Massive Cerebral Cortical Necrosis in Suckling Rats
Inoculated Intracerebrally with Mouse
Hepatitis Virus, A59 Strain

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(Received for publication October 1, 1979)

Abstract. A necrotizing encephalopathy with massive cerebral cortical necrosis was
produced in suckling rats inoculated intracerebrally with MHV-A59 strain. After inoculation
at 3 days of age most rats died on 6 to 13 days postinoculation showing a central
nervous symptom. At autopsy the cornus ammonis was visible through the edematous
leptomeninges owing to liquefactive necrosis of the cerebral cortex with increased cere-
brosinal fluid in the lesion. By immunofluorescence viral antigen was detected in neurons
of the cerebral cortex on day 2 or later postinoculation. Histopathologically degeneration
or necrosis of neurons and glial cells were recognized in some liquefactive foci. After
inoculation of the same virus at 7 days of age all suckling rats survived, while they had
necrotic lesions in the brain, which tended to have repairing process consisting of glia-
mesenchymal scarring. There were little changes in the brain of rats inoculated at 2
weeks of age.

Mouse hepatitis virus (MHV) is known to
cause either hepatitis or encephalitis not
only in mice but also in hamsters and rats
[1, 14, 21], while the pathogenic ability
varied between MHV strains [10, 17, 20, 22].

Recently, we revealed that massive necrosis
and disappearance of the cerebral cortex
were produced in suckling rats, which were
inoculated with MHV-A59 and survived
from the infection [8]. Though the lique-
factive necrosis in the cortex was described
in the brain of MHV-JHM infected suck-
ling mice [5], there have been no reports on
the production of massive necrosis of the
cerebral cortex as observed in the present
study.

The present paper deals with histopatho-
logy of massive necrosis of the cerebral cor-
tex in suckling rats infected with MHV-
A59, which might provide a useful model
for cerebral anomalies due to perinatal viral
infection in men and domestic animals.

Materials and Methods

Virus: Mouse hepatitis virus A59 strain (MHV-
A59) was kindly supplied by Dr. J. C. Parker, Micro-
biological Associates, Inc., Bethesda, Md., U.S.A.,
and it was propagated in SR-CD-DBT (DBT) cells
[7]. Virus titration was carried out by counting
plaque forming unit (PFU) on DBT cells as pre-
viously described [9]. Mice were inoculated intra-
cerebrally (i.c.) with 10⁵ PFU in 0.02 ml.

Animals: Fischer-CDF rats at 2 weeks of preg-
nancy were purchased from a commercial breeder
(Charles River Japan Co., Atsugi). Offsprings were
nursed by their own dams and they were inoculated
at 3 days, 1 and 2 weeks after birth.

Tissues of the brain, liver, spleen, lung, heart,
thymus and mandibular glands were taken from
dead or killed rats and fixed in Bouin’s fixation
solution, and embedded in paraffin. Four to 6 μm
sections were made, and stained with hematoxylin-eosin, Klüver-Barrera’s Luxol fast blue, periodic acid-Schiff (PAS). According to Watanabe or Loots [13], silver impregnation method was applied to brain preparations.

Immunofluorescence: Brain tissues were taken from suckling rats inoculated at 3 days of life and killed 12 to 96 hr postinoculation (p.i.) and from those killed at 24 hr to 6 days p.i. They were immediately frozen in n-hexane chilled in acetone-dry ice mixture, 5 μm sections were fixed in acetone for 5 min, and treated at 4°C overnight with anti-MHV-2 rabbit IgG conjugated with fluorescein-isothiocyanate (FITC). The preparation of the FITC-labeled antibody was made according to a method described earlier [4].

Results
Most of suckling rats inoculated at 3 days of age with 10⁵ PFU MHV-A59 died in 6 to 13 days, while some developed a central nervous symptoms and survived (Table 1). In all dead cases there was marked increase in cerebrospinal fluid. The cornu ammonis was visible through the edematous leptomeninx, which had severe hemorrhage in some cases. The liquefactive necrosis of the cerebral cortex was prominent in all cases. The distribution of the lesions is shown in Fig. 1.

In suckling which were inoculated at 3 days of age and died in 8 days or later the whole cerebral cortex showed severe liquefaction with the high amounts of fluid substance filling the lesion (Fig. 2).
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In those survived the acute stage and killed at day 12 p.i. necrosis and partial tissue destruction of the cerebral cortex were recognized with infiltration of macrophages and neutrophils. Though a part of the diencephalon was involved, changes were seen in neither any other parts of brain stem, cerebellum, spinal cord nor visceral organs.

The rats inoculated with the same virus dose at 7 days of age showed ataxia and trembling. The clinical symptoms became less serious in 10 days or later and all of the inoculated rats survived. In rats inoculated at 14 days of age, there were little lesions in the brain.

In the next experiment suckling rats were inoculated intracerebrally at 3 days of age and they were killed and examined for occurrence of brain lesions at intervals of 12 hr.

Slight dilatation of the lateral ventricles and hemorrhage in subependymal area were already seen at 12 hr p.i. There appeared polykaryocytes in the subependymal area of the olfactory ventricle (Fig. 3) as well as partial proliferation of the leptomeninges with fibrous pili. At 36 hr p.i. the cerebral cortical tissue showed granular appearance. At 48 hr p.i. there was no gross change but atrophy of neurons with rounding and pyknosis was recognized. A few neutrophils and mononuclear cells infiltrated around small vessels of the leptomeninges or the brain parenchyma (Fig. 4). At this stage of infection faint specific immunofluorescence was seen in occasional neurons of the cerebral cortex.

At 72 hr p.i., the rarefaction of the tissue with accumulation of neutrophils and mononuclear cells having a rod shaped nucleus appeared in every part of the cerebral cortex, especially in perivascular area. Specific immunofluorescence was seen in most neurons in the cerebral cortex (Fig. 5). In a part of the cerebral cortex and corpus geniculatum mediale degenerative or necrotic lesions with cell infiltration and liquefactive loss of the tissues were seen (Fig. 6).

At 96 hr p.i., extensive liquefactive destruction of the cerebral cortex was recognized (Fig 7). At 144 hr p.i. or later, the changes became severer. Pyknosis, karyorrhexis and cellulolysis of neurons and glial cells were prominent with degeneration of infiltrated cells in the cerebral cortex as well as a part of the diencephalon.

At 6 days or later neutrophils decreased in number while there was increased in macrophages containing debris which were stained dark with both luxol fast blue and PAS (Fig. 8). Only faint immunofluorescence was recognized in severely necrotized lesions. Many thrombi were formed in vessels of the meninge and ependyma (Fig. 9). No demyelination was observed.

In sucklings inoculated at 7 days of age the occurrence of brain lesions up to 48 hr was similar to those seen in rats inoculated at 3 days of age. However, in rats inoculated at 7 days of age, there were only some wedge-shaped or semispherical necrotic lesions with a number of mononuclear cells appearing in the cerebral cortex. Mononuclear cells and hemorrhage were present in the submeningeal areas and thickened leptomeninges.

At 6 days p.i. some necrotic areas appeared in the diencephalon with infiltration of a number of mononuclear cells and a few neutrophils. Around the necrotic foci in the cerebral cortex or diencephalon a considerable number of microglial cells with rod-shaped nuclei were scattered, and there were accumulations of macrophages within loose network of reticular fibers, along with the thickened leptomeninges (Fig. 10).
At 8 days p.i. the proliferation of reticular and collagen fibers was prominent in submeningeal cortical lesions, especially around the vessels. In the deep of the cerebral cortex there was proliferation of astrocytes and macrophages around atrophic and depressed necrotic lesions, while in submeningeal lesions a few astrocytes were recognized (Fig. 11).

At 10 days p.i. cell debris mostly disappeared and formation of reticular fiber network denoted glialenchymal scars or mesenchymal scars. Fibrosis was not so prominent (Fig. 12). In other areas of the cerebral cortex and mesencephalon a few glial nodules were formed and, in many cases, microglial cells were diffusely scattered.

At 16 days p.i. the network were replaced by fibrous tissues with precipitation of PAS-positive granules.

Fig. 13 is a scheme showing the sites of brain lesions after MHV-A59 inoculation in suckling rats.

**Discussion**

Hydrocephalus is a congenital anomaly frequently produced in men and animals [6, 25], and most cases are of the internal hydrocephalus characterized by dilatation of the lateral ventricles due to marked accumulation of cerebrospinal fluid. Less frequently, however, there have been also some cases of hydranencephaly or porencephaly with extensive necrosis resulting in loss of the cerebral cortex [2, 24].

On the other hand, the hydrocephalus can be produced experimentally by inoculation with neurotropic viruses. For example, the destruction of the subcortical white matter in hydranencephaly or porencephaly was reported in lambs which had received Bluetongue vaccine virus [15].

Among various strains of mouse hepatitis virus. Both MHV-JHM and MHV-A59 strains were reported to be encephalitogenic in suckling rats [8]. Although MHV-JHM can produce destructive encephalitis or persistent infection in suckling rats [14], MHV-A59 was found to cause diffuse liquefactive necrosis in the cerebral cortex, resulting in hydranencephaly which have been described in men and domestic animals [12, 25].

By immunofluorescence rat brain neurons were demonstrated to be target cells for MHV-A59 as observed in infected mice [4]. Massive necrosis of the cerebral cortex observed in rats infected with MHV-A59 had
never been observed in acute and fatal infection in mice.

In MHV-A59 infected suckling rats viral antigen was detected only in neurons but not in ependymal cells of the lateral ventricles nor vascular endothelial cells [8]. This suggests that an anomaly might result from massive destruction of immature neuronal tissues during the brain development.

The resistance to intracerebral inoculation with MHV-A59 increased with age, and rats were no more susceptible to the virus at 2 weeks of age. Such a resistance may not be related to immunological maturation, because the immune system is known to be still immature at this age in rats [19]. On the other hand, the structural immaturity of the cerebral cortex in suckling rats, showing dense populations of immature neurons with unmyelinated axons and glial cells, may provide a favorable conditions for spreading and replication of the virus.

During a repairing process of necrotic lesions in suckling rats, glia-mesenchymal scarring was shown to be produced with reactions of astrocytes, perivascular mesenchymal cells and macrophages from the blood stream. These scars were formed in the deep of the cerebral cortex and diencephalon. Besides, another type of mesenchymal scars without proliferation of astrocytes was present involving the pia mater and the submeningeal lesions, consisting of reticular fiber network from the pia mater and mesenchymal cells. The two kinds of scars were different each other in localization but not in the process of formation. The repair process was similar to that seen in cerebral softening with hemorrhage followed by cavitation in man [3] as well as in experimentally produced brain injury in rabbit [18]. While the lesions persisted, viral antigen was shown to disappear at an early stage of infection.

Experimentally, hydranencephaly or porencephaly was reported to be produced in lambs after inoculation with Bluetongue vaccine during pregnancy [16]. In this case necrotizing process occurred first in neuroblasts and neuropia cells, which consequently failed to migrate and mature in the cerebral cortex. The present study showed that undifferentiated neurons are initially infected with MHV-A59, leading to massive liquefactive necrosis of the immature cerebral cortex resulting in hydranencephaly or false porencephaly. Experimentally produced hydrocephalus was described in suckling hamsters and mice inoculated with myxoviruses. These, however, seem to be different from the present cases in terms of that neurons remained unaffected while there were dilatation of the lateral ventricles and severe spongy degeneration in the cerebral cortex and medulla [11].

Recently, congenital abnormality of calves with arthrogryposis and hydranencephaly was reported. As for the hydranencephaly, the necrosis of the immature central nervous tissue caused by inflammatory stimuli were stressed in pathogenesis [12].

The observations in suckling rats inoculated with MHV-A59 strain might tend to elucidate pathogenesis of congenital or perinatal cerebral anomalies, e.g., hydranencephaly or porencephaly, in both men and animals.

References


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Explanation of Figures

Figs. 2-6, tissue sections were from suckling rats inoculated at 3 days of age and Fig. 7-11, those were inoculated at 7 days of age.

Fig. 2. Massive liquefactive tissue destruction of the cerebral cortex and dorsal thalamic areas. Coronal section. Dead at 8 days p.i. Hematoxylin-eosin (H-E) stain. ×10.

Fig. 3. Polykaryocytes in subependymal area of the olfactory ventricle. 48 hr p.i. H-E stain. ×420.

Fig. 4. Swelling or pyknosis in neurons with infiltration of a few neutrophils and mononuclear cells in the cerebral cortex. Killed at 48 hr p.i. H-E stain. ×420.

Fig. 5. Virus specific immunofluorescence in neurons of the cerebral cortex. Killed at 72 hr p.i. ×800.

Fig. 6. Extensive liquefactive destruction of the cerebral cortical tissues. Killed at 72 hr p.i. H-E stain. ×100.

Fig. 7. Extensive liquefactive destruction of the cerebral cortical tissues. Killed at 96 hr p.i. ×100.

Fig. 8. Accumulation of many macrophages containing PAS positive debris in the submeningeal lesion. Killed at 6 days p.i. PAS. ×800.

Fig. 9. Venous thrombus in the pia mater. Killed at 6 days p.i. H-E stain. ×200.

Fig. 10. Perivascular and tissue infiltration of macrophages in the submeningeal cortical area. Killed at 6 days p.i. H-E stain. ×420.

Fig. 11. Astrocytes proliferation around the necrotic lesions. Killed at 8 days p.i. H-E stain. ×1000.

Fig. 12. Reticular fiber network in the submeningeal area of the cerebral cortex. Killed at 10 days p.i. Watanabe's silver impregnation. ×1000.