Immunosuppressive Therapy in Myocarditis

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Although there is general agreement on the favorable effect of immunosuppression in eosinophilic, granulomatous, giant-cell myocarditis and in lymphocytic myocarditis associated with connective tissue disorders and with rejection of a transplanted heart, its therapeutic role in lymphocytic inflammatory cardiomyopathy (ICM) is still debated. Previous retrospective studies reported a relevant clinical benefit in 90% of patients with virus-negative ICM and no response or cardiac impairment in 85% of those with virus-positive ICM following immunosuppression. Other studies identified cardiomyocyte HLA upregulation as an additional indicator of ICM susceptibility to immunosuppressive therapy. Recently in a single-center randomized prospective double-blind trial using a combination of prednisone and azathioprine in addition to supportive treatment in 85 virus-negative ICM patients, a significant improvement in left ventricular (LV) ejection fraction and a significant reduction in LV dimensions in 88% of 43 treated patients compared with 42 patients receiving placebo who showed a cardiac impairment in 83% of cases (TIMIC study) was reported. These data confirm the efficacy of immunosuppression in virus-negative ICM. Lack of response in 12% of cases suggests the presence of unscreened viruses or mechanisms of damage and inflammation not susceptible to immunosuppression. Recovery of cardiac function in responders to immunosuppression was associated with inhibition of cardiomyocyte death, increased cell proliferation and with newly synthesized contractile material. (*Circ J* 2015; **79**: 4–7)

Key Words: Heart failure; Immunosuppressive therapy; Myocarditis; Viruses

Myocarditis is an inflammatory disease of the heart caused by viral, bacterial and fungal infections, systemic diseases, autoimmune dysregulation, drugs and toxins. From the clinical point of view, it ranges from subclinical paucisymptomatic forms to life-threatening arrhythmias,¹ cardiogenic shock and sudden death.

Although in approximately 40% of cases the acute myocarditis resolves spontaneously,² in the remaining patients it evolves to a chronic phase as a consequence of an abnormal immune response, with ventricular dilation, reduced contractility, and clinical progression to heart failure.^{3,4}

Despite the advancement of diagnostic techniques in defining the etiology of myocarditis, a specific standardized treatment is not yet available. This is mainly related to the still unknown mechanisms regulating the normal or abnormal host immune response leading either to virus elimination and spontaneous resolution of the inflammatory process or to immune-mediated damage persisting with or without viral clearance. In addition, the type of infectious agent and its prevalent mechanism of cell damage (ie, directly cytopathic or immune-mediated) could also affect the evolution of myocardial inflammation.

In particular, the use of immunosuppressive treatment for lymphocytic myocarditis is still controversial,⁵ both in children^{6,7} and adults^{8,9} presenting with either cardiac arrhythmias¹⁰

or heart failure.¹¹ Indeed, in the absence of specific markers of eligibility for this treatment, a large trial by Mason et al in the past produced misleading results showing the absence of evident improvement of survival in myocarditis patients treated with immunosuppressive drugs vs. placebo.⁹ For this reason, the use of immunosuppressive therapy is still confined to the treatment of eosinophilic,¹² granulomatous¹³ and giant-cell myocarditis,¹⁴ as well as lymphocytic myocarditis associated with connective tissue diseases¹⁵ or with rejection of a transplanted heart. More recently Wojnicz et al¹⁶ in a randomized placebo-controlled study suggested that upregulation of HLA antigens in the myocardial tissue of patients with lymphocytic myocarditis may identify a homogeneous subgroup of inflammatory dilated cardiomyopathy sustained by an autoimmune mechanisms of damage, and may represent a marker of susceptibility to the treatment. However, in that study the presence of a viral genome in the myocardium was not investigated.

Our group, in both retrospective and prospective studies, identified the characteristics of patient responders to immunosuppressive therapy and the cellular and molecular mechanisms of cardiac recovery after immunosuppression.

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Figure. Echocardiographic and histologic evaluation of a 42-year-old man with chronic virus-negative inflammatory cardiomyopathy. Echocardiographic apical 4-chamber view (A) and long-axis view (B) showing severe LV dilation (EDD=90mm) and dysfunction (EF=18%), with remarkable mitral regurgitation (C, color Doppler imaging). These findings improved significantly after 6 months of immunosuppressive therapy, with EF increasing to 36% (D, apical 4-chamber view), mitral regurgitation reducing to mild (E, color Doppler imaging) and LVEDD reducing to 73 mm (F, M-mode imaging). Histology at baseline showed flord active lymphocytic myocarditis (G, H&E, x200) with overexpression of HLA-DR, denoting immune-mediated mechanism of damage (H, immunoperoxidase, x200), progressing to healed myocarditis (I, Masson's trichrome, x200) after immunosuppression. Of note, the ECG recordings under the echocardiographic images show an intraventricular conduction disturbance (A-C) that disappeared after therapy (D-F). EDD, end-diastolic dimension; EF, ejection fraction; LV, left ventricular.

Retrospective Study

In our retrospective study,¹⁷ the virologic and immunologic profiles of patients with active lymphocytic myocarditis and chronic heart failure, responders and nonresponders to immunosuppressive therapy, were analyzed. The study group comprised 41 patients with a histological diagnosis of active myocarditis and characterized by progressive heart failure with an ejection fraction (EF) <40%, lasting >6 months despite conventional supportive therapy. All patients were similar in terms of the duration and severity of cardiac disease, histological findings and poor response to full supportive therapy. They received immunosuppressive therapy consisting of

1 mg · kg⁻¹ · day⁻¹ prednisone for 4 weeks followed by 0.33 mg · kg⁻¹ · day⁻¹ for 5 months and 2 mg · kg⁻¹ · day⁻¹ azathioprine for 6 months. Patients were classified as a responder if they had a decrease of at least 1 NYHA class and an improvement in EF ≥10% compared with baseline measures or a nonresponders if NYHA class and EF failed to improve or deteriorated or there were major events such as cardiogenic shock, heart transplantation or cardiac death. Among the 41 patients, 21 responded with a prompt improvement of EF and showed evidence of healed myocarditis in a control biopsy. Conversely, 20 patients failed to respond, and 12 of them remained unchanged, 3 underwent cardiac transplantation and 5 died, showing histological evolution towards dilated cardiomyopathy. Retro-

spective PCR on frozen endomyocardial samples and evaluation of circulating cardiac autoantibodies on patients' sera showed that nonresponders had a high prevalence of viral genomes in the myocardium (85%) and no detectable autoantibodies in the serum, whereas 90% of responders were positive for autoantibodies, with only 3 (15%) presenting viral genomes on PCR analysis. Among the nonresponders, the myocardial persistence of enterovirus and adenovirus or their combination was associated with the worst clinical outcome. These data indicate that the absence of cardiac viral genomes is a prerequisite for the clinical use of immunosuppression while suggests the potential effect of antiviral agents for patients with virus-positive inflammatory cardiomyopathy (ICM). Interestingly, serology for cardiotropic viruses failed to predict the presence of viral genome in the myocardium. This result, also confirmed in a recent study,¹⁸ suggests that this tool cannot be used as an alternative to endomyocardial tissue PCR to diagnose viral myocarditis.

Prospective Study

To confirm our results in a prospective manner, we performed a randomized, double-blind, placebo-controlled single-center trial enrolling patients with myocarditis and chronic heart failure and submitting all patients with no evidence at PCR of a myocardial viral infection to immunosuppressive treatment.¹⁹ The 85 patients were treated prednisone $1 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 4 weeks followed by $0.33 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 5 months and azathioprine $2 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ for 6 months (43 patients, Group 1) or placebo (42 patients, Group 2) in addition to conventional therapy for heart failure. Primary outcome was the 6-month improvement in LV function. Group 1 showed a significant improvement in LVEF and a significant decrease in LV dimensions and volumes compared with baseline (Figure). Specifically, 38 of 43 patients on immunosuppressive therapy (88%) showed an improvement in cardiac function and dimensions. The remaining 5 patients maintained a stable clinical picture and cardiac function parameters. Remarkably, even patients with severe, advanced disease (LVEDD $\leq 90 \text{ mm}$ and LVEF $< 20\%$, Figure) significantly improved, being able to resume their previous work. The duration of heart failure did not correlate with the extent of recovery. None of the Group 2 patients at 6-month follow-up showed improvement in LVEF, which was significantly worsened compared with baseline. In particular, 35 of the Group 2 patient (83%) showed further impairment of cardiac function while the remaining 7 patients remained unchanged. No major adverse reactions as a result of immunosuppression were registered. Histological analysis showed an active myocarditis with diffuse inflammatory infiltrates associated with focal necrosis of the adjacent myocytes (meeting the Dallas criteria) with interstitial and focal replacement fibrosis in most of the left and right ventricular specimens from all patients (Figure). The infiltrates included mainly activated T cells (CD45RO+, CD3+) with a moderate number of cytotoxic lymphocytes (CD8+) and macrophages (CD68+).

Morphometric analysis showed no differences in terms of the extent of fibrosis and the number of inflammatory cells between Group 1 and Group 2 patients. Control histology at 1 and 6 months showed, in the 38 Group 1 patients who improved with immunosuppression, a healed myocarditis with disappearance of inflammatory infiltrates associated with interstitial and focal replacement fibrosis (Figure). In the 5 Group 1 patients who did not improve, myocardial inflammation had reduced or disappeared in the control biopsies but some de-

generative changes of myocardiocytes were observed. In Group 2 patients, control biopsies were not dissimilar from baseline, showing persistence of myocarditis as well as expansion of interstitial and replacement fibrosis.

The results of this trial confirmed the positive effect of immunosuppression on recovery of LV function in a high percentage (88%) of patients with virus-negative ICM. Remarkably, a striking improvement occurred even in patients with extreme LV dilatation and dysfunction. In this group of patients, myocardial inflammation was most likely the result of an immune-mediated injury towards segregated (ie, myosin) or new antigens shared with viral components (ie, antigenic mimicry).

The efficacy of immunosuppressive therapy in patients with myocarditis was confirmed in a recent meta-analysis²⁰ of randomized controlled trials on immunosuppressive treatment for myocarditis in a selected 9 reports from 1966 to 2013 in which 342 patients were in the immunosuppressive treatment group and 267 patients in conventional treatment group. The immunosuppressive treatment group showed a significant improvement in LVEF at both short-term (≤ 3 months) and long-term follow-up.

Finally, the recent position statement of the European Society of Cardiology²¹ and the JSC Guidelines²² both recommend the use of immunosuppression only after ruling out active infection on endomyocardial biopsy by PCR, as well as in proven autoimmune forms of myocarditis.

In our study, the lack of response in 12% of cases suggests the presence of unscreened viruses or mechanisms of damage and inflammation not susceptible to immunosuppression. With regard to undetected viral genomes, metagenomic assessment of the myocardial virome, including DNA and RNA extraction from PCR-negative endomyocardial biopsies and use of the GS-FLX platform, may identify new infectious agents and provide indications.

Cellular Mechanisms of Cardiac Recovery

The cellular mechanisms of cardiac recovery in patients with ICM treated with immunosuppression were analyzed,²³ including cell death, activation of cell proliferation and reconstitution of cell myofibrillar content to clarify the effect of cell repair vs. cell proliferation or the possible contribution of cell death inhibition. The 10 responders, all showing the presence of circulating cardiac autoantibodies and absence of viral genomes in the myocardium at PCR analysis, and 10 nonresponders, characterized by worsening of LV dysfunction, absence of circulating cardiac autoantibodies, and by the presence of myocardial viral genomes¹³ were retrospectively studied in order to analyze the cellular events associated with the opposite clinical outcome. In all patients before treatment, transmission electron microscopy studies showed large cytoplasmic areas apparently empty or filled with fine granular material as a result of reduced myofibrillar content (myofibrillolysis). After 6-month immunosuppressive treatment, responders showed recovery of myofibrillar mass and architecture, whereas in the myocytes of nonresponders there was a further reduction of myofibrillar content. Further evidence of a strong activation of contractile protein synthesis comes, in responding patients, from molecular biology studies of the α - and β -isoforms of myosin heavy chain (MHC). The increased expression of α -MHC and inhibition of β -MHC synthesis, with an enhanced α/β MHC ratio after effective treatment, strongly suggests gene activation of fetal protein isoforms that typically become operative in the process of cell repair. Both apoptotic and ne-

crotic cell death of myocardiocytes were greater in baseline biopsies of responders and nonresponders than in controls, showing that myocyte loss is an important mechanism of myocardial damage in myocarditis with cardiac dysfunction. Importantly, after 6 months of effective immunosuppressive therapy, apoptosis and necrosis decreased by 85% and 62%, respectively, but further increased by 42% and by 46%, respectively, in follow-up biopsies of nonresponders. The number of cycling myocytes in baseline myocardial tissue of both responders and nonresponders was greater than in controls and significantly increased after immunosuppression in both groups, suggesting that in chronic myocarditis, as in other forms of heart failure, there is activation of myocyte regeneration in an attempt to compensate for cell loss. Thus, our study suggests that recovery of cardiac function in patients with myocarditis responding to immunosuppression is associated with remarkable cellular events, including strong inhibition of cell degeneration and death, activation of cell proliferation and mostly newly synthesized contractile elements.

Conclusions

Immunosuppressive therapy is an important resource in the management of chronic virus-negative ICM. Lack of identification of new or unconventional viral agents remains a major limit of this therapeutic approach, explaining the minor cohort of nonresponders. Future objective will be the development of molecular programs (ie, metagenomic assessment of the myocardial virome) able to detect elusive genome sequences.

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