Pathogenesis and Preventive and Therapeutic Trials in an Animal Model of Dilated Cardiomyopathy Induced by a Virus

AKIRA MATSUMORI, M.D., CHUICHI KAWAI, M.D., CLYDE S. CRUMPACKER, M.D.*
AND WALTER H. ABELMANN, M.D.*

An animal model of dilated cardiomyopathy following encephalomyocarditis (EMC) virus has been developed. Virus was isolated from mouse hearts and viral antigens were detected in the myocardium until the second week of infection, but neither was found thereafter. Differences were found among different strains of mice in the frequency of occurrence and severity of myocarditis, and even in the character of the pathologic lesions. Thus, genetic factors may play an important role in the pathogenesis.

Autoantibodies against heart developed and the distribution of cardiac myosin isoenzymes was altered during the course of myocarditis. Virus, vaccine, maternal vaccination, recombinant interferon alpha A/D and ribavirin were effective in protecting the mice from developing myocarditis.

This animal model is suitable for studying the pathogenesis of viral myocarditis and evaluating preventive and therapeutic interventions of the condition.

RECENTLY, we developed animal models of viral myocarditis, using encephalomyocarditis (EMC) virus, in which congestive heart failure developed in the acute to subacute stages. Dilatation and hypertrophy, as seen in dilated cardiomyopathy, developed in the chronic stage. We have investigated the natural history and pathogenesis of viral myocarditis and assessed therapeutic and preventive interventions in these models.

An Animal Model of Dilated Cardiomyopathy

We observed a severe myocarditis in an inbred strain of DBA/2 mice inoculated with the M variant of EMC virus. Mice with severe myocarditis died of congestive heart failure in the acute stage. In the surviving mice with myocarditis, both the heart weight and the HW/BW ratio were significantly increased and the cavity dimensions of the left ventricle were enlarged in the chronic stage. Myocardial fibrosis was prominent and hypertrophy of myocardial fibers was evident. There was no mononuclear cell infiltration at this stage. Congestion of the lungs and liver was observed in both the acute and chronic stage. These findings suggest that congestive cardiomyopathy may develop as early as three months after virus infection. Dilatation and hypertrophy of the heart persisted up to the eighth month after inoculation with EMC virus in C3H/He and DBA/2 mice.

EMC virus was isolated from the hearts and EMC virus antigen was detected in the myocardium until the second week of infection (Fig. 1), but neither was found thereafter. 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. In EMC virus infection, differences were found in...
positive granular fluorescence was seen on day 5 after infection and persisted to day 90, most marked in myocytes near foci of myocardial fibrosis and calcification. Thus, the distribution of cardiac isoenzymes was altered following viral myocarditis.

**Prevention and Treatment of Viral Myocarditis in an Animal Model**

*Virus Vaccine*

EMC virus, $5 \times 10^6$ plaque-forming units (pfu/ml), was inactivated with $1:4,000$ formalin $^6$ Four-week-old CD-1 mice were injected subcutaneously and then intravenously with vaccine. Seven days after the second vaccine, 100 pfu of EMC virus were inactivated intraperitoneally. None of 10 mice treated with vaccine and infected with EMC virus died or showed myocardial lesions. All of 10 mice without vaccine died 5-7 days after EMC virus inoculation and showed severe myocardial lesions. Thus, virus vaccine prevented development of myocarditis.

**Preventive Effect of Maternal Immunization upon Myocarditis in Offspring**

Viral myocarditis has a predilection for infants, and outbreaks in newborns have been reported. Therefore, the effect of maternal vaccination on viral myocarditis in offspring was studied in our animal model of viral myocarditis. EMC virus, $5 \times 10^6$ plaque-forming-units (pfu)/ml, was inactivated with $1:4,000$ formalin and incubated at $37^\circ C$ for seven days. Four-week-old female CD-1 mice (non-pregnant) were injected subcutaneously and seven days later intravenously with 0.1 ml of inactivated virus vaccine. Four weeks later the mice were mated. After delivery, litters of mice were inoculated at the age of 2, 10 or 21 days, with 100 pfu of EMC virus and observed up to seven days. Age-matched mice delivered from non-immunized mothers were injected with virus in the same manner and served as controls (each group, n = 10). Among the offspring from non-immunized mothers, all of the 2-day-old mice died on day 2, all of the 10-day-old mice died on day 3 and all of the 21-day-old mice died before day 5. None of the offspring from immunized mothers died. Myocardial virus titers of the offspring from non-immunized mother at the time of death were $3.1 \pm 2.3 \times 10^6$ (2-day-old), $1.8 \pm 1.1 \times 10^5$ (10-day-old) and $1.6 \pm 1.0 \times 10^4$ (21-day-old) pfu/mg (mean $\pm$ S.D.), respectively.

Anti-heart Antibodies

Anti-heart autoantibodies were found in the sera from DBA/2 mice infected with EMC virus $^4$ Frozen sections of the hearts of uninfected mice were incubated with mouse sera and stained with anti-mouse IgG. Positive granular staining was first seen on day 4 and persisted to day 90; the titer was highest on day 21. The demonstration of anti-heart antibody in this model of viral myocarditis suggests a pathogenetic role in the previously demonstrated later cardiomyopathy.

Distribution of Cardiac Myosin Isoenzymes as Determined by Selective Monoclonal Antibodies

Frozen sections of the hearts were stained with the monoclonal antibody specific for the myosin isoenzyme $V_2$ (R11D10). R11D10 did not stain myocytes of uninfected mice, whereas
virus was isolated from the hearts of the offspring from immunized mothers (Table I). In this model, vaccination of mothers before pregnancy completely inhibited myocardial virus replication and had a protective effect on EMC virus infection in offspring.

**Ribavirin**

Ribavirin is a drug with broad antiviral activity against RNA and DNA viruses. Chemically, ribavirin is a synthetic nucleoside analog, structurally related to inosine and guanosine. Clinically, its efficacy has been demonstrated in measles, influenza and respiratory syncytial virus infection. We investigated the effect of ribavirin on experimental myocarditis caused by EMC virus? Four-week-old DBA/2 mice were inoculated with 10 pfu of EMC virus. Ribavirin in a dose of 100 (group 1, n = 20), 200 (group 2, n = 10), or 400 mg/kg/day (group 3, n = 10) was administered subcutaneously on days 0 to 12 after virus inoculation, and animals were observed for 12 days. Mice treated with ribavirin survived longer than controls (mean survival 6.7 days for group 1, 7.4 days for group 2, 7.7 days for group 3, and 5.2 days for control; p < 0.005). Myocardial virus titers on days 6 to 8 were significantly lower in group 2 (3.24 ± 0.49 log_{10} pfu/mg; p < 0.005) and in group 3 (1.70 ± 0.65 log_{10} pfu/mg; p < 0.001) compared with controls (4.09 ± 0.57 log_{10} pfu/mg). Histologic examination showed extensive myocardial necrosis and cellular infiltration in untreated groups; there was less infiltration in groups 2 and 3 (p < 0.01) and less severe necrosis in group 3 (p < 0.01). Thus ribavirin effectively inhibited myocardial virus replication and reduced the inflammatory response and myocardial damage in an experimental model of viral myocarditis.

**Recombinant Human Leukocyte Interferon alpha A/D**

The effects of recombinant human leukocyte interferon alpha A/D on experimental myocarditis due to EMC virus were investigated. Four-week-old male DBA/2 mice were inoculated intraperitoneally with 10 pfu of EMC virus. Interferon alpha A/D, 10^4 (group 1) u/g/day, was administered subcutaneously daily, starting one day before the infection. Interferon alpha A/D was also given starting the same day (group 2). Animals were sacrificed on day 4 for evaluation. Myocardial virus titers were significantly lower in group 1 (8.2 ± 25.2 x 10^2 pfu/mg; p < 0.05) and in group 2 (3.0 ± 5.5 x 10^3 pfu/mg; p < 0.05) compared with controls (5.6 ± 4.1 x 10^4 pfu/mg). Histologic examination showed extensive myocardial necrosis and cellular infiltration in all mice of an untreated group, but no myocardial necrosis or cellular infiltration was evident in group 1 and less severe necrosis and infiltration in group 2. Thus, interferon alpha A/D, when given before and simultaneously with virus inoculation, effectively inhibited myocardial virus replication and reduced the inflammatory response and myocardial damage in an experimental model of viral myocarditis.

Interferon and ribavirin have several advantages in that they are broadly active antiviral and therefore should be of great advantage in diseases of multiple etiology, such as viral myocarditis. At first sight, the demonstrated beneficial effects of recombinant interferon alpha A/D and ribavirin before and at the time of virus inoculation may appear to be of little clinical value. However, clinical relevance may lie in the protection of individuals infected in the laboratory or exposed to epidemic outbreaks, especially in infants, such as has been repeatedly reported to occur in nurseries. Therefore, it is our opinion that the results reported justify further study of the effects of interferon alpha A/D and ribavirin against other cardiotropic virus infections.

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**Table 1: Mortality And Virus Titer Of The Heart**

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<tr>
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<th>Mice from immunized mother</th>
<th>Mice from non-immunized mother</th>
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<tbody>
<tr>
<td></td>
<td>2-day-old</td>
<td>10-day-old</td>
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<tr>
<td>Mortality</td>
<td>0</td>
<td>0</td>
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<tr>
<td>(n = 10)</td>
<td>(day 2)</td>
<td>(day 3)</td>
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<tr>
<td>Virus titer of the heart</td>
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<td>(pfu/mg)</td>
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REFERENCES


