Heart Fail Rev (2013) 18:683–702
DOI 10.1007/s10741-012-9357-4

Viral myocarditis: from experimental models to molecular diagnosis in patients

Sabine Pankuweit · Karin Klingel

Published online: 16 October 2012

Abstract Cardiotropic viruses have been implicated as major pathogenetic agents in acute and chronic forms of myocarditis. By the introduction of molecular tools, such as (RT-) polymerase chain reaction ((RT-) PCR) and in situ hybridization in the diagnosis of inflammatory heart disease, genomes of various RNA and DNA viruses comprising enteroviruses, adenoviruses, parvovirus B19 (B19V) and herpesviruses (EBV, HHV6, HCMV) were detected in endomyocardial biopsies of patients with myocarditis and dilated cardiomyopathy. Meanwhile, it is known that the outcome of a virus infection in the heart resulting in myocarditis is determined by genetic host factors as well as by the viral pathogenicity which considerably varies in the different virus infections. A considerable portion of our knowledge about the etiopathogenetic mechanisms in viral heart disease is derived from animal studies. Whereas the evolvement of cardiac inflammation in enterovirus infections is guided by viral cytotoxicity and virus persistence, in herpesvirus infections, the pathophysiology is rather determined by primary immune-mediated pathogenicity. By investigation of immunocompetent and gene-targeted mice, valuable new insights into host and virus factors relevant for the control of cardiac viral infection and inflammation were gained which are reviewed in this paper.

S. Pankuweit
Department of Cardiology, University Hospital Gießen & Marburg, 35043 Marburg, Germany
e-mail: pankuwei@staff.uni-marburg.de

K. Klingel (✉)
Department of Molecular Pathology, University Hospital Tuebingen, Liebermeisterstr. 8, 72076 Tuebingen, Germany
e-mail: karin.klingel@med.uni-tuebingen.de

Keywords Viral myocarditis · Molecular diagnosis · Animal models · Virus prevalence

Introduction

In the past years in patients with acute and chronic forms of myocarditis, a variety of RNA and DNA viruses have been detected in endomyocardial biopsies by molecular biological techniques such as in situ hybridization and PCR. Besides, enteroviruses (EV), including coxsackieviruses of group B (CVB), adenoviruses (ADV), parvovirus B19 (B19V), human herpesvirus-6 (HHV6) and Epstein–Barr virus (EBV), were found in a significant number of patients with myocarditis [1–5]. In addition, genomes of other virus infections including influenzaviruses, HIV, human herpesvirus type 1 (HSV1) and human cytomegalovirus (CMV) were amplified by (RT-) PCR in inflamed hearts [6, 7]. However, whereas the etiopathogenetic role of enteroviruses and especially of CVB in the induction and progression of acute myocarditis to postviral cardiomyopathy was substantially confirmed by observations in CVB3-infected mice, it is rather unclear by which mechanisms herpesviruses, adenoviruses or parvovirus B19 might contribute to cardiac damage and inflammation.

In order to improve the knowledge about the pathophysiology of viral myocarditis, diverse animal models of DNA and RNA virus infections have been established. By investigation of infected immunocompetent as well as gene-targeted mice, valuable new insights into virus pathogenicity and the host immune response were gained. It is important to note that considerable differences in the outcome and course of myocarditis in dependency of virus-induced pathogenicity and genetic factors of the host are present in these animal models [Fig. 2; for review see 8].

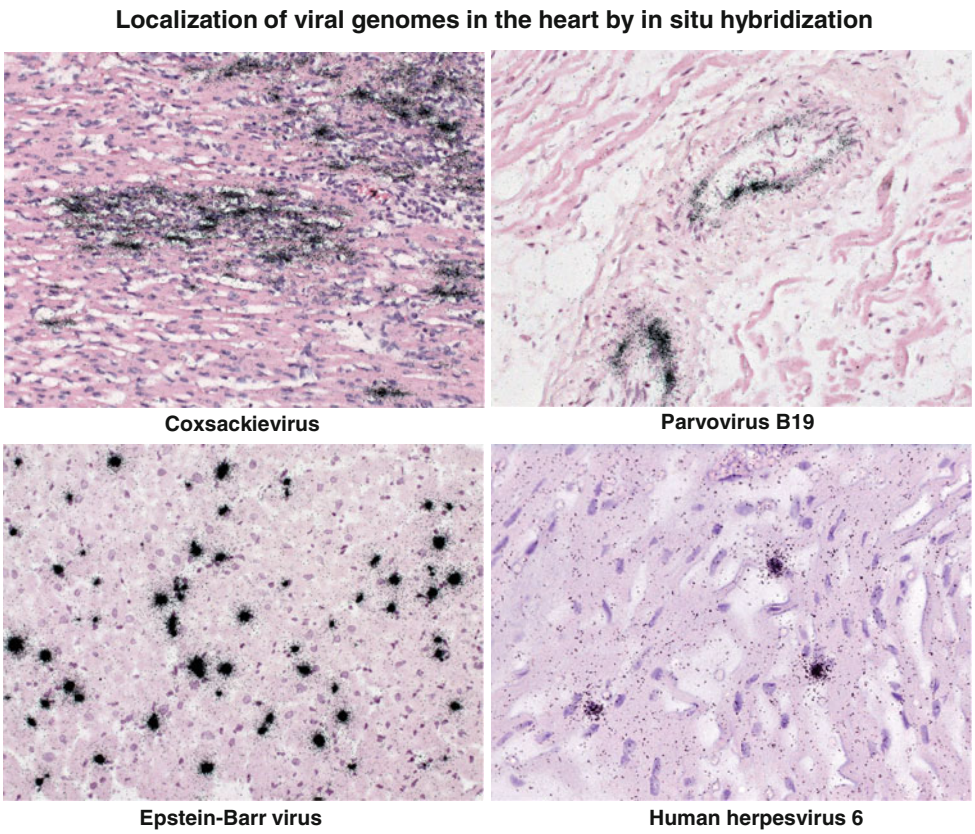

Fig. 1 Localization of viral genomes in different cardiac cell types in patients with acute myocarditis by radioactive in situ hybridization (virus genomes are indicated by black silver grains). Coxsackieviruses infect cardiomyocytes whereas parvovirus B19 DNA is exclusively found in endothelial cells. Epstein–Barr virus and human herpesvirus 6 genomes are present in interstitial immune cells (T cells, B cells, macrophages) but not in cardiomyocytes

Thus, it is highly probable that also the different course of myocarditis in humans is determined by individual immune reactions on infection of specific cardiac cell types by the various RNA and DNA viruses. By means of light and electron microscopic in situ hybridization experiments, it was possible for the first time to allocate specific virus infections to particular cardiac cell types in the human heart as well as in murine hearts [9, 10]. Coxsackieviruses belonging to the enteroviruses were found to infect primarily cardiomyocytes, and due to extensive virus replication, a rapid cytolysis of these cells occurs [11]. The consecutive antiviral immune response which involves NK cells, macrophages, and CD4+ and CD8+ T lymphocytes aims to eliminate CVB from the myocardium and is successful in most humans but some patients develop a chronic myocarditis on the basis of viral genome persistence [9]. At later stages of the disease, the virus-induced cytolysis may also trigger autoimmune reactions which are primed by the release of specific cellular antigens from necrotic myocytes such as to beta1-adrenergic receptors [12], myosin or M2 muscarinic receptors, which have also been observed in some animal models of myocarditis [13]. Further studies are needed to address the contribution of autoimmune reactions in comparison with those induced by persistent virus infections in the outcome of viral heart disease.

In contrast to enteroviruses, all other viruses often detected in the human heart cannot infect myocytes, for example, due to absence of the correspondent viral receptors. Instead, some cardiotropic viruses infect exclusively endothelial cells as we have shown by radioactive in situ hybridization for B19V. This virus was exclusively found in endothelial cells of children and adult patients with myocarditis (Fig. 1). On the other hand, herpesviruses including HHV6 and EBV which also do not infect cardiomyocytes were detected in cardiac inflammatory cells (macrophages, T or B lymphocytes) in patients with myocardits (Fig. 1). Thus, numerous cardiotropic viruses

do not damage the heart via cytolysis of cardiomyocytes but most likely via expression of cardiotoxic chemokines and cytokines from infected endothelial or immune cells, contributing to further attraction of potentially harmful immune cells into the heart. It is known that, for example, HHV6 may induce the expression of the proinflammatory cytokine IL-6 which is decisive for the invasion of T cells into infected organs [14]. In order to delineate the differences between the cellular and molecular mechanisms in acute and chronic myocarditis induced by different viral triggers, various animal models are discussed in the following chapters.

Murine models of coxsackievirus myocarditis

The murine model of CVB3 myocarditis is by far the most thoroughly investigated animal model of viral myocarditis as genetically diverse mouse strains perfectly reflect the different course of enteroviral myocarditis in patients [15]. The induction of enteroviral myocarditis is mediated by the entry of the virus into the cardiomyocytes via internalization using the transmembrane receptor CAR (coxsackievirus and adenovirus receptor) and the deflecting protein decay accelerating factor (DAF) as a coreceptor. CAR has nicely been shown to be critical for infectivity of the heart as absence of this receptor completely prohibited infection of cardiomyocytes and the subsequent cardiac inflammation [16]. CVB are able to lyse myocytes in vivo very quickly due to pronounced viral replication as shown in Fig. 2 [10, 11]. In in vitro experiments, it was shown that upon infection, cardiomyocytes undergo cytolysis within 16 h [17]. In CVB3-infected mice, myocytolysis due to virus replication was proven by electron microscopic in situ hybridization studies, demonstrating replicative RNA intermediates in close spatial association with vacuoles within myocytes [10]. Also, the presence of extensive myocyte damage in SCID mice lacking mature B and T lymphocytes support the view that virus replication induces early myocyte damage [18]. Transgenic mice which express a replication competent but not infectious full-length CVB3 cDNA reveal severe loss of myocytes and scarring, indicating that expression of viral proteins mediates cardiac dysfunction [19].

The most relevant molecular mechanism by which enteroviruses contribute to the pathogenesis of myocarditis was described by Badorff et al. [20], demonstrating that CVB3 cleaves dystrophin via the viral proteinase 2A resulting in the disruption of cytoskeleton in myocytes. More recently, the intracellular protein degradation systems comprising the ubiquitin–proteasome and lysosome pathways have been identified as crucial factors of virus infectivity. Luo et al. [21] found that treatment of cells with proteasome inhibitors significantly decreased virus titers and prevented virus-induced cell death. Investigations in infected mouse cardiomyocytes suggested that the integrin-linked kinase ILK plays a critical role in CVB3 pathogenesis by modulating virus replication and virus-induced cellular injury via an Akt-dependent mechanism [22]. CVB3 was also found to activate the host's autophagy machinery to enhance its replication [23].

As a consequence of viral replication in myocytes, the innate immune response is triggered, the first line of defense against invading pathogens. Pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor (TNFα) and interferons (type I and II) are released from resident cardiac cells, which consecutively activate macrophages. A major impact for the course of the disease emerged to be the type I interferon system. CVB3-infected type I-IFNR-deficient mice died within 2–4 days post infection [24]. In mice deficient for IFN-beta, a downregulation of IFN-stimulated gene targets as well as increased cardiomyocyte injury was noted [25].

Various TLR's which are expressed on immune cells, comprising NK cells, DCs and macrophages, have been implicated to be involved in the early immune response against enteroviruses. CVB3-infected TLR3-knockout (ko) mice were found to develop a severe ongoing myocarditis underlining the view that TLR3 plays a central role in the effective control of the infection [26]. Investigations in CVB3-infected TLR-9 ko mice suggested that the MyD88/TNF-alpha axis due to TLR9 activation in the heart contributes to the development of acute myocarditis but not of chronic myocarditis [27].

Following the activation of the innate immunity, the adaptive immune response evolves around 6 days post infection (pi). CD4+ and CD8+ T lymphocytes as well as B lymphocytes were found to contribute to the elimination of CVB3 in the heart. As shown in CVB3-infected beta-2 microglobulin- [28] and CD8-deficient mice [29], the severity of disease was magnified, demonstrating protective effects of CD8+ T cells in the propagation of viral myocarditis. On the other hand, B-cell-deficient mice, which are characterized by agammaglobulinemia, were found to establish a chronic CVB3 infection in a variety of organs, including the heart [30].

Depending on the genetic background, susceptible animals such as A/J, ABY/SnJ, ASW/J, SWR/J, Balb/c develop a chronic myocarditis which may last for several months [15]. The failure to resolve viral RNA from the heart can be deleterious and results in ongoing myocarditis (Fig. 2) [15]. However, when the virus is completely cleared as observed in C57BL/6 mice 2 weeks pi, the downregulation of the inflammation in the heart occurs which is mediated by the production of anti-inflammatory cytokines such as transforming growth factor beta (TGFβ) and IL-10 by regulatory T cells and alternatively activated (M2) macrophages [31].

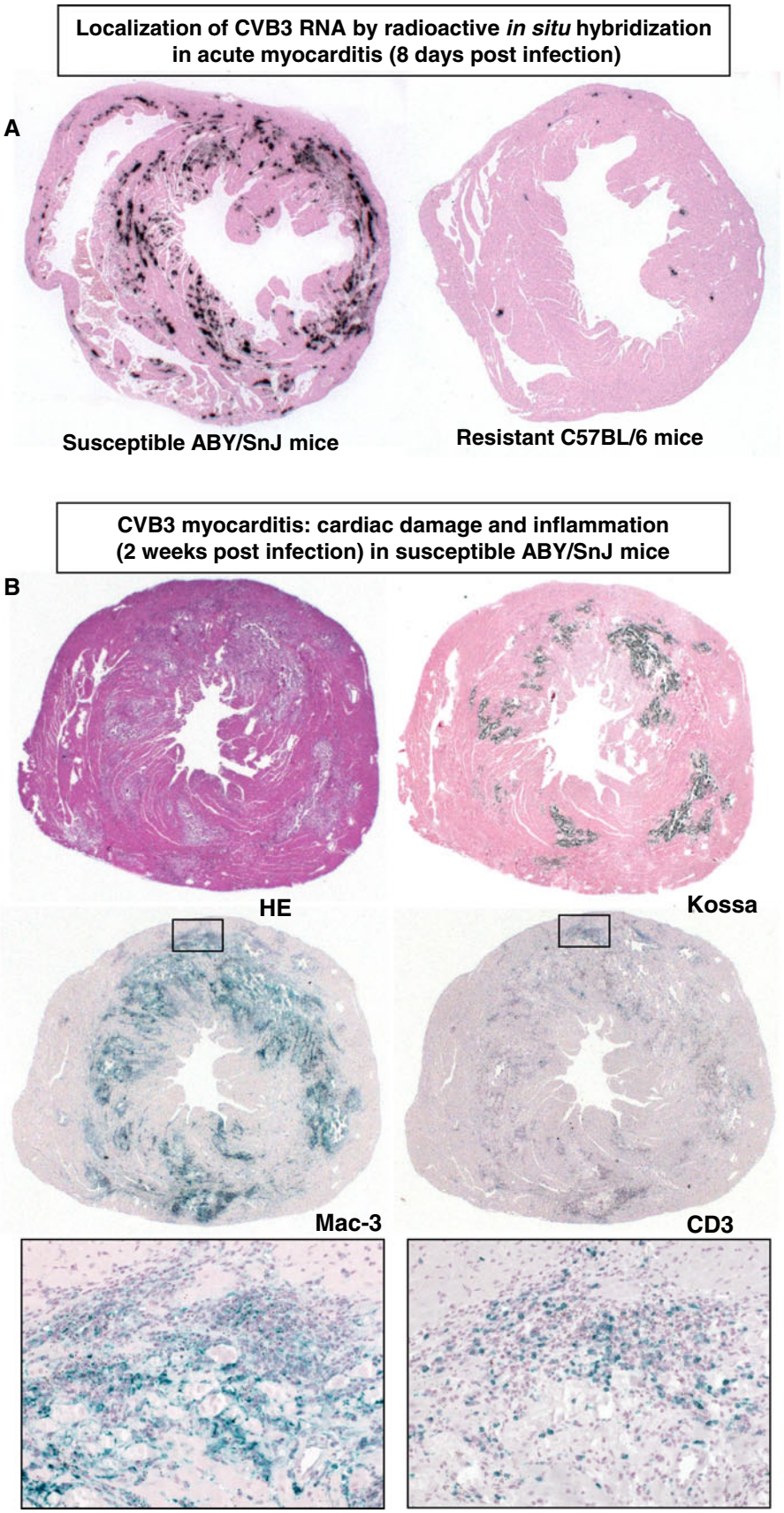

Fig. 2 CVB3 replication in myocytes, cardiac damage and inflammation is extensive in susceptible ABY/SnJ (H-2^b) mice compared to resistant C57BL/6 (H-2^b) mice which do not get a chronic myocarditis (**a**). Consecutive tissue sections of an ABY/SnJ heart 2-week pi CVB3 reveals massive calcification (Kossa staining) in areas of necrotic myocytes (HE) and ongoing inflammation by MAC-3+ macrophages and CD3+ T cells (**b**)

Susceptible mice revealing chronic inflammation were found to have a delayed IFN-γ secretion and a highly diminished IL-10 production [32]. Findings in IL-10-deleted mice confirmed the regulatory role of IL-10 in the outcome of CVB3 myocarditis [32]. Recently, Li et al. [33] showed that the protection of female mice to excessive cardiac damage in the coxsackieviral mouse model is attributed to a larger presence of M2 macrophages in comparison with male mice. The consequences of chronic inflammation following CVB3 infection are cardiac fibrosis with remodeling of the extracellular matrix (EMC) which may finally result in dilated cardiomyopathy and heart failure. Important regulators of the ECM are matrix metalloproteinases (MMPs) which can degrade the different components in the interstitum. MMP-2, MMP-9 and MMP-12 transcription were increased during acute myocarditis, and the tissue inhibitors of metalloproteinases TIMP-3 and TIMP-4 expression were found to be downregulated, indicating that cardiac remodeling is at least partially mediated via activation of MMPs [34]. On the other hand, results from MMP-9 ko mice suggest that MMP-9 is obviously necessary to prevent virus spread in the heart, to promote proper immune infiltration and fibrosis, thus preserving heart function [35]. A crucial role for urokinase-type plasminogen activator (uPA) and MMPs in mediating cardiac inflammation and necrosis in CVB3-induced viral myocarditis was shown by investigations in uPA ko mice which exhibited a reduction in cardiac dilatation and dysfunction [36]. Another protein that has been described to be involved in inflammatory responses and in the maintenance or reconfiguration of tissue integrity is osteopontin (OPN). In contrast to resistant C57BL/6 and OPN gene-deficient mice, transcription levels of matrix metalloproteinase-3, TIMP1, uPA and transforming growth factor (TGF) beta1 were elevated in susceptible mice, and as a consequence, procollagen-1 mRNA expression and fibrosis were considerably enhanced but could be successfully treated with a vitamin D analog [37]. In addition to OPN also connective tissue growth factor (CTGF), a member of the CCN protein family was found to be associated with the development of fibrosis in ongoing enteroviral myocarditis. CTGF which is known to be basically mediated by TGF-β was found to be extensively upregulated in CVB3-infected susceptible mice [38]. Interestingly, the matricellular protein Cyr61, another CCN protein, was found not only to be linked with tissue repair but also to function as a modulator of immune cell migration as shown in a murine model of autoimmune myocarditis. The CCN1-driven modulation of immune cell migration is mimicked in part by cyclic RGD peptides which might offer a therapeutic option for the treatment for inflammatory heart diseases [39].

Finally, common epitopes among viruses and cellular antigens are supposed to play a role in repeated episodes or chronicity of myocarditis by the mechanisms of T-cell mimicry [40, 41]. In coxsackievirus-induced myocarditis, the cardiac myosin mimicking M protein peptide NT4 was found to induce tolerance and prevent coxsackievirus-induced myocarditis, suggesting T-cell mimicry between coxsackieviruses and streptococcal M protein, both of which are associated with inflammatory heart disease [40]. Prophylactic nasal administration of cardiac myosin (CM) major histocompatibility class (MHC) II peptides $CM_{947-960}$ and $CM_{735-747}$ and blockade of OX40 in CVB3-infected Balb/c mice was found to reduce myocarditis and mortality by enhancing Treg and IL-10 induction [42].

Murine models of encephalomyocarditis myocarditis

Encephalomyocarditis virus (EMCV) is another single-stranded Picornavirus of the *Enterovirus* genus which has been studied to evaluate pathogenetic mechanisms in enteroviral myocarditis. Similar to CVB, EMCV was found to induce a necrotic myocarditis in mice but was also detected in the heart of young Rhesus macaques [43]. Whether the transmission of EMCV to humans occurs is unclear. However, in 2009, EMCV was obviously isolated two patients with fever, nausea, headache and dyspnea supporting a role for EMCV in human infection and febrile illness [44].

In order to identify molecular mechanisms in EMCV myocarditis, mice lacking functional TLR3 were investigated. Correspondent to findings in CVB3-infected TLR3 ko mice [26], EMCV-infected TLR3 ko mice were found to be unable to control the proliferation of EMCV, subsequently resulting in increased cytopathogenic effects in cardiac myocytes and early death. The findings in this study implicate the importance of TLR3 signaling and antiviral effects of TNF-alpha and IL-6 in the very early stages of the heart disease [45]. On the other hand, it is well known that inflammatory cytokines including TNF-alpha may accelerate the pathology of EMCV myocarditis and negatively influence the cardiac function [46]. In order to identify the underlying mechanisms, the responses to TNF-alpha in mice revealing an overexpression of the human IL-6 gene in EMCV myocarditis were investigated. Treatment with recombinant human TNF-alpha significantly improved viral clearance, indicating that persistent expression of IL-6 accelerates myocardial injury in the inflammatory stage of myocarditis [47]. Interestingly, activated mast cells were found as source of IL-6, TNF-alpha and also of mediators increasing extracellular matrix such as MMPs in EMCV-infected hearts [48]. In addition, findings in EMCV-infected TNF $^{ARE/+}$ mice which are unable to downregulate TNF expression following infection indicate that the duration and degree of activation of the innate immune system plays a critical role in the outcome of EMCV myocarditis [49].

Using cyclooxygenase-2 (COX-2) gene-deficient mice, it was shown that the inhibition of COX-2 may enhance myocardial damage via the reciprocal expression of TNF-alpha and adiponectin in EMCV-infected mice [50].

Treatment trials of EMCV myocarditis revealed that human leukocyte IFN-alpha A/D inhibited the multiplication of virus in the heart and protected the mice from myocarditis. Also, an inhibitor of NF-kB (SUN-C8079) lowered mortality, attenuated myocardial necrosis and cellular infiltration, and decreased the intracardiac production of IL-1 and TNF-alpha but without significantly changing viral replication. In contrast, immunosuppressive therapy with prednisolone or cyclosporine was found to increase mortality in EMCV-infected mice, indicating that immunosuppression might be deleterious in enteroviral myocarditis [for review see 51].

Murine models of reovirus myocarditis

Reoviruses which are enteric non-enveloped viruses with a double-stranded RNA genome have been widely used as model systems to study viral pathogenesis in the central nervous system, liver and heart. The morphology of reovirus-induced myocarditis is generally characterized by mild inflammatory infiltrates but extensive myocardial necrosis. Comparable to findings in coxsackievirus infections, also, reovirus was found to induce a myocarditis in SCID mice, illustrating that reovirus myocarditis is primarily not an immune-mediated disease [52]. Correspondent to observations in CVB3 myocarditis [15], the extent of viral RNA synthesis during replication but not generation of infectious virus was found to be a determinant of reovirus-induced acute myocarditis [53].

Differences in the tropism and virulence have been linked to sensitivity of type I interferons [54] and various components of the innate and adaptive immunity [55]. However, in contrast to enterovirus infections, TLR3 was found not to be required for limiting reovirus infection in mice [56]. Instead, the retinoic acid inducible gene I (RIG-I) and the RIG-I adaptor were found to be necessary for the activation of antiviral transcription factors including interferon regulatory factor 3 (IRF-3) [57] and NF-κB [58]. There is firm evidence that IRF-3 but not CD8+ T cells and serum IgG contribute to the rapid clearance of virus infection from the heart [59]. In addition, interferon-β was identified as an important determinant of protection against reovirus myocarditis [54]. Proteomic analyses of myocytes infected with myocarditic and non-myocarditic reoviruses revealed that heat shock protein Hsp25 is differentially modulated in this system, indicting that Hsp25 is not only a marker of cytopathology but may mediate protection in the myocardium [60]. Using the same experimental approach, it was also demonstrated that myocarditic reoviruses preferentially increased apoptosis in myocytes relative to fibroblasts. On the other hand, fibroblasts infected with a non-myocarditic reovirus variant were found to express more cytokines (IL-1β, IL-4, IL-6, IL-10, TNF-alpha) than those infected with a myocarditic strain, indicating that differential cytokine expression is important for the outcome of reovirus myocarditis [61]. Further investigations on the apoptotic signaling pathways in reovirus myocarditis revealed that in contrast to death receptors, inhibition of mitochondrial apoptotic signaling had no effect on reovirus-induced cardiac myocyte apoptosis [62].

Murine models of adenovirus myocarditis

Adenovirus infections in humans are known to involve different organs including the respiratory tract, the gastrointestinal tract and the conjunctiva. Less common is the infection of the myocardium which has mainly been reported in children on the basis of PCR results [63]. Despite the fact that adenoviruses 2 and 5 use the same receptor (CAR) for entry into the host cells as coxsackieviruses 1–6, no data are available illustrating that adenoviruses replicate in cardiomyocytes in patients with myocarditis. Thus, it cannot be excluded that the PCR detection of adenoviruses in the myocardium just reflects the presence of viral genomes in the blood or in blood cells present in the heart of systemically infected patients.

The study of human adenoviruses, which are non-enveloped viruses with a double-stranded linear DNA genome, in animal models has been hampered by the species specificity of these viruses. Thus, non-human adenoviruses offer an alternative approach to study the pathogenesis of this infection in animal models. The murine adenovirus type 1 (MAV-1) induces a lethal disease in newborn mice with focal necrosis in various organs comprising the heart. Early electron microscopic studies suggested that MAV-1 induces cytopathic effects due to multiplication of the virus in the myocardium, endocardium and heart valves. Viral intranuclear inclusion bodies were noted between day 6 and 12 pi, whereas the areas of damage with necrosis, inflammation and dystrophic calcification were most prominent from days 17 to 21 pi [64]. Later performed in situ hybridization and immunohistochemistry studies revealed a tropism for endothelial cells [65]. Guida et al. [66] demonstrated that depending on the genetic background, adult C57BL/6 mice but not Balb/c mice are susceptible for a fatal MAV-1 infection with highest levels of viral RNA in the brain and spinal cord and less pronounced viral RNA content in the heart, spleen and lungs. Activated microglia secretes IL-1β, IL-6 and NO which is responsible for pathological changes in the CNS

and might also be relevant for the damage in the heart. In addition to endothelial cells, also, macrophages have been implicated as target cells for MAV-1 [67]. The finding that depletion of macrophages increased virus replication in spleens of resistant Balb/c mice suggests that these cells may play a role in dissemination of the virus to the brain and spleen after ip inoculation, contributing to the pathogenesis of the virus infection [67].

Animal models of parvovirus myocarditis

Human parvovirus B19 (B19V), the only human pathogenic parvovirus, is the causative agent of a wide spectrum of human diseases, including fifth disease (erythema infectiosum), hydrops fetalis in pregnant women and transient aplastic crisis in patients. Numerous reports demonstrating the presence of B19V in the heart of patients with acute and chronic myocarditis further suggest that this virus may be associated with inflammatory heart disease. Our current understanding about the mechanisms by which B19V regulates disease progression is rather limited, also due to the lack of adequate animal models as infection of mice with B19V does not induce myocarditis. Only very recently, the first mouse models on B19V myocarditis were published, illustrating that mice which were treated with a recombinant VP1 protein of the human P19V reveal myocardial injury with increased levels of the functional enzymes aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine kinase isoenzyme (CK-MB) [68]. In a second study, NZB/W F1 mice which received antibodies against human parvovirus B19 VP1 unique region were described to develop cardiac injury by induction of inflammatory but not of myocardial infarction-associated proteins via activation of phosphorylated p-38 and NF-κB signaling [69].

Other animal models with non-human parvoviruses including canine parvovirus (CPV), feline panleukopenia virus (FPV) and mink enteritis virus (MEV) are known for a long time. Parvoviruses infect only actively dividing cells; thus, the clinical manifestation of the evolving diseases is strongly depending on the age of the host. In animals older than 4 weeks, parvoviruses mainly replicate in bone marrow, lymph nodes, spleen and progenitor cells of the intestine [for review see 70]. Parvoviruses as pathogenic agents in myocarditis have been reported from cats infected with FPV [71] and from puppies infected with the canine parvovirus type 2 (CPV-2). The cardiac lesions in dogs were characterized by severe diffuse myocardial degeneration and necrosis with occasional massive mineralization and intranuclear inclusion bodies, suggesting that CPV-2 may replicate not only in endothelial cells but also in cardiomyocytes [72].

Murine models of herpesvirus myocarditis

Epstein–Barr virus has been observed in the hearts of up to 8 % of the patients with inflammatory heart disease [2, 63]. The processes explaining cardiac inflammation and injury in EBV infection are uncertain mainly due to the absence of suitable animal models [22, 73]. One animal model which might mimic in some aspects human EBV infection is infection of mice with the murine gamma herpesvirus MHV-68. Both viruses can induce a latent infection of B cells [74]. With regard to myocardial infection, MHV-68 seems to replicate in the heart of immunocompetent mice, showing a maximum replication between 5 and 10 days. Myocardial necrosis and focal inflammation, consisting mainly of T lymphocytes, occur after 10–12 days and 33–35 days, respectively. B- and T-cell-deficient B6-(Rag1)^{TM} mice revealed high myocardial viral loads but no myocardial necrosis, indicating that viral replication is not sufficient to explain myocardial damage. However, in this model, it is still unclear which cells are infected in the heart and which molecular mechanisms lead to myocardial necrosis in BALB/c mice but not in C57BL/6 [74].

The evolvement of myocarditis has also been reported in mice infected with another herpesvirus, the murine cytomegalovirus (MCMV) [75]. The hearts of MCMV-infected BALB/c mice were found to be more susceptible than those of C57BL/6 for cardiac infiltration, which mainly consist of CD8+ and CD4+ T cells, macrophages, B cells and neutrophils. In this model, MCMV titers in the heart were low and replicative virus could not be isolated beyond the first week pi. Correspondent to human infection with cytomegalovirus (HCMV), also in cardiac MHV-68 and MCMV infection, the direct lysis of myocytes due to virus replication in vivo was not proven [75]. In another study of MCMV-induced myocarditis in BALB/c mice, it was shown that myocarditis-related pathological changes and increase in viral load were greatest at day 8 p.i., corresponding with peak cytokine transcription of TNF-alpha, IL-6 and IFN-γ, as well as of IL-10 mRNA transcripts [76]. Interestingly, treatment of MCMV-infected mice with IFNA6, A9 and B inhibited acute myocarditis, and IFNA6 was even found to reduce chronic cardiac inflammation, supporting the hypothesis that acute MCMV myocarditis does not reflect virus load but rather the immunomodulatory responses to this infection [77].

Animal models of influenza virus myocarditis

Reports on the prevalence of myocardial involvement in human influenza A virus infections ranges from 0 to 11 % dependent on the diagnostic criteria applied [78]. In the past years, several cases of acute myocarditis especially in

juvenile patients have been reported in association with pandemic H1N1 influenzavirus infections [6, 79]. Genomes of influenza A/H1N1 virus were detected by RT-PCR analysis in blood as well as in myocardial tissue in a patient with lethal influenzavirus infection [6]. So far, no in situ hybridization data are available to illustrate which cell types in the myocardium might be infected and whether influenza A viruses can replicate in the heart. Due to the missing direct effects of viral replication in the myocardium, cytokine-mediated signaling pathways are presumed to be responsible for the clinical symptoms and impaired cardiac function in influenza A virus-associated myocarditis [80].

In the literature, only a few animal models are reported which investigated the effects of a systemic influenza virus infection on the heart. Most recently, the study of naturally influenza A/H5N1 ("bird flu") virus-infected chicken and ducks revealed that, in addition to many other organs, like the pancreas, brain, lungs and muscle also the hearts show a multifocal lymphohistiocytic inflammation in both species. Viral nucleoprotein was consistently present in areas with histopathological changes in the heart as convincingly demonstrated by immunohistochemistry [81]. These results suggest that natural influenza A/H5N1 virus infection in chicken and ducks induces a systemic infection with viral replication and inflammation also in the myocardium, which probably contributes to heart failure and high mortality observed especially in domestic ducks [81]. In contrast to chicken and ducks, immunocompetent mice severely infected with several types of influenza A viruses including H5N1, H1N1 and H7N7 subtypes did not reveal any signs of myocarditis [82, 83]. Accordingly, in situ hybridization experiments performed in these animals were consistently negative for the detection of influenza A (H5N1, H1N1, H7N7) RNA in the hearts in contrast to the lungs, confirming that murine hearts are no target for influenza A virus replication and damage [82, 83]. Correspondently, in ponies challenged with high doses of equine influenza virus (EIV) influenza, only transient increases in cTnI but no histopathological abnormalities were detectable in the hearts of the horses [84], indicating that EIV does not induce a severe myocarditis in the animals.

Virus myocarditis in fishes

Finally, it has to be mentioned that not only mammals but also fishes can suffer from myocarditis upon virus infection. Most recently, a necrotizing myocarditis was reported in farmed and wild Atlantic salmons which is based on the infection with a double-stranded RNA virus with the suggested name piscine myocarditis virus, belonging to the family of Totiviridae. Viral genomes were detected by in situ hybridization in degenerated cardiomyocytes within myocardial lesions from clinical cases with cardiomyopathy syndrome (CMS) which is obvious 6 weeks post infection [85]. The identification of this virus as etiopathogenetic agent in this disease will be an important step toward a control of the economically highly relevant CMS and the development of a vaccine.

In addition, most recently from an eel of the Lake Constance, a new picornavirus (eel picornavirus (EPV F15/05) was isolated from the heart of the fish. This virus is pathogenic in experimentally infected glass eels and induces a high mortality [Roland Zell, University of Jena, Germany, Europic Meeting 2010]. More experimental studies are required to evaluate whether this virus or other picornaviruses of the marine ecosystems, like the bottlenose dolphins or seal picornavirus, can also induce inflammatory heart diseases.

Diagnosis of viral myocardial infections in humans: early attempts

After the first description of myocarditis in man by Fiedler in 1899 numerous case reports of this disease came to our knowledge [86]. A long list of viral, bacterial and other agents have been suggested as etiologic factors in acute and chronic forms of myocarditis [87]. Starting from 1950 enteroviruses and especially coxsackieviruses of group B were increasingly recognized as a main cause of perimyocarditis in children as well as in adult patients [88–92]. Apart from some systemic infectious diseases with cardiac involvement, such as rubeola or mumps virus infections, which are clinically characteristic, specific laboratory tests are needed to substantiate the diagnosis of viral heart disease [87].

Virological diagnoses were mainly based on three approaches, which were introduced by Lerner in 1973 [93]. The most important method was the isolation of the appropriate virus from a patient specimen obtained during the first days of illness, which upon inoculation replicates in cultured cells, embryonated eggs or animals. Characteristic cytopathic effects induced by the replicating virus or positive immunohistological stainings were used to identify the viral agents. However, negative results do not exclude a causative role in the underlying heart disease, and, on the other hand, a positive result does not automatically imply cardiopathogenicity of the isolated virus, especially if a systemic infection could not be excluded [87, 93]. Another method which was introduced for the detection of infectious agents was the direct investigation of fluids or tissues by light and electron microscopy, as it was established, for example, for cytomegalovirus infection revealing intracellular virus-induced inclusion bodies

[94]. In addition, direct and indirect immunofluorescence techniques visualizing viral antigens were applied to different tissues and fluids of patients [95].

Most often, the virological diagnosis with regard to inflammatory heart disease based on a fourfold or greater rise in neutralizing antiviral antibody titers or an unusual high antibody titer without significant fluctuation [87, 92]. The WHO surveillance data 1975–1985 showed for the first time a strong association of elevated antibody titers against entero-, adeno-, cytomegalo- and herpesviruses with inflammatory heart diseases [96]. In addition, the characterization of the Ig class of viral antibodies was found to be helpful for determining the stage of infection. Unfortunately, the specificity of antibodies detected in different tests considerably varied, and thus, patients are often referred for diagnostics and medical treatment with a significant delay from the onset of the initial infection [73]. Recently, Mahfoud et al. [97] showed in a cohort of 124 patients that sensitivity for serological detection of a virus detected by PCR in EMB was 9 % and the specificity 77 %. In addition, diagnostic value of antibody testing is limited by the fact that almost all viruses involved in the pathogenesis of myocarditis are detected with a high prevalence in the general population [97]. Often, the presence of antiviral antibodies reflects rather the status of infection during lifetime of a patient and not an acute infection.

Molecular biological methods used in the diagnosis of viral myocarditis

The diagnosis of virus-associated myocarditis was clearly facilitated by the introduction of endomyocardial biopsy techniques by Sakakibara and Konno in 1962 [98] in addition to the development of polymerase chain reaction (PCR) by Mullis in 1982 [99]. The combination of both methods allowed for the first time the detection of viral genomes directly within the affected myocardial tissue in a patient with suspected myocarditis. A wide range of different PCR assays have been developed, which are suitable to identify different cardiac RNA and/or DNA viruses with a higher sensitivity in comparison with standard immunohistochemical methods used for the detection of viral proteins [for review 100; 101–103].

By these molecular approaches, enteroviruses have been identified as highly relevant pathogenic agents in myocarditis [104–112]. Moreover, the presence of genomes from adenoviruses (ADV), parvovirus B19 (B19V) [113], herpesviruses (human herpes virus 6 (HHV6), cytomegalovirus (CMV), Epstein–Barr virus (EBV), herpes simplex virus type 1 (HSV1) [114], chlamydia pneumoniae [115], borrelia burgdorferi [116, 117], as well as other infectious agents [118] was reported in patients with inflammatory heart disease.

One of the major problems associated with the analysis of cardiotropic agents by PCR is the fact that this technique allows only the detection of viral genomes without differentiating potentially infected cardiac cell types. In addition, active replication of the virus is generally not investigated by PCR [119]. Thus, in order to substantiate an etiopathogenetic role of an infectious agent, PCR must be carefully evaluated in the context of clinical, histological and immunohistochemical findings of endomyocardial biopsies.

To overcome this diagnostic gap, the in situ hybridization technique was established which is capable to attribute viral sequences to specific cells types in the heart as shown in Fig. 1. Also, as shown for coxsackieviruses, in situ hybridization allows the detection viral plus-strand RNA as well as the replicative minus-strand RNA intermediates which are of particular interest for the diagnosis of active myocardial infections [106–108, 120].

Starting from 2002, fluorescence-based real-time PCR assays were established for the evaluation of the viral load in the heart. Regarding the quantification of B19V genomes, real-time PCR assays have been developed for the use of the LightCycler system [121], fluorescence resonance energy transfer probes [122]) as well as for the ABI Prism system [123, 124].

Generally, sequencing of PCR products from patients in comparison with distinct positive controls is needed to confirm specificity of PCR results and to exclude contaminations during the isolation and processing of nucleic acids. In the case of B19V, sequencing of PCR products allowed the identification of three genotypes [125]. Genotype 1 seems to be the most common genotype in middle Europe, whereas genotype 2 has been reported preferentially in skin biopsies [126] and genotype 3 in patients with transient aplastic anemia in France [127, 128]. In 2005, a PCR method was developed which is suitable for routine diagnostics and allows the sensitive detection of all three genotypes of B19V in one common assay [129]. From the clinical point of view, this differentiation of the B19V genotypes is not required as clinical symptoms are similar in all genotypes, but it may offer the possibility to detect potential differences in long-time follow-up of patients [5].

Prevalence of cardiotropic viruses in endomyocardial biopsies assessed by molecular tools

Viral genomes were identified in a varying subset of patients with acute and chronic myocarditis and DCM, but the impact of these viral genomes on cardiac function and clinical outcome is still controversial [130]. The overall prevalence of cardiotropic viruses amplified by (RT-)PCR

Table 1 Prevalence of enterovirus RNA in endomyocardial biopsies of patients with dilated cardiomyopathy (DCM), myocarditis (M), inflammatory cardiomyopathy (DCMi) and controls (C)

References	No/pts	Methods	Diseases	% positive
Bowles et al. [131]	21	Slot blot (sb)	DCM	29
	8		M	50
	19		C	5
Tracy et al. [132]	19	ISH (in situ hybridization)	M	18
	16	ISH	DCM	50
Jin et al. [133]	25	PCR (polymerase	DCM	12
	5	chain reaction)	M	40
	13		C	0
Weiss et al. [134]	5	PCR	M	20
	11		DCM	0
	21		C	0
Kandolf et al. [135]	29	ISH	DCM	17
	25		M	24
	30		C	0
Petitjean et al. [136]	45	PCR	DCM	76
	10		M	30
	23		C	39
Martin et al. [105]	34	PCR	M	21
	17		C	0
Pauschinger et al. [137]	128	PCR + sb	DCM	28
			M	57
Bowles and Towbin [138]	199	PCR	M	16
	132		DCM	8
	65		C	2
Talwar et al. [139]	15	PCR		
DCM + M	53			
	6		C	33
Maisch et al. [140]	191	PCR	DCM	4
	71		Chronic M	6
	31		Acute M	3
	45		C	0
Pauschinger et al. [141]	51	PCR + sb	M	41
Bowles et al. [63]	624	PCR	M	14
Kühl et al. [142]	245	PCR	DCM	9
	172	PCR	M	33
Pankuweit et al. [143]	584	PCR	DCM	0.5
	282	PCR	DCMi	2.8
	816	PCR	M	1.5
Mahrholdt et al. [144]	87	nPCR (nested PCR)	M	1
Kandolf et al. [145]	3,219	nPCR	M/DCM	9.7

Table 2 Prevalence of cytomegalovirus DNA in endomyocardial biopsies of patients with dilated cardiomyopathy (DCM), myocarditis (M), inflammatory cardiomyopathy (DCMi) and controls (C)

References	No/pts	Methods	Diseases	% positive
Maisch et al. [149]	52	ISH	DCM	14
	62		M	6
	31		C	2
Schönian et al. [150]	29	ISH	DCM	40
	18		M	38
	15		C	2
Martin et al. [105]	34	PCR + sb	M	3
	17		C	0
Towbin et al. [151]	199	PCR + sb	M	3
	132		DCM	0
	65		C	0
Maisch et al. [152]	67	PCR	M	4
	179		DCM	9
	25		C	0
Bowles et al. [63]	624	PCR	M	3
Kühl et al. [142]	245	PCR	DCM	<1
Pankuweit et al. [143]	584	PCR	DCM	0.8
	282	PCR	DCMi	3.9
	816	PCR	M	3.1
Kytö et al. [148]	40	PCR	M	42.5
Mahrholdt et al. [144]	87	PCR	M	0
Kandolf et al. [145]	3,219	PCR	M/DCM	2.1

in endomyocardial biopsies of these patients differs widely: Enteroviral genomes were detected in 3–53 %, cytomegalovirus DNA in 3–40 % and adenovirus DNA in 3–23 % in the myocardium of patients with inflammatory heart disease. In addition to the previous summary [100], Tables 1, 2, 3, 4, 5, 6 summarize the wide range of results that have been obtained by different molecular methods also with regard to newer cardiotropic viruses such as HHV6, EBV and B19V.

Prevalence of enterovirus genomes in patients with myocarditis and DCM

The most reliable data for a direct role of viral infection of the myocardium leading to myocarditis and dilated cardiomyopathy as long-term sequela in humans were derived from CVB3 infections [17, 87, 130]. The prevalence of enteroviral RNA in endomyocardial biopsies from patients with myocarditis by RT-PCR or nested RT-PCR (see Table 1) ranged from 3 % (low) in the analyses by Maisch et al. [140] to 50 and 53 % (high) published by Bowles et al. and Talwar et al [131, 139]. In patients with DCM, the prevalence of enteroviral genomes ranged from 0 %

Table 3 Prevalence of adenovirus DNA in endomyocardial biopsy of patients with dilated cardiomyopathy (DCM), myocarditis (M), inflammatory cardiomyopathy (DCMi) and controls (C)

References	No/pts	Methods	Diseases	% positive
Towbin et al. [151]	199	PCR + sb	M	23
	132		DCM	20
	65		C	0
Hufnagel et al. [157]	84	PCR	DCM	3
	75		M	12
	85		C	0
Bowles et al. [63]	624	PCR	M	23
Kühl et al. [142]	245	PCR	DCM	2
	172		M	8
Pankuweit et al. [143]	584	PCR	DCM	1.2
	282	PCR	DCMi	2.1
	816	PCR	M	1.5
Mahrholdt et al. [144]	87	PCR	M	0
Kandolf et al. [145]	3,219	PCR	M/DCM	1.1

Table 4 Prevalence of parvovirus B19 DNA in endomyocardial biopsy of patients with dilated cardiomyopathy (DCM), myocarditis (M), inflammatory cardiomyopathy (DCMi) and controls (C)

References	No/pts	Methods	Diseases	% positive
Pankuweit et al. [167]	36	PCR	M	19.5
	18		DCM	16
	13		DCMi	23
	26		C	4
Bowles et al. [63]	624	PCR	M	23
Klein et al. [168]	80	PCR	M	11
	49		DCM	10
	36		C	0
Kühl et al. [142]	245	PCR	DCM	51
	172		M	37
Pankuweit et al. [143]	584	PCR	DCM	17.6
	282	PCR	DCMi	33
	816	PCR	M	20.4
Mahrholdt et al. [144]	87	PCR	M	56
Kandolf et al. [145]	3,219	PCR	M/DCM	36.7

(low) in the report of Weiss et al. [134] to 76 % (high) in the analyses by Petitjean et al. [136]. Kandolf et al. [135] found a prevalence of 24 % in patients with myocarditis and 17 % in patients with DCM by radioactive in situ hybridization. Astonishingly, Pauschinger et al. [141] published in patients with heart failure but without active myocarditis a prevalence of enteroviral genomes in 41 %. By the use of a strand-specific RT-PCR, enteroviral plus-strand RNA was detected in endomyocardial biopsies of 40 % of patients with suspected myocarditis, whereas minus-strand RNA indicative for active viral replication

Table 5 Prevalence of Epstein–Barr virus DNA in endomyocardial biopsy of patients with dilated cardiomyopathy (DCM), myocarditis (M) and controls (C)

References	No/pts	Methods	Diseases	% positive
Bowles et al. [63]	624	PCR	M	0.5
Kühl et al. [142]	245	PCR	DCM	2
Mahrholdt et al. [144]	87	PCR	M	1
Kandolf et al. [145]	3,219	PCR	M/DCM	7.4

Table 6 Prevalence of human herpesvirus 6 DNA in endomyocardial biopsy of patients with dilated cardiomyopathy (DCM), myocarditis (M) and controls (C)

References	No/pts	Methods	Diseases	% positive
Kühl et al. [142]	245	PCR	DCM	22
	172		M	11
Mahrholdt et al. [144]	87	PCR	M	18
Kandolf et al. [145]	3,219	PCR	M/DCM	20.1

was detected in only 56 % of these plus-strand RNA-positive patients [141, 146].

Data from 2003 until now indicate a decreasing prevalence of enteroviral RNA in endomyocardial biopsies, showing enteroviral positivity in 14 % in patients with myocarditis [63], in 9 % in patients with DCM [142], 10 % in patients with clinically suspected myocarditis [145] and about 2.5–1 % in patients with inflammatory heart disease in investigations from Pankuweit and Mahrholdt [143, 144]. In contrast, Kühl et al. [1] showed that in 56/172 patients with histology proven myocarditis enteroviral, RNA is detectable (32.6 %). In 50 % of those patients, viral RNA was eliminated spontaneously with a significant improvement of ejection fraction, whereas in the remaining 28 patients, persistence of viral genomes was correlated with a significant decrease in ejection fraction.

Importantly, the application of molecular tools in the diagnosis of inflammatory heart disease has significantly contributed to the observation that direct effects by viral replication as well as chronic inflammation in the heart are relevant for the outcome of the disease [147]. Further implementation of host factors comprising the innate and adaptive immune system leading to virus elimination or chronic (auto)immune reactions is required especially with regard to possible treatment decisions.

Prevalence of cytomegalovirus genomes in patients with myocarditis and DCM

In most of the early serological investigations in patients with suspected myocarditis, elevated antibody levels against human cytomegalovirus have been shown [96]. In

addition, in several cases of CMV-associated fatal myocarditis in children and adults, CMV DNA was detected in endomyocardial biopsies of patients [148]. The overall prevalence of CMV DNA in endomyocardial biopsies from patients with myocarditis by PCR or nested PCR (see Table 2) ranged from 3 % (low) in the analyses by Martin et al. [105] and Towbin et al. [151] to 38 % (high) in the report of Schönian et al. [150]. More recently, the prevalence of CMV DNA in endomyocardial biopsies of patients with cardiac dysfunction was <3 % [145]. Nevertheless, there seems to be a correlation of the cytomegalovirus infection with fatal myocardial inflammation as reported by Kytö et al. [148], indicating that CMV infection may contribute to the development of myocarditis in rare cases.

Prevalence of adenoviral genome in patients with myocarditis and DCM

The assumption that adenoviruses may be causative agents of myocarditis in children and adults is based on the finding that some adenoviruses (types 2 and 5) use the same receptor CAR as coxsackieviruses 1–6 of group B [153, 154]. In a study presented by Bowles [63], analysis of a large number of samples identified adenoviral genome frequently (23 %) in the myocardium of patients with myocarditis of DCM (see Table 3). In the 1990s, Towbin et al. [151] reported the presence of adenovirus DNA in 23 % of children with myocarditis and in 20 % of patients with DCM and Hufnagel et al. [155] showed a prevalence of adenovirus DNA in 12 and 3 %, respectively, of biopsies from patients with myocarditis and DCM. In patients with heart failure without active myocarditis, Pauschinger et al. [156] reported in 1999 a prevalence of adenoviral genomes in 13 %. More recently, adenovirus-positive endomyocardial biopsies in patients with suspicion for myocarditis were detected in <2 % of patients investigated [145]. Together with the decreasing prevalences of cardiac enteroviral and cytomegaloviral genomes, the data pinpoint toward a shift of the viral spectrum toward other cardiotropic viruses such as parvovirus B19, human herpesvirus 6 and Epstein–Barr virus.

Prevalence of parvovirus B19 genomes in patients with myocarditis and DCM

Parvovirus B19 (B19V) has been implicated as a possible etiologic agent in myocarditis and DCM with inflammation (DCMi) and without inflammation [113, 157–159]. Infection with B19V may lead to erythema infectiosum, arthropathy, hydrops fetalis, acute hepatitis and myopericarditis [160–163]. In fetuses with hydrops, numerous cardiac cells but no cardiomyocytes were found to contain B19V genomes as shown by radioactive in situ hybridization [164]. The blood group P antigen, which serves as receptor for B19V, has been identified on proliferating red blood cell precursors [165] as well as on endothelial cells and fetal myocardial cells [164, 166]. In 1999, Schowengerdt et al. [113] reported the presence of B19V DNA in 1 % of pediatric endomyocardial biopsy samples from children with myocarditis and in 3 % of children with heart rejection, suggesting a possible involvement of the B19V in the pathogenesis of inflammatory heart disease. Investigations in adult patients with inflammatory heart diseases revealed a prevalence of B19V DNA in 19.5 % of patients with myocarditis, 23 % in patients with DCMi and 16 % in patients with DCM [167]. Prevalences for PVB19 genomes detected in patients with myocarditis or DCM ranged from 11 to 56 % in patients with myocarditis and 10–51 % in patients with DCM (see Table 4). As reported for enteroviruses, also, persistence of B19V in patients with LV dysfunction was found to be associated with a progressive impairment of LVEF, whereas spontaneous viral elimination was associated with a significant improvement in LV function [1]. However, in contrast to enteroviruses, spontaneous virus elimination of B19V was observed in only 22 % of patients. These results suggest that persisting cardiac viral infections may constitute a major cause of progressive LV dysfunction in patients with past myocarditis or DCM. Interestingly, it was shown in 24 patients presented with acute onset of angina pectoris and ST segment elevations or T-wave inversion mimicking acute myocardial infarction that histological analysis excluded mostly active or borderline myocarditis, but B19V, EV and ADV genomes were detected in the myocardium of 12, 3 and 2 patients, respectively [169]. Virus genomes were demonstrated in 71 % of patients with normal coronary anatomy, clinically mimicking acute myocardial infarction, an observation which was first published by Bültmann et al. [170]. In a female patient with clinical signs of myocardial infarction and histopathological fulminant myocarditis in situ hybridization studies of the autopsy heart revealed the presence of B19V genomes exclusively in endothelial cells of the smaller intramyocardial vessels. Immunohistochemical stainings exhibited marked expression of E-selectin by endothelial cells, a finding indicative of endothelial dysfunction. These processes are likely to lead to disturbances in the coronary microcirculation and may explain the observation that many patients with B19V-associated myocarditis present with the clinical signs that are typical of ischemic heart disease.

However, the causal relationship of B19V infections with cardiac disease has been questioned, mainly because epidemiological data demonstrated a lifelong persistence of B19V genomes in various organs, apart from the heart

[171, 172] and the fact that B19V DNA was also detected in heart tissue from patients without clinical manifestations of inflammatory cardiomyopathy [171, 173–175].

Nevertheless, parvovirus replication in myocardial endothelial cells was substantiated by the detection of B19V RNA replicative intermediates in the myocardium only of inflamed hearts, whereas viral RNA was not detected in chronic dilated cardiomyopathy without inflammation or in control hearts [4]. On the basis of these data, it was suggested that viral loads of more than 500 genome equivalents per microgram isolated nucleic acid in endomyocardial biopsies are the clinically relevant threshold for the maintenance of myocardial inflammation. In a study of Schmidt-Lucke et al. [176], data were presented supporting the hypothesis that B19V persistence in endothelial cells contributes to ongoing vascular injury which can be improved by immunomodulation using IFNβ. Most recently, an association between LVEF changes during follow-up and the underlying genotype pattern in patients with B19V positive dilated cardiomyopathy was described [5]. Left ventricular function in this small patient cohort improved only in patients with genotype 1, whereas the mean LVEF even decreased in patients with genotype 2. This finding may show that genetic variants of the B19V may influence the course of myocardial function within clinical short-time follow-up. However, the molecular mechanisms responsible for a possible reactivation of B19V, the influence of the immune system triggering B19V replication and immune-independent viral pathogenesis in uninflamed hearts are the remaining gaps in our understanding of B19V pathogenicity in heart diseases [4].

Prevalence of Epstein–Barr virus and human herpesvirus 6 in patients with myocarditis and DCM

In immunocompetent patients, herpesviruses including EBV and HHV6 infections rarely induce cardiac symptoms. For example, EBV-linked acute pericarditis or myocarditis is only reported in some immunocompetent patients [177, 178]. Also, HHV6-induced myocarditis has been published in a small number of patients, but sometimes with a fatal outcome [179, 180]. Investigation into autopsy material showed diffuse myocarditis with a granulocytic and monocytic infiltrate, necrotizing arteritis of the coronary arteries, and fulminant hepatitis with microvesicular steatosis and necrosis together with the detection of HHV6 genome in heart, liver, lung and spleen [179]. In the larger series of patients with inflammatory heart diseases, analyses for HHV6 and EBV were always included (see Tables 5, 6). Prevalences for HHV6 genomes detected in patients with myocarditis or DCM ranged from 8 to 20 % and for EBV genomes from 0 to 8 %. Nevertheless, the pathophysiological mechanisms of herpesviruses in acute myocarditis and especially the possible relevance of HHV-6 reactivation for the development of chronic cardiomyopathies remain to be assessed.

Prevalence of influenza virus RNA in patients with myocarditis and DCM

As stated earlier, several cases of acute myocarditis especially in juvenile patients have been reported in association with pandemic H1N1 influenzavirus infections. Genomes of influenza A/H1N1 virus were detected by RT-PCR analysis in blood as well as in myocardial tissue in patients with a lethal influenzavirus infection [6]. So far, only one systematic investigation linking influenza virus infections with myocarditis was performed. In more than 3,000 patients, influenzavirus genomes were detected in <1 % of patients with inflammatory heart disease [143]. Nevertheless, fulminant myocarditis caused by H1N1 infection seems to be a rare but severe and often lethal complication not only in children [79, 181].

Prevalence of 'double or triple infections' in patients with myocarditis and DCM

In a larger series of patients with myocarditis and dilated cardiomyopathy investigated by Kühl et al. [142] and Kandolf et al. [145], it has been shown that the detection of two or more cardiotropic viruses by PCR in the myocardium is rather common. In a series of 245 patients with DCM, multiple infections were found in 27.3 % of patients. Most often, HHV6 + B19V (10.6 % of cases) and B19V + EV (3.7 % of cases) genomes were amplified in parallel by PCR [142]. Comparably, in a published study of 3,219 patients with cardiac dysfunction and suspected myocarditis in 11.6 % of the 20 % patients with multiple infections, HHV6 and B19V genomes were concurrently detected in the heart [145]. However, there are no data available, whether clinical symptoms or cardiac histopathology differ in patients with multiple infections or whether prognosis in those patients is worse or different when compared to patients with only one virus type in the heart.

Further diagnostic perspectives and conclusions

There is convincing evidence from animal models and investigations in humans that viral infections may induce a significant damage of cardiomyocytes through direct virus-mediated injury and secondary immune reactions, finally leading to chronic myocarditis and dilated cardiomyopathy.

In addition, viral endomyocardial infections have also been reported as an independent predictor of graft loss in pediatric cardiac transplant recipients [182]. However, the question why some individuals develop a severe chronic heart disease upon infection while others do not is still unsolved. Clinically, one-third of the patients with viral myocarditis will spontaneously recover, in one-third, we observe an ongoing disease, and in another third of the cases, the viral heart disease deteriorates. It is important to mention that in a relevant portion of patients, the presence of viral genomes in the myocardium is not associated with inflammatory reactions or signs of heart failure. However, in a part of patients with inflammatory cardiomyopathy, the phenotype of DCM is assumed to be the end stage of a multifactorial virus-induced pathophysiology. Designating factors include genetic predisposition for viral infections and consecutive (auto)immune reactions as well as environmental parameters [183, 184].

In the past years, it has been shown in animal models of viral myocarditis that viruses can trigger the activation of the innate immune system via a family of Toll-like receptors (TLR), which can abrogate the cardiac infection and improve the clinical outcome of the disease by in time release of proinflammatory and antiviral cytokines [26, 185, 186]. There is also firm evidence that dysregulation of Toll-like receptor signaling may contribute to disease progression in heart failure of patients. A rare TLR3 variant was identified in a patient, which was found to alter the innate immune response and to influence host susceptibility resulting in increased cardiac pathology after experimental infection with cardiotropic viruses [187].

In addition to TLR-dependent mechanisms, various other regulatory pathways including the MyD-88/nuclear factor-κB axis, the SOCS system, JAK/STAT and PI3K/Akt depending pathways have been shown to influence the innate and adaptive immune response to enhance host survival, attenuate the virus, or both [185]. In addition to positive regulators of the host defense responses, there are also systems of negative modulators that will counteract an efficient early immunity. Unfortunately, activation of innate immunity can also have adverse effects by production of cytokines and costimulation of T cells, leading to clonal expansion and enhanced inflammatory infiltrates [188]. Excessive and/or prolonged inflammation leads to the paradoxical destruction of the myocardium by expression of cardiotoxic NO, TNFα molecules, etc., leaving the patient probably worse off than without antiviral cellular immune response [32, 46, 185]. In experimental CVB3 myocarditis, it has been shown that excessive activation of the MyD-88/nuclear factor-B adaptor molecule axis leads to increased inflammation and higher mortality rates [189], whereas activation of interferon regulator factor-3 and interferons is protective for the host, thus reducing the mortality rate [25].

In a recent study using a gene array comprising of 59 innate immune genes, 32 patients with ischemic cardiomyopathy (ICM), 26 patients with idiopathic dilated cardiomyopathy (DCM) and 7 patients with viral cardiomyopathy (VCM), as well as 14 non-failing (NF) were investigated [190]. It was shown that different genes and pathways of the innate immune system are differentially expressed which raises the idea that the innate immune system is regulated in different ways in response to the nature of the pathological tissue injury pattern [190].

Despite the exponential growth of information regarding the pathophysiological mechanisms in viral heart disease, our knowledge of viral and genetically defined host factors influencing the outcome of myocarditis is rather limited. The dissection of protective and pathogenic factors determining the efficiency of innate and adaptive immunity in the individual is required to identify hosts being susceptible or resistant for chronic viral heart disease. It is important to state that elimination of different viruses from the heart obviously requires different policies as shown recently. Enteroviruses and adenoviruses but not B19V and HHV6 were successfully eliminated from the heart by treatment of myocarditis patients with IFNβ [191]. Thus, future projects will have to investigate whether antiviral pharmacological or rather immune-modulating approaches will be feasible to interrupt the signaling pathways leading to the development of heart failure in virus-infected patients.

Acknowledgments This work was partially funded by the Federal Ministry of Education and Research 01GI 0205—TP9a to SP and 01EZ0817 to KK and the Deutsche Forschungsgemeinschaft (SFB-TR19) to KK.

References

1. Kühl U, Pauschinger M, Seeberg B, Lassner D, Noutsias M, Poller W, Schultheiss HP (2005) Viral persistence in the myocardium is associated with progressive cardiac dysfunction. Circulation 112:1965–1970
2. Kindermann I, Kindermann M, Kandolf R, Klingel K, Bültmann B, Müller T et al (2008) Predictors of outcome in patients with suspected myocarditis. Circulation 118:639–648
3. Schultz JC, Hilliard AA, Cooper LT Jr, Rihal CS (2009) Diagnosis and treatment of viral myocarditis. Mayo Clin Proc 84:1001–1009
4. Bock CT, Klingel K, Kandolf R (2010) Human parvovirus B19-associated myocarditis. N Engl J Med 362:1248–1249
5. Ruppert V, Meyer T, Balbach A, Richter A, Müller HH, Maisch B, Pankuweit S (2011) German heart failure network. Genotype-specific effects on left ventricular function in parvovirus B19-positive patients with dilated cardiomyopathy. J Med Virol 83:1818–1825
6. Gdynia G, Schnitzler P, Brunner E, Kandolf R, Bläker H, Daum E, Schnabel P, Schirmacher P, Roth W (2011) Sudden death of an immunocompetent young adult caused by novel (swine origin) influenza A/H1N1-associated myocarditis. Virchows Arch 458:371–376

7. Barbaro G (2005) HIV-associated myocarditis. Heart Fail Clin 1:439–448 (Review)
8. Esfandiarei M, McManus BM (2008) Molecular biology and pathogenesis of viral myocarditis. Annu Rev Pathol Mech Dis 3:127–155 (Review)
9. Kandolf R, Klingel K, Mertsching H, Canu A, Hohenadl C, Zell R, Reimann BY, Heim A, McManus BM, Foulis AK et al (1991) Molecular studies on enteroviral heart disease: patterns of acute and persistent infections. Eur Heart J 12(Suppl D):49–55 (Review)
10. Klingel K, Rieger P, Mall G, Selinka HC, Huber M, Kandolf R (1998) Visualization of enteroviral replication in myocardial tissue by ultrastructural in situ hybridization: identification of target cells and cytopathic effects. Lab Invest 78:1227–1237
11. McManus BM, Chow LH, Wilson JE, Anderson DR, Gulizia JM, Gauntt CJ, Klingel KE, Beisel KW, Kandolf R (1993) Direct myocardial injury by enterovirus: a central role in the evolution of murine myocarditis. Clin Immunol Immunopathol 68:159–169
12. Jahns R, Boivin V, Hein L, Triebel S, Angermann CE, Ertl G, Lohse MJ (2004) Direct evidence for a beta1-adrenergic receptor-directed autoimmune attack as a cause of idiopathic dilated Cardiomyopathy. J Clin Invest 113:1419–1429
13. Caforio AL, Tona F, Bottaro S, Vinci A, Dequal G, Daliento L, Thiene G, Iliceto S (2008) Clinical implications of anti-heart autoantibodies in myocarditis and dilated cardiomyopathy. Autoimmunity 41(1):35–45 (Review)
14. Ogata M, Satou T, Kawano R, Takakura S, Goto K, Ikewaki J, Kohno K, Ikebe T, Ando T, Miyazaki Y, Ohtsuka E, Saburi Y, Saikawa T, Kadota J (2010) Correlations of HHV-6 viral load and plasma IL-6 concentration with HHV-6 encephalitis in allogeneic stem cell transplant recipients. Bone Marrow Transpl 45:129–136
15. Klingel K, Hohenadl C, Canu A, Albrecht M, Seemann M, Mall G, Kandolf R (1992) Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: quantitative analysis of virus replication, tissue damage, and inflammation. Proc Natl Acad Sci USA 89:314–318
16. Shi Y, Chen C, Lisewski U, Wrackmeyer U, Radke M, Westermann D, Sauter M, Tschöpe C, Poller W, Klingel K, Gotthardt M (2009) Cardiac deletion of the Coxsackievirus-adenovirus receptor abolishes Coxsackievirus B3 infection and prevents myocarditis in vivo. J Am Coll Cardiol 53:1219–1226
17. Herzum M, Ruppert V, Küytz B, Jomaa H, Nakamura I, Maisch B (1994) Coxsackievirus B3 infection leads to cell death of cardiac myocytes. J Mol Cell Cardiol 26:907–913
18. Chow LH, Beisel KW, McManus BM (1992) Enteroviral infection of mice with severe combined immunodeficiency. Evidence for direct viral pathogenesis of myocardial injury. Lab Invest 66:24–31
19. Wessely R, Klingel K, Santana LF, Dalton N, Hongo M, Jonathan Lederer W, Kandolf R, Knowlton KU (1998) Transgenic expression of replication-restricted enteroviral genomes in heart muscle induces defective excitation-contraction coupling and dilated cardiomyopathy. J Clin Invest 102:1444–1453
20. Badorff C, Lee GH, Lamphear BJ et al (1999) Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. Nat Med 5:320–326
21. Luo H, Zhang J, Cheung C, Suarez A, McManus BM, Yang D (2003) Proteasome inhibition reduces coxsackievirus B3 replication in murine cardiomyocytes. Am J Pathol 163:381–385
22. Esfandiarei M, Suarez A, Amaral A, Si X, Rahmani M, Dedhar S, McManus BM (2006) Novel role for integrin-linked kinase in modulation of coxsackievirus B3 replication and virus-induced cardiomyocyte injury. Circ Res 99:354–361
23. Wong J, Zhang J, Si X, Gao G, Mao I, McManus BM, Luo H (2008) Autophagosome supports coxsackievirus B3 replication in host cells. J Virol 82:9143–9153
24. Wessely R, Klingel K, Knowlton KU, Kandolf R (2001) Cardioselective infection with coxsackievirus B3 requires intact type I interferon signaling: implications for mortality and early viral replication. Circulation 103:756–761
25. Deonarain R, Cerullo D, Fuse K, Liu PP, Fish EN (2004) Protective role for interferon-beta in coxsackievirus B3 infection. Circulation 110:3540–3543
26. Weinzierl AO, Szalay G, Wolburg H, Sauter M, Rammensee HG, Kandolf R, Stevanović S, Klingel K (2008) Effective chemokine secretion by dendritic cells and expansion of cross-presenting CD4-/CD8+ dendritic cells define a protective phenotype in the mouse model of coxsackievirus myocarditis. J Virol 82:8149–8160
27. Riad A, Westermann D, Escher F, Becher PM, Savvatis K, Lettau O, Heimesaat MM, Bereswill S, Volk HD, Schultheiss HP, Tschöpe C (2010) Myeloid differentiation factor-88 contributes to TLR9-mediated modulation of acute coxsackievirus B3-induced myocarditis in vivo. Am J Physiol Heart Circ Physiol 298(6):H2024–H2031
28. Klingel K, Schnorr JJ, Sauter M, Szalay G, Kandolf R (2003) beta2-microglobulin-associated regulation of interferon-gamma and virus-specific immunoglobulin G confer resistance against the development of chronic coxsackievirus myocarditis. Am J Pathol 162:1709–1720
29. Opavsky MA, Penninger J, Aitken K, Wen WH, Dawood F, Mak T, Liu P (1999) Susceptibility to myocarditis is dependent on the response of alphabeta T lymphocytes to coxsackieviral infection. Circ Res 85:551–558
30. 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 155:1205–1215
31. Frisancho-Kiss S, Coronado MJ, Frisancho JA, Lau VM, Rose NR, Klein SL, Fairweather D (2009) Gonadectomy of male BALB/c mice increases Tim-3(+) alternatively activated M2 macrophages, Tim-3(+) T cells, Th2 cells and Treg in the heart during acute coxsackievirus-induced myocarditis. Brain Behav Immun 23:649–657
32. Szalay G, Sauter M, Hald J, Weinzierl A, Kandolf R, Klingel K (2006) Sustained nitric oxide synthesis contributes to immunopathology in ongoing myocarditis attributable to interleukin-10 disorders. Am J Pathol 169:2085–2093
33. Li K, Xu W, Guo Q, Jiang Z, Wang P, Yue Y, Xiong S (2009) Differential macrophage polarization in male and female BALB/c mice infected with coxsackievirus B3 defines susceptibility to viral myocarditis. Circ Res 105:353–364
34. Cheung C, Luo H, Yanagawa B, Leong HS, Samarasekera D, Lai JC, Suarez A, Zhang J, McManus BM (2006) Matrix metalloproteinases and tissue inhibitors of metalloproteinases in coxsackievirus-induced myocarditis. Cardiovasc Pathol 15:63–74
35. Cheung C, Marchant D, Walker EK, Luo Z, Zhang J, Yanagawa B, Rahmani M, Cox J, Overall C, Senior RM, Luo H, McManus BM (2008) Ablation of matrix metalloproteinase-9 increases severity of viral myocarditis in mice. Circulation 117:1574–1582
36. Heymans S, Pauschinger M, De Palma A, Kallwellis-Opara A, Rutschow S, Swinnen M, Vanhoutte D, Gao F, Torpai R, Baker AH, Padalko E, Neyts J, Schultheiss HP, Van de Werf F, Carmeliet P, Pinto YM (2006) Inhibition of urokinase-type plasminogen activator or matrix metalloproteinases prevents cardiac injury and dysfunction during viral myocarditis. Circulation 114:565–573

37. Szalay G, Sauter M, Haberland M, Zuegel U, Steinmeyer A, Kandolf R, Klingel K (2009) Osteopontin: a fibrosis-related marker molecule in cardiac remodeling of enterovirus myocarditis in the susceptible host. Circ Res 104:851–859
38. Lang C, Sauter M, Szalay G, Racchi G, Grassi G, Rainaldi G, Mercatanti A, Lang F, Kandolf R, Klingel K (2008) Connective tissue growth factor: a crucial cytokine-mediating cardiac fibrosis in ongoing enterovirus myocarditis. J Mol Med (Berl) 86:49–60
39. Rother M, Krohn S, Kania G, Vanhoutte D, Eisenreich A, Wang X, Westermann D, Savvatis K, Dannemann N, Skurk C, Hilfiker-Kleiner D, Cathomen T, Fechner H, Rauch U, Schultheiss HP, Heymans S, Eriksson U, Scheibenbogen C, Poller W (2010) Matricellular signaling molecule CCN1 attenuates experimental autoimmune myocarditis by acting as a novel immune cell migration modulator. Circulation 122:2688–2698
40. Cunningham MW (2004) T cell mimicry in inflammatory heart disease. Mol Immunol 40:1121–1127
41. Li Y, Heuser JS, Cunningham LC et al (2006) Mimicry and antibody-mediated cell signaling in autoimmune myocarditis. J Immunol 177:8234–8240
42. Fousteri G, Dave A, Morin B, Omid S, Croft M, von Herrath MG (2011) Nasal cardiac myosin peptide treatment and OX40 blockade protect mice from acute and chronic virally-induced myocarditis. J Autoimmun 36:210–220
43. Masek-Hammerman K, Miller AD, Lin KC, Mackey J, Weissenböck H, Gierbolini L, Burgos A, Perez H, Mansfield KG. (2011) Epizootic myocarditis associated with encephalomyocarditis virus in a group of rhesus macaques (Macacamulatta). Vet Pathol 28 [Epub ahead of print]
44. Oberste MS, Gotuzzo E, Blair P, Nix WA, Ksiazek TG, Comer JA, Rollin P, Goldsmith CS, Olson J, Kochel TJ (2009) Human febrile illness caused by encephalomyocarditis virus infection, Peru. Emerg Infect Dis 15:640–646
45. Hardarson HS, Baker JS, Yang Z, Purevjav E, Huang CH, Alexopoulou L, Li N, Flavell RA, Bowles NE, Vallejo JG (2007) Toll-like receptor 3 is an essential component of the innate stress response in virus-induced cardiac injury. Am J Physiol Heart Circ Physiol 292:H251–H258
46. Yamada T, Matsumori A, Sasayama S (1994) Therapeutic effect of anti-tumor necrosis factor-alpha antibody on the murine model of viral myocarditis induced by encephalomyocarditis virus. Circulation 89:846–851
47. Tanaka T, Kanda T, McManus BM, Kanai H, Akiyama H, Sekiguchi K, Yokoyama T, Kurabayashi M (2001) Overexpression of interleukin-6 aggravates viral myocarditis: impaired increase in tumor necrosis factor-alpha. J Mol Cell Cardiol 33:1627–1635
48. Higuchi H, Hara M, Yamamoto K, Miyamoto T, Kinoshita M, Yamada T, Uchiyama K, Matsumori A (2008) Mast cells play a critical role in the pathogenesis of viral myocarditis. Circulation 118:363–372
49. Huang CH, Vallejo JG, Kollias G, Mann DL (2009) Role of the innate immune system in acute viral myocarditis. Basic Res Cardiol 104:228–237
50. Takahashi T, Zhu SJ, Sumino H, Saegusa S, Nakahashi T, Iwai K, Morimoto S, Kanda T (2005) Inhibition of cyclooxygenase-2 enhances myocardial damage in a mouse model of viral myocarditis. Life Sci 78:195–204
51. Matsumori A (2007) Treatment options in myocarditis: what we know from experimental data and how it translates to clinical trials. Herz 32:452–456 (Review)
52. Sherry B, Li XY, Tyler KL, Cullen JM, Virgin HW 4th (1993) Lymphocytes protect against and are not required for reovirus-induced myocarditis. J Virol 67:6119–6124
53. Sherry B, Baty CJ, Blum MA (1996) Reovirus-induced acute myocarditis in mice correlates with viral RNA synthesis rather than generation of infectious virus in cardiac myocytes. J Virol 70:6709–6715
54. Sherry B, Torres J, Blum MA (1998) Reovirus induction of and sensitivity to beta interferon in cardiac myocyte cultures correlate with induction of myocarditis and are determined by viral core proteins. J Virol 72:1314–1323
55. Virgin HW, Dermody TS, Tyler KL (1998) Cellular and humoral immunity to reovirus infection. Curr Top Microbiol Immunol 233:147–161 (Review)
56. Edelmann KH, Richardson-Burns S, Alexopoulou L, Tyler KL, Flavell RA, Oldstone MB (2004) Does Toll-like receptor 3 play a biological role in virus infections? Virology 322:231–238
57. Holm GH, Zurney J, Tumilasci V, Leveille S, Danthi P, Hiscott J, Sherry B, Dermody TS (2007) Retinoic acid-inducible gene-I and interferon-beta promoter stimulator-1 augment proapoptotic responses following mammalian reovirus infection via interferon regulatory factor-3. J Biol Chem 282:21953–21961
58. O'Donnell SM, Holm GH, Pierce JM, Tian B, Watson MJ, Chari RS, Ballard DW, Brasier AR, Dermody TS (2006) Identification of an NF-kappaB-dependent gene network in cells infected by mammalian reovirus. J Virol 80:1077–1086
59. Holm GH, Pruijssers AJ, Li L, Danthi P, Sherry B, Dermody TS (2010) Interferon regulatory factor 3 attenuates reovirus myocarditis and contributes to viral clearance. J Virol 84:6900–6908
60. Li L, Sevinsky JR, Rowland MD, Bundy JL, Stephenson JL, Sherry B (2010) Proteomic analysis reveals virus-specific Hsp25 modulation in cardiac myocytes. J Proteome Res 9:2460–2471
61. Miyamoto SD, Brown RD, Robinson BA, Tyler KL, Long CS, Debiasi RL (2009) Cardiac cell-specific apoptotic and cytokine responses to reovirus infection: determinants of myocarditic phenotype. J Card Fail 15:529–539
62. DeBiasi RL, Robinson BA, Leser JS, Brown RD, Long CS, Clarke P (2010) Critical role for death-receptor mediated apoptotic signaling in viral myocarditis. J Card Fail 16:901–910
63. Bowles NE, Ni J, Kearney DL, Pauschinger M, Schultheiss HP, McCarthy R, Hare J, Bricker JT, Bowles KR, Towbin JA (2003) Detection of viruses in myocardial tissues by polymerase chain reaction. Evidence of adenovirus as a common cause of myocarditis in children and adults. J Am Coll Cardiol 42:466–472
64. Blailock ZR, Rabin ER, Melnick JL (1968) Adenovirus myocarditis in mice. An electron microscopic study. Exp Mol Pathol 9:84–96
65. Kajon AE, Brown CC, Spindler KR (1998) Distribution of mouse adenovirus type 1 in intraperitoneally and intranasally infected adult outbred mice. J Virol 72:1219–1223
66. Guida JD, Fejer G, Pirofski LA, Brosnan CF, Horwitz MS (1995) Mouse adenovirus type 1 causes a fatal hemorrhagic encephalomyelitis in adult C57BL/6 but not BALB/c mice. J Virol 69:7674–7681
67. Ashley SL, Welton AR, Harwood KM, Van Rooijen N, Spindler KR (2009) Mouse adenovirus type 1 infection of macrophages. Virology 390:307–314
68. Nie X, Zhang G, Xu D, Sun X, Li Z, Li X, Zhang X, He F, Li Y (2010) The VP1-unique region of parvovirus B19 induces myocardial injury in mice. Scand J Infect Dis 42:121–128
69. Tzang BS, Lin TM, Tsai CC, Hsu JD, Yang LC, Hsu TC (2011) Increased cardiac injury in NZB/W F1 mice received antibody against human parvovirus B19 VP1 unique region protein. Mol Immunol 48:1518–1524
70. Hoelzer K, Parrish CR (2010) The emergence of parvoviruses of carnivores. Vet Res 41:39
71. Meurs KM, Fox PR, Magnon AL, Liu S, Towbin JA (2000) Molecular screening by polymerase chain reaction detects panleukopenia virus DNA in formalin-fixed hearts from cats with

idiopathic cardiomyopathy and myocarditis. Cardiovasc Pathol 9:119–126
72. Agungpriyono DR, Uchida K, Tabaru H, Yamaguchi R, Tateyama S (1999) Subacute massive necrotizing myocarditis by canine parvovirus type 2 infection with diffuse leukoencephalomalacia in a puppy. Vet Pathol 36:77–80
73. Kawai C (1999) From myocarditis to cardiomyopathy: mechanisms of inflammation and cell death: learning from the past for the future. Circulation 99:1091–1100 (Review)
74. Häusler M, Sellhaus B, Scheithauer S, Gaida B, Kuropka S, Siepmann K, Panek A, Berg W, Teubner A, Ritter K, Kleines M (2007) Myocarditis in newborn wild-type BALB/c mice infected with the murine gamma herpesvirus MHV-68. Cardiovasc Res 76:323–330
75. Lenzo JC, Fairweather D, Cull V, Shellam GR, James Lawson CM (2002) Characterisation of murine cytomegalovirus myocarditis: cellular infiltration of the heart and virus persistence. J Mol Cell Cardiol 34:629–640
76. Ritter JT, Tang-Feldman YJ, Lochhead GR, Estrada M, Lochhead S, Yu C, Ashton-Sager A, Tuteja D, Leutenegger C, Pomeroy C (2010) In vivo characterization of cytokine profiles and viral load during murine cytomegalovirus-induced acute myocarditis. Cardiovasc Pathol 19:83–93
77. Cull VS, Bartlett EJ, James CM (2002) Type I interferon gene therapy protects against cytomegalovirus-induced myocarditis. Immunology 106:428–437
78. Mamas MA, Fraser D, Neyses L (2008) Cardiovascular manifestations associated with influenza virus infection. Int J Cardiol 130:304–309
79. Bratincsák A, El-Said HG, Bradley JS, Shayan K, Grossfeld PD, Cannavino CR (2010) Fulminant myocarditis associated with pandemic H1N1 influenza A virus in children. J Am Coll Cardiol 55:928–929
80. Pan HY, Yamada H, Chida J, Wang S, Yano M, Yao M, Zhu J, Kido H (2011) Up-regulation of ectopic trypsins in the myocardium by influenza A virus infection triggers acute myocarditis. Cardiovasc Res 89:595–603
81. Woo GH, Kim HY, Bae YC, Jean YH, Bak EJ, Kim MJ, Hwang EK, Joo YS (2011) Comparative histopathological characteristics of highly pathogenic avian influenza (HPAI) in chickens and domestic ducks in 2008 Korea. Histol Histopathol 26:167–175
82. Gabriel G, Klingel K, Planz O, Bier K, Herwig A, Sauter M, Klenk HD (2009) Spread of infection and lymphocyte depletion in mice depends on polymerase of influenza virus. Am J Pathol 175:1178–1186
83. Gabriel G, Klingel K, Otte A, Thiele S, Hudjetz B, Arman-Kalcek G, Sauter M, Shmidt T, Rother F, Baumgarte S, Keiner B, Hartmann E, Bader M, Brownlee GG, Fodor E, Klenk HD (2011) Differential use of importin-α isoforms governs cell tropism and host adaptation of influenza virus. Nat Commun 2:156
84. Durando MM, Birks EK, Hussey SB, Lunn DP (2011) Cardiac troponin I concentrations in ponies challenged with equine influenza virus. J Vet Intern Med 25:339–344
85. Haugland O, Mikalsen AB, Nilsen P, Lindmo K, Thu BJ, Eliassen TM, Roos N, Rode M, Evensen O (2011) Cardiomyopathy syndrome of atlantic salmon (Salmo salar L.) is caused by a double-stranded RNA virus of the Totiviridae family. J Virol 85:5275–5286
86. Fiedler A (1900) Über akute interstitielle Myokarditis. Zentralbl f Med 21:212–213 (Cited by Scott RW, Saphir O (1929–30) Acute isolated myocarditis. Am Heart J 5:129–141.)
87. Woodruff JF (1980) Viral Myocarditis. Am J Pathol 101:428–479
88. Sainani GS, Dekate MP, Rao CP (1975) Heart disease caused by Coxsackie virus B infection. Br Heart J 37:819–823
89. Gordon RB, Lennette EH, Sandrock RS (1959) The varied clinical manifestations of Coxsackie virus infections. Arch Int Med 103:63–75
90. Null FC, Castle CH (1959) Adult pericarditis and myocarditis due to coxsackie virus, group B, type 5. N Engl J Med 26i: 937–942
91. Smith WG (1970) Coxsackie B myocarditis in adults. Am Heart J 80:34–46
92. Bell EJ, Grist NR (1970) Further studies of enterovirus infections in cardiac disease and pleurodynia. Scan J Infect Dis 2:1–6
93. Lerner AM, Wilson FM (1973) Virus myocardiomyopathy. Prog Med Virol 15:63–91
94. Medearis DN Jr (1957) Cytomegalic inclusion disease: an analysis of the clinical features based on the literature and 6 additional cases. Pediatrics 19:467–480
95. Lenette DA, Specter S, Thompson KD (eds) (1979) Diagnosis of viral infections: The role of clinical laboratory. University Park Press, Baltimore
96. Grist NR, Reid D (1993) Epidemiology of viral infections of the heart. In: Banatvala JE (ed) Viral infections of the heart. Hodder and Stoughton, London, pp 23–31
97. Mahfoud F, Gärtner B, Kindermann M, Ukena C, Gadomski K, Klingel K, Kandolf R, Böhm M, Kindermann I (2011) Virus serology in patients with suspected myocarditis: utility or futility? Eur Heart J 32:897–903
98. Sakakibara S, Konno S (1962) Endomyocardial biopsy. Jpn Heart J 3:537–543
99. Saiki RK, Scharf S, Faloona F, Mullis KB, Horn GT, Ehrlich HA, Arnheim N (1985) Enzymatic amplification of β3-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. Science 230:1350–1354
100. Pankuweit S, Portig I, Eckhardt H, Crombach M, Hufnagel G, Maisch B (2000) Prevalence of viral genome in endomyocardial biopsies from patients with inflammatory heart muscle disease. Herz 25(3):221–226
101. Jackson DP, Lewis FA, Taylor GR, Boylston AW, Quirke P (1990) Tissue extraction of DNA and RNA and analysis by polymerase chain reaction. J Clin Pathol 43:499–504
102. Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162:156–159
103. Maniatis T, Fritsch EF, Sambrook J (1992) Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor
104. Rotbart HA (1990) Enzymatic RNA amplification of enteroviruses. J Clin Microbiol 28:438–442
105. Martin AB, Webber S, Fricker J, Jaffe R et al (1994) Acute myocarditis: rapid diagnosis in children. Circulation 90:330–339
106. Kandolf R, Ameis D, Kirschner P, Canu A, Hofschneider PH (1987) In situ detection of enteroviral genomes in myocardial cells by nucleic acid hybridization: an approach to the diagnosis of viral heart disease. Proc Natl Acad Sci USA 84:6272–6276
107. Klingel K, Stephan S, Sauter M, Zell R et al (1996) Pathogenesis of murine enterovirus myocarditis: virus dissemination and immune target cells. J Virol 70:8888–8895
108. Klump WM, Bergmann I, Müller BC, Ameis D, Kandolf R (1990) Complete nucleotide sequence of infectious coxsackievirus B3 cDNA: two initial 5′ uridine residues are regained during plus-strand RNA synthesis. J Virol 64:1573–1583
109. Archard L, Khan M, Soteriou BA et al (1997) Characterisation of coxsackie B virus RNA in myocardium from patients with dilated cardiomyopathy by nucleotide sequencing of reverse transcription-nested polymerase chain reaction products. Hum Pathol 29:578–584
110. Chapman NM, Tracy S, Gauntt CJ, Fortmüller U (1990) Molecular detection and identification of enteroviruses using

enzymatic amplification and nucleic acid hybridisation. J Clin Microbiol 28:843–850
111. Grasso M, Arbustini E, Silini E et al (1992) Search for Coxsackievirus B3 RNA in idiopathic dilated cardiomyopathy using gene amplification by polymerase chain reaction. Am J Cardiol 69:658–664
112. Tracy S, Chapman NM, McManus BM et al (1990) A molecular and serologic evaluation of enteroviral involvement in human myocarditis. J Mol Cell Cardiol 22:403–414
113. Schowengerdt K, Ni J, Denfield S et al (1997) Association of parvovirus B19 genome in children with myocarditis and cardiac allograft rejection. Diagnosis using the polymerase chain reaction. Circulation 96:3549–3554
114. Piiparinen H, Vaheri A (1991) Genotyping of herpes simplex viruses by polymerase chain reaction. Arch Virol 119:541–548
115. Weiss SM, Roblin PM, Gaydos CA et al (1996) Failure to detect chlamydia pneumoniae in coronary atheromas of patients undergoing atherectomy. J Infect Dis 173:957–962
116. Goodman JL, Jurkovic P, Kramber JM, Johnson RC (1991) Molecular detection of persistent borrelia burgdorferi in the urine of patients with active Lyme disease. Infect Immunol 59: 269–278
117. Stanek G, Klein J, Bitnner R, Glogar D (1990) Isolation of borrelia burgdorferi from the myocardium of a patient with longstanding cardiomyopathy. N Engl J Med 322:249–252
118. Nakhleh RE, Copenhaver CM, Werdin K (1991) Lack of evidence for involvment of Epstein-Barr virus in the development of 'Quilty' lesion of transplanted hearts: an in situ hybridization study. J Heart Lung Transpl 10:504–507
119. Klingel K, Sauter M, Bock CT, Szalay G, Schnorr JJ, Kandolf R (2004) Molecular pathology of inflammatory cardiomyopathy. Med Microbiol Immunol (Berl) 193(2–3):101–107
120. Kandolf R, Hofschneider PH (1985) Molecular cloning of the genome of a cardiotropic coxsackie B3 virus: full-length reverse-transcribed recombinant cDNA generates infectious virus in mammalian cells. Proc Natl Acad Sci USA 82: 4818–4822
121. Manaresi E, Gallinella G, Zuffi E, Bonvicini F, Zerbini M, Musiani M (2002) Diagnosis and quantitative evaluation of parvovirus B19 infections by real-time PCR in the clinical laboratory. J Med Virol 67:275–281
122. Harder TC, Hufnagel M, Zahn K, Beutel K, Schmitt H-J, Ullmann U et al (2001) New LightCycler PCR for rapid and sensitive quantification of parvovirus B19 DNA guides therapeutic decision-making in relapsing infections. J Clin Microbiol 39: 4413–4419
123. Aberham C, Pendl C, Gross P, Zerlauth G, Gessner M (2001) A quantitative, internally controlled real-time PCR Assay for the detection of parvovirus B19 DNA. J Virol Methods 92:183–191
124. Gruber F, Falkner FG, Dorner F, Hammerle T (2001) Quantitation of viral DNA by real-time PCR applying duplex amplification, internal standardization, and two-color fluorescence detection. Appl Environ Microbiol 67:2837–2839
125. Servant A, Laperche S, Lallemand F, Marinho V, De Maur G, Meritet JF, Garbarg-Chenon A (2002) Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 76:9124–9134
126. Hokynar K, Soderlund-Venermo M, Pesonen M, Ranki A, Kiviluoto O, Partio EK, Hedman K (2002) A new parvovirus genotype persistent in human skin. Virology 302:224–228
127. Nguyen QT, WongS HeegaardED, Brown KE (2002) Identification and characterization of a second novel human erythrovirus variant A6. Virology 301:374–380
128. Heegaard ED, Brown KE (2002) Human parvovirus B 19. Clin Microbiol Rev 15:485–505
129. Liefeldt L, Plentz A, Klempa B, Kershaw O, Endres AS, Raab U, Neumayer HH, Meisel H, Modrow S (2005) Recurrent high level parvovirus B19/genotype 2 viremia in a renal transplant recipient analyzed by real-time PCR for simultaneous detection of genotypes 1 to 3. J Med Virol 75(1):161–169
130. Yajima T, Knowlton KU (2009) Viral myocarditis: from the perspective of the virus. Circulation 119:2615–2624
131. Bowles NE, Rose ML, Taylor P et al (1989) End-stage dilated cardiomyopathy persistence of enterovirus RNA in myocardium at cardiac transplantation and lack of immune response. Circulation 80:1128–1136
132. Tracy S, Wiegand V, McManus B et al (1990) Molecular approaches to enteroviral diagnosis in idiopathic cardiomyopathy and myocarditis. JACC 15:1688–1694
133. Jin O, Sole M, Butany JW et al (1990) Detection of enterovirus RNA in myocardial biopsies from patients with myocarditis and cardiomyopathy using gene amplification by polymerase chain reaction. Circulation 82:8–16
134. Weiss L, Mohaved L, Billingham ME, Cleary ML (1991) Detection of coxsackie B3 RNA in myocardial tissues by polymerase chain reaction. Am J Pathol 138:497–503
135. Kandolf R, Klingel K, Zell R et al (1993) Molecular pathogenesis of enterovirus-induced myocarditis: virus persistence and chronic inflammation. Intervirology 35:140–150
136. Petitjan J, Kopecka H, Freymuth F et al (1992) Detection of enteroviruses in endomyocardial biopsy by molecular approach. J Med Virol 37:76–82
137. Pauschinger M, Dörner G, Meissner G et al (1994) Enteroviral RNA detection by polymerase chain reaction (PCR) in cardiac muscle biopsies of patients with myocarditis or dilated cardiomyopathy. J Am Coll Cardiol 2:880–884
138. Bowles N, Towbin J (1996) Viral heart muscle disease in children. Newslett Sci Counc Cardiomyopathies 11:4–5
139. Talwar KK, Thatai D, Kannath P et al (1996) Enteroviral detection by polymerase chain reaction technique in endomyocardial biopsy specimen: an initial study. Indian Heart J 48: 479–480
140. Maisch B, Schönian U, Hufnagel G, Pankuweit S (1997) Enteroviral persistence in myocarditis and dilated cardiomyopathy. Eur Heart J 18:593
141. Pauschinger M, Phan MD, Dörner A et al (1997) Detection of enteroviral RNA replicative intermediates in endomyocardial biopsies: indication of viral replication in the human heart. Eur Heart J 18:594A
142. Kuhl U, Pauschinger M, Noutsias M, Seeberg B, Bock T, Lassner D, Poller W, Kandolf R, Schultheiss HP (2005) High prevalence of viral genomes and multiple viral infections in the myocardium of adults with idiopathic left ventricular dysfunction. Circulation 111:887–893
143. Pankuweit S, Ruppert V, Eckhardt H, Strache D, Maisch B (2005) Pathophysiology and etiological diagnosis of inflammatory myocardial diseases with a special focus on parvovirus B 19. J Vet Med B Infect Dis Vet Public Health 52(7–8):344–347
144. Mahrholdt H, Wagner A, Deluigi CC, Kispert E, Hager S, Meinhardt G, Vogelsberg H, Fritz P, Dippon J, Bock CT, Klingel K, Kandolf R, Sechtem U (2006) Presentation, patterns of myocardial damage, and clinical course of viral myocarditis. Circulation 114(15):1581–1590
145. Kandolf R, Bültmann B, Klingel K, Bock CT (2008) Molecular mechanisms and consequences of cardiac viral infections. Pathologe 29(Suppl 2):112–117
146. Pauschinger M, Dörner A, Kühl U et al (1999) Enteroviral replication in the myocardium of patients with left ventricular dysfunction and clinically suspected myocarditis. Circulation 99:889–895

147. Chapman M, Kim KS (2008) Persistent coxsackievirus infection: enterovirus persistence in chronic myocarditis and dilated cardiomyopathy. Curr Top Microbiol Immunol 323:275–292
148. Kytö V, Vuorinen T, Saukko P, Lautenschlager I, Lignitz E, Saraste A, Voipio-Pulkki LM (2005) Cytomegalovirus infection of the heart is common in patients with fatal myocarditis. Clin Infect Dis 40(5):683–688
149. Maisch B, Schönian U, Crombach M et al (1993) Cytomegalovirus associated inflammatory heart muscle disease. Scan J Infect Dis suppl 88:135–148
150. Schönian U, Crombach M, Maisch B (1993) Assessment of cytomegalovirus DNA and protein expression in patients with myocarditis. Clin Immun Immunpathol 68:229–233
151. Towbin JA (1996) Diagnosis and management of myocarditis in children. Newsletter of the Scientific Council on Cardiomyopathies 11:8
152. Maisch B, Pausch R, Hufnagel G, Schönian U (1997) The neglected role of DNA viruses in chronic myocarditis and dilated cardiomyopathy. Eur Heart J 18:515
153. Bergelson JM, Cunningham JA, Droguett G et al (1997) Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. Science 275:1320–1323
154. Tomko RP, Xu R, Philipson L (1997) HCAR and MCAR: the human and mouse cellular receptors for subgroup C adenoviruses and group B coxsackieviruses. Proc Natl Acad Sci USA 94:3352–3356
155. Hufnagel G, Busse C, Ische B, Maisch B (1997) Detection of adenoviral DNA in endomyocardial biopsies of adult patients with myocarditis and dilated cardiomyopathy. Eur Heart J 18:515
156. Pauschinger M, Bowles NE, Fuentes-Garcia FJ et al (1999) Detection of adenoviral genome in the myocardium of adult patients with idiopathic left ventricular dysfunction. Circulation 99:1348–1354
157. Rohayem J, Dinger J, Fischer R et al (2001) Fatal myocarditis associated with acute parvovirus B19 and human herpesvirus 6 coinfection. J Clin Microbiol 39:4585–4587
158. Heegaard ED, Eiskjaer H, Baandrup U et al (1998) Case report: parvovirus B19 infection associated with myocarditis following adult cardiac transplantation. Scand J Infect Dis 30:607–610
159. Chia JKS (1996) Myopericarditis due to parvovirus B19 in an adult. Clin Infect Dis 23:200–201
160. Schwarz TF, Wiersbitzky S, Pambor M (1994) Case report: detection of parvovirus B19 in a skin biopsy of a patient with erythema infectiosum. J Med Virol 43:171–174
161. Gabriel SE, Espy M, Erdman DD et al (1999) The role of parvovirus B19 in the pathogenesis of giant cell arteritis. Arthritis Rheum 42:1255–1258
162. Nigro G, Bastianon V, Colloridi V et al (2000) Human parvovirus B19 infection in infancy associated with acute and chronic lymphocytic myocarditis and high cytokine levels: report of 3 cases and review. Clin Infect Dis 31:65–69
163. Sokal EM, Melchior M, Cornu C et al (1998) Acute parvovirus B19 infection associated with fulminant hepatitis of favourable prognosis in young children. Lancet 352:1739–1741
164. Enders M, Klingel K, Weidner A, Baisch C, Kandolf R, Schalasta G, Enders G (2010) Risk of fetal hydrops and non-hydropic late intrauterine fetal death after gestational parvovirus B19 infection. J Clin Virol 49:163–168
165. Brown KE, Anderson SM, Young NS (1993) Erythrocyte P antigen: cellular receptor for B19 parvovirus. Science 262:114–117
166. Porter HJ, Quantrill AM, Fleming KA (1998) B19 parvovirus infection of myocardial cells. Lancet 1:535–536
167. Pankuweit S, Moll R, Baantrup U, Portig I, Hufnagel G, Maisch B (2003) Prevalence of the parvovirus B19 genome in endomyocardial biopsy specimens. Hum Pathol 34:497–500
168. Klein RM, Jiang H, Niederacher D, Adams O, Du M, Horlitz M, Schley P, Marx R, Lankisch MR, Brehm MU, Strauer BE, Gabbert HE, Scheffold T, Gülker H (2004) Frequency and quantity of the parvovirus B19 genome in endomyocardial biopsies from patients with suspected myocarditis or idiopathic left ventricular dysfunction. Z Kardiol 93(4):300–309
169. Kühl U, Pauschinger M, Bock T, Klingel K, Schwimmbeck CP, Seeberg B, Krautwurm L, Poller W, Schultheiss HP, Kandolf R (2003) Parvovirus B19 infection mimicking acute myocardial infarction. Circulation 108(8):945–950
170. Bültmann BD, Klingel K, Sotlar K, Bock CT, Baba HA, Sauter M, Kandolf R (2003) Fatal parvovirus B19-associated myocarditis clinically mimicking ischemic heart disease: an endothelial cell-mediated disease. Hum Pathol 34:92–95
171. Kuethe F, Lindner J, Matschke K, Wenzel JJ, Norja P, Ploetze K, Schaal S, Kamvissi V, Bornstein SR, Schwanebeck U, Modrow S (2009) Prevalence of parvovirus B19 and human bocavirus DNA in the heart of patients with no evidence of dilated cardiomyopathy or myocarditis. Clin Infect Dis 49: 1660–1666
172. Lotze U, Egerer R, Glück B, Zell R, Sigusch H, Erhardt C, Heim A, Kandolf R, Bock T, Wutzler P, Figulla HR (2010) Low level myocardial parvovirus B19 persistence is a frequent finding in patients with heart disease but unrelated to ongoing myocardial injury. J Med Virol 82:1449–1457
173. Corcioli F, Zakrzewska K, Rinieri A, Fanci R, Innocenti M, Civinini R, De Giorgi V, Di Lollo S, Azzi A (2008) Tissue persistence of parvovirus B19 genotypes in asymptomatic persons. J Med Virol 80:2005–2011
174. Schenk T, Enders M, Pollak S, Hahn R, Huzly D (2009) High prevalence of human parvovirus B19 DNA in myocardial autopsy samples from subjects without myocarditis or dilative cardiomyopathy. J Clin Microbiol 47:106–110
175. Stewart GC, Lopez-Molina J, Gottumukkala RV, Rosner GF, Anello MS, Hecht JL, Winters GL, Padera RF, Baughman KL, Lipes MA (2011) Myocardial parvovirus B19 persistence: lack of association with clinicopathologic phenotype in adults with heart failure. Circ Heart Fail 4(1):71–78
176. Schmidt-Lucke C, Spillmann F, Bock T, Kühl U, Van Linthout S, Schultheiss HP, Tschöpe C (2010) Interferon beta modulates endothelial damage in patients with cardiac persistence of human parvovirus b19 infection. J Infect Dis 201:936–945
177. Zafrir B, Aviv A, Reichman N, Flatau E (2005) Epstein-Barr virus associated pericarditis and pericardial effusion: case report and diagnostic aspects. Eur J Intern Med 16:528–530
178. Roubille F, Gahide G, Moore-Morris T, Granier M, Davy JM, Vernhet H, Piot C (2008) Epstein Barr virus (EBV) and acute myopericarditis in an immunocompetent patient: first demonstrated case and discussion. Intern Med 47(7):627–629
179. Chang YL, Parker ME, Nuovo G, Miller JB (2009) Human herpesvirus 6–related fulminant myocarditis and hepatitis in an immunocompetent adult with fatal outcome. Hum Pathol 40(5):740–745
180. Leveque N, Boulagnon C, Brasselet C, Lesaffre F, Boutolleau D, Metz D, Fornes P, Andreoletti L (2011) A fatal case of Human Herpesvirus 6 chronic myocarditis in an immunocompetent adult. J Clin Virol 52(2):142–145
181. Liao YC, Hsieh YC, Chang WC, Huang JL, Ting CT, Wu TJ (2011) Fulminant myocarditis in an adult with 2009 pandemic influenza A (H1N1 influenza) infection. J Chin Med Assoc 74(3):130–133
182. Moulik M, Breinholt JP, Dreyer WJ, Kearney DL, Price JF, Clunie SK, Moffett BS, Kim JJ, Rossano JW, Jefferies JL, Bowles KR, O'Brian Smith E, Bowles NE, Denfield SW, Towbin JA (2010) Viral endomyocardial infection is an independent predictor and potentially treatable risk factor for graft

loss and coronary vasculopathy in pediatric cardiac transplant recipients. J Am Coll Cardiol 56(7):582–592
183. Shaw T, Elliot P, McKenna WJ (2002) Dilated cardiomyopathy: a genetical heterogeneous disease. Lancet 360:654–655
184. Mason JW (2003) Myocarditis and dilated cardiomyopathy: an inflammatory link. Cardiovasc Res 60:5–10
185. Maekawa Y, Ouzounian M, Opavsky MA, Liu PP (2007) Connecting the missing link between dilated cardiomyopathy and viral myocarditis: virus, cytoskeleton, and innate immunity. Circulation 115(1):5–8
186. Linde A, Mosier D, Blecha F, Melgarejo T (2007) Innate immunity and inflammation–new frontiers in comparative cardiovascular pathology. Cardiovasc Res 73(1):26–36
187. Gorbea C, Makar KA, Pauschinger M, Pratt G, Bersola JL, Varela J, David RM, Banks L, Huang CH, Li H, Schultheiss HP, Towbin JA, Vallejo JG, Bowles NE (2010) A role for Toll-like receptor 3 variants in host susceptibility to enteroviral myocarditis and dilated cardiomyopathy. J Biol Chem 285:23208–23223
188. Mann DL (2011) The emerging role of innate immunity in the heart and vascular system: for whom the cell tolls. Circ Res 108(9):1133–1145
189. Fuse K, Chan G, Liu Y, Gudgeon P, Husain M, Chen M, Yeh WC, Akira S, Liu PP (2005) Myeloid differentiation factor-88 plays a crucial role in the pathogenesis of coxsackievirus B3 induced myocarditis, and influences type I interferon production. Circulation 112:2276–2285
190. Mann DL, Topkara VK, Evans S, Barger PM (2010) Innate immunity in the adult mammalian heart: for whom the cell tolls. Trans Am Clin Climatol Assoc 121:34–50
191. Schultheiss HP, Kühl U, Cooper LT (2011) The management of myocarditis. Eur Heart J 32:2616–2625