Lone Hepatitis C Virus Myocarditis Responsive to Immunosuppressive Therapy*

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Objectives: This study analyzes the causal role of hepatitis C virus (HCV) in patients with lone myocarditis, and its susceptibility to immunosuppression.

Background: Prevalence of HCV in lone myocarditis, its mechanism of damage, and possible treatment are still unknown.

Methods: Among 48 consecutive patients with myocarditis serologically screened for HCV and other cardiotropic viruses, 3 patients had anti-HCV antibodies. Clinical manifestation was heart failure in two cases, and left bundle-branch block with moderate cardiac dysfunction was present in patient 3. The three patients underwent two-dimensional echocardiography, coronary angiography, and endomyocardial biopsy. Nested polymerase chain reaction (PCR) for positive and negative strands of HCV on sera and myocardial samples, and PCR for the most common cardiotropic viruses were performed. HCV in the myocardium was detected by TORDJI-22 antibody.

Results: At histology, a lymphocytic myocarditis associated with myocytes positively stained by TORDJI-22 was shown in all. Cardiac autoantibodies were detected in all cases. Nested PCR showed both positive and negative strands of HCV RNA in serum and myocardium; other viral genomes were absent. Patients were treated with prednisone and azathioprine for 6 months, with recovery of cardiac volumes and function. At 4-week control biopsy, myocarditis progressed to a healed phase, though HCV RNA was still detectable in the serum and myocardium. Cardiac improvement was maintained at the 12-month overall follow-up.

Conclusions: HCV can be detected in the myocardium of as many as 6% of patients with lone myocarditis; HCV myocarditis can benefit from immunosuppression despite persistence of viral genome, suggesting an immunomediated mechanism of damage.

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Key words: hepatitis C virus infection; immune system; myocarditis

Abbreviations: ACE = angiotensin-converting enzyme; ANA = antinuclear antibodies; ANCA = antineutrophil cytoplasm antibodies; HCV = hepatitis C virus; LBBB = left bundle-branch block; LV = left ventricular; LVEF = left ventricular ejection fraction; PCR = polymerase chain reaction; RT = reverse transcriptase

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reatment of myocarditis remains a major challenge possibly because of the multiplicity and the heterogeneity of its etiologic and pathogenetic mechanisms. In particular, the results of immunosuppressive treatment have been controversial1–3; therefore, its efficacy is still speculative and considered contraindicated when a viral genome is demonstrated in the myocardocytes.4 Although enteroviruses are considered the most common pathogens responsible for viral myocarditis,5,6 preliminary reports suggest that hepatitis C virus (HCV) infection may be associated with several myocardial diseases, including chronic myocarditis.7–9 In this study, we report three cases of HCV-related myocarditis demonstrated by histologic and molecular biology techniques, which responded to immunosuppressive therapy despite evidence of persistent replication of viral genomes in cardiac myocytes.

MATERIALS AND METHODS

Patient Population

Serologic tests for HCV in addition to the most common cardiotropic viruses (echovirus, Coxsackievirus A and B, cyto-
megalovirus, herpes simplex viruses, Epstein-Barr virus, parvovirus B19, adenovirus, influenza virus A and B, and parainfluenza virus) were performed in 48 consecutive patients (32 men and 16 women; mean ± SD age, 45 ± 14.9 years) with a clinical and histologic diagnosis of myocarditis, hospitalized from September 1998 to March 2000 in our institution. Three of these patients (two women and one man, 6.25%) had positive findings for serum anti-HCV antibodies. None had a history of chronic hepatitis or previous evidence of the infection. Patient 1 was a 35-year-old woman in whom, during the second week after the delivery of her first child, progressive dyspnea and palpitation developed. Her previous clinical history was uneventful, and no infectious disease was recorded during the pregnancy. Patient 2 was a 18-year-old boy admitted to the hospital because of severe cardiac failure (New York Heart Association class IV), with palpitation and progressive dyspnea appearing 1 year earlier in spite of treatment with digitalis, diuretic, and angiotensin-converting enzyme (ACE) inhibitors. He had received large doses of adriamycin, 750 mg/m² body surface, at the age of 10 years for a lymphoma with complete remission; he also had received blood transfusions. Patient 3 was a 62-year-old woman with ulcerative colitis, with a recent history of recurrent episodes of chest pain lasting up to 20 min, occurring at rest and occasionally during effort, unresponsive to sublingual nitroglycerin administration, and angiographic evidence of normal coronary arteries. The patient was admitted to the hospital because of the persistence of chest pain and the appearance of a left bundle-branch block (LBBB) associated with a moderate left ventricular (LV) dilatation and dysfunction.

Clinical Investigations

All patients underwent cardiac catheterization, biplane left and right ventriculography, coronary angiography, and biventricular endomyocardial biopsy. In patient 3, because of the presence of angina-like symptoms with normal coronary arteries, an intracoronary ergonovine test was performed as previously described.10

After informed consent, endomyocardial biopsies (three to four for each ventricular chamber) were performed using a Bipal Byoptome Cordis (Johnson & Johnson; Miami, FL), approached by a 7F (501–613 and 501–613A) sheath in the septal-apical region of both ventricles. Two samples were immediately frozen in optimal cutting temperature compound with isopentane cooled in liquid nitrogen for molecular studies, and the remaining were fixed in 10% buffered formalin, and embedded in paraffin wax. Blood samples were collected at the time of cardiac catheterization, and the separated serum was stored at −80°C.

Two groups of patients were studied as control subjects: (1) two patients with anti-HCV antibodies in the serum and a cardiac disease other than myocarditis (ie, cardiac hemochromatosis [control subject 1] and chronic stable angina [control subject 2]); and (2) three patients with negative HCV serologic findings, affected by chronic ischemic heart disease (control subjects 3 and 4) and amyloidosis (control subject 5). Blood and myocardial samples were collected at the time of cardiac catheterization with endomyocardial biopsy (control subjects 1 and 5) or during coronary artery bypass grafting (control subjects 2, 3, and 4).

Histologic and Immunohistochemical Analysis

Four to six endomyocardial samples obtained from each patient were processed for histologic and immunohistochemical studies, as previously described.11 Dallas criteria12 were employed for histologic diagnosis of myocarditis. Immunohistochemical detection of HCV antigen was performed by the monoclonal antibody TORDJ1-22 (1:60; Biogenex Laboratories; San Ramon, CA) specific for the HCV c100 protein, using as chromogen 3-amino-9-ethylcarbazole. Appropriate positive and negative controls were used.13

Serologic Studies

All three patients underwent serologic tests for HCV and for the above-mentioned cardiotropic viruses and immunologic studies (antinuclear antibody [ANA], anti-DNA, and anticardiolipin, antiscarcodermal, and antinoncolomerlal antibodies; antineutrophil cytoplasm antibodies [ANCA]; circulating immune complexes; C3c, C4). HCV antibodies were detected by means of a third-generation enzyme-linked immunosorbent assay (Ortho Diagnostic Systems; Raritan, NJ) and confirmed by a recombinant immunoblot assay (Ortho Diagnostic Systems). Patients’ sera were also tested for the presence of cardiac autoantibodies through the use of standard indirect immunofluorescence as previously described.14

Molecular Analysis

Myocardial samples (two for each patient and control subjects) were used for the detection of cardiotropic viruses, through polymerase chain reaction (PCR) analysis, as previously described.15 In particular, a nested PCR for the highly conserved 5’ noncoding region of HCV was performed for detection of this virus (positive and negative strands).

Sera collected at the time of endomyocardial biopsies (patients 1, 2, and 3, and control subjects 1 and 5) and of cardiac surgery (control subjects 2, 3, and 4) was also analyzed for the presence of HCV RNA in the same way. Patients’ sera were also analyzed after 1 month of immunosuppressive therapy and after 1 month from the interruption of immunosuppression. HCV types were determined on the basis of variation in nucleotide sequence within restricted regions in the putative C (core) gene of HCV virus. All values are expressed as mean ± SD.

RESULTS

Angiographic Study

Invasive studies confirmed the global cardiac dysfunction in patients 1 and 2 (Figs 1, 2) with an increase in left ventricular (LV) end-diastolic and mean pulmonary pressures. Coronary angiography ruled out coronary stenoses in all cases. The ergonovine test, performed in patient 3, failed to show a spastic response.

Histology and Immunohistochemistry

In all three cases, histology showed an active myocarditis with diffuse inflammatory infiltrates associated with focal necrosis of adjacent myocytes, meeting the Dallas criteria12 consistently in all right ventricular and LV specimens (Figs 1, 2). In all patients, the inflammatory changes were associated with interstitial and focal replacement fibrosis. In patients 1 and 2, myocyte hypertrophy and endocardial thickening were also present.
Immunophenotypical characterization of the inflammatory cells showed the presence of activated T lymphocytes (CD45RO+), including a moderate amount of cytotoxic lymphocytes (CD8+). Focal intracytoplasmic positivity for TORDJI-22 was observed in myocytes of all patients (Fig 3).

Serologic Studies

Serologic tests for cardiotropic viruses revealed in all three cases a positivity for IgG anti-HCV, and were not indicative of other active infections in all cases (less than fourfold rise in IgG titers in paired
The sera of all three patients were positive for cardiac autoantibodies, with diffuse cytoplasmic immunofluorescence staining of myocytes at titer of 1:10. Immunologic studies showed a positivity for ANA with a titer of 1:80 in patients 1 and 2. In patient 2, a positivity for circulating immunocomplexes (6.5 μg/mL; normal value < 5.1 μg/mL) was also present. Patient 3 had a mild positivity for ANCA (18 UI/mL; normal value < 11 UI/mL).

**Molecular Data**

In all patients, the presence of sufficient target nucleic acid was confirmed by amplification of β-
globin for DNA and 3GPDH for RNA. Both positive and negative strand HCV RNA were present in the serum of patients with active myocarditis before and after 1 month from the onset of immunosuppressive treatment. Otherwise, in serum of control subjects 1 and 2, with positive HCV serologic findings, only the positive strand of the virus was detected (Fig 4). HCV titer (genomes per milliliter) and HCV type are shown in Table 1. In particular, with immunosuppression there was an increase in HCV titer in all cases (Table 1), with an evident reduction after 1 month from treatment withdrawal (0.7 \times 10^6 in patient 1, 0.09 \times 10^6 in patient 2, 1.2 \times 10^6 in patient 3).

Search of viral genomes in the myocardium revealed the presence of HCV RNA (both positive and negative strands) in patients with myocarditis, before and during immunosuppression, but in none of control groups included in the study (with and without serologic evidence of HCV infection; Fig 4). Genomes of the other investigated viral agents were absent both in patients and in control subjects.

Treatment and Follow-up

Patients 1 and 2 were receiving full therapy with ACE inhibitors, diuretics, and digitalis, while patient 3 had received no cardiac medication. All three patients, because of the histologic evidence of an active myocarditis associated to the presence of cardiac autoantibodies, received prednisone (1 mg/kg/d for 4 weeks followed by 0.33 mg/kg/d for 5 months) and azathioprine (2 mg/kg/d for 6 months). The patients were followed up clinically and with ECG and two-dimensional echocardiography weekly on the first month and every 2 weeks for the remaining 5 months. One week after the beginning of immunosuppression, all patients had a
visible improvement of symptoms, with reduction of heart rate and disappearance of gallop rhythm in patients 1 and 2 (heart rate, 70 beats/min and 80 beats/min, respectively) and disappearance of chest pain in patient 3. The ECG showed a positivity of T waves in the precordial leads and in D1-aVL in patient 1, an increase in voltages and disappearance of repolarization abnormalities in patient 2, and the disappearance of the LBBB in patient 3 (Fig 5). In all patients, echocardiography showed an increase in LV function (LV ejection fraction [LVEF] rose to 55%, 45%, and 56%, respectively) and a reduction of left atrial and LV dimensions. The patients at 1, 3, and 6 months of follow-up again underwent cardiac catheterization, angiography, and endomyocardial biopsy, which showed considerable improvement in right ventricular and LV function with a reduction in volumes and intracavity pressures (Table 1). Histology revealed a healed myocarditis with disappearance of inflammatory infiltrates and of myocytolysis (Figs 1, 2).

Serology as early as 3 months of immunosuppressive therapy showed disappearance of ANA, ANCA, and circulating cardiac autoantibodies. Laboratory tests during immunosuppression failed to show an increase of aminotranferases. On echocardiography, the liver appeared of normal dimension and morphology in all patients before, during, and after the therapy, with no signs of liver involvement, so that the opportunity of a liver biopsy was ruled out. At 16 months (patient 1), 10 months (patient 2), and 11 months (patient 3) of follow-up (mean, 12.3 ± 3.2 months), the patients remained asymptomatic, with preserved improvement of cardiac volumes and function.

**Discussion**

Growing evidence derived from myocardial molecular biology studies suggests the possible associa-

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**Table 1—Clinical and Molecular Data of Patients With HCV Myocarditis Before and Following 1 Month of Immunosuppressive Therapy**

<table>
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<th>Variables</th>
<th>Patients</th>
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<td>On-I</td>
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<td>On-I</td>
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<td>On-I</td>
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</table>

*Pre-I = before immunosuppressive therapy; On-I = during immunosuppressive therapy; NYHA = New York Heart Association; LVEDD = LV end-diastolic diameter; PASP = pulmonary artery systolic pressure; LVEDP = LV end-diastolic pressure; AST = aspartate aminotransferase; ALT = alanine aminotransferase; aM = active myocarditis; hM = healed myocarditis.

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**FIGURE 4. Case 1. Immunohistochemistry for TORDJI-22. Note the typical clustered red perinuclear granules in myocyte indicative for HCV (original × 160).**
HCV as Causal Agent of Myocarditis

The ability of HCV to induce a myocarditis has been recognized in patients with hypertrophic cardiomyopathy as well as dilated cardiomyopathy.\(^{15,16}\) Its prevalence seems to vary from different populations, as it has been found very low in German people\(^{17}\) and much higher in the Japanese. This may be due to variability in the genetic predisposition or to different methods of assessment of viral myocardial infection. In this regard, some concerns have been expressed about the genome analysis and need for confirmation by \textit{in situ} hybridization.\(^{9,17}\)

In our study, the possible causal role of HCV is supported by the presence of both positive (genomic) and negative (replicative) strand of HCV RNA in the myocardium of all three patients and by the absence of other cardiotropic viruses at PCR analysis. Positive and negative HCV RNA was also present in the serum of the three patients, and the HCV titer ranged from $1.4 \times 10^6$ to $3.1 \times 10^6$ genomes per milliliter, denoting a high viral load. The risk of a false-positivity of the negative strands, due to the possible action of the positive-strand HCV RNA as template for the synthesis of false-negative strand, suggested by previous studies,\(^{18}\) was minimized by using heat denaturation after reverse transcriptase (RT) incubation and a ribonuclease digestion after complementary DNA synthesis to degrade the remaining positive-strand genomic sequences, applied in our molecular analysis.

Possible contamination with HCV material from the serum or circulating infected cells was ruled out by using two control subjects with HCV infection but without myocarditis (\textit{i.e.}, control subjects 1 and 2). In these control subjects, the positivity of HCV RNA in the serum (HCV titer $0.28 \times 10^6$ in control subject 1, and $1.5 \times 10^6$ in control subject 2) was not associated with a positivity in the myocardium. Moreover, the absence of HCV RNA in the myocardium of infected control subjects who did not have myocardial inflammatory changes suggests the possibility that the myocardial localization of HCV may play a causative role in myocarditis, supporting recent preliminary reports.\(^{7–9}\)

The observation of a myocyte immunohistochemical positivity for HCV in our patients with myocarditis supports the recent findings of Takeda et al.,\(^{19}\) who showed, by \textit{in situ} hybridization, the cardiotropism of the virus. Although not indicative of the replicative status of the virus, immunohistochemical staining with TORDJI-22 monoclonal antibody has demonstrated a 70% sensitivity and an 84% specificity compared with RT-PCR in the detection of the virus.\(^{13}\) The evidence of a myocyte positivity for TORDJI-22 together with the presence of a myocardial HCV replication by PCR may be considered a demonstration that this virus can replicate in the myocytes.

In conclusion, the causal role of HCV in our three patients is suggested by the following: (1) the histologic evidence of a myocarditis not associated with the myocardial detection of other cardiotropic viruses; (2) the absence of HCV in the myocardium of control subjects with cardiac diseases other than myocarditis, even in presence of HCV in serum; and
(3) the detection of HCV antigens in cardiac myocytes associated with the evidence of HCV intracellular replication.

**HCV Myocarditis and Immunosuppressive Therapy**

All patients were treated with prednisone and azathioprine with prompt and relevant clinical improvement. Immunosuppression was indicated by the presence of cardiac autoantibodies, suggesting an immune-mediated mechanism of cell injury, and by the absence of cardiotoxic viruses other than HCV. HCV infection is often associated with hepatic and extrahepatic autoimmune disease (ie, essential mixed cryoglobulinemia, lymphocytic sialoadenitis, membranoproliferative glomerulonephritis, panarteritis nodosa)\(^20,21\) and with the presence of autoantibodies.\(^22\) Although none of our patients with HCV myocarditis had other autoimmune diseases, all had circulating cardiac autoantibodies and other autoimmune serologic manifestation, such as ANA (patients 1 and 2), ANCA (patient 3), and circulating immune complexes (patient 2). In patients with HCV infection and autoimmune manifestations, corticosteroids are the first-choice therapy, even if these drugs may exacerbate HCV replication, while the use of interferon has resulted in disease worsening.\(^23,24\) In patients with chronic HCV myocarditis, interferon and ribavirin were used as a second-line therapy, with prompt and relevant clinical improvement.\(^25\) Immunosuppression, a phenomenon that is not reversible, despite full therapy with digitalis, diuretics, and ACE inhibitors. In patient 3, a LBBB disappeared soon after the introduction of immunosuppression, a phenomenon that is not reported in spontaneously healing myocarditis. On the contrary, this could be explained with resolution of interstitial edema by steroid administration or alternatively with repair or even regeneration of the conduction tissue once myocardial inflammation has been removed as appears on control biopsies.

**HCV and Myocardial Injury in Myocarditis**

The exact mechanisms of myocyte injury by HCV are still unclear. At the moment the evidence of a direct cytopathic effect of HCV remains unproven and may not play a major role in myocyte damage.\(^26\) Other mechanisms, such as antiviral and autoimmune inflammatory reaction, appear to be prevalent.

The presence of cardiac autoantibodies and the favorable response to immunosuppression, associated with an initial increase of HCV RNA titers that decreased below pretreatment values after its discontinuation,\(^26\) also support that an immunemediated mechanism may play a major role than a direct viral cytotoxic effect of HCV on the myocardium.

**Clinical Implication**

The experience herein reported suggests that myocarditis may be the first and isolated clinical manifestation of HCV infection and that this agent should be searched for when an inflammatory heart muscle disease is diagnosed by endomyocardial biopsy, as HCV myocarditis may account for up to 6% of these patients and benefit from immunosuppressive therapy. Cardiac recovery is persistent and may occur even in the presence of replicating viral genome in the myocardiocytes. Future therapeutic strategies might associate the immunosuppressive treatment with new antiviral agents, such as antisense oligonucleotides or ribozyme, directly inhibiting viral replication without enhancing the host immune response.\(^27\)

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