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HDAC11 regulates interleukin-13 expression in CD4+ T cells in the heart

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ABSTRACT

Background and aims: Immune deregulation is a causative factor in pathogenesis of myocarditis. Histone deacetylases (HDAC) involve multiple biochemical activities in the cell. This study aims to elucidate the role of HDAC11 in the regulation of interleukin (IL)-13-expression in $CD4^+$ T cells of heart tissue in patients with myocarditis (MCD).

Methods: After heart transplantation, surgically removed hearts were collected from patients with advanced heart failure and MCD or dilated cardiomyopathy (DCM). $CD4^+$ T cells were isolated from the heart samples and analyzed by immune assay. The association between IL-13 over production by $CD4^+$ T cells in heart tissue and the pathogenesis of MCD was analyzed.

Results: T helper (Th) 2-biased inflammation was observed in hearts tissue of MCD patients with advanced heart failure. $CD4^+$ T cells isolated from MCD heart tissue showed lower levels of HDAC11 expression than that isolated from DCM heart tissue. HDAC11 was negatively correlated with IL-13 expression in the $CD4^+$ T cells. A complex of HDAC11 and E4 binding protein-4 (E4BP4; the transcription factor of *IL13*) was detected in the $CD4^+$ T cells, which restricted the binding between E4BP4 and the *Il13* promoter to repress the *Il13* gene transcription. Reconstitution of HDAC11 in MCD $CD4^+$ T cells reduced the expression of IL-13, while inhibition of HDAC11 in DCM $CD4^+$ T cells increased the IL-13 expression.

Conclusions: HDAC11 is a regulatory molecule in Th2 response and plays a critical role in the restriction of the biased IL-13 expression in $CD4^+$ T cells of the heart.

1. Introduction

Myocarditis, the inflammation of the heart muscle, can develop into dilated cardiomyopathy, cardio arrest and heart failure. The prevalence of myocarditis is unknown. It was found that about 1–9% of all patients had evidence of myocardial inflammation in a series of routine autopsies and up to 20% of all cases of sudden death are due to myocarditis [1]. Myocarditis may be primarily induced by viral infection, bacterial infection, or autoimmunity; it may be also secondary to other causes, such as heart attack. The treatments of myocarditis mainly include systemic administration of corticosteroids. Digoxin and diuretics may be used to control cardiac symptoms. Inotropes or angiotensin-converting-enzyme (ACE) inhibitors may be used in the cardiac severe dysfunction. To date, the treatment of myocarditis is unsatisfactory. The pathogenesis of myocarditis is not fully understood yet.

Using the myosin heavy chain as the specific antigen can induce myocarditis-like cardiomyopathy in animals [2]. Clinical studies indicate that administration with immune suppressors can better control myocarditis [3]. The fact suggests that autoimmunity plays an important role in the pathogenesis of myocarditis [4]. Autoimmunity is the system of immune responses against its own cells and tissues. Thus, it is accepted that at least a portion of myocarditis is resulted from autoimmunity and can be called autoimmune myocarditis [4]. The pathological features of autoimmune response is that the aberrant aggregation of immune cells in the local tissues; these immune cells highly produce proinflammatory cytokines, such as the Th2 cytokines [5], tumor necrosis factor [6] and interleukin (IL)-17 [7], to cause injury in the local tissues [8, 9]. Yet, mechanisms causing the aberrant high production of proinflammatory cytokines in heart tissue during myocarditis are not fully understood.

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Skewed cytokine production by immune cells in the heart tissue is an important factor in the pathogenesis of myocarditis [10]. IL-13 is a member of the Th2 cytokines; it plays a critical role in the pathogenesis of Th2-biased inflammation [11]. Besides Th2 cells, IL-13 can also be produced by Th1 cells, eosinophils, basophils, and natural killer T cells [12]. It was speculated that the transcription pathway of IL-13 was the same as IL-4. However, published data show the production of IL-13 can occur together with the Th1 cytokine interferon (IFN)-γ in asthma patients [13], indicating that alternative pathways exist for the expression of IL-13. Recent studies indicate that the transcription factor E4-binding protein 4 (E4BP4) can initiate the *Il13* gene transcription [12]. E4BP4 is also called nuclear factor interleukin-3-regulated (NFIL3) and can be induced by circadian clock [14], glucocorticoids, insulin, and cAMP in various cell types and tissues [15], while whether the activation of E4BP4-induced IL-13 production plays a role in myocarditis has not been investigated.

Gene transcription is a key procedure in any cytokine production. Overproduction of proinflammatory cytokines in myocarditis indicates that gene transcription of these cytokines may be highly activated. Histone acetylation and deacetylation at gene promoter loci of certain cytokines play a key role in the process of cytokine gene transcription; this event is tightly regulated by a group of enzymes, including histone acetyltransferases and histone deacetylases (HDAC). The HDACs are involved in the regulation of both innate immunity and adaptive immunity [16]. It was reported that the aberrant HDAC activities were found in myocarditis [17]. E4BP4 is involved in the induction of immune inflammation [14]. E4BP4 can initiate the *Il13* gene transcription [12]. Whether HDACs and E4BP4 acetylation are involved in the modulation of proinflammatory cytokine, such as IL-13, production during myocarditis remains to be further investigated.

Based on the information above, we hypothesize that the aberrant activities of HDACs promote the production of IL-13 in the heart, which may be association with the pathogenesis of myocarditis. To test this, we collected surgically removed myocarditis hearts after heart transplantation. The heart tissues were analyzed to elucidate the role of HDACs in the regulation of $CD4^+$ T cell activities in the heart. The results showed that the expression of HDAC11 was markedly lower in $CD4^+$ T cells of myocarditis hearts. The insufficient expression of HDAC11 resulted in highly IL-13 expression in heart $CD4^+$ T cells. Reconstitution of HDAC11 expression restored the homeostasis of IL-13 expression in $CD4^+$ T cells of the myocarditis heart.

2. Materials and methods

2.1. Reagents

The HDAC11 shRNA kit, antibodies of HDAC11 (C-5), E4BP4 (A-9), acetylated histone (acH)3 (AH3-120, for Lys 9), acH4 (B-10), RNA polymerase II (Pol II; B6-1) and ac Lysine (B-10) were purchased from Santa Cruz Biotech (Santa Cruz, CA). The ELISA kits of IL-4 (C050), IL-5 (C159), IL-13 (C088), IFN-γ (RGP010R) and anti-myosin IgG (EBRK1265H1) were purchased from the Biomart (Beijing, China). The immune cell isolated kits were purchased from Miltenyi Biotech (San Diego, CA). The reagents for RT-qPCR, Western blotting, gene transfection were purchased from Invitrogen (Carlsbad, CA). The reagents for immunoprecipitation (IP) and chromatin IP were purchased from Sigma Aldrich (St. Louis., MO).

2.2. Heart tissue collection

The hearts were collected from 12 patients with myocarditis and advanced heart failure undergone heart transplantation (MCD heart, in short). Patients with any of the following conditions were excluded: Allergic diseases; autoimmune diseases; using steroids or other immune suppressors and other severe organ diseases. Ten hearts with dilated cardiomyopathy (DCM) at the advanced heart failure were collected from the heart transplantation surgery and used as controls; these patients did not have records of myocarditis. The diagnosis of MCD and DCM was carried out by our doctors. The demographic data are presented in Table 1. The using human tissue in the present study was approved by the Human Ethics Committee at Beijing Fuwai Hospital. An informed written consent was obtained from each human subject with heart transplantation. For the ethic reason, heart tissue from healthy subjects was not used in the present study.

Table 1
Demographic data of the patients.

Baseline characteristics of the MCD and DCM patients		DCM group
Characteristics	MCD group	
Number	12	10
Demography		
Age (year)	34.4 ± 15.5	38.6 ± 10.2
Male (no. %)	9 (75%)	5(50%)
Body-mass index	20.4 ± 3.2	20.8 ± 5.5
Clinical characteristics		
Diagnosis to transplantation (months)	52.0 ± 44.7	46.0 ± 20.8
SBP (mMCDg)	99.6 ± 8.8	108.6 ± 23.8
Heart rate	80.6 ± 17.4	90.5 ± 32.8
Medication history		
Antiplatelet	3 (25%)	4(40%)
Digoxin	10 (83.3%)	10 (100%)
ACEI/ARB	4 (33.3%)	8(80%)
Diuretic	12 (100%)	120(100%)
Beta blockers	10 (83.3%)	10 (100%)
Antiarrhythmic	4 (33.3%)	6(60%)
Cardiac function		
I	0	0
II	2 (16.7%)	1(10%)
III	2 (16.7%)	0
IV	8 (66.7%)	9(90%)
Hypertension	1 (8.3%)	3(30%)
Diabetes	0	4(40%)
Hyperlipidemia	1 (8.3%)	3(30%)
ECG		
AF	5 (41.7%)	6(60%)
LBBB	0	2(20%)
RBBB	3 (25%)	4(40%)
Paroxysmal ventricular tachycardia	5 (41.7%)	3(30%)
UCG		
Left atrium diameter (mm)	46.6 ± 11.5	53.5 ± 16.9
LVEDD (mm)	70.4 ± 14.9	67.4 ± 10.8
EF (%)	30.6 ± 14.7	29.6 ± 10.7
Moderately to severe MR (%)	9 (75%)	8 (80%)

ECG: electrocardiogram; AF: atrial fibrillation; LBBB: left bundle branch block; RBBB: right bundle branch block; LVEDD: Left ventricular end diastolic diameter; EF: Ejection fraction; MR: Mitral regurgitation.

2.3. Histology

The heart tissue was fixed with 4% formalin overnight. The tissues were processed for paraffin sections. The sections were stained with hematoxylin and eosin and observed with a light microscope.

2.4. Isolation of mononuclear cells from heart tissue

The heart tissue was cut into small pieces (2 × 2 × 2 mm) and incubated with collagenase IV (1 mg/ml) for 2 h at 37 °C with mild agitation. Single cells were passed through cell strainers (100 μm first, then 70 μm) and collected by centrifugation. The mononuclear cells were isolated from the single cells by Percoll gradient density centrifugation. The cells were cultured in RPMI1640 medium overnight and then handed over further experiments.