Apoptotic Changes in the Thymus of Mice Infected with Mouse Hepatitis Virus, MHV-2

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ABSTRACT. In mice infected with MHV-2, histopathological changes of the thymus was studied. Extensive cell lysis with pyknotic nuclear debris appeared at 48 hr postinfection, and cortico-medullary border was indistinguishable. Electron microscopy revealed vacuolation and shrinkage of the cytoplasm of lymphoid cells, margination of nuclear chromatin, and fragmentation of nuclei. Virus particles were detectable in the lymphoid and reticular epithelial cells, being immunohistochemically positive for viral antigen. By DNA electrophoresis thymocytes showed DNA fragmentation with a laddering pattern characteristic of apoptosis.---KEY WORDS: apoptosis, MHV-2, thymus.

In mouse hepatitis virus (MHV) infection in mice, a model for human viral hepatitis, the host resistance to the infection strongly depends on the genetic background and immune function. The lymphotropism of MHV has been suggested by the viral isolation from lymphoid organs of infected animals [15], the ability of MHV to replicate in cultured lymphocytes [11] and the persistent infection in mouse lymphoid cell lines [12]. The hepatopathogenicity of MHV and its ability to cause lymphoid injuries [13, 14] and selective T lymphocyte depletion in the spleen and thymus resulting in progressive immunodeficiency, were reported [13, 14, 17].

Apoptosis is considered as a physiologic process leading to cell death acting as a part of homeostatic regulation during the normal embryonic development [7, 20]. However, apoptosis-mediated cell death was observed also in some pathological conditions, such as toxic changes and viral infection, and apoptosis-mediated T lymphocyte lysis was reported to be induced in a few viral infections [1, 6, 10, 16, 18].

The present study was conducted to see the morphological changes of thymocytes including apoptosis during acute MHV-2 infection, providing useful information for the immunological process of hepatocellular death in viral hepatitis.

MATERIALS AND METHODS

Mice and virus inoculation: Four-week-old female ICR mice were purchased from an MHV-free commercial breeder (Charles River Japan, Atsugi), and through this experiment, they were kept in metal cages and fed with autoclaved pellets (MF, Orient Yeast Co., Tokyo) and water ad libitum. Mice were inoculated intraperitoneally (i.p.) with $1 \times 10^5$ PFU of MHV-2 grown and titrated on the DBT cell line [8].

Histopathology: Mice were necropsied at 24, 48 and 72 hr postinoculation (p.i.), and the thymus was fixed in Bouin’s solution and processed for a routine light microscopy. The tissue processing for electron microscopy was the same as previously described [5].

Virus titration: Thymus tissues from infected mice stored at $-80\textdegree$C were homogenized in phosphate buffered saline containing kanamycin (PBS-kanamycin) and the homogenates were centrifuged at 3,000 rpm for 10 min. The supernatants were titrated on DBT cells as described by Hirano et al. [8].

Immunohistochemistry: For the detection of viral antigen in tissues avidin-biotin-complex (ABC) method was applied. Bouin-fixed and paraffin embedded tissue sections were incubated with 0.3% H$_2$O$_2$ in methanol and treated with 5% normal goat serum for blocking non-specific reaction. Sections were then treated overnight at 4$\textdegree$C with a polyclonal anti-MHV-2 mouse serum (1:100) produced at this laboratory. After the second treating with biotinylated antisem to mouse IgG (1:100) (KPL, Gaithersburg, MD) and ABC reagent (Vector Lab., Burlingame, CA), sections were exposed to 3,3'-diaminobenzidine tetrachloride (0.5 mg/ml, DAB, Sigma, St. Louis, MO) in 0.05 M Tris-HCl buffer (pH 7.6) containing 0.01% H$_2$O$_2$ and counterstained with Mayer’s hematoxylin.

DNA electrophoresis: Thymus tissue was sampled from MHV-2 infected mice and homogenized in 5 mM Tris-HCl (pH 7.4) containing 0.5% SDS, 2 mM EDTA, and 0.05% proteinase K (Sigma), and the homogenate was incubated at 37$\textdegree$C for 1 hr. RNase (Sigma) was added at a concentration of 50 mg/ml, and further incubation was made at 37$\textdegree$C for 1 hr. DNA was extracted with phenol-chloroform and precipitated in ethanol. The extracted DNA was dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) containing 0.1% ethidium bromide and electrophoresed in 1.0% agarose gel.

RESULTS

Virus titer in the thymus was evaluated at times p.i. (Table 1), indicating productive replication of MHV-2 in the lymphoid cells. The thymus of mice infected with
Table 1. Alterations of the thymus during MHV-2 infection

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<th>Hours after infection</th>
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<tbody>
<tr>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Cell lysis</td>
<td>–</td>
</tr>
<tr>
<td>Viral antigen</td>
<td>++</td>
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<td>Virus titers</td>
<td>3.87±0.43^o</td>
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a) By immunohistochemistry.  
b) Log_{10}PFU/0.2g

MHV-2 showed atrophy and extensive cell lysis with pyknotic nuclear debris on day 2 p.i. (Fig. 1A). The cortex and medulla were indistinguishable because of hypocellularity in the cortex. Viral antigen was expressed in a small number of lymphoid cells, vascular endothelial cells, and/or reticular epithelial cells (Fig. 1B). As shown in Fig. 2, a multiple laddering pattern indicating DNA nucleosomal fragmentation was observed in MHV-2 infected thymocytes, but not in uninfected control.

By electron microscopy, degenerative changes of thymic lymphoid cells were revealed such as, cytoplasmic vacuolation, swelling of mitochondria and margination of nuclear chromatin. There was nuclear fragmentation, and round electron dense apoptotic bodies formed (Fig. 3A and B). Such type of changes was seen also in non-infected mice, while much less severe. Virus particles were detected within the cisternae of smooth vesicles of some lymphoid or reticular cells (Fig. 3A and D), or on the surface of some lymphoid cells (Fig. 3C), as well as within vascular endothelial cells (Fig. 3E). Virus particles were round in shape and 90–110 nm in size with distinct double membranes. The inner diameter was 70–80 nm. In some lymphoid cells, unusual cytoplasmic aggregates, which had morphological similarity with reticular inclusions, as well as the budding of virions into the cytoplasmic vesicles near to such inclusions, were observed.

**Discussion**

In MHV infection in mice, the lesions of the spleen, lymph nodes, and thymus have been studied by light and electron microscopy [2, 9]. The spleen of the virus infected mice showed widespread pyknosis and karyorrhexis in follicular lymphocytes while the marginal mantle were well-preserved [2]. East et al. [3] described consistent and severe damage in the thymus of newborn mice after MHV infection, while it was uncertain whether the cortical destruction was caused specifically by the virus or not. In the present study the MHV-infected thymus showed severe lymphoid cell destruction with starry sky-like appearance.

**Fig. 1.** Severe cell lysis in the cortical area (A; HE stain, ×280) and large reticular epithelial (arrow heads) and small lymphoid (arrows) cells positive for viral antigen (B; ABC immunohistochemistry, ×510) in an MHV-2 infected thymus. 48 hr p.i.

**Fig. 2.** DNA electrophoresis of the MHV-2 infected thymus homogenate. 72 hr p.i. 
Several virus species [1, 6, 10, 16, 18, 19] have been known to induce apoptosis in infected cells. Chicken anemic virus (CAV), experimentally causing depletion of lymphoid cells in the thymus of chickens [4, 10, 21] as well as human immunodeficiency virus (HIV) were reported to cause apoptosis-mediated lymphocyte lysis in vivo. In the MHV infected severely damaged thymic cells, there were margination and increased density of the nuclear chromatin and mitochondrial swelling, and more frequently, shrinkage and intense osmophilia of both nuclear and cytoplasmic elements were seen in the absence of viral particles. In the present study, apoptotic changes were shown by DNA electrophoresis in lymphoid cells but not in stromal or epithelial cells, nor in interdigitating dendritic cells, as described in case of CAV infection.

Some steroid hormones, poisons and radiation are known to induce apoptotic changes of thymic lymphoid cells, which might easily become apoptotic. Of importance is whether such type of apoptotic changes in thymocytes is
viral specific or not, since no virus particles were detectable in apoptotic thymic lymphoid cells.

REFERENCES


