Encephalomyocarditis (EMC) Virus-Induced Myocarditis by Different Virus Variants and Mouse Strains

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Abstract. The mode of occurrence of encephalomyocarditis (EMC) virus-induced myocarditis in mice was pathologically and virologically investigated using 2 virus variants (highly diabetogenic EMC-D and non-diabetogenic EMC-B) and 2 mouse strains (diabetes-susceptible BALB/c and diabetes-resistant C57BL/6). Mice were inoculated with 10^5 PFU/head of the virus intraperitoneally and observed up to 7 days post inoculation (7 DPI). As compared with EMC-B-infected BALB/c and EMC-D-infected C57BL/6 mice, EMC-D-infected BALB/c mice developed marked myocarditis and exhibited a heart virus titer of more than 100 times above that of the others after 4 DPI. Electron microscopically, small aggregations of virus-like particles, with 20–25 nm in diameter, were found in the cytoplasm of degenerated cardiomyocytes showing mitochondrial and myofibrillar degeneration in EMC-D-infected BALB/c mice. —key words: BALB/c mouse, C57BL/6 mouse, EMC-B, EMC-D, myocarditis.


Encephalomyocarditis (EMC) virus is a cardiovirus which belongs to the family Picornaviridae. In the field of veterinary science, it is well known as an important causative agent of fetal death [7] and acute necrotizing myocarditis in pigs [1]. On the other hand, in the field of experimental medicine, EMC virus has been attracting great attention as a useful tool for the investigation of viral diabetes since Craighead and McLane [4] showed that the M variant of EMC virus (EMC-M) isolated from pigs with myocarditis could produce diabetes mellitus-like syndrome in mice. Recently, the highly diabetogenic (EMC-D) and non-diabetogenic variant of EMC virus (EMC-B) were established by repeated plaque-purification of EMC-M [11], and these variants are now being widely used to study the pathogenesis of viral diabetes.

It is generally said that EMC-B is non-diabetogenic in any of the mouse strains and that EMC-D induces diabetes only in particular strains of mice (e.g., BALB/c, DBA/2, SJL, ICR: Swiss etc.) [6]. We also confirmed these points using a combination of 2 virus variants (EMC-B and EMC-D) and 2 representative mouse strains (diabetes-susceptible BALB/c and diabetes-resistant C57BL/6) in the preliminary examinations. However, there are few reports concerning the modification of myocarditis by different EMC virus variants and mouse strains.

The first purpose of this study is to examine whether or not there are any EMC virus variant- and mouse strain-dependent differences in the mode of occurrence of myocarditis. The second is to clarify the ultrastructural characteristics of cardiac lesions in EMC-D-infected mice, on which there are also few reports.

Materials and Methods

Animals: Sixty 8-week-old BALB/c male mice (Japan SLC Inc., Shizuoka) and 30 age-matched C57BL/6 male mice (Charles River Japan Inc., Kanagawa) were used. Animals were housed in an air conditioned room (temperature, 23±2°C, relative humidity, 55±5%) and fed commercial pellets (Oriental MF, Oriental Yeast Co., Ltd., Tokyo) and water ad libitum.

Virus: Plaque-isolated D (EMC-D) and B variant of EMC virus (EMC-B) (gifts from Dr. Ji-Won Yoon, the University of Calgary, Calgary, Alberta, Canada) were cultured on mouse L-929 cells and stored at –80°C until used. The titers of these virus stocks determined by plaque assay on L-929 cell cultures were 6×10^7 plaque forming units (PFU)/ml for EMC-D and 1.3×10^6 PFU/ml for EMC-B, respectively. The virus stocks were diluted with 0.01 M phosphate buffered saline (PBS) and adjusted to 10^6 PFU/ml just before use, and 0.1 ml of the dilution (10^5 PFU/head) of EMC-D was inoculated intraperitoneally (i.p.) into 25 mice each of BALB/c and C57BL/6 strains (EMC-D-BALB and EMC-D-C57BL groups). In addition, 10^5 PFU/head of EMC-B was inoculated i.p. into additional 25
BALB/c mice (EMC-B-BALB group). The remaining mice were inoculated i.p. with 0.1 ml/head of PBS in the same way and served as control.

Five mice of each groups were sacrificed by exsanguination under ether anesthesia at 1, 2, 3, 4 and 7 days post inoculation (DPI), respectively. Control mice were sacrificed at 7DPI in the same way.

Clinical signs and mortality: During the experimental period, particular changes in behavior or appearance and mortality of mice were recorded daily.

Blood glucose level: Non-fasting blood glucose levels were colorimetrically determined on each serum sample collected at autopsy using the Glucose C-test kit (Wako Pure Chemical Industries Inc., Tokyo). An infected mouse was scored as hyperglycemic if the non-fasting glucose level was more than 3 standard deviations above the mean of uninfected controls.

Virus titer: Virus titration by plaque assay on L-929 cell cultures was done on the heart and blood according to the method of Matsuzaki et al. [8].

Histopathology: At autopsy, the heart was fixed in 10% neutral buffered formalin. Paraffin sections (4 μm) were stained with hematoxylin and eosin (HE).

For electron microscopy, small pieces of the heart of mice of EMC-D-BALB group sacrificed at 7DPI were fixed in 5% glutaraldehyde in 0.1 M phosphate buffer, 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 1200 EX electron microscope (JEOL, Tokyo).

RESULTS

Clinical signs and mortality: Except for one mouse each of EMC-D-BALB and EMC-D-C57BL groups which died at 4DPI, no mice showed particular changes in the behavior or appearance during the experimental period.

Blood glucose level: As shown in Fig. 1, marked hyperglycemia developed in EMC-D-BALB group from 3DPI, while no significant change in blood glucose level was observed in EMC-B-BALB group. In EMC-D-C57BL group, only one mouse exhibited hyperglycemia at 4DPI.

Virus titer: Although virus titer in the blood was higher in EMC-D-BALB group than in the other 2 groups, titers in all groups reached their maximum levels at 1DPI and no more viral replication was detected at 4DPI (Fig. 2a).

In the heart, after reaching their maximum levels at 3DPI or 4DPI, the virus titers decreased. During the first 3 days, the virus titer in EMC-D-C57BL

Fig. 1. Changes in blood glucose levels.

EMC-D-BALB, EMC-B-BALB, BALB/c control and C57BL control group. DPI: Days post inoculation. Values are expressed as mean±SD of 5 mice.

Fig. 2a. Changes in virus titers of blood.

EMC-D-BALB, EMC-B-BALB, and EMC-D-C57BL group. DPI: Days post inoculation. Average titers of 5 mice at each point were examined. Values are expressed as average of 5 mice.
group was the highest among 3 groups. Thereafter the titer in EMC-D-BALB group was significantly higher than that in the other 2 groups, and it was more than 100 times above that in the other 2 groups even at 7DPI.

**Histopathological findings:** Minimal lesions consisting of degenerated myocardium and inflammatory cells were sporadically observed in a small number of mice of all 3 groups at 2DPI (Fig. 3), and focal myocardial necrosis developed at 4DPI (Fig. 4). At 7DPI, myocardial lesions extended and myocardial cell necrosis with subsequent replacement by immature granulation tissue was conspicuous in many mice in EMC-D-BALB/c group (Fig. 5). On the other hand, there was no extension of myocardial lesions in the other 2 groups after 4DPI (Fig. 6).

**Electron microscopic findings:** Concentration and/or destruction of mitochondria with deposition of electron-dense particles in their matrix and distortion and disruption of myofibrils were observed in cardiomyocytes (Figs. 7 and 8). In addition, small aggregations of virus-like particles with 20–25 nm in diameter were found in the cytoplasm of some degenerated cardiomyocytes (Fig. 9).
DISCUSSION

In the present study, it was clarified that the mode of occurrence of myocarditis differed with EMC virus variants and mouse strains in the same manner as previously proposed concerning EMC virus-induced diabetes [11]. There was no difference in myocardial lesions among the 3 groups during the first 4 days. Thereafter the lesion extended in EMC-D-BALB group while it did not develop further in EMC-B-BALB and EMC-D-CS7BL groups. On the other hand, the heart virus titer in EMC-D-BALB group was more than 100 times above that in the other 2 groups during the latter half of the observation period. These histopathological and virological findings suggest that the extension of cardiac lesions in EMC-D-BALB group might be caused by long-lasting active replication of
EMC virus in cardiomyocytes.

Electron microscopically, degeneration of mitochondria with deposition of electron-dense particles in their matrix was most conspicuous and it was often accompanied by distortion and disruption of myofibrils. Such ultrastructural characteristics in EMC-D-infected BALB/c mice were almost similar to those in EMC-M-infected mice [3] and EMC-D-infected Mongolian gerbils [8], though they were different from those in EMC-D-infected guinea pigs which showed prominent intracellular oedema [9]. In addition, we found small aggregations of virus-like particles in the cytoplasm of degenerated cardiomyocytes. These particles were 20–25 nm in diameter being consistent with the size of EMC virus reported previously [5]. Moreover, they were differentiated from intracellular organella and glycogen granules based on their size and mode of appearance. Therefore, it seems reasonable to consider that these particles may be EMC viruses. In the previous reports of EMC virus-infection, virus particles in the affected cells were detected unexceptionally in a form of crystalline arrays [2, 3, 6, 10].

It is generally supposed in EMC virus infection in mice that either immunogenicity of viruses or immunoresponsibility of mice may play an important role in the pathogenesis of pancreatic beta cell damage as well as myocarditis. In this connection, we found recently that macrophage activity and macrophage-derived IFN were the key factors in the early immunological phenomena of EMC virus infection in mice (data not shown). Further investigations using various immunomodulators are now in progress.

REFERENCES