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Cardiac and pancreatic lesions in guinea pigs infected with encephalomyocarditis (EMC) virus

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Summary. Cardiac and pancreatic lesions were observed in guinea pigs infected with 2 variants (B and D) of encephalomyocarditis (EMC) virus. Cardiac changes were characterized by focal myocardial necrosis and subsequent replacement by immature granulation tissue, and the pancreatic ones by vacuolar degeneration of acinar cells. In the electron microscopic examinations, the affected cardiomyocytes showed intracellular oedema, swelling and/or partial destruction of mitochondria, and distortion and disruption of myofibrils. Intracellular vacuolization and dilatation of rough-surfaced endoplasmic reticulum (rER) were conspicuous in the damaged pancreatic acinar cells. In addition, intracisternal granules were found in dilated rER with a high frequency. These changes were common to animals infected with the B and D variants. On the contrary, B cell alterations; i.e. degranulation and degeneration of insulin granules, were detected only in animals infected with the D variant.

Key words: Guinea pig, EMC virus, Heart, Pancreas

Introduction

Encephalomyocarditis (EMC) virus was first isolated from nonhuman primates (Helwig and Schmidt, 1945) and then from pigs (Murane et al., 1960) and is now considered to be an important causative agent of spontaneous myocarditis in pigs, in which small rodents are suspected to participate as reservoir hosts or carriers. In addition, Craighead and McLean (1968) showed that the M variant of EMC virus (EMC-M) could induce diabetes mellitus-like syndrome in particular strains of mice. Later Yoon et al. (1980) established the highly diabetogenic (EMC-D) and non-diabetogenic (EMC-B) variants by repeated plaque-purification of EMC-M, and now there are many reports focused on EMC-D-induced diabetes in mice (Yoon et al., 1982; Doi et al., 1989).

However, reports of EMC virus infection in small rodents other than mice are rare (Matsuzaki et al., 1989a), and there are only 2 reports concerning EMC virus infection in guinea pigs. One described microscopic heart lesions characterized by myocardial necrosis with marked inflammatory cell infiltration (Schmidt, 1948), and the other represented the insusceptibility of guinea pigs to EMC virus (Dickenson and Griffiths, 1966). Recently, we observed pancreatic lesions as well as cardiac ones in guinea pigs infected with EMC-B and EMC-D. This paper principally describes the ultrastructural characteristics of the cardiac and pancreatic lesions.

Materials and methods

Twenty-one 6-week-old male guinea pigs of the Hartley strain weighing about 350g were obtained from Nippon Bio-Supply Center (Tokyo). Animals were housed in an animal room under controlled condition and fed BR pellets (Oriental Yeast Co. Ltd., Tokyo) and water *ad libitum* throughout the experimental period.

EMC virus used was grown in cultures of L-cells and stored at 80°C. Virus titer of infected L-cell supernatant was 5×10^5 plaque forming units (PFU)/ml for EMC-B and 5×10^7 PFU/ml for EMC-D by plaque assay on L-cells according to Matsuzaki et al. (1989b).

Six animals were infected intraperitoneally (i.p.) with 10^5 PFU/head of EMC-B, and 12 animals with 10^5 or 10^7 PFU/head of EMC-D. Half of them were killed by exanguination under ether anesthesia days after inoculation (3 DAI) and the rest were killed 4 days later (7 DAI). The remainining 3 animals were killed 7 DAI and served as controls. In the preliminary examination, recovery of virus from the heart and pancreas was confirmed in guinea pigs infected with these concentrations of EMC viruses and killed 3 DAI.

Organs were fixed in 10% neutral buffered formalin,

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and 4 μm paraffin sections were stained with hematoxylin and eosin (HE). For electron microscopy, small pieces of the heart and pancreas were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), post-fixed in 1.0% osmium tetroxide in the same buffer, and embedded in Poly/Bed 812 (Polyscience Co. Ltd., Warrington, PA). Ultrathin sections were double stained with uranyl acetate and lead citrate and observed under a JEM-1200 EX electron microscope (JEOL Co. Ltd., Tokyo).

Results

No deaths occurred to any animals throughout the experimental period, and no macroscopic changes were observed in any animals at necropsy. Histopathological lesions were detected only in the heart and pancreas.

Light microscopic findings

Heart: Focal myocardial necrosis (3 DAI) and subsequent replacement by immature granulation tissue (7 DAI) were observed in EMC-B (10^5 PFU/head) and EMC-D (10^7 PFU/head) groups (Fig. 1).

Pancreas: In EMC-B group, focal vacuolar degeneration of acinar cells was observed 3 DAI. This change became more prominent and was sometimes accompanied by pyknosis 7 DAI (Fig. 2). Inflammatory response was never detected. In EMC-D group, similar but only slight acinar cell change was detected in animals infected with 10^7 PFU/head and killed 7 DAI. On the other hand, islet cells showed no light microscopical alterations in any groups.

Electron microscopic findings

Heart: Intracellular oedema, swelling and partial destruction of mitochondria, and distortion and disruption of myofibrils were common to the damaged cardiomyocytes (Fig. 3).

Pancreas: Intracellular vacuolization and dilatation of rough-surfaced endoplasmic reticulum (rER) were conspicuous in the affected acinar cells, which were sometimes accompanied by swelling of mitochondria and condensation and atrophy of nucleus (Fig. 4). Dilated cisternae of rERs often contained granules with moderate to high electron density (Fig. 4, Inset). Similar intracisternal granules were also detected in acinar cells of control animals, but their frequency was significantly lower than that of infected animals. In addition, dilated

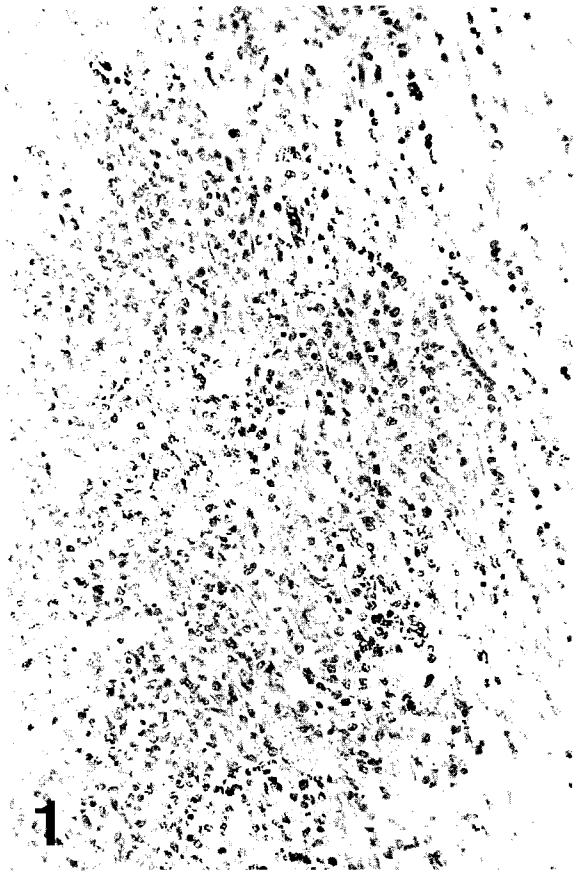

Fig. 1. Heart of a guinea pig infected with 10^5 PFU/head of EMC-B and killed 7 DAI. Myocardial necrosis with replacement by immature granulation tissue. HE × 200

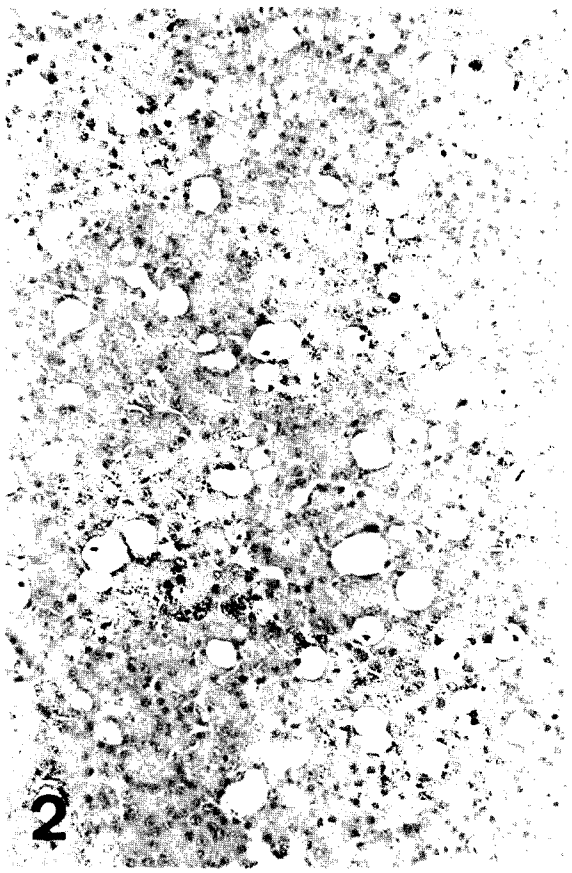

Fig. 2. Pancreas of the same animal as Fig. 1. Vacuolar degeneration of acinar cells. HE × 200

rERs and large intracytoplasmic vacuoles showed, in places, a close spacial relationship with each other (Fig. 4).

Fig. 3. Cardiomyocyte of a guinea pig infected with 10^7 PFU/head of EMC-D and killed 3 DAI. Intracytoplasmic oedema, swelling of mitochondria and disruption of myofibrils. × 6,000

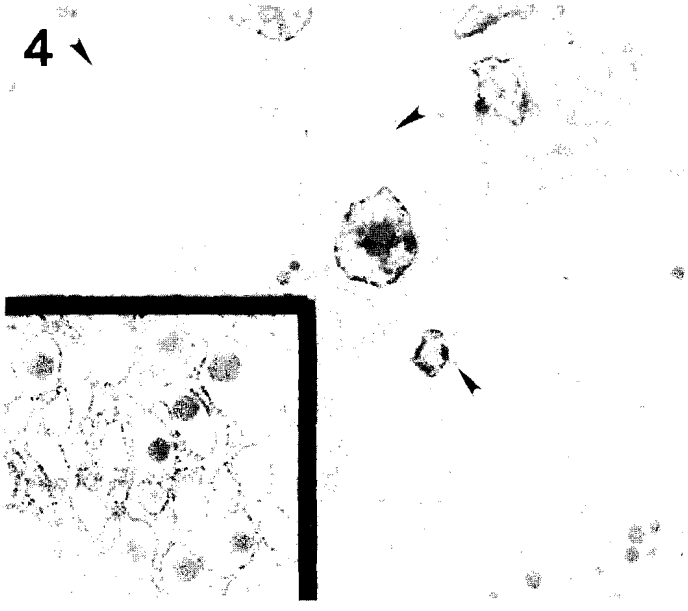

Fig. 4. Pancreatic acinar cells of a guinea pig infected with 10^5 PFU/head of EMC-B and killed 3 DAI. Intracellular vacuolization, dilatation of rERs, and increase in number of intracisternal granules (arrow heads). 3,000. Inset: Higher magnification of intracisternal granules in rERs. × 24,000

In the pancreatic islets of EMC-D group, degranulation and degeneration of insulin granules of B-cells were characteristically observed (Fig. 5A). Dilatation of rERs and swelling of mitochondria were also not infrequently found in these affected B-cells (Fig. 5B). A- and D-cells were however always normal. In EMC-B group, there was no ultrastructural alteration in any types of islet cells.

Discussion

Histopathological nature of heart lesions in guinea pigs was the same as that in mice (Craighead, 1966; Tui et al., 1971; Doi et al., 1988) and gerbils (Matsuzaki et al., 1989a). Electron microscopically, intracellular oedema was more conspicuous while mitochondrial damage was less severe in guinea pigs than in mice and gerbils (Burch and Rayburn, 1977; Matsuzaki et al., 1989b).

Apart from its severity, histopathological nature of pancreatic acinar cells was common to EMC-B and EMC-D groups and was characterized by vacuolar degeneration. Although this change was also reported in mice, hamster and gerbils, coagulative necrosis of acinar cells with marked inflammatory exudation was far more prominent in these species (Matsuzaki et al., 1989a,b).

Ultrastructural changes of acinar cells were characterized by intracytoplasmic vacuolization and dilatation of rERs, and at least some of the large intracytoplasmic vacuoles seem to be formed by fusion of markedly dilated and partially disrupted rERs. Dilated rERs of the affected acinar cells often contained intracisternal granules with moderate to high electron density. Although appearance of similar granules was also detected in normal guinea pigs as previously described by Palade (1956), its frequency was significantly lower in comparison with that in the affected animals. In addition, appearance of intracisternal granules of rERs in pancreatic acinar cells was noticed under various pathological conditions in the other animal species (Watari, 1974; Kodama and Mori, 1983), and Watari (1974) considered that the intracisternal granules were the result of a block in the intracellular transport of secretory proteins from rER to condensing vacuoles. In the EMC virus-infected guinea pigs, similar functional alteration might induce an increase in number of these granules.

Although we could not use higher dose of EMC-B than 10^5 PFU/head because of low titer of EMC-B stock, pancreatic islet B-cell changes were observed only in EMC-D group as previously reported in mice (Yoon et al., 1980). In comparison with those in mice of susceptible strains (Craighead and Steinke, 1971; Munterfering, 1972; Craighead et al., 1974; Yoon et al., 1980; Doi et al., 1989; Matsuzaki et al., 1989a), the B-cell changes in guinea pigs were slight, and detectable only by

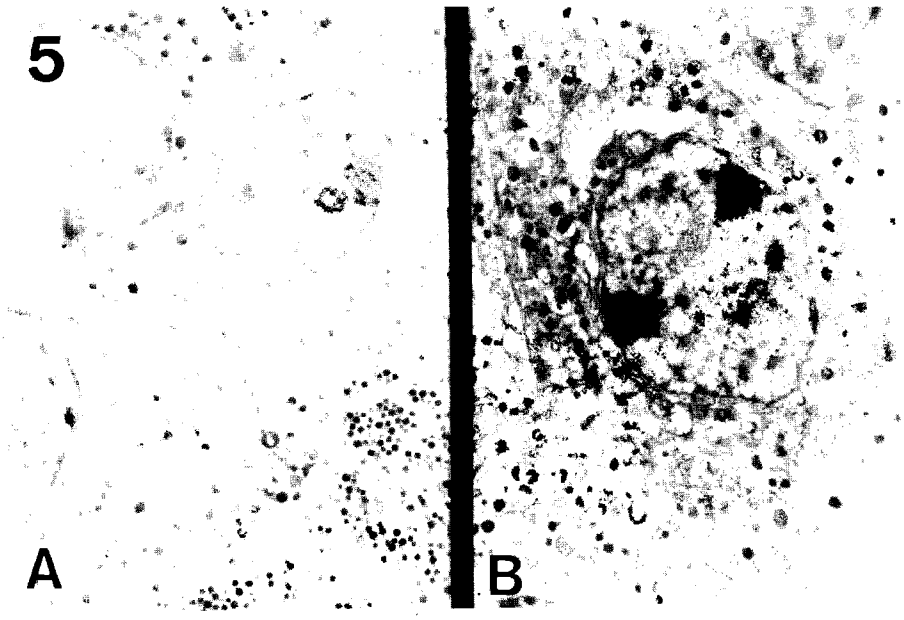

Fig. 5. Pancreatic islet cells of a guinea pig infected with 10^7 PFU/head of EMC-D and killed 3 DAI. A: Degranulation of B-cells. × 1,860. B: Dilatation of rERs and swelling of mitochondria in B-cells. × 2,200

electron microscopy. Even in electron microscopic examinations, differing from those in mice (Munterfering, 1972; Burch et al., 1974), destructive changes of intracytoplasmic organella were not prominent in pancreatic islet B-cells.

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