day 21 disclosed foci of massive neuronal cell necrosis and loss, often accompanied by pseudocalcification. Such changes were commonly seen in the olfactory bulb and the subthalamic nucleus. Vacuolar change of the mesencephalic and pontine reticular formation was most extensive in the mice sacrificed on day 21.

The spinal cord: Development of the viral antigen was apparently retarded in the spinal cord. Although as early as 3 days after virus inoculation, the viral antigen was detected in a few neurons residing in the vicinity of the central canal and root entry zones, the majority of spinal neurons remained Ag-negative until day 7. The number of Ag-positive neurons reached its peak on day 10 and then decreased. Except for few swollen chromatolytic neurons in both the anterior and posterior horns, histopathological change of the spinal cord was inconspicuous.

Unlike the infection in the brain, however, groups of Ag-positive cells were found in the white matter on day 10 and thereafter. These Ag-positive cells were often clustered near the surface and mostly lined up along the myelinated fibers (Fig. 5a), but they were not necessarily accompanied by myelin degeneration. Most of them had a scanty cytoplasm and seemed to be GFAP negative (Fig. 5b). In all of 4 mice sacrificed on day 21, there were many foci of focal vacuolar degeneration in the white matter (Fig. 5c). These vacuoles were mostly associated with swollen axons and Ag-positive mass was occasionally localized to these vacuolar lesions (Fig. 5d).

Fig. 5. Vacuolar change and viral antigens in the spinal white matter. Most of Ag-positive cells lining along myelinated fibers (a) appear to be GFAP-negative (b). Many vacuoles in the white matter (c) are apparently associated swollen axons and often contained Ag-positive mass(d).

DISCUSSION

Vacuolar change of the CNS tissue in mouse hepatitis virus (MHV) infection has long been recognized but the study on its mechanism was often hampered by a high mortality of the infection in mice. Unlike other strains of MHV, the JHM-CC virus infection in adult mice is not fatal and yet constantly induces vacuolar degeneration in the CNS tissue. In the present study, Cy-treatment effectively suppressed inflammatory reactions at least for 3 weeks at the expense of an increased mortality rate up to 40%. In the absence of inflammatory cell infiltration, Cy-treated mice developed vacuolar degeneration in the CNS. Although the absence of inflammatory cells gave superficially an impression of milder pathological changes in Cy-treated mice than those in immunocompetent mice, the severity and temporal and spatial sequences of vacuolar degeneration in Cy-treated mice was comparable to those in immunocompetent mice. Therefore, host immune response does not seem to be playing any major role in the pathogenesis of vacuolar degeneration induced by this JHM-CC virus.

Cy-treatment also resulted in the protraction of virus clearance from the CNS tissue, and this provided an opportunity to chase the spread of virus in the CNS for an extended period of time. After intracerebral virus inoculation, a portion of the inoculum apparently spread along the passage of the CSF resulting in the development of sporadic infections in the ventricular and paraventricular tissues. However, early and exclusive development of a large number of Ag-positive cells in the bilateral olfactory system and the brainstem reticular formation could be indicative of possible neural spread of the virus within the CNS as reported in an A59 virus infection in mice (3). Distribution of the viral antigen in the present study seemed to support the possibility of virus spread through the habenular nucleus, the fasciculus retroflexus and the interpeduncular nucleus to the brainstem reticular formation.

In the brain, the viral antigen was solely localized to neurons except for rare infection of ependymal cells at an early stage of the infection. Susceptibility of neuronal cells to this strain of virus is seemingly diverse. No viral antigens were detected in the internal granule cell layer of the olfactory bulb despite the severe infection and extensive necrosis in the neighboring mitral cell layer. Similarly, in the cerebellum, only Purkinje and Golgi cells were selectively infected leaving the granule cells totally uninvolved. The fate of infected cells was also variable. Comparing the number of Ag-positive cells with that of necrotic cells, many of the infected cells seemed to survive. Massive neural necrosis was found only in the mitral cell and molecular layers of the olfactory bulb, the subthalamic nucleus, the entopeduncular nucleus, and when they were involved, it occurred always bilaterally.

While pyknotic cells with karyorrhexis were occasionally found in the brain, the majority of the infected cells were rendered to marked swelling of both the perikarya and neuritic processes; a unique phenomenon in virus infection. The process was apparently slow but it was severe enough to make a substantial contribution to the development of vacuolar degeneration in the midbrain and pons. Similar vacuolar changes also involved small fiber tracts, anatomically related to the sites of severe infection, such as the fasciculus retroflexus and the olfactory tract. On the other hand, the callosal radiation and the pyramidal tract remained intact even on day 21. Therefore, the white matter does not seem to be the primary target of the infection. Nevertheless, swelling and vesicular degeneration of infected cells were a unique feature of this virus infection.

In the spinal cord, the viral antigen was mostly localized to

neurons, and many neurons in both the anterior and posterior horns were strongly Ag-positive on days 7 and 10. On day 10 and thereafter, however, there were a number of Ag-positive cells in the white matter. They tended to locate near the surface of the cord or in the vicinity of the root entry zones. Most of the Ag-positive cells had a scanty cytoplasm and lined up in a row along myelinated fibers. They were most likely to be oligodendrocytes. No particular white matter lesion, however, was associated with the presence of these Ag-positive cells, and the implication of this finding remains to be elucidated. On the other hand, there were many small foci of vacuolar change in the white matter on day 21. These lesions were apparently formed by swollen axons, and the viral antigens were often localized to the degenerated axons. The presence of Ag-positive neurons in the dorsal root ganglion, although it was rare, suggested a centrifugal spread of the virus.

Although the diversity of pathogenesis of mouse hepatitis virus has been well recognized (4), much attention has been paid to the demyelinating activity of mouse hepatitis virus. The present experiment provided further evidence for the importance of neuronal degeneration in the development of vacuolar degeneration which sometimes mimicks the pathology of demyelination.

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