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Coxsackievirus B3 infection leads to the generation of cardiac myosin heavy chain-α-reactive CD4 T cells in A/J mice

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Abstract Enteroviruses like coxsackievirus B3 (CVB3) are common suspects in myocarditis/dilated cardiomyopathy patients. Autoimmunity has been proposed as an underlying mechanism, but direct evidence of its role is lacking. To delineate autoimmune response in CVB3 myocarditis, we used IA^k dextramers for cardiac myosin heavy chain (Myhc)-α 334–352. We have demonstrated that myocarditis-susceptible A/J mice infected with CVB3 generate Myhc-α-reactive CD4 T cells and such a repertoire was absent in naïve mice as measured by proliferative response to Myhc-α 334–352 and IA^k dextramer staining. We also detected Myhc-α 334–352 dextramer$^+$ cells in the hearts of CVB3-infected mice. The autoreactive T cell repertoire derived from infected mice contained a high frequency of interleukin-17-producing cells capable of inducing myocarditis in naïve recipients. The data suggest that CVB3, a *bona fide* pathogen of cardiovascular system that

Abbreviations 7-AAD, 7-aminoactinomycin D; ANT, adenine nucleotide translocator; BCKD, branched chain α-ketoacid dehydrogenase; CPE, cytopathic effect; Con-A, concanavalin-A; cpm, counts per minute; CVB3, coxsackievirus B3; DCM, dilated cardiomyopathy; EAM, experimental autoimmune myocarditis; FC, flow cytometry; GM-CSF, granulocyte macrophage-colony stimulating factor; IFN, interferon; IL, interleukin; IRBP, interphotoreceptor retinoid-binding protein; LN, lymph nodes; MHC, major histocompatibility complex; MNC, mononuclear cells; Myhc-α, cardiac myosin heavy chain-α; NASE, neuraminidase; PI, postinfection; PMA, phorbol 12-myristate 13-acetate; RNase, ribonuclease; $TCID_{50}$, 50% tissue culture infective dose; Th, T helper; TNF, tumor necrosis factor; TNI, cardiac troponin I.

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primarily infects the heart can lead to the secondary generation of autoreactive T cells and contribute to cardiac pathology.

1. Introduction

Heart failure is the leading cause of death in the United States. It is estimated that ~80 million American adults have some form of cardiovascular disease, with a projected incidence of 40% by 2030 [1]. The potential causes of heart failure include pericardial, coronary artery, or heart valve diseases, idiopathic cardiomyopathy, chronic ischemia, and myocarditis.

Myocarditis is an inflammation of the myocardium, but only ~10% of those affected show clinical manifestations of the disease [2]. Nonetheless, myocarditis is still regarded as an important cause of heart failure in children and adolescents, particularly athletes, indicating that a low degree of inflammation could be present in the hearts of apparently healthy individuals. This hypothesis was supported by a study of autopsies in more than 12,000 accidental deaths, which showed evidence of lymphocytic myocarditis in 1.06% of individuals [3], raising a question as to the trigger of inflammation in heart tissues. Furthermore, as a sequel to myocarditis, affected individuals can develop dilated cardiomyopathy (DCM) [4]. Approximately half of patients with DCM undergo heart transplantations due to the lack of effective chemotherapy. Enteroviruses are commonly suspected in DCM patients because the genomic material can be detected in up to 70% of patients, and serologically, virus-reactive neutralizing antibodies can be found in ~50% of patients [2]. The question to be addressed is how virus infection can promote DCM. Autoimmunity is one possible mechanism, as evidenced in patients with myocarditis/DCM by the presence of autoantibodies to various cardiac antigens, such as cardiac myosin heavy chain (Myhc)-α, adenine nucleotide translocator (ANT), β-adrenergic receptor-1, branched chain α-ketoacid dehydrogenase (BCKD), laminin, and muscarinic receptor; Myhc-α is a well-characterized antigen [5,6].

Based on cellular infiltrations, forms of myocarditis have been classified as lymphocytic, giant cell, and eosinophilic, and various infectious (viruses, bacteria, protozoa, helminthes) and noninfectious (drugs, metals, chemicals) agents have been implicated in the causation of myocarditis. Prominent among the infectious causes are viruses, importantly enteroviruses like coxsackievirus B3 (CVB3). To study the immune events of myocardial injuries in CVB3 infection, various rodent models have been developed. For example, the virus induces myocarditis in susceptible strains of A/J (H-2^a) and Balb/C (H-2^d) mice, the histologic features of which resemble those in human disease [7]. The disease course has two distinct stages that occur in continuum: the acute phase, in which infectious virus is present causing damage to cardiac myocytes; and the chronic phase, in which inflammation persists, although the extent of virus replication is much reduced due to selection of a defective virus [8,9]. This is consistent with the observation that infectious CVB3 cannot be isolated in cardiac tissues from patients with DCM [2,10]. The long-standing question is whether this inflammatory process occurs due to an autoimmune response to cardiac antigens. Importantly, the same strains of mice that are susceptible to chronic myocarditis and DCM after viral infection also are susceptible to disease induction by immunization with Myhc-α. In contrast, the strains that develop only limited acute myocarditis are resistant to Myhc-α-induced disease [11]. In addition, susceptible mice made tolerant to Myhc-α fail to develop myocarditis after viral infection [12]. It was previously shown that lymphocytes isolated on day 7 postinfection (PI) from Balb/C mice infected with CVB3 were lytic to cardiomyocytes, but their putative target antigens were not characterized [13,14]. Furthermore, these cytotoxic lymphocytes induced the disease in naïve mice infected with or without CVB3 prior to the transfer [14,15]. While these observations point to a role for autoreactive T cells in the mediation of CVB3 myocarditis, it is critical to delineate both the mechanisms of their generation and their target antigens in cardiac tissues.

With the establishment of experimental autoimmune myocarditis (EAM) disease models induced with various cardiac antigens, it is now generally accepted that autoreactive CD4 T helper (Th) cells play a pivotal role in the mediation of autoimmune myocarditis [16]. The relevance of autoreactive CD4 T cells is further emphasized by the fact that histologic features of EAM closely mimic those of postinfectious myocarditis induced with CVB3 [6,8]. Therefore, to study the role of autoreactive CD4 T cells in the occurrence of CVB3 myocarditis, we recently created major histocompatibility complex (MHC) class II/IA^k dextramers for Myhc-α 334–352, which permitted us to enumerate the frequencies of antigen-specific T cells in infected mice. Using these reagents, we demonstrated that A/J mice infected with CVB3 develop myocarditis accompanied with the generation of Myhc-α-specific CD4 T cells and they infiltrate into the hearts of infected animals. We noted that the autoreactive T cells obtained from CVB3-infected mice contain a significant proportion of interleukin (IL)-17-producing cells capable of inducing myocarditis in naïve mice.

2. Materials and methods

2.1. Mice

Six-to-eight-week-old A/J and B10.A mice were procured from the Jackson Laboratory (Bar Harbor, ME). The mice were maintained according to the animal protocol guidelines of the University of Nebraska-Lincoln, Lincoln, NE.