Encephalomyocarditis (EMC) Virus Myocarditis in DBA/2 Mice

I. Acute Stage

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Severe myocarditis was induced in inbred DBA/2 mice inoculated with M variant of EMC virus. Yellowish-white patches were seen on the surface of the ventricles and atria, and histologically, myocardial necrosis and calcification was evident on the fourth day after virus inoculation. Myocardial lesions appeared earlier and were more extensive than we observed in previous myocarditis induced by Coxsackie B viruses.

Spontaneous perimyocardial lesions were observed in control DBA/2 mice, which were exclusively limited to the right ventricle; however, these lesions were different in localization from lesions observed in infected mice in which extensive myocardial lesions were noted.

This animal model is considered to be excellent for studies on the pathogenesis and natural history of viral myocarditis.

To elucidate the possible role of virus infection in the pathogenesis of idiopathic cardiomyopathy, especially of dilated (congestive) type, we have studied on experimental coxsackievirus myocarditis in mice¹⁻⁴. Following acute inflammation, we found significant myocardial fibrosis and calcification persisted long after inoculation with the virus, but dilatation or hypertrophy of the heart was not observed.

In this study, DBA/2 mice inoculated with encephalomyocarditis (EMC) virus, developed marked myocarditis in the ventricles, inter-ventricular septum and atria. This animal model considered to be excellent for studies on viral myocarditis in humans.

Key Words:

Encephalomyocarditis virus
Myocarditis
Cardiomyopathy
Myocardial Disease

MATERIALS AND METHODS

Mice: Inbred DBA/2 mice were obtained from Shizuoka Agricultural Cooperative Association, Japan. This strain has been maintained continuously by brother-sister matings.

Virus: M variant of EMC virus was kindly obtained from Dr. K. Hayashi. Hearts from intraperitoneally inoculated 3 to 4-week-old mice were removed aseptically on the fourth day and homogenized to make a 10% suspension in Eagle's minimum essential medium (MEM). Virus suspension was clarified by centrifugation at 6,000 rpm for 30 min. Virus stock has a titer of $10^{6.0}$ TCID₅₀ (tissue culture infective dose) titrated by tissue culture of Girardi heart cells.

Experimental infections: At 3 to 4 weeks of age, mice were inoculated intraperitoneally with 0.1 ml of virus suspension containing 100 TCID₅₀/0.1 ml and the animals were sacrificed 3, 4, 5, 6, 7, 10 and 12 days after the inoculation. Gross inspection of the heart was made for

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Fig.1. Twelve days after inoculation with EMC virus. Yellowish-white patches are evident on the surface of the ventricles. Scale: 1mm

Fig.2. Four days after the inoculation (left ventricle). There is fine calcification of necrotic myocardial cell. Inflammatory cells were sparse at this stage. Hematoxylin and eosin stain (HE), $\times 160$

Fig.3. Six days after the inoculation. Myocardial lesions are noted not only in the right and left ventricles, and interventricular septum, but also in the atria. HE stain, $\times 14$

Fig.4. Six days after the inoculation (interventricular septum). Extensive myocardial necrosis and calcification are evident. HE stain, $\times 160$

Fig.5. Twelve days after the inoculation (left ventricle). Prominent myocardial calcification was noted. Mononuclear cell infiltrations are evident. HE stain, $\times 160$

Fig.6. The heart of a 6-week-old control mouse (right ventricle). Calcified lesion is noted in the epicardial side of the right ventricle. HE stain, $\times 160$

any alteration in myocardial appearance. After inspection, hearts were processed for histologic or virologic studies.

Virus isolation from the hearts: Hearts were ground with seasand and 1% suspension was prepared in MEM. The suspension was centrifuged and 0.1 ml of supernatant was inoculated into tube culture of Girardi heart cells containing of 1.0 ml of MEM supplemented with 2% fetal calf serum. Tubes were observed daily for the appearance of characteristic cytopathic effect over a period of 7 days.

Histologic examination: Tissues were fixed in 10% formalin solution, embedded in paraffin and stained with hematoxylin and eosin, von Kossa, van Gieson and Mallory-azan stains.

Control experiments: Thirty-eight DBA/2 mice ranging from 4 to 8 weeks of age were used as non-treated controls.

RESULTS

Forty-two of 87 mice (48.3%) inoculated with virus died. Virus was isolated from the heart in 10 of 10 mice on the fourth day after inoculation with EMC virus.

Gross pathology: Yellowish-white patches were seen on the surface of the right and left ventricles of the heart from the fourth day through the twelfth day after inoculation with the virus (Fig. 1).

Histopathology: On the third day, myocardial fibers were intensely eosinophilic and vacuoles were noted in them. Cross striations were not evident and nuclei appeared pyknotic. On the fourth day, fine dystrophic calcification was seen in the necrotic fibers stained with von Kossa. Inflammatory cells were sparse at this stage (Fig. 2). Thereafter, pathologic changes became more extensive and calcification became more prominent. These myocardial lesions were seen not only in the right and left ventricles, and interventricular septum, but also in the atria (Figs. 3 and 4). On the twelfth day, prominent myocardial calcification was noted and cellular infiltrations, consisting mainly of mononuclear cells, were evident (Fig. 5). Cumulative incidence of myocardial lesions in the infected mice was 28 of 63 (44.4%).

Control experiments: In 5 of 38 mice (16.1%), there were yellowish-white patches on the surface of the right ventricle. However, these lesions were limited within the epicardial side of the right ventricle (Fig. 6) and different in their

localization from the lesions observed in mice infected with EMC virus, which involved left ventricle, interventricular septum and atria.

DISCUSSION

In our previous study, we showed that coxsackieviruses B3 and B4 produced significant myocarditis in a random-bred strain of ddY mice, followed by myocardial calcification and fibrosis. However, there was considerable variation in number and severity of myocardial lesions among the different groups of litters.¹ Thereafter, we used inbred strains of mice in experimental viral myocarditis.

Recently, we found a severe perimyocarditis in an inbred strain of BALB/c mice induced by coxsackievirus B3 which was exclusively limited to the right ventricle. Marked perimyocardial fibrosis with calcification was observed up to the twelfth month after inoculation with the virus, and this animal model was considered to be an excellent model for studying on the natural history of perimyocarditis of viral etiology and its possible sequels, chronic or constrictive pericarditis in humans?²⁻⁴

EMC virus was a picornavirus biologically similar to the group B coxsackieviruses. EMC virus was first isolated in 1945 from primates after sudden death⁵ and thereafter from rodents and swine.

In experimental studies conducted on different animals after inoculation with the virus, the pathologic lesions which were especially lethal in the animals were mainly encephalitis and myocarditis. Two variants of EMC virus which differ in pathogenicity for mice were described by Craighead.⁶ The E variant is highly neurotrophic and produces a rapidly fatal infection in 12-week-old mice. The M variant usually causes a mild, non-fatal illness and widespread myocytolysis in the heart but few signs of central nervous system involvement. Organs other than the heart and brain may also display pathologic lesions in experimental mice inoculated with EMC virus.

Recently, interest has focused on the ability of EMC infection to produce a diabetes mellitus-like syndrome in certain strains of inbred mice.⁷

There are only a few reports regarding experimental EMC virus myocarditis.^{6,8,9} In this study, we found a severe myocarditis in DBA/2 mice inoculated with M variant of EMC virus. Myocardial lesions appeared earlier and were

more extensive than we observed in previous experimental myocarditis induced by coxsackieviruses. These pathologic lesions were seen not only in the right and left ventricles, and inter-ventricular septum, but also in the atria. This animal model is considered to be excellent for studies on the pathogenesis and natural history of viral myocarditis. EMC virus myocarditis, in the later stage, may even develop into a lesion such as is seen in dilated (congestive) cardiomyopathy. Further studies on the possible role of EMC virus infection in the pathogenesis of idiopathic cardiomyopathy are being undertaken in our laboratory.

Ball et al.¹⁰ reported spontaneous myocardial lesions in DBA mice. In DBA/2 mice used in our experiment, perimyocardial lesions, which were exclusively limited to the right ventricle, were detected in 5 of 38 non-treated 4- to 8-week-old mice. These lesions were different in localization from myocarditis observed in mice infected with EMC virus in which extensive myocardial lesions were found.

Although a few isolations of EMC virus have been made from sick persons,^{11,12} and serologic evidence of infection has been reported in various human populations,^{13,14} little is known about the epidemiology or disease potential of EMC virus infection in man. Studies are necessary to elucidate a possible role in the pathogenesis of myocarditis in humans.

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