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EDITORIAL

Statins as a New Therapeutic Perspective in Myocarditis and Postmyocarditis Dilated Cardiomyopathy

Editorial to "Pitavastatin Regulates Helper T-Cell Differentiation and Ameliorates Autoimmune Myocarditis in Mice" by K. Tajiri et al.

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Myocarditis is an inflammatory disease of the myocardium characterized by mononuclear cell infiltration with myocyte necrosis and degeneration [1].

The disease represents a relevant social problem worldwide, as it is one of the leading causes of dilated cardiomyopathy (DCM), particularly in young, previously healthy, individuals [2]. In fact, an underlying myocarditis is found in about 10 % of patients with unexplained heart failure, and follow-up studies in subjects with acute myocarditis have reported the occurrence of DCM in 21 % of the cases over a period of 3 years [1, 2]. Moreover, the disease is associated with a high arrhythmic risk, as demonstrated by post-mortem data identifying myocarditis in up to 12 % of cases of sudden death in patients under 40 years of age [3].

Viruses are the most common primary cause of myocarditis in Western countries, predominantly enteroviral coxsackievirus B3 (CVB3), adenovirus, parvovirus B19, and human herpes virus 6 [1]. Although the pathogenesis of the disease remains largely unclear, there is substantial evidence that a virus-triggered autoimmune response to heart antigens, particularly cardiac myosin, critically contributes to the development and progression of myocarditis [1, 2]. On this basis, two animal models of experimental myocarditis have been developed and currently used for studying the disease, i.e. infection-induced myocarditis, such as CVB3 infection, and experimental autoimmune myocarditis (EAM) induced by cardiac myosin immunization [2]. These models have significantly contributed to increase our knowledge of the pathogenesis of the disease, schematically consisting of three phases: (i) an *acute phase*, characterized by acute injury of the cardiomyocytes induced by virus replication, (ii) a *subacute phase*, critically driven by a CD4+ T cell-mediated immune response triggered by the exposure of intracellular antigens as a consequence of myocyte injury, and (iii) a *chronic phase*, characterized by myocardial repair and remodelling, with the possible progression to DCM [1, 2, 4]. In this scenario, it is widely accepted that the mainstay actor is the T-helper (Th)1 cell, crucially involved in orchestrating the immune response required for the defence of the virus-infected heart, but also responsible for the organ damage [4]. Nevertheless, increasing evidence strongly suggests that also interleukin (IL)-17 producing Th17 cells play a key role in the process, particularly in the progression of myocarditis to DCM [5, 6].

On the basis of the increasing evidence for the involvement of the immune system in the pathogenesis of the disease, more than 20 treatment clinical trials using immunosuppressive, immunomodulating or anti-inflammatory agents as well as immunoadsorption therapy have been conducted [1]. However, although some of these studies demonstrated a benefit, particularly in chronic, virus-negative inflammatory cardiomyopathy, no standard management strategies targeting the immune-mediated pathogenesis of the disease could be defined as yet. As a consequence, current standard treatment schemes in myocarditis remain limited to the therapy of heart failure in most cases, and this fact is absolutely not satisfactory [1].

Statins are a widely employed class of cholesterol-lowering drugs acting by inhibiting the hydroxy-methyl-glutaryl Coenzyme A (HMG-CoA) reductase, a key enzyme involved in the conversion of HMG-CoA in the cholesterol precursor mevalonate [7, 8]. A large body of evidence demonstrated that statins reduce the cardiovascular risk to a greater extent to that expected on the basis of the blood cholesterol-lowering effect alone, as a result of a broad range of adjunctive activities, collectively known as pleiotropic effects, mainly mediated by

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Table 1 Effects of statins on animal models of myocarditis

Author, year	Animal model	Administration	Compound used	Daily dose	Main findings	Mechanisms
Azuma et al., 2004 [16]	EAM, rat	oral	fluvastatin	3.75 or 7.5 mg/Kg for 3 weeks	Significant improvement in heart weight/body weight ratio, echocardiography and histopathology findings in high-doses (7.5 mg/Kg/day)-treated vs. untreated animals	(i) suppression of myosin-induced T cell proliferation, (ii) inhibition of the activation of myocardial NFκB, (iii) reduced production of Th1-type (IFNγ, IL-2), Th2-type (IL-4,IL-10) and pro-inflammatory cytokines (TNFα, IL-1β) in the myocardium
Liu et al., 2005 [17]	EAM, rat	oral	atorvastatin	1 or 10 mg/Kg for 3 weeks	Significant improvement in heart weight/body weight ratio, echocardiography and histopathology findings in treated vs. untreated animals	inhibition of Th1 polarization together with enhancement of Th2 development (decreased expression level of IFNγ and IL-2, increased production of IL-4 and IL-10)
Li et al., 2006 [18]	EAM, rat	oral	atorvastatin	1 or 10 mg/Kg for 3 weeks	Significant improvement in echocardiography and histopathology findings in treated vs. untreated animals	(i) downregulation of the expression of type IV CIITA promoter and MHC class II molecules on the cardiomyocytes, (ii) inhibition of Th1 polarization with enhancement of Th2 development (decreased expression level of IFNγ and IL-2, increased production of IL-4 and IL-10)
Tang et al., 2007 [19]	EAM, mouse	oral	atorvastatin	10 mg/Kg for 3 weeks	Significant improvement in heart weight/body weight ratio, echocardiography and histopathologyfindings, and electrophysiological abnormalities (APD prolongation) in treated vs. untreated animals	(i) reduction of cardiac levels of IFNγ and TNFα, (ii) attenuation of the decrease of outward potassium currents in cardiomyocytes
Wu et al., 2008 [20]	EAM, rat	oral	simvastatin	8 mg/Kg for 45 days	Significant improvement in echocardiography and histopathology findings in treated vs. untreated animals; inhibition of adoptive transfer of EAM in vivo	(i) inhibition of the cross-talk between CD4+ lymphocytes and APCs by reducing co-stimulatory molecule expression (CD28 in lymphocytes, CD80 and CD86 in APCs), (ii) reduced TNFα production in CD4+ lymphocytes and APCs
Zhang et al., 2010 [21]	CVB3-induced myocarditis, mouse	oral	atorvastatin	5 or 10 mg/Kg for 2 weeks	Significant improvement in survival rate and histopathology findings in high-doses (10 mg/Kg/day)-treated vs. untreated animals	(i) restoration of Cx43 and Cx45 distribution pattern in the heart, (ii) reduced myocardial expression and circulating levels of TNFα and IFNγ
Guan et al., 2010 [22]	CVB3-induced myocarditis, mouse	oral	atorvastatin	10 mg/Kg for 2 weeks	Significant improvement in survival rate, circulating cTnI levels, echocardiography and histopathology findings in treated vs. untreated animals	inhibition of the induction of Fas expression in the myocardium with reduced apoptosis of cardiac cells
Liu et al., 2012 [23]	EAM, mouse	oral	rosuvastatin	1 or 10 mg/Kg for 3 weeks	Significant improvement in heart weight/body weight ratio, echocardiography and histopathology findings in treated vs. untreated animals	(i) decrease in circulating levels of TNFα and IL-6, (ii) reduced expression of active caspase-3 in the myocardium with reduced apoptosis of cardiomyocytes

Table 1 (continued)

Author, year	Animal model	Administration	Compound used	Daily dose	Main findings	Mechanisms
Tajiri et al., 2013 [24]	EAM, mouse	oral	pitavastatin	5 mg/Kg[a] for 3 weeks	Significant improvement in heart weight/body weight ratio, circulating cTnI levels and histopathology findings in treated vs. untreated animals; inhibition of adoptive transfer of EAM in vivo	(i) inhibition of T-cell differentiation into Th1 and Th17 cells, with reduced production of IFNγ and IL-17, by suppressing STAT3 and STAT4 phosphorilation, and inhibiting the transcription of T-bet and RORγT, respectively, (ii) decrease of the amount of proiflammatory cytokines (IL-6,IL-1β) and chemokines (CCL2,CCL3,CCL5,CCL17, CCL20, CXCL10) in the heart.

[a] In some experiments, EAM mice were treated with 0.05, 0.5 or 5 mg/kg of pitavastatin

EAM experimental autoimmune myocarditis; *NF-κB* nuclear factor-κB; *IFNγ* interferon-γ; *IL-2* interleukin-2; *IL-4* interleukin-4; *IL-10* interleukin-10; *TNFα* tumor ncrosis factor-α; *IL-1β* interleukin-1β; *CIITA* class II transactivator; *MHC* major histocompatibility complex; *APD* action potential duration; *APCs* antigen presenting cells; *CVB3* coxsackievirus B3; *Cx43* connexin 43; *Cx45* connexin 45; *cTnI* cardiac troponin I; *Fas* FS7-associated cell surface antigen; *IL-6* interleukin-6; *IL-17* interleukin-17; *STAT3* signal transducer and activator of transcription 3; *STAT4* signal transducer and activator of transcription 4; *T-bet* T-box transcription factor; *RORγT* Retinoic acid-related orphan receptor γ T; *CCL2* chemokine (C-C motif) ligand 2; *CCL3* chemokine (C-C motif) ligand 3; *CCL5* chemokine (C-C motif) ligand 5; *CCL17* chemokine (C-C motif) ligand 17; *CCL20* chemokine (C-C motif) ligand 20; *CXCL10* chemokine (C-X-C motif) ligand 10

the interference on the protein (iso)prenylation processes [7]. In fact, mevalonate also represents the precursor of the isoprenoid derivatives, such as farnesyl pyrophosphate (FPP), and geranylgeranyl pyrophosphate (GGPP), which are involved in the regulation of several cellular mechanisms (signal transduction, cell proliferation and differentiation, cytoskeletal assembly and vescicle transport) by adding to specific intracellular proteins of the Ras, Rho, and Rab families [9]. Mounting evidence from basic and clinical studies has been demonstrating that the pleiotropic effects of statins include a wide variety of anti-inflammatory and immunomodulating properties that besides participating in the reduction of the cardiovascular risk, may also be useful in the treatment of some autoimmune diseases, particularly rheumatoid arthritis [10] and multiple sclerosis [11].

Notably, many recent in-vitro and in-vivo studies showed that statins are able to deeply affect, directly or indirectly, the function of the T-lymphocyte. Indirect effects include inhibition of T-cell homing, migration, and activation by targeting leukocytes and endothelial cells (reduced expression of chemokines, chemokine receptors, and adhesion molecules), as well as antigen presenting cells, APCs (inhibition of MHC class II antigen and costimulatory molecules expression; interference in the intracellular mechanisms of antigen processing and presentation). On the other hand, statins can also directly influence the T-lymphocyte by a) inhibiting cell activation: direct binding and block of lymphocyte function associated antigen-1, LFA-1, and reduced expression of the activation markers CD69 and CD25; b) inhibiting cell proliferation: disruption of T-cell receptor, TCR, signalling cascade at the critical steps regulated by small Ras-like GTPase; c) modulating Th1/Th2 differentiation: suppression of signal transducer and activator of transcription-4, STAT-4, with inhibition of Th1 polarization [12–14]. Finally, recent works demonstrated how statins are also effective in antagonizing Th17-mediated response, not only by reducing IL-6 and IL-23 production by APCs, thereby inhibiting Th17 cell differentiation, but also by directly suppressing the IL-17 gene expression and protein secretion in CD4+ cells [15].

On the basis of the above data and in consideration of the key role played by T helper cells in the pathogenesis of myocarditis, in the last years several authors [16–24] investigated the therapeutic potential as well as the putative underlying mechanisms of statin administration in animal models of the disease (Table 1). The results of these studies, in which different molecules were used (mainly atorvastatin, but also fluvastatin, simvastatin, rosuvastatin and pitavastatin), clearly and consistently demonstrated the ability of this class of drugs to significantly improve both cardiac function, as assessed by echocardiography, and the histopathological severity of the disease, with respect to untreated animals. Moreover, these studies provided relevant information about some potential immunomodulatory mechanisms responsible for the beneficial

effects of statins in myocarditis. In particular, the effectiveness of statins seems to be primarily related to a series of activities on the T-lymphocyte, including suppression of cell proliferation, inhibition of Th1 polarization, and interference in the cross-talk with APCs. In turn, these effects finally result in a marked reduction of the amount of cardiac Th1-type and pro-inflammatory cytokines, thus attenuating their harmful consequences on the myocardium, i.e. cardiomyocyte apoptosis, and structural and electrophysiological remodelling, which are chiefly involved in the severe depression of cardiac function, and the high arrhythmic risk characterizing myocarditis and inflammatory DCM [16–24].

In this issue of *Cardiovascular Drugs and Therapy*, Tajiri and colleagues [24] demonstrate that in BALB/c mice developing EAM after immunization with murine α-myosin heavy chain, pitavastatin administration significantly reduces the pathophysiological severity of the disease by targeting the function of the T-cell. The paper, besides providing convincing data further supporting the view that statins are protective in this pathological condition, also gives new original information contributing to clarify the mechanisms of action by which these drugs counteract myocarditis development and may reduce the associated risk of progression to DCM.

In particular, the authors show for the first time that the beneficial effects of statins on EAM are associated not only with the previously reported inhibition of Th1 polarization, but also include a concomitant suppressive activity on Th17 differentiation, which seems to be even stronger as it occurs for doses lower than those required to inhibit the differentiation of Th1 cells. This effect may be of particular relevance in the clinical setting as increasing evidence indicates that although Th17 cells, and in particular IL-17A, have only a mild effect on the severity of myocarditis during the acute phase in which the major role seems to be played by the Th1 cell [2, 4], however they are essential for the long-term progression to DCM [5, 6]. In fact, it has been demonstrated that IL-17A-deficient mice, while developing myocarditis with similar incidence and severity to wild-type controls, were protected from post-myocarditis remodelling and did not develop DCM. Notably, these mice showed reduced interstitial myocardial fibrosis and downregulated expression and activity of matrix-metalloproteinases in the heart [5]. Moreover, treatment of BALB/c mice with anti-IL17A monoclonal antibody administered after the onset of EAM abrogated myocarditis-induced cardiac fibrosis and preserved ventricular function [5]. These observations, together with the evidence that IL-17A is able to directly signal the function of the cardiac fibroblast [25–27], indicate that this cytokine critically contributes to myocardial fibrosis and remodelling of the extracellular matrix, thus driving the progression to DCM.

On this basis, the novel data of Tajiri and colleagues [24] suggest that by modulating the function of the T-cell, statins exert multistep beneficial effects in myocarditis, including suppression of the Th1-driven autoimmune response during the subacute phase as well as inhibition of the Th17-dependent postmyocarditis cardiac remodelling occurring in the chronic phase, thus potentially representing an attractive new therapy for the human disease.

At the moment, no clinical studies investigating the effects of statins in patients with myocarditis are available. Moreover, although more than 10 studies have been so far conducted in patients with established heart failure, including DCM, overall reporting the advantages of statin therapy [28], however they were in most cases small clinical studies and, particularly, only one of these involved patients with biopsy-proven inflammatory DCM [29]. In this randomized study including 74 patients, a 6 month-therapy with atorvastatin (40 mg/day) was associated with a significant improvement in both clinical and echocardiographic parameters as compared to conventional treatment. Interestingly, a significant reduction in the frequency of T-cells and macrophages in myocardial infiltrates, paralleled with a decrease in the expression of the class II MHC antigens, was detected in repeated biopsies in the atorvastatin group, but not in controls [29].

In conclusion, mounting data from in-vivo studies provide evidence that statins are highly effective in reducing the severity of experimental myocarditis in animal models through a multi-level, sequential interference on the T-cell-mediated immune response critically involved in the development and the progression of the disease. Large randomized clinical trials are warranted and needed to verify whether this basic data may be transposed into the clinical setting, thus defining if statins may take a place in the currently unsatisfactory therapeutic strategy of patients with myocarditis and inflammatory DCM.

Conflict of Interest The authors declare that they have no conflict of interest.

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