An Animal Model of Congestive (Dilated) Cardiomyopathy: Dilatation and Hypertrophy of the Heart in the Chronic Stage in DBA/2 Mice with Myocarditis Caused by Encephalomyocarditis Virus

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SUMMARY To investigate whether lesions that develop in the chronic stage of viral myocarditis are similar to those seen in congestive (dilated) cardiomyopathy, we studied myocarditis in inbred strains of DBA/2 mice infected with encephalomyocarditis (EMC) virus. Myocardial necrosis with calcification appeared on day 4. Thereafter, myocardial necrosis became more extensive and mononuclear cell infiltration was evident and was most marked on day 14. On day 90, cellular infiltration had decreased and myocardial fibrosis was prominent. At this stage, the heart weight was significantly greater in the infected mice than in the controls (0.190 ± 0.028 g vs 0.122 ± 0.013 g, mean ± SD) (p < 0.001) and the cavity dimensions of the left ventricle were larger (1.67 ± 0.29 mm vs 1.11 ± 0.20 mm) (p < 0.001). The diameters of myocardial fibers of the right ventricle, the interventricular septum and the left ventricle were significantly larger than those of the controls (right ventricle, 16.6 ± 1.8 vs 13.4 ± 1.5 μm; interventricular septum, 17.8 ± 1.5 vs 13.8 ± 1.5 μm; left ventricle, 19.4 ± 1.7 vs 14.8 ± 1.1 μm) (p < 0.001).

This study demonstrates that in viral myocarditis in the chronic stage, lesions develop that resemble those seen in congestive cardiomyopathy.

CARDIOMYOPATHIES are defined as heart muscle diseases of unknown cause. On the basis of the morphologic and functional abnormalities, Goodwin classified cardiomyopathies as congestive, hypertrophic and restrictive or obliterative. The term congestive cardiomyopathy is derived from the common late clinical manifestation of congestive heart failure. However, dilatation of the cardiac chambers (particularly the left ventricle) is an earlier and more prominent feature; thus, the term "dilated cardiomyopathy" is better.

Congestive (dilated) cardiomyopathy might be considered the final common pathway of many different disorders. Although viral infection, alcohol, puerperium and other conditions are regarded only as possible risk factors, whether these conditions potentiate the underlying cardiomyopathy, unmask it or cause it is unclear.

A relation between viral infection and cardiomyopathy has been suggested, but complete evidence is still lacking. We found congestive heart failure after experimental encephalomyocarditis (EMC) virus infection in BALB/c mice in the acute stage of the disease. However, dilatation or hypertrophy was not seen in this strain of mice in the chronic stage. In this study, we found marked dilatation and hypertrophy in the chronic stage of EMC virus myocarditis in DBA/2 mice. This is the first documentation that viral myocarditis causes lesions similar to those seen in congestive cardiomyopathy.

Methods

Experimental Infections

The M variant of EMC virus was used; 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 10^6.5 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 DBA/2 mice were obtained from the Shizuoka Agricultural Cooperative Association. This strain has been maintained continuously by brother-sister matings. At 4 weeks of age, 174 mice were inoculated intraperitoneally with 0.1 ml of virus suspension containing 100 TCD50 per 0.1 ml. Mice were observed daily for 90 days after inoculation.

After gross inspection of the heart for alterations in myocardial appearance, the hearts were processed for histologic or virologic studies.

The mice were weighed to the nearest 0.1 g and hearts to the nearest 0.1 mg, and the heart weight/body weight ratio (HW/BW) was calculated.

Seventy control 4-week-old mice were inoculated intraperitoneally with 0.1 ml of virus-free FL cell culture fluids and killed on days 5 and 90. Their hearts were processed and examined in the same manner as those of the infected mice.

Pathologic Study

Hearts were fixed in 10% formalin solution, sectioned transversely (perpendicular to the long axis) at the middle portion of the ventricle, embedded in paraffin and stained with hematoxylin-eosin, von Kossa and Mallory-azan stains. The thickness of the walls of the right and left ventricles and of the interventricular septum as 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.

In the lateral wall of the right and left ventricles and the interventricular septum, myocardial fiber diameter was determined by measuring, in the stained cross-sectional areas, the shortest diameter at the level of the nucleus of 50 myocardial fibers using an ocular micrometer.

Virologic Study

For virus isolation, hearts were ground with sea 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 observed daily for 7 days for the appearance of a characteristic cytopathic effect.

Statistical Analysis

Statistical analysis of the data was performed by an analysis of variance with multiple comparisons by Neuman-Keuls' method. Values are mean ± sd.

Results

Mortality and Incidence of Myocarditis

Four days after the inoculation, infected mice appeared ill. Some developed spastic paralysis, but some of the sick mice survived and appeared well after 14 days. One hundred one of the 174 inoculated mice (58.0%) died. Eighty-nine of these 101 (88.1%) died between the third and seventh days after the inoculation. Gross myocardial lesions were seen on the surface of the ventricles of the heart in 115 of the 174 mice (66.1%). These myocardial lesions were observed in 82 of the 101 dead mice and in 33 of the 73 (45.2%) surviving mice. No mice died after day 60. On day 90, myocardial lesions were found in 11 of 30 mice (36.7%).

The mortality rate was highest on the fourth day and then decreased gradually. Mice that died after day 7 showed pleural effusion, ascites and congestion of the lungs and liver; therefore, the cause of death seemed to be congestive heart failure.

Body Weight, Heart Weight and the HW/BW Ratio (fig. 1)

Heart weight, body weight and the HW/BW ratio were measured in the mice with myocarditis and in the controls in the acute stage (days 4–5), when myocardial necrosis became apparent, and in the chronic stage (day 90), when dilatation and hypertrophy of the heart developed. By days 4–5, the body weight of infected mice (n = 40) was significantly lower (10.9 ± 2.8 g) than in controls (n = 40) (18.0 ± 2.4 g) (p < 0.001), but on day 90 there was no significant difference between infected mice (n = 11) (23.4 ± 2.9 g) and controls (n = 30) (22.1 ± 3.0 g).

The heart weight was not significantly greater in infected mice on days 4–5 (0.101 ± 0.018 g vs 0.108 ± 0.016 g in controls), but was significantly greater on day 90 (0.190 ± 0.028 vs 0.122 ± 0.013 g in controls, p < 0.001).

The HW/BW ratios were greater in infected mice than in controls both on days 4–5 (9.43 ± 1.64 × 10⁻³ vs 6.04 ± 1.01 × 10⁻³, p < 0.001) and on day 90 (8.27 ± 1.72 × 10⁻³ vs 5.55 ± 0.63 × 10⁻³, p < 0.001).

Cavity Dimension and Wall Thickness (fig. 2)

Cavity dimension and wall thickness were measured on day 90. The cavity dimension of the left ventricle in the 11 infected mice was significantly greater than that in the 20 controls (1.67 ± 0.29 mm vs 1.11 ± 0.20 mm) (p < 0.001). The cavity dimension of the right ventricle was 0.44 ± 0.15 mm, not significantly different from that of the controls, 0.36 ± 0.14 mm. The wall thickness of the right ventricle, the interventricular septum and the left ventricle was 0.55 ± 0.14 mm.
Thereafter, myocardial fiber diameter (fig. 3) was prominent and hypertrophy of myocardial cells was evident. Myocardial calcification still persisted, but there was no mononuclear cell infiltration (figs. 4F and G). Congestion of the lungs was noted at this stage (fig. 4H).

Spontaneous calcification, limited to the epicardial side of the right ventricle, was found in six of 40 control mice on day 5 and in 15 of 30 on day 90 after the inoculation of virus-free FL cell culture fluids. However, lesions were not found in other parts of the myocardium, so the location of the lesions differed from that in mice infected with EMC virus.

Virus Isolation

Virus was isolated from the hearts of all 20 mice inoculated with EMC virus on days 4–5, but from none of 11 mice on day 90. No virus was recovered from the hearts of control mice.

Discussion

Heart disease occurs as a complication of several viral infections.9–13 The course of acute viral myocarditis is usually considered benign if the patient can survive the acute phase of illness.

Kline and Saphir14 reviewed their autopsy series of 225 cases of myocarditis. Six cases deteriorated in a protracted course, for 6 weeks to 6 years, interrupted by remissions and exacerbations with progressive heart failure. There was interstitial myocarditis with replacement fibrosis. Although no cause was established in any of these cases, they preceded the era of availability of viral studies.

There are a few follow-up studies of patients with proved viral myocarditis, as well as clinically acceptable acute and chronic myocarditis. Smith15 reported a series in which although 82% of 42 adult patients with coxsackievirus B myopericarditis made a complete clinical recovery, in 12 patients symptoms persisted for 3 months or longer, seven patients had one or more recurrences of cardiac illness and six had persistent abnormal ECGs. Two patients died after a recurrence and three had residual cardiomegaly.
Fuster and colleagues reported 104 patients with dilated cardiomyopathy; 20% had a severe influenza-like syndrome shortly before the first manifestation of cardiomyopathy. Significantly higher titers of antibodies against coxsackievirus B have been found in patients with cardiomyopathy than in the general population.

Such observations have given rise to speculation that some cases of congestive cardiomyopathy might represent the end stage of an earlier acute myocarditis, either subclinical or clinically manifested. However, complete evidence is lacking. Even in experimental viral myocarditis, direct evidence of the transition from acute myocarditis to cardiomyopathy is lacking. After studies on experimental coxsackievirus myocarditis, we found a severe myocarditis in inbred strains of BALB/c mice inoculated with the M variant of EMC virus. Congestive heart failure developed in the acute stage, but dilatation or hypertrophy of the heart was not seen in the chronic stage.

In this study, we found a severe myocarditis in inbred strains of DBA/2 mice inoculated with the M variant of EMC virus. On days 4–5, the HW/BW ratio of infected mice was greater, but this was because of a
decrease in body weight. Mice with severe myocarditis died of congestive heart failure in the acute stage. In the surviving mice with myocarditis, on day 90, both the heart weight and the HW/BW ratio were significantly increased and the cavity dimension of the left ventricle was enlarged. Myocardial fibrosis was prominent and hypertrophy of myocardial fibers was evident. There was no mononuclear cell infiltration at this stage. Congestion of the lungs was observed in both the acute and chronic stages. These findings suggest that congestive cardiomyopathy may develop as early as 3 months after virus infection. The wall thickness of the right ventricle, interventricular septum and left ventricle was not increased. Hypertrophy was masked by dilatation, as in congestive cardiomyopathy.20

Ball and Williams21 reported spontaneous myocardial lesions in DBA/2 mice. In our DBA/2 mice, perimyocardial calcification, limited to the right ventricle, was seen in six of 40 control mice on days 4–5, and in 15 of 30 controls on day 90. However, these lesions were in a different location from those in myocarditis of mice infected with EMC virus, in which extensive myocardial lesions were found.

Genetic factors may play a role in susceptibility to infection and severity of the disease, and even in the difference in the character of the pathologic lesions. Differences were found in the frequency of occurrence of myocarditis in inbred strains of A/J, BALB/c, C3H/He, C57BL/6 and DBA/2 mice. Also, myocardial calcification was less prominent in BALB/c mice than in DBA/2 mice (report in preparation).

How the host reacts to viral infection may determine the course of an illness as much as the virulence of the infective agents. The absence of inducible in vitro suppressor cell function22 may influence the course of viral infection and may play some role in developing con-
gestive cardiomyopathy. Information is needed that can help to determine if a certain group is particularly susceptible to viral myocarditis. The relationship of HLA types to clinical disease might be of value in identifying such individuals in view of evidence that cell-mediated immunity has been implicated in the pathogenesis. 

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References

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