



## Association between Histological Changes and Clinical Manifestations of Fulminant Myocarditis

Chen Chen and Dao Wen Wang

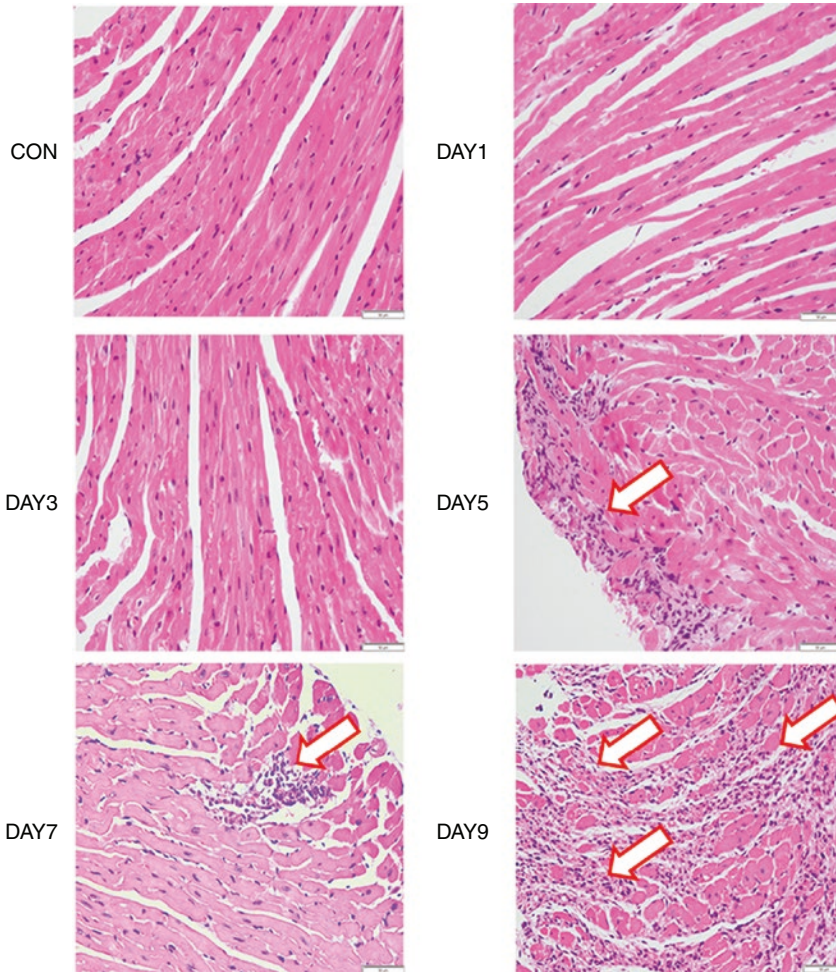
Myocarditis is an inflammatory disorder of the myocardium, usually involving mononuclear cells infiltrating the myocardium. The lesions can be focal or diffuse. The core mechanism of myocarditis is the pathophysiological changes caused by an inflammatory response [1]. Fulminant myocarditis, an acute form of myocarditis, involves the rapid generation of numerous inflammatory cells that invade the heart [2] (Fig. 9.1).

Based on the type of infiltrating cells, myocarditis can be classified as lymphocytic, giant cell, eosinophilic, or cardiac sarcoidosis [2], while based on the clinical course of the disease, it can be classified as fulminant, acute, chronic active, or chronic persistent [3]. In general, the pathophysiological processes of the different types of myocarditis are similar. Viral infections are the

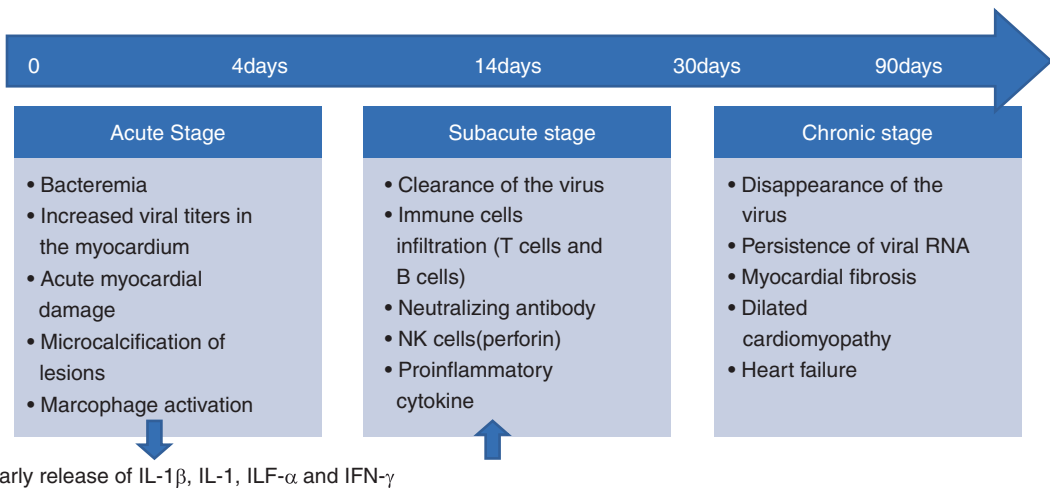
main cause of myocarditis, and enterovirus infections are the most thoroughly studied ones, especially the Coxsackievirus B3 (CVB3) infection [4]. At the cytological level, the pathophysiological process of myocarditis is divided into three stages, acute: viral colonization and replication, subacute: inflammatory cell infiltration, and chronic: ventricular remodeling (Fig. 9.2) [5]. Most viruses that cause myocarditis are localized in cardiomyocytes, but some viruses can also infect the non-cardiomyocyte cells of the heart such as endothelial cells and lymphocytes (Table 9.1). Cardiac damage during viral myocarditis occurs mainly through two aspects: direct damage caused by virus infection and indirect damage caused by the immune response of the host [1].

---

C. Chen (✉) · D. W. Wang (✉)  
Division of Cardiology, Department of Internal  
Medicine and Hubei Key Laboratory of Genetics and  
Molecular Mechanisms of Cardiological Disorders,  
Tongji Hospital, Tongji Medical College, Huazhong  
University of Science and Technology, Wuhan, China  
e-mail: [chenchen@tjh.tjmu.edu.cn](mailto:chenchen@tjh.tjmu.edu.cn);  
[dwwang@tjh.tjmu.edu.cn](mailto:dwwang@tjh.tjmu.edu.cn)



**Fig. 9.1** Disordered myocardial cells infiltrated by numerous inflammatory cells (blue blobs indicated by arrows), as detected by hematoxylin and eosin staining of myocardium of mice with fulminant myocarditis



**Fig. 9.2** Three stages of viral myocarditis in mice

**Table 9.1** Target cells of different pathogenic viruses in myocarditis

Virus	Main target cells
Adenovirus	Myocardial cell, fibroblast, endothelial cell
Enterovirus/ Coxsackie virus	Myocardial cell
Parvovirus	Myocardial cell, endothelial cell
Human herpes virus	Endothelial cell?
Cytomegalovirus	Myocardial cell
EB virus	Lymphocyte
Influenza virus	Macrophage, lymphocyte
Hepatitis C virus	Myocardial cell
HIV	Myocardial cell

## 9.1 Lymphocytic Myocarditis

Lymphocytic myocarditis is the most common type of myocarditis, and viral myocarditis mostly manifests as lymphocytic myocarditis. The pathology of lymphocytic fulminant myocarditis is characterized by a huge infiltration of lymphocytes in the myocardial and perivascular interstitium, along with the presence of edema (fluid trapped in gaps between cardiomyocytes) and plasma cells (Fig. 9.3). Immunohistochemical studies followed by classification of the infiltrating lymphocytes identified CD3<sup>+</sup>, CD4<sup>+</sup>, and CD8<sup>+</sup> cells as well as CD20<sup>+</sup> B lymphocytes. Furthermore, an equivalent number of CD56<sup>+</sup> macrophages as well as MPO<sup>+</sup> monocytes are also presented (Fig. 9.3).

The classification of the inflammatory cells provides important clues regarding the mechanism of action of the pathogenic molecules; the molecules act on the pattern recognition receptors of cardiomyocytes, resulting in the production of cytokines and chemokines and the infiltration of monocytes or macrophages. This is followed by the initiation of the adaptive immune response via a series of mechanisms that include production of lymphocytes and plasma cells, which leads to an immoderate immune activation and inflammatory storm [6].

In clinical practice, 32 patients of lymphocytic fulminant myocarditis, as confirmed by endomyocardial biopsy, who initially appeared healthy, experienced a sudden onset of symptoms

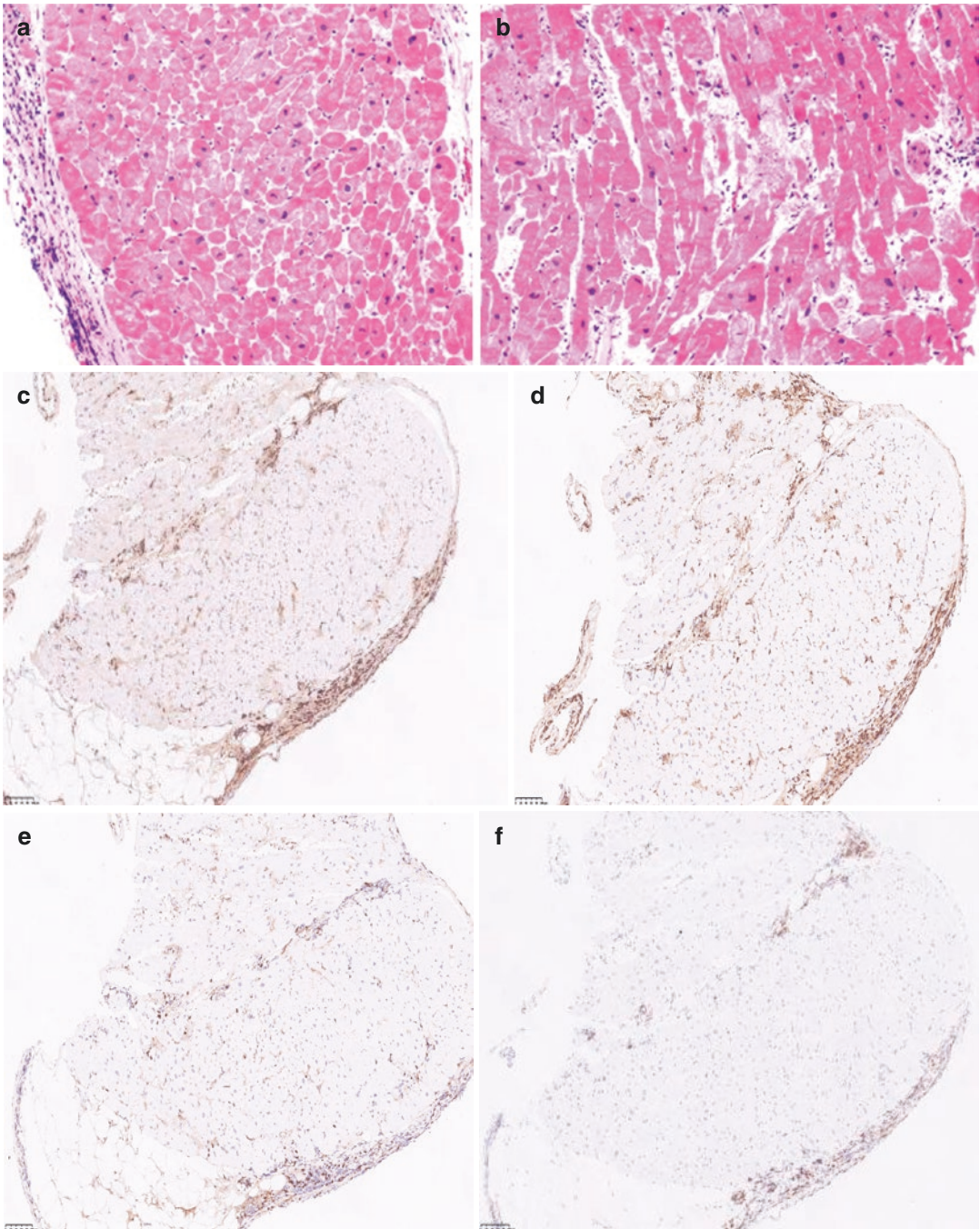
after prolonged stress and arrived at the hospital within 3–5 days in a state of hypotension, shock, and with recurrent syncope.

It has long been recognized that myocarditis is primarily caused by viruses; studies have identified CVB3 receptors in cardiomyocytes and established mouse models of myocarditis accordingly [7]. Although viruses were not detected in most patients, the role of viral infections as initiators of the immune response cannot be excluded. The results of the study in the CVB3 mouse model are briefly introduced below.

Myocarditis caused by CVB3 infection is divided into a pre-infection period (stage 0) followed by three post-infection periods (stages 1, 2, and 3) [7]. The pre-infection period (stage 0) is very important for the prevention of fulminant myocarditis, which can be effectively prevented if susceptibility to viral infection is rapidly identified and appropriate measures are taken. Stage 1 comprises the acute phase during which the virus actively replicates in myocardial cells; most patients with fulminant myocarditis present in this phase. Stage 2 is the subacute phase during which viral replication ceases. However, the viral genome continues to be expressed in the myocardial cells and can effectively recruit inflammatory cells to infiltrate and induce an immune response that damages the myocardium. Stage 3 is the chronic phase, during which the viral genes have been cleared, but the immune response persists, and the heart remodels. There is a high probability that the virus will not be detected in a Stage 3 myocarditis patient.

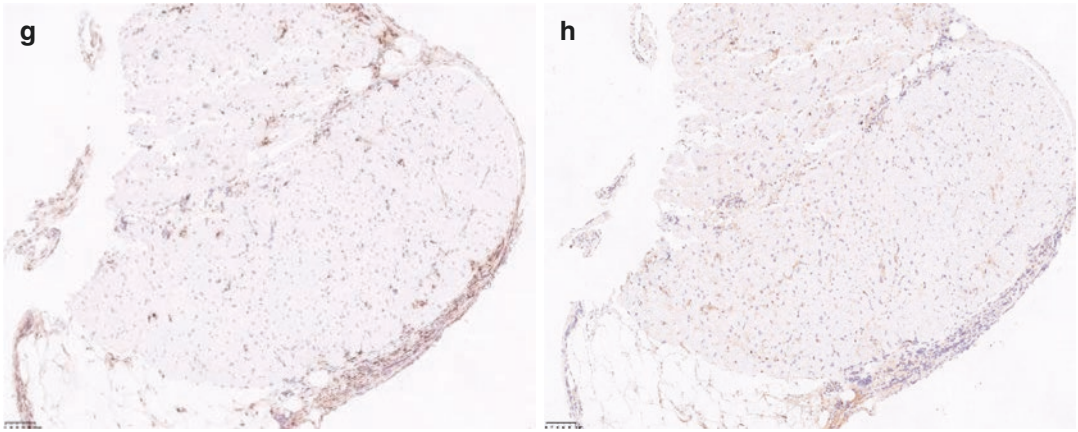
### 9.1.1 Stage 0 (Pre-Infection Stage)

In terms of clinical value, prevention of myocarditis reduces its morbidity and mortality radically, and should therefore be prioritized. Advances in immunization against enteroviruses have proven to be effective in preventing myocarditis [8–10]. However, since the morbidity and mortality rates of various types of viral myocarditis, especially fulminant myocarditis, are currently unclear, the risk-benefit ratio of using viral vaccines to prevent myocarditis is unknown.



**Fig. 9.3** The pathology of endocardial biopsy sample of a patient with lymphocytic fulminant myocarditis. Hematoxylin and eosin staining (**a, b**) showing infiltration of neutrophils and lymphocytes in endocardium and myocardial interstitium, degeneration and partial lytic necrosis

cardiomyocytes, and interstitial edema; as well as immunohistochemical staining of CD3 (**c**), CD4 (**d**), CD8 (**e**), CD20 (**f**), CD56 (**g**) and MPO (**h**) showing the infiltration of various classes of lymphocytes and neutrophils



**Fig. 9.3** (continued)

Hence, they are not yet suitable for promotion in the general population.

Factors that influence the susceptibility of the heart to viral infections are not completely understood yet. Why do some people contract viral infections after exposure while others do not, despite living in the same closed environment? Why do individual symptoms of infection vary from mild discomfort to acute myocarditis or even to fulminant myocarditis? The influence of genetic as well as environmental factors on the development of myocarditis, especially fulminant myocarditis, deserves further investigation.

Normally, the non-pathogenic strain of coxsackievirus, CVB3/0, does not attack the heart, but the occurrence of genetic mutations in six nucleobases renders its genome (CVB3/0Se-) identical to that of pathogenic strains (CVB3/M1 or CVB3/20), causing it to develop pathogenicity and increasing the incidence of viral myocarditis in selenium-deficient mice [11].

Susceptibility to viral myocarditis is affected not only by the genetic variation of viruses but also by the genetic polymorphism of the host. In 2010, genes of 57 patients who had positive endomyocardial biopsies for enterovirus infection were sequenced, which led to the identification of genetic variants of the *TLR3* (Toll-like

receptor 3) gene that increased host susceptibility to viral cardiomyopathy [12]. Mutations in the *TLR3* gene (P554S or L412F) inhibits the NF- $\kappa$ B and type I interferon signaling pathways, attenuates cardiac autophagy and repair functions, enhances viral replication in the heart, and activates abnormal immune responses, ultimately resulting in impaired cardiac function [12].

There are limited genetic studies on myocarditis, most of which have been disseminated as small sample reports. Although polymorphisms in genes, such as *HLA-DQ* and *CD45*, have been reported to be associated with the risk of developing myocarditis, validation of the sites of these genetic variances using large multicenter samples and biological studies to determine the specific mechanisms by which these polymorphisms lead to myocarditis, especially fulminant myocarditis, are required [13, 14].

### 9.1.2 Stage 1 (Acute Stage)

This stage comprises the period from the beginning of the viral infection in the heart to the cessation of viral replication, which is usually within 2 weeks of infection; fulminant myocarditis is typically presented at this stage. The pathophysiological processes include: (i) entry of the virus

into cardiomyocytes, (ii) virus replication within the cardiomyocytes, (iii) direct viral damage to cardiomyocytes, and (iv) transcellular viral infection of adjacent cardiomyocytes. The treatment of viral myocarditis could target these key processes.

### 9.1.2.1 Entry of Virus into Cardiomyocytes

It has been previously demonstrated that coxsackieviruses and adenoviruses are mediated into host cells by the Coxsackievirus and adenovirus receptor (CAR) [15]. Additionally, these viruses can infect host cells via decay-accelerating factor (DAF, CD55) or integrin ( $\alpha v\beta 3$  and  $\alpha v\beta 5$ ) that act as receptors [16].

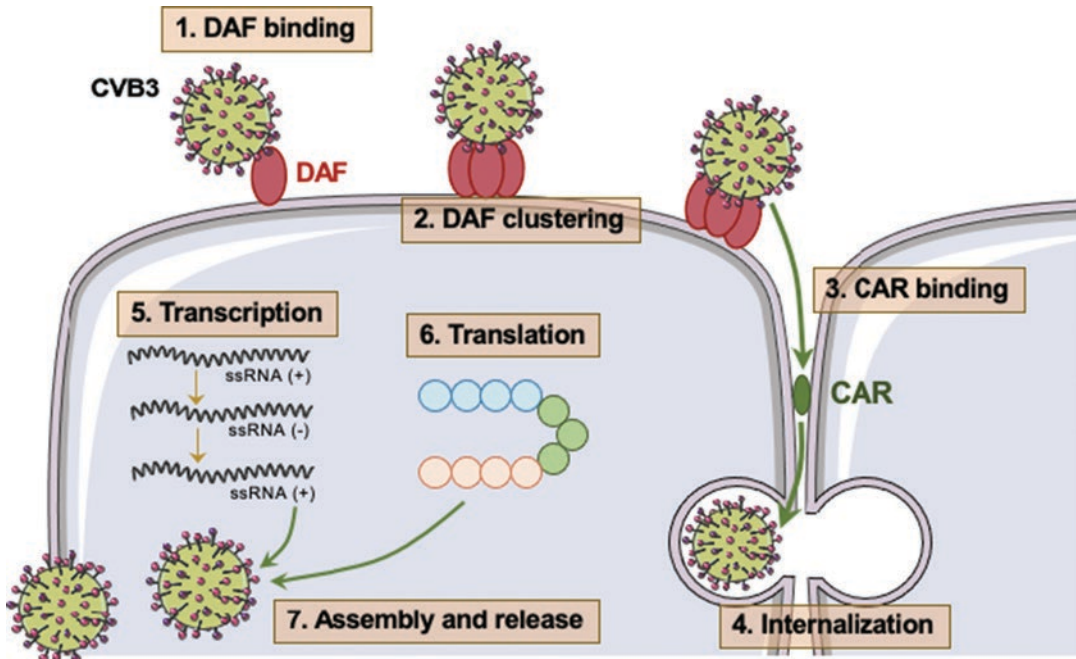
The CAR is a transmembrane protein belonging to the intercellular adhesion molecule family that mediates rhinovirus and enterovirus infection in host cells. It has a region comprising two extracellular immunoglobulin structural domains that forms an inverse parallel dimer by binding to another CAR on an adjacent cell (Fig. 9.4). In the physiological state, CARs are localized in the AV node and mediate electrical conduction; there-

fore, their impairment by the virus leads to the development of different forms of arrhythmia in patients of myocarditis.

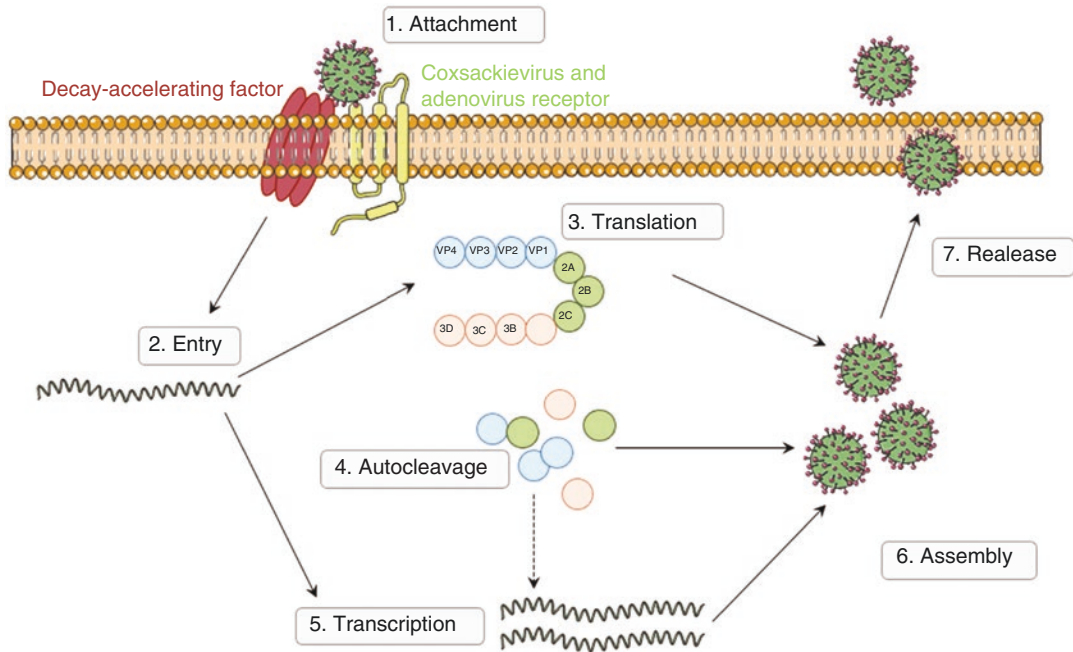
### 9.1.2.2 Replication of Virus

Once the virus enters the host cell, it utilizes various host cell molecules for replication, including proteins and non-coding RNAs, thus further aggravating the damage to cardiac function that could even progress to dilated cardiomyopathy. Various signaling pathways in the host cell have been identified to play important roles in the different stages of the viral life cycle, such as viral entry, replication, release into other cells, and evasion of the host's immune response (Fig. 9.5) [17].

Entry of viruses into epithelial cells via tight junctions is mediated by Fyn and Abl kinases [18]. Coxsackievirus infection activates the tyrosine protein kinase p56lck, MAPK1/2 kinase, and protein kinase C (PKC) signaling pathways, which in turn activate the host cell immune response while promoting viral replication [19]. In addition, the virus-induced p38 MAPK as well as glycogen synthase kinase (GSK-3 $\beta$ ) pathways promote apoptosis and necrosis, which then pro-



**Fig. 9.4** Entry of CVB3 into cardiomyocytes via CAR



**Fig. 9.5** Life cycle of CVB3

mote the release of the virus from the cell, facilitating subsequent cell invasion and infection [20]. Administration of p38 MAPK inhibitors to mice with coxsackievirus infection-induced myocarditis reduced viral replication in cardiomyocytes, attenuated myocardial injury, and improved cardiac function [21]. Notably, these pathways are unable to mediate the injurious effects of viruses on the heart independently and require interaction with each other. Furthermore, viruses utilize different signaling pathways of host cells at various life cycle stages to ensure their continued survival.

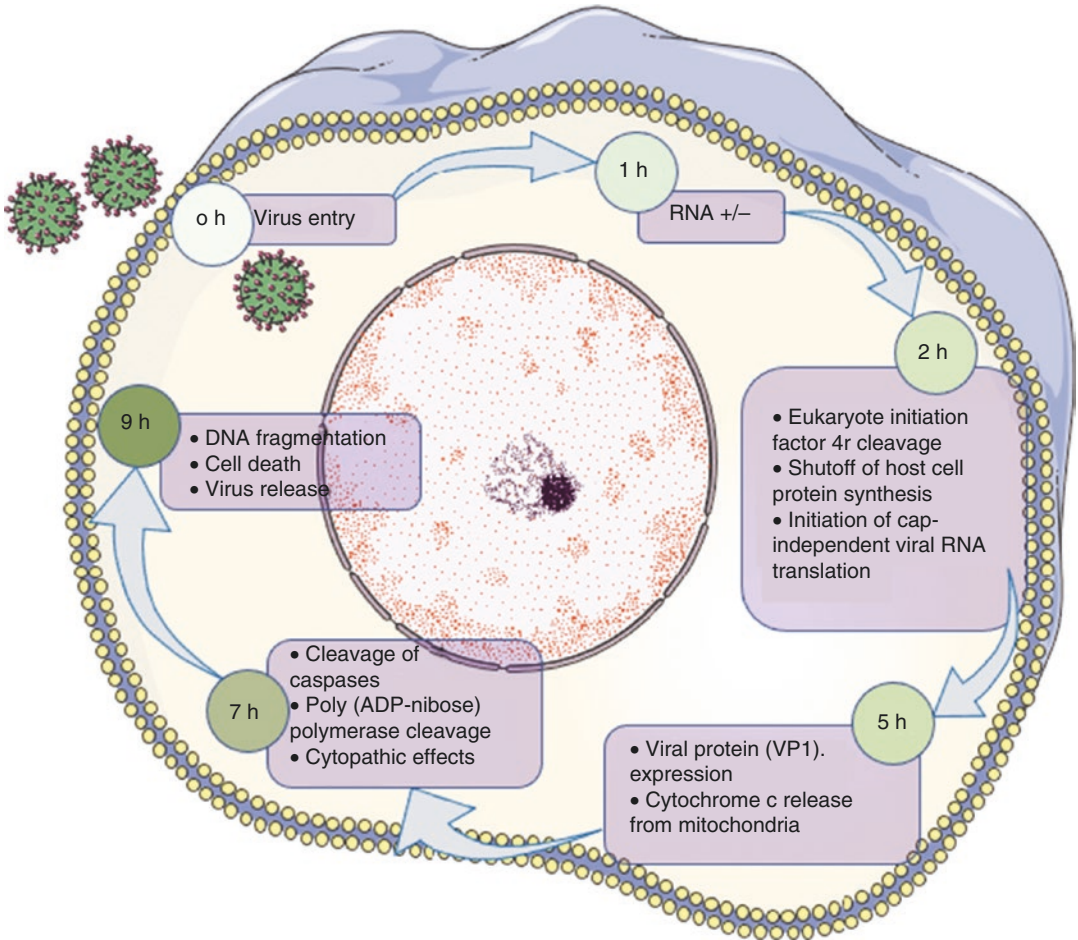
Viruses are able to exploit non-coding RNAs, especially microRNAs (miRNAs), present in host cells to promote their own replication and inflict damage on the host cells. Infection with coxsackieviruses promotes the expression of miR-141, which was the first miRNA identified to be associated with coxsackievirus replication. MiR-141 blocks the expression of various proteins in host cells by inhibiting eIF4E [22]. Host-derived miRNAs can also interact with viral genomes; host miR-10a-3p promotes viral syn-

thesis by interacting with a sequence of coxsackievirus RNA [23].

Although most virus-induced host miRNAs promote viral replication and host cell destruction, some of them, such as miR-221 and miR-222, protect the host from viral invasion. These miRNAs, whose expression is significantly elevated in coxsackievirus-induced myocarditis, inhibit viral replication as well as host cell inflammatory responses, and reduce host cell apoptosis, mainly by suppressing host genes such as interferon regulatory factor 2 (*IRF2*), C-X-C Motif Chemokine Ligand 12 (*CXCL12*), and B-cell lymphoma 2 (*bcl-2*) [24].

### 9.1.2.3 Direct Viral Damage to Cardiomyocytes

After a successful evasion from the host's innate immune response, the virus begins to replicate in host cells and produces viral proteins that directly attack the cardiomyocytes (Fig. 9.6). Therefore, viral infection of immunodeficient mice led to severe symptoms that manifested as fulminant myocarditis [25].



**Fig. 9.6** Direct cell damage by CVB3 after cell entry

The enterovirus genome encodes protease 2A and protease 3C, which process the viral polyproteins into separate structural or nonstructural proteins that are essential to complete the entire viral life cycle. These proteases inhibit the formation of translation initiation complexes and prevent the production of cell membrane repair proteins [26]. In addition, protease 2A specifically affects many host proteins related to cardiomyocyte structure, signal transduction, and contractile function. It is capable of shearing the host eukaryotic initiation factor-4G (eIF4G), a key initiation factor required for translation, and inhibiting the synthesis of contractile proteins in cardiomyocytes [27]. It also shears the hinge 3 region of the cell repair-related protein dystrophin. This dis-

rupts the integrity of the myocardial membrane, increases cell permeability, promotes virus spread to neighboring cells, and increases the susceptibility of the heart to the virus [28, 29].

Autophagy is an important mechanism by which cells repair themselves. During CVB3 infection, cell function is damaged due to shearing of glycine at position 241 in Sequestosome 1 (SQSTM1), an important protein in the autophagic response, which leads to the impair of autophagic response, reduction in the clearance of damaged proteins and subsequent accumulation of misfolded proteins [30].

To investigate the role of proteinase 2A, transgenic mice with high expression of proteinase 2A in cardiac myocytes were developed. These mice



displayed rapid enlargement of heart chambers, decreased cardiac function, cardiac fibrosis, disturbed cardiomyocyte arrangement, and impaired cardiac cytoskeletal structure, suggesting that proteinase 2A causes direct damage to the structure and function of cardiac myocytes [31].

During viral infection, proteinase 2A also shears the melanoma differentiation-associated protein 5 (MDA5) of the host, inhibits the production of type I interferon, and weakens the host's virus-clearance mechanism [32]. In addition

to shearing proteins, proteinase 2A and proteinase 3C activate the exogenous (caspase-8-mediated) and the endogenous (mitochondrial) apoptotic pathways, directly causing cardiomyocyte apoptosis or necrosis.

These studies demonstrated that virus-encoded proteases can directly attack cardiomyocytes in several ways (Table 9.2), suggesting that effective inhibition of viral protease function is necessary for the treatment of viral myocarditis [33].

**Table 9.2** Targets of enterovirus proteases

Type	Protease	Virus	Target genes
Cytophylaxis	2A	PV, EV71	MDA5
	2A, 3C	CVB3, HRV, EV71, PV	MAVS
	3C	CVB3, PV, EV71	RIG-I
	2A, 3C	EV71	NLRP3
	3C	EV71, CVB3	TRIF
RNA/protein synthesis	2A	PV	DCP1a
	3C	PV	La/SSB
	2A	CVB3	p62/SQSTM1
	2A, 3C	CVB3	NBR1
Cell integrity	3C	CVB3	RIP3
	2A	CVB3	Dysferlin
	2A	CVB3	Dystrophin
	2A	HRV	Cytokeratin 8
Gene transcription	3C	PV	MAP-4
	3C	PV	Oct-1
	2A	PV	TBP
	3C	PV	TFIIIC
	3C	PV	CREB
	2A	CVB3	SRF
	3C	CVB3	AUF1
	3C	PV	PTB
	3C	PV	hnRNP M
	2A	PV	Gemin3
Protein translation	3C	CVB3	TDP-43
	2A	CVB3, PV, EV71, RV	eIF4GI, eIF4GII
	2A, 3C	CVB3, PV	PABP
	2A	CVB3	DAP5
	3C	CVB3, PV, HRV	eIF5B
	3C	PV	PCBP
	3C	PV, HRV	p65-RelA
Others	3C	CVB3	IKBa
	2A, 3C	CVB3	GAB1
	2A	HRV	Nup
	3C	PV, CVB3, EMCV	G3BP1

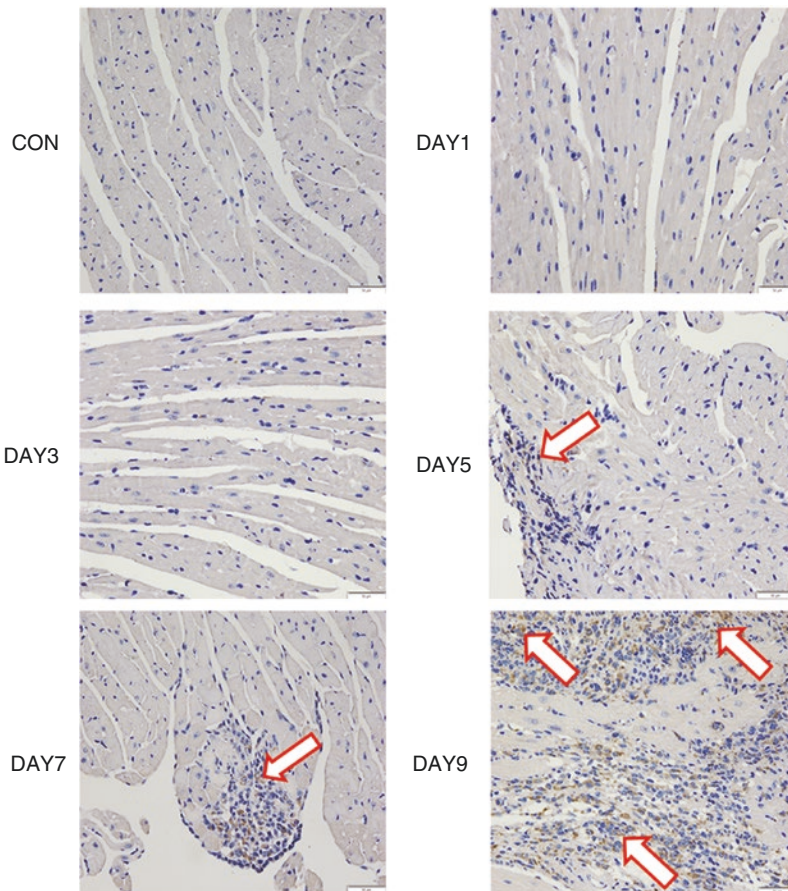
CVB3 Coxsackie virus, EMCV encephalomyocarditis virus, EV enterovirus, HRV human rhinovirus, PV poliovirus

### 9.1.2.4 Host Defense—Innate Immune Response

Once a virus is successfully bound to a receptor, it confronts the host's immune response; this is inevitable and crucial for the clinical regression of myocarditis, especially for fulminant myocarditis. The immune system of higher vertebrates is divided into two main categories: innate and acquired immunity. Innate immune responses are not antigen-specific and can occur rapidly after pathogen stimulation of the immune system, whereas acquired immune responses are antigen-specific. In addition to pathogens such as viruses, other non-infectious etiologies can activate innate and acquired immunities in the host via various mechanisms.

The innate immune response is highly conserved in interspecies evolution and is the host's

first defense against invading pathogens. Upon activation by TLRs, the innate immune response induces macrophages and natural killer (NK) cells, which constitute the main inflammatory cells that infiltrate the heart in lymphocytic myocarditis (Fig. 9.7). During the first 4–5 days of viral infection in the myocardium, acquired immune response is not activated, and the innate immune response induces the release of a variety of cytokines, including interleukin-1 (IL-1), IL-6, and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ), to limit viral replication and dissemination. Previous studies have suggested that activation of endoplasmic reticulum stress inhibits macrophage infiltration in the hearts of mice with viral myocarditis, thereby reducing inflammatory factors released by macrophages and improving cardiac function [34, 35]. Recently, it has been reported



**Fig. 9.7** Immunohistochemical staining showing infiltration of macrophages (brownish areas marked by arrows) and NK cells in the heart of mice with fulminant myocarditis

that the inflammatory response induces neutrophils to migrate to the heart, promote cardiomyocytes to undergo pyroptosis, and impairs cardiac function [36].

However, by the time the innate immune response manages to remove the virus in fulminant myocarditis, the immune system becomes over-activated and recruits a high number of inflammatory cells to the heart, all of which release large amounts of inflammatory factors. The resultant “inflammatory storm” magnifies the damage to the patient’s heart, leading to a dramatic decrease in cardiac function. Simultaneously, the “inflammatory storm” also induces the release of large amounts of vasoactive substances that dilate blood vessels and result in shock (see Chap. 3, Pathogenesis of fulminant myocarditis: The role of cytokine storm in the development of fulminant myocarditis). Therefore, proper control of the “inflammatory storm” to limit the damaging effects of the immune response is one of the priorities in the early stages of fulminant myocarditis treatment. In case the immune response fails to clear the virus completely, the disease becomes chronic and may develop into dilated cardiomyopathy.

### Interferons

This is a class of powerful antiviral cytokines and important effector molecules of the innate immune response. Type I interferons include interferons  $\alpha$  and  $\beta$ , while type II includes interferon  $\gamma$ . In vitro cellular assays have demonstrated that exogenous type I and type II interferons inhibit coxsackievirus replication in cells, while exogenous type I interferons improve the impaired cardiac function in mice with coxsackievirus-induced myocarditis [37]. To investigate the role of endogenous interferon molecules in the pathophysiology of viral myocarditis, mice with type I or type II interferon receptor deficiency were infected with coxsackievirus. Mice with type I interferon receptor deficiency displayed a significant increase in viral replication in the liver and in mortality. However, there was no increase in viral RNA titers in the heart, suggesting that the increase in mortality was not secondary to heart infection. Similar results were observed after coxsackievirus infec-

tion in mice with interferon  $\beta$  deficiency. This suggests that the endogenous type I interferon receptor signaling pathway plays a critical role in organ damage due to viral infection, such as liver damage, but not in viral replication in the heart during the early stages of infection. The absence of the endogenous type II interferon signaling pathway resulted in a slight increase in viral titers in the heart and liver but did not have any effect on mortality. These suggest that there may be other molecules that mediate the role of interferon-related signaling pathways in myocarditis, thus necessitating further investigation.

### TLRs

TLRs are important molecules in the innate immune response that can recognize pathogen-associated molecular patterns (PAMPs) and initiate innate immune defense mechanisms. Various pathogens can activate TLR signaling pathways via different ligands. Ten and thirteen TLR isoforms have been identified in humans and mice, respectively. It has been reported that mutation of the human *TLR3* gene at P554S or L412F decreases its function, inhibits host NF- $\kappa$ B and type I interferon signaling pathways, attenuates cardiac autophagy repair, enhances viral replication in the heart, and activates abnormal immune responses, ultimately impairing cardiac function [12]. In addition, polymorphisms in *TLR2* and *TLR4* genes have been reported to be associated with bacterium-induced septic shock. TLR2, TLR3, TLR4, TLR7, TLR8, and TLR9 are capable of mediating the antiviral effects of type I interferons (Table 9.3). Compared with other tissues, TLR3 and TLR4 expression is relatively low and TLR7, TLR8, and TLR9 are barely expressed in the human heart. Therefore, there is a high

**Table 9.3** TLRs and viral infections

TLRs	Virus ligands
2	Envelope proteins of measles virus, cytomegalovirus, and herpes simplex virus type I
3	Viral double stranded RNA
4	Respiratory syncytial virus F protein, mouse mammary tumor virus envelope protein
7/8	Viral single-stranded RNA
9	CpG DNA

probability of other signaling pathways in the heart that mediate the antiviral effects of interferon in addition to the TLR signaling pathway.

The TLR3 and TLR7/8 signaling pathways are activated by double-stranded RNA (dsRNA) and single-stranded RNA (ssRNA), respectively. However, the genome of the enterovirus is an ortho-stranded ssRNA. Following entry into the host cell, viral RNA is released from the capsid protein and used as a template to form dsRNA. Infection of TLR3-deficient mice with ssRNA viruses leads to the development of severe myocarditis with significantly higher levels of viral replication in the heart, greater myocardial damage, and ultimately, a significant increase in early mortality [38]. Morphological examination of the heart on days 3 and 5 post viral infection revealed that viral titers as well as inflammatory responses were significantly higher in the hearts of TLR3-knockout mice than in the hearts of wild-type mice. This suggests that by inhibiting viral replication in the heart, TLR3 plays an important role in the host's antiviral response. Interestingly, interferon expression was significantly higher in the hearts of TLR3-deficient mice, suggesting that TLR3 may activate the innate immune response via an interferon non-dependent pathway.

TLR4 mainly recognizes lipopolysaccharides of gram-negative bacilli. Coxsackieviruses, among others, activate TLR4 in macrophages, promoting the secretion of inflammatory factors and increasing viral replication in cardiomyocytes [39]. However, the specific mechanism by which viruses activate the TLR4 signaling pathway remains poorly understood. TLR9 primarily recognizes CpG DNA regions of bacteria and viruses. The role of other TLRs in early viral replication in the heart has not been clarified. Myeloid differentiation factor-88 (MyD88) is an important molecule that mediates TLR2, TLR4, TLR5, TLR7, and TLR9 signaling pathways. MyD88-deficient mice revealed significantly lower viral titers in the hearts on days 4, 7, and 10, along with higher survival rates, after coxsackievirus infection as compared to wild-type mice [40]. These results demonstrate the importance and complexity of TLR signaling pathways in cardiac regulation and suggest that viruses

could mediate their actions in the heart via non-classical TLR signaling pathways. The potential roles of different TLRs are inconsistent and require further investigation.

### RNA Helicases

Although the majority of TLRs are localized on the surface of the cell membrane, TLR3, TLR7/8, and TLR9 are localized in the cellular endosome wherein they recognize viral nucleic acids following viral entry into the host cell [41]. Since viral nucleic acid replication products may also be present in the cytoplasm outside the endosome, the TLR signaling pathway in the endosome may not interact with all viral nucleic acid molecules in the host cell. It has been demonstrated that intracellular viral dsRNA can be recognized by two RNA helicases, retinoic acid-induced protein I (RIG-I) and MDA-5 [42, 43]. Further, intracellular viral DNA can be recognized by the DNA-dependent activator of interferon regulatory factor (DAI, also known as DLM-1/ZBP1) [44]. By constructing RIG-I- or MDA-5-deficient mice, the anti-RNA virus properties of RNA helicases have been confirmed, but the anti-DNA viral role of DAI *in vivo* remains to be confirmed. RIG-I recognizes paramyxoviruses, influenza viruses, and epidemic B encephalitis viruses, while MDA-5 recognizes encephalomyocarditis viruses and dsRNA viruses. Both RIG-I and MDA-5 contain two important structural domains: the caspase activation and recruitment domain (CARD) and the RNA helicase domain. The RNA helicase domain recognizes and binds to dsRNA, enabling RIG-I and MDA-5 to form a dimer having an altered structure, thus facilitating the binding of CARD to downstream signaling molecules and activating a series of signaling pathways. In recent years, mitochondrial antiviral signaling (MAVS) has been found to mediate the activation of downstream signaling pathways of RIG-I and MDA-5 [45]. The N-terminal region of MAVS is the CARD, and the C-terminal is the mitochondrial transmembrane structural domain. The complex formed by binding of dsRNA with RIG-I or MDA-5 attaches to the N-terminal CARD of MAVS and subsequently activates a series of transcription factors, such as NF- $\kappa$ B and

interferon regulatory factors 3 and 7, ultimately producing a series of innate immune responses, including type I interferon release. Compared to wild-type mice, the viral titer in the hearts of MDA-5- or MAVS-deficient mice at 48 h after infection with encephalomyocarditis virus was more than 1000-fold higher than that in wild-type mice, suggesting that RNA helicase plays an important role in the removal of intracellular viral nucleic acids [46, 47].

**9.1.2.5 Host Defense–Acquired Immune Response**

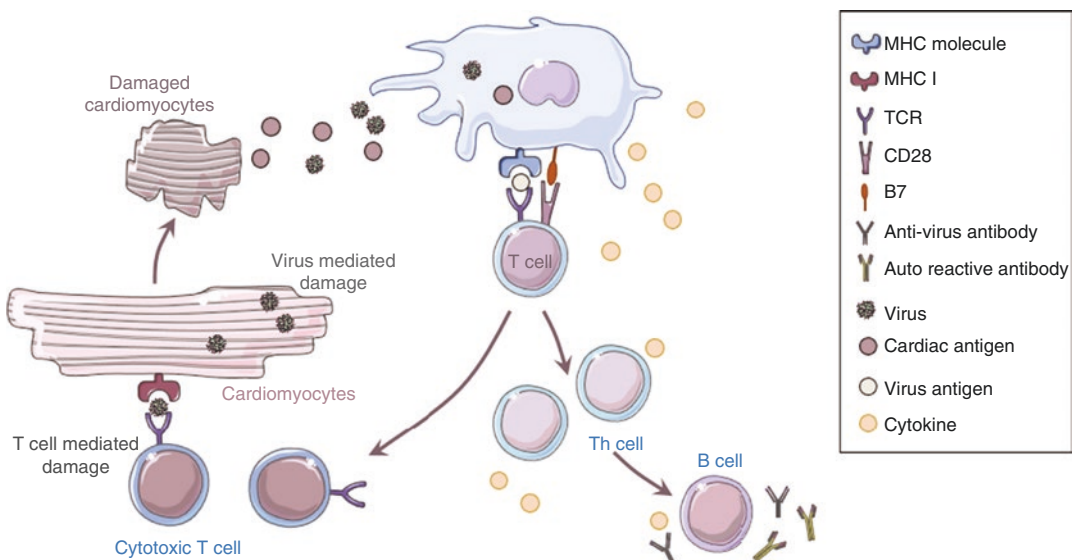
After 6–7 days of viral infection, the acquired immune response is activated and T-lymphocytes begin to infiltrate the heart, which indicates the terminal phase of Stage 1. The peak of T lymphocyte infiltration usually occurs between the seventh and the 14th day post viral infection [48]. However, in fulminant myocarditis, the acquired immune response is activated rapidly, within 2–3 days after infection, or even on the day of infection, recruiting T-lymphocytes to the heart and triggering an “inflammatory storm,” which forms the core pathophysiological mechanism underlying the rapid onset and critical nature of fulminant myocarditis (Fig. 9.8).

Infiltration of T-lymphocytes leads to two distinct effects: the advantageous removal of virally

infected cardiomyocytes, and the disadvantageous effect of damage or necrosis of cardiomyocytes. In a severely and acutely injured heart, upon significant infiltration of terminally differentiated and non-dividing T-lymphocytes, the cellular damage caused by the removal of virally infected cells by T-lymphocytes, cannot be compensated for by the proliferation of normal non-infected cells, which ultimately leads to impaired cardiac function. A previous study had reported that following infection with coxsackievirus, the morbidity and mortality of myocarditis were significantly reduced in CD4+ (helper T-lymphocytes) and CD8+ T-lymphocytes (cytotoxic T-lymphocytes) double-knockout mice as compared to those in wild-type mice [49]. Interestingly, despite complete knock-down of both CD4+ and CD8+ T-lymphocytes, the titer of coxsackievirus in the hearts of double-knockout mice did not change significantly as compared to that in wild-type mice, suggesting the existence of a T lymphocyte-independent viral clearance mechanism in the heart.

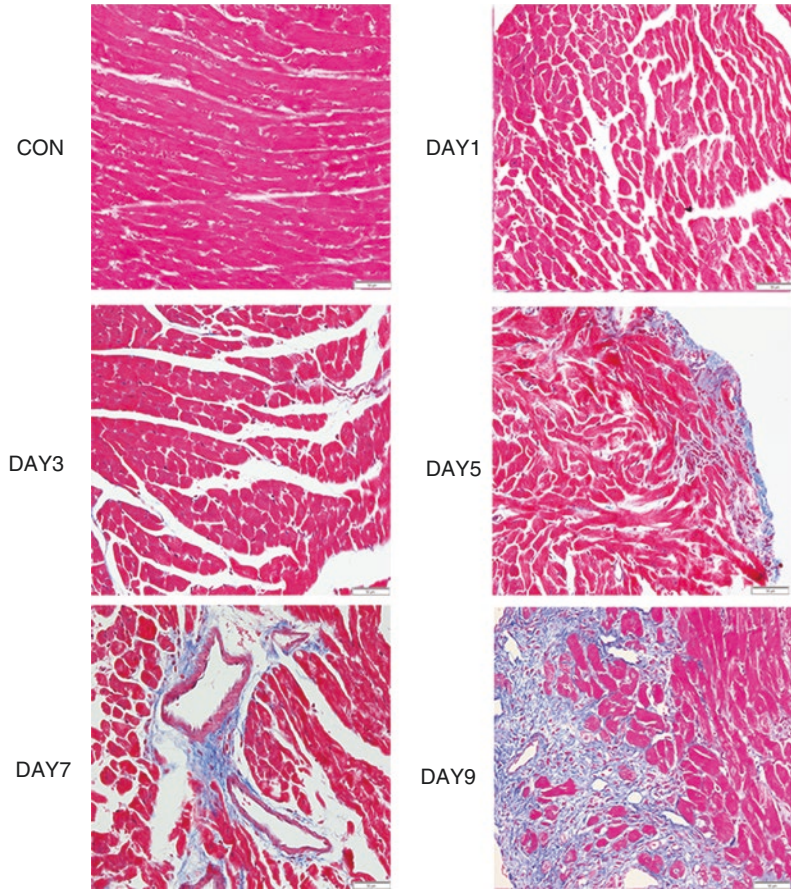
**9.1.2.6 Damage to Host Cells by Host Immune Response**

In the absence of proper control of the host immune response, the damaging effects of the “inflammatory storm” caused by the over-activation of the immune response are stronger



**Fig. 9.8** Immune activation-mediated cellular injury during the course of viral myocarditis

**Fig. 9.9** Masson staining confirms the presence of severe fibrosis (blue stains) in the hearts of mice with fulminant myocarditis



than its beneficial effects of virus clearance, ultimately leading to myocardial fibrosis, ventricular remodeling, and even heart failure (Fig. 9.9).

Regulatory T cells (Tregs) suppress the inflammatory response and reduce host cell immune damage, whereas T helper 17 (Th17) cells enhance the immune inflammatory response and promote host cell damage; together they maintain a delicate yet dynamic balance in the development and regression of myocarditis [50]. Although Tregs promote the release of anti-inflammatory factors, such as transforming growth factor-beta (TGF- $\beta$ ) and IL-10, these factors are unable to inhibit the massive amount of pro-inflammatory factors (such as TNF- $\alpha$  and IL-1) consistently released by host cells during infection, thus leading to irreversible myocardial injury and even acute heart failure [51, 52].

The conversion between M1 pro-inflammatory macrophages and M2 anti-inflammatory macrophages can also influence the regression of myo-

carditis [53]. Comparison of inflammatory responses to coxsackievirus infection in mice of different sexes showed a higher expression of M1 macrophages in the hearts of males and higher expression of M2 macrophages in the hearts of females [54]. More importantly, the myocardial inflammatory response in male as well as female mice was boosted by exogenous M1 macrophages, whereas M2 macrophages altered the inflammatory factor profile of the heart, increased anti-inflammatory factors, promoted Treg differentiation, and significantly reduced the cardiac inflammatory response in male mice [54].

After the activation of the acquired immune response, the massive infiltrating lymphocytes not only removes the infected myocardial cells, but also causes damage to normal myocardial cells. In the 1970s, researchers have observed a significant decrease in the cardiac inflammatory response in T lymphocyte-deficient mice infected with coxsackievirus, as well as in the extent of

myocardial injury and mortality as compared to those in wild-type mice [55].

Further, it is not just the virus that induces an immune response in the host; the host itself produces antigens that induce an autoimmune response. Viral infection in cardiac myocytes damages intracellular autoantigens (such as cardiac myosin), which cross-react with coxsackievirus antigens and induce autoimmune responses, which further activates B lymphocytes, produces large amounts of cardiac autoantibodies and inflammatory factors, and ultimately causes damage to the cardiac myocytes [56].

Since current pathological diagnostic criteria define only the type and number of infiltrating inflammatory cells without a distinction of subtypes or functional types, they are relatively less suitable for prognostic assessment. Further analysis of various cell subtypes and functions is required in the future.

### 9.1.3 Stage 2 (Subacute Stage)

This stage ranges from a few weeks to a few months after viral infection. Studies on animal models have shown that coxsackieviruses persist in cardiomyocytes for an extended period following infection, even without replication. In order to investigate whether the persistence of the non-replicating genome of coxsackievirus in cardiomyocytes promotes the development of dilated cardiomyopathy, cardiac-specific coxsackievirus-mutant transgenic mice were constructed, in which low levels of intact coxsackievirus were expressed in the heart. However, these were incapable of forming intact viral particles and were therefore unable to replicate. The lack of intact viral particles also prevented the production of corresponding antibodies in the host. Histomorphological examination depicted a series of typical dilated cardiomyopathy phenotypes such as interstitial fibrosis of cardiomyocytes, myocardial hypertrophy, and cardiomyocyte degeneration in the hearts of these transgenic mice [57]. In clinical practice, viral genome expression is detected in myocardial biopsy specimens of patients of myocarditis or dilated cardiomyopathy; however, replicative

state viruses are rarely detected in the specimens from patients of myocarditis. These results suggest that the mere presence of the viral genome is sufficient for the development and progression of cardiomyopathy.

### 9.1.4 Stage 3 (Chronic Stage)

This stage mostly ranges from several months to years after viral infection. Since the majority of the patients at this stage fail to detect the presence of the virus or viral genome in the myocardium, they are often diagnosed and treated for other types of heart disease. Even in cases where dilated cardiomyopathy is diagnosed, the cause remains undetermined since the previous infection is unconfirmed. Therefore, the importance of diagnosis early in the infection is even more emphasized along with an urgent need to design tests that are more specific, as well as sensitive, to the detection of viral infection.

---

## 9.2 Giant Cell Myocarditis

Giant cell myocarditis (GCM) is a rare and extremely critical myocarditis that was first reported in 1905 [58]. The onset of the disease is acute, with rapid deterioration of cardiac function. Prior to the introduction of heart transplantation or immunomodulatory drugs, almost all patients of GCM perished within a few days of onset, relying solely on autopsy findings for diagnosis. The onset age of GCM patients is about 40 years old, and 20% of patients have a history of autoimmune diseases [59]. Most patients of GCM present with acute heart failure, with approximately 50% of them developing ventricular tachycardia, different degrees of atrioventricular block, and having a median survival time of 3 months without resorting to heart transplantation or treatment with immunomodulatory drugs [60].

GCM is a T lymphocyte-mediated autoimmune disease; gene expression profiling of myocardial tissue from patients of GCM showed an abnormally enhanced immune response in their hearts, particularly a marked increase in the expression of chemokines associated with Th1 activation in T

helper (Th) cells [61]. In contrast to viral myocarditis, cardiac autoantibodies are uncommon in patients of GCM, suggesting that the pathogenesis of GCM is dominated by T-lymphocytes, rather than B lymphocyte-mediated autoimmune responses. The main pathological feature of GCM is the formation of multinucleated giant cells following massive lymphocyte infiltration is the main pathological feature of GCM, with highly activated T cells, leading to an inflammatory storm.

Monotherapy with glucocorticoid-based immunosuppressants does not improve the prognosis of GCM. However, the combination of concurrent T cell-targeted immunosuppressants effectively mitigates GCM progression. Nevertheless, drug therapy is less effective than heart transplantation and is associated with multiple cardiovascular complications. Moreover, even after heart transplantation, GCM recurs in approximately 25% of post-transplant patients [60].

---

### 9.3 Eosinophilic Myocarditis

Eosinophilic myocarditis is caused by a variety of etiologies and comprises eosinophil infiltration into the heart followed by myocardial endocardial fibrosis. Eosinophils comprise cytoplasmic granules which release cytotoxic proteins in response to immunogenic stimulation, thereby inducing oxidative stress and leading to apoptosis or necrosis. Furthermore, eosinophils can directly damage cardiomyocytes.

One of the etiologies of eosinophilic myocarditis is drug allergy, associated with the use of drugs (antibiotics, such as penicillin, diuretics, or dopamine) or vaccines (such as smallpox vaccine), with a very rare onset. Usually, the onset occurs at the time of administering the drug; however, in rare cases, the onset occurs after several years of drug administration [62].

---

### 9.4 Sarcoidosis Myocarditis

Sarcoidosis is an inflammatory disease involving multiple organs and systems (such as the eyes, skin, lungs, or heart). The pathogenesis of sar-

coidosis has not yet been elucidated; histomorphologically, it manifests as an accumulation of T-lymphocytes, mononuclear phagocytes, and non-neoplastic granulomas. The main pathological feature that differentiates sarcoidosis from GCM is the presence of granulomas which appear as follicular structures composed of tightly packed lymphocytes (especially CD4<sup>+</sup> T cells), giant cells, and epithelial cells, and surrounded by fibroblasts and more lymphocytes (including B cells, CD4<sup>+</sup> T cells, and CD8<sup>+</sup> T cells) [63]. In the early stages of the nodal disease, Th1 cells are stimulated to mediate the activation of the inflammatory response in the heart, as well as to interact with antigen-presenting cells (APCs) to form granulomas. Subsequently, Th2 cells are activated, leading to fibrosis. Sarcoidosis patients have increased levels of Th1 cell-associated cytokine expression in the heart [64]. Myocardial tissue biopsies of sarcoidosis myocarditis patients had revealed a high number of CD209<sup>+</sup> dendritic cells as well as CD68<sup>+</sup> macrophages, whereas the number of CD163<sup>+</sup> M2 macrophages was low [65], suggesting a higher number of injurious macrophages. Although the pathogenesis of sarcoidosis myocarditis is clearly different from that of lymphocytic myocarditis or GCM, there are no specific biomarkers available yet for the clinical diagnosis of sarcoidosis myocarditis [66].

---

### 9.5 Connective Tissue Disease-Induced Autoimmune Myocarditis

Multiple connective tissue disorders (CTDs), such as systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), scleroderma, or dermatomyositis are complicated by myocarditis [67]. Approximately 10% of SLE patients show clinical manifestations of myocarditis, mostly lymphocytic; RA patients have a comparatively lower incidence of myocarditis, which is mostly interstitial or sarcoid [68].

SLE causes monocyte infiltration, formation of immune complexes, as well as complement deposition in the heart tissues of patients of myocarditis. In RA patients, anti-citrullinated protein antibodies are produced following the conversion



of arginase to citrulline. A study using cardiac magnetic resonance technique found that RA patients showing high levels of anti-citrullinated protein antibodies had a higher left ventricular weight index than those with low levels, suggesting that RA may induce myocardial injury [69]. The formation of these anti-autoantibodies in massive numbers followed by their deposition in the heart activates autoimmune responses and impairs cardiac function.

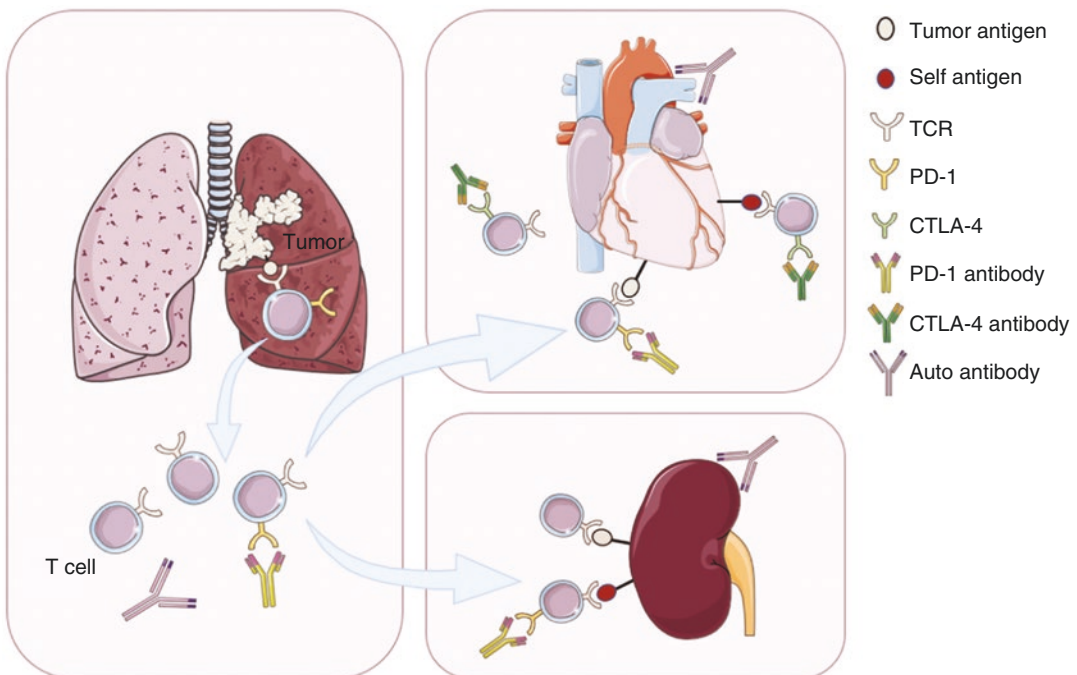
Notably, CTDs also indirectly damage cardiac function by impairing blood vessels.

## 9.6 Immune Checkpoint Inhibitor-Induced Myocarditis

Myocarditis induced by immune checkpoint inhibitors (ICIs) is the most common side effect of ICIs in the cardiovascular system. Infiltration of T-lymphocytes in massive numbers has been observed in the hearts of cytotoxic T lymphocyte-associated protein 4 (CTLA-4) and programmed cell death protein

1 (PD-1) knockout mice, leading to autoimmune dilated cardiomyopathy [70]. Several studies have shown that PD-1 knockout mice are more susceptible to autoimmune myocarditis and suffer greater cardiac damage than the wild type [71–73].

The mechanisms by which ICIs cause myocarditis have not been completely elucidated yet; multiple impairment effects (as follows) may synergize to affect cardiac function: (i) ICIs act as monoclonal antibodies that bind directly to antigens (such as CTLA-4) on the surface of normal cells, leading to T lymphocyte infiltration and complement activation, thus causing myocardial tissue damage. (ii) ICI treatment enhances the off-target effect by promoting the function of T-lymphocytes, which then recognizes the tumor antigen or healthy tissue expressing the antigen through the circulatory system. (iii) ICIs increase the circulating as well as tissue levels of cytokines and promote the infiltration of inflammatory molecules in non-targeted tissues. (iv) ICIs promote autoimmune-associated antibody production, leading to autoimmune responses (Fig. 9.10) [74].

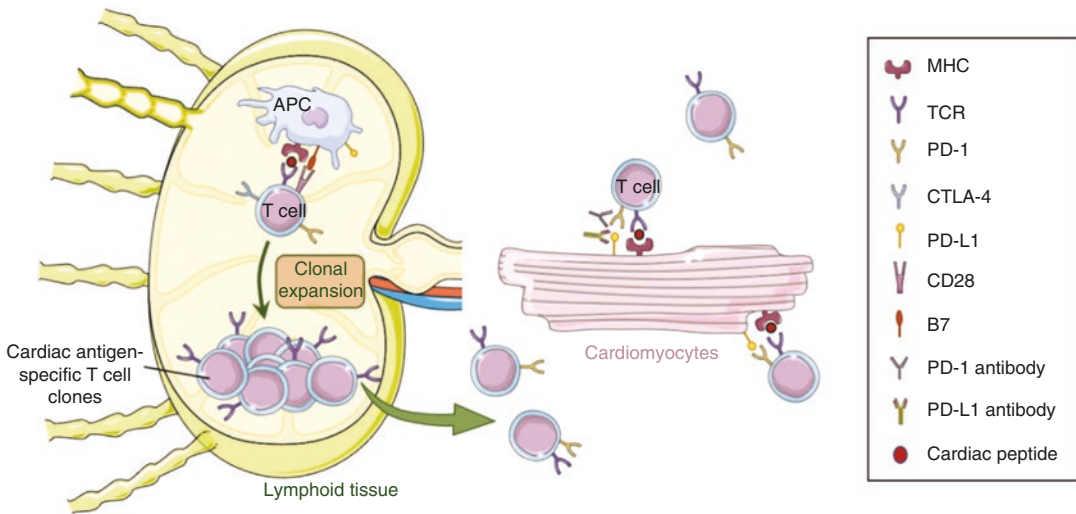


**Fig. 9.10** Mechanisms by which immune checkpoint inhibitors damage the heart and kidney

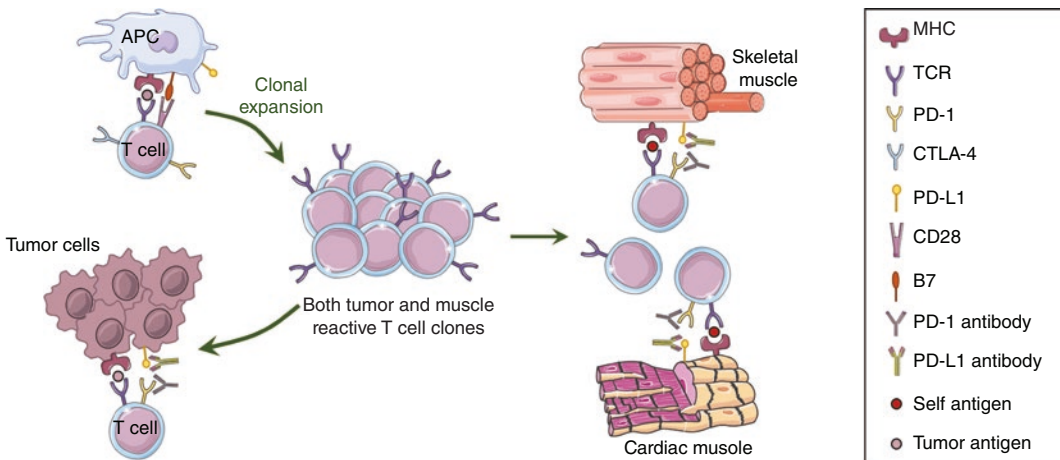
The CTLA-4 monoclonal antibody affects the binding of CTLA-4 (present on the surface of T cells) to B7 (present on the surface of APCs), thus reducing the threshold of cardiac T cell activation. CTLA-4 monoclonal antibody interacts with CTLA-4-expressing Treg cells, thereby affecting the inhibitory function of Tregs *in vivo* and leading to enhanced T cell activity in the heart. PD-1 antibodies damage the heart by blocking the binding of PD-1 and its ligands to APCs and cardiomyocytes, thus inducing T cell activation (Fig. 9.11).

In addition, T cells attack common antigens of tumors and the heart. In patients of ICI-

induced myocarditis, some of the T cells infiltrating the myocardial tissue were identical to those present in tumor cells or skeletal muscle, suggesting that these T cells respond to a common antigen. Furthermore, an abnormal increase in the expression of muscle-specific antigens (troponin and junctional proteins) was observed in the tumor tissues of these patients. This suggests that T cells target antigens common to the tumor and heart; ICIs damage the heart by enhancing the action of such T cells, leading to fulminant myocarditis (Fig. 9.12).



**Fig. 9.11** Immune checkpoint inhibitors disrupt the body’s immune tolerance to the heart



**Fig. 9.12** Tumors, skeletal muscle, and myocardium share antigenic epitopes. Immune checkpoint inhibitors induce a massive expansion of T cells targeting shared antigenic epitopes, leading to T cell attack on skeletal and cardiac muscle

All pathological types of fulminant myocarditis present with rapid cardiac dysfunction and lethal arrhythmias. In general, lymphocytic myocarditis is more common. However, giant cell as well as eosinophilic myocarditis have a comparatively rapid onset and are often more severe. Therefore, a pathologic diagnosis is essential for deciding the treatment strategy.

### Key Points

1. Myocarditis is an inflammatory disorder of myocardial cells in which mononuclear cells infiltrate the myocardium.
2. The pathophysiological process of myocarditis is divided into three stages at the cellular level: acute stage of viral colonization and replication, subacute stage of inflammatory cell infiltration, and chronic stage of ventricular remodeling.
3. In fulminant myocarditis patients, by the time the virus is removed via the immune response, the immune system is over-activated and inflammatory cells will be infiltrated. Meanwhile, massive inflammatory factors are stimulated and released, causing an “inflammatory storm” that amplifies the damage to the patient’s heart caused by pathogenic erosion, resulting in a decrease in myocardial contractility and a sharp decline in cardiac function, ultimately leading to cardiogenic shock and arrhythmia. Inflammatory storms also induce the release of numerous vasoactive substances, which dilate blood vessels and aggravate the state of shock.

### References

1. Yajima T, Knowlton KU. Viral myocarditis: from the perspective of the virus. *Circulation*. 2009;119:2615–24.
2. Ammirati E, Veronese G, Brambatti M, Merlo M, Cipriani M, Potena L, Sormani P, Aoki T, Sugimura K, Sawamura A, Okumura T, Pinney S, Hong K, Shah P, Braun O, Van de Heyning CM, Montero S, Petrella D, Huang F, Schmidt M, Raineri C, Lala A, Varrenti M, Foa A, Leone O, Gentile P, Artico J, Agostini V, Patel R, Garascia A, Van Craenenbroeck EM, Hirose

K, Isotani A, Murohara T, Arita Y, Sionis A, Fabris E, Hashem S, Garcia-Hernando V, Oliva F, Greenberg B, Shimokawa H, Sinagra G, Adler ED, Frigerio M, Camici PG. Fulminant versus acute nonfulminant myocarditis in patients with left ventricular systolic dysfunction. *J Am Coll Cardiol*. 2019;74:299–311.

3. Lieberman EB, Hutchins GM, Herskowitz A, Rose NR, Baughman KL. Clinicopathologic description of myocarditis. *J Am Coll Cardiol*. 1991;18:1617–26.
4. Klingel K, Sauter M, Bock CT, Szalay G, Schnorr JJ, Kandolf R. Molecular pathology of inflammatory cardiomyopathy. *Med Microbiol Immunol*. 2004;193:101–7.
5. Liu PP, Mason JW. Advances in the understanding of myocarditis. *Circulation*. 2001;104:1076–82.
6. Gong T, Liu L, Jiang W, Zhou R. DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat Rev Immunol*. 2020;20:95–112.
7. Shi Y, Chen C, Lisewski U, Wrackmeyer U, Radke M, Westermann D, Sauter M, Tschöpe C, Poller W, Klingel K, Gotthardt M. Cardiac deletion of the coxsackievirus-adenovirus receptor abolishes Coxsackievirus B3 infection and prevents myocarditis in vivo. *J Am Coll Cardiol*. 2009;53:1219–26.
8. Dan M, Chantler JK. A genetically engineered attenuated coxsackievirus B3 strain protects mice against lethal infection. *J Virol*. 2005;79:9285–95.
9. Chapman NM, Kim KS, Tracy S, Jackson J, Hoffing K, Leser JS, Malone J, Kolbeck P. Coxsackievirus expression of the murine secretory protein interleukin-4 induces increased synthesis of immunoglobulin G1 in mice. *J Virol*. 2000;74:7952–62.
10. Henke A, Zell R, Ehrlich G, Stelzner A. Expression of immunoregulatory cytokines by recombinant coxsackievirus B3 variants confers protection against virus-caused myocarditis. *J Virol*. 2001;75:8187–94.
11. Beck MA, Levander OA, Handy J. Selenium deficiency and viral infection. *J Nutr*. 2003;133:1463S–7S.
12. 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. A role for toll-like receptor 3 variants in host susceptibility to enteroviral myocarditis and dilated cardiomyopathy. *J Biol Chem*. 2010;285:23208–23.
13. Lozano MD, Rubocki RJ, Wilson JE, McManus BM, Wisecarver JL. Human leukocyte antigen class II associations in patients with idiopathic dilated cardiomyopathy. Myocarditis Treatment Trial Investigators. *J Card Fail*. 1997;3:97–103.
14. Tchilian EZ, Gil J, Navarro ML, Fernandez-Cruz E, Chapel H, Misbah S, Ferry B, Renz H, Schwitzer R, Beverley PC. Unusual case presentations associated with the CD45 C77G polymorphism. *Clin Exp Immunol*. 2006;146:448–54.
15. Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, Horwitz MS, Crowell RL, Finberg RW. Isolation of a common receptor for coxsackie B viruses and adenoviruses 2 and 5. *Science*. 1997;275:1320–3.

16. Wickham TJ, Mathias P, Cheresh DA, Nemerow GR. Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell*. 1993;73:309–19.
17. Garmaroudi FS, Marchant D, Hendry R, Luo H, Yang D, Ye X, Shi J, McManus BM. Coxsackievirus B3 replication and pathogenesis. *Future Microbiol*. 2015;10:629–53.
18. Coyne CB, Bergelson JM. Virus-induced Abl and Fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. *Cell*. 2006;124:119–31.
19. Opavsky MA, Martino T, Rabinovitch M, Penninger J, Richardson C, Petric M, Trinidad C, Butcher L, Chan J, Liu PP. Enhanced ERK-1/2 activation in mice susceptible to coxsackievirus-induced myocarditis. *J Clin Invest*. 2002;109:1561–9.
20. Yuan J, Zhang J, Wong BW, Si X, Wong J, Yang D, Luo H. Inhibition of glycogen synthase kinase 3beta suppresses coxsackievirus-induced cytopathic effect and apoptosis via stabilization of beta-catenin. *Cell Death Differ*. 2005;12:1097–106.
21. Marchant D, Dou Y, Luo H, Garmaroudi FS, McDonough JE, Si X, Walker E, Luo Z, Arner A, Hegele RG, Laher I, McManus BM. Bosentan enhances viral load via endothelin-1 receptor type-A-mediated p38 mitogen-activated protein kinase activation while improving cardiac function during coxsackievirus-induced myocarditis. *Circ Res*. 2009;104:813–21.
22. Ho BC, Yu SL, Chen JJ, Chang SY, Yan BS, Hong QS, Singh S, Kao CL, Chen HY, Su KY, Li KC, Cheng CL, Cheng HW, Lee JY, Lee CN, Yang PC. Enterovirus-induced miR-141 contributes to shutoff of host protein translation by targeting the translation initiation factor eIF4E. *Cell Host Microbe*. 2011;9:58–69.
23. Tong L, Lin L, Wu S, Guo Z, Wang T, Qin Y, Wang R, Zhong X, Wu X, Wang Y, Luan T, Wang Q, Li Y, Chen X, Zhang F, Zhao W, Zhong Z. MiR-10a\* up-regulates coxsackievirus B3 biosynthesis by targeting the 3D-coding sequence. *Nucleic Acids Res*. 2013;41:3760–71.
24. Xu HF, Ding YJ, Shen YW, Xue AM, Xu HM, Luo CL, Li BX, Liu YL, Zhao ZQ. MicroRNA-1 represses Cx43 expression in viral myocarditis. *Mol Cell Biochem*. 2012;362:141–8.
25. Chow LH, Beisel KW, McManus BM. Enteroviral infection of mice with severe combined immunodeficiency. Evidence for direct viral pathogenesis of myocardial injury. *Lab Invest*. 1992;66:24–31.
26. Chau DH, Yuan J, Zhang H, Cheung P, Lim T, Liu Z, Sall A, Yang D. Coxsackievirus B3 proteases 2A and 3C induce apoptotic cell death through mitochondrial injury and cleavage of eIF4GI but not DAP5/p97/NAT1. *Apoptosis*. 2007;12:513–24.
27. Lamphear BJ, Yan R, Yang F, Waters D, Liebig HD, Klump H, Kuechler E, Skern T, Rhoads RE. Mapping the cleavage site in protein synthesis initiation factor eIF-4 gamma of the 2A proteases from human Coxsackievirus and rhinovirus. *J Biol Chem*. 1993;268:19200–3.
28. Badorff C, Lee GH, Lamphear BJ, Martone ME, Campbell KP, Rhoads RE, Knowlton KU. Enteroviral protease 2A cleaves dystrophin: evidence of cytoskeletal disruption in an acquired cardiomyopathy. *Nat Med*. 1999;5:320–6.
29. Xiong D, Lee GH, Badorff C, Dorner A, Lee S, Wolf P, Knowlton KU. Dystrophin deficiency markedly increases enterovirus-induced cardiomyopathy: a genetic predisposition to viral heart disease. *Nat Med*. 2002;8:872–7.
30. Shi J, Wong J, Piesik P, Fung G, Zhang J, Jagdeo J, Li X, Jan E, Luo H. Cleavage of sequestosome 1/p62 by an enteroviral protease results in disrupted selective autophagy and impaired NFkB signaling. *Autophagy*. 2013;9:1591–603.
31. Xiong D, Yajima T, Lim BK, Stenbit A, Dublin A, Dalton ND, Summers-Torres D, Molckentin JD, Duplain H, Wessely R, Chen J, Knowlton KU. Inducible cardiac-restricted expression of enteroviral protease 2A is sufficient to induce dilated cardiomyopathy. *Circulation*. 2007;115:94–102.
32. Feng Q, Langereis MA, Lork M, Nguyen M, Hato SV, Lanke K, Emdad L, Bhoopathi P, Fisher PB, Lloyd RE, van Kuppeveld FJ. Enterovirus 2Apro targets MDA5 and MAVS in infected cells. *J Virol*. 2014;88:3369–78.
33. Fung G, Luo H, Qiu Y, Yang D, McManus B. Myocarditis. *Circ Res*. 2016;118:496–514.
34. Cai Z, Shen L, Ma H, Yang J, Yang D, Chen H, Wei J, Lu Q, Wang DW, Xiang M, Wang J. Involvement of endoplasmic reticulum stress-mediated C/EBP homologous protein activation in coxsackievirus B3-induced acute viral myocarditis. *Circ Heart Fail*. 2015;8:809–18.
35. Chen J, Lai J, Yang L, Ruan G, Chaugai S, Ning Q, Chen C, Wang DW. Trimetazidine prevents macrophage-mediated septic myocardial dysfunction via activation of the histone deacetylase sirtuin 1. *Br J Pharmacol*. 2016;173:545–61.
36. Chen J, Wang B, Lai J, Braunstein Z, He M, Ruan G, Yin Z, Wang J, Cianflone K, Ning Q, Chen C, Wang DW. Trimetazidine attenuates cardiac dysfunction in endotoxemia and sepsis by promoting neutrophil migration. *Front Immunol*. 2018;9:2015.
37. Karupiah G, Xie QW, Buller RM, Nathan C, Duarte C, MacMicking JD. Inhibition of viral replication by interferon-gamma-induced nitric oxide synthase. *Science*. 1993;261:1445–8.
38. Hardarson HS, Baker JS, Yang Z, Purevjav E, Huang CH, Alexopoulou L, Li N, Flavell RA, Bowles NE, Vallejo JG. Toll-like receptor 3 is an essential component of the innate stress response in virus-induced cardiac injury. *Am J Physiol Heart Circ Physiol*. 2007;292:H251–8.
39. Fairweather D, Yusung S, Frisancho S, Barrett M, Gatewood S, Steele R, Rose NR. IL-12 receptor beta 1 and toll-like receptor 4 increase IL-1 beta- and IL-18-associated myocarditis and coxsackievirus replication. *J Immunol*. 2003;170:4731–7.

40. Fuse K, Chan G, Liu Y, Gudgeon P, Husain M, Chen M, Yeh WC, Akira S, Liu PP. Myeloid differentiation factor-88 plays a crucial role in the pathogenesis of coxsackievirus B3-induced myocarditis and influences type I interferon production. *Circulation*. 2005;112:2276–85.
41. Takeda K, Akira S. Toll-like receptors in innate immunity. *Int Immunol*. 2005;17:1–14.
42. Yoneyama M, Kikuchi M, Natsukawa T, Shinobu N, Imaizumi T, Miyagishi M, Taira K, Akira S, Fujita T. The RNA helicase RIG-I has an essential function in double-stranded RNA-induced innate antiviral responses. *Nat Immunol*. 2004;5:730–7.
43. Yoneyama M, Kikuchi M, Matsumoto K, Imaizumi T, Miyagishi M, Taira K, Foy E, Loo YM, Gale M Jr, Akira S, Yonehara S, Kato A, Fujita T. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. *J Immunol*. 2005;175:2851–8.
44. Lee S, Choi J, Mohanty J, Sousa LP, Tome F, Pardon E, Steyaert J, Lemmon MA, Lax I, Schlessinger J. Structures of beta-klotho reveal a 'zip code'-like mechanism for endocrine FGF signalling. *Nature*. 2018;553:501–5.
45. Seth RB, Sun L, Ea CK, Chen ZJ. Identification and characterization of MAVS, a mitochondrial antiviral signaling protein that activates NF-kappaB and IRF 3. *Cell*. 2005;122:669–82.
46. Kato H, Takeuchi O, Sato S, Yoneyama M, Yamamoto M, Matsui K, Uematsu S, Jung A, Kawai T, Ishii KJ, Yamaguchi O, Otsu K, Tsujimura T, Koh CS, Reis e Sousa C, Matsuura Y, Fujita T, Akira S. Differential roles of MDA5 and RIG-I helicases in the recognition of RNA viruses. *Nature*. 2006;441:101–5.
47. Kumar H, Kawai T, Kato H, Sato S, Takahashi K, Coban C, Yamamoto M, Uematsu S, Ishii KJ, Takeuchi O, Akira S. Essential role of IPS-1 in innate immune responses against RNA viruses. *J Exp Med*. 2006;203:1795–803.
48. Kishimoto C, Kuribayashi K, Masuda T, Tomioka N, Kawai C. Immunologic behavior of lymphocytes in experimental viral myocarditis: significance of T lymphocytes in the severity of myocarditis and silent myocarditis in BALB/c-nu/nu mice. *Circulation*. 1985;71:1247–54.
49. Opavsky MA, Penninger J, Aitken K, Wen WH, Dawood F, Mak T, Liu P. Susceptibility to myocarditis is dependent on the response of alphabeta T lymphocytes to coxsackieviral infection. *Circ Res*. 1999;85:551–8.
50. Martinez NE, Sato F, Kawai E, Omura S, Chervenak RP, Tsunoda I. Regulatory T cells and Th17 cells in viral infections: implications for multiple sclerosis and myocarditis. *Future Virol*. 2012;7:593–608.
51. Huber SA, Sartini D. Roles of tumor necrosis factor alpha (TNF-alpha) and the p55 TNF receptor in CD1d induction and coxsackievirus B3-induced myocarditis. *J Virol*. 2005;79:2659–65.
52. Lim BK, Choe SC, Shin JO, Ho SH, Kim JM, Yu SS, Kim S, Jeon ES. Local expression of interleukin-1 receptor antagonist by plasmid DNA improves mortality and decreases myocardial inflammation in experimental coxsackieviral myocarditis. *Circulation*. 2002;105:1278–81.
53. Frisancho-Kiss S, Coronado MJ, Frisancho JA, Lau VM, Rose NR, Klein SL, Fairweather D. 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*. 2009;23:649–57.
54. Li K, Xu W, Guo Q, Jiang Z, Wang P, Yue Y, Xiong S. Differential macrophage polarization in male and female BALB/c mice infected with coxsackievirus B3 defines susceptibility to viral myocarditis. *Circ Res*. 2009;105:353–64.
55. Woodruff JF, Woodruff JJ. Involvement of T lymphocytes in the pathogenesis of coxsackie virus B3 heart disease. *J Immunol*. 1974;113:1726–34.
56. Rose NR. Learning from myocarditis: mimicry, chaos and black holes. *F1000Prime Rep*. 2014;6:25.
57. Wessely R, Klingel K, Santana LF, Dalton N, Hongo M, Jonathan Lederer W, Kandolf R, Knowlton KU. Transgenic expression of replication-restricted enteroviral genomes in heart muscle induces defective excitation-contraction coupling and dilated cardiomyopathy. *J Clin Invest*. 1998;102:1444–53.
58. S S. Ueber diffuse myokarditis. *Virchows Arch Pathol Anat Berl*. 1905:1–39.
59. Kandolin R, Lehtonen J, Salmenkivi K, Raisanen-Sokolowski A, Lommi J, Kupari M. Diagnosis, treatment, and outcome of giant-cell myocarditis in the era of combined immunosuppression. *Circ Heart Fail*. 2013;6:15–22.
60. Cooper LT Jr, Berry GJ, Shabetai R. Idiopathic giant-cell myocarditis—natural history and treatment. Multicenter Giant Cell Myocarditis Study Group Investigators. *N Engl J Med*. 1997;336:1860–6.
61. Kittleson MM, Minhas KM, Irizarry RA, Ye SQ, Edness G, Breton E, Conte JV, Tomaselli G, Garcia JG, Hare JM. Gene expression in giant cell myocarditis: altered expression of immune response genes. *Int J Cardiol*. 2005;102:333–40.
62. Haas SJ, Hill R, Krum H, Liew D, Tonkin A, Demos L, Stephan K, McNeil J. Clozapine-associated myocarditis: a review of 116 cases of suspected myocarditis associated with the use of clozapine in Australia during 1993-2003. *Drug Saf*. 2007;30:47–57.
63. Agostini C, Adami F, Semenzato G. New pathogenetic insights into the sarcoid granuloma. *Curr Opin Rheumatol*. 2000;12:71–6.
64. Terasaki F, Ukimura A, Tsukada B, Fujita S, Katashima T, Otsuka K, Otsuka K, Kanzaki Y, Shimomura H, Fujita M, Tanaka T, Kitaura Y. Enhanced expression of type 1 helper T-cell cytokines in the myocardium of active cardiac sarcoidosis. *Circ J*. 2008;72:1303–7.

65. Honda Y, Nagai T, Ikeda Y, Sakakibara M, Asakawa N, Nagano N, Nakai M, Nishimura K, Sugano Y, Ohta-Ogo K, Asaumi Y, Aiba T, Kanzaki H, Kusano K, Noguchi T, Yasuda S, Tsutsui H, Ishibashi-Ueda H, Anzai T. Myocardial immunocompetent cells and macrophage phenotypes as histopathological surrogates for diagnosis of cardiac sarcoidosis in Japanese. *J Am Heart Assoc.* 2016;5.
66. Lassner D, Kuhl U, Siegismund CS, Rohde M, Elezkurtaj S, Escher F, Tschöpe C, Gross UM, Poller W, Schultheiss HP. Improved diagnosis of idiopathic giant cell myocarditis and cardiac sarcoidosis by myocardial gene expression profiling. *Eur Heart J.* 2014;35:2186–95.
67. Trachtenberg BH, Hare JM. Inflammatory cardiomyopathic syndromes. *Circ Res.* 2017;121:803–18.
68. Apte M, McGwin G Jr, Vila LM, Kaslow RA, Alarcon GS, Reveille JD, Group LS. Associated factors and impact of myocarditis in patients with SLE from LUMINA, a multiethnic US cohort (LV). [corrected]. *Rheumatology (Oxford).* 2008;47:362–7.
69. Geraldino-Pardilla L, Russo C, Sokolove J, Robinson WH, Zartoshti A, Van Eyk J, Fert-Bober J, Lima J, Giles JT, Bathon JM. Association of anti-citrullinated protein or peptide antibodies with left ventricular structure and function in rheumatoid arthritis. *Rheumatology (Oxford).* 2017;56:534–40.
70. Nishimura H, Okazaki T, Tanaka Y, Nakatani K, Hara M, Matsumori A, Sasayama S, Mizoguchi A, Hiai H, Minato N, Honjo T. Autoimmune dilated cardiomyopathy in PD-1 receptor-deficient mice. *Science.* 2001;291:319–22.
71. Tarrío ML, Grabie N, Bu DX, Sharpe AH, Lichtman AH. PD-1 protects against inflammation and myocyte damage in T cell-mediated myocarditis. *J Immunol.* 2012;188:4876–84.
72. Lucas JA, Menke J, Rabacal WA, Schoen FJ, Sharpe AH, Kelley VR. Programmed death ligand 1 regulates a critical checkpoint for autoimmune myocarditis and pneumonitis in MRL mice. *J Immunol.* 2008;181:2513–21.
73. Grabie N, Gotsman I, DaCosta R, Pang H, Stavrakis G, Butte MJ, Keir ME, Freeman GJ, Sharpe AH, Lichtman AH. Endothelial programmed death-1 ligand 1 (PD-L1) regulates CD8+ T-cell mediated injury in the heart. *Circulation.* 2007;116:2062–71.
74. Sury K, Perazella MA, Shirali AC. Cardiorenal complications of immune checkpoint inhibitors. *Nat Rev Nephrol.* 2018;14:571–88.