An Experimental Model for Congestive Heart Failure After Encephalomyocarditis Virus Myocarditis in Mice

AKIRA MATSUMORI, M.D., AND CHUICHI KAWAI, M.D.

SUMMARY Severe myocarditis was induced in inbred BALB/c mice inoculated with the M variant of encephalomyocarditis (EMC) virus. The mortality rate was maximal on the fourth day, then decreased gradually, but increased again between the eleventh and fourteenth days. Gross myocardial lesions were seen on the surface of the ventricles in 62 of 125 mice (49.6%) after the fifth day. These myocardial lesions were observed more frequently in the dead mice (46 of 49, 93.9%).

Cavity dimensions and wall thickness were measured in two groups of mice with myocarditis. On days 5-7, the cavity dimensions of the right (RV) and left (LV) ventricles in inoculated mice (0.92 ± 0.51 mm and 1.21 ± 0.18 mm, respectively) were significantly larger than those in controls (RV 0.54 ± 0.17, LV 1.01 ± 0.15; p < 0.05). The wall thickness of the RV (0.46 ± 0.09, controls 0.64 ± 0.11; p < 0.001) and the LV (0.97 ± 0.13, controls 1.12 ± 0.19; p < 0.05) was significantly decreased. On days 8-14, dilatation of the LV was more pronounced (1.48 ± 0.37, p < 0.005) than during days 5-7, and the interventricular septum was also thinner. Pleural effusion, ascites and congestion of the lungs and liver were noted, and death seemed due to congestive heart failure.

This study is the first documentation of congestive heart failure after viral infection in an experimental animal.

HEART DISEASE occurs as a complication of several viral infections, particularly coxsackieviruses.1, 2 Viral infections are often subclinical, and only a very few result in obvious cardiac involvement, so it may be possible for congestive (dilated) cardiomyopathy to develop after an asymptomatic infection.

To elucidate the possible role of viral infection in the pathogenesis of idiopathic cardiomyopathy, we studied experimental coxsackievirus myocarditis in mice3-4 and found significant myocardial fibrosis and calcification persisting long after virus infection, but dilatation or hypertrophy was not observed.

We carried out studies in which inbred BALB/c mice were inoculated with encephalomyocarditis (EMC) virus. These mice developed congestive heart failure, a previously undocumented complication of experimental viral myocarditis.

Methods

Experimental Infections

The virus stock was prepared in cultures of FL (human amnion) cells in Eagle's minimum essential medium (MEM). Virus suspensions were centrifuged after the cytopathic effect had developed. Virus stock had a titer of 10⁶.⁴ TCD₅₀ (50% tissue culture infective dose) determined in tissue cultures of FL cells. Control fluids from FL cell cultures were also prepared. Both virus and control fluids were stored at -70°C until use.

Inbred BALB/c mice were obtained from Charles River, Japan. This strain has been maintained continuously by brother-sister matings. At 4 weeks of age, the mice were inoculated intraperitoneally with 0.1 ml of virus suspension containing 100 TCD₅₀ per 0.1 ml. The mice were observed daily for 28 days after inoculation.

After gross inspection of the heart for alterations in myocardial appearance, the hearts were processed histologic or virologic studies. The mice were weighed to the nearest 0.1 g and the hearts to the nearest 0.1 mg, and the ratio of heart weight (HW) to body weight (BW) was calculated.

Forty-one 4-week-old control mice were inoculated intraperitoneally with 0.1 ml of virus-free FL cell culture fluids and killed on days 5-14, so that they were age-matched to infected mice. Their hearts were processed and examined in the same manner as those infected mice.

Pathologic Study

The hearts were fixed in 10% formalin solution, sectioned transversely at the midportion of the ventricle, embedded in paraffin and stained with hematoxylin-eosin, von Kossa and Mallory-azan stains. The wall thickness of the right ventricle (RV), the left ventricle (LV) and the interventricular septum was measured to the nearest 0.01 mm with an ocular micrometer. The lungs, livers, kidneys and other organs were also sectioned and stained with hematoxylin-eosin.

Virologic Study

For virus isolation, the hearts were ground with sand in 2.0 ml of MEM. The suspension was centrifuged and 0.1 ml of each supernatant was inoculated into tube cultures of FL cells containing 1.0 ml of MEM supplemented with 2.0% fetal calf serum. The tubes were examined daily for 7 days for the appearance of a characteristic cytopathic effect.
CHF CAUSED BY EMC VIRUS MYOCARDITIS/Matsumori and Kawai

Statistical Analysis

Statistical analysis of the data was performed by an analysis of variance with multiple comparisons by Neuman-Keul's method. The results were expressed as mean ± SD.

Results

Mortality and Incidence of Myocarditis

Four days after the inoculation, the infected mice appeared ill and moved sluggishly. Some of the mice developed spastic paralysis, but some sick mice survived and appeared well after day 14. Sixty-five of 150 inoculated mice (43.3%) died. Forty-one of these 65 (63.1%) died 3-7 days after inoculation. Gross myocardial lesions were seen on the surface of the ventricles in 62 of 125 mice (49.6%) after the fifth day. These myocardial lesions were observed in 46 of 49 dead mice (93.9%) and in 16 of 76 surviving mice (21.1%).

The mortality rate was highest on the fourth day and then decreased gradually, but increased again between days 11 and 14 (fig. 1). Mice that died on days 8-14 showed pleural effusion, ascites and congestion of the lungs and liver. The cause of death seemed to be congestive heart failure.

Body Weight, Heart Weight and HW/BW Ratio

Body weight, heart weight and HW/BW ratio were measured on days 5-7 when myocardial necrosis became apparent and on days 8-14 when congestive heart failure developed (fig. 2). After the fifth day, the body weight of infected mice decreased significantly (p < 0.005). Weight loss was greatest in mice with myocardial lesions. These mice did not regain lost body weight. The heart weight of mice with myocarditis was significantly increased (p < 0.05) on days 5-7, but not on days 8-14. The HW/BW ratio of control mice was 5.82 ± 0.32 x 10^-4 on days 5-7 and 5.37 ± 0.31 x 10^-4 on days 8-14. HW/BW ratios of infected mice without myocardial lesions were 5.87 ± 0.62 x 10^-4 and 6.51 ± 1.40 x 10^-4, respectively (NS). The HW/BW ratios of mice with myocardial lesions were markedly increased in both groups (p < 0.001 vs controls or infected mice without myocarditis).

Figure 1. Number of dead mice after inoculation with encephalomyocarditis virus. Mortality rate is maximal on day 4, decreases gradually, then increases again between days 11 and 14.

Figure 2. Body weight (BW), heart weight (HW) and HW/BW ratio. (+) = with; (-) = without myocardial lesions; n = number of mice. Values are mean ± SD. The decrease of BW was most pronounced in mice with myocardial lesions. The HW of mice with lesions increased on days 5-7. The HW/BW ratios of mice with myocarditis increased markedly on days 5-7 and days 8-14.
FIGURE 3. Cavity dimension and wall thickness in mice with myocarditis. Right ventricular (RV) and left ventricular (LV) cavity dimension increased and wall thickness of both ventricles decreased on days 5–7. Dilatation of the LV cavity became more pronounced and the thickness of the interventricular septum (IVS) decreased on days 8–14.
Figure 4. Pathologic findings in hearts of mice inoculated with encephalomyocarditis virus. (A) After day 5, yellowish white patches are seen on the surface of the ventricles. Scale = 1 mm. On day 5, myocardial necrosis localizes frequently to the subendocardial myocardium (B) and interstitial edema and mononuclear cell infiltration are present (C). (D) On day 7, myocardial necrosis with calcification is often seen on the epicardial side of the right ventricle. Ten days after inoculation, aneurysmal dilatation of the right ventricle is present (arrow) (E). Extensive myocardial necrosis with calcification and mononuclear cell infiltration is evident (F). (G) Heart of control mouse 14 days after inoculation with virus-free control fluid. On day 14, dilatation of both ventricular cavities and decreased wall thickness are evident (H). Myocardial necrosis and cellular infiltration are evident (I, interventricular septum). Marked congestion of the lung (J) and liver (K) is also present at this stage. (L) On day 28, myocardial fibrosis is present. Hematoxylin-eosin stain; magnification: (B) × 33, (C) × 185, (D, F and I–L) × 82, (E, G and H) × 10.

Cavity Dimension and Wall Thickness

Cavity dimension and wall thickness were also measured on the transverse (perpendicular to long axis) section of the middle of the ventricles in the two groups of mice with myocarditis (fig. 3). On days 5–7, the cavity dimensions of the RV and LV (0.92 ± 0.51 mm and 1.21 ± 0.18 mm, respectively) were significantly greater than those of the controls (RV 0.54 ±
0.17, LV 1.01 ± 0.15; p < 0.05). The wall thickness of the RV (0.46 ± 0.09, controls 0.64 ± 0.11; p < 0.001) and the LV (0.97 ± 0.13, controls 1.12 ± 0.19; p < 0.05) was significantly decreased. On days 8–14, dilatation of the LV cavity was increased (1.48 ± 0.37, p < 0.005 vs days 5–7), and the interventricular septum was thinner.

Pathologic Findings

Yellowish white patches were seen on the surface of the ventricles of the heart after the fifth day (fig. 4A). Changes were first noted in the myocardium 3 days after inoculation with the virus. Initially these consisted of focal myocytolysis localized frequently to the subendocardial myocardium. On day 5, necrotic foci appeared in the myocardium with small mononuclear cell infiltrations, and interstitial edema was evident (figs. 4B and 4C). After day 7, myocardial necrosis became more extensive and myocardial lesions were seen not only in the RV, LV and interventricular septum, but also in the atria. Myocardial necrosis with calcification was often present in the subepicardial regions of the RV (fig. 4D).

Dilatation of the ventricular cavity became prominent after day 8. Aneurysmal dilatation of the RV was seen in one mouse on day 10 (fig. 4E). Extensive myocardial necrosis with calcification was evident on days 10–14 (fig. 4F). Marked dilatation of the ventricles was seen at this stage (figs. 4G–I). Pleural effusion, ascites and congestion of the lungs (fig. 4J) and liver (fig. 4K) were noted at this stage and death seemed to be due to congestive heart failure. Myocardial fibrosis was present on day 28 (fig. 4L).

In one of 41 control mice (2.4%), there were small yellowish patches on the surface of the RV that were limited to the pericardium and different in location from the lesions in mice infected with EMC virus.

Virus Isolation

EMC virus was isolated from the hearts of all 10 mice examined on days 3, 5 and 7, from the hearts of four of 10 mice on day 14, and from none of 10 on day 28. No virus was recovered from the hearts of control mice.

Discussion

In our previous study, we showed that coxsackieviruses B3 and B4 produced significant myocarditis in random-bred strains of ddY mice, followed by myocardial calcification and fibrosis. These findings were similar to the changes reported by Wilson et al. There was considerable variation in number and severity of myocardial lesions in the different litters. Thereafter, we used inbred strains of mice for experimental viral myocarditis.

In inbred strains of BALB/c mice we found severe perimyocarditis induced by coxsackievirus B3; perimyocarditis was limited to the RV. Marked perimyocardial fibrosis and calcification were present through the twelfth month after virus inoculation. This animal model was excellent for studying the natural history of viral perimyocarditis and its possible sequelae, similar to chronic or constrictive pericarditis in humans. However, congestive heart failure is a previously undocumented complication of experimental viral myocarditis.

EMC virus is a picornavirus biologically similar to coxsackievirus. EMC virus was first isolated in 1945 from primates after sudden death. In experimental studies of different animals after inoculation with the virus, the pathologic lesions which were especially lethal were encephalitis and myocarditis. Two variants of EMC virus that differ in pathogenicity for mice were described by Craighead. The E variant is highly neurotrophic and produces a rapidly fatal infection in 12-week-old mice. The M variant usually causes a mild, nonfatal illness and widespread myocytolysis in the heart, but few signs of central nervous system involvement. Recently, interest has focused on the ability of EMC infection to produce a diabetes mellitus-like syndrome in certain strains of inbred mice.

There are only a few reports regarding experimental EMC virus myocarditis. and these describe only acute changes. In this study, we found a severe myocarditis in inbred BALB/c mice inoculated with the M variant of EMC virus. The heart weight and HW/BW ratios of infected mice with lesions increased acutely. On days 5–7, interstitial edema was evident histologically; therefore, an increase in heart weight at this stage seemed due to interstitial edema. However, heart weight did not increase on days 8–14 when interstitial edema had decreased compared with days 5–7. Myocardial lesions appeared earlier and were more extensive than those previously found in myocarditis induced by coxsackievirus. Dilatation of the ventricular cavities, pleural effusion, ascites and congestion of the lungs and liver were present, and death seemed to be due to congestive heart failure. Myocardial calcification in the present study was less marked than with coxsackievirus myocarditis. This animal model seems to be better for studying viral myocarditis.

El-Khatib et al. reported the experimental production in three instances of aneurysmal dilatation of the LV 17–23 days after the onset of acute myocarditis in suckling mice infected with coxsackievirus B1 or B4. We found aneurysmal dilatation of the right ventricle in one mouse 10 days after inoculation with EMC virus. These results suggest that viral myocarditis in humans may lead to aneurysm of the RV or LV.

Myocarditis can develop rapidly and progress to circulatory collapse or to congestive heart failure. Congestive heart failure has been reported as accompanying chickenpox, mumps, psittacosis, poliomyelitis and coxsackievirus infection in man. However, few data on the transition from acute myocarditis to congestive cardiomyopathy have been reported. Obeyesekere and Hermon demonstrated the development of cardiomyopathy in patients suffering from myocarditis after dengue and chikungunya fever caused by arbovirus in Ceylon. They concluded that arbovirus infections play a significant role in the etiology of cardiomyopathies in Ceylon.
Kawai\textsuperscript{18} found a higher incidence of complement fixing antibodies to coxsackievirus B and herpes simplex virus and neutralizing antibodies to coxsackievirus B in patients with cardiomyopathy than in controls. Recently, Cambridge et al.\textsuperscript{14} demonstrated that high neutralization titers to coxsackievirus B were more common among patients with congestive cardiomyopathy than among controls and that high titers were more common in those with a short history and when there had been a febrile illness at the onset of symptoms. These results support the theory that viral infection can cause congestive cardiomyopathy.

Although a few isolations of EMC virus have been made from sick persons,\textsuperscript{17,18} and serologic evidence of infection has been reported in human populations,\textsuperscript{19,20} little is known about the epidemiology or disease potential of EMC virus infection in man. Studies are necessary to elucidate the possible role of EMC virus in the pathogenesis of myocarditis in man.

Acknowledgment

We thank Drs. K. Hayashi and K. Otaki for providing the EMC virus, Dr. T. Sakurai for help with the statistical analysis, and Dr. A. Cary for reading the manuscript.

References

An experimental model for congestive heart failure after encephalomyocarditis virus myocarditis in mice.
A Matsumori and C Kawai

Circulation. 1982;65:1230-1235
doi: 10.1161/01.CIR.65.6.1230
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/65/6/1230