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ULTRASTRUCTURAL STUDY OF MOUSE THYMUS VIRUS REPLICATION

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Summary. - With the exception of thymocytes, no other cell types have been reported to be involved in mouse thymus virus (MTV) infection. The ultrastructure of thymuses of mice infected with MTV were examined. The earliest sign of infection was detected 5 days p.i.; lymphocytes, epithelial and phagocytic (macrophages) reticular cells were shown to be affected. Viral particles and filamentous structures were present in both the nucleus and the cytoplasm of these cells. At more severe stages of cellular necrosis, 6 and 7 days p.i., cytoplasmic granulation as well as loss in definition of cytoplasmic organelles became apparent. This was followed by nuclear degradation and aggregation of cells. After 9 days p.i. necrotic lesions were still observed but viral particles were no longer detectable. This study provides evidence of the susceptibility of macrophages to MTV.

Key words: mouse thymus virus; electron microscopy

Introduction

MTV is a naturally occurring enzootic virus of laboratory and wild mice which induces a nonfatal disease in newborn mice associated with extensive necrosis of the thymus (Cross *et al.*, 1979; Parker *et al.*, 1973; Rowe and Capps, 1961). Although morphological and serological characteristics of MTV have been described (Athanassious *et al.*, 1990; Cross *et al.*, 1979; Lussier *et al.*, 1988*a*; Parker *et al.*, 1973; Rowe and Capps, 1961), not much attention has been paid to its effect on thymus cells; emphasis was placed on the structure of the virus itself rather than on the infected cells (Athanassious *et al.*, 1990; Parker *et al.*, 1973. Epithelial cells were thought to be resistant, although it was suggested that macrophages may be secondary sites of attack (Morse, 1988). In the present study, attention has been paid to the degree of damage and the cell types affected by MTV.

Materials and Methods

Stock virus was prepared as previously described (Lussier *et al.*, 1988*b*). MTV isolated in our laboratory was shown to be serologically identical to the original strain characterized by Cross *et al.* (1979).

Newborn CD-1 mice (i.e. less than 1 day-old) were inoculated intraperitoneally with approximately 10^3 ID_{50} of the virus. Animals were sacrificed everyday up to the 7th day and thereafter on days 9, 14, 17, 21 and 56 p.i. and the thymuses were processed for ultrastructural study.

Tissues were fixed overnight at 4 °C in a mixture of 2 % glutaraldehyde and 1 % paraformaldehyde in 0.1 mol/l phosphate buffer pH 7.4, followed by three 1-hr washes with the same buffer containing 3 % sucrose. Post-fixation was carried out with 1 % osmium tetroxide prepared with the washing buffer. Tissues were dehydrated in an ethanol series and embedded in low viscosity Spurr resin. Ultrathin sections, prepared with a LKB Ultratome III, were collected on 200 mesh, coated copper grids and stained with uranyl acetate and lead citrate. Sections were examined with a Phillips EM 300 electron microscope at 80 kV.

Results

Thymuses up to 4 days p.i. were free of lesions. Mild lesions were first apparent on the 5th day p.i. They were found in localized areas of the thymus and in a limited number of cells, in both the cortical and medullary regions. Nuclear degradation was observed in lymphocytes. Capsids with electron lucent and electron dense cores were also present in the cytoplasm and nuclei of these cells (Fig. 1A).

By 6–7 days p.i., the number of infected cells had increased and cellular breakdown was apparent (Fig. 1B). This resulted in the release of viral particles into surrounding structures (Fig. 1C). The nuclei of lymphocytes were at a more advanced stage of degradation. Cellular organelles also showed signs of disorganization: vacuolation of Golgi apparatus (Fig. 1B), change in structure of mitochondrial membranes (Fig. 1C), and granulation of cytoplasm (Fig. 1C). Most expressed necrosis was found to correspond to maximum viral production (Fig. 1D). Although reticular cells showed disturbance of cell structure, damage was less pronounced (Fig. 2A, 2B). At this stage, viral particles were observed in the nuclei of lymphocytes and in the cytoplasm of both lymphocytes and reticular cells (Fig. 2A, 2B). By 7 days p.i., severe necrosis was observed. In some areas this was manifested by disruption of lymphocytes; this included destruction of membranes, nuclear aggregation, and presence of undefined cytoplasmic organelles. In other areas, the number of lymphocytes was reduced and viral particles were present in the remaining broken cells. A certain number of lymphocytes appeared resistant to the infection and remained structurally unaffected.

Damage to epithelial reticular cells was less marked (Fig. 2A, 2B), while phagocytic reticular cells were strongly damaged (Fig. 2C). Affected epithelial reticular cells contained filaments in both nucleus and cytoplasm and viral particles were often found in association with the filaments (Fig. 2D).

After 9 days p.i., necrotic lesions were still observed but viral particles were no longer detectable.

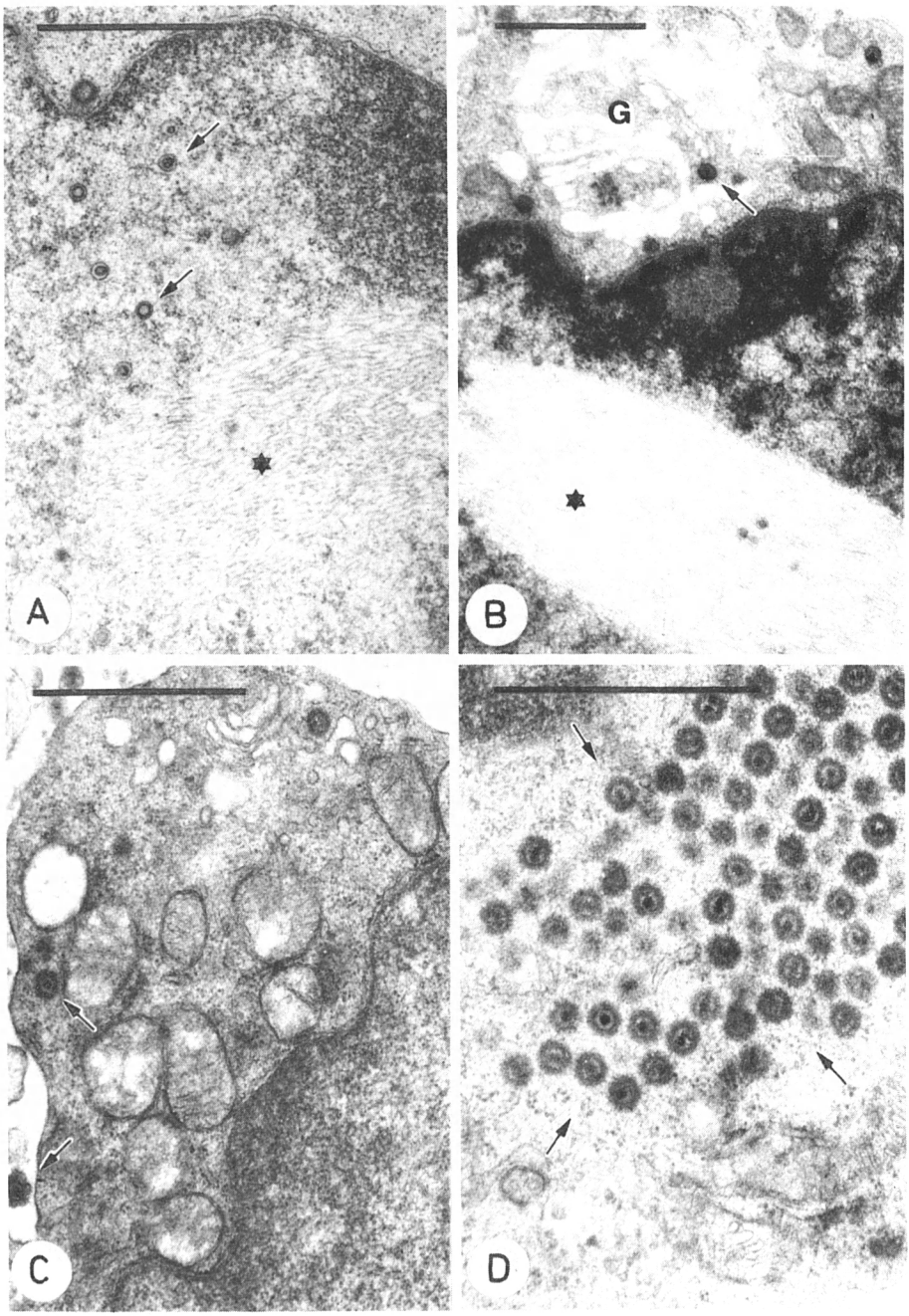

Fig. 1
For legend see page 179

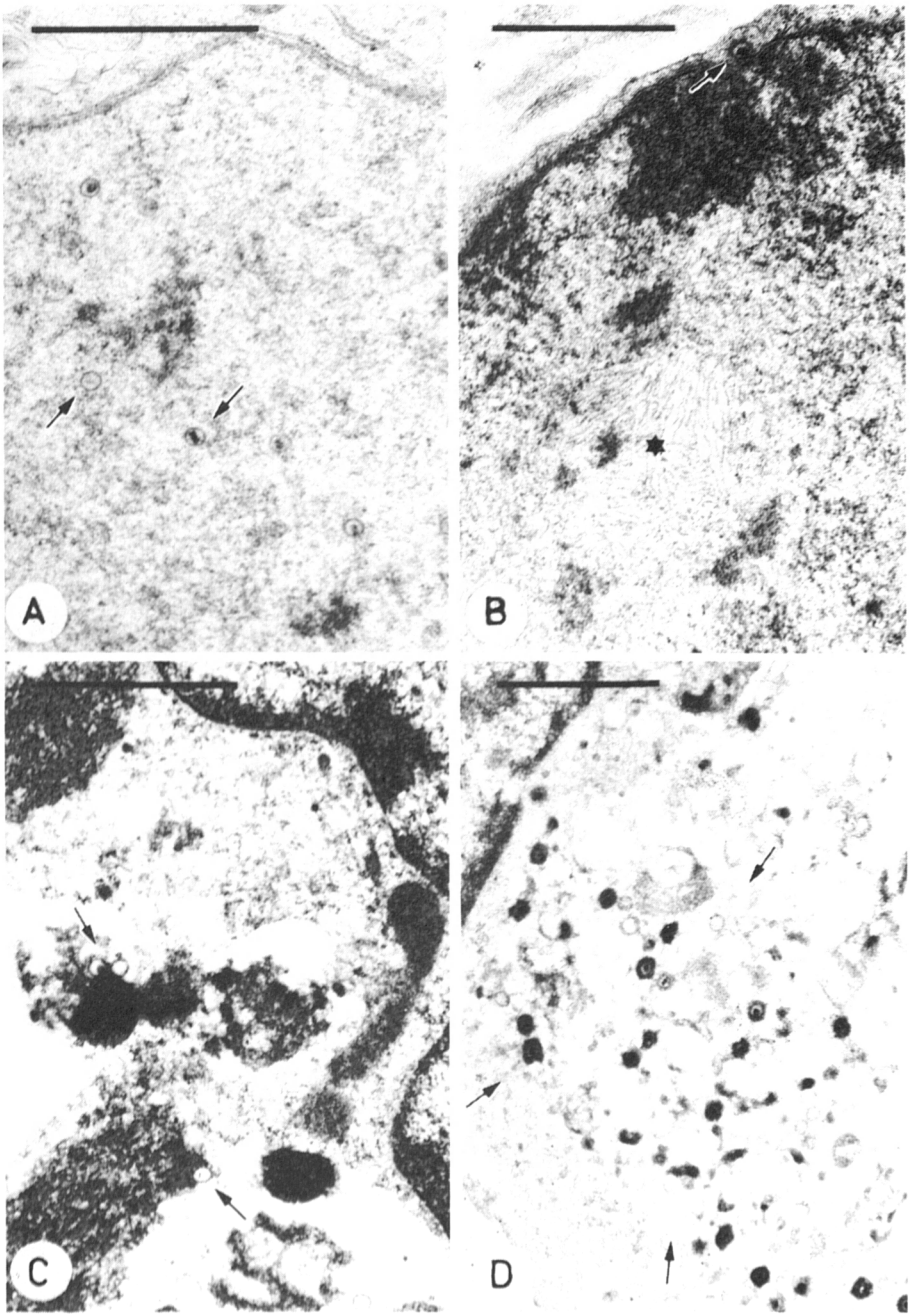

Fig. 2
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Discussion

The present study supports previous studies on the early signs of infection, histology and pathogenesis of MTV infection in newborn mice showing that the destruction of thymus lymphocytes is a special feature of this infection (Cross *et al.*, 1979; Morse, 1987; Rowe and Capps, 1961). Viral particles and filamentous structures are present in both the nucleus and cytoplasm of medullary and cortical cells, nuclear breakdown is also evident. Although the majority of lymphocytes is affected, some remain intact. This selective effect on lymphocyte subpopulations supports earlier reports that functionally most of the affected cells are helper T lymphocytes (Cohen *et al.*, 1975; Guignard *et al.*, 1989; Morse and Valinsky, 1989) with alterations in surface antigens.

In addition to lymphocytes, epithelial reticular and phagocytic reticular cells (macrophages) are also affected, although the number of lymphocytes affected and their rate of breakdown far exceeds that of the other cells. Prior to the present study, no cell types other than lymphocytes had been shown to be affected by MTV; although Morse (1988) had suggested that macrophages may act as a secondary cell type in which MTV could persist in the absence of lymphocytes. Our study provides direct ultrastructural evidence of the presence of MTV particles in macrophages. The early damage induced by MTV in reticular cells is similar to that seen in lymphocytes; i.e. presence of viral filamentous structures and nuclear breakdown.

Fig. 1

Lesions in thymus lymphocytes

A. Five days p.i. Filament formation in the nucleus of a lymphocyte (asterisk). Note presence of capsids (arrow) with electron translucent and electron dense cores in association with filaments.
B. Six days p.i. A lymphocyte with its nucleus at an advanced stage of breakdown. Note vacuolation of Golgi apparatus (G). The ultrastructural changes are associated with presence of viral particles (arrow) in nucleus and cytoplasm.
C. Six days p.i. Lymphocyte with granulation of cytoplasm, degradation of nucleus, and changes in structure of mitochondrial membranes. At this stage, intra- and extra- cellular viral particles (arrow) can be observed.
D. Seven days p.i. Note the large number of viral particles (arrow) within the lymphocyte. Viral particles with electron dense cores can be seen within the cytoplasm.
(Bar = 1 μm)

Fig. 2

Lesions in thymus reticular cells

A. Five days p.i. Reticular epithelial cell containing naked capsids (arrow) with electron translucent and electron dense cores.
B. Six days p.i. Capsids with electron dense core (arrow) in cytoplasm of phagocytic reticular cell. Note presence of filaments (asterisk) in nucleus.
C. Seven days p.i. Note breakdown of phagocytic reticular cell. Naked capsids with electron translucent cores (arrow) are associated with ingested cellular debris.
D. Seven days p.i. Note the number of viral particles (arrow) in cytoplasm of reticular epithelial cell.
(Bar = 1 μm)

It has been suggested that the suspectibility of thymus lymphocytes and macrophages to MTV is age-related. Surface antigens of lymphocytes change with age and this has been correlated with resistance to MTV (Mosier *et al.*, 1977); on the other hand, the susceptibility of immature macrophage function of newborn mice is affected by herpes simplex virus (Johnson, 1964). Our results are in agreement with these suggestions, lymphocytes as well as macrophages of neonatal mice are susceptible to MTV.

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