The Quest for Antiinflammatory and Immunomodulatory Strategies in Heart Failure

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Intensive research over the last 3 decades has unequivocally demonstrated the relevance of inflammation in heart failure (HF). Despite our current and ever increasing knowledge about inflammation, the clinical success of antiinflammatory and immunomodulatory therapies in HF is still limited. This review outlines the complexity and diversity of inflammation, its reciprocal interaction with HF, and addresses future perspectives, calling for immunomodulatory therapies that are specific for factors that activate the immune system without the risk of nonspecific immune suppression.

Heart failure (HF) is a complex syndrome involving different organs and systems beyond the heart, including the immune system. Since the recognition in the 1990s that cytokines play an important role in the pathogenesis of HF (cytokine hypothesis), inflammation has increasingly been recognized as being relevant in HF,[1,2] and, as a consequence, as being an important therapeutic target for the treatment of HF.[3,4] The disappointing results from past clinical trials testing tumor necrosis factor (TNF)-α antagonists highlight the diversity and complexity of inflammation and the difficulties and challenges in developing treatment modalities that can modulate the cytokine network in patients with HF. Those findings indicated that further research was, and still is, required to be able to more precisely identify the most important "actors" in the immunopathogenesis of HF and thereby improve the immunomodulatory treatment regimens for this disorder.[3] Thanks to state-of-the-art immune-phenotyping approaches, adoptive transfer of splenocytes, knockdown animals, and lineage-tracing cell imaging among other techniques, our understanding about the inflammatory process in HF has grown over the past decades. Inspired by the precision medicine initiative, the need for stratification of patients to better guide therapeutic interventions has also reached the cardiology field.[5] The outcome of the landmark Canakinumab Antiinflammatory Thrombosis Outcome Study (CANTOS) trial[6] is partly built on the specific inclusion of patients with post–myocardial infarction (MI) with a residual inflammatory risk mirrored by high sensitivity (hs) C-reactive protein (CRP) levels above 2 mg/L, reflecting high interleukin (IL)-1ß levels, and their hereto treatment with an antibody against IL-1ß. Chronic life-long suppression of the immune system, as envisioned in the CANTOS trial, may lead to severe side effects, whereas discontinuation of the therapy causes an unanticipated rebound of inflammation, as shown with another IL-1ß antibody.[7] These limitations make a nonspecific antiinflammatory therapy less feasible and desirable in clinical practice and indicate the need to search further for specific antiinflammatory and immunomodulatory strategies. This review outlines the complexity and diversity of inflammation, its reciprocal interaction with HF, and discusses how, despite our established and ever increasing knowledge of inflammation, clinical success of antiinflammatory and immunomodulatory therapies in HF is still limited. Future perspectives are addressed, calling for immunomodulatory therapies that are specific for the relevant signals that engage pathological inflammation without the risk of nonspecific immune suppression.

INFLAMMATION

Inflammation is an essential immune response that aims to resolve the source of the disturbance (infection or injury) and to maintain tissue homeostasis. The inflammatory response requires fine-tuning and precise regulation and should be limited by an antiinflammatory response, which is fast, reversible, localized, flexible to changes, and integrated by the nervous system. Persistence of the inflammatory trigger disables an appropriate induction of the resolution phase and leads to a state of chronic low-grade inflammation, which can contribute to further disease progression.[8] The complexity, diversity of inflammation, and its different forms (low grade vs. high grade, acute vs. chronic, and systemic vs. local) in HF and HF underlying comorbidities are outlined in **Figure 1**. The diversity of inflammation highlights the need to understand precisely when to counteract or modulate inflammatory pathways and accentuates that a "one size fits all strategy" may not account for all forms of HF.

INFLAMMATION TRIGGERS HF

Inflammation triggers HF in its different aspects. Inflammation affects pathological substrates (endothelial dysfunction and atherosclerosis),[9] and comorbidities (diabetes and obesity)[10] underlying HF, and influences the progression and outcome of acute [11,12] and chronic HF.[13,14] The relevance of inflammation in HF follows from the findings that patients with systemic inflammatory disorders, including rheumatoid disorders as well as diabetes mellitus and obesity, have an increased risk of cardiovascular events,

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which is associated with the degree of inflammation.[15] Patients with cancer are also more prone to develop HF,[16] whereas patients with HF have recently been shown to have an increased risk to develop cancer,[17] corroborating the relevance of inflammation as a common contributor to cancer and HF.

The correlation between elevated serum concentrations of proinflammatory cytokines and adverse clinical outcomes is common to both the main forms of HF: HF with reduced ejection fraction (HFREF) and HF with preserved ejection fraction (HFPEF).[13,18] However, how inflammation contributes to the pathogenesis of both forms of HF is postulated to be different. According to the postulated paradigm, a systemic proinflammatory state induced by comorbidities is the origin of microvascular endothelial cell inflammation, which subsequently triggers HFPEF-specific problems (i.e., concentric, cardiac remodeling, and dysfunction),[1] whereas in HFREF, cardiomyocyte damage induced by, for example, MI, ischemia, or cardiotoxicity, is the initial trigger underlying inflammation, and subsequent cardiac remodeling and dysfunction. Conform to the paradigm, circulating biomarkers of systemic inflammation, including hs-CRP and IL-6,[19] are higher in patients with HFPEF than in HFREF, whereas markers of myocyte stress and injury, brain natriuretic peptides, and hs troponin T are higher in HFREF vs. HFPEF.[19]

Beyond triggering chronic cardiac remodeling processes, cytokines, which can be from cardiac origin (cardiokines; produced by cardiomyocytes, cardiac endothelial cells, cardiac fibroblasts, cardiac tissue macrophages, and cardiac infiltrated immune cells, originating from lymphoid organs as the spleen and the bone marrow)[20] or from extra-cardiac tissues, including adipose tissue, gut, and lymphoid organs, can contribute to atrial fibrillation and sudden death.[12] Inflammatory cytokines promote structural and electrical atrial remodeling via impairment of gap junctions by changes in connexins and via inducing intracellular Ca^{2+}-handling abnormalities and atrial fibroblast activation, leading to impaired atrial conduction.[21]

HF TRIGGERS INFLAMMATION

HF as the end result of virtually all forms of cardiac disease is a complex syndrome. HF induces sterile inflammation in the heart itself via wall stress and signals released by malfunctioning, stressed, or dead cells secondary to HF. Additionally, HF induces inflammation in various peripheral tissues in a direct (inflammatory) and indirect (hemodynamic) manner, as previously reviewed in detail.[2] Cardiac cells release regulatory peptides and cardiokines in response to changes in the cardiac environment. These cardiokines affect the heart and also exert physiological and pathological effects in organs distal to the heart, such as the spleen, bone marrow, adipose tissue, and muscle, which affects cell death, growth, fibrosis, remodeling, metabolism, and inflammation. For example, IL-1ß released upon MI induces leukopoiesis in the bone marrow and at extramedullary sites, including the spleen.[22] Beyond cardiokines, HF-associated activation of the renin angiotensin system boosts the release of monocytes from their splenic reservoir,[23] which can be overcome by angiotensin-converting enzyme inhibition. HF-associated stimulation of the ß adrenergic nervous system activates bone marrow progenitor cells following MI. The pain and acute stress of the acute MI promote local catecholamine synthesis in the bone marrow and the systemic release of ß3-adrenergic stimulants.[24] The impact of adrenergic signaling in monocytopoiesis follows from findings showing that patients undergoing acute coronary syndromes who were allocated to ß-blockers before the acute coronary syndrome had significantly lower leukocyte and monocyte counts compared with those who had never used ß-blockers.[24] In alignment with the cytokine hypothesis and the release of cytokines upon HF, ß blockers reduce TNF-α and IL-1ß.[25]

HF-associated decreased cardiac output can decrease intestinal perfusion and cause mucosal ischemia. This can ultimately lead to a disrupted intestinal mucosa and increased gut permeability and subsequent leakage of bacteria and bacterial toxins into the blood, which can contribute to systemic inflammation and further exacerbate HF.[26] The HF-associated gut luminal hypoxia and decrease in mucosal pH[27] can also change the microbiota to pathogenic microbiota (dysbacteriosis), which further contributes to increased gut permeability[26] and subsequent systemic inflammation. Modulation of gut microbiota with probiotics, diet, or nonlethal microbial enzyme inhibitors with the aim to alter immune system composition are all areas of active research in HF.[28]

With respect to pathophysiological processes in the heart, there is accumulating evidence that mechanoreceptive intracellular pathways, including signaling via mechanosensitive integrins, which depend on matrix mechanical compliance and cyclic strain, are involved in cardiac fibroblast proliferation and matrix remodeling. HF-activated myofibroblasts, which can be activated by mechanical or inflammatory stress, are capable of inducing the inflammatory response via (i) release of chemokines that attract immune cells to the heart, (ii) induction of the expression of adhesion molecules on the endothelium, (iii) stimulation of monocytes to express gelatinases that facilitates their transmigration through the basolateral membrane,[29,30] and (iv) activity of their NLRP3 inflammasome and IL-1ß release.[31] In this manner, a vicious cycle is induced that supports chronic inflammation in the heart. Integration of mechanical cues into the complex humoral control of cardiac fibroblast activation in the failing heart offers not only a new level of understanding of the molecular mechanisms underlying HF, it also provides new hopes for selective antifibrotic/antiinflammatory treatments based on reverting or "desensitizing" pathologic cardiac fibroblast mechanosensation. This includes pharmacological (antiintegrin antibodies) as well as mechanical (left ventricular unloading; Impella)[32] approaches.

The complex and reciprocal interaction between HF and inflammation, which involves different cells, organs, and systems, explains how an initial inflammatory response may develop toward a chronic low-grade inflammatory response. The complexity of the interactions underlying the interplay between HF and inflammation also reveals why it is so difficult to counteract the inflammatory response due to the different systems and organs involved (e.g., increased monocytopoiesis post-MI) has been shown to accelerate coronary plaque growth after the first MI (cardiovascular continuum) and may be responsible for the high secondary event rates.[20,24] It also clarifies how conventional HF therapy, including ß-blockers and angiotensin converting enzyme inhibitors, is associated with antiinflammatory effects.

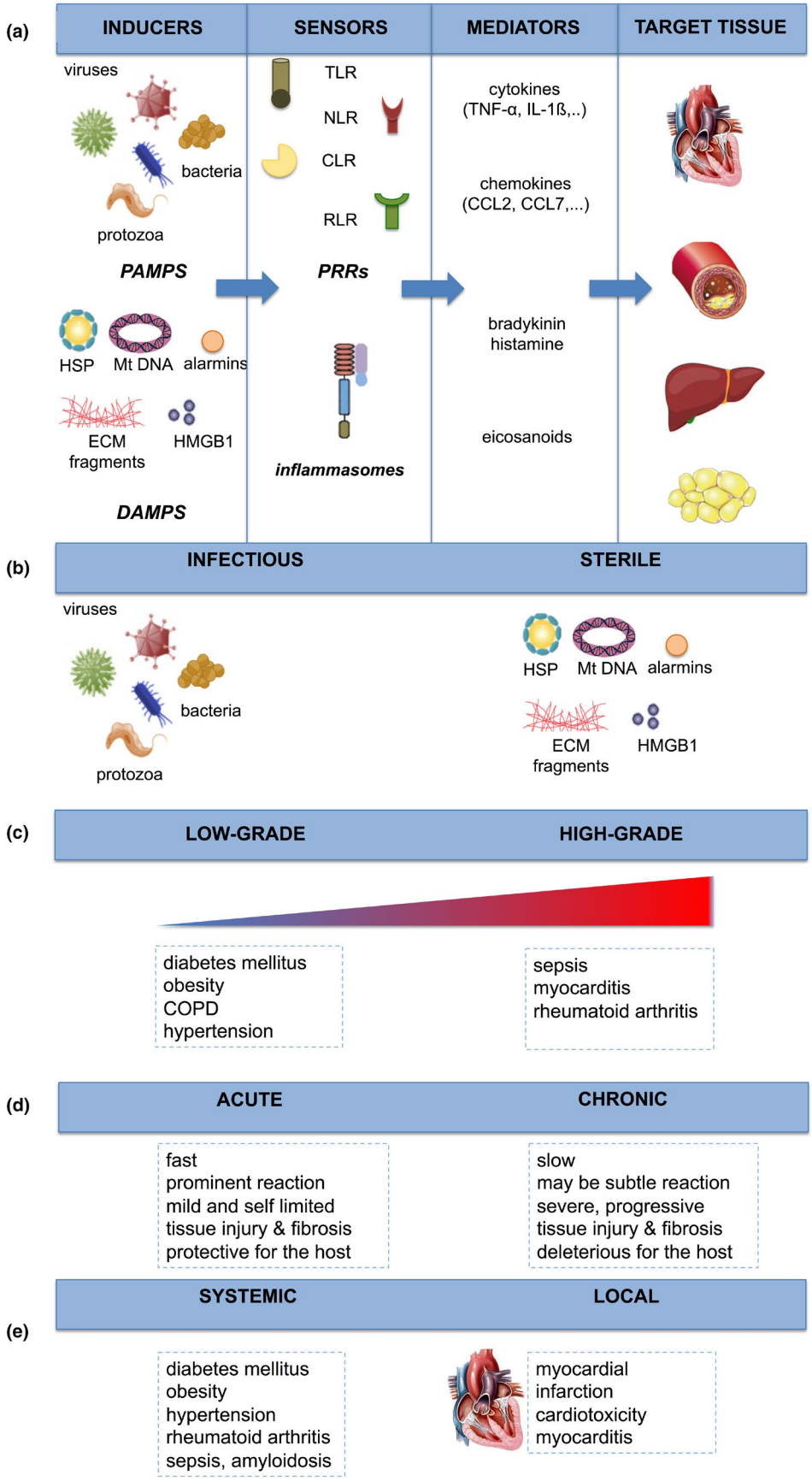
(a)
INDUCERS
SENSORS
MEDIATORS
TARGET TISSUE
viruses
bacteria
protozoa
PAMPS
HSP
Mt DNA
alarmins
ECM fragments
HMGB1
DAMPS
TLR
NLR
CLR
RLR
PRRs
inflammasomes
cytokines (TNF-α, IL-1ß,..)
chemokines (CCL2, CCL7,...)
bradykinin
histamine
eicosanoids
(b)
INFECTIOUS
STERILE
viruses
bacteria
protozoa
HSP
Mt DNA
alarmins
ECM fragments
HMGB1
(c)
LOW-GRADE
HIGH-GRADE
diabetes mellitus
obesity
COPD
hypertension
sepsis
myocarditis
rheumatoid arthritis
(d)
ACUTE
CHRONIC
fast
prominent reaction
mild and self limited
tissue injury & fibrosis
protective for the host
slow
may be subtle reaction
severe, progressive
tissue injury & fibrosis
deleterious for the host
(e)
SYSTEMIC
LOCAL
diabetes mellitus
obesity
hypertension
rheumatoid arthritis
sepsis, amyloidosis
myocardial infarction
cardiotoxicity
myocarditis

Figure 1 Inflammation is diverse and complex. (**a**) The inflammatory response comprises (i) inducers including pathogen-associated molecular patterns (PAMPs; of viruses, bacteria, and protozoa), and damage-associated molecular patterns (DAMPs; heat shock proteins (HSP), mitochondrial DNA (mt DNA), extracellular matrix (ECM) fragments, alarmins, and high-mobility group protein B1 (HMGB1)), (ii) sensors that detect them including pattern recognition receptors (PRRs; Toll-like receptor (TLR), Nod-like receptor (NLR), C-type lectin receptor (CLR), and RIG-I-like receptor (RLR)) and inflammasomes, (iii) the inflammatory mediators induced by the sensors including cytokines, chemokines, bradykinin, histamine, and eicosanoids, and (iv) the cells, tissues, and the functional states, which are affected by the inflammatory mediators. One distinct (**b**) infectious vs. sterile inflammation induced by infectious (viruses, bacteria, and protozoa) and noninfectious (HSP, mt DNA, ECM fragments, alarmins, and HMGB1) inducers, respectively; (**c**) low-grade vs. high-grade inflammation; (**d**) acute vs. chronic inflammation, by which acute inflammation is characterized by a fast, prominent reaction, which leads to mild and self-limited tissue injury and fibrosis and is overall protective for the host. In contrast, chronic inflammation is slow, may be a subtle reaction, which leads to severe and progressive tissue injury and fibrosis, and is largely deleterious to the host; and (**e**) systemic vs. local inflammation.

ANTIINFLAMMATORY AND IMMUNOMODULATORY STRATEGIES

Lessons from an old flame

Pioneer work from Levine *et al*.[33] in 1990 demonstrated that the levels of circulating TNF-α are increased in patients with severe chronic HF and with increasing New York Heart Association (NYHA) class. Further investigations by Deswal *et al*.[13] showed that circulating levels of TNF-α and soluble TNF receptors (TNFRs) are independent predictors of mortality in patients with HF. These observations formed the basis for the cytokine hypothesis of HF and suggested that TNF-α antagonism held a therapeutic promise, which was further supported by experimental studies illustrating cardioprotection of TNF-α antagonism in rats subjected to continuous TNF-α infusion[34] and in mice with cardiac-restricted TNF-α overexpression.[35,36] However, phase II and III clinical trials in patients with HF that have attempted to antagonize TNF-α with soluble TNF-α antagonists (etanercept) or by neutralizing antibodies (infliximab) were negative[37,38] and, in some patients, TNF-α antagonism even resulted in worsening of HF and/or death. Although the precise reason for the worsening of HF in the Randomized Etanercept North AmerIcan Strategy to Study AntagoNism of CytokinEs (RENAISSANCE), Research into Etanercept Cytokine Antagonism in Ventricular Dysfunction (RECOVER), and phase II Anti-TNF-α Therapy Against Congestive HF (ATTACH) trial[38] is not known, different potential explanations have been postulated, including the intrinsic toxicity of etanercept[39] and infliximab,[40] or the beneficial effects of TNF-α in the setting of HF.

The genetically engineered TNFR (etanercept) acts as a decoy to prevent TNF-α from binding to its TNF-α receptors on target cells, however, it can stabilize TNF-α, increasing its bioactivity, and hereby acts as an agonist (referred to as a stimulating antagonist).[39] At an early stage of rheumatoid arthritis, wherein TNF-α is encapsulated within a joint space and peripheral circulating TNF-α levels are relatively low compared with HF, this stabilizing effect might not be problematic. Whereas in patients with HF and increased TNF-α levels, the accumulation of high concentrations of immunoreactive TNF-α in the peripheral circulation may worsen HF due to the known biological properties of TNF-α.

The monoclonal antibody infliximab, consisting of a genetically engineered anti-TNF-α murine Fab fragment fused to a human fragment crystallizable portion of human IgG1, is directly cytotoxic to cells expressing TNF-α on their membrane due to complement fixation. This effect is beneficial in eliminating activated T cells that have invaded the gastrointestinal mucosa of patients with Crohn's disease. However, in the setting of HF, in which failing cardiomyocytes express TNF-α on their cell membranes,[41] complement fixation leads to sustained myocarditis as well as cardiac myocyte lysis mediated by the complement membrane attack complex.[40] The finding that the increased rates of mortality and HF hospitalization in the ATTACH trial[38] were particularly prominent in the group who received the highest dose of infliximab corroborate a dose-dependent cardiotoxic effect of infliximab.

The explanation involving beneficial effects of TNF-α in the setting of HF builds further on the dichotomous nature of TNF-α, which has partly been discovered after the disappointing results of the clinical HF trials.[42] TNF-α signals through the TNFR1, which induces persistent NF-κB activation and accelerates remodeling, whereas signaling through TNFR2 counterbalances these effects.[43] At a high concentration, TNF-α promotes endothelial cell apoptosis and inflammation,[9] whereas, at a low concentration, TNF-α induces angiogenesis.[42] The proangiogenic effects of TNF-α at physiological levels contribute to the beneficial effects during acute ischemic injury.[42] TNF-α antagonism during acute ischemic injury would, therefore, worsen HF, indicating the importance of administrating TNF-α antagonists within a specific timeframe during HF. However, based on these findings, it is impossible to predict how TNF-α antagonism would affect the chronic situation. Retrospective characterization of the positive responders, negative responders, and nonresponders from these TNF-α trials would have been helpful to understand the detrimental effects of TNF-α antagonists in chronic HF.

In conclusion, despite a well-characterized target and the use of pharmacological agents, which are successfully applied to treat patients with rheumatic disorders,[44] TNF-α antagonists were not beneficial in patients with HF. This highlights the fact that further insights are needed in relation to how the source of TNF-α (cardiac vs. noncardiac) may explain discrepancies in etanercept-induced or infliximab-induced toxicity in patients with HF vs. patients with rheumatic disorders and, in general, that repurposing of drugs needs to be performed with caution. It also addresses the need to evaluate whether due to redundancy of inflammatory cascades, foreseen outcomes of treatment cannot be reached and combination therapies or step-up strategies are required.[45] The later discovery that TNF-α may exert cardioprotective effects in acute HF indicates that further understanding and characterization of inflammation (here, TNF-α) and development of biomarkers during the pathogenesis of HF is needed to allow the stratification of patients and tailored therapies. It further accentuates the need for more translational preclinical animal testing before embarking on human trials.

The disappointing results from TNF-α antagonists in the clinical setting have further boosted the search for not only novel targets but also alternative strategies. These include strategies involving a broader spectrum of inflammatory mediators rather than a single target, strategies that trigger endogenous repair, and strategies focused on underlying immune and inflammatory cell networks that serve as important sources and targets of cytokines. In light of this, extensive research has been performed during the last decade investigating the role of specific immune cells in HF, a topic that has been reviewed in detail elsewhere.[46,47]

In brief, to counteract or modulate complex and diverse inflammatory reactions, different strategies have been developed over the years. Depending on their specificity (targeted vs. nontargeted), their spectrum (narrow vs. broad), and their response (constitutive vs. responsive), one can group these therapies into different classes: (i) targeted antiinflammatory approaches (e.g., TNF-α antagonists (etanercept, infliximab, and adalimumab) and IL-1ß antagonists (anakinra and canakinumab)), (ii) targeted strategies with a broad spectrum of antiinflammatory properties (e.g., pentoxifylline, thalidomide (or its analogues), and statins), (iii) immunomodulatory strategies that activate antiinflammatory pathways (e.g., immune modulation therapy: intravenous immunoglobulin (IVIG)), and (i.v.) immunomodulatory strategies, which are primed by the environment (e.g., mesenchymal stromal cells (MSCs)). Antiinflammatory and immunomodulatory strategies comprise antibodies, small molecules, cells, and devices. Below, some antiinflammatory and immunomodulatory strategies, including targeted antiinflammatory approaches as well as broad strategies, will be discussed.

Targeted antiinflammatory approaches

S100A8/A9 inhibitors. Accumulating evidence demonstrates an involvement of the damage-associated molecular pattern S100A8/A9 in cardiovascular disorders, like coronary artery disease[48] and Coxsackievirus B3 (CVB3)-induced myocarditis.[49] Several studies have illustrated its relevance as a biomarker for future MIs, and in rheumatoid arthritis and Crohn's disease, S100A8/A9 serum levels are used as a therapy-monitoring tool and included into appropriate guidelines.[50] Several pharmacological agents are available counteracting S100A8/A9: Quinoline-3-carboxamides (Q-compounds), including paquinimod, tasquinimod, and laquinimod, which bind to S100A9, preventing S100A9 binding to toll-like receptor (TLR) 4 and the receptor for advanced glycated end products. These compounds are currently in clinical development for both autoimmune diseases and cancer. Recent findings from Vogl *et al*.[51] demonstrate how S100A8/A9 released by activated phagocytes is only temporarily active in the local extracellular microenvironment due to a mechanism of autoinhibition that is induced by calcium-dependent tetramerization of S100A8/A9. Oligomer formation of two heterodimers blocks the ability of the heterodimer to bind to TLR4/MD2, thus restricting the activity of these alarmins at local sites of inflammation. Loss of this control mechanism results in fatal TNF-α-driven inflammation *in vivo*. In terms of therapy development, specifically blocking the TLR4-binding site of the active dimeric form of S100A8/A9 may represent a promising approach for local suppression of inflammatory diseases, minimizing systemic side effects.[51] Such a specific S100A8/A9 antagonizing therapy preceded by screening of S100A8/A9 serum levels for diagnosis and further therapy-monitoring form the optimal conditions to achieve a specific target-directed antiinflammatory strategy.

Canakinumab and anakinra. The IL-1ß antibody canakinumab has recently been shown to successfully reduce cardiovascular events in patients with previous MI and residual inflammatory risk.[6] Although all-cause mortality was not increased, fatal infection was raised, indicating that suppression of the immune system may lead to severe side effects. The IL1-receptor antagonist, anakinra, has been evaluated in clinical trials in patients with acute MI, acute decompensated HF, HFPEF, and idiopathic recurrent pericarditis with mixed results. Anakinra blunted the acute inflammatory response associated with ST-segment elevation and acute MI and numerically lowered the incidence of HF.[52] In HFPEF, anakinra treatment for 14 days significantly reduced the systemic inflammatory response and improved the aerobic exercise capacity of patients with HFPEF and elevated plasma CRP levels,[53] whereas treatment with anakinra for 12 weeks in a group of obese patients with HFPEF failed to improve peak Vo_2.[54] This emphasizes the importance of the duration of treatment and the patient cohort when considering treatment therapies and their efficacy. In patients with colchicine resistance and corticosteroid-dependent recurrent pericarditis, anakinra reduced the risk of recurrence over a median of 14 months.[55] Similar to canakinumab, anakinra may lead to increased infections due to nonspecific immune suppression.

NLRP3 inhibitors. The nucleotide oligomerization domain-containing, leucine-rich repeat-containing, and pyrin domain-containing protein (NLRP)3 inflammasome, an innate immune signaling complex, is the key mediator of IL-1 family cytokine production and considered necessary for initiating a profound sterile inflammatory response. Beyond its significance in atherosclerosis,[6,56] evidence from NLRP3 gene silencing in mice[57,58] and NLRP3-deficient mice[31,59,60] illustrates that NLRP3 is relevant in HF and that its regulation, activation, and impact on HF depends on the underlying cardiac disorder and timepoint during the pathogenesis of HF.[61] Furthermore, Toldo *et al*.[62] showed that formation of the inflammasome in acute myocarditis is predictive for the NYHA class and outcome. To date, clinical treatment of NLRP3-related diseases targets IL-1ß with IL-1ß antibodies like canakinumab (e.g., CANTOS trial, or IL-1ß receptor antagonists, like anakinra). For strategies targeting IL-1ß, it should be noted that IL-1ß secretion is not the only product of NLRP3 inflammasome activation. Other proinflammatory cytokines, including HMGB1 and IL-18, may be involved in the pathogenesis of these diseases.[63] On the other hand, IL-1ß can be produced by inflammasome-independent

pathways or other inflammasomes. Therefore, IL-1ß inhibitors may lead to unintended immunosuppressive effects besides preventing NLRP3 inflammasome activation and thus lead to increased infections as observed in CANTOS.[6] Pharmacological inhibitors specific to NLRP3 inflammasome may be the best choice for treatment of NLRP3-related diseases.[64] Inhibitory agents that are specific to NLRP3 have been identified and validated in *in vitro* and *in vivo* experimental models. Among others, selective NLRP3-inflammasome inhibition has been shown to reduce infarct size and preserve cardiac function in a randomized, blinded, translational large animal MI model.[65] The potential of these agents for the treatment of patients with specific HF needs to be further explored.

Immunomodulation

An alternative approach to targeting specific components of the inflammatory cascade is to use strategies that result in a decrease in the systemic inflammatory response. This approach includes the use of "immune modulation therapy," IVIG, immunoadsorption, and antimetabolites demolishing inflammatory cells (e.g., methotrexate (MTX), as well as cell therapies, including T regulatory cells (Tregs) and MSC).

General immunomodulation

Immune modulation therapy. Immune modulation therapy (celecade) is a technology that involves the administration of autologous blood following the *ex vivo* exposure to physicochemical stressors. The patients' own blood is stressed to induce cell death, and then the mixture of apoptotic cells is intramuscularly injected into the same patient. This strategy builds further on the knowledge that macrophages that phagocytose apoptotic cells, downregulate proinflammatory cytokines and upregulate antiinflammatory cytokines. This technology was evaluated in the Advance Chronic Heart Failure Clinical Assessment of Immune Modulation (ACCLAIM) trial, which enrolled 2,426 patients with NYHA II–IV.[66] In patients with nonischemic cardiomyopathy and more mild symptoms, a subtotal of 919 subjects, there was a 39% reduction in all-cause mortality and a reduction in cardiovascular admission.

MTX. The disease-modifying antirheumatic drug, MTX is a folic acid antagonist that decreases antigen-dependent T cell proliferation and triggers the release of adenosine, a molecule with antiinflammatory properties. MTX therapy in patients with rheumatoid arthritis is associated with a 70% reduction in cardiovascular mortality.[67] However, the results from the recently completed Cardiovascular Inflammation Reduction Trial (CIRT) do not support the findings in patients with rheumatoid arthritis. Patients enrolled in CIRT, which was comprised of 4,786 patients with previous MI or multivessel coronary artery disease who additionally had either type 2 diabetes mellitus or metabolic syndrome, were treated with low-dose MTX therapy. Patients were subsequently evaluated for the antiinflammatory and cardiovascular effects of low-dose MTX therapy.[68] Low-dose MTX did not reduce levels of IL-1β, IL-6, or CRP, and did not result in fewer cardiovascular events than placebo. Evaluation of MTX in patients with HF has not been performed so far.

Numerous small trials suggest potential promise of other non-pharmacological as well as pharmacological immunomodulatory strategies in HF, but large pragmatic clinical trials are lacking. Strategies targeting anticardiac antibodies, such as immunoadsorption and IVIG, have been used in clinical practice with positive outcomes. A pooled meta-analysis of small trials using pentoxifylline, a nonselective phosphodiesterase inhibitor, which affects the synthesis of inflammatory mediators by blocking their transcriptional activation, demonstrated a decrease in all-cause mortality in patients with nonischemic and ischemic cardiomyopathy.[69] Treatment with the plant alkaloid, colchicine, which has been used for centuries to treat gout, has been shown to improve cardiac outcomes in inflammatory cardiac disorders, including pericarditis, coronary artery disease, and postpericardiotomy syndrome.[70]

Tregs. Recent evidence has demonstrated the pathophysiological role of T cells in HF. $CD4^+$ T lymphocytes are globally expanded and activated in chronic ischemic HF, with Th2 (vs. Th1) and Th17 (vs. Treg) predominance in failing hearts.[71] In contrast, a wealth of evidence from experimental and clinical studies has indicated that CD4CD25FOXP3+ Tregs might have an important role in protecting against cardiovascular disease, including MI, myocarditis, dilated cardiomyopathy, and HF.[72,73] In HF, Tregs are qualitatively and quantitatively impaired independently of the etiology and are consequently ineffective in regaining immune homeostasis. Restoration of the dysregulated Treg/Th17 balance by Treg therapy[73] or IL-2 agonists,[74,75] promoting Treg cell production and mature Treg cell survival and suppressor function could be an option for the treatment of HF in the future.

Primed immunomodulation

MSCs. MSCs are attractive cells for use in the treatment of HF due to their ability to regulate inflammatory processes. When exposed to an inflammatory environment, MSCs can orchestrate local and systemic innate[76,77] and adaptive immune responses[78] through the release of various mediators, including immunosuppressive molecules, growth factors, exosomes, chemokines, complement components, and various metabolites. They reduce cardiac and systemic NLRP3 inflammasome activity,[77] increase systemic Tregs,[78] and modulate the cardiosplenic axis.[76] An exceptional characteristic of MSCs is that they know "when" and "where" they are needed. MSCs have immunosuppressive properties, although only upon "priming" of MSCs by a specific inflammatory milieu,[79,80] avoiding potential negative side effects. In addition, MSCs home to injured, inflamed tissue[81] upon intravenous injection.[78] Because the immunomodulatory capabilities of MSCs are not constitutive but rather are licensed by inflammatory cytokines, the net outcomes of MSC activation might vary depending on the levels and the types of inflammation within the tissues in which they reside. In some cases, MSCs will be programmed to suppress the immune response and in others to enhance it.[82] This plasticity of immunoregulation by MSCs is an important safety feature, avoiding infection due to a general

immunosuppressive effect. On the other hand, given this plasticity of the immunoregulatory phenotype of MSCs, inflammatory status and concurrent use of immunosuppressants should be considered when administering MSCs for the treatment of HF.

REFINEMENT AND FUTURE PERSPECTIVES

Translational advanced preclinical models

In the search for novel antiinflammatory and immunomodulatory strategies, optimal experimental models should be searched for and the limitations of traditional rodent models should be taken into account. Given the multiorgan complexity of HF,[2] animal experiments remain essential to understand the fundamental mechanisms underlying its pathogenesis. However, for disorders such as HF, in which inflammation and immunity play a central role, results from rodent models should be interpreted and the results extrapolated with caution. Depending on the strain,[83] sex,[84] and importantly also housing conditions,[85] the immune status of rodents differ. Because the immune status is of relevance in HF, this consequently affects the outcome of the experiments. Rodents are kept within "specific pathogen free" surroundings in animal facilities. Specific pathogen free-housed mice have a predominantly naive immune system and consequently, detrimental effector T cells are not present. With increasing antigen exposure (immune ageing), the effector and effector memory pool within the adaptive immunity of an individual increases, while simultaneously the naïve lymphocyte pool diminishes. This process of immune aging is greatly influenced by time but not *per se* comparable among individuals, specifically if they have seen different immune challenges.[85] Considering the immune system's experience level in an individual will likely allow patients to be stratified and treated more effectively than previously.[85] Experimental studies related to bone homeostasis have convincingly demonstrated substantial differences in healing capacity and maintenance of bone homeostasis in mice with an experienced compared with a naïve immune composition.[85] These findings support the idea that to improve translation of experimental results into a clinical setting, mice with an experienced rather than with a naive immune system should be used to better reflect the immune status of patients with HF. Differences in therapy responsiveness due to differences in immune composition could, therefore, be identified at the experimental level, and subsequently used to stratify patients into responding vs. nonresponding cohorts based on their immune profile.

Beyond appreciation of the relevance of the immune experience in animal models of HF, further factors need to be considered for animal models to be used in a valuable and effective way to understand the pathogenesis of HF and to evaluate the therapeutic potential of novel strategies. For example, as a model of virus-induced myocarditis, the CVB3-induced myocarditis model is the most commonly used and the best characterized. However, in contrast to humans, these mice develop severe pancreatitis in addition to myocarditis, thereby disabling a direct extrapolation of the findings into the clinical setting.[78] A model with cardio-specific CVB3 targeting is on its way. During the last few decades, a shift in cardioviral prevalence has taken place, with parvovirus B19 being the most prominent virus present in the hearts of patients with myocarditis.[86] Currently, it is not clear whether parvovirus B19 plays a causal role in inflammatory cardiomyopathy or is a bystander.[87] Humanized mouse models with parvovirus B19 infection, which so far are not available, would allow this question to be answered. Thus far, the impact of anticancer drugs on cardiotoxicity and HF has largely been studied in nontumor-bearing mice. Because inflammation is a common trigger for cancer and HF,[16] the use of tumor-bearing mice, with the cancer corresponding to the evaluated therapy, are needed to allow extrapolation of the findings to the human setting. Additionally, most experimental studies use juvenile mice, whereas patients with HF are mainly middle-aged or older. Beyond biological age, comorbidities, such as diabetes and hypertension, as well as comedications, known to interfere with cardioprotection, should also be taken into consideration. The well-known impact of sex on inflammation and the immune response,[88] as well as on HF,[89] further accentuates the need to conduct experiments in both sexes to avoid bias into translational findings, clinical concepts, and drug development. Concepts of sex-specific analysis in basic research have largely been neglected so far. Research funding agencies approached this issue but implementation of policy changes in the scientific community is still limited.[90]

To provide further evidence supporting the paradigm that the pathogenesis of HFPEF, which in contrast to HFREF, is triggered by a systemic low-grade inflammatory status,[1] experiments evaluating systemic overexpression of inflammatory mediators are required. These experiments would enable the evaluation of the sole (chronic) effect of a circulating inflammatory marker on the heart, which is not possible in animal models of diabetes mellitus and obesity, in which the metabolic burden also directly affects the heart. In the context of chronic HF, conditional deletion or overexpression of a gene would help to evaluate the role of a specific gene during (a specific phase in) the pathogenesis of HF. This will further allow us to gain insights into which components of the inflammatory response are physiological (protective) or pathological (harmful) in chronic HF.

In brief, animal models are needed to understand complex syndromes like HF involving different organs, and have provided, and continue to provide, useful information for the understanding of the pathogenesis of HF. However, to enable better translation of experimental findings into the clinic, advanced animal models are needed that better represent the cardiac condition and consider immune experience, and also exposure to environmental factors (caloric excess, intake of processed foods, antibiotic usage, and physical inactivity) common to Western lifestyle as well as disease (e.g., cancer when evaluating therapies to counteract anticancer drug-induced HF). Recognition of the multiple and complex interactions between the different arms of the immune system during different phases in the pathogenesis of HF via these HF murine models will help us to solve the inflammatory puzzle.

Refined approaches

Despite the complex nature of HF and the inflammatory processes involved, the antiinflammatory strategies used so far have

been mainly directed against a single component of the inflammatory response, be it against a specific cytokine or a particular immune cell type. This single component approach may explain why the results from clinical trials have been disappointing. Similarly, as for cardioprotective strategies against acute myocardial ischemia/reperfusion,[91] one now realizes that multitarget strategies taking into account the alterations in inflammatory status during the pathogenesis of HF should be considered. Assessment of the inflammatory status via biomarkers or imaging is, therefore, essential. For example, for MI with pronounced inflammatory status, antiinflammatory cytokines could be administered in the proinflammatory phase followed by subsequent administration of profibrotic factors or antiinflammatory monocytes, boosting the reparative response.[47] Combinations of immune suppressive therapies with prolonged use of axial flow pumps like the Impella systems (PROPELLA-concept) might be a viable treatment option for patients with severe HF due to fulminant myocarditis in the setting of bridge-to-transplant or bridge-to-recovery.[32] Beyond providing adequate circulatory support, the axial flow pump can unload the left ventricle, decrease cardiac wall stress, and mitigate inflammatory responses. The reduction of the inflammatory response by mechanical unloading further supports the concept that integrin receptors are potential new targets to block the mechanical load-induced inflammatory response.

Stratification of patients

Rather than a continuous disease spectrum with a uniform pathogenesis, HF has multiple phenotypes with different underlying pathophysiologic features. This heterogeneity of HF may explain why diagnosing and treating HF is so challenging and why clinical trials with antiinflammatory therapies have, thus far, largely failed. The challenge now is to establish clinical phenotypic characterizations to direct therapy. Phenomapping, a process of using machine-learning algorithms applied to clinical data sets, including age and sex, has been used to identify phenotypically distinct and clinically meaningful HF groups and to distinguish subclasses in HFPEF.[92] Particularly important in stratification is the recognition of sex and age, because both are known to influence inflammation and immunity[88,93] and affect HF.[89,93] HFREF occurs more often in men than women and HFPEF occurs more in older women. Recent findings specified that younger patients (age ≤55 years) with HFPEF are more often obese, nonwhite men, whereas older patients with HFPEF are more often white women with a higher prevalence of atrial fibrillation, hypertension, and chronic kidney disease.[94] Underrepresentation of women in cardiovascular trials, probably due to older age at HF onset and the poor inclusion of sex-specific data in trial reports, hinder the identification of gender differences in the efficacy and safety of cardiovascular medications, and may hereby contribute to the limited clinical success of antiinflammatory and immunomodulatory therapies in HF. The increasing prevalence of the women-dominated HF subtype with preserved ejection fraction adds impetus to this issue, as the underlying mechanism of this syndrome seems to exhibit sex differences, and therapies are lacking.[95]

Stratification of patients based on phenotypic profile, including inflammatory status, would allow for more direct selection of the optimal therapy and for matching targeted therapies with specific HF subtypes. An example illustrating how patients with a specific immune profile may profit from a specific immune cell-targeted strategy follows from patients with virus-negative inflammatory cardiomyopathy with endomyocardial biopsies positive for CD20-positive B cells. Those patients are known not to respond to a classical immunosuppressive therapy with prednisolone and azathioprine, whereas treatment with rituximab, a chimeric monoclonal antibody against the pan-B cell surface molecule CD20, has been described as a potential therapy.[96] Related to patients with inflammatory cardiomyopathy by whom analysis of endomyocardial biopsies allows the identification and quantification of specific immune cells in the heart, etiology-specific treatment strategies are, despite long year research, still in their infancy.[70] So far, diagnosis of inflammatory cardiomyopathy is based on the quantification of immune cells in endomyocardial biopsies via immunohistochemistry (≥ 14 leukocytes/mm^2 including up to 4 monocytes/mm^2 with the presence of CD3-positive T-lymphocytes ≥ 7 cells/mm^2).[97] Evidence from profound immunophenotyping, sophisticated adoptive transfer, and lineage tracing studies during the last decade illustrate the diversity of macrophages in the heart and their contribution to the development and progression of cardiovascular disease.[98] This calls for further defined and standardized evaluation of immune cells subtypes, including proinflammatory vs. antiinflammatory monocytes/macrophages, in the heart via flow cytometry, gene expression profiles, or imaging to better mirror the cardiac immune homeostasis in those patients and to allow better stratification.

Currently, a plethora of biomarkers are already available.[99] However, the question still remains how those can help us in stratifying patients with HF and finding patient-specific therapies. Immune profiles, signatures[100] or ratios (Treg/Teff; proinflammatory/antiinflammatory monocytes) better reflect the immune status and do not restrict on one specific marker or target, which, due to redundancy of inflammation, may be compensated via other inflammatory signaling pathways. Therefore, stratification of patients with HF based on systemic or cardiac (in case of patients with inflammatory cardiomyopathy) immune profiles, signatures, or ratios, rather than on sole biomarkers, may be the future way to go.

CONCLUSIONS

Intensive research over the last 3 decades has unequivocally demonstrated the relevance of inflammation in HF. The strong reciprocal interaction between inflammation and HF highlights the difficulties in counteracting inflammation and HF once this vicious cycle has begun. Additionally, there is a need to control the inflammatory process at an early stage, avoiding chronic inflammation and HF. To understand this complex interaction involving different organs and systems, valid animal models are needed that better reflect the immune status of patients with HF, which more closely resemble the human disease setting, and which bring us further insights in the immune cells involved at the different stages of HF. Immune experience and exposure to environmental factors common to Western lifestyle and disease should, therefore, be taken into consideration when designing animal experiments. In addition to furthering our understanding of the pathogenesis of

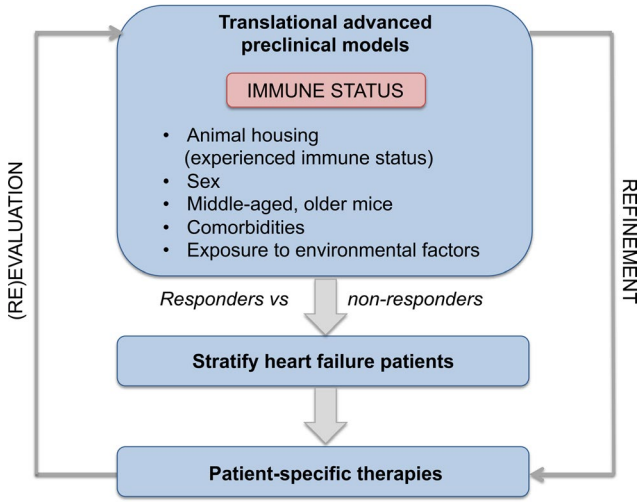

Figure 2 Requirements to achieve refined antiinflammatory or immunomodulatory strategies for the treatment of heart failure (HF). To obtain patient-specific antiinflammatory or immunomodulatory strategies for the treatment of HF, (i) translational advanced preclinical models are required, which better represent the immune status of the patient with HF via considering animal housing conditions, sex, age, comorbidities, and exposure to environmental factors. Those models allow a more precise understanding of the complex pathogenesis of HF and enable to identify differences in therapy responsiveness due to variations in immune composition at the experimental level. These findings can subsequently be used to (ii) stratify patients into responding vs. nonresponding cohorts based on their immune profile and hereby lead to (iii) patient-specific therapies. Those therapies can be (re)evaluated in advanced animal models and further refined.

HF and to identify novel targets, the challenge over the next years, and possibly decades, will be to effectively use the antiinflammatory and immunomodulatory strategies that are already clinically available in stratified patient cohorts, and to develop new biomarkers or immune signatures allowing the stringent follow-up of their inflammatory status. Characterization and differentiation of inflammatory processes will allow for the stratification of patients and enable the provision of tailored, target-specific therapies. The need for such a differentiated approach follows from the disappointing results of antiinflammatory strategies used in patients with HF so far[37,38,68] and is in agreement with the growing appreciation of precision medicine in cardiology.[5,6] Successful stratification of patients based on their inflammatory status will require the availability of valuable biomarkers or immune signatures reflecting this state. These biomarkers, combination of biomarkers, imaging, endomyocardial biopsy, and computational analysis, will not only be of relevance in stratification, but also in diagnosis, prognosis, decision making for the start of therapy, and therapy follow-up.[70] The specific biomarkers and analyses will depend on the cardiac disorder underlying HF.

Many antiinflammatory and immunomodulatory strategies are currently already available, due in part to repurposing of existing drugs, and new therapeutic targets have already been defined. However, refinement of therapeutic strategies is required to avoid drugs with intrinsic biological activity,[39] and to circumvent side effects and general immune suppression.[51] A more nuanced understanding of the actions of these therapeutics may further allow for the refinement of their dosage and timing of administration. Further knowledge of pharmacodynamics and pharmacokinetics of HF drugs in women vs. men also needs to be considered. In conclusion, much research related to inflammation and HF has already been performed with relatively low translational success. In the search for specific antiinflammatory and immunomodulatory therapies, refinement is a requirement at different levels starting from experimental design, including advanced animal models, through to refined therapeutic strategies and stratification of patients (**Figure 2**).

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