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**REVIEW**

# Inflammation as a therapeutic target in heart failure? A scientific statement from the Translational Research Committee of the Heart Failure Association of the European Society of Cardiology

**Stephane Heymans*, Emilio Hirsch, Stefan D. Anker, Pal Aukrust, Jean-Luc Balligand, Jan W. Cohen-Tervaert, Helmut Drexler, Gerasimos Filippatos, Stephan B. Felix, Lars Gullestad, Denise Hilfiker-Kleiner, Stefan Janssens, Roberto Latini, Gitte Neubauer, Walter J. Paulus, Burkert Pieske, Piotr Ponikowski, Blanche Schroen, Heinz-Peter Schultheiss, Carsten Tschöpe, Marc Van Bilsen, Faiez Zannad, John McMurray, and Ajay M. Shah**

Experimental & Molecular Cardiology, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Universiteitssingel 50, 6229 ER Maastricht, The Netherlands

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The increasing prevalence of heart failure poses enormous challenges for health care systems worldwide. Despite effective medical interventions that target neurohumoral activation, mortality and morbidity remain substantial. Evidence for inflammatory activation as an important pathway in disease progression in chronic heart failure has emerged in the last two decades. However, clinical trials of 'anti-inflammatory' therapies (such as anti-tumor necrosis factor-α approaches) have to date failed to show benefit in heart failure patients. The Heart Failure Association of the European Society of Cardiology recently organized an expert workshop to address the issue of inflammation in heart failure from a basic science, translational and clinical perspective, and to assess whether specific inflammatory pathways may yet serve as novel therapeutic targets for this condition. This consensus document represents the outcome of the workshop and defines key research questions that still need to be addressed as well as considering the requirements for future clinical trials in this area.

**Keywords** Inflammation • Heart failure • Treatment

## Introduction

The increasing prevalence of heart failure (HF) poses enormous challenges for health care systems worldwide. More than 7 million Europeans have HF and it is a leading cause of hospitalization.[1,2] The major risk factors for HF are hypertension, ischaemic heart disease, dyslipidaemia, diabetes, obesity, genetic predisposition, viruses, and toxins.[1] The common syndrome resulting from these different causes is characterized by evidence of cardiac systolic and/or diastolic dysfunction and clinical signs of dyspnoea, fatigue, and fluid retention. A large number of randomized clinical trials have shown that treatment with β-blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor antagonists, and mineralocorticoid receptor blockers reduces overall mortality and improves clinical symptoms of chronic HF. Major common detrimental pathways that are believed to be targeted by the above agents are activation of the renin–angiotensin–aldosterone system and sympathetic activation (so-called neurohumoral activation). In addition, mechanical interventions such as resynchronization therapy also have proven benefit in sicker patients. However,

* Corresponding author. Tel: +31 43 3877097, Fax: +31 43 3871055, Email: s.heymans@cardio.unimaas.nl

mortality and morbidity remain substantial in HF patients despite the use of the above treatments, underscoring the need for additional therapeutic options. In particular, there may be significant potential in therapies that target other pathophysiological pathways. Such therapeutic strategies are however sorely lacking.

Research over the last two decades or more has provided strong evidence implicating inflammatory activation as an important pathway in disease progression in chronic HF. Seminal clinical data found raised plasma levels of cytokines in HF patients,[3,4] and subsequent animal experiments suggested that certain anti-inflammatory therapies may be beneficial. This led to relatively swift attempts to translate these findings to humans through large clinical trials.[5,6] However, the approaches tested so far have been largely disappointing due either to neutral findings or even a worsening of HF.[7,8] These discouraging results have raised a number of important questions regarding the role that inflammation may play in the pathogenesis of HF and whether anti-inflammatory approaches are likely to be beneficial.

The Heart Failure Association of the European Society of Cardiology recently organized an expert workshop that brought together clinicians, clinical trialists, and basic and translational scientists to address this topic. Several major questions were addressed. Is there a common 'inflammatory pathway' that characterizes HF in general or should we stratify different forms and grades of HF and define the underlying inflammatory mechanisms relevant to each of these? Are the inflammatory pathways that are activated similar in acute HF [e.g. soon after myocardial infarction (MI)] vs. chronic HF? Should different therapies be tested in different patient groups? Are the previous targets that have been tested, such as tumour necrosis factor alpha (TNFα), still relevant or should we look for novel targets? If so, what are these novel targets? The present document is a Consensus Statement summarizing the main conclusions of the workshop. Besides analysing previous clinical trials and why they may have been unsuccessful, it attempts to provide new perspectives on patient stratification as well as novel 'anti-inflammatory' targets evolving from basic research, which could eventually form the basis for new clinical trials.

The document will first address the need for precise patient selection when starting a new clinical trial targeting inflammation in HF. Next, it will summarize what we have learned from previous clinical trials, and finally provide a flavour of some of the emerging mechanisms that could be targeted to treat adverse inflammation in the failing heart.

# Sub-groups of heart failure patients and related inflammation

A first consensus was that the testing of anti-inflammatory therapies for HF in clinical trials may require more careful and precise patient selection. For example, inflammatory activation may be different in HF occurring in the early stages after acute MI compared with chronic HF, and it would be worthwhile undertaking different clinical trials in these patient groups. Similarly, the diversity of different forms of HF such as diabetic, ischaemic, hypertensive, viral, and idiopathic cardiomyopathy, and also gender differences should be taken into account when considering specific inflammatory pathways to target. Related to these points was a second point of consensus, namely that pre-clinical data in animal experiments have been mainly obtained in relatively acute models of MI, hypertension, diabetes, or viral myocarditis, whereas most patients enrolled in trials for the anti-inflammatory strategies tested to date had chronic HF.

## Ischaemic heart failure

Despite aggressive primary therapy after MI, prognosis remains poor in patients with large infarction and severe left ventricular dysfunction. Acute sustained coronary occlusion causes rapid death of cardiac myocytes in the ischaemic heart. Following such injury, removal of irreparably damaged or dead cells and repair of the infarct through scar formation are essential for maintenance of cardiac integrity. An influx of inflammatory cells into the infarct area is thought to be an essential component of the very early wound healing process. However, inflammation may persist beyond the initial repair phase and later also extend into the non-infarcted remote myocardium, playing a role in longer-term adverse ventricular remodelling. Anti-inflammatory approaches might theoretically be of value in preventing and/or treating left ventricular dysfunction following large infarction,[9,10] although the timing of such treatment is likely to be critical in view of the beneficial role of inflammatory cells in very early wound repair.

Supportive evidence for anti-inflammatory therapy in chronic ischaemic HF is based on evidence of inflammatory activation (such as histological evidence in pre-clinical studies or increased plasma levels of pro-inflammatory cytokines in patients) together with an impressive range of pre-clinical studies of a variety of anti-inflammatory approaches, both genetic and pharmacological.[5,6,8,11–14] However, it is important to note that the vast majority of animal studies have been performed in relatively acute models (e.g. early remodelling after acute experimental MI), where the most prominent inflammatory response takes place within the infarct tissue.[10,15,16] As such, the healing infarct is the main target of anti-inflammatory therapy in this setting.[10] Therefore, it seems likely that beneficial effects reported, in terms of cardiac geometry (dilatation) and function (ejection fraction), may have been to a large extent dependent on factors related to the infarct healing process, such as infarct expansion. The role of inflammation—and thus the beneficial effect of anti-inflammatory therapies—in the delayed remodelling of the non-infarcted myocardium that takes place over many weeks and months may be more difficult to establish in such models.

Two further areas of consensus emerged. First, therapeutic approaches that appear promising based on the type of pre-clinical studies discussed earlier[17–25] should be tested clinically in the acute post-MI population rather than patients with chronic HF. Hindering early infarct expansion and adverse remodelling by therapeutic interventions on the inflammatory response relatively soon after acute MI may hold promise to prevent early expansion of the infarcted heart. Second, there is an urgent need to develop better pre-clinical models of chronic HF that may inform the choice of therapeutic approaches to test in the chronic HF population.

## Viral heart disease

For viral heart disease, four distinct phenotypes related to viral infection and inflammation have been described, that may

require different therapeutic approaches: (i) absence of viral persistence and no histological evidence of inflammation, consistent with healed myocarditis; (ii) presence of inflammation without viral persistence, suggestive of autoimmune disease; (iii) existence of inflammation and viral persistence consistent with insufficient viral clearance; and (iv) viral persistence without inflammation.[26,27] With the introduction of new molecular techniques, such as PCR and *in situ* hybridization in endomyocardial biopsies, many different viruses have been found to be associated with myocarditis, diastolic dysfunction, sudden death after MI, and idiopathic dilated cardiomyopathy (DCM).[28–35] In particular, a high prevalence of parvovirus B19 (PVB19) and human herpesvirus 6 (HHV6) besides the well-known adenoviruses and enteroviruses has been described. The prevalence of PVB19 and HHV6 ranges from 15 to 60% and from 8 to 30%, respectively, using PCR analyses in endomyocardial biopsies of patients with immunohistologic myocarditis. A similar prevalence in patients with idiopathic DCM[33] compared with acute myocarditis was found.[31,33,36]

Viral proteases can directly cleave the dystrophin–sarcoglycans complex, leading to cardiac dilatation.[37,38] Viruses also activate innate immunity—for example through STAT3 and gp130 signalling pathways—for systemic clearance of the virus.[39] However, this innate immune response can have adverse consequences in the heart via the production of cytokines and destructive inflammation.

Although more quantitative data and data on protein expression of the actual viruses are mostly lacking, these findings suggest a pathogenic process where viruses may trigger long-term cardiac injury and dysfunction. A recent observational study indicated that immunohistological signs of inflammation, rather that viral presence *per se*, is related to poor outcome in patients with suspected myocarditis and persistent cardiac dysfunction.[40] Therefore, the development of an adverse inflammatory response appears to be a requisite to develop clinical symptoms of myocarditis, and/or progression to DCM following virus infection in the heart.[40–42]

In view of these findings, studying virus presence and immunostaining of inflammation in endomyocardial biopsies is likely to be useful in informing subsequent therapeutic decisions in patients with unexplained HF or myocarditis with persistent dysfunction, in line with current European and American guidelines for endomyocardial biopsy.[43,44]

Open questions are the prevalence of viral persistence in other forms of HF or in normal hearts, and whether and how these common cough viruses may trigger HF in the long term. Future research should also focus on the understanding of the underlying genetic susceptibility and related immune alterations that explain why some people are susceptible to developing myocarditis following viral infection, whereas others 'spontaneously' improve or progress to an 'idiopathic' DCM or diastolic dysfunction afterwards. Treatment may need to target viral persistence, adverse inflammation, and auto-immune responses causing HF.

## Hypertensive heart disease

In the pre-clinical setting, left ventricular overload can result in myocardial inflammation evident from myocardial expression of proinflammatory cytokines and leucocyte infiltration into the myocardium.[6,11,45] Various observational studies in animals and patients have indicated a relationship between the presence of pro-inflammatory markers and pressure-overload induced hypertrophy and fibrosis.[46–49] The fact that the relative degree of inflammation (as assessed by levels of cytokine expression) is often substantially higher in patients with overload and compensated LV function compared with those with decompensated function[50] may challenge the concept that cytokine activation *per se* is necessarily detrimental in the chronically pressure overloaded heart. There is also very little knowledge regarding the implications of inflammation in the acutely overloaded heart. Increased oxidative stress,[51] proteolytic degradation of the extracellular matrix,[52–54] and cytokines and growth factors,[47,50,55,56] all potentially leading to increased inflammation, have been implicated in HF due to acute pressure overload. There is a need to investigate whether anti-inflammatory approaches may have utility in clinical settings with predominant acute or chronic pressure overload.

## Obesity and diabetes

Obesity, metabolic syndrome, and diabetes are expanding health care problems that increase the incidence of cardiovascular diseases. Growing evidence indicates that a systemic inflammatory response induced by obesity results in deleterious inflammation and microvascular dysfunction.[57] Obesity appears to involve some unique mechanisms of inflammation, most notably adipokines (such as leptin, resistin, TNFα, adiponectin, angiotensinogen, and IL-6) and insulin resistance. These factors cause the microvasculature to develop an inflammatory phenotype that may render the heart more susceptible to cardiac injury and dysfunction.[58,59] For instance, adiponectin exerts anti-inflammatory properties in the small vessels, by attenuating the induction of endothelial cell adhesion molecules and inhibiting the activity of nuclear factor kappa-B.[60–62] Plasma levels of adiponectin are decreased in obese subjects, which may cause microvascular inflammation and dysfunction.[63]

Insulin resistance and type II diabetes often accompany obesity. While the dependence of insulin resistance on obesity-related inflammatory mediators remains unknown, the evidence for a contribution of insulin resistance to inflammation in the heart, and in particular in the microvessels is growing. Insulin resistance causes an increased production of reactive oxygen species, adhesion molecules and chemokines, and suppresses the activation of nuclear factor kappa-B.[64,65]

Thus, reducing microvascular inflammation in HF patients with obesity and/or diabetes merits consideration as a therapeutic target.

# What have we learned from previous clinical trials?

## Cytokine-targeting therapy

Based on studies identifying a potential role of the proinflammatory cytokine TNFα as a mediator of disease progression in the failing heart, several randomized placebo-controlled trials of anti-TNFα therapies have been performed. The RENEWAL programme included two trials, RENAISSANCE and RECOVER, both of which tested etanercept, whereas the ATTACH trial used infliximab (reviewed in[6,8,11,12]). None of these studies

showed a beneficial effect and there were even indications of higher rates of mortality with such treatment. There may be several non-mutually exclusive explanations for the failure of anti-TNFα therapy trials in HF. First, the chimeric anti-TNFα antibody (infliximab), which was tested in some of the studies, directly binds to the transmembrane form of the TNFα receptor and could have resulted in detrimental effects on TNFα-expressing cardiomyocytes via antibody-dependent cellular toxicity, complement-dependent cytotoxic effector mechanisms, and induction of apoptosis.[66] Whereas such damage to TNFα-expressing inflammatory cells and synovial cells may be beneficial in disorders such as inflammatory bowel disease and rheumatoid arthritis,[66,67] similar effects on cardiomyocytes would clearly be detrimental in the heart. Secondly, low physiological levels of TNFα may be required for adequate tissue remodelling and repair, and may prompt cytoprotective responses during acute myocardial injury.[11,67] In keeping with this, a number of experimental studies suggest that physiological levels of TNFα induce cytoprotective responses in the heart during acute ischaemic injury.[11,12] It is clear that the plasma levels of infliximab achieved in the above clinical trials were quite high for a period of 19 weeks in both the 5 and 10 mg/kg infliximab treatment groups,[12,68] and may have reduced TNFα concentrations to below the levels required for physiologically beneficial effects. High dosages may also have resulted in a dose-dependent toxicity during anti-TNFα therapy.[8] Finally, in patients with HF, it may be that those with the most advanced HF (i.e. NYHA functional class IV) have higher TNFα levels.[13] However, only 3% of the population in the RENEWAL trial and 5% of the population in the ATTACH trial were in NYHA class IV and the numbers were too small to make any comment on the efficacy of anti-TNFα therapy.[7,8] For these reasons, future research should clarify the best type of anti-TNFα therapy, the optimal dosage, and what subgroups of chronic HF to treat before any firm conclusions regarding efficacy in chronic HF can be drawn. Other approaches to inhibiting TNFα-dependent inflammatory activation, such as inhibition of its synthesis or release, also merit investigation.

It is possible that anti-TNFα therapy may be valuable in patients with inflammatory cardiomyopathies secondary to viral infection or systemic diseases such as sarcoidosis. However, further research in this area will also have to identify other factors in the immunopathogenesis of HF. Other cytokines such as IL-1, IL-6, IL-18, macrophage inflammatory protein-1α, monocyte chemoattractant protein 1, and cardiotrophin-1 have also been found to promote HF (reviewed in[6,11,13,69]), and these could also merit consideration.

## Immunomodulation therapy: the ACCLAIM trial

Recently, the Advanced Chronic Heart Failure Clinical Assessment of Immune Modulation Therapy (ACCLAIM) trial tested the effects of immunomodulation therapy in patients with chronic HF. The rationale was to have a non-specific but broad immunomodulatory effect by reducing pro-inflammatory and increasing anti-inflammatory cytokines.[70,71] The study was based on a device where a blood sample is exposed to an *ex vivo* oxygen/ozone gas mixture at a temperature of 42.5°C for about 20 min, after which the treated blood sample is administered by intragluteal injection in an attempt to evoke beneficial immune responses.[70] The trial did not find any significant reduction in mortality or cardiovascular hospitalization.[70] However, it should be noted that the precise mechanism(s) of action of 'immunomodulation' remain unclear and that no assessment of cytokine activation was undertaken in the trial. In addition, two pre-specified subgroups of patients, those without a history of previous MI and those with NYHA II HF, had a significant reduction in their primary endpoint—suggesting that there could be benefit in certain subgroups. More studies are therefore needed to unravel the biological effects of this treatment and to clarify possible positive effects.

## Immunoglobulin or interferon treatment

The underlying biological effects are also unclear for treatment based on the administration of intravenous immunoglobulins (IVIg) Most of the studies to date have been conducted in patients with myocarditis and dilated non-ischaemic cardiomyopathy.[72–74] A beneficial effect of IVIg in HF was first reported in an uncontrolled study of 10 patients with myocarditis and acute cardiomyopathy.[74] Next, a randomized trial was performed in 62 patients with recent onset cardiomyopathy or myocarditis, but this did not confirm a beneficial effect of IVIg compared with placebo: both IVIg and placebo treatments improved cardiac function to a similar extent.[75] Whereas spontaneous resolution of HF and effective standard anti-HF treatment may in part explain the results, it should be noted that there was no endomyocardial biopsy data on histology, inflammation or viral persistence in these patients, which could have obscured the possible beneficial effects of IVIg in the patients most in need of it. In contrast, a double-blind, placebo-controlled study showed that IVIG significantly enhanced LV ejection fraction in HF patients, with the same pattern in both ischaemic and non-ischaemic cardiomyopathy. This beneficial effect significantly correlated with systemic anti-inflammatory effects of IVIg (e.g. up-regulation of IL-10 and IL-1 receptor antagonist). The reason for these discrepancies is at present unclear, but it is noteworthy that when maintenance therapy was given there was evidence of improvement of cardiac function.[73] A recent follow-up study showed that most of the HF patients in the IVIg group had a decrease in ejection fraction 1 year after termination of the study, suggesting that maintenance therapy is needed for an extended period of time as in other chronic inflammatory disorders.[6] Nevertheless, further clinical studies are necessary to define the possible utility of this therapy; such studies require the establishment of the role of virus persistence and in particular of parvovirus B19, classification of inflammatory infiltration based on histology on endomyocardial biopsies, and the role of autoantibodies.[76] Furthermore, patient selection will be mandatory in view of the limited supply, potential side effects and high costs of this treatment modality.

In 2003, a pilot study in 22 patients showed that the use of interferon-β in enteroviral or adenoviral persistence-related cardiac dysfunction resulted in virus elimination and a significant improvement in cardiac function.[77] These results evolved into the 'Betaferon in Chronic Viral Cardiomyopathy' (BICC) study, a randomized phase II trial that evaluates the use of interferon-β1b (INFβ-1b) in the treatment of patients with adenovirus,

enterovirus, and/or parvovirus persistence-related DCM—recently presented at the American Heart Association scientific sessions.[78] A total of 143 patients were randomized in a 1:1:1 fashion to either 8 million IU IFNβ-1b injection every other day subcutaneously (high dose), 4 million IU IFNB-1b injection every other day subcutaneously (low dose), or placebo for 24 weeks. The majority of patients had New York Heart Association (NYHA) class II symptoms. The results revealed a significant reduction in the primary endpoint of viral load lowering in the IFNβ-1b groups vs. placebo, although total virus elimination was observed in only a minority. NYHA class at 12 weeks and quality of life at 24 weeks were also significantly better in the IFNβ-1b group but objective parameters (such as echocardiographic and haemodynamic parameters and 6 min walk test) were not significantly improved. The value of this treatment strategy remains uncertain and a larger phase III trial is required.

## Immuno-adsorption

Auto-antibodies are detected in a significant proportion of patients with idiopathic DCM and their relatives.[79,80] A variety of cardiac antibodies has now been described in the literature, but without establishing any one group of antibodies as being responsible for clinical HF. Animal models, however, indicate that DCM can be triggered by auto-antibodies against the β1-adrenergic receptor[81,82] or troponin-I.[83]

In order to investigate the importance of the humoral immune system in HF, clinical studies to reduce levels of auto-antibodies by immunoadsorption have been conducted. Immunoadsorption removes circulating auto-antibodies, including those against the β1-adrenergic receptor.[84,85] Randomized studies involving a limited number of DCM patients have shown that immunoadsorption induces improvement of LV function[86,87] and decreases myocardial inflammation.[88] Immuno-adsorption leads to an improvement of LV function only in patients where cardiodepressant auto-antibodies have been demonstrated before treatment, but not in those where the auto-antibodies did not have a cardiodepressant effect.[89] Taken together, these findings suggest that immunoadsorption may be a promising therapeutic approach in DCM patients who have evidence of cardiodepressant auto-antibodies, and a larger randomized controlled clinical trial including over 200 patients is currently underway.[90]

## Anti-oxidant therapies?

Significant clinical and pre-clinical data implicate oxidative stress as being important in the development and/or progression of chronic HF,[51,69,91] and oxidative stress, redox signaling, and inflammation are known to be closely intertwined.[51,92–94] Given the above background, it may be envisaged that 'anti-oxidant approaches' may be beneficial in HF. Potential approaches include anti-oxidant vitamins or targeting specific sources of oxidative stress. A small double-blind randomized controlled trial investigating the use of vitamin E in 56 patients with NYHA class III or IV HF, did not demonstrate any significant improvements in prognostic or functional indexes.[95] A recent successful trial, the African-American Heart Failure Trial (AHeFT),[96] used hydralazine and isosorbide dinitrate, both reported to have anti-oxidant effects. Here, it was speculated that an anti-oxidant mechanism may be involved, but whether this would also involve an anti-inflammatory effect is unknown.

An alternative approach to inhibit oxidative stress generated by xanthine oxidase has recently been tested in a clinical trial using oxypurinol. However, this trial did not find evidence of a significant reduction in morbidity, mortality, or quality of life, although *post-hoc* analysis suggested that patients with elevated uric acid levels might receive benefit.[97] Whether the degree of serum uric acid elevation could be used as a biomarker, to select patients suitable for xanthine oxidase inhibition remains to be tested.

Despite the potential for anti-oxidant therapy, several limitations of such approaches should also be recognized. First, most patients will already be on therapies that are known to have anti-oxidant actions, e.g. angiotensin-converting enzyme inhibitors, angiotensin-receptor blockers, statins, and aldosterone antagonists. Secondly, non-specific anti-oxidant approaches such as the use of vitamins may interfere with beneficial pathways that depend on redox signalling, for example hypoxia sensing, angiogenesis and antioxidant defence pathways. Finally, there may be significant variation according to ethnicity—with evidence of increased oxidative stress in black populations and gender—with evidence of increased anti-oxidant reserve in women.

# Emerging anti-inflammatory targets

In addition to the treatments discussed earlier, there are several novel potential therapeutic options to reduce inflammation in HF that are emerging from pre-clinical discoveries. These target novel cytokines, chemokines, and mediators of oxidative stress, and target cell signalling pathways and the inflammatory cell response. Some of the most promising approaches are considered here, a sample of continuous active research in the field.

## Pentraxins

Pentraxins (PTX) were identified in the 1990s as cytokine-inducible genes or molecules expressed in specific tissues and cells including endothelial cells, smooth muscle cells, adipocytes, fibroblasts, mononuclear phagocytes, and dendritic cells. PTX3 production is induced by primary inflammatory signals, such as IL-1, TNFα, and lipopolysaccharides (LPS) (reviewed in[98]). The high levels of PTX3 expression in the heart during inflammatory reactions, its production by vascular cells in response to inflammatory signals and oxidized-LDL,[99] and the occurrence of PTX3 in atherosclerotic lesions,[100] prompted studies on PTX3 levels in acute MI. In a recent study in a cohort of 748 patients, plasma PTX3 levels when measured along with established markers including CRP emerged as the only independent predictor of mortality.[101]

The *in vivo* role for PTX3 in inflammatory conditions has been investigated using PTX3 transgenic and knockout mice. In a model of MI, the absence of PTX3 in mice exacerbated heart damage, which was associated with a greater no-reflow area, increased neutrophil infiltration, decreased number of capillaries, and increased number of apoptotic cardiomyocytes.[102]

Administration of exogenous PTX3, however, rescued the phenotype of increased myocardial damage in PTX3 knockout mice.[102]

Taken together, these data reveal that PTX3 may be a reliable biomarker for the prediction of mortality after MI and may also represent a novel therapeutic tool to prevent early cardiac damage after MI.

## PI3Kγ inhibitors

The recruitment of white blood cells from the blood stream into tissues—a key aspect of inflammation—involves three main steps: rolling, firm adhesion, and diapedesis leading to tissue penetration.[103] Recently, the enzyme PI3Kγ has become recognized as a crucial component of signal transduction controlling leukocyte migration.[104] Indeed, neutrophils and macrophages from mice lacking PI3Kγ show dramatically impaired recruitment to inflammation sites, thus suggesting that inhibition of the catalytic activity of this enzyme could provide a new tool to fight inflammatory diseases. Testing of first generation PI3Kγ inhibitors in pre-clinical models of inflammatory diseases, such as rheumatoid arthritis, has demonstrated significant therapeutic efficacy.[105]

The finding of PI3Kγ expression not only in leukocytes but also in the heart raised concerns about the safety of its inhibition, particularly considering potential cardiovascular side effects.[106] Interestingly, mice lacking PI3Kγ show normal blood pressure and heart rate but slightly increased cardiac contractility. In addition, when PI3Kγ -deficient mice are subjected to increased cardiac workload by transverse aortic banding, they display rapid loss of cardiomyocytes, left ventricular dilation, and death. Unexpectedly, mice expressing a catalytically inactive PI3Kγ do not show this phenotype, suggesting that the cardiac function of PI3Kγ does not depend on its catalytic activity. In agreement, PI3Kγ is found as a part of a multi-protein complex containing a phosphodiesterase: PI3Kγ, thus, plays a role as a crucial scaffold protein necessary for negative regulation of cardiac contractility.[107]

The above findings strongly suggest that drugs targeting the kinase activity of PI3Kγ can be safe for the heart and can be useful to treat cardiac inflammation. Based on these results, a compound inhibiting PI3Kγ catalytic domain was used to inhibit the recruitment of inflammatory cells after ischaemia and reperfusion of the myocardium.[108] Initial results in pre-clinical models of cardiac ischaemia/reperfusion in rats and pigs show protective effects of PI3Kγ inhibitors even if administered 3 h after the ischaemic insult.

Taken together, anti-PI3Kγ drugs appear to be a promising therapeutic tool in a variety of inflammatory conditions of the heart including ischaemia/reperfusion or fibrosis. A 'perfect' PI3Kγ inhibitor has not been found yet and ongoing studies from several pharmaceutical companies will likely soon provide new molecules with improved therapeutic margins, efficacy and safety profile.

## Matrix modulation

LV remodelling after MI and in response to pressure overload leads to progressive ventricular dilatation, fibrosis, and decreased cardiac performance.[109–113] The degree of the detrimental remodelling leading to congestive HF predicts morbidity and mortality.[114,115] Based on extensive evidence regarding the involvement of the matrix metalloproteinase (MMP) system in remodelling of the failing heart, one may consider using MMP-inhibitors for targeting remodelling. However, the main challenge and difficulty of MMP-inhibition in acute HF is to target a specific portfolio of MMPs at specific time points during acute HF.[116] The first clinical trial in patients with acute MI, the PREMIER trial[113] failed to show a treatment benefit when using a synthetic inhibitor. However, the MMP-inhibitor used targeted different MMPs, which may have resulted in unwanted side effects and off-target effects. Cardiac imaging or follow-up of plasma levels of MMP-inhibition was lacking, and therefore the therapeutic effects of MMP-inhibition remained unclear. In a recent randomized, placebo-controlled study, the beneficial effects of thalidomide in chronic HF was significantly associated with a down-regulatory effects of plasma levels of MMP-2, but the effect of this drug may involve several other potential mechanisms.[117] Future clinical studies should use specific MMP-blocking agents to target single MMPs during a limited time period, by preference in patients with acute HF where increased MMP-expression relates to acute dilatation and failure.[116]

Whereas MMPs increase cardiac failure by degrading the matrix and facilitating inflammation, increased expression of matricellular proteins in the cardiac matrix protects against HF by reinforcing matrix integrity and decreasing inflammation.[10,118] The matricellular proteins are a group of non-structural extracellular glycoproteins that are involved in interactions between cardiac cells and the matrix. Their expression is low to absent in the normal heart, but reappears at high levels during cardiac stress or injury.[10,118–120] Most of these matricellular proteins, including thrombospondins,[24,121] osteopontin,[119,122] periostin,[123,124] and tenascins[125,126] protect against HF by improving matrix integrity and favouring proper infarct healing and overall cardiac remodelling. They protect the heart by interacting with integrin pathways[122,127–129] that increase myocyte survival, by altering cytokine expression and by promoting the formation of a qualitative and mature collagen matrix.[121,129,130] In particular, increased levels of thrombospondin-2 *per se* reduce MMP-2 and -9 activity via scavenging of the MMP-TIMP-2-thrombospondin-2 complex through the lipoprotein-related protein receptor,[131] pointing towards a cross-talk between matricellular proteins and the MMP-system. Therapeutic use of these matricellular proteins as recombinant proteins could potentially be promising for the treatment of acute HF.

## Mannose-binding lectins

Glycoprotein-130 (gp130) is the common receptor of IL-6 and activates downstream JAK-STAT, SHP2-Ras-ERK, and PI3/Akt in response to inflammation, pressure overload, and ischaemic injury.[132] Targeted deletion of gp130 in mice results in increased mortality and HF in response to increased biomechanical stress.[133] In a search for altered downstream effectors, microarray data revealed a specific up-regulation of mannose-binding lectin (MBL), a lectin involved in complement activation, in infarcted ERK activation-dead mice (D Hilfiker-Kleiner, unpublished data). Although MBLs and the lectin complement pathway are known to be involved in the primary defence against microorganisms, there are emerging lines of evidence to suggest an active proinflammatory

role for MBLs in different chronic diseases[134] and cardiovascular injury.[135,136] Epidemiological studies have suggested that genetically determined alterations in the lectin MBL complement system influence the susceptibility to and the course of different types of infectious, autoimmune, metabolic and cardiovascular diseases.[136–138] Reports on the role of MBL in ischaemic heart disease are rather controversial. Whereas high MBL may predict decreased likelihood of MI in individuals at high risk[136] and low MBL levels measured 1 month after MI are associated with a higher incidence of re-infarction in patients with post-MI HF,[139] another study indicates that genetically elevated levels of MBL seem to be related to an increased risk of future coronary artery disease in apparently healthy men.[140] Concordant with the latter study, high serum levels of MBL together with high serum levels of agalactosyl IgG are associated with increased risk of ischaemic heart disease, MI, and premature death in patients with rheumatoid arthritis.[141] The reason for these apparent discrepancies is not clear, but may reflect the complex role of MBL in inflammation. On the one hand, MBL is an acute-phase protein, and the decrease in MBL levels after MI may reflect a decreased acute-phase response. On the other hand, low MBL levels, and in particularly MBL deficiency, may increase the risk and duration of inflammatory responses to various infectious stimuli.

Taken together, these findings indicate that IL-6 cytokines and downstream signalling are strongly implicated in cardiovascular diseases. Intervention on the downstream lectin MBL complement pathway may represent a novel therapeutic target to tackle inflammation following cardiac injury.

## Conclusions

HF is a major epidemiological burden in the industrialized world. Chronic inflammation interplaying with increased oxidative stress, cytokine production, proteolytic matrix degradation, and autoimmunity, is implicated in HF pathophysiology by increasing cardiac injury, fibrosis, and dysfunction. However, the initial trials of strategies to target these processes in the overall HF population have had very limited success. The most successful trials have been those where small very carefully selected groups of patients have been treated, notably with immuno-adsorption for auto-immune-related cardiomyopathy.[79] The idea of a common inflammatory pathway that characterizes all different forms of HF appears unrealistic. It will probably be important to design specific anti-inflammatory approaches for different types and stages of HF. In particular, anti-inflammatory approaches may need to differ markedly in acute vs. chronic HF. Determination of the specific inflammatory pathways in different forms of HF may be essential. Translation of findings to clinical trials requires pre-clinical studies where the appropriate animal model is used for either acute or chronic HF. Novel treatment options include the use of specific inhibitors of oxidative stress, cytokines, MMPs, or PI3K signalling pathways. However, sufficient knowledge of basic mechanisms, in-depth pre-clinical studies in different forms of acute vs. chronic HF, and adequate patient selection for clinical trials will be prerequisites for the development of successful anti-inflammatory approaches in HF patients.

## Consensus summary

- Testing of anti-inflammatory therapies for HF in clinical trials requires more careful and precise patient selection.
  - Different inflammatory pathways may be involved in diabetic, ischaemic, hypertensive, and viral heart disease, and in different genders.
- Pre-clinical testing of new therapeutic modalities should occur in appropriate animal models.
  - Therapeutic approaches that appear promising in pre-clinical models of HF after acute MI should be tested clinically in the acute post-MI population rather than on patients with chronic HF.
  - Better pre-clinical models of chronic HF that may inform the choice of therapeutic approaches to test in the chronic HF population are required.
- Understanding of the underlying mechanisms that explain why some people are susceptible to develop fulminant myocarditis or progress to an 'idiopathic' DCM with virus persistence is essential.
  - Endomyocardial biopsies should be considered in patients with unexplained chronic HF or acute myocarditis with persistent cardiac dysfunction.
  - Treatment should either target viral presence or reduce adverse inflammation and auto-immune responses.
  - Immunoadsorption may be a promising therapeutic approach in patients with cardiodepressant auto-antibodies.
- Anti-TNFα therapy cannot yet be discarded.
  - Clarification of the best type and optimal dosage of anti-TNFα therapy, as well as the specific patient subgroups that may benefit, is needed before any firm conclusions regarding its application in chronic HF can be drawn.
- There are several emerging novel therapeutic targets to tackle inflammation in the heart (e.g. pentraxin-3, mannose-binding lectin, PI3Kγ, matricellular proteins) that merit further assessment.

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