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INVITED SPOTLIGHT REVIEW

Cardiovascular consequences of viral infections: from COVID to other viral diseases

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Abstract

Infection of the heart muscle with cardiotropic viruses is one of the major aetiologies of myocarditis and acute and chronic inflammatory cardiomyopathy (DCMi). However, viral myocarditis and subsequent dilated cardiomyopathy is still a challenging disease to diagnose and to treat and is therefore a significant public health issue globally. Advances in clinical examination and thorough molecular genetic analysis of intramyocardial viruses and their activation status have incrementally improved our understanding of molecular pathogenesis and pathophysiology of viral infections of the heart muscle. To date, several cardiotropic viruses have been implicated as causes of myocarditis and DCMi. These include, among others, classical cardiotropic enteroviruses (Coxsackieviruses B), the most commonly detected parvovirus B19, and human herpes virus 6. A newcomer is the respiratory virus that has triggered the worst pandemic in a century, SARS-CoV-2, whose involvement and impact in viral cardiovascular disease is under scrutiny. Despite extensive research into the pathomechanisms of viral infections of the cardiovascular system, our knowledge regarding their treatment and management is still incomplete. Accordingly, in this review, we aim to explore and summarize the current knowledge and available evidence on viral infections of the heart. We focus on diagnostics, clinical relevance and cardiovascular consequences, pathophysiology, and current and novel treatment strategies.

Keywords Viral infections • Myocarditis • Inflammatory cardiomyopathy • Advanced diagnostics • Treatment strategies

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1. Introduction

Infectious agents are the major causes of myocarditis and inflammatory cardiomyopathy (DCMi).[1–4] The clinical presentation is extremely heterogeneous, the natural history is unpredictable, and prognosis also varies according to the underlying aetiology, environmental factors—most commonly initiated by a virus—and genetic predispositions.[5,6] This fact, in conjunction to the lack of non-invasive specific diagnostic methods, makes it an underdiagnosed entity.

To date, several cardiotropic viruses have been implicated as causes of myocarditis and DCMi. The main viruses associated are enteroviruses (EVs), including Coxsackievirus B3 (CVB3), and adenoviruses (ADVs), the most commonly detected parvovirus B19 (B19V), influenza (A, B), human herpesvirus 6 (HHV6), human immunodeficiency virus (HIV), hepatitis C virus (HCV), human cytomegalovirus (CMV), and Epstein–Barr virus (EBV) (Table 1).[7,8] All these viruses can cause myocarditis with similar inflammatory features (*Figure 1*).[5,10]

In December 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified as the causative agent of a cluster of suspicious pneumonia cases in Wuhan, Hubei, China. The incredible fast worldwide spread of the coronavirus disease 2019 (COVID-19) prompted the World Health Organization to declare COVID-19 a

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Table 1 List of viral species detected in EMB samples

Viruses in EMB	Viral genome organization
Adenovirus	dsDNA
Arenavirus	ssRNA
Coronavirus (including Sars-CoV-2)	ssRNA
Coxsackievirus (A, B)	ssRNA
Cytomegalovirus	dsDNA
Dengue virus	ssRNA
Echovirus	ssRNA
Encephalomyocarditis virus	ssRNA
Epstein–Barr virus	dsDNA
Hepatitis B virus	dsDNA
Hepatitis C virus	ssRNA
Herpes simplex virus	dsDNA
Human herpesvirus-6	dsDNA
Human immunodeficiency virus	ssRNA
Influenza (A, B) virus	ssRNA
Measles virus	ssRNA
Metapneumovirus	ssRNA
Mumps virus	ssRNA
Parvovirus B19	ssDNA
Polio virus	ssRNA
Rabies virus	ssRNA
Respiratory syncytial virus	ssRNA
Rubella virus	ssRNA
Vaccinia virus	dsDNA
Varicella-zoster virus	dsDNA
Variola virus	dsDNA
Zika virus	ssRNA

ds, double stranded; ss, single stranded.

pandemic on 11 March 2020. Epidemiological data from the present coronavirus pandemic demonstrate a significant relationship between COVID-19 and cardiovascular disease (CVD). Whether there is direct myocardial damage caused by SARS-CoV-2 or if it is primarily an endothelial disease is currently under investigation.[11–14] A recent landmark study by Bailey *et al.*[15] employing human autopsy tissues, human pluripotent stem cell-derived cardiomyocytes, and engineered heart tissues has provided evidence that SARS-CoV-2 directly infects cardiomyocytes and does not infect cardiac macrophages, fibroblasts, or endothelial cells. They also found that infection of cardiomyocytes resulted in cytokine induction, sarcomere disassembly, and cell death. Beyond a broad spectrum of previous clinical studies into the multiple other aspects of COVID-19, these data provide important additional insight into specific SARS-CoV-2 pathology within the heart.

To better understand the frequently unpredictable progression of viral myocarditis and DCMi, one has to address the underlying pathophysiological processes.[16,17] Currently, there is consensus that both—immune-mediated and viral cytotoxic mechanisms—play a significant role in this regard.[7] Beyond virus cytotoxicity, chronic immune stimulation or autoimmunity in DCMi results from incompletely cleared virus infection, or in response to the preceding virus- or immune-mediated chronic tissue damage, respectively, even in the absence of infectious viral particles.[5,6]

It is considered possible that at some point in progression, multiple aetiologic types confluence into a common autoimmune pathogenic process that leads to chronic inflammation, tissue remodelling, and fibrosis, ultimately progressing to the clinical phenotype of dilated cardiomyopathy (DCM). A consistent progression from myocarditis to DCM is described in about 30% of myocarditis patients. Any diagnostics started at this time often cannot elucidate the initial causes of the disease.[18–24]

Understanding the underlying molecular mechanisms is required in order to be able to estimate the prognosis of the patients and is fundamental to proper management and specific treatments.[20,25] Viral diagnostics and antiviral treatment should be started early before irreversible myocardial damage has developed.[25,26]

In this review, we discuss common viral infections and various stages of disease. We assess pathogenesis and mechanisms, clinical relevance and consequences, and outline patient-specific therapeutic options that are based on an accurate diagnosis, covering current and novel treatment strategies.

2. SARS-CoV-2

SARS-CoV-2 is a novel coronavirus that was identified as the cause of COVID-19 in early 2020 (*Coronaviridae Study Group of the International Committee on Taxonomy of Viruses*[27]). Infection with SARS-CoV-2 can lead to viral pneumonia and acute respiratory distress syndrome and is accompanied by an increased risk of morbidity and mortality.[28] Besides respiratory complications, SARS-CoV-2 can trigger cytokine storm and coagulation abnormalities, leading to thromboembolic events up to multiorgan damage.[14,29] Strikingly, there is a strong connection between CVD and severity of COVID-19. Initial clinical data suggested that both, susceptibility and clinical cause are highly dependent on cardiovascular comorbidities.[30]

2.1 Virological background

SARS-CoV-2 is a membrane-enveloped positive-sense, single-stranded RNA virus with a diameter of ~80–140 nm. Infection with human coronaviruses mainly results in respiratory and enteric diseases ranging from mild 'cold-like' symptoms up to severe life-threatening respiratory pathologies and lung injuries.[27] The infection of host cells with SARS-CoV-2 involves specific binding of viral spike (S) protein to the cellular entry receptor angiotensin-converting enzyme 2 (ACE2).[31] In addition, fusion of viral particles is dependent on the proteolytic cleavage of the S protein by the host cell surface serine protease TMPRSS2.

Host organism's innate immune response plays a major role in the cause of COVID-19. Thus, several SARS-CoV-2 accessory proteins have been suggested to affect the innate immune response.[32] Abnormal pro-inflammatory cytokine levels and immune cell infiltration have been associated with the severity of tissue damage and morbidity of coronavirus infection.[33,34] Aberrant infiltration of pro-inflammatory macrophages, cytotoxic T-cells, and neutrophils has been observed in COVID-19.[35,36] Thus, dysregulation of host immune response and elevated cytokine release seem to be crucial factors for the severity of COVID-19.

2.2 Cardiovascular involvement

A meta-analysis involving more than 6000 COVID-19 patients indicates an incidence of cardiac injury ranging from 15% to 42% depending on age and disease severity.[37] Post-mortem analysis of cardiac tissue of 39 patients who died as a consequence of coronavirus infection, revealed an incidence of 61.5% positive SARS-CoV-2 RNA detection in the heart.[38] A recent meta-analysis and literature screening revealed hypertension

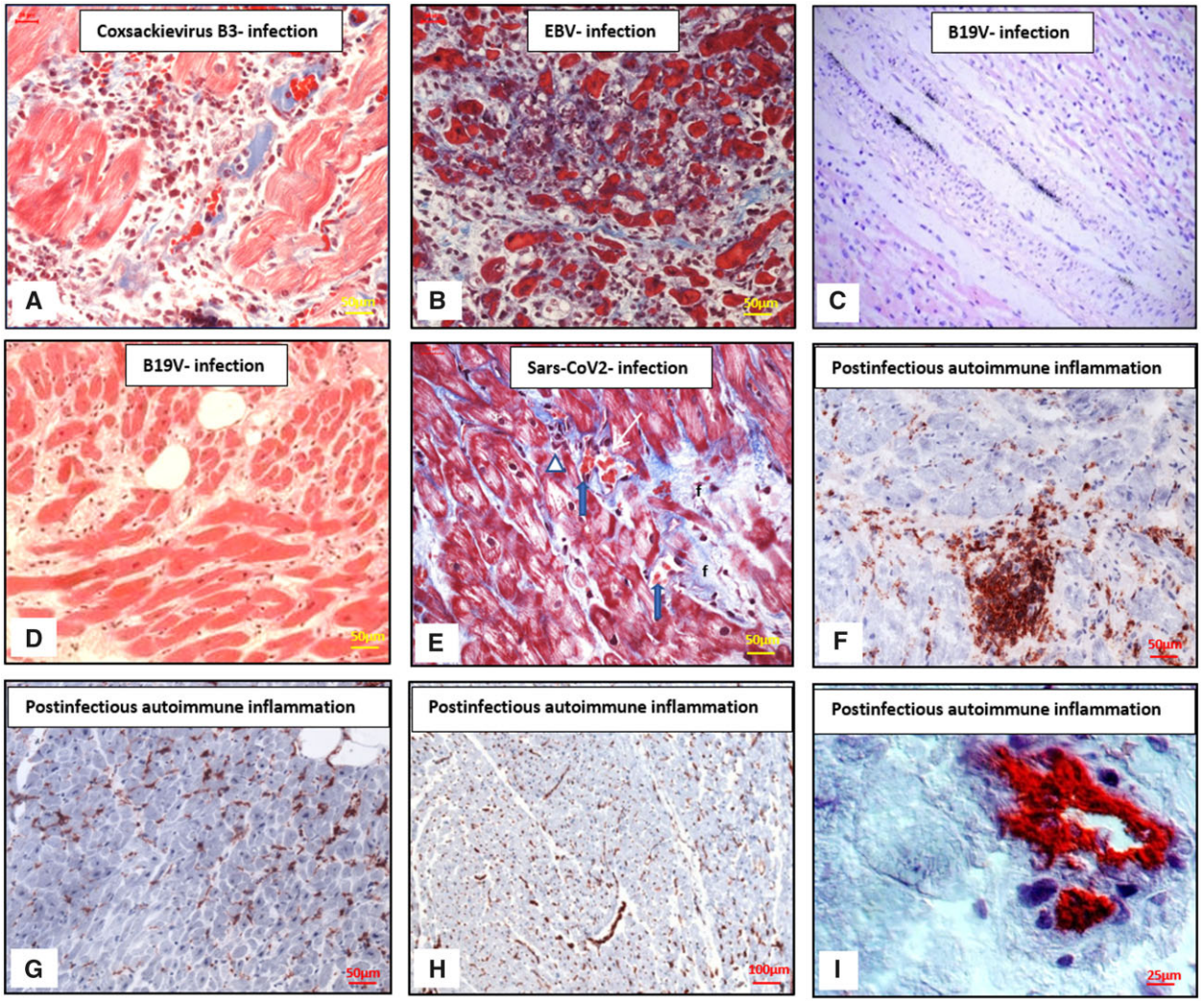

Figure 1 (Immuno-)Histological manifestations of myocarditis and inflammatory cardiomyopathy. (*A*) CVB3-positive patient, histological analysis of active myocarditis with massively infiltrating cells and myocytolysis. Azan staining. Scale bars: 50 µm. (*B*) Active myocarditis in a case of EBV infection with dense infiltration of inflammatory cells, necrosis, and dissolution of myocytes in the centre of the panel. Azan stain. Scale bars: 50 µm. (*C*) Detection of B19V in the endothelial layer of an intramyocardial vessel in the heart (radioactive *in situ* hybridization, original high-power magnification, haematoxylin, and eosin) obtained at autopsy from an infant who died from myocarditis. Reprinted with permission from Bock *et al.*[9] (*D*) Enhanced fibrosis in a B19V positive patient with transcriptional activity. H&E stain. Scale bars: 50 µm. (*E*) Histological analysis in a patient with positive proof of SARS-CoV-2 genomes in EMB. In the periphery of a fibrosis (f) capillaries (white arrow) with sinus-like structure contain aggregated erythrocytes (blue arrows), unstructured protein, and lack endothelial cells. Adjacent some round cells (white triangle). Myocytes distended without signs of damage. Azan stain. Scale bars: 50 µm. (*F*) Enhanced focal post-infectious autoimmune inflammation, IH staining of focal infiltration of CD3-positive T-lymphocytes. Scale bars: 50 µm. (*G*) Post-infectious autoimmune inflammation, IH staining of diffuse infiltration of CD45R0-positive T-memory cells. Scale bars: 50 µm. (*H*) Post-infectious autoimmune inflammation, IH staining of increased HLA-DR isotype—expression. Scale bars: 100 µm. (*I*) Post-infectious autoimmune inflammation, IH staining of increased VCAM-1 expression. Scale bars: 25 µm. B19V, parvovirus B19; EBV, Epstein–Barr virus; HLA-DR, human leukocyte antigen-DR; IH, immunohistochemistry; VCAM-1, vascular cell adhesion protein 1.

(28%), diabetes (14%), and CVD (12%) to be the most prevalent comorbidities in COVID-19 patients and thus, independent risk factors for mortality.[39] Moreover, a study including 40 SARS-CoV-2 positive patients confirmed the relationship between the presence of COVID-19 and acute cardiac damage.[40] However, whether cardiac injury is directly induced by SARS-CoV-2 infection is not clarified yet.[41] There are numerous hypotheses assessing the impact of SARS-CoV-2 infection on cardiovascular manifestations. These range from direct myocardial injury by disturbance of the ACE2 signalling, over systemic inflammatory damage (including cytokine storm) to cardiometabolic issues, arrhythmias, and ischaemia.[13]

Infection has been proven for SARS-CoV-2 in cardiomyocytes and organoids.[15] Noteworthy, endotheliitis has been suggested to be involved in SARS-CoV-2-mediated cardiac damage.[13,42,43] A study on cardiac autopsy tissue from COVID-19 positive patients identified strong ACE2/TMPRSS2 expression in capillaries of the heart and endotheliitis of small vessels with prevalence of $CD4^+$ and $CD68^+$ inflammatory cells.[44] Another post-mortem analysis of nine COVID-19 patients, who died

due to cardiogenic shock, revealed the involvement of all compartments of the heart including intramural vessels, conduction tissue, and subepicardial ganglia.[45] There is accumulating evidence that SARS-CoV-2 S protein directly interacts with myocardial Toll-Like Receptor (TLR)4 leading to activation of the TLR4 signalling cascade (including pro-inflammatory cytokines and type I interferons) and even to up-regulation of ACE2 surface expression.[46]

2.3 Myocarditis and inflammatory cardiomyopathy

First detection of SARS-CoV-2 genomes was provided in endomyocardial biopsies (EMBs) of patients with suspected myocarditis or unexplained heart failure.[47] Ultrastructural analysis of EMB of a 69-year-old patient positively tested for SARS-CoV-2 identified viral particles within the interstitial cells of the myocardium.[48] Cardiac magnetic resonance imaging in patients recently recovered from Sars-CoV-2 infection identified 78% with cardiac involvement and 60% with an ongoing myocardial inflammation.[49] Further studies must show how long the effects last. EMB analysis of two patients with a history of upper airway infection of unknown origin and clinical signs of myocarditis revealed positive detection of SARS-CoV-2 genome in combination with elevated inflammatory cell infiltration.[50] Since nasopharyngeal swabs tested negative for SARS-CoV-2 in these patients, it is likely that cardiac inflammation development is delayed following previous infection. Further case reports documented left ventricular dysfunction and inflammation of the heart related to direct Sars-CoV-2-infection with a latency period of 4 weeks after the onset of pulmonary symptoms.[51] Autopsy cases from COVID-19 victims confirm lymphomononuclear infiltrates in the myocardium with focal necrosis of adjacent myocytes, in the pericardium as well as in intramural vessels with necrosis of the vascular wall.[45]

2.4 Acute coronary syndrome

As known for other infectious diseases, SARS-CoV-2 is assumed to trigger acute coronary syndrome (ACS). However, the incidence of ASC in COVID-19 patients is still illusive and detailed mechanisms of SARS-CoV-2 contribution remain speculative. Putative involvement of COVID-19 in the development of ACS includes plaque rupture, coronary spasm or micro-thrombi induced by cytokine storm, and endothelial or vascular injury by direct infection of these cells with SARS-CoV-2.[52]

3. Human B19V

Human parvovirus (B19V) genomes are the most frequently detected viral species in EMBs of patients with suspected heart failure (*Figure 2*).[53–55] Infection with B19V can start during childhood and continues throughout adulthood, such that between 70% and 88% of adults show serologic evidence of past infections (*Table 1*).

3.1 Virological background

B19V is a non-enveloped single-stranded linear DNA virus of 20–24 nm in diameter. Its ~5.6 kb genome encodes for two major proteins, the non-structural protein (NS1) and VP1/2 protein (capsid protein), and the small accessory 11 and 7.5 kDa proteins of largely unknown function. The NS1 protein transactivates viral transcription and host genes, induces cell cycle arrest and DNA damage response, in order to facilitate viral replication and host cell apoptosis to release viral progeny.[56,57] Various molecular mechanisms, such as NS1 induced apoptosis may be responsible for direct cytotoxicity.[58]

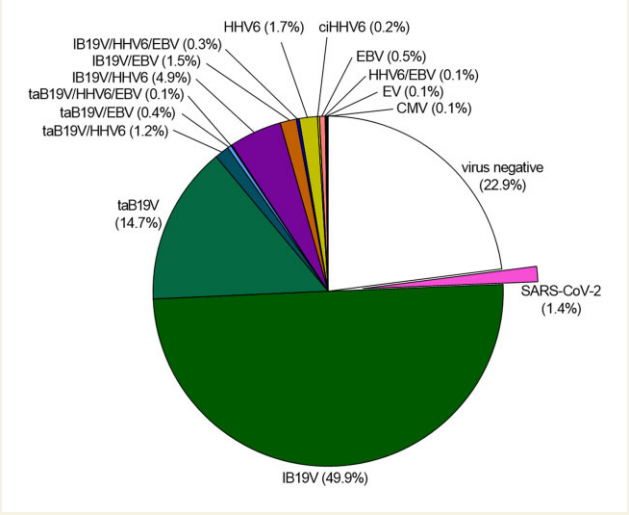

Figure 2 Distribution of viral genomes in EMBs of $n = 1132$ consecutive patients in 2020 with suspicion of myocarditis or unexplained heart failure. B19V infection is divided into latent (lB19V) and transcriptional active (taB19V) infection. For SARS-CoV-2, $n = 364$ EMBs were analysed, of which $n = 5$ (1.4%) were positive for SARS-CoV-2 genomes. B19V, parvovirus B19; ciHHV6, chromosomal integrated human herpesvirus 6; CMV, cytomegalovirus; EBV, Epstein–Barr virus; EMB, endomyocardial biopsy; EV, enterovirus; HHV6, human herpesvirus 6.

3.2 Cardiovascular involvement

The association of myocardial B19V genome detection to heart diseases is still a matter of controversial discussion.[9,55,59] B19V DNA genomes were detected in ~73% of patients EMBs (*Figure 2*) and were also found in 55% of healthy donor hearts suggesting no causal relationship.[60] However, these studies did not differentiate between latent (inactive) and transcriptional active (positive mRNA) viral infection. In contrast to latent B19V infection, expression of B19V viral mRNA and proteins in the myocardium was demonstrated to be of significance,[61] and replicative active B19V in the myocardium is related to adverse clinical outcome.[9,54]

We identified different cell types belonging to the heterogenous group of bone marrow-derived circulating angiogenic cells with similarities to endothelial and erythroid lineage, to be targets for B19V infection.[62] In chronic B19V-associated disease, cardiomyocytes, which are devoid of B19V receptors, are precluded from infection.[63] Endothelial dysfunction is a consequence of impaired endothelial regeneration during cardiac B19V infection, leading to impaired coronary microcirculation and results in secondary cardiac myocyte damage.[62,64,65] Besides direct cytopathic effects, B19V potentially induces autoimmunity[66] possibly triggered by phospholipase activity of VP1u domain.[67,68]

4. Further cardiotropic viral pathogens

Besides B19V, primary cardiotropic viruses are EV including CVB3 and echoviruses, ADV, the *Herpesviridae* genus, such as HHV6, EBV, and

CMV, all of which may cause or trigger myocarditis and DCMi[53,69] (*Figure 2*). Influenza-(IAV/IBV), HCV, and HIV infections are associated with an increased incidence of cardiac complications.[70–72] In addition to the cardiotropic viruses mentioned above, there are sporadic reports, most often as case reports, identifying varicella zoster virus (VZV), Zika virus, Dengue virus, and Chikungunya virus being linked to viral myocarditis. Similar observations have been made for rabies, rubella, mumps, and measles virus.[8] In general, proof of viral genomes in the myocardium is independent from the severeness of myocardial dysfunction.[53] Whether all of these viruses can be causative for the development of viral myocarditis or just being an incidental finding has to be determined.

4.1 Enteroviruses

EVs (*Picornaviridae*) are small, single-stranded, positive-sense RNA viruses. Non-enveloped EVs are common human pathogens responsible for lower and upper respiratory tract infections that are transmitted via the faecal–oral route targeting the heart secondarily. Twenty years ago, frequency of enteroviral infection accounts for up to 10% of heart failure patients who underwent EMB. However, recent studies report less frequent finding of CVB-3, which might be associated with regional and temporal patterns.[73,74] EV and ADV enter cardiomyocytes via binding the transmembrane Coxsackievirus and adenovirus receptor (CAR), which represents a potential antiviral target.[75,76] Direct cardiac damage during acute phase is a consequence of viral replication and impaired cellular translation, induction of apoptosis, and oxidative stress followed by cell lysis.[75,77,78] During sub-acute phase of CVB-3 infection, an unbalanced immune response and immune-mediated destruction of cardiac tissue or induction of autoimmune processes may occur.[79,80] EV persistence in the myocardium is associated to a significant higher mortality.[81] Genotyping revealed a strong correlation between the CCR5 mutation and spontaneous virus clearance with improved outcomes.[82]

During the chronic phase, CVB-3 might be eliminated or viral persistence may result in the progression to DCM characterized by cytoskeletal disruption and compromised contractility often associated with virus mediated immune response[80, 83] (*Figure 3*).

4.2 Human herpesviruses

HHV6 (subtype A and B), as the most frequently found herpesvirus in the myocardium (*Figure 2*), primarily infects $CD4^+$ T lymphocytes.[84,85] HHV6 is a double stranded enveloped DNA virus with a genome of ~170 kb that encodes for various viral proteins, including a viral DNA polymerase, further proteins and microRNAs (miRNAs), that are involved in the control of viral latency, host cell cycle and evasion of immune response. Infection is usually acquired during childhood in the absence of clinical symptoms or it may manifest as *Exanthema subitum* and results in lifelong persistence with a seroprevalence of >90%.[84] Re-activation after latency occurs by unknown mechanisms and is mostly asymptomatic in immunocompetent individuals while leading to sub-acute clinical symptoms in the immunocompromised patients. Clinical relevance of HHV6 infection of the myocardium has been shown in particular for paediatric patients after heart transplantation.[86] There is strong evidence that co-infection with other viruses, in particular with B19V, contributes to cardiac dysfunction since exclusive cardiac infection with HHV6 occurs only rarely.[87]

The HHV6 genome may integrate into the telomere region of somatic cells or germ line cells [chromosomally integrated HHV6 (ciHHV6)]. The prevalence of ciHHV6 is ~0.8–1.5% of HHV-6-positive EMBs.[88]

4.3 CMV, EBV, and VZV

Only few case reports describe findings of CMV, EBV, and VZV in the myocardium that are associated with a pathological phenotype.[69,89,90] Molecular mechanism of CMV and EBV infection of the myocardium remain to be elucidated, however pathophysiological effects most probably result from immune-mediated damage or endothelial dysfunction as a consequence of CMV replication (*Figure 3*).

4.4 Hepatitis C virus

Accumulating evidence suggests that HCV, a globally widespread RNA virus that mainly affects the liver, may also play a role in the pathogenesis of heart diseases including myocarditis and DCM.[72,91] Besides hepatitis C, chronic HCV infection is associated with various extrahepatic manifestations, like glomerulonephritis, myositis, and others. Extrahepatic manifestations are believed to be due to the lymphotropism of HCV with accumulation of circulating immune complexes, modulation of host immune response, and activation of autoimmune responses.[92] In recent multicentric studies, Matsumori *et al.*[72,93] identified a significant higher seroprevalence of anti-HCV antibodies in patients suffering from myocarditis, DCM, and heart failure than in the general population. Additionally, HCV RNA genomes could be also detected in anti-HCV positive sera and EMBs from patients with myocarditis and DCM. The pathogenesis of HCV-induced myocarditis and DCM is still poorly investigated; however, a recent study provides evidence that of mononuclear cells a major target of HCV could be leukocytes and especially CD68 positive monocytes/macrophages.[94] These cells induced by HCV infection may cause inflammation in the organs including the heart muscle leading to myocarditis, DCM, and other cardiomyopathies.

5. Clinical presentation

Myocarditis und DCMi present with heterogeneous clinical signs and symptoms, ranging from subclinical disease to refractory cardiogenic shock with substantial morbidity and mortality.[17,95] A virus-specific phenotype of myocardial diseases does not exist. Patients present with uncharacteristic complaints, such as angina, dyspnoea, fatigue, reduced physical ability, or arrhythmias in the presence of a preserved or impaired systolic or diastolic ventricular function.[96] A viral infection of the respiratory or the gastrointestinal tract, may precede the onset of cardiac symptoms, although the occurrence of such a viral syndrome is highly variable. In acute disease, sudden onset of chest pain, dyspnoea, and heart failure with normal or enlarged ventricular chambers, ventricular arrhythmias, and abnormal ST-elevation changes in the presence of elevated cardiac enzymes are highly suspicious for an acute viral myocarditis, if a coronary artery disease has been excluded.[1,2,4,10]

6. Diagnostics of viral infections

The initial evaluation of acute myocarditis and DCMi includes a detailed history and physical examination in which possible features suggestive of aetiology may provide clues.

Cardiac serum biomarkers, specifically troponin I and troponin T, can help to confirm the diagnosis, but lack sensitivity. Other inflammatory serum markers, including white blood cell count, erythrocyte sedimentation rate, and C-reactive protein levels, may be elevated in acute myocarditis, but are neither sensitive nor specific in terms of determining the presence or absence of active myocardial inflammation with or

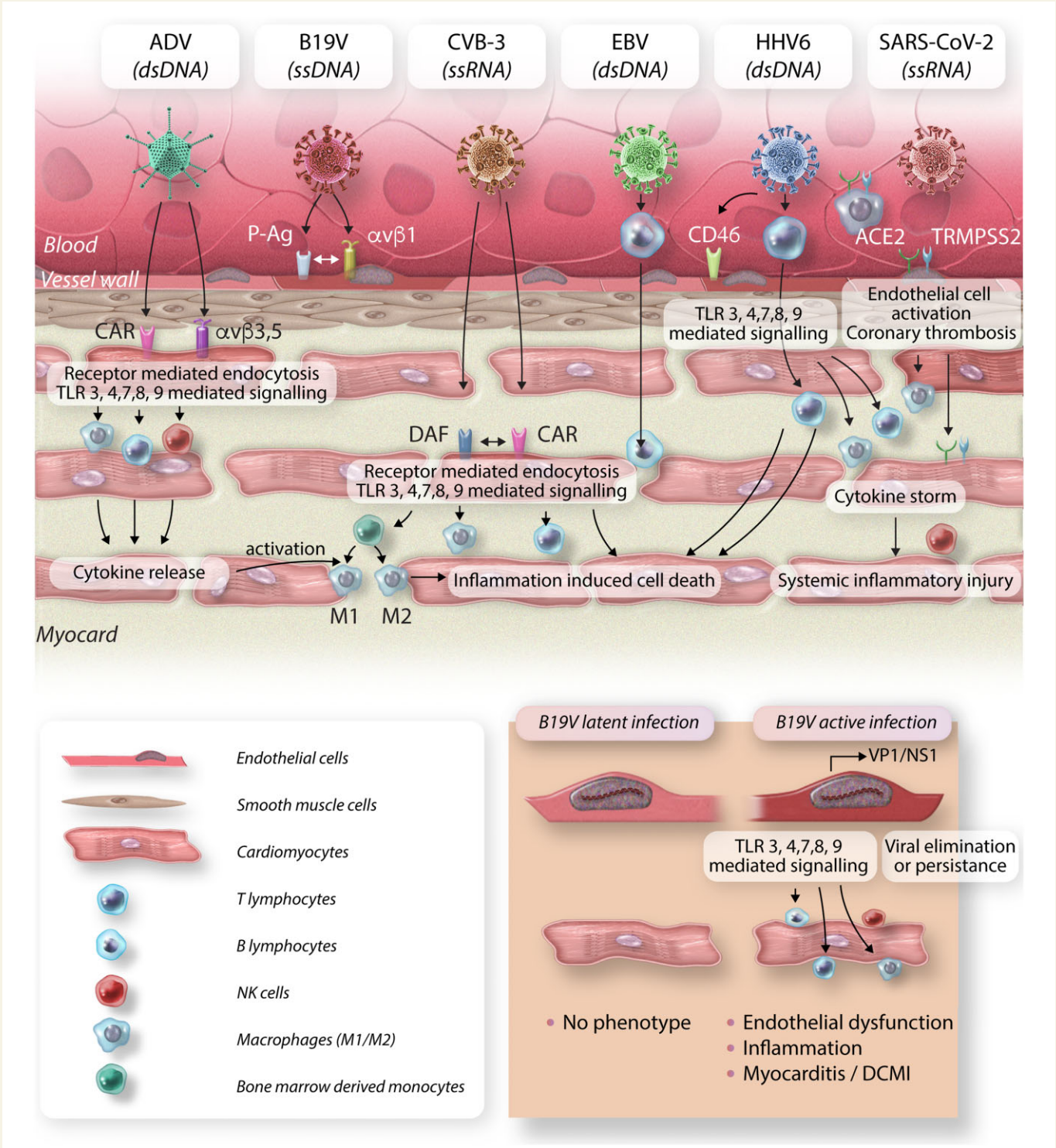

Figure 3 Most abundant cardiotropic viruses and their target cells in the heart. CVB-3 and ADV enter cardiomyocytes via binding the transmembrane CAR. In addition, decay-accelerating factor serves as CVB-3 receptor. Integrins (αvβ3 and αvβ5) promote ADV internalization. B19V targets endothelial cells by binding to erythrocyte P antigen and integrin αvβ1 as co-receptor. EBV efficiently infects resting human B lymphocytes, whereas HHV6 primarily targets CD4+ T lymphocytes. Using CD46 as cellular receptor, HHV6 can directly infect endothelial cells and subsequently enter adjacent tissues. SARS-CoV-2 cellular entry involves specific binding to the ACE2 receptor as well as proteolytic cleavage by the host cell surface serine protease TMPRSS2. For SARS-CoV-2, several cardiac targets including vascular endothelial cells and cardiomyocytes are proposed. Moreover, pulmonary-derived macrophages are suggested carrying the virus into the myocardium. As a consequence of viral infection, TLR3, 4, 7, 8, and 9 signalling cascade is initiated, followed by infiltration of several inflammatory cells including T and B lymphocytes, natural killer cells and bone-marrow derived monocytes, which differentiate into M1 and M2 macrophages. B19V infection can be differentiated into latent infection without myocardial damage and active infection characterized by VP1 and/or NS1 mRNA detection. The later can result in severe endothelial dysfunction, followed by immune cell infiltration and development of DCMi. ds, double stranded; ss, single stranded.

without viral infection.[8,97] Serologic testing has often been used in the past to identify pathogens in viral myocarditis. However, these methods lack direct correlation between viral infection and myocarditis.[10,98]

Electrocardiographic findings in myocarditis patients include T-wave and ST-segment changes, including ST-segment elevation mimicking acute myocardial infarction. However, these changes are neither sensitive nor specific for the diagnosis of myocarditis and DCMi. Echocardiography is a valuable tool in detecting global or regional wall motion abnormalities, with myocardial strain patterns adding a special value.[10]

Magnet resonance imaging (MRI) with extracellular contrast agent can be valuable for mapping tissue hyperaemia associated with the intense inflammatory response of acute myocarditis. Imaging techniques, such as MRI provide accurate non-invasive tissue characterization but not genesis clarification of an infectious agent because they cannot detect or quantify different viral types and loads or subtypes of immune response.[99]

EMB is the gold- standard method to distinguish directly infectious agent-mediated from multiple types of immune-mediated injury of tissue and can provide specific aetiologic information with significant consequences for management and differential therapy.[1,8,10,97] The 2013 European Society of Cardiology position paper recommends characterization of cardiac inflammation and infection by immunohistochemistry and viral analysis using quantitative PCR methods (real-time PCR and nested PCR with reverse transcription).

Viral presence does not always satisfy the Dallas criteria of myocarditis perhaps due to different timepoints in diagnosing. Therefore, beside histological and immunohistological evaluation, molecular analysis of EMB is a prerequisite to establish viral infection and persistence.[100,101]

State-of-the-art molecular virological diagnostics of EMBs for pathogen detection should not be restricted to the PCR proof of viral RNA or DNA genomes alone, but further include the quantification of viral loads and transcriptional activity.[1,53] Recent data show that testing of replicative status is clinically relevant and is, therefore, a prerequisite for further therapeutic decisions.[54,61] Additionally, virus genotypes and variants may be detected by next-generation sequencing.[102]miRNAs are important epigenetic regulators of the immune response in the heart. Epigenetic factors influence the expression of different genes as well as the genetic susceptibility to the development of myocarditis and DCMi.[103,104] A panel of miRNAs in serum provides a new non-invasive diagnostic perspective to identify patients with unexplained heart failure, who should undergo an EMB due to intramyocardial inflammation and/or viral persistence.[105]

The expression of eight miRNAs was significantly increased in samples from patients with advanced heart failure and viral persistence with or without inflammation.[106] Thus, miRNAs can serve as a non-invasive, additional tool for indication of EMB decision making.

7. Treatment options

Symptomatic heart failure therapy may improve clinical symptoms and hemodynamic situation. However, a specific antiviral or anti-inflammatory therapy is not covered by this.[17]

7.1 SARS-CoV-2

Several antiviral therapies are currently being investigated for patients with COVID-19, including strategies to prevent viral entry into the host cell (e.g. chloroquine and hydroxychloroquine), protease inhibitors (lopinavir-ritonavir and darunavir), RNA polymerase inhibitors (remdesivir), and anti-cytokine agents [e.g. interleukin (IL)-6 receptor antagonists], all of which relate to general treatment strategies.[107]

Negative results were obtained for clinical trials of newly developed HIV protease inhibitors, such as lopinavir/ritonavir [Randomized Evaluation of COVid-19 thERapY (RECOVERY) Trial] and darunavir/cobicistat for COVID-19, with no significant impact on mortality or length of hospital stay.[108] Clinical trials with ribavirin against MERS showed high levels of toxicity.[109] In a Phase 3 clinical trial, remdesivir was not associated with clinical improvement.[110] Chloroquine or hydroxychloroquine does not seem to show significant improvement in mortality. In COVID-19, elevated IL-6 levels have been correlated with increased mortality, sparking interest in the use of tocilizumab—a recombinant, monoclonal antibody against the IL-6 receptor—for COVID-19 therapy. A randomized, placebo-controlled trial in patients with severe COVID-19 demonstrated that tocilizumab did not reduce mortality or intubation rates.[111] Convalescent plasma from recovered COVID-19 patients contains naturally produced antibodies that can provide temporary protection against the worst effects of the disease. Synthetic anti-SARS-CoV-2 antibody cocktails are highly enriched specific antibodies against the SARS-CoV-2 S glycoprotein that prevent cell entry. The antibody cocktails are currently being clinically tested as part of the RECOVERY Collaborative Group Trials. As COVID-19 triggers a procoagulatory state that increases the risk for thromboembolic events, first studies indicate an improved outcome under antithrombotic treatment.[112,113]

7.2 EV, ADV, and B19V

Spontaneous enteroviral clearance is associated with significant improvement of LVEF while persistence leads to progressive heart failure and is associated with significantly higher risk of death.[17,74] A non-randomized study was started treating EV and ADV positive patients with interferon-ß (IFN-ß). Upon IFN-ß treatment complete elimination of EV and ADV genome was proved by follow-up EMBs after finishing of the antiviral therapy.[81] Virus clearance was paralleled by an improvement of mean LVEF and an amelioration of heart failure symptoms and improvement of survival. Thereafter, a Phase 2 study—betaferon in a chronic viral cardiomyopathy—trial was initiated.[114] Patients with symptoms of heart failure and biopsy-proven EV, ADV, and/or B19V genomes were randomly assigned to double-blinded treatment. Compared to the placebo, virus elimination and/or virus load reduction was higher in the IFN-ß groups. IFN-ß treatment was associated with significant improvement on NYHA functional class improvement and in quality of life. IFN-β treatment has proven less effective in clearing B19V infection. However, no differentiation between latent B19V infection and viral transcriptional activity was made in this study. In a pilot study, endothelial dysfunction improved with treatment of IFN-ß due to suppression of viral replicative intermediates, suggesting that this treatment option may improve endothelial viability.[63] Innovative therapy and prevention strategies to control B19V transcriptional activity are currently under investigation. Telbivudine is an antiviral nucleoside analogue reverse transcriptase inhibitor with pleiotropic immunomodulatory effects that has been described to be effective in retroviral and pararetroviral (hepatitis B virus) infections by preventing dysregulation of BIRC3 and thus suppresses induction of apoptotic pathways.[115] Clinical improvement and reduction of transcriptional activity has been shown after Telbivudine treatment in a non-randomized study.[116] Intravenous immunoglobulin therapy did not result in clinical

improvement of B19V-associated chronic DCM, however, transcriptional activity was not evaluated.[117]

7.3 HIV, HCV, and HHV6

Patients with HIV-associated myocarditis or DCMI are treated by antiretroviral therapy with clear survival benefits although with cardiac side effects of medication.[118,119] Patients with HCV-associated DCMi were treated by combination therapies employing ombitasvir, paritaprevir, ritonavir, and dasabuvir.[70,120]

Treatment with aciclovir, ganciclovir, or valaciclovir might be considered for herpesvirus infections, although their efficacy has not been directly evaluated in patients with myocarditis. Persistently high loads of HHV6 genomes in blood cells or tissues confirm the presence of ciHHV6. Elimination of the chromosomally integrated virus is impossible, but the transcriptional activity of ciHHV6 may be reduced by treatment with valganciclovir.[88]

7.4 Post-viral autoimmunity

Myocardial inflammation or systemic autoimmunity persisting despite virus elimination warrants immunosuppressive treatment, in order to prevent later immune-mediated myocardial injury. However, viral genomes have to be excluded prior to immunosuppressive therapy as analysis of patients with DCMi showed that patients with persistent viruses did not improve or even deteriorated upon immunosuppressive therapy, while virus-negative patients improved significantly.[121,122] Treatment approaches for these patients with post-infectious chronic myocarditis/inflammatory cardiomyopathy consist of corticosteroids, azathioprine, or cyclosporine A, in addition to optimal heart failure medication.[123]

T_H17 cell response seems to be one of the keys in the progression to chronic damage, cardiac fibrosis, and loss of cardiac function in autoimmune processes.[5] The potential of an anti-IL-17 therapy still needs to be evaluated.

The Phase 3, multicentre double-blind, placebo-controlled, randomized-withdrawal study *RHAPSODY* provided evidence of the potential efficacy and safety of rilonacept, an IL-1α and β inhibitor in chronic pericarditis.[124] This agent may also be considered as a potential therapeutic option for post-viral inflammation processes.

8. Perspectives

8.1 Prophylaxis

Whereas conventional antiviral vaccine development methods[125] have proven efficient against SARS-CoV-2, the most recent virus of immense medical impact, novel, and entirely RNA-based vaccines have yielded exceptionally good results against this agent.[126–128] The revolutionary method successfully used to develop the *BioNTec© and Moderna©* vaccine was never before employed at scale, and indeed the RNA modification/stabilization/purification methods[129,130] as well as the associated nanoparticle delivery tools[131] are of recent origin. Importantly, as emphasized by the authors of the landmark paper reporting the results of the *BioNTec©* vaccine trial,[126] they could start the development of the vaccination RNA sequence immediately after the publication of the genome sequence of the new virus,[28] which was derived soon after the recognition of COVID-19 as a new disease entity.[132,133] Speed and adaptation to entirely new or variant viruses, which unfortunately are most likely to emerge in the future, are significant advantages beyond the current pandemic.[134]

8.2 New treatment strategies

Whereas prophylaxis was and will of course always be superior to any possible treatment, a spectrum of novel nucleic acid-based therapeutics against molecular targets that cannot be sufficiently or optimally addressed using traditional small molecule drugs or antibodies, has recently successfully entered the clinical arena. In the field of cardiovascular medicine,[135,136] several large-scale clinical trials have proven clinical efficacy of RNA-targeted therapeutics for gene silencing (ASO antisense oligonucleotides; RNA interference-inducing siRNAs). Long-acting ASO and/or siRNA molecules lower apo(a), PCSK9, apoCIII, ANGPTL3, or transthyretin (TTR) for the prevention and treatment of patients with atherosclerotic CVD or TTR amyloidosis. Further approaches of interest are miRNA-modulating and epigenetic therapies, as well as methods based on CRISPR-Cas systems. The latter are of particular interest for the field of virology, too, since they are highly adaptable to essentially all viruses and their individual key molecular therapeutic targets. While below, we focus on SARS-CoV-2, all other cardiotropic viruses are amenable to the same strategies.

It is also important to note that the incidence of cardiovascular/myocardial infections with several viruses is known to be highly variable over decades. Since detection of myocardial viral infections is far more difficult compared to systemic ones, this epidaemia-like rise and fall of viruses, such as CVB3 could only be detected by large-scale in-depth myocardial diagnostics, which are not commonly conducted.[74] This is an unfortunate situation since at times CVB3 or ADVs caused a large fraction of all heart failure cases among children and adults, whereas their incidence is currently low. Conversely, if a large epidemic or even pandemic with a highly cardiotropic virus 'free' of systemic signs on infection would be rapidly spreading, clinical recognition of this wave could be critically delayed until a rather high number of heart failure cases arises in a population without recognizable risk factors. In fact, this was the way by which the first viral myocarditis/heart failure 'outbreak' with CVB3 was discovered in the small city of Coxsackie, in New York state. On the other hand, recent CRISPR-based technological breakthroughs including massively multiplexed nucleic acid detection using the CARMEN-Cas13 system[137] now enable more comprehensive virome screening than prior PCR-based approaches.

8.2.1 CRISPR-based methods

A recent landmark paper reported the development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza.[138] The authors demonstrate a CRISPR-Cas13-based strategy, prophylactic antiviral CRISPR in human cells (PAC-MAN), for viral inhibition that can degrade RNA from both SARS-CoV-2 sequences and live influenza A viruses in human lung epithelial cells. Importantly, their bioinformatic analysis showed that a group of only six crRNAs can target more than 90% of all coronaviruses. They conclude that with the development of a safe and effective system for respiratory tract delivery, PAC-MAN has the potential to become an important pan-coronavirus inhibition strategy.

8.2.2 ASO- and RNAi-based methods

siRNA molecules for silencing nucleocapsid phosphoprotein and surface glycoprotein gene of SARS-CoV-2 have been designed.[139,140] Other groups[141,142] have determined the structural landscape of SARS-CoV-2 RNA and regulatory untranslated regions of SARS-CoV-2 and other coronaviruses. They found ASOs targeting the structural elements and FDA-approved drugs inhibiting the SARS-CoV-2 RNA-binding proteins dramatically reduced SARS-CoV-2 infection in cells derived from human liver and lung tumours. These studies shed light on ASO candidate therapeutics.

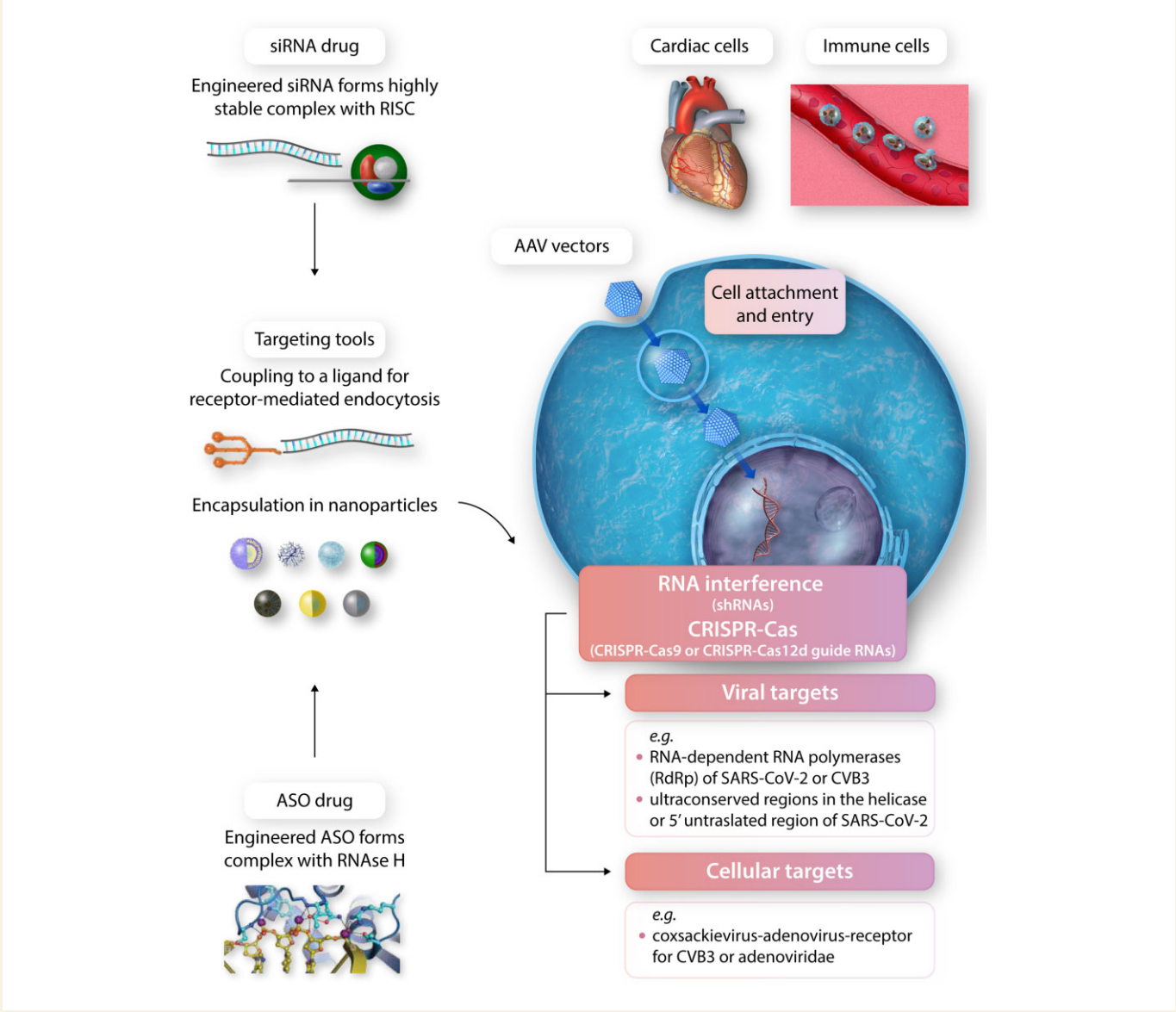

Figure 4 Innovative antiviral strategies. The continuing need for the development of innovative antiviral strategies is strikingly illustrated by the catastrophic SARS-CoV-2 pandemic, which suddenly arose by transmission of an animal virus to man and is difficult to control, amongst other problems, due to sequential accumulation of mutations. The recent introduction of novel therapeutic approaches based on biological antiviral defence systems (RNA interference, CRISPR-Cas) or antisense drugs (ASOs) is most welcome in this context. Although technically demanding, RNAi and ASO drugs have entered cardiovascular clinical practice when the key problem of their liver-directed targeting was solved by ligand-coupling and nanoparticle encapsulation (to the left). Further development of ASO, RNAi 141, 142 and CRISPR-Cas 140, 163 antiviral drugs justifies major efforts since essentially any viral or cellular target (examples are given for Cosackieviruses and SARS-CoV-2) may be addressed by these highly flexible tools once efficient delivery to the affected tissue is enabled. In that regard, a recent pioneering study by Bailey *et al.*[15] is of interest. Decades after similar work on CVB3 myocarditis in humans, this article dealing with SARS-CoV-2 finds similarly restricted cellular tropism (cardiomyocytes but not cardiac macrophages, fibroblasts, or endothelial cells) and mechanistic sequelae of SARS-CoV-2 infection (innate immune activation with cytokine induction, sarcomere disassembly, and cell death). Whereas recombinant AAV vectors (to the right) were successfully employed for RNAi and anti-miR therapy of myocardial disorders in animal models, this approach has not yet entered the clinical arena. Global efforts, significantly driven by the current pandemic, are currently being devoted to fully exploit the clinical potential of these new antiviral strategies.

Cardiomyocyte-targeted RNAi has been investigated to inhibit cardiotropic viruses[143,144] including human CVB3[145,146] and human ADV[147] in cardiomyocytes. Of note, B19V may be transactivated by adenoviral helper functions in vascular endothelial cells[148] illustrating the impact of intercurrent viral co-infections upon clinical course. In addition to directly antiviral approaches, RNAi was also evaluated regarding its potential to suppress pathogenic cardiac inflammation.[149] RNAi against a single cellular target was able to block multiple interacting pro-inflammatory and profibrotic pathways in cardiac fibroblasts. Successful clinical translation of these approaches, as well as of recombinant expression of virus receptor traps[150] critically depends on the availability of clinically safe and efficient drug delivery systems (*Figure 4*).

8.2.3 Non-coding RNA (ncRNA) targets

ncRNAs including miRNAs are deeply involved in the host cells' innate antiviral immune response.[151] There are multiple targets for human miRNAs on SARS-CoV-2 RNA, most of which are located in the 5' and 3' untranslated regions. Mutations of the viral genome that result in the creation or loss of miRNA-binding sites may therefore have substantial effects on the pathogenicity of SARS-CoV-2.[152] Thus, Alam *et al.*[153] have shown that human miRNA-122, a previously known cofactor of another RNA virus, HCV, whose genome it binds as a prerequisite for pathogenesis, can also bind the RNA genome of SARS-CoV-2 with high affinity. This opens the possibility of using RNA-based drugs against HCV, such as Miravirsen, to treat COVID-19.

Relevance of miRNAs for the clinical course of infections has also been documented for human cardiomyopathies associated with B19V[154] or CVB3. In the latter, differential cardiac miRNA expression closely predicted the clinical course. The most highly expressed miRNAs associated with rapid progression and an adverse outcome could possibly constitute RNAi targets.

8.2.4 Remaining challenges

We recently discussed that the high potential of CRISPR and other nucleic acid drugs needs to be weighed against potential risks, but from the clinical practice viewpoint the delivery issue of the nucleic acid drugs to target organs is only partially solved. Current nanoparticle vehicles employing the Gal-NAc system have efficiently delivered ASO and RNAi drugs to the liver as documented in several landmark trials in the cardiovascular field.[135] Progress has also been achieved towards aerosol delivery of nucleic acid drugs to the lung including a combination treatment using an inhalable GapmeR oligonucleotide and recombinant ACE2 for COVID-19.[155,156]

Biologically efficient delivery of nucleic acid therapeutics (siRNAs) to the myocardium has been achieved by recombinant expression from AAV viral vectors in animal models.[157,158] Whereas this delivery approach has not yet entered the clinical arena.[159,160] AAV-based as well as non-viral delivery of a broad spectrum of novel antiviral nucleic acids drugs would thus become available for treatment trials of viral cardiomyopathies. In summary and synopsis with a recent comprehensive review by Le *et al.*[161] on nucleic acid-based technologies targeting coronaviruses, it is evident that possible clinical success of any nucleic acid drug is critically dependent on the technological challenge of efficient and focused drug delivery.

8.3 Need for highly versatile antiviral tools

Importantly, the above new therapeutic approaches offer extremely high versatility to adapt to essentially *any* coding or non-coding, viral or host cell, molecular target. Further, their large-scale production will follow similar (i.e. RNA, DNA, and XNA) synthetic pathways, enabling massive up-scaling of therapeutics production if required.

The current pandemic, originating from transmission of a mutated animal virus to man, has heightened concerns and awareness that amongst the vast number of animal viruses others may cross the species barrier to humans.[162,163] Therefore, foresighted expansion of our antiviral arsenal appears warranted.

The combination of genetic factors that increase susceptibility to cardiomyopathy combined with acquired causes of cardiomyopathy, such as viral infection and/or autoimmunity, may be an explanation for the variable penetrance and severity of DCMi. The availability of novel techniques and novel insights into pathophysiology will help to address knowledge gaps in the future. Efficacy of therapeutic approaches needs to be evaluated in large, controlled, randomized trials to facilitate the development of personalized treatment options.

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Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

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