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The Role of Viruses, Inflammation and Myocardial Macrophages in the Development of Idiopathic Arrhythmia

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Abstract. We studied viral antigens, inflammation, and macrophages in the endomyocardial biopsies of patients with idiopathic arrhythmias. Immunohistological study was performed to identify the antigens of cardiotropic viruses and the types of lymphocytes and macrophages. We observed the presence of viral antigens in the myocardium of patients with and without histological criteria of myocarditis. Heart failure and ventricular arrhythmias were associated with small focal infiltration of the myocardium with macrophages. The presence of viral antigens in the myocardium was associated with fewer number of myocardial M2 macrophages. Severity of myocardial interstitial fibrosis correlated with small-focal infiltration of M2 macrophages.

INTRODUCTION

The most frequent cause of idiopathic arrhythmias [1, 2, 3] is viral myocarditis. Myocarditis is diagnosed in 34.7% of adult autopsies in cases of sudden death and in 30-40% of myocardial biopsy samples in patients with dilated cardiomyopathy [4]. Persistence of viral genome is associated with cardiac dysfunction [5]. Innate immune system plays an important role in antiviral defense and pathogenesis of inflammation. Macrophages are the most important cells of the innate immune system. However, the role of macrophages in myocardial pathology has not been well defined. Our study focuses on the role of viruses, inflammation and macrophages macrophages in the development of arrhythmias.

MATERIAL AND METHODS

We studied biopsies and medical records of 25 patients aged 44.08±16.47 years with idiopathic arrhythmias. Invasive coronary angiography was performed for exclusion of coronary artery disease. Valvular heart diseases were excluded by echocardiography. Three bioptic samples from each patient have been taken from the right ventricular part of the interventricular septum, right part of the apex, and the outflow tract through the femoral vein approach

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(bioptome BiPAL 7, Cordis Corporation, USA). Endomyocardial samples were fixed in buffered 10%-formalin at room temperature. Light microscopy examination (microscope AxioLab A1, Zeiss) was performed by using formalin-fixed and paraffin-embedded serial sections stained with hematoxylin-eosin, picrofuchsin, toluidine blue and immunohistochemical staining.

For assessment of inflammatory infiltrate, CD45 and CD3 antibodies were used. To detected macrophage phenotype, CD68 (common marker of macrophages) and stabilin-1 (marker of M2 macrophages) antibodies were used [6]. To identify viral antigens, we performed immunohistochemical studies with monoclonal antibodies against VP2 protein of parvovirus B19, VP1 protein of enteroviruses, cytomegalovirus early nuclear protein, LMP antigen of Epstein-Barr virus, and adenovirus and with polyclonal antibodies against capsid antigens of herpes viruses 2 types. HRP-DAB detection system (Spring BioScience) was used for antigen visualization. Morphological verification of myocarditis was based on World Heart Federation Consensus Definition of Inflammatory Cardiomyopathy, 1997 (Tab. 1) [7]. Histopathological analysis was based on semi-quantitative histological criteria, including inflammatory cell types, semi-quantitative assessment of myocyte damage and inflammation and semi-quantitative assessment of fibrosis [8]. The numbers and distribution of CD68+ and stabilin-1+ macrophages were assessed. Statistical analyses were performed with Statistica for Windows 10.0.

TABLE 1. Inflammatory Cardiomyopathy Classification: Grading and Staging

	Parameters	0 [Score]	1 [Score]	2 [Score]	3 [Score]
Grading [Maximum Score 5]	Myocytes Damage	Absent	Focal	Multifocal	
	Interstitial Inflammation	<7 T [cells/mm²]	7 to ≤ 14 T [cells/mm²]	>14 T [cells/mm²]	
	Endocardial Involvement (Inflammation, Thrombosis)	Absent	Present		
Staging [Maximum Score 5]	Interstitial/Replacement Fibrosis	Absent	10 to <20 %	20 to ≤40 %	>40%
	Subendocardial Fibrosis	Absent	Present		
	Endocardial Fibroelastosis	Absent	Present		

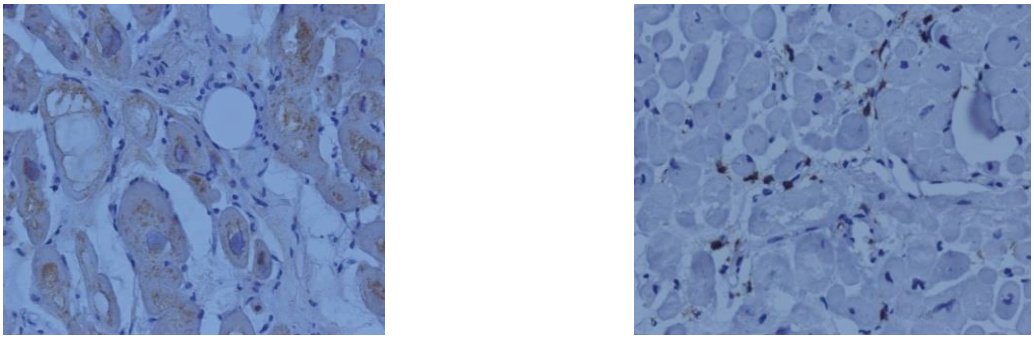
RESULTS

Morphologically verified myocarditis was present in 14 patients. Myocardial fibrosis without inflammation was observed in biopsies of 11 patients. All patients had supraventricular arrhythmias. Holter monitoring recorded ventricular arrhythmias in 4 patients with myocarditis and in 1 patient with myocardial fibrosis. Two patients with myocarditis and 2 patients without myocarditis had heart failure of 2-3 NYHA functional class. Viral antigen expression was detected in the myocardium of 19 patients. Among them, 13 patients had morphological criteria of myocarditis (Fig. 1, 2) and 6 patients had only fibrosis of the myocardium. No viral antigens were found in the biopsies of 1 patient with myocarditis and 5 patients with myocardial fibrosis. CD68+ and stabilin-1+ macrophages were presented in the myocardial biopsies of patients with myocarditis and patients with myocardial fibrosis. Small focal infiltrates of CD68+ macrophages were observed in the myocardium of 35.71% patients with myocarditis and 45.45% patients without it. Stabilin-1+ macrophages formed small focal infiltrates in the myocardium of 21.43% patients with myocarditis and 36.36% patients with myocardial fibrosis (Tab. 2).

In the absence of viral antigens in the myocardium, the fine-focal infiltrates of stabilin-1+ macrophages were found more often than in the presence of viral infection ($R=-0.48$ $p=0.014$). A positive correlation between the numbers of T-lymphocytes and stabilin-1+ macrophages was detected ($R=0.63$ $p=0.021$). Moreover, in patients without myocarditis, the presence of small focal infiltrates of stabilin-1+ macrophages correlated with severity of the interstitial fibrosis ($R=0.63$ $p=0.036$) (Fig. 3).

TABLE 2. Patient's Characteristics

	Patient With Morphological Proven Myocarditis n=14	Patient With Myocardial Fibrosis n=11	All Patients n=25	p
Age [years]	44.08±16.47r	39.43±16,94	45.57±15.89	NS
Supraventricular Arrhythmias	14 [100%]	11 [100%]	25 [100%]	NS
Ventricular Arrhythmias	4 [28.57%]	1 [9.09%]	5 [20%]	NS
Heart failure (NYHA 2-3)	2 [14.29%]	2 [18.18%]	4 [16%]	NS
Morphological Features				
The Presence of Viral Antigens	13 [92.86%]	6 [54.55%]	19 [76%]	NS
The Presence of CD 68+ Macrophages	13 [92.86%]	8 [72.73%]	21 [84%]	NS
Small Focal Infiltration of CD 68+ Macrophages	5 [35.71%]	5 [45.45%]	10 [40%]	NS
The Presence of Stabilin-1+ Macrophages	9 [64.29%]	6 [54.55%]	15 [60%]	NS
Small Focal Infiltration of Stabilin-1+ Macrophages	3 [21.43%]	4 [36.36%]	7 [28%]	NS



(a) (b)

FIGURE 1. The presence of enterovirus VP1 protein in the cytoplasm of cardiomyocytes, x400 (a). Morphologically verified myocarditis: CD45+ lymphocytes infiltration of the myocardium, x400 (b), immunohistochemistry.

(a) (b)

FIGURE 2. The presence of Epstein-Barr LMP antigen in the myocardium (brown staining in the nucleus and cytoplasm) (a). Negative control (b), IHC, x400.

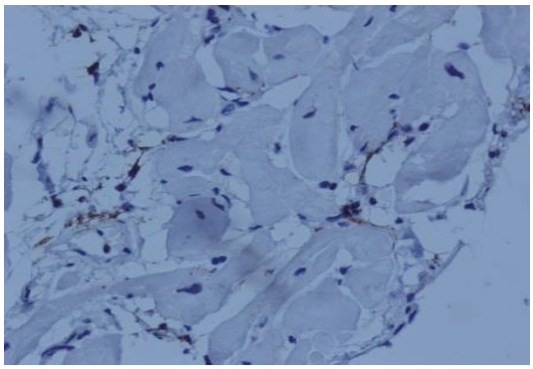


FIGURE 3. Small focal infiltrate of stabilin-1+ macrophages in the fibrosis focus, immunohistochemistry, x400.

Regardless of the presence of myocarditis, small focal infiltrates of CD68+ macrophages were found more often in the myocardium of patients with ventricular arrhythmias and heart failure of II-III functional class (NYHA) (R=0.45 p=0.039; R=0.51 p=0.012, respectively).

CONCLUSION

The presence of viral antigens in the myocardium in the absence of histological criteria of myocarditis suggested the preceding miocarditis. The negative correlation between the presence of viral antigens and small-focal infiltration with M2 macrophages implied that activation of M2 macrophages contributed to elimination of the viruses from the myocardium. The activation of M2 macrophages without myocarditis resulted in the progression of myocardial interstitial fibrosis.

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