Therapy with cyclosporine in experimental murine myocarditis with encephalomyocarditis virus

E. SCOTT MONRAD, M.D., AKIRA MATSUMORI, M.D., JAMES C. MURPHY, D.V.M., PH.D., JAMES G. FOX, D.V.M., M.S., CLYDE S. CRUMPACKER, M.D., AND WALTER H. ABELMANN, M.D.

ABSTRACT To explain the progression from infectious viral myocarditis to congestive cardiomyopathy an infection/immune hypothesis has been proposed stating that the primary viral process incites an excessive or disordered immunologic response against the myocardium. To test whether one form of immunosuppressive therapy might ameliorate this process, we used cyclosporine in a murine preparation of infectious myocarditis (encephalomyocarditis [EMC] virus), which has been shown to result in a congestive cardiomyopathy pathologically similar to that seen in man. Eight-week-old male DBA-2 mice were infected with EMC virus and randomized to a treatment or control group. Cyclosporine (25 mg/kg/day) was administered subcutaneously for 3 weeks, starting (1) at 1 week after infection during viral replication, and (2) at 3 weeks after infection, after the period of active viral replication. In mice treated during viral replication there was a significantly higher mortality rate compared with that of control mice (15/21 vs 9/29, p = .01). There was no evident reduction in myocardial pathology (inflammation, necrosis, or calcification) in the treated compared with the control groups. In mice treated after the period of viral replication, there was no improvement in mortality (8/22 vs 2/19, NS) compared with control. Treated mice showed no reduction in myocardial histopathologic lesions. Furthermore, treated mice had significantly greater heart weight/body weight ratios (1.3 ± 0.4% vs 1.0 ± 0.3%, p < .005), lung weight/body weight ratios (1.1 ± 0.5% vs 0.8 ± 0.3%, p < .05), and liver weight/body weight ratios (6.0 ± 0.8% vs 5.4 ± 0.6%, p < .005) than control mice, suggesting more severe myocardial failure. Thus, the use of immunosuppressive therapy with cyclosporine in this murine preparation of acute viral myocarditis was associated with greater mortality when administered early in the illness, and greater myocardial failure when administered during the early recovery period, without evident reduction in pathologic indexes of myocardial injury to suggest possible longer term benefit. Clinical trials of cyclosporine therapy for inflammatory myocarditis secondary to an acute viral infection should be carried out with great caution and only in the setting of a carefully controlled clinical trial.


THERE IS increasing evidence that chronic congestive cardiomyopathy may result from prior viral myocarditis.1–7 Encephalomyocarditis (EMC) virus is an entero-virus that can cause an acute myocarditis in a number of animal species,8 and that may also infect man.9–12 We have previously shown that EMC infection in the mouse produces a severe, acute myocarditis that may be followed by development of cardiomyopathy and congestive heart failure.13, 14 This preparation is characterized pathologically by myocardial dilatation and hypertrophy, and so is similar to the congestive cardiomyopathy seen in man.

It is unclear whether this evolution from the acute illness to chronic myocardial failure occurs primarily because of the direct cytopathic effect of the virus on myocardial cells or because of an infection-induced, host-mediated inflammatory response against myocardial tissue.15–17 If it is the latter, appropriate immunosuppressive therapy may ameliorate the degree of myocardial injury. Consonant with this are the reports of several investigators that, in certain patients with heart failure in association with myocarditis, both the inflammation and myocardial dysfunction may respond to immunosuppressive therapy.18, 19 Whether

From the Charles A. Dana Research Institute and the Harvard-Thorndike Laboratory of the Beth Israel Hospital, Department of Medicine (Cardiovascular Division), Beth Israel Hospital and Harvard Medical School, Boston, and the Division of Comparative Medicine, Massachusetts Institute of Technology, Cambridge.

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Address for correspondence: Walter H. Abelmann, M.D., Harvard-Thorndike Laboratory, Department of Medicine, Beth Israel Hospital, 330 Brookline Ave., Boston, MA 02215.

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these reported cases represent a primary autoimmune process, or rather an autoimmune process triggered by a primary viral myocarditis is unclear.

Cyclosporine is a fungal metabolite with potent and unique immunosuppressive properties. Predominantly a suppressor of the T cell helper system, it has been used in the therapy of several diseases of an autoimmune origin and in the suppression of rejection of allograft transplants (including heart transplants). In this study we examined the effect of immunosuppressive therapy with cyclosporine on the clinical course and on the pathologic severity of myocardial injury in the murine preparation of EMC viral myocarditis.

Methods

**Infection protocol.** The virus stock of the M variant of EMC virus was prepared in cultures of FL (human amnion) cells in Eagle’s minimum essential medium. Virus suspensions were centrifuged after the cytopathic effect had developed, and viral stock had a titer of $10^8$ plaque-forming units (PFU) per milliliter, determined in tissue cultures of FL cells.

Eight-week-old male, inbred DBA-2 mice (Jackson Laboratories) that were certified virus-free were studied under a protocol approved by the Beth Israel Hospital Animal Research Committee and in conformance with the principles of the American Physiological Society. They were maintained in laminar-flow isolation rooms throughout the study. They were inoculated intraperitoneally with 0.1 ml of virus suspension containing 10 PFU per 0.1 ml.

**Treatment protocol.** Cyclosporine was kindly supplied by David Winter, M.D., Sandoz Inc., Hanover, NJ. Because of its strongly hydrophobic nature, the cyclosporine was dissolved in 0.1 ml of a solution of 10% ethanol/90% olive oil and administered subcutaneously at a dose of 25 mg/kg/day—a dose chosen because previous reports demonstrated this to be both well tolerated and effectively immunosuppressive in animals.

**Experiment 1—early protocol.** Fifty mice surviving to 1 week after inoculation were randomly assigned either to treatment with cyclosporine (21 mice), or to no cyclosporine treatment (29 mice). Nine of the latter 29 were sham-injected subcutaneously with 0.1 ml of 10% ethanol/90% olive oil for the treatment period. Starting with day 8 after inoculation, treatment was administered for 21 days. The mice were observed daily, and autopsies were performed immediately on those mice found dead. Mice surviving to the end of the treatment period were killed and autopsies were performed at that time.

An additional control group of un inoculated mice (n = 9) treated for 21 days with cyclosporine starting at 9 weeks of age was also studied.

**Experiment 2—late protocol.** Forty-one mice surviving to 3 weeks after inoculation, at which time virus can no longer be isolated from either blood or myocardium, were randomly assigned to either treatment with cyclosporine (administered as above; 22 mice) or no treatment (sham-injected subcutaneously with 0.1 ml of 10% ethanol/90% olive oil; 19 mice). In the treatment group, treatment was administered for 21 days until 42 days after inoculation, when surviving mice were killed and autopsies were performed. The mice were observed daily, and autopsies were performed on all mice that died during the course of the experiment.

**Pathologic examination.** The hearts were graded for gross evidence of involvement of the left ventricle. The body organs were weighed and then fixed in buffered 10% formalin. Hearts were sectioned in the long axis through both atria and ventricles. Tissue was processed by standard methods, embedded in paraffin, cut into 5 μm thick sections, and stained with hematoxylin-eosin. Myocardial sections were graded by two of the authors (W. H. A. and J. C. M., who were blinded to the respective treatment groups) for severity of inflammation, necrosis, and calcification of the left ventricle.

The pathologic criteria for grading of the severity of myocardial inflammation, necrosis, or calcifications were as follows: grade 1 (mild), one or two small foci; grade 2 (slight), several small foci; grade 3 (moderate), multiple small foci or several large foci; grade 4 (severe), multiple large foci or diffuse inflammation, necrosis, or calcification.

Because of the recognized propensity of DBA-2 mice for spontaneous dystrophic calcification of the right ventricle, this parameter was not included in the analysis. The other organs were evaluated for evidence of viral or other pathologic processes.

**Virologic study.** To assess the time course of viral replication in this preparation tissue from 21 hearts of “control” mice (including one mouse that died on the sixth day after inoculation) and from 21 hearts of mice treated with cyclosporine were homogenized in 2.0 ml of Eagle’s minimal essential medium. The suspensions were centrifuged, 0.1 ml of each supernatant was inoculated into plate cultures of FL cells, and plaque assays were performed as previously described.

**Statistical analysis.** Survival data were analyzed by the chi-square method with the Yates correction. Primary comparison of the other data was performed on results from all the mice studied, control vs treatment groups, by the unpaired t test for parametric data and the Mann-Whitney test for nonparametric data. Because of the disparity between control and treatment groups with respect to the time of death, secondary paired comparisons were made (1) of mice in the early treatment protocol that died the same or similar lengths of time after inoculation (to match for similar stage and severity of illness), (2) of mice in both early and late treatment protocols paired for initial weight (to more tightly control for initial state), and (3) of mice in the late treatment protocol that survived to the end of the treatment period and that were sacrificed (again, to match for stage and severity of illness and minimize postmortem artifact). Statistical analysis in the paired subgroups was performed by the paired t test for parametric data and by Wilcoxon’s signed-rank test for nonparametric data; in the unpaired subgroups, statistical analysis was performed as for the primary groups.

**Results**

Infection with EMC produced a clinical and pathologic picture similar to that reported previously. By 4 days after inoculation, the mice appeared ill. Some of the mice developed spastic paralysis. Mice that died showed pleural effusion and ascites. Grossly, the myocardium had pale yellow patches and calcareous foci that correlated with the inflammation, necrosis, and calcification seen microscopically. Atrial and ventricular thrombi were common. One mouse had severe myocardial necrosis with formation of a left ventricular aneurysm, a finding reported in other murine preparations of viral myocarditis.

Cytopathic assay of myocardial viral titers showed peak viral titers at days 7 to 10 after inoculation. No virus was isolated from the myocardium after day 15.
TABLE 1

Results of early treatment protocol (during the period of viral replication)

<table>
<thead>
<tr>
<th></th>
<th>All animals</th>
<th>Animals paired for body weight</th>
<th>Animals paired for time of death/sacrifice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uninfected controls treated</td>
<td>Infected controls</td>
<td>Infected treated</td>
</tr>
<tr>
<td>(n = 9)</td>
<td>(n = 29)</td>
<td>(n = 21)</td>
<td>(2 vs 3)</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>24.5 ± 2.3</td>
<td>22.2 ± 1.9</td>
<td>21.7 ± 2.5</td>
</tr>
<tr>
<td>Weight (g)</td>
<td>26.6 ± 2.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>23.3 ± 4.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.6 ± 3.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HW/BW (%)</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>LuW/BW (%)</td>
<td>0.7 ± 0.1</td>
<td>1.1 ± 0.5</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>LiW/BW (%)</td>
<td>6.0 ± 0.4</td>
<td>5.4 ± 0.7</td>
<td>5.6 ± 0.8</td>
</tr>
<tr>
<td>Gross LV</td>
<td>0.1 ± 0.3</td>
<td>2.0 ± 1.1</td>
<td>2.0 ± 1.3</td>
</tr>
<tr>
<td>Inflammation</td>
<td>0.0 ± 0.0</td>
<td>1.6 ± 1.0</td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td>Necrosis</td>
<td>0.4 ± 0.5</td>
<td>2.4 ± 1.3</td>
<td>2.9 ± 0.9</td>
</tr>
<tr>
<td>Calcification</td>
<td>0.4 ± 0.5</td>
<td>2.6 ± 1.0</td>
<td>3.0 ± 0.8</td>
</tr>
</tbody>
</table>

Values are mean ± SD. p value denotes the statistical significance of the observed differences between the infected controls and the treatment group. weight<sub>1</sub> = the weight at entry into the study; weight<sub>2</sub> = the weight at death or sacrifice; HW/BW = heart weight/body weight ratio (%); LuW/BW = the lung weight/body weight ratio (%); LiW/BW = the liver weight/body weight ratio (%); Gross LV = pathologic score (0 to 4) for gross left ventricular lesions; Inflammation/Necrosis/Calcification = pathologic score (0 to 4) of left ventricular lesions (microscopically).

<sup>a</sup>p < .005 weight, vs weight<sub>1</sub>; <sup>b</sup>p = NS weight, vs weight<sub>1</sub>; <sup>c</sup>p < .001 weight, vs weight<sub>1</sub>.

Uninfected/treatment protocol (table 1; figure 1). In this group, which had received cyclosporine for 3 weeks, there were no deaths, and all mice gained weight (mean ± SD, 24.5 ± 2.3 to 26.6 ± 2.8 g, p < .0005). There was no microscopic evidence of inflammation. However, five of the mice showed "minimal" microscopic evidence of dystrophic calcification. One of these had a small lesion evident on gross inspection of the left ventricle.

Experiment 1—early treatment protocol (table 1). In the control group, nine of 29 mice died, while in the cyclosporine-treated group 15 of 21 mice died (p = .01). Body weight at death or sacrifice (table 1) did not differ significantly from baseline in the control group (22.2 ± 1.9 vs 23.3 ± 4.6 g, NS), while it fell in the cyclosporine-treated group (21.7 ± 2.5 vs 18.6 ± 3.7 g, p < .001).

There was no evident reduction in the score for gross myocardial lesions (2.0 ± 1.1 vs 2.0 ± 1.3, NS), in myocardial hypertrophy as assessed by heart weight/body weight ratio (1.1 ± 0.4% vs 1.2 ± 0.3%, NS), or in the extent of myocardial inflammation (1.6 ± 1.0 vs 2.0 ± 0.8, NS), necrosis (2.4 ± 1.3 vs 2.9 ± 0.9, NS), or calcification (2.6 ± 1.0 vs 3.0 ± 0.8, NS) on microscopic assessment. Notably, although it did not achieve statistical significance (p < .07), there was a trend to greater inflammation in the treatment group. This lack of evidence of benefit from treatment with cyclosporine was also noted when the data were analyzed by pairing the mice so as to control for time of death or to control for initial body weight.

Experiment 2—late treatment protocol (table 2; figure 1). In the control group, two of 19 mice died, while in the cyclosporine treatment group eight of 22 mice died, a difference that was not significant.

At the time of randomization to treatment at 3 weeks after inoculation, the weights of the mice were below baseline for both the treatment (23.9 ± 2.8 to 21.0 ± 3.9 g, p < .005) and control (23.5 ± 2.9 to 22.1 ± 3.3 g, NS) groups, although the change in the latter did not achieve statistical significance (p = .06). By the end of the sixth week, the weights of the control mice had increased (at death or sacrifice) above baseline (25.6 ± 4.6 g, p < .05); however, the weights of the cyclosporine-treated mice remained below baseline (21.2 ± 4.4 g, p < .05).

With later cyclosporine treatment, there was no significant reduction in the score for gross myocardial lesions (control compared with treatment group) (1.7 ± 0.7 vs 2.1 ± 1.2, NS), or in the microscopic extent of inflammation (1.4 ± 1.0 vs 1.4 ± 0.9, NS), necrosis (2.7 ± 0.6 vs 2.7 ± 0.9, NS), or calcification (2.6 ± 0.6 vs 2.8 ± 0.8, NS). Furthermore, the cyclosporine group had a significantly greater heart weight/body weight ratio (1.0 ± 0.3% vs 1.3 ± 0.4%, p < .005), lung weight/body weight ratio (0.8 ± 0.3% vs 1.1 ± 0.5%, p < .05), and liver weight/body weight ratio (5.4 ± 0.6% vs 6.0 ± 0.8%, p < .005), suggesting more severe heart failure (although the interpretation of the liver weight/body weight data is complicated by the tendency of mice, when ill, to lose weight).

Similar findings were also present when the mice
were paired to control for initial body weight. When only data from those mice surviving to the end of the late treatment protocol were analyzed, there also was no evidence of improvement in pathologic indexes of myocardial injury after therapy with cyclosporine, but the evidence suggesting more severe heart failure was no longer not present. However, in this analysis, data from only two of 19 mice in the control group were

**TABLE 2**

Results of late treatment protocol (after the period of viral replication)

<table>
<thead>
<tr>
<th></th>
<th>All animals</th>
<th>Animals paired for body weight</th>
<th>Animals surviving to end of protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infected controls (n = 19)</td>
<td>Infected treated (n = 22)</td>
<td>p value (1 vs 2)</td>
</tr>
<tr>
<td>Weighti (g)</td>
<td>23.5 ± 2.9</td>
<td>23.9 ± 2.8</td>
<td>NS</td>
</tr>
<tr>
<td>Weightii (g)</td>
<td>22.1 ± 3.3</td>
<td>21.0 ± 3.9</td>
<td>NS</td>
</tr>
<tr>
<td>Weightiii (g)</td>
<td>25.6 ± 4.6</td>
<td>21.2 ± 4.4</td>
<td>.005</td>
</tr>
<tr>
<td>HW/BW (%)</td>
<td>1.0 ± 0.3</td>
<td>1.3 ± 0.4</td>
<td>.005</td>
</tr>
<tr>
<td>LuW/BW (%)</td>
<td>0.8 ± 0.3</td>
<td>1.1 ± 0.5</td>
<td>.05</td>
</tr>
<tr>
<td>LiW/BW (%)</td>
<td>5.4 ± 0.6</td>
<td>6.0 ± 0.8</td>
<td>.005</td>
</tr>
<tr>
<td>Gross LV</td>
<td>1.7 ± 0.7</td>
<td>2.1 ± 1.2</td>
<td>NS</td>
</tr>
<tr>
<td>Inflammation</td>
<td>1.4 ± 1.0</td>
<td>1.4 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Necrosis</td>
<td>2.7 ± 0.6</td>
<td>2.7 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>Calcification</td>
<td>2.6 ± 0.6</td>
<td>2.8 ± 0.8</td>
<td>NS</td>
</tr>
</tbody>
</table>

`Weighti = weight at entry into the study; weightii = the weight at randomization to treatment or control groups 3 weeks after inoculation; weightiii = the weight at death or sacrifice; other abbreviations are as in table 1.

`ap = NS weighti vs weightii; b p < .005 weighti vs weightiii; c p < .001 weighti vs weightii; d p = NS weighti vs weightii; e p = NS weighti vs weightii; f p = NS weighti vs weightii; g p = NS weighti vs weightii; h p = NS weighti vs weightii; i p = NS weighti vs weightii; j p = NS weighti vs weightii; k p = NS weighti vs weightii; l p = NS weighti vs weightii; m p = NS weighti vs weightii; n p = NS weighti vs weightii; o p = NS weighti vs weightii; p p = NS weighti vs weightii; q p = NS weighti vs weightii; r p = NS weighti vs weightii; s p = NS weighti vs weightii; t p = NS weighti vs weightii; u p = NS weighti vs weightii; v p = NS weighti vs weightii; w p = NS weighti vs weightii; x p = NS weighti vs weightii; y p = NS weighti vs weightii; z p = NS weighti vs weightii.`

FIGURE 1. The effect of cyclosporine on survival in murine (DBA-2) myocarditis with EMC virus. When drug was administered early in the illness, during the period of demonstrable viral replication (experiment 1), there was a significantly greater (p = .01) mortality in the mice treated with cyclosporine (closed circles) than in control mice (x). Even when administered later in the illness (experiment 2), during the period of early recovery (open circles), no significant improvement (NS) compared with that in control mice (+) resulted. See text for details.
excluded, while those from eight of 22 in the treatment group were excluded, resulting in a bias in favor of the treatment group.

Other organ involvement. Overall, pancreatic lesions of likely viral origin were noted in six control mice (three of which died) and five treatment mice (four of which died), and these may have contributed to the observed clinical courses. No viral lesions were noted in the brain, liver, or kidneys. One mouse in the late treatment group was noted to have fungi invading the intestinal wall.

Discussion

With more widespread use of endomyocardial biopsy, inflammatory myocarditis—acute, subacute, and chronic—has been recognized increasingly as one of the causes of congestive cardiomyopathy.1-7 It is likely that the pathologic presentation of inflammatory myocarditis results from several causes, with “inflammation” as a common pathologic response, inasmuch as inflammatory lesions in the myocardium may be seen after various insults, including infection,3 autoimmune diseases,26-28 and hypersensitivity reactions.29

Viral myocarditis in man may result from any of several viral agents. It is usually a self-limited process,2,15,16 but may follow either a fulminant course with rapid progression to heart failure, or lead to myocardial fibrosis with later stage myocardial dysfunction and heart failure. It has been speculated that progressive myocardial injury may result from an exaggerated and persistent immunologic response30-32 to neoantigens induced or exposed (e.g., sequestered antigens) by the viral genome. In fact, in two preparations of immunodeficient mice, the severity of the myocardial lesions in experimental Coxsackie viral myocarditis was less than that in immunocompetent mice,”33,34 and immunostimulation with levamisole has been shown to exacerbate experimental murine Coxsackie myocarditis.35 Because of this evidence, we investigated the effects of immunosuppression with cyclosporine in an enterovirus preparation of murine myocarditis (EMC virus) that has been shown to progress to congestive cardiomyopathy with chronic myocardial failure.

In congestive cardiomyopathy in man, supporting the above hypothesis of the immunopathogenesis of myocardial failure after viral myocarditis, several immunologic abnormalities have been reported.36-39 Predominant among these has been defective T cell suppressor function, suggesting a loss of immunologic tolerance to host myocardial tissue either through altered responsiveness to intrinsic host antigens or as a response to neoantigens induced by viral or other processes. Furthermore, other diseases with an “autoimmune” basis and causing abnormalities of suppressor T cell function40 may be accompanied by an inflammatory myocardial process.27

For those lesions resulting from a disordered or excessive immune response, immunosuppressive therapy might ameliorate the inflammatory process and the progression to congestive cardiomyopathy. There is evidence for this in the report of two uncontrolled series of patients with inflammatory myocarditis and heart failure who responded favorably to immunosuppressive therapy with corticosteroids and azathioprine.48,49 However, with the variability of the clinical course that patients with inflammatory myocarditis and/or congestive cardiomyopathy may follow, a more controlled evaluation is required to assess the overall role of immunosuppressive therapy in patients with these disorders.

Cyclosporine30-32 is a fungal metabolite with unique immunosuppressive properties that make it potentially a particularly suitable agent for the therapy of autoimmune disorders characterized by a deficiency of T cell suppressor function. This agent has been shown to preferentially inhibit T cell helper function, probably through inhibition of interleukin-2 production, while relatively sparing T cell suppressor function, the primary deficiency of which may be responsible for the autoimmune process. Therefore, therapy with cyclosporine might restore a normal helper/suppressor balance.

EMC, an enterovirus of the family Picornaviridae, may infect many mammals,8 including man.9-12 We have previously shown that EMC infection in the mouse may be followed by congestive cardiomyopathy and congestive heart failure, with pathologic changes similar to those seen in man.11,14 In this study we investigated the effect of therapy with cyclosporine on the course of EMC myocarditis in this preparation. When administered early in the disease process (1 week after inoculation), in the midst of the viremic phase of the illness, cyclosporine led to a significantly higher mortality rate than that seen in the control population of inoculated mice, and there was no pathologic evidence of a reduction in myocardial injury. Administration of cyclosporine after the time of demonstrable viral replication led to no benefit with regard to survival or pathologic indexes of myocardial injury, and was associated with increased heart, lung, and liver to body weight ratios, suggesting impaired myocardial function and heart failure.

These results are consonant with those of other investigators, who, using different preparations of, and
therapies for, acute infectious myocarditis, have generally found deleterious effects of immunosuppressive therapy. In murine chagasic myocarditis, while salicylate therapy (which may have direct antitrypanosomal effects in addition to its anti-inflammatory effects) was shown to reduce the severity of myocardial lesions, immunosuppression with cyclophosphamide led to uniformly increased mortality and severity of myocardial lesions. In murine preparations of Coxsackie viral myocarditis several investigators have shown deleterious effects of pharmacologic immunosuppression with corticosteroids and cyclophosphamide and a persistence of viral replication in myocardial tissue of immunocompromised mice. The mechanisms by which cyclosporine might exert the observed deleterious effects are several. First, it may have led to a suppression of an appropriate immunologic response with resultant worsening of myocardial injury. Second, immunosuppression may result in secondary “opportunistic” infections—induced fungal invasion of the bowel wall was noted in one treated mouse. Third, cyclosporine has been noted to have nonimmunologic side effects that may have contributed to our findings, the most notable of which is induction of renal insufficiency. The absence of microscopic renal pathology is common with cyclosporine-induced renal insufficiency, and animals with myocardial failure may prove even more sensitive to this. Also, hypertension may be seen after initiation of cyclosporine therapy, and although the mean time for onset of this in man is 50 days, it may be seen as early as 6 days, and animals with myocardial injury would tolerate especially poorly any increase in afterload. Finally, altered renal handling of electrolytes, in particular potassium, may be seen with cyclosporine therapy and may predispose to the development of arrhythmias in injured myocardium, which also may have contributed to the adverse clinical outcomes observed. These nonimmunologic effects may be of particular relevance to our findings in the late treatment protocol, and are important when considering the potential role of this agent for the treatment of inflammatory myocarditis.

There are several limitations to the present study. EMC virus may result in significant disease of other organs (in particular the pancreas and the brain), which may affect the observed outcomes. However, the strain of EMC used was the “M (or myocarditic) variant,” which results predominantly in myocardial disease, and pathologic evidence of pancreatitis and central nervous system infection was infrequent. The frequent occurrence of spontaneous calcification of the right ventricle in DBA-2 mice limited analysis of right ventricular pathology, which may predominate in cases of myocarditis/cardiomyopathy in man. EMC is a virus that only infrequently has been associated with clinically evident disease in man; thus, the results of studies in this preparation may not be directly applicable to myocarditis and cardiomyopathy in man. However, EMC is an enterovirus, related to those organisms predominantly responsible for viral myocarditis in man, and in this murine preparation it has been shown to lead to pathologic changes similar to those seen in cardiomyopathy in man. Also, only one dosing regimen of cyclosporine was used, and other regimens might have led to different results. Finally, the results of the present study apply only to the use of cyclosporine in the acute or subacute phases of viral myocarditis and may not be extrapolated to its use in the chronic phases of the illness.

The implication of the present study with respect to inflammatory myocarditis in man is that therapy with cyclosporine, although still potentially useful for myocarditis secondary to primary autoimmune disorders, may be deleterious when the disease results from an acute viral infection, at least in the early period of viral replication and shortly thereafter. In this setting, cyclosporin should be used only under the auspices of a carefully controlled trial, which is needed to define its potential role in the therapy of inflammatory myocarditis in man.

We are indebted to Ms. Kathryn A. Thorp for technical assistance, and to Bernard J. Ransil, M.D., for assistance with the statistical analyses.

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