Mononuclear cell secretome in autoimmune myocarditis

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This paper was guest edited by Prof. Filippo Crea, Dipartimento di Scienze Cardiovascolari, Universita Cattolica Rome, Italy.

This editorial refers to ‘Mononuclear cell secretome protects from experimental autoimmune myocarditis’¹, by K. Hoetzenecker et al., on page 676.

Myocarditis is defined as inflammation of cardiac muscle with or without myocyte necrosis. Both infectious and non-infectious aetiologies for myocarditis are known, but the most common is virus-induced myocarditis often followed by post-infectious inflammatory cardiomyopathy.¹ Many RNA (picorna-, orthomyxo-, paramyxvo-, and flaviviruses) as well as DNA (adeno-, erythro-, herpes-, and retroviruses) viruses are capable of infecting the heart and inducing direct cytopathic injury.² During the last 20 years, important new observations and insights regarding the pathophysiological mechanisms of the disease have been made by employing two murine models of myocarditis, one induced by Coxsackievirus B3 (CVB3) infection, and the other by immunization with cardiac myosin or troponin.³ These studies resulted in novel therapeutic interventions such as immunosuppressive and immunomodulatory treatments.⁴⁻⁵ However, because of the heterogeneity of clinical presentation and incomplete understanding of human immunopathology, therapeutic options are still limited.

Congestive heart failure is frequently caused by virus infection that has been implicated as an important causal factor responsible for the development of dilated inflammatory cardiomyopathy (DCMi). Viral infection triggers an inflammatory process by expression of cytokines, chemokines, and abundant infiltration with T cells and macrophages, that outlasts the initial replicative phase and may lead to post-viral autoimmunity. Chronic inflammation then leads to tissue injury, endothelial dysfunction, and cardiac remodelling, resulting in deterioration of myocardial contractility and left ventricular ejection fraction (Figure 1). Therefore, the immune response, characterized by immunohistologically proven inflammatory infiltrates and viral genomes in biopsy specimens, plays a crucial role in the diagnosis as well as the outcome of DCMi.⁶⁻⁵

In many patients, the innate and adaptive immune response results in elimination of the viral agents followed by termination of inflammation. However, disarrangement of the immune response or molecular mimicry often triggers exposition of autoantigens followed by a chronic autoimmune disorder called inflammatory cardiomyopathy. Immunosuppressive therapy in the absence of virus together with symptomatic heart failure medication is the mainstay of clinical therapy and has been shown in prospective clinical trials to protect cardiac tissue from inflammatory injury.⁶⁻⁸ However, virus particles including enterovirus are often detected in cardiac tissue.⁹ Specific treatments encompass antiviral agents such as Val-/Gan-/Aciclovir (human herpes virus 6, cytomegalovirus, herpes zoster virus, herpes simplex virus), interferon-beta (entero-/adenovirus), or interferon-alpha and ribavirin (hepatitis C virus).¹⁰⁻¹¹

Hoetzenecker et al.¹¹ report a possible new therapeutic option for virus-negative autoimmune cardiomyopathy. They used an approach originally employed in stem cell therapy, injecting the secretome (i.e. secreted proteins) of mononuclear cells into mice with experimental autoimmune myocarditis (EAM), and were thus able to limit cardiac inflammation in this T cell-dependent model of autoimmune myocarditis (Figure 1).

Experimental autoimmune myocarditis in mouse mimics certain aspects of human inflammatory cardiomyopathy. In particular the autoimmune response and chronic inflammation of the heart can be studied by immunizing susceptible mice (e.g. BALBc) with cardiac-specific α-myosin heavy chain peptides. The autoimmune inflammation characterized by mononuclear cell infiltration peaks at day 21 post-immunization. Hoetzenecker et al. injected the secretome (i.e. a cocktail of several secreted proteins from mononuclear cells) at different time points in order to suppress autoimmune inflammation since monocytes are known to inhibit CD4⁺ cell-dependent inflammation in this model.¹²

The immune response to pathogens or other tissue injury relies on the concerted release of cytokines and proteins with biological
activity important for host protection, host defence, and wound healing. Consequently, the secretome of immune cells provides a promising resource for the discovery of specific molecular markers and targets for pharmacological intervention. Surprisingly, one high concentration injection of the secretome almost completely abrogated cardiac autoimmune inflammation on day 21, associated with lack of apoptotic and necrotic lesions within the heart. Moreover, autoantibody levels and concentrations of inflammatory cytokines were not significantly different compared with medium-treated controls. However, the proliferation of splenocytes isolated from immunized mice was significantly inhibited by mononuclear secretome injections. The authors further determined a mononuclear cell secretome-induced apoptosis in CD4+ cells by the external apoptotic pathway via caspase 8 and support those findings by determining a decreased CD4+/CD8+ ratio in secretome-treated animals. This study significantly advances our understanding of the mechanisms of cardiac autoimmune disease.

Despite the advantage of testing new and promising therapeutic targets in the presently best available animal model mimicking autoimmune mechanisms of inflammatory heart disease, one has to be careful when interpreting the data from animal studies and drawing conclusions for human disease. First, only one of several time points investigated showed the desired effect of suppressing cardiac inflammation on day 21. The authors try to explain this by time dependency of CD4+ suppression by proteins of the secretome with a supposedly short half-life. However, effects on distinct immunological mechanisms are another explanation, since, for example, antigen presentation by and the function of dendritic cells, a crucial cell type in this model of autoimmune myocarditis, was not influenced by the mononuclear secretome. Therefore, there appears to be a time- or stage-dependent effect of the secretome without influence on immune cell priming. Thus, it might be difficult to define the best time for therapy in patients with different onsets and courses of the disease, and presenting with a multitude of symptoms with or without viral infection at different stages of cardiac inflammation. However, immunohistological and virological diagnostics in myocardial biopsy specimens is mandatory for differential treatment. The effects on chronic myocarditis, remodelling, and inflammatory cardiomyopathy were not studied in this model, although EAM mice develop DCM later on. Those data would have further supported and enhanced the findings of the present study. The authors conclude that most probably multiple injections of the protein cocktail would be necessary for effective suppression of chronic myocarditis/inflammatory cardiomyopathy in humans. In this regard, it is important to realize that immunosuppression in clinical trials has been given for 3–6 months.

Importantly, the specific components of the secretome responsible for suppression of cardiac inflammation are not yet known. In this regard, endogenous modulators of immune cell migration and immunomodulation and their effects in inflammatory cardiomyopathy have been recently described. Further research to characterize those proteins/peptides or their concerted action will certainly enhance our understanding of the mechanisms leading to chronic inflammation. The authors expand and confirm observations from earlier studies that CD11+ monocytes suppress the CD4+-dependent autoimmune response. Supporting evidence that secretome injections might affect patients is provided by a similar cytokine profile of mononuclear preparations in human subjects. However, the mouse model of EAM and chronic human inflammatory cardiomyopathy differ in certain immunological aspects, and proof of principle in humans is not yet available.

Taken together, being aware of the aforementioned limitations, high dose mononuclear secretome injections have shown a significant immunomodulating effect in EAM. Further studies to characterize the specific factors involved as well as the applicability to
human disease are needed to validate the method as a possible new approach for future immunosuppressive therapy in a subset of patients with virus-negative chronic myocarditis and autoimmune inflammatory cardiomyopathy.

**Conflicts of interest:** None declared.

**References**