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Immunosuppressive therapy in virus-negative inflammatory cardiomyopathy

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Abstract

While there is general agreement on the favorable impact of immunosuppression in eosinophilic, granulomatous, giant cell myocarditis, in lymphocytic myocarditis associated with connective tissue disorders and with rejection of the transplanted heart, its therapeutic role for lymphocytic inflammatory cardiomyopathy (ICM), is still debated. Previous retrospective studies reported a relevant (≥10% left ventricular ejection fraction; LVEF) clinical benefit in 90% of patients with virus-negative ICM and no response or cardiac impairment in 85% of patients 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 (1 mg/kg a day for 4 weeks followed by 0.33 mg/kg a day for 5 months) and azathioprine (2 mg/kg a day for 6 months) in addition to supportive treatment in 85 virus-negative ICM patients, a significant improvement was reported in LVEF and a significant reduction in left ventricular dimensions in 88% of 43 treated patients compared with 42 patients receiving placebo who showed a cardiac impairment in 83% of cases (TIMIC study). These data confirm the efficacy of immunosuppression in virus-negative ICM. Lack of response in 12% of cases suggests the presence of viruses that were not screened for 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, an increase in cell proliferation and with newly synthesized contractile material. ■ Heart Metab; 2014;62:18–21

Keywords: Heart failure; immunosuppressive therapy; myocarditis; recovery of function; viruses.

Introduction

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

Although in about 40% of cases acute myocarditis may resolve spontaneously [1], in the remaining patients it evolves to a chronic phase as a consequence of an abnormal immune response, with

Abbreviations

EF: ejection fraction; **ICM:** inflammatory cardiomyopathy; **LV:** left ventricular; **LVEF:** left ventricular ejection fraction; **MHC:** myosin heavy chain; **NYHA:** New York Heart Association; **PCR:** polymerase chain reaction

ventricular dilation, reduced contractility and clinical progression to heart failure.

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 an 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 the myocardial inflammation.

In particular, the use of immunosuppressive treatment for lymphocytic myocarditis is still controversial, both in children [2, 3] and in adults [4, 5] presenting with either cardiac arrhythmias [6] or heart failure [7]. Indeed, in the absence of specific markers of eligibility for this treatment, a large trial by Mason et al [5] in the past produced misleading results showing the absence of an evident improvement of survival in myocarditis patients treated with immunosuppressive drugs compared with placebo. For this reason the use of immunosuppressive therapy is still confined to the treatment of eosinophilic [8], granulomatous [9] and giant-cell myocarditis [10], as well as lymphocytic myocarditis associated with connective tissue diseases [11] or with the rejection of a transplanted heart. More recently, Wojnicz et al [12] in a randomized placebo controlled study suggested that an 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 mechanism 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 retrospective and prospective studies has identified the characteristics of patient responders to an immunosuppressive therapy and the cellular and molecular mechanisms of cardiac recovery after immunosuppression.

Retrospective study

In a retrospective study, the virological and immunological profiles of patients with active lymphocytic myocarditis and chronic heart failure, responders and non responders to immunosuppressive therapy, were analyzed [13]. Forty-one patients with a histological diagnosis of active myocarditis and characterized by progressive heart failure with an ejection fraction (EF) of less than 40%, lasting over 6 months in spite of conventional supportive therapy were studied. They received immunosuppressive therapy including 1 mg/kg a day of prednisone for 4 weeks followed by 0.33 mg/kg a day for 5 months and 2 mg/kg a day of azathioprine for 6 months. Patients were classified as responders if they had a decrease of at least one New York Heart Association (NYHA) class and an improvement in EF of 10% or greater compared with baseline measures, and non responders if NYHA class and EF failed to improve or deteriorated or in the presence of 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 at control biopsy. Conversely, 20 patients failed to respond, and 12 of them remained stationary, three underwent a cardiac transplantation and five died, showing a histological evolution towards a dilated cardiomyopathy. Retrospective polymerase chain reaction (PCR) on frozen endomyocardial samples and evaluation of circulating cardiac autoantibodies on patient sera showed that non responders 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 three (15%) presenting with viral genomes on PCR analysis. Among the non responders the myocardial persistence of enterovirus and adenovirus or their combination was associated with the worst clinical outcome. These data indicate in the absence of cardiac viral genomes a prerequisite for the clinical use of immunosuppression, while they also suggest a potential impact of antiviral agents for patients with virus-positive inflammatory cardiomyopathy.

Prospective study

To confirm this result in a prospective manner, we performed a randomized, double-blind, placebo

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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 [15]. Eighty-five patients were treated with prednisone 1 mg/kg a day for 4 weeks followed by 0.33 mg/kg a day for 5 months and azathioprine 2 mg/kg a day for 6 months (43 patients, group 1) or placebo (42 patients, group 2) in addition to conventional therapy for heart failure. The primary outcome was the 6-month improvement in left ventricular (LV) function. Group 1 showed a significant improvement in the left ventricular ejection fraction (LVEF) and a significant decrease in LV dimensions and volumes compared with baseline (*Figure 1(a, b)*). In

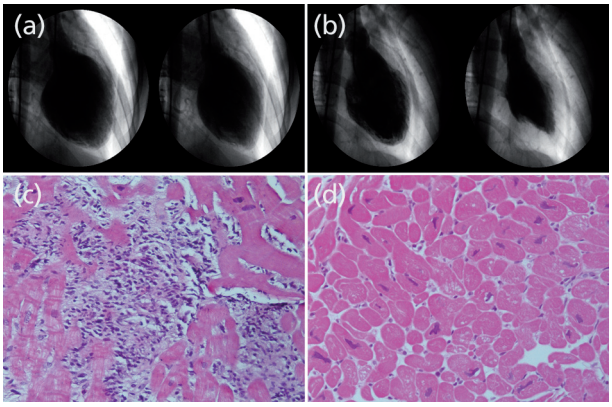

Fig. 1 *Severe LV dilatation and dysfunction; (a) angiographic systole in right panel and diastole in left panel due to virus-negative active lymphocytic myocarditis (c) that responds remarkably to 6 months immunosuppressive therapy with normalization of LV dimensions and function; (b) angiographic systole in right panel and diastole in left panel and disappearance of inflammatory infiltrates at control biopsy (d). LV, left ventricular.*

particular, 38 out of 43 patients on immunosuppressive therapy (88%) showed an improvement in cardiac function and dimensions. The remaining five patients maintained a stable clinical picture and cardiac function parameters. Remarkably, even patients with severe advanced disease (LV end-diastolic diameter up to 90 mm and LVEF of 20%) 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 the 6-month follow-up showed an improvement in LVEF, which significantly worsened compared with baseline. In particular, 35 of 42 group 2 patients (83%) showed further impairment of cardiac function, while the remaining seven patients remained the same. No major adverse reaction was registered as a result of immunosuppression. Histological analysis showed 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 1(c)*). The infiltrates included mainly activated T cells (CD45RO positive, CD3 positive) with a moderate amount of cytotoxic lymphocytes (CD8 positive) and macrophages (CD68 positive).

Morphometric analysis showed no differences in terms of the extent of fibrosis and the amount 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 the inflammatory infiltrates associated with interstitial and focal replacement fibrosis (*Figure 1(d)*). In the five group 1 patients who did not improve, myocardial inflammation reduced or disappeared in the control biopsies, but some degenerative changes in 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 impact of immunosuppression on the recovery of LV function in a high rate (88%) of patients with virus-negative inflammatory cardiomyopathy. 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). Lack of response in 12% of cases suggests the presence of viruses that had not been screened for or mechanisms of damage and inflammation not susceptible to immunosuppression. With regard to undetected viral genomes, metagenomic assessment of myocardial virome, including DNA and RNA extraction from PCR-negative endomyocardial biopsies and the use of the GS-FLX platform, may identify new infectious agents and provide indications for a more selective administration of immunosuppressive therapy.

Cellular mechanisms of cardiac recovery

Cell mechanisms of cardiac recovery in patients with inflammatory cardiomyopathy treated with immunosuppression were analyzed, including cell death, activation of cell proliferation and reconstitution of cell myofibrillar content [16] to clarify the impact of cell repair against cell proliferation or the possible contribute of cell death

inhibition. Ten responders, all showing the presence of circulating cardiac autoantibodies and the absence of viral genomes in the myocardium at PCR analysis, and 10 non responders, characterized by the worsening of LV dysfunction, the absence of circulating cardiac autoantibodies and by the presence of myocardial viral genomes [13] were retrospectively studied in order to analyze the cellular events associated with the opposite clinical outcome. Transmission electron microscopy studies showed in all patients before treatment large cytoplasmic areas apparently empty or filled with fine granular material as the result of the reduction of myofibrillar content (myofibrillolysis). After 6 months of immunosuppressive treatment, in responders myofibrillar mass and architecture recovered while in the myocytes of non responders there was a further reduction of myofibrillar content. Myocardiocyte apoptotic and necrotic cell death were greater in baseline biopsies of responders and non responders 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, while they further increased by 42% and by 46%, respectively, in follow-up biopsies of non responders. The number of cycling myocytes in baseline myocardial tissue of both responders and non responders 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 an activation of myocyte regeneration in the attempt to compensate cell loss.

Conclusion

Immunosuppressive therapy is an important resource in the management of chronic virus-negative inflammatory cardiomyopathy. Lack of identification of new or unconventional viral agents remains a major limit of this therapeutic approach explaining the minor cohort of non responders. Future objectives will be the development of molecular programmes (ie, metagenomic assessment of myocardial virome) able to assess elusive genome sequences. ■

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