ISSN: 0976-3104
REGULAR ISSUE
Li et al.

THE IIOAB JOURNAL

REVIEW ARTICLE OPEN ACCESS

EVIDENCES OF INTERACTION BETWEEN PANCREAS, HEART AND SPLEEN IN THE PATHOGENESIS OF COXSACKIEVIRUS CARDIOMYOPATHY

Minghui Li, Yeqing Xie, Xinggang Wang, Yunzeng Zou, Junbo Ge, Ruizhen Chen*

Key Laboratory of Viral Heart Diseases, Ministry of Public Health, Shanghai Institute of Cardiovascular Diseases, Zhongshan Hospital, Fudan University, Shanghai-200032, CHINA

ABSTRACT

Pathogenesis of coxsackievirus cardiomyopathy has not yet been clarified. Evidences from clinical practices and laboratory researches have hinted a pathologic association among organs of pancreas, spleen and heart, underlying a probable new pathogenesis. We summarized these evidences here and proposed the probable mechanism, which might bring about some changes to the diagnosis and treatment of viral cardiomyopathy.

Received on: 1st-Feb-2012
Revised on: 16th-Feb-2012
Accepted on: 22nd-Feb-2012
Published on: 10th-Apr-2012

KEY WORDS

coxsackievirus, pathogenesis, cardiomyopathy, pancreas, spleen

***Corresponding author:** Email: chen.ruizhen@zs-hospital.sh; Tel: 86-21-64041990 ext. 2104; Fax: 86-21-64223006

[I] INTRODUCTION

Cardiomyopathy is a major cause of sudden unexpected death in young patients, it contributes significantly to the societal burden of heart failure. Now significant evidences from animal models and clinical studies suggests that viral myocarditis is important in the etiology of dilated cardiomyopathy. Enterovirus, and especially Coxsackieviruses of group B (CVB), is the most common infectious agent [1, 2].

Enteroviruses (family Picornaviridae) are nonenveloped icosahedral viruses that contain a single plus-strand RNA genome of about 7,500bp. The CVB are typical enteroviruses and 5' end of the genome is not capped but is linked covalently to the viral protein, VPg. The virus receptor, human coxsackievirus and adenovirus receptor (CAR), a protein of the immunoglobulin superfamily [3-5], most likely interacts with virus capsid in the depression that surrounds the 5-fold axes of symmetry.

In clinical, progression of coxsackievirus myocarditis to dilated cardiomyopathy has always been a deteriorating outcome which usually resorts to heart transplant [1]. Pathogenesis of coxsackievirus cardiomyopathy hasn't yet been clarified and the diagnosis is conventionally based on clinical presentations [2]. Although endomyocardium biopsy (EMB) evidences have been the gold standard, this Dallas criteria has been questioned in practice [6, 7]. New pathogenesis viewpoint may help in diagnosis and treatment of the disease. Based on current evidences of a pathologic association between pancreas, spleen and heart, such a viewpoint is proposed as a hypothesis in this review.

[II]COEXISTENCE OF PANCREAS DISEASES AND MYOCARDIAL INJURY IN CLINICAL PRACTICE

The phenomenon of myocardial injury accompanying pancreas diseases has been noticed for a long time in clinical practice. A wide spectrum of the related pancreas diseases have been mentioned including cystic fibrosis [8], pancreas carcinoma [9] and acute pancreatitis caused by toxic substances [10]. A large sample autopsy in children covering 2,000 cases carried out by Nezelof C et al. [11] found that children's pancreatic diseases were usually associated with multifocal myocardial necrosis and fibrosis, in which the pancreatic diseases include cystic fibrosis, pancreatic lipomatosis and extensive small bowel resection.
Pancreas injuries resulting from various reasons usually accompany heart inflammation. This is also the case with regard to coxsackievirus cardiomyopathy.

[III] EXISTENCE OF A PATHOLOGIC ASSOCIATION BETWEEN PANCREAS AND HEART WITH REGARD TO COXSACKIEVIRUS

THE IIOAB JOURNAL
ISSN: 0976-3104

INFECTION

Among the various causative agents of cardiomyopathy, enterovirus infection, especially coxsackievirus infection is the most common reason [2]. Coincidentally, coxsackievirus infection of the heart is often accompanied by pancreas infection. In 1998, an outbreak of enterovirus 71 infection was evoked in Taiwan, a comprehensive study on death cases revealed that hearts and pancreas suffered infection at the same time [12]. A case report of neonate death in Germany in 2006 was attributed eventually to enterovirus myocarditis complicated by pancreatitis [13]. Early in the 1970's, it has been found that coxsackievirus infection could lead to myocarditis and necrosis of the endocrine part of pancreas causing the simultaneous secondary diabetes [14]. An autopsy research [15] found that enterovirus infection, while causing the onset of myocarditis, almost simultaneously led to the inflammation of pancreas. These evidences are all implying a correlation between pancreas diseases and myocardium injury in the background of virus infection.

Such a correlation was also observed in animals. Gómez RM et al. [16] infected Balb/c mouse with coxsackievirus B3 and 1 week post infection all mouse were found pancreas glandular duct inflammation and focal myocarditis. Such organ injuries were restricted in pancreas and heart selectively and was absent in other organs like liver etc. This "targeted-organ-injury" property of coxsackievirus was attributed to at least one important cell structure component- coxsackie and adenovirus receptor (CAR) [17, 18]. After the depletion of CAR from pancreas and heart, coxsackievirus titers decreased significantly both in vitro and in vivo and the related organs injury ameliorated as well[19]. Besides coxsackievirus, another myocarditis-causing agent encephalomyocarditis virus (EMV) could also injury pancreas and lead to the secondary diabetes [20].

Mouse infected with fatal doses of CVB3 after expressing IFNγ in pancreas via transgenic method survived without suffering myocarditis [21]. Suppressing the replication of virus in the pancreas prevented the onset of myocarditis. There must be some connection between pancreas and heart pathologically. Tracy et al. [22] used 8 different strains of CVB3 to infect mouse respectively, and 3 strains induced myocarditis. All strains replicated and persisted in pancreas 8 days post infection, but the cardiotropic strains of CVB3 tended to reach a higher titer level in the early phase and persist longer in serum, pancreas and heart than the non-cardiotropic strains.

From the above evidences, we could see that pancreas diseases and myocardium injuries associated with each other.

[IV] PANCREAS: RESERVOIR OF COXSACKIEVIRUS COMPARING WITH HEART

A study [23] focusing on coxsackievirus B 5's injury to mouse organs revealed that virus clearance rate was slow in both pancreas and heart than in liver. And virus RNA reached to peak value more earlier in pancreas than in heart (2.5 days V.S. 4 days), and the pathologic injury was also more serious (acute pancreatitis V.S. marginal myocarditis). Cheung et al. [24] found that as for the mouse 4 weeks and 8 weeks post infection, heart and pancreas were the most seriously injured organs, which implied the susceptibility to CVB5 infection of both organs. However, along with age growing susceptibility of the heart tissue decreased gradually. "Comparatively, susceptibility of the pancreas exocrine part to the virus didn't change. Pancreas is vulnerable to virus infection and favors virus replication. This is true not only for the enteroviruses but also for the encephalomyocarditis virus (EMV), which was supported by the fact that 28 days post EMV infection virus antigen could still be detected in the pancreas and pancreas suffered from chronic obstructive pancreatitis, but the inflammation focuses were almost healed completely except for some virus antigen detected in the valves.

Various recombinant CVBs had been used to clarify the pathogenesis of viral myocarditis. Henke et al. [25] designed a recombinant CVB3 expressing IFNγ and IL10. This recombinant virus could infect mouse, but its appearance is only restricted in pancreas rather than heart tissue. Pancreas seemed to be friendlier to CVB instinctively. Similarly, Slifka et al. [26] inserted one of the cytotoxic T-lymphocyte (CTL) epitopes from lymphocytic choriomeningitis virus (LCMV) into the genome of CVB3 and infected wild type neonate mouse and LCMV immunized mouse"respectively with this recombinant virus. The authors found that the latter displayed significant anti-virus effect with a 50 fold decrease of virus titer in the heart but only 6 fold in the pancreas. Besides, the authors found that the inserted epitope of this recombinant virus disappeared in vivo organ specifically with only 0～1.8% maintained in heart and rather more in pancreas. Although the authors addressed the immune pathogenesis of virus infection, these data emphasized on the other hand that pancreas is more vulnerable to CVB3 than heart and more compatible with the invaded virus. Another experiment using a recombinant CVB3 (CVB3-PL2-Ad2L1) [27] concluded that the recombinant coxsackievirus evoked viremia without inducing significant pathological changes in pancreas and heart, but in the pancreas rather than heart the authors found the persistent replication of this virus. This also indicated that pancreas seemed to be friendlier than heart to CVBs. Pancreas tends to be a reservoir of CVBs.

Why pancreas is seemed to be friendlier than heart to CVBs? The mechanism has not yet been clarified. Evidence hinted that it might be associated with the pattern pancreas activates the immune response. Vella et al. [28] studied pancreas sensitivity of 8 different mouse genotypes to CVB4 and found that the innate immunity, represented by NK cells, could inhibit virus replication in the early phase while the humoral response in the late phase failed inhibiting the virus replication. Further research found an inability of pancreatic

THE IIOAB JOURNAL
ISSN: 0976-3104

acinar cells to express the MHC I molecules. So it seemed that the comparative inability of the pancreas immunity pattern determines its compatibility with CVBs. Besides, low doses of virus infection of NK cell-depleted C3H/HeJ mouse led to pancreatitis in all mouse but myocarditis only in one case, which also implied a more vulnerable property of pancreas than heart.

Summarily, pancreas' susceptibility to coxsackievirus and compatibility with its proliferation seemed to be an intrinsic property determined by some mechanisms not yet clarified. The above evidences direct us to regard pancreas as a reservoir of CVBs.

[V] THE HEART IS MORE VULNERABLE THAN THE PANCREAS TO THE IMMUNOPATHOLOGIC INJURY AROUSED BY COXSACKIEVIRUS INFECTION

In the late phase of cardiomyopathy, viral genome may persist in many cases [29]. However the deterioration of heart function couldn't merely be explained by direct injury effect of virus and brought out the mechanism of pathologic immunity [30, 31]. Comparing with pancreas, heart is more vulnerable to pathologic immunity attack with regard to CVB infection. And related mechanisms have been summarized in a serious of reviews elsewhere [32-36].

One of the mechanisms we focused here is the autoimmunity mechanism, which is still controversial. Although some investigator questioned the role of autoimmunity in the pathogenesis coxsackievirus cardiomyopathy [32], heart reactive autoantibodies indeed exist like autoantibodies to β1 receptor [37], muscarinic acetylcholine receptor-2 [38], α-and β-cardiac myosin heavy chain [39] and ADP/ATP carrier (adenine nucleotide translocator, ANT) [40].One of the mechanisms involved in the production of these autoantibodies is antigenic mimicry [34]. Some CVB protein components are structurally similar with related heart proteins. For example, the amino acid residues of ANT protein on 27-36 position are homologous to that of CVB3 capsid protein on 1218-1228 position [41].

Immune cells' intracellular signal transduction might also be associated with susceptibility of heart to myocarditis [42]. Inhibiting the NFAT in CD4+ T cells could prevent the onset of myocarditis but not pancreatitis. Virus titers in wild type mouse and NFAT-inhibited mouse were in the same level in pancreas but increased in NFAT-inhibited mouse's heart, indicating the difference of these two organs' responses to virus infection, in which pancreas' injury seemed not owning to immune factors like the heart was involved.

[VI] COMPROMISE OF SPLEEN B CELLS WITH COXSACKIEVIRUS

Basically, B cell humoral immunity plays a dual role in the injury of coxsackievirus to heart. On one hand, humoral immunity plays a certain role in defense against virus infection. Agamaglobulinemia patients are most vulnerable to coxsackievirus infection because of inability to produce antibody due to abnormal genes [43]. Despite B cells' lacking CARs on membrane surface, researches [44, 45] found that CVBs could infect spleen B cells in a passive way of been phagocytized in the procedure of antigen processing and presentation.

On the other hand, humoral immunity could facilitate the dissemination and replication of the infected CVBs [46, 47]. In mouse whose B cells were knocked out, virus dissemination and replication was delayed and chronic infection were observed in various organs including heart, liver, brain, kidney, lung, pancreas and spleen, which demonstrate B cells' roles in facilitating CVB replication and dissemination besides their killing effect[44].

Considering the compatibility of pancreas with CVBs and vulnerability of heart to pathologic immune injuries, such a compromise between B cells and CVBs might promote the formation of a vicious circle.

[VII] SUMMARY AND HYPOTHESIS: CAUSE AND EFFECT CHAIN OF "PANCREAS-SPLEEN-HEART"

In summary **[Figure–1]**, we hypothize that coxsackievirus invade the digestive tract, infect pancreas, and invade myocardium via viremia causing myocarditis and pancreatitis. After the acute phase, virus in the heart are cleared while still persist in pancreas. Anatomically adjoining, immune cells in spleen, especially B cells, are activated continually or at intervals by virus or virus components from pancreas, which results in synthesis and release of anti-virus products like antibodies to the peripheral circulation. Due to the similarity of heart structure proteins to virus epitopes, heart tissue is injured simultaneously. Besides, compatibility of pancreas with virus keeps on the above malignant events and forms the vicious cycle in specific individuals causing the progression of myocarditis to cardiomyopathy eventually. In short, cause and effect chain of "pancreas virus infection- spleen immunity activation- heart tissue pathologic immune injury" finally leads to the irreversible heart insufficiency.

THE IIOAB JOURNAL
ISSN: 0976-3104

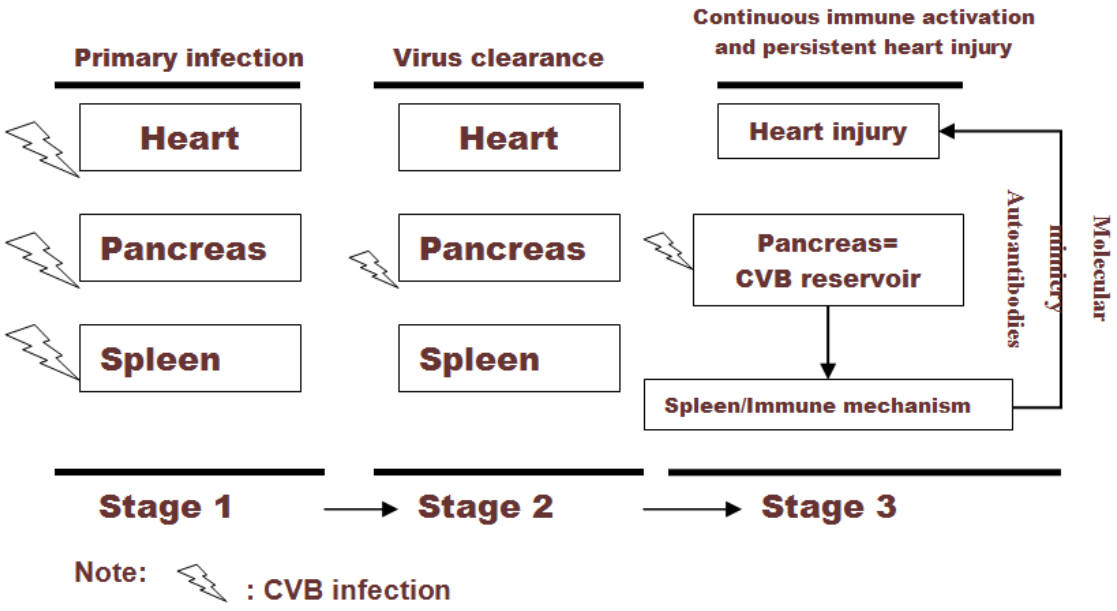

Fig: 1. Probable mechanism of interaction between heart, pancreas and spleen causes continuous heart injury after virus clearance in the heart via immune mechanisms. At stage 1, heart, pancreas and spleen are all infected by CVBs. Immue system is evoked and viruses are cleared at namely stage 2 completely in heart and spleen and only partially in pancreas. CVBs incubate in pancreas for a certain period and active immune system represented by spleen functions continuously or at intervals at stage 3. Via molecular mimicry mechanism, autoantibodies to heart proteins are also produced continuously or at intervals and cause persistent heart injury leading to the evolvement of myocarditis to cardiomyopathy.

FINANCIAL DISCLOSURE

This project was supported by grants from National Natural Science Foundation of China (No. 31070786), International Cooperation and Exchanges NSFC (No.81010007) and Research Fund for the Doctoral Program of Higher Education of China (No.20090071110076).

CONFLICTS OF INTEREST

All authors declare that we have no conflicts of interest.

REFERENCES

[1] Cooper LT Jr. Myocarditis. [2009] *N Engl J Med* 360(15):1526–1538.
[2] Schultz JC, Hilliard AA, Cooper LT Jr, Rihal CS. [2009] Diagnosis and treatment of viral myocarditis. *Mayo Clin Proc* 84(11):1001–1009.
[3] Kim DS, Nam JH. [2011] Application of attenuated coxsackievirus B3 as a viral vector system for vaccines and gene therapy. *Hum Vaccine* 7(4):410–416.
[4] Pan J, Narayanan B, Shah S, Yoder JD, Cifuente JO, et al. [2011]Single amino acid changes in the virus capsid permit coxsackievirus B3 to bind decay-accelerating factor. *J Virol* 85(14):7436–7443.
[5] Carson SD, Chapman NM, Hafenstein S, Tracy S. [2011] Variations of coxsackievirus B3 capsid primary structure, ligands, and stability are selected for in a coxsackievirus and adenovirus receptor-limited environment. *J Virol* 85(7):3306–3314.
[6] Hauck AJ, Kearney DL, Edwards WD. [1989] Evaluation of postmortem endomyocardial biopsy specimens from 38 patients with lymphocytic myocarditis: implications for role of sampling error. *Mayo Clin Proc* 64(10):1235–1245
[7] Baughman KL. [2006] Diagnosis of myocarditis: death of Dallas criteria. *Circulation* 113(4):593–595.
[8] Oppenheimer EH, Esterly JR. [1973] Myocardial lesions in patients with cystic fibrosis of the pancreas. *Johns Hopkins Med J* 133(5):252–261.
[9] Williams JO, Pollitzer RS, Green HD. [1952] Acute interstitial myocarditis associated with carcinoma of the body of the pancreas; report of a case. *N C Med J* 13(3):147–150.
[10] Verma SK, Ahmad S, Shirazi N, Barthwal SP, Khurana D, et al. [2007] Acute pancreatitis: a lesser-known complication of aluminum phosphide poisoning. *Hum Exp Toxicol* 26(12):979–981.
[11] Nezelof C, LeSec G. [1979] Multifocal myocardial necrosis and fibrosis in pancreatic diseases of children. *Pediatrics* 63(3):361–368.
[12] Yan JJ, Wang JR, Liu CC, Yang HB, Su IJ. [2000]An outbreak of enterovirus 71 infection in Taiwan 1998: a comprehensive pathological, virological, and molecular study on a case of fulminant encephalitis. *J Clin Virol* 17(1):13–22.
[13] Dettmeyer RB, Padosch SA, Madea B. [2006] Lethal enterovirus-induced myocarditis and pancreatitis in a 4-month-old boy. *Forensic Sci Int* 156(1):51–54.
[14] Foulis AK, McGill M, Farquharson MA, Hilton DA. [1997] A search for evidence of viral infection in pancreases of newly diagnosed patients with IDDM. *Diabetologia* 40(1):53–61.
[15] Gladisch R, Hofmann W, Waldherr R. [1976] Myocarditis and insulitis following coxsackie virus infection. *Z Kardiol* 65(10):837–849.
[16] Gómez RM, Lopez Costa JJ, Pecci Saavedra G, Berria MI. [1993] Ultrastructural study of cell injury induced by coxsackievirus B3 in pancreatic and cardiac tissues. *Medicina (B Aires)* 53(4):300–306.
[17] Raschperger E, Thyberg J, Pettersson S, Philipson L, Fuxe J, Pettersson RF. [2006] The coxsackie- and adenovirus receptor

(CAR) is an in vivo marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis. *Exp Cell Res* 312(9):1566–1580.

[18] Ashbourne Excoffon KJ, Moninger T, Zabner J. [2003] The coxsackie B virus and adenovirus receptor resides in a distinct membrane microdomain. *J Virol* 77(4):2559–2567.

[19] Kallewaard NL, Zhang L, Chen JW, Guttenberg M, Sanchez MD, Bergelson JM. [2009] Tissue-specific deletion of the coxsackievirus and adenovirus receptor protects mice from virus-induced pancreatitis and myocarditis. *Cell Host Microbe* 6(1):91–98.

[20] Ujevich MM, Jaffe R. [1980] Pancreatic islet cell damage. Its occurrence in neonatal Coxsackievirus encephalomyocarditis. *Arch Pathol Lab Med* 104(8):438–441.

[21] Horwitz MS, La Cava A, Fine C, Rodriguez E, Ilic A, Sarvetnick N. [2000] Pancreatic expression of interferon-gamma protects mice from lethal coxsackievirus B3 infection and subsequent myocarditis. *Nat Med* 6(6):693–697.

[22] Tracy S, Höfling K, Pirruccello S, Lane PH, Reyna SM, Gauntt CJ. [2000]Group B coxsackievirus myocarditis and pancreatitis: connection between viral virulence phenotypes in mice. *J Med Virol* 62(1):70–81.

[23] Moon MS, Joo CH, Hwang IS, Ye JS, Jun EJ, et al. [2005] Distribution of viral RNA in mouse tissues during acute phase of Coxsackievirus B5 infection. *Intervirology* 48(2-3):153–160.

[24] Cheung PK, Yuan J, Zhang HM, Chau D, Yanagawa B,et al. [2005] Specific interactions of mouse organ proteins with the 5'untranslated region of coxsackievirus B3: potential determinants of viral tissue tropism. *J Med Virol* 77(3):414–424.

[25] Henke A, Zell R, Ehrlich G, Stelzner A. [2001] Expression of immunoregulatory cytokines by recombinant coxsackievirus B3 variants confers protection against virus-caused myocarditis. *J Virol* 75(17):8187–8194.

[26] Slifka MK, Pagarigan R, Mena I, Feuer R, Whitton JL. [2001] Using recombinant coxsackievirus B3 to evaluate the induction and protective efficacy of CD8+ T cells during picornavirus infection. *J Virol* 75(5):2377–2387.

[27] Höfling K, Tracy S, Chapman N, Kim KS, Smith Leser J. [2000] Expression of an antigenic adenovirus epitope in a group B coxsackievirus. *J Virol* 74(10):4570-4578.

[28] Vella C, Festenstein H. [1992] Coxsackievirus B4 infection of the mouse pancreas: the role of natural killer cells in the control of virus replication and resistance to infection. *J Gen Virol* 73:1379–1386.

[29] Chapman NM, Kim KS. [2008] Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. *Curr Top Microbiol Immunol* 323:275–292.

[30] Olson JK, Croxford JL, Miller SD. [2001] Virus-induced autoimmunity: potential role of viruses in initiation, perpetuation, and progression of T-cell-mediated autoimmune disease. *Viral Immunol* 14(3):227–250.

[31] Richer MJ, Horwitz MS. [2008] Viral infections in the pathogenesis of autoimmune diseases: focus on type 1 diabetes. *Front Biosci* 13:4241–4257.

[32] Fujinami RS, von Herrath MG, Christen U, Whitton JL. [2006] Molecular mimicry, bystander activation, or viral persistence: infections and autoimmune disease. *Clin Microbiol Rev* 19(1):80–94.

[33] Horwitz MS, Sarvetnick N. [1999] Viruses, host responses, and autoimmunity. *Immunol Rev* 169:241–253.

[34] Münz C, Lünemann JD, Getts MT, Miller SD. [2009] Antiviral immune responses: triggers of or triggered by autoimmunity? *Nat Rev Immunol* 9(4):246–258.

[35] Olson JK, Ercolini AM, Miller SD. [2005] A virus-induced molecular mimicry model of multiple sclerosis. *Curr Top Microbiol Immunol* 296:39–53.

[36] Olson JK, Croxford JL, Miller SD. [2001] Virus-induced autoimmunity: potential role of viruses in initiation, perpetuation, and progression of T-cell-mediated autoimmune disease. *Viral Immunol* 14(3):227–250.

[37] Limas C, Goldenberg I, Limas C. [1991] Effect of anti β1 receptor antibodies in dilated cardiomyopathy on the cycling of cardic beta receptors. *Am Heart J* 122:108–114.

[38] Fu L ,Magnusson Y, Bergh C, et al. [1993] Localization of a functional autoimmune epitope on the muscarinic acetylcholine receptor-2 in patients with idiopathic dilated cardiomyopathy. *J Clin Invest* 91:1964-1968.

[39] Caforio A ,Grazzini M, Mann J ,et al. [1992] Identification of α- and β-cardiac myosin heavy chain isoforms as major autoantigens in dilated cardiomyopathy. *Circulation* 85:1734–1742.

[40] Schulze K, Becker BF, Schultheiss HP. [1989] Antibodies to the ADP/ATP carrier, an autoantigen in myocarditis and dilated cardiomyopathy, penetrate into myocardial cells and disturb energy metabolism in vivo. *Circ Res*.64(2):179–192.

[41] Caforio AL, Vinci A, Iliceto S. [2008] Anti-heart autoantibodies in familial dilated cardiomyopathy. *Autoimmunity* 41(6):462–469.

[42] Huber SA, Rincon M. [2008] Coxsackievirus B3 induction of NFAT: requirement for myocarditis susceptibility. *Virology* 381(2):155-160.

[43] Winkelstein JA, Marino MC, Lederman HM, Jones SM, Sullivan K, et al. [2006] X-linked agammaglobulinemia: report on a United States registry of 201 patients. *Medicine (Baltimore)* 85(4):193–202.

[44] Mena I, Perry CM, Harkins S, Rodriguez F, Gebhard J, Whitton JL. [1999] The role of B lymphocytes in coxsackievirus B3 infection. *Am J Pathol* 155(4):1205–1215.

[45] Klingel K, Stephan S, Sauter M, Zell R, McManus BM, Bültmann B, Kandolf R. [1996] Pathogenesis of murine enterovirus myocarditis: virus dissemination and immune cell targets. *J Virol* 70(12):8888–8895.

[46] Kemball CC, Alirezaei M, Whitton JL. [2010] Type B coxsackieviruses and their interactions with the innate and adaptive immune systems. *Future Microbiol* 5(9):1329–1347.

[47] Huber S. [2008] Host immune responses to coxsackievirus B3. *Curr Top Microbiol Immunol* 323:199–221.