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REVIEW

The innate immune system in chronic cardiomyopathy: a European Society of Cardiology (ESC) scientific statement from the Working Group on Myocardial Function of the ESC

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Activation of the immune system in heart failure (HF) has been recognized for over 20 years. Initially, experimental studies demonstrated a maladaptive role of the immune system. However, several phase III trials failed to show beneficial effects in HF with therapies directed against an immune activation. Preclinical studies today describe positive and negative effects of immune activation in HF. These different effects depend on timing and aetiology of HF. Therefore, herein we give a detailed review on immune mechanisms and their importance for the development of HF with a special focus on commonalities and differences between different forms of cardiomyopathies. The role of the immune system in ischaemic, hypertensive, diabetic, toxic, viral, genetic, peripartum, and autoimmune cardiomyopathy is discussed in depth. Overall, initial damage to the heart leads to disease specific activation of the immune system whereas in the chronic phase of HF overlapping mechanisms occur in different aetiologies.

Keywords Immune system • Macrophage • T-cell • Ischaemic cardiomyopathy • Hypertensive cardiomyopathy • Diabetic cardiomyopathy • Toxic cardiomyopathy • Viral cardiomyopathy • Genetic cardiomyopathy • Peripartum cardiomyopathy • Autoimmune cardiomyopathy

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Introduction

Heart failure (HF) is a common disease, requiring significant primary care, with high hospital admissions and mortality rates. Despite major advances in treating and managing HF in recent years, further therapeutic options are needed to improve patient prognosis.

The pathophysiology of HF is complex and not completely understood. Besides the role of signalling molecules, such as cyclic nucleotides, phosphoinositide 3-kinase (PI3K) isoforms and calcium, the recognition in 1990 that patients with HF have elevated cytokine levels[1] opened a new field of research, which then revealed that the immune system has an important role in the progression of HF. More specifically, a pathological signalling cascade initiated within the injured myocardium has the ability to activate innate immune receptors. Subsequent research has investigated different aspects of the innate immune response including cellular (especially monocytes/macrophages) and non-cellular immune responses. For example, after an acute myocardial infarction (MI), an inflammatory response is required to resolve the necrotic myocardium; however, the immune system can become chronically activated, leading to adverse myocardial effects.

Current knowledge of immune activation in HF has certain shortcomings:

- To date, most results rely on acute HF models in mice or rats. We understand that acute cardiac stress or injury goes through a sequence of phases: first a pro-inflammatory stage, followed by healing (fibrosis), and finally remodelling.[2] Most generated data, summarized in excellent recent review articles,[3–5] were from the first two phases. Data are sparse for the chronic remodelling phase (over 8 weeks after HF) even though this stage is equally important, particularly with regard to chronic treatment options in the clinic.
- Most of the animal models used have non-inducible gene defects. Thus, genetic alterations are already present before induction of MI, making it difficult to separate early and late effects. Unfortunately, these genetic models do not replicate clinical reality.
- The human aetiology of HF is diverse, including MI, hypertension, myocarditis, gene mutations, toxic triggers, and arrhythmias. Immune mechanisms might be different for distinct aetiologies.
- The syndrome of HF is complex and often comprises a corollary of pro-inflammatory co-morbidities rather than a singular pathology.
- We have currently (too) little human data since these are difficult to be obtained. However, it should be kept in mind that there are substantial differences between an immune system of a mouse and a human. For example, monocyte/macrophage subtypes are different between the species. Animals in animal facilities have no contact to germs or viruses, what is essential for the development of the immune system in adults. Furthermore, co-morbidities have an impact on the immune system as e.g. atherosclerosis, what is difficult to mimic in an animal experiment.

Seeking to address the challenges summarized above, we specifically discuss common and different features in the immune response for ischaemic, hypertensive, diabetic, toxic, viral, genetic, peripartum, and autoimmune cardiomyopathy.

The immune system

Innate immune system

Pattern recognition receptors

The innate immune system is activated through receptors that either recognize patterns shared by invading pathogens, e.g. lipopolysaccharide (LPS), or indicate danger. This is in contrast to adaptive immune receptors that recognize only specific epitopes. These innate immune receptors, called pattern recognition receptors (PRRs), are constitutively expressed on most cardiac cells, while adaptive immune receptors are not. The most important receptors include Toll-like receptors (TLRs), NOD-like receptors (NLRs), RIG-I-like receptors (retinoic acid inducible), pentraxins, and C-type lectin receptors (CLRs).

Toll-like receptors, the first PRRs identified, are a family of transmembrane or cytosolic receptors.[6] TLRs recognize a wide range of patterns like double-stranded RNA (TLR3), LPS (TLR4), single-stranded RNA (TLR7 and 8), and DNA (TLR9). TLR activation induces the release of inflammatory cytokines, chemokines, and type I interferon (IFN) (for a in-depth review see Kawai and Akira[6]). All 10 human TLRs are expressed in the heart.[7] TLRs are upregulated in HF.[8]

NOD-like receptors are cytosolic innate immune receptors. They are activated by microbial products and danger signals. NLR activation lead to activation of inflammatory responses, inflammasome assembly [multiprotein platform that activates caspase-1 with subsequent activation of interleukin (IL)-1β and IL-18], and transcriptional activity.[9] Five NLRs are expressed in the heart.[10]

The pentraxin family has two members in humans: C-reactive protein (CRP) and amyloid P. Pentraxins are soluble and have a function mainly in the defence against bacteria. They can also recognize damaged tissue.[11]

Finally, *CLRs* are a family of proteins with one or more C-type lectin domains. CLRs recognize different molecules. Little is known about their cardiac function.[12]

Danger-associated molecular patterns

Because most HF aetiologies have no infectious origin, PRRs are instead activated by so-called danger-associated molecular patterns (DAMPs). DAMPs are either released by injured cells/necrosis or damaged extracellular matrix.[12] Classical DAMPs include heat shock proteins (HSP60, HSP72, HSC70, etc.); HSP60, for example, can activate TLR2 and 4. Another important DAMP is HMGB1, a nuclear DNA-binding protein that can activate different TLRs.

Effector systems of innate immunity

Several effector systems mediate the innate immune response. They can be categorized as either non-cellular (cytokines,

chemokines, complement) or cellular (neutrophils, monocytes/macrophages).

Pro-inflammatory cytokines with major roles in the innate immune response include tumour necrosis factor (TNF), IL-1β, IL-6, and IL-8. Cytokines activate the endothelium and lymphocytes and mediate local tissue injury. *Chemokines* are a subgroup of cytokines that are chemoattractants and have a small molecular weight. Four chemokine groups can be differentiated depending on cysteine residue position (CC, CXC, C, CX3C). Over 50 different chemokines have been described. *Complement* is a group of serum proteins that can be activated through a reaction cascade. Complement activation leads to the formation of the membrane attack complex that facilitates lysis of bacterial membranes. Complement is also important for opsonisation, initiation of phagocytosis, and inflammatory cell activation.

Neutrophils and monocytes/macrophages are the most important cells of the innate immune system. *Neutrophils* demarginate from the vessel wall upon stress and infiltrate tissue in response to chemokines and cytokines. Circulating neutrophils express selectin ligands, interact with the endothelium, and induce conformational changes that subsequently lead to transmigration. Tissue neutrophils release proteolytic enzymes that amplify the immune response with direct cytotoxic effects. In humans, three *monocyte* subsets have been reported: classical ($CD14^{++}$ $CD16^{-}$), intermediate ($CD14^{++}$ $CD16^{+}$), and non-classical ($CD14^{+}$ $CD16^{++}$). Mature murine monocytes are classified by either Ly-$6C^{high}$ or Ly-$6C^{low}$ expression. *Macrophages* reside in tissue. Most macrophages are known to arise from circulating blood monocytes, though more recently innate resident macrophages were reported to exist within the normal myocardium. These innate resident macrophages have a different configuration than those migrating from blood after being triggered by an inflammatory response.[13] The lifespan of a macrophage varies from hours in different disease states to months under steady state conditions. Macrophages have diverse functions ranging from phagocytosis, cytotoxicity, and production of inflammatory cytokines to very specialized functions such as the macrophage-like osteoclasts for bone remodelling, microglia in the brain, and Kupffer cells in the liver.

Adaptive immune system

A detailed description of the adaptive immune system is beyond the scope of this review. In contrast to the innate immune system, the adaptive immune system acquires pathogen-specific receptors by which it creates immunologic memory. The adaptive immune system also has humoral and cellular components (B- and T-lymphocytes). It interacts with the innate immune system.

Immune mechanisms in heart failure pathophysiology

Although the aetiology of HF may vary, pathophysiologic mechanisms that influence immune activation can be identical (*Figure 1*). Here, we highlight the possible influence the autonomic system, natriuretic peptides, renin–angiotensin–aldosterone system (RAAS), and the extracellular matrix have on innate immunity.

Autonomic nervous system

Although an association between immune status and autonomic nervous system activation has been empirically observed for many years, the underlying mechanism has only recently been clarified. Innate immune cells originate from hematopoietic stem cells (HSC) in the bone marrow, where their self-renewal, proliferation, and differentiation are tightly controlled in the stem cell niche. Recent work has illustrated the importance of cross-talk between HSC and specific stromal cells, i.e. nestin-positive mesenchymal stem cells (MSC). These quiescent $Nestin^{+}$-MSC are necessary for homing and maintaining HSC in their niche. $Nestin^{+}$-MSC are a main source of CXCL12 (or stromal-derived factor-1), which prevents HSC egress out of the bone marrow and thus also controls the number of progenitors of different lineages in the peripheral circulation and tissues.

The bone marrow has numerous autonomic nerve terminals; tyrosine hydroxylase-positive, norepinephrine-producing sympathetic post-ganglionic nerve cells; and Schwann cells. These cells are closely apposed to the $Nestin^{+}$-MSC in the stem cell niches, where sympathetic innervation not only promotes $Nestin^{+}$-MSC quiescence but also inhibits their differentiation to osteoblasts.[14] Beta3AR expressed on $Nestin^{+}$-MSC convey critical survival signals, ensuring an optimal niche environment for the HSC. However, increased sympathetic activation (reproduced by directly administering catecholamines) also forces HSC exit from the niche.[15] This HSC egress results from β-adrenergic receptor mediated downregulation of genes (e.g. CXCL12) that promote HSC maintenance and attraction in the niche following β-adrenergic receptor stimulation.[14] Although such gene regulation was initially attributed to β2-adrenergic receptor activation,[15] more recent research showed cooperation between β2- and β3-adrenergic receptors, which have higher expression on $Nestin^{+}$-MSC,[16] at least in mice.

Building on these initial paradigms, studies addressed the effects of chronic psychological stress (and consequent sympathetic nervous system activation) on HSC proliferation, egress, and the resulting concentration of circulating leucocytes. Both humans and mice had higher peripheral leucocyte counts during stress. HSC proliferation also increased, thereby resulting in higher numbers of macrophage and lymphoid progenitors in the bone marrow of stressed mice.[17] This and other[18] studies illustrate mechanistic links among sympathetic nervous system activity (i.e. in response to psychological stress, a known cardiovascular risk factor), innate immunity, and peripheral inflammation, with potential implications for cardiovascular diseases with a chronic inflammatory component.

Natriuretic peptides

Natriuretic peptides increase during all forms of HF and are predominantly produced by cardiac myocytes. Inhibiting natriuretic peptide degradation may increase survival in chronic HF patients, as suggested in the randomized controlled PARADIGM-HF trial.[19] Immune cells contain natriuretic peptides and carry their receptors.[20] The cytokines TNF and IL-1β can induce the B-type natriuretic peptide (BNP) in neonatal rat ventricular myocytes.[21] Conversely, atrial natriuretic peptide (ANP) inhibits the

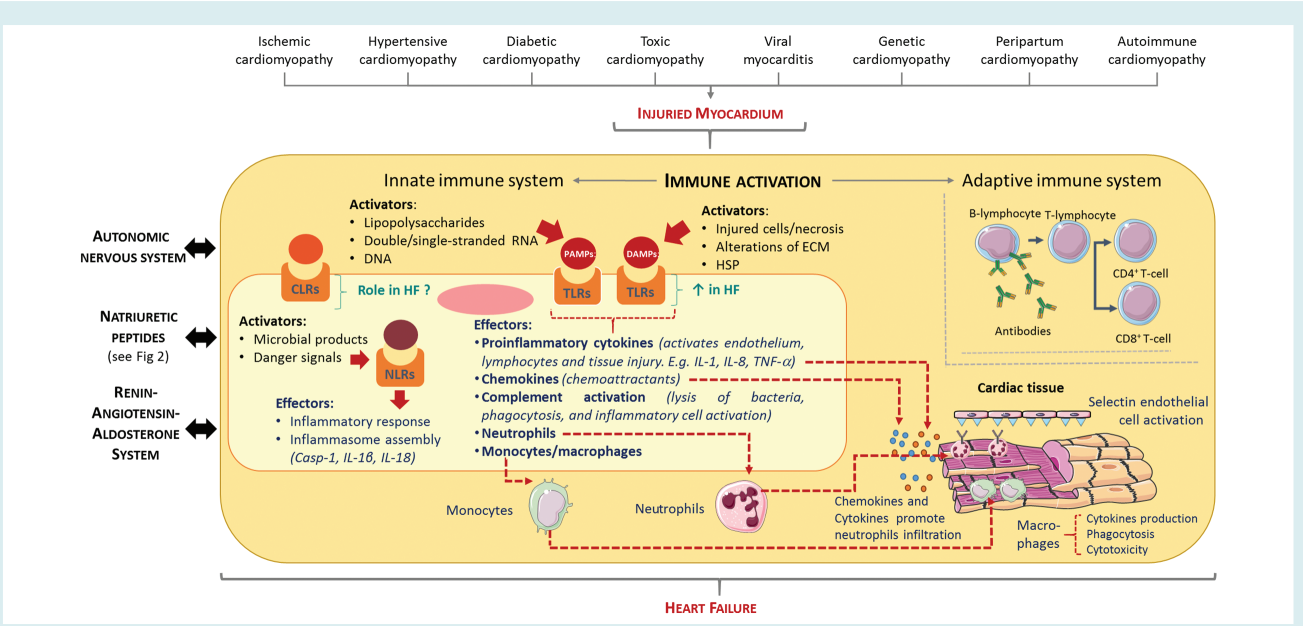

Figure 1 Interaction between select neurohormonal systems and components of innate immunity. Casp-1, caspase-1; CLR, C-type lectin receptor; DAMP, danger-associated molecular pattern; ECM, extracellular matrix; HF, heart failure; HSP, heat-shock protein; IL, interleukin; NLR, NOD-like receptor; PAMP, pathogen-associated molecular pattern; TLR, Toll-like receptor; TNF, tumour necrosis factor.

transcription factor nuclear factor kappa B (NF-κB).[22] ANP, via its guanylyl cyclase-A (GC-A) receptor, reduces TNF-induced endothelial hyperpermeability[23] and neutrophil adhesion.[24] Mice lacking endothelial GC-A are protected early after MI, an effect characterized by reduced neutrophil infiltration due to altered cyclic adenosine monophosphate/cyclic guanosine monophosphate (cAMP/cGMP) cross-talk.[25] These observations suggest that natriuretic peptides have anti-inflammatory effects *in vivo* (*Figure 2*).

Renin–angiotensin–aldosterone system

One of HF's major pathophysiologic mechanisms is chronic activation of the RAAS. The interaction between the RAAS and immune activation has been known for many years. Aldosterone, for example, increases the expression of pro-inflammatory cytokines in macrophages.[26] A macrophage-specific knockout for the mineralocorticoid receptor (MR) protects mice against cardiac fibrosis induced by hypertensive deoxy-corticosterone acetate plus high salt intake.[27] Furthermore, MR antagonism, especially by the selective MR antagonist eplerenone, skewed the monocyte/macrophage population towards higher numbers of healing-promoting Ly6C^{low} cells.[28]

Extracellular matrix

Degradation of the structural collagen fibres—over 90% of the total cardiac matrix components—as well as non-structural glycoproteins by matrix metalloproteinases (MMP-2 and -9) will result in the generation of pro-inflammatory collagen, glycosamin, and hyaluron fragments.[29] Secondly, the expression of glycoproteins and proteoglycans – also called matricellular proteins – strongly increases during diabetes and hypertension. Those secreted matricellular proteins include small leucin- and cystein-rich proteoglycans such as osteoglycin, asporin, osteonectin (SPARC), and lumican, as well as glycoproteins such as thrombospondins, osteopontin, periostin, and osteoprotegerin, among many others. Their increased expression during cardiac stress strongly affects cardiac inflammation through activation or inhibition of membrane receptors, growth factors, and cytokines, and direct stimulation of fibroblasts mainly via the transforming growth factor (TGF)-β receptor pathway.[30] Those matricellular proteins also affect collagen production and quality, leading to decreased cardiac dilatation in HF with reduced left ventricular (LV) function, but increased diastolic dysfunction in hypertension.

Diabetes and hypertension also directly activate a fibrogenic programme, first with accumulation of advanced glycation end-products (AGEs) that cross-link extracellular matrix proteins, and transduce fibrogenic signals through reactive oxygen species generation, or through activation of receptor for AGE-mediated pathways. Thus, AGEs together with matricellular proteins not only modulate cardiac inflammation but also stimulate collagen production and its cross-linking. The innate immune cells, and macrophages in particular, will thus promote interstitial fibrosis in chronic cardiac conditions of stress or injury.

Immune activation in different heart failure aetiologies

Myocardial infarction

Experimental MI is the most widely used HF model worldwide and has been extensively reviewed by others. Herein, we therefore

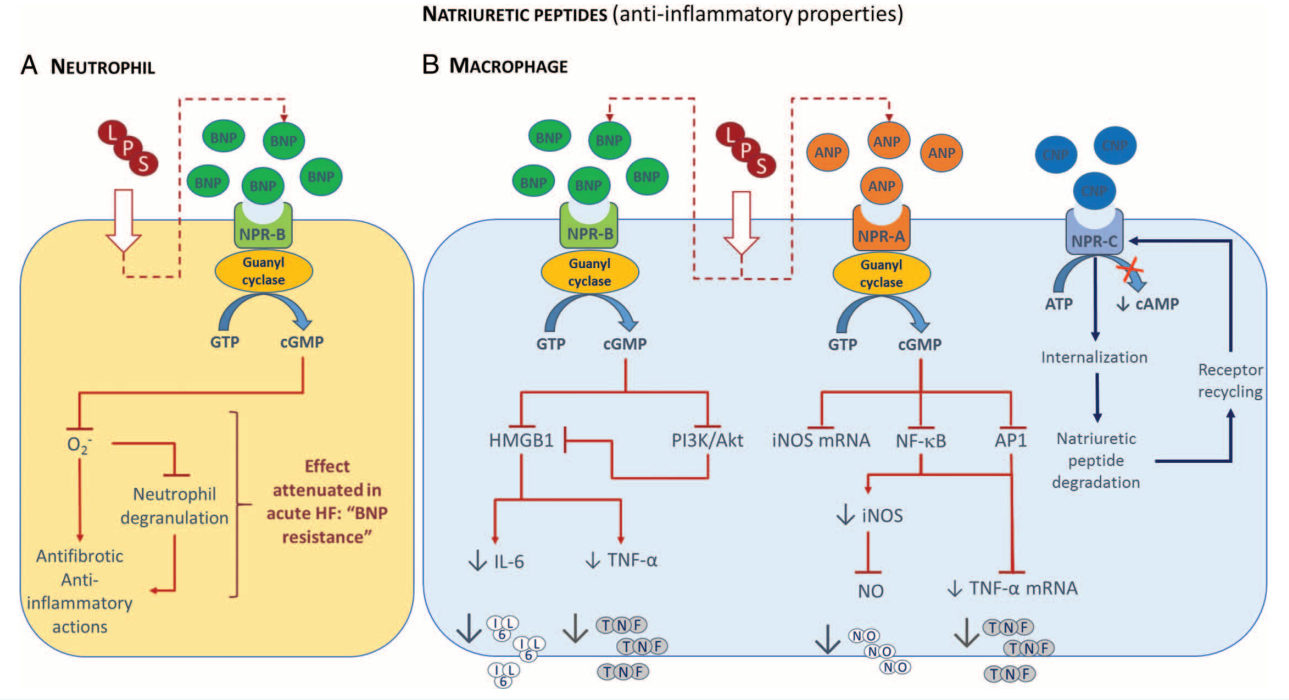

Figure 2 Role of natriuretic peptides in the innate immune system, showing its anti-inflammatory actions in neutrophils (*A*, yellow) and in macrophages (*B*, blue). (*A*) Recent data have suggested that the anti-inflammatory effect of B-type natriuretic peptide (BNP) is attenuated in acute heart failure patients. (*B, left section*) In ischaemia/reperfusion, post-conditioning of BNP protects against myocardial injury. (*B, middle section*) The lipopolysaccharide (LPS)-induced expression of tumour necrosis factor (TNF)-α and inducible nitric oxide synthase (iNOS) is inhibited by atrial natriuretic peptide (ANP). (*B, right section*) C-type natriuretic peptide receptors (NPR-C) or clearance receptors are coupled to adenylyl cyclase inhibition through a subsequent decrease in intracellular levels of cyclic adenosine monophosphate (cAMP) and/or to phospholipase C (PLC) activation. Akt, protein kinase B; AP1, activator protein 1; ANP, atrial natriuretic peptide; ATP, adenosine triphosphate; CNP, C-type natriuretic peptide; cGMP, cyclic guanosine monophosphate; GTP, guanosine triphosphate; HF, heart failure; HMGB1, high mobility group box 1 protein; IL, interleukin; NF-κB, nuclear factor kappa B; NO, nitric oxide; NPR-A, atrial natriuretic peptide receptor; NPR-B, B-type natriuretic peptide receptor; O_2^-, superoxide; PI3K, phosphoinositide 3-kinase.

focus only on a few essential receptors and inflammatory cells. Following coronary artery ligation, neutrophils are the first to invade the heart (hours to days) and start the pro-inflammatory phase. The subsequent healing (sometimes also called proliferative) phase is characterized by infiltration of macrophages, resolution of dead tissue, and the beginning of scar formation (days 3–30). This is followed by the remodelling phase, during which the inflammatory response should decrease and the non-infarcted myocardium undergoes pathophysiologic changes.[2]

The innate immune system is activated by cardiac injury through PRRs. The most thoroughly investigated PRRs after experimental MI are TLRs. TLR4 is elevated in myocardium of animals and patients with ischaemic HF.[8] However, functional data in transgenic mice exist only up to 4 weeks after MI. TLR2$^{-/-}$[31] and TLR4$^{-/-}$[32] mice have improved LV remodelling and reduced mortality rates after MI. TLR downstream signalling components, like the transcription factor NF-κB, are chronically activated. NF-κB inhibition improves remodelling up to 8 weeks after MI.[33]

NF-κB itself can activate different cytokines that could play a role in HF. Indeed, the first cytokine that was shown to increase in chronic HF was TNF-α.[1] Transgenic mice with cardiac TNF-α overexpression develop a HF phenotype.[34] Rats treated with TNF-α to a concentration comparable to that measured in patients with chronic HF, develop LV dysfunction.[35] This adverse effect is most likely mediated by changes in extracellular matrix remodelling. Studies using anti-TNF-α therapy in chronic HF were negative, and anti-TNF-α even increased mortality in chronic HF; these results reflect the incomplete understanding of TNF-α involvement in the chronic condition.

Cytokines and innate immune receptors regulate the invasion of inflammatory cells after MI. Most of them have different subpopulations with varying functions. They are essential for debris clearance, adequate healing, and functional restoration.

Neutrophils are recruited by transmigration very early and usually disappear in the heart 3 to 7 days after MI. These cells are pro-inflammatory, contribute to dead cell clearance and macrophage polarization after MI. Neutrophil ablation leads to worsening heart function and fibrosis.[36] They are early-phase effector cells; their chronic roles have not been described.

In the first phase, pro-inflammatory Ly6C^{high} *monocytes* are recruited to the heart as early as 30 min after MI. Recruitment relies on MCP-1/CCR2 chemokine/chemokine receptor interaction.[37] In the proliferation phase, Ly6C^{low} monocytes become abundant and regulate angiogenesis and myofibroblast formation. Yet data are limited on the chronic remodelling phase. A dominant anti-inflammatory M2 macrophage infiltration characterizes the myocardium 8 weeks after experimental MI,[38] but chronic macrophage activation seems to worsen myocardial function. Splenectomy 8 weeks post-MI protected cardiac remodelling, while adoptive transfer of splenocytes worsened it.[38] We lack data on human myocardium for the chronic phase. The precise function of monocytes/macrophages in the remodelling phase is still unclear, but they seem to play important roles in angiogenesis, extracellular matrix remodelling, and cross-talk with myocytes. Interestingly, very recent data demonstrate that resident tissue macrophages are established during embryonic development and maintained through self-renewal.[39] Furthermore, they may play a role in tissue healing, very likely in the neonatal heart but probably also in adults, though this has not been evaluated so far.[40]

Mast cells populate the normal heart. They can release histamins, proteases, cytokines, and growth factors. Mast cells accumulate after MI. Most of the data suggest that mast cells promote fibrous tissue deposition (for a review see Kong *et al.*[41]).

Lymphocytes, members of the adaptive immune system, are linked to the innate immune response and only recently gained attention. CD4$^+$ lymphocytes accumulate and are essential for adequate healing after MI.[42,43] Different genetic animal models lacking T helper cells had adverse LV remodelling and increased rates of LV rupture.[44] These phenomena are mediated by regulatory T-cells: regulatory T-cell activation improved healing after MI. Interestingly the effect of T-cells seems to be mediated by an interaction with macrophages. Regulatory T-cells secrete paracrine factors like TGF that lead to a preferential recruitment of anti-inflammatory M2 macrophages to the myocardium with beneficial effects.[45] Also, B-cells can enhance tissue injury via Ccl7-dependent Ly6C^{hi} monocyte recruitment up to 2 weeks after MI.[46] However, all of these investigations looked at the early phase after MI; long-term effects of T- or B-cells and their interaction with macrophages are still unknown.

Despite very promising research on the early phase after MI, we have only limited data for the chronic phase (*Table 1*).[31,32,34–36,38,44,47] The available data suggest that a chronic pro-inflammatory response might be detrimental, whereas it is essential for healing after MI. Different subpopulations of inflammatory cells have different functions during this process. Targets for suppressing this pro-inflammatory mechanism long term have not yet been identified.

Heart failure with preserved ejection fraction

The most common aetiologies for HF with preserved ejection fraction (HFpEF) are obesity, hypertension, diabetes, and metabolic syndrome. HFpEF is characterized by cardiomyocyte hypertrophy and increased interstitial fibrosis leading to increased cardiac stiffness and diastolic dysfunction. Cardiomyocyte hypertrophy is the first step in the development of HFpEF, whereas fibrosis occurs at a later stage (*Figure 3*). Inflammation and macrophage recruitment in liver steatosis, vessel atheroma, and nephropathy are key pathogenic features of obesity and diabetes. Cardiac macrophages—and related oxidative stress—are also involved in inducing cardiomyocyte death and interstitial fibrosis in the HFpEF heart.[48,49] The latest findings support the concept that macrophages and their secreted proteins are directly involved in cardiomyocyte hypertrophy during diabetes and hypertension. Depleting cardiac macrophages by splenectomy,[50] chronic clodronate administration,[51] or indirectly by adoptive T-regulatory cell transfer,[52] all reduced hypertrophic cardiomyocyte growth in hypertension. Macrophage depletion also reduced cardiac dysfunction in lipotoxic cardiomyopathy.[53] In both acute and chronic HFpEF, monocyte quality and quantity are related to HFpEF. Particularly in human patients, monocytosis and pro-inflammatory M2 macrophage activation are associated with chronic diastolic dysfunction and progression to HFpEF.[54] Yet how macrophage modulate cardiomyocyte hypertrophy is an open question. Recently, a proof of principle for macrophage secretome-related cardiomyocyte growth was shown for miRNA-155.[55]

Type 2 diabetes mellitus is a significant risk factor for HFpEF. The progression from diastolic dysfunction to HFpEF is characterized by insulin resistance, impaired cardiac insulin signalling, mitochondrial dysfunction, impaired calcium handling, abnormal coronary microcirculation, inappropriate neurohumoral activation, and altered immune responses. Diabetic cardiomyopathy can be promoted by dysregulation of the innate and adaptive immune systems.[43,56] The visceral adipose tissue shows a higher CD8$^+$:CD4$^+$ T-cell ratio.[57] Macrophage M1 polarization is a chronic pro-inflammatory response in obesity and insulin-resistant states,[58] with pro-inflammatory cytokines contributing to HFpEF progression.[59]

Chronic metabolic activation of the innate immunity in the heart involves three important systems: (i) the PRRs, including TLRs, (ii) the NLRP3 inflammasome (nucleotide-binding and oligomerization domain-like receptor family, pyrin domain containing protein 3), and (iii) the NF-κB/PPAR-α pathway, inducing the expression of pro-inflammatory cytokines, such as TNF-α, IL-6, pro-IL-1β, and pro-IL-18 (also reviewed in Nishida and Otsu[60]). Cross-talk happens between those systems, e.g. NF-κB also induces NLRP3 expression. TNF-α- and IL-1β-expressing immune cells are increased in streptozotocin (STZ)-induced type 1 diabetic rat model[61] and TNF-α antagonism attenuates the development of diabetic cardiomyopathy in this model.[62] Knockdown of NLRP3 improves cardiac function and reduces mature IL-1β expression in diabetic rat models with a combination of high fat diet and STZ injection.[63]

The inflammatory state—in particular IL-6 and TNF-α—induced by the metabolic syndrome is predictive of incident HFpEF but not of incident HF with reduced ejection fraction (HFrEF).[64] Cross-sectional study of HFpEF patients revealed the same cytokines, on top of soluble ST2 and pentraxin 3 to be elevated in HFpEF patients.[65]

Table 1 Comparison of acute vs. chronic effects of immune components after experimental myocardial infarction

	<4 weeks after MI	>4 weeks after MI
Humoral immune response		
TNF	Decreased LV function[35]	Chronic overexpression leads to heart failure[34]
TLR	TLR 2$^{-/-}$ protected[31]	Data lacking
	TLR 4$^{-/-}$ protected[32]	Data lacking
Cellular immune response		
Neutrophils	Depletion maladaptive[36]	Data lacking
Macrophages	Depletion maladaptive[47]	Splenectomy protective[38]
T-cells	T helper cell depletion maladaptive[44]	Data lacking

LV, left ventricular; MI, myocardial infarction; TLR, Toll-like receptor; TNF, tumour necrosis factor.

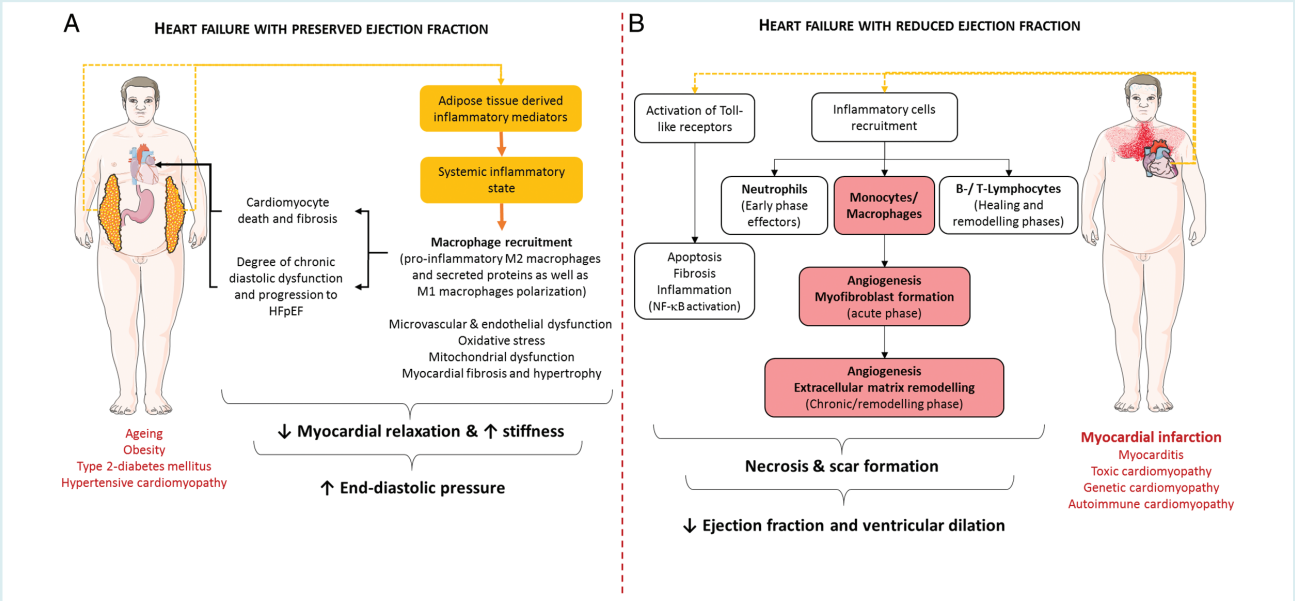

Figure 3 Immune activation in different heart failure aetiologies. (*A*) The impact of systemic inflammation on the progression of heart failure with preserved ejection fraction (HFpEF). (*B*) The immune mechanisms associated with myocardial infarction as an example of heart failure with reduced ejection fraction. NF-κB, nuclear factor kappa B.

All together, these data point to macrophages and their secreted proteins playing a central role in cardiomyocyte hypertrophy and chronic HFpEF progression. Different macrophage lineages are involved: whereas resident macrophages may protect against chronic harm induced by risk factors or chronic injury, recruited macrophages allow cardiac repair after ischaemia but also cause cardiac hypertrophy and fibrosis during hypertension or diabetes.

Toxic cardiomyopathy

Chemotherapeutic drugs cause cardiac toxicity. The mechanisms of cardiotoxicity differ substantially between chemotherapeutic medications leading to different phenotypes (acute vs. chronic, reversible vs. non-reversible, etc.) that have to be treated specifically. Some of their adverse effects are mediated by interference with the immune system.

TLRs in particular sense damage caused by anthracycline. In TLR2 knockout mice, LV function was partially preserved and survival improved compared to wild-type animals in a model of acute doxorubicin treatment.[66] The mechanisms are unclear, though one possibility is that TLR2 may sense oxidative stress. Doxorubicin might also increase DAMP HMGB1, which activates different TLRs. Indeed, pharmacologically inhibiting either HMGB1 or TLR protected against anthracycline-induced myocyte apoptosis, cardiac fibrosis, and inflammation.[67] Moreover, inflammatory cell recruitment depends on TLR2 (and TLR9) and the downstream effector myeloid differentiation primary response gene 88 (MyD88) but not TLR4 after anthracycline challenge.[68] The role of TLR4 in anthracycline-mediated cardiomyopathy is unclear. TLR4 knockout mice were protected against anthracycline cardiotoxicity;[69] however, antibodies neutralizing TLR4 had opposing effects.[67]

Antineoplastic agents' anti-tumour effects are derived in part from their ability to inhibit inflammatory signalling cascades in tumour cells. More specifically, these agents target TNF-α and IL-6-induced MAPK, PI3K-Akt, and JAK/STAT signalling. This might also affect cardiac fate. For example, doxorubicin reduces cardiomyocyte STAT3 expression,[70] and overexpression of gp130-STAT3 signalling has been shown to protect against doxorubicin-induced cardiotoxicity.[71]

Monoclonal antibodies against immune checkpoints revolutionized cancer therapy for many tumour types. The two most prominent checkpoint blockers target cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and the interaction between programmed cell death 1 (PD-1) and programmed death-ligand 1 (PD-L1). Unfortunately, checkpoint inhibitors have been associated with a broad spectrum of adverse events called immune-related adverse events (IRAEs). Immune-mediated cardiotoxicity induced by checkpoint inhibitors has been generally reported in single cases with variable presentations (myocarditis, pericarditis, etc.).[72] A recent article reported severe cases of cardiotoxicity during checkpoint inhibitor treatment.[72] Interestingly, PD-1-deficient mice developed dilated cardiomyopathy and sudden death by congestive HF.[73,74] In addition, PD-1 protects T-cell-mediated myocarditis,[75] with PD-1 deficiency resulting in fatal myocarditis in mice genetically predisposed to autoimmunity.[76] To date, there is no published research on late-onset IRAEs. The rate of myocarditis with combined PD-1 and CTLA-4 inhibitor treatment is at least double the rate with either inhibitor used alone. Patients receiving long-term checkpoint inhibition should be carefully monitored for cardiac adverse events.

Taken together, the innate and adaptive immune systems seem to be important for the development of toxic cardiomyopathy. In particular, TLRs seem to sense anthracycline-mediated damage. The immune system is also implicated in cardiotoxicity, including immune-related adverse cardiac pathologies such as myocarditis and pericarditis, developed after anticancer-immune checkpoint inhibitors.

Viral myocarditis

Acute myocarditis, chronic inflammatory cardiomyopathies, and post-cardiac transplantation-mediated rejections refer to acute or chronic inflammatory cardiac responses to environmental or endogenous triggers, most commonly viruses, toxic agents, autoimmunity, or rejection-based immune effects. Most of our current understanding comes from experiments in susceptible murine strains. Human myocarditis is mimicked in mice through infection with human coxsackie virus B3 (CVB3) or by injection of cardiac antigens that induce an autoimmune response.[77,78] The latter non-infectious models mainly provide insights into the latter phases of myocarditis.

The transition from acute viral infection through active inflammation to cardiac dysfunction is a multiphase process. First, viral entry into cardiomyocytes leads to the production of type 1 IFN within hours and myocyte death through apoptosis and autophagy.[79] Virus entry activates inflammatory signalling pathways in cardiac myocytes while also altering—mainly increasing—viral virulence and replication.[80,81] The initial cardiomyocyte damage leads to the presentation of cardiac protein fragments—antigens—that may induce autoimmune reactions.[82]

TLRs play a key role in early activation of the innate immune response against viral and other infections. So far, specific roles in inflammatory heart disease and myocarditis have been identified for TLR2, TLR3, TLR4, TLR7, and TLR9 along with their downstream adaptors MyD88 and TRIF.[80,83–88] Enteroviruses, which cause myocarditis and/or dilated cardiomyopathy, activate TLR3 signalling. Accordingly, TLR3-deficient mice show markedly increased mortality after infection with enteroviruses.[89] Interestingly, a common TLR3 polymorphism has been found in patients with enteroviral myocarditis/cardiomyopathy.[90] Patients with viral myocarditis have elevated TLR4 transcripts, and TLR4 deficiency in mice reduced cardiac inflammation in CVB3 myocarditis.

The monocyte and macrophage lineages, mainly derived from circulating monocytic precursors expressing Ly6C and CCR2, comprise/make up/are the majority of infiltrating cells in myocarditis.[91,92] Functional differentiation of monocyte precursors is strongly influenced by cytokines and chemokines released from $CD4^+$ T-cells.[93] The Th1 cytokine IFN-γ potentiates microbicidal activity by increasing lysosomal proteolytic enzymes and peroxide production while also promoting antigen presentation. Th1 cytokine-activated macrophages can be categorized as M1-type macrophages.[94] In experimental autoimmune myocarditis, most heart-infiltrating cells express CD11b, which points to their myeloid lineage, but are Gr1-negative, which excludes the possibility that they are neutrophils.[91] $Ly6C^{low}$ resident macrophages activate myofibroblasts.[95]

After the acute phase, immune cells from the adaptive immune system also accumulate in the infected heart, including both T- and B-lymphocytes. $CD8^+$ cytotoxic T-cells directly bind and eliminate infected cells through recognizing MHC class I antigens on infected cardiomyocytes, assisted by the effects of TNF-α and IFN-γ which promote MHC class I presentation and facilitate cell–cell contact between T-cells and myocytes.[96] $CD4^+$ T helper cells do not directly kill infected cells but are important mediators between professional antigen presenting cells and cytotoxic T-cells as well as B-cells, which produce neutralizing antibodies to limit viral replication. During the subacute phase, intense activity of the immune system eliminates infected and dead cells, but also significantly contributes to irreversible cardiac damage.

The third stage of viral myocarditis therefore starts after complete elimination of the virus and is characterized by cardiac repair and remodelling. In absence of pro-inflammatory triggers, anti-inflammatory cytokines such as TGF-β and IL-10 secreted by regulatory T-cells and alternatively activated (M2) macrophages promote resolution of the immune response and replacement of dead tissue by a fibrotic scar.[97] Depending on the extent of myocardial damage, cardiac dilatation and compensatory hypertrophy will lead to long-term cardiac dysfunction. Alternatively, failure to completely clear virus from the heart may also result in chronic inflammation and accelerated progression to dilated cardiomyopathy.

The chronic phase of myocarditis, also called inflammatory cardiomyopathy, is characterized by an immune shift towards an adaptive immune response with inflammatory cellular infiltration.

Herein, T-cells may target the host's organs by molecular mimicry, in which antibodies can cross-react with cardiac myosin, eventually inducing autoimmunity. If this (auto)-immune response prevails, ongoing myocardial inflammation and fibrosis, along with adverse cardiac remodelling, will lead to chronic cardiac dysfunction and dilatation.

In conclusion, TLRs modulate early activation of the innate immune response against viral infection. Chronic inflammatory cardiomyopathy may evolve from acute viral myocarditis, but little is known about the inflammatory pathways in the transition from acute to chronic detrimental inflammation in the human heart after infection. Autoantigens may induce autoimmunity in the heart, but we still lack both direct proof of their direct involvement and targeted therapy against them.

Genetic cardiomyopathy

Genetic cardiomyopathies present phenotypically as dilated, hypertrophic, or arrhythmogenic cardiomyopathies affecting the left and/or right ventricle.[98,99] The penetrance of any particular pathogenetic mutation varies among the different genes, with lamin A/C, phospholamban, and RBM-20 mutations being the most malignant. The pathogenicity of a gene mutation and its prognostic relevance also vary by age, hormones, and additional acquired, environmental factors such as hypertension, diabetes, toxic agents, ischaemia, viruses, and (auto)-immune factors.[100] Increased cardiac inflammation—mainly innate macrophages—is generally seen in dilated, arrhythmogenic, or hypertrophic cardiomyopathies. Whether those invading or resident cardiac immune cells are primary or secondary, i.e. related to disease progression, often remains unclear.

An additional acquired disease such as an autoimmune disease, active viral infection, or toxic agents that activate innate immunity may enhance cardiac inflammation in a person with 'genetic' cardiomyopathy. Complete clinical history, blood sampling, and endomyocardial biopsies with detailed immune staining will help identify those acquired cardiac immune activators. The addition of this primary inflammation on top of the pathogenic gene mutation will lead to an adverse prognosis.[100] Further, inflammation may also be caused by the gene mutation itself, as suggested in preclinical animal models. For example, the MYPBC3 gene mutation in mice resulted in significantly increased total and classically-activated pro-inflammatory macrophages in dilated cardiomyopathy compared to wild-type hearts.[101] Still, the pro-inflammatory response in hypertrophic and dilated hearts—independent of other acquired immune activators—is most likely a response to myocyte damage. When myocyte damage occurs in the left ventricle, as in hypertrophic or dilated cardiomyopathy, then inflammation will trigger enhanced cardiac fibrosis. In contrast, when the right ventricle is affected, fibrofatty replacement goes along with enhanced cardiac inflammation. It is well accepted that this inflammatory response may trigger disease progression, not only by either activating fibrosis in the left ventricle or fibrofatty degeneration in the right ventricle but also by activating the humoral immune system, which produces circulating cardiac autoantibodies that can be cleared away trough immune adsorption.[102] Whether gene mutations themselves cause enhanced cardiac inflammation, independent of acquired triggers or cardiac tissue damage, requires further investigation. As cardiomyocyte hypertrophy in HF accompanies a shift towards pro-inflammatory cytokine profiles in myocytes, it is very likely that myocyte hypertrophy in dilated or hypertrophic cardiomyopathy may cause a change in the behaviour of resident macrophages, increase oxidative stress, and as such promote myocyte hypertrophy, fibrosis, and overall disease progression.

In conclusion, beside its prognostic relevance, since increased cardiac inflammation is associated with a worse prognosis, immune activation supports fibrosis and fibrofatty degeneration. Little is known about direct causative links between inflammation and genetic cardiomyopathies, which should be addressed in future research.

Peripartum cardiomyopathy

In women dying of HF in the peripartum period, enlarged hearts with focal areas of necrosis and fibrosis suggest that infection during pregnancy may have spread to the heart and subsequently caused myocarditis. Indeed, several studies have addressed viral myocarditis in pregnant women diagnosed with peripartum cardiomyopathy (PPCM). The prevalence of myocarditis in PPCM patients was highly variable, ranging from 8.8% to 78%.[103] Moreover, some studies showed that myocarditis was clearly associated with HF in the postpartum period, while others observed no correlation between inflammation in histological examination and the outcome in PPCM patients.[104] In fact, the incidence of virus-positive endomyocardial biopsies varied greatly and seems similar to that in control populations.[104] Recently, a genetic predisposition, namely *titin* gene mutations, was found in PPCM with similar prevalence to idiopathic dilated cardiomyopathy.[105]

Pre-existing cardiac viral infection increases the severity of postpartum myocardial damage compared with non-pregnant control mice.[106] In this regard, primary viral infection in childhood can lead to a latent life-long viral persistence without pathological significance until an impairment in the immune defence occurs.[103] Such an immune-compromised situation may arise in pregnancy, thus allowing the reactivation of persistent virus infections and making a pregnant woman more susceptible to reactivated viral activity and more general pathogen infection.[103] Such a scenario likely explains viral myocarditis in some patients with peripartum HF; therefore, further studies are required to investigate the impact of infectious diseases on the development and the prognosis of PPCM patients. It seems that gene–environmental interactions (i.e. gene mutations, viruses, etc.) may all act in concert to cause PPCM.

Several studies analysed blood levels of pro-inflammatory cytokines in patients with peripartum HF. Elevated levels of TNF-α, CRP, IL-6, and IFN-γ have been reported in blood samples from PPCM patients compared with healthy age- and pregnancy-matched controls.[107,108] TNF-α also seems to be either a causative or driving factor for PPCM; one study reports improved outcome in PPCM patients with elevated TNF-α serum levels after treatment with TNF-α-neutralizing antibodies on top of conventional HF therapy.[109,110]

In PPCM patients with adverse outcomes, there is a strong positive correlation between oxidative stress and the kinetics of serum levels of IFN-γ, N-terminal pro-BNP (NT-proBNP), and prolactin.[107] This suggests chronic systemic inflammation may play a role in the progression of PPCM. Whether the inflammation is pathogen-triggered or caused by autoimmune processes is not clear. Genetic involvement, which is estimated to be present in about 15% of patients, may also be important.[105] Interestingly, experimental studies showed that IFN-γ and prolactin together accelerate cardiac inflammation.[111] Moreover, blocking prolactin with the dopamine 2D receptor agonist bromocriptine could completely prevent inflammation as well as onset of peripartum HF in a mouse model of PPCM.[111] A small pilot study showed that bromocriptine strongly improved healing in patients with PPCM.[112]

Foetal microchimerism persists in women's serum after pregnancy, and may also trigger peripartum HF. Foetal cells remain in circulation as a consequence of the naturally suppressed immunologic state of the mother during pregnancy.[103] With postpartum immune recovery, these cells may be recognized as non-self and cause a pathological immune response and cardiac inflammation.[113] As such, autoantibodies against cardiac tissue may also cause peripartum HF.[114–116] One hypothesis is that rapid uterine remodelling after delivery releases various proteins into maternal circulation. Autoantibodies formed against such proteins may subsequently cross-react with similar proteins in the myocardium, leading to cardiomyopathy in the peripartum period.[103,113] Indeed, women with PPCM have high titres of autoantibodies against selected cardiac tissue proteins such as cardiac myosin heavy and light chains, human cardiac transaldolase, human cardiac actin chain, adenine nucleotide translocator, branched chain αketo-acid dehydrogenase, and the β1-adrenergic receptor.[103,115,116] The titres of some of these antibodies, such as specific IgG and subclasses, correlated with disease severity and/or outcome even with stratification for treatment options.[104,115–117] Some case reports suggest autoimmune disorders, such as systemic lupus erythematosus (SLE), might be a risk factor for peripartum HF.[103] Although intravenous γ-globulin and immunoabsorption have been discussed as treatment options for peripartum HF, these have not yet been studied. Moreover, it is still unclear whether autoimmune processes play a causal role in the development and progression of peripartum HF and PPCM or whether they increase as a consequence of the disease state.

Taken together, these studies show that pro-inflammatory cytokines may be elevated in patients with PPCM and might be useful as both biomarkers for risk stratification and targets for potential immune modulatory treatments. However, the cause of increased systemic and cardiac inflammation is largely unknown. In this regard, *de novo* or reactivated infection by pathogens, hormonal influence, and autoimmunity should be further explored with regard to peripartum HF.

Inflammatory co-morbidities

All autoimmune diseases increase the risk of developing either accelerated atherosclerosis or (inflammatory) non-ischaemic cardiomyopathy, the latter also independent of coronary artery disease. Cardiovascular disease is one of the most important extra-articular causes of morbidity and mortality in patients with rheumatoid arthritis (RA). These patients have an excess risk of developing congestive HF not explained by traditional cardiovascular risk factors than persons without RA.[118] This risk is higher in patients positive for rheumatoid factor and anti-citrullinated protein antibody (ACPA). Several studies have linked IL-6 with coronary heart disease in patients with RA.[119,120] Tocilizumab (TCZ), an anti-IL-6 receptor monoclonal antibody approved for treating RA, elevates total cholesterol and high density lipoprotein (HDL).[121] In patients treated with TCZ, changes in RA disease activity, but not in lipid levels, are associated with adverse cardiovascular events.[121] Several medications used in RA patients might worsen traditional cardiovascular risk factors. Nonsteroidal anti-inflammatory drugs, glucocorticoids, and cyclosporine all are associated with an increased risk of hypertension. Glucocorticoid use in RA is associated with a dose-dependent increase in mortality rates, with a daily threshold dose of 8 mg.[122]

SLE, granulomatosis and giant cell myocarditis (GCM) are the main autoimmune diseases with cardiac involvement. SLE is an autoimmune disease affecting mainly female patients. Although pericarditis is the most common cardiovascular manifestation, occurring in approximately 15% of SLE patients, either coronary heart disease or non-ischaemic cardiomyopathy occurs in over 10% of patients.[123] Atherosclerosis is accelerated in patients with SLE, arising from specific SLE-related factors, including autoantibodies, anti-phospholipid autoantibodies, anti-oxidized low-density lipoprotein, anti-apolipoprotein A-1, pro-inflammatory small HDL particles, dysregulation of cytokines and chemokines, homocysteine, C3, and C4.[124] Cardiac involvement is a frequent cause of death in systemic sclerosis as well.[125] Here, disturbed myocardial microcirculation—in addition to coronary artery disease—is an important factor contributing to cardiac dysfunction.[126,127] This vascular insufficiency is associated with increased myocardial fibrosis causing chronic HF and conduction system abnormalities.[128]

Eosinophilic granulomatosis with polyangiitis (EGPA), previously known as Churg–Strauss syndrome, is a disease at the intersection of primary systemic vasculitis and hypereosinophilic disorders. EGPA commonly presents with eosinophilia, asthma, peripheral neuropathy, and cardiac and skin lesions. Cardiac involvement occurs in 27–47% of patients with EGPA[129–131] and represents the major cause of early death. Cardiac involvement, closely related to the degree of eosinophilia, is due to the cytotoxic effects of cationic proteins released by eosinophils.[132] Eosinophil cationic protein and major basic protein activate human cardiac mast cells[133] and platelets that promote atherosclerosis.[134] LV dysfunction, myocardial ischaemia, and arrhythmias are the main clinical features. Also, chronic eosinophilic myocarditis may lead to impaired LV function.[131] Granulomatosis with polyangiitis (GPA), the nephew of EGPA, is characterized by granulomatous inflammation and necrotizing vasculitis. GPA is associated with anti-neutrophil cytoplasmic antibodies directed against the neutrophil- and monocyte-derived proteinase 3 (PR3). Membrane-associated PR3 triggers the secretion of cytokines and chemokines, which contribute to immune

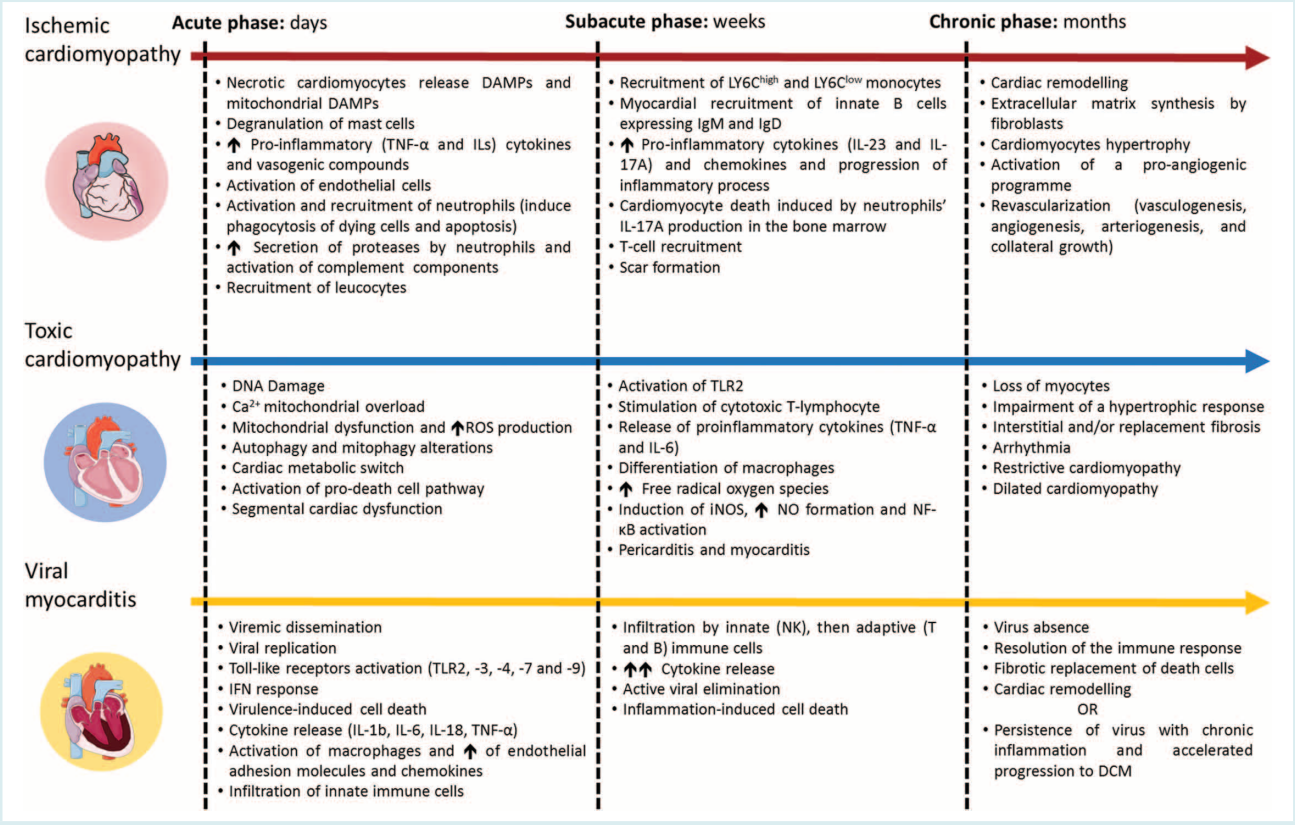

Figure 4 Immune mechanisms in different phases in ischaemic, toxic cardiomyopathy and viral myocarditis. DAMP, danger-associated molecular pattern; DCM, dilated cardiomyopathy; IFN, interferon; IL, interleukin; iNOS, inducible nitric oxide synthase; NO, nitric oxide; ROS, reactive oxygen species; TLR, Toll-like receptor; TNF, tumour necrosis factor.

dysregulation in GPA and increased incidence of cardiovascular events.[130] However, the prevalence of cardiac involvement and its prognostic relevance are lower in GPA as compared to EGPA patients.[130]

Idiopathic GCM is a rare cardiac inflammatory disorder characterized by infiltration of the ventricular myocardium by lymphocytes and multinucleated giant cells, eosinophils, cardiomyocyte necrosis, and, eventually fibrosis. The diagnosis of GCM rests on microscopy of the endomyocardial biopsy. Gene expression profiling may improve the diagnosis.[135] This disorder, attributed to a T-lymphocyte-mediated cardiac inflammation, is associated with autoimmune disorders in approximately 20% of cases.[136] These associations support an autoimmune aetiology, although autoantigens in GCM remain undefined. A possible role for T-lymphocytes provided the rationale for using a monoclonal antibody targeting these cells in combination with high-dose glucocorticoids and cyclosporine.[137] Despite improvement in treatment approaches, mortality remains high.

All in all, cardiovascular diseases are one of the most important causes of morbidity and mortality in most autoimmune diseases. Cardiovascular specialists and immunologists should therefore work together for better comprehensive assessment of these patients.

Clinical studies

Given the preclinical data summarized above, clinical investigators have started several clinical studies to improve HF by modulation of the immune system. An in-depth discussion of the clinical studies is beyond the scope of this article (for a review see Mann[3]). Shortly, three forms of clinical strategies can be distinguished: (i) anti-inflammatory; (ii) immunomodulatory; (iii) autoimmune strategies. All of them were used in chronic and not in acute HF. Whether novel anti-inflammatory therapy, such as selective inhibition of TLR2 and TLR4, PPAR-α, IL-1 or NLPR3, might also be beneficial in the human situation still remains to be determined.

Anti-inflammatory strategy

Antagonists to pro-inflammatory factors have been tested in phase II and III clinical trials. Pentoxifilline modulates different cytokines. It had clinical beneficial effects; however, the studies were small. Antibodies directed against TNF had no beneficial effects on HF progression.[3] However, the field got a positive 'push' in the last months by publication of the CANTOS trial,[138] where over 10 000 patients with previous MI and high CRP were randomized to placebo or canakinumab, a monoclonal antibody targeting IL-1β. This led to a reduction of cardiovascular events and in patients

with substantial CRP depression to a mortality benefit. Although CANTOS is clearly not a HF trial, for the first time it implemented protective cardiovascular effects with an anti-inflammatory therapy. This might be due to the treatment strategy, where only patients with high CRP were selected, thus with a potential high inflammatory load. This could also be a model for HF trials.

Immunomodulatory strategy

Due to the multiple parts of the immune system that may be involved in progression of HF, there were also trials with broad-based immunologic modifiers. However, the results for intravenous immunoglobulins, bortezomib, and methotrexate are not conclusive so far.

Autoimmune strategy

In this strategy, different autoantibodies against cardiac proteins have been targeted. However, also these results have not been conclusive so far.

Conclusion—implications for translation

This overview of the innate immune system's role in different HF aetiologies shows that different cardiomyopathies have different developmental phases. The initial phases seem to be specific to each disease with distinct immune mechanisms involved (*Figure 4*), e.g. acute inflammation in viral myocarditis and acute ischaemic injury will completely differ. In the chronic phase, pathophysiologic mechanisms may converge, with a common activation of the innate immunity through the autonomous nerve system, natriuretic peptides, the RAAS, and autoantibodies. Herein subpopulations of macrophages and lymphocytes can promote healing and restore immune balance. Therefore, similar interventions aimed at immune modulation might be useful in chronic HF with different aetiologies. Unfortunately, most data obtained in mouse cardiomyopathy models describe acute changes. We lack data on the chronic immune phase, not only in mice but also in large animals or humans. Moreover, it would be helpful to define in humans if an immune mechanism plays a role. We should have definitions of an 'inflammatory cardiomyopathy', e.g. by the number of CD3-positive cells in the myocardium. The use of biomarkers could circumvent the problem of myocardial biopsy and help to individualize a therapy as it is happening for atherosclerosis with CRP levels in the CANTOS trial. Therefore, we need a better understanding and definition of chronic immune activation and the identification of markers of an 'inflammatory cardiomyopathy' to adequately designing clinical trials with appropriate pharmacologic targets.

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