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A central role for Monocyte - Platelet Interactions in Heart Failure

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Conflict of interest

Drs. Ledwidge and Gilmer report board membership and shares in Solvotrin Therapeutics and are named inventors on several patents relating to isosorbide prodrugs of aspirin.

Abstract

Heart Failure (HF) is an increasingly prevalent and costly multifactorial syndrome with high morbidity and mortality rates. The exact pathophysiological mechanisms leading to the development of HF are not completely understood. Several emerging paradigms implicate cardio-metabolic risk factors, inflammation, endothelial dysfunction, myocardial fibrosis, and myocyte dysfunction as key factors in the gradual progression from a healthy state to HF. Inflammation is now a recognized factor in disease progression in HF and a therapeutic target. Furthermore, the monocyte – platelet interaction has been highlighted as an important pathophysiological link between inflammation, thrombosis, endothelial activation, and myocardial malfunction. The contribution of monocytes and platelets to acute cardiovascular injury and acute HF is well established. However their role and interaction in the pathogenesis of chronic HF is not well understood. In particular, the crosstalk between monocytes and platelets in the peripheral circulation and in the vicinity of the vascular wall in the form of monocyte – platelet complexes may be a crucial element, which influences the pathophysiology and progression of chronic heart disease and HF. In this review, we discuss the role of monocytes and platelets as key mediators of cardiovascular inflammation in HF, the mechanisms of cell activation, and the importance of monocyte-platelet interaction and complexes in HF pathogenesis. Finally, we summarize recent information on pharmacological inhibition of inflammation and studies of anti-thrombotic strategies in the setting of HF that can inform opportunities for future work. We discuss recent data on monocyte – platelet interactions and the potential benefits of therapy directed at monocyte-platelet complexes, particularly in the setting of HF with preserved ejection fraction.

Keywords

Monocytes; platelets; monocyte-platelet complexes; heart failure; pharmacological intervention

Heart Failure and inflammation

Cardiovascular disease is the number one cause of death in the world and chronic heart failure (HF) is an increasingly prevalent and costly multifactorial syndrome with high morbidity and mortality rates. HF affects approximately 1–3% of the population in developed countries and its prevalence rises to ≥10% in people over 65 years of age [1,2]. There are predictions of an increase in HF prevalence by more than 40% by the year 2030 [3] predominantly due to ageing populations, increased population prevalence of cardio-metabolic abnormalities and improved survival post myocardial infarction.

HF is classified as either HF with preserved (HFPEF) or reduced (HFREF) ejection fraction and a cut-off of left ventricular ejection fraction (LVEF) above or below 50% is often used to differentiate these subtypes. The precise pathophysiological interactions, causes, and sequence of events leading to the development of HF have not been fully elucidated although HFREF is often associated with myocardial ischemia and infarction and is modifiable by several classes of pharmacologic and device therapy. HFPEF has a community prevalence at least as high as HFREF and is associated with older age, metabolic abnormalities and chronic hypertension leading to vascular and myocardial dysfunction [4].

Several emerging pathophysiological paradigms implicate cardiometabolic risk factors, endothelial dysfunction (peripheral vascular, coronary vascular, and endocardial), inflammation, cardiomyocyte dysfunction, and myocardial fibrosis as key factors in the progression from healthy state to HF, including HFPEF [5-9]. Such hypotheses suggest that in early stage HF cardiac injury is driven by systemic inflammation and assisted by heightened platelet activation and oxidative stress arising from comorbid conditions such as hypertension, obesity, diabetes mellitus, iron deficiency, and chronic pulmonary and kidney disease [5-7,9-15]. The heightened systemic inflammatory response affects the peripheral and

heart vasculature and promotes endothelial inflammation (such as coronary microvascular endothelial inflammation [5], endocardial endothelial dysfunction [16]), and oxidative stress.. This triggers a series of events including progressive invasion of pro-inflammatory cells through dysfunctional, "leaky" endothelium into the myocardium, disrupted endothelial nitric oxide (NO) bioavailability, further endothelial imbalances, phosphorylation deficits of major cardiomyocyte proteins such as titin, and increased cardiomyocyte stiffness. The aggravating imbalances promote myocardial dysfunction by altering the composition of the myocardial extracellular matrix, resulting in collagen deposition and imbalances in matrix metalloproteinases (MMP) and their tissue inhibitors (TIMP). In advanced HF myocardial damage occurs and is amplified by the concurrent exposure to imbalanced systemic, endothelial, and paracrine inflammatory mediators such as tumor necrosis factor alpha (TNFα), interleukins (IL) 1 and 6, C-reactive protein (CRP), monocyte chemotactic protein 1 (MCP1), reactive oxygen species (ROS), NO, transforming growth factor beta (TGFβ) and MMP [5,17,18].

Inflammation is a recognized factor in disease progression in both HFREF and HFPEF and a therapeutic target in this setting [19]. However, initial clinical trials utilizing broad anti-inflammatory therapies such as anti-TNFα agents in chronic HF patients (predominantly HFREF) have shown limited success (reviewed in Mann 2005 [20] and Mann 2015 [21]). Indeed there is some evidence from *post-hoc* analyses of the anti-TNF therapy trial that those patients receiving higher dose therapy or longer duration of treatment had more adverse outcomes including heart failure and cardiovascular events [22]. One solution proposed may be to focus again on the role of innate immunological responses in HF [21]. Another may be individualize therapy according to the etiology, severity and even subtype of HF (HFREF or HFPEF) due to the different nature of underlying causative and pathophysiological factors, associated comorbidities, and clinical presentation of the disease. For example, it is

noteworthy that anti-inflammatory therapy using injections of modified autologous blood to non-specifically downregulate pro-inflammatory cytokines and increase production of anti-inflammatory cytokines, showed benefits in patients with non-ischemic cardiomyopathy or patients with milder heart failure (NYHA class II) despite overall neutral results [23]. A further approach might borrow from several large scale clinical trials which have been initiated in the setting of atherosclerosis, testing the effect of specific inhibitors of the IL1-TNFα-IL6 pathway and related inflammatory pathways (oxidized-low density lipoprotein, P-selectin, phospholipase A2) that act to reduce inflammation in damage-sensitive systems such as the vessel wall, the monocyte/macrophage system, the adipose tissue, and the liver (reviewed in Ridker 2014 [24]). The results of these trials may advance the case for new, targeted anti-inflammatory therapies for chronic cardiovascular diseases including HF. While, on one hand, a more effective therapeutic approach may benefit from targeting specific inflammatory mediators (such as monocytes) and/or specific inflammatory pathway(s) or components within this pathway(s) which have significant contribution to HF pathogenesis and progression, there has been limited success of targeted approaches to date. Undoubtedly further research is required to help define the relevant specific cellular partners and inflammatory pathways with the biggest potential impact for HF immunopathogenesis. Currently, anti-inflammatory therapy in HF patients is prescribed based on comorbidities and syndromes including atherosclerosis, acute coronary syndrome (ACS), previous MI, ischemic and non-ischemic CAD, myocardial ischemia, and atrial fibrillation. It involves, for example, the use of statins, anti-coagulants (aspirin, warfarin), corticosteroids, and non-steroidal anti-inflammatory drugs (e.g. ibuprofen, naproxen) and while some studies have observed benefits associated with anti-inflammatory therapies to date, in many cases large scale studies of these therapies have resulted in neutral or even adverse effects in the failing heart [21,25,26].

Alternatively, an approach for beneficially modulating inflammation in HF may be the use of combination therapy to target platelet activation, endothelial dysfunction and/or oxidative stress alongside inflammation and inflammatory cell activation. Benefits of restoring NO signalling (using, for example, endothelial NO synthase enhancers or NO donors) which modulate the pathway at the center of endothelial dysfunction, and of restoring endothelial function and normalizing platelet function have been shown to improve cardiac function in experimental animal models of cardiac stress, injury and HF(PEF) [27-30]. A novel endothelial therapeutic approach with potential application in the HF setting utilized Protective Antioxidant Carriers for Endothelial Targeting (PACkET) to facilitate endothelial-targeted delivery of antioxidant enzymes (catalase and superoxide dismutase). This therapy provided vascular anti-oxidant and anti-inflammatory protection in animal models of inflammation and oxidative stress [31]. In addition, inhibition of platelet activation was shown to prevent cardiac inflammation, fibrosis and adverse HF remodelling in response to angiotensin II insult in mouse models [32], and had beneficial effects (not confined to prevention of thromboembolic complications) on post chronic MI HF remodelling in rats with coronary ligation [33].

Building on these promising non-clinical data, an approach targeting endothelial function, platelet activation, with or without anti-inflammatory therapy may improve HF treatment and prevention. Few studies have reported on this strategy in the clinic. In practice, similar to the situation with anti-inflammatory therapies in HF patients, pharmacological agents aimed at improving endothelial function and regulating platelet activity are only being used to treat patients with HF if they present with concomitant peripheral, cardiac or cerebral vascular disease, congestion, atrial fibrillation, or stroke. In this regard, retrospective observations about the beneficial impact of chronic, low dose antiplatelet therapy in HF have been made [34] and disputed [35]. Furthermore, among patients with reduced LVEF who were in sinus rhythm, there was no significant overall difference in outcome between treatment with warfarin and

treatment with aspirin [36]. Therefore, existing clinical studies on pharmacological inhibition of inflammation, leukocyte/monocyte, and platelet function and data examining the monocyte-platelet interaction and its antagonism in HF are presented and discussed in this review. Overall, in the management of inflammation in HF there may be a need not only for further evaluation of novel pharmacological agents, but also novel therapeutic strategies which are able to regulate and target inflammatory cell-cell interactions and communication in the circulation. This may be of particular value in the coronary vasculature where endothelial dysfunction, inflammatory cell interactions and inflammatory mediator release from those cells (e.g. TNFα, IL6) may be critical to the development and progression of HF.

Monocytes and platelets as mediators of cardiovascular inflammation in Heart Failure

Platelets and monocytes are the principal cellular mediators of hemostasis in response to cardiovascular injury (reviewed in Rondina 2013 and Fernandez-Velasco 2014 [37,38]). Platelets, however, also play a major role in pathogenic thrombosis as a result of plaque rupture and endothelial dysfunction in atherothrombotic vascular diseases such as ACS, CAD, MI, cerebral ischaemia and cerebral ischaemic attack. Platelets are mediators of inflammation and atherogenesis via interactions with leukocytes (monocytes, lymphocytes, neutrophils, basophils, and eosinophils) and the endothelium. A mechanistic role for platelets in the development of acute and chronic HF has been described [39,40]. HF patients were shown to have higher mean platelet volume, increased whole blood aggregation, and higher levels of adhesion proteins including soluble and platelet-bound P-selectin and soluble CD40 ligand (sCD40L) [41-47]. Yet, despite the robust platelet activation and increase in activation markers, three studies in HF patients have shown that these may not modulate HF directly, but rather relate to future cardiovascular events via associated comorbidities [47-49]. In the first study,

Chung *et al.* reported increased levels of markers of platelet activation (soluble P-selectin, platelet surface P-selectin, and CD63) in stable congestive HFREF patients (compared to healthy controls but not compared to CAD patients with normal LVEF > 50% [47]. However, since none of the platelet markers in HF and CAD patients were predictive of future events, platelet abnormalities in HF were claimed to relate to associated comorbidities. A second study in ambulatory HFREF patients also showed heightened platelet activity unaffected by aspirin therapy compared to healthy controls [48]. The degree of platelet activation was similar in ischemic and non-ischemic HF patients and was not related to disease severity or to outcome. Similarly, results from the congestive HF EPCOT trial which sought to assess the diagnostic utility of the platelet function analyzer (PFA-100) in HF, showed no significant differences when patients were divided by incidence of vascular events, emergency revascularization needs, survival, or HF etiology, suggesting that platelet abnormalities do not reliably predict clinical outcomes in this population [49]. Furthermore, in three trials of aspirin versus warfarin in patients without concomitant anticoagulant or antiplatelet therapy and without a definite indication for antiplatelet therapy, there have been inconsistent results. The WASH and WATCH trials identified an increased risk of cardiovascular and HF events in aspirin users compared with warfarin users [50,51], whereas the WARCEF trial did not demonstrate a benefit of aspirin compared with warfarin use in this population [36].

From a mechanistic viewpoint, a major role is anticipated for activated platelets in boosting systemic inflammatory responses, enhancing endothelial permeability and malfunction, and influencing subsequent tissue damage in cardiovascular disease and HF. These processes are regulated by platelet-induced activation of blood leukocytes and endothelial cells, enhanced platelet and leukocyte adhesion to endothelial cells, and enhanced leukocyte invasion into affected tissues.

Leukocytes, particularly monocytes, play important roles in various cardiovascular (patho)-physiological conditions including cardiovascular inflammation, wound healing, atherosclerosis, MI, ischemia, hypertension, and HF (reviewed in Swirski 2013 and Ghattas 2014 [52,53]). The inflammatory phase of acute and chronic cardiac damage is characterized by inflamed myocardial tissue and endothelium of the adjacent coronary microvasculature. This results in chemo-attraction of monocytes, both of myeloid bone marrow [54] or splenic [55,56] origin, via chemotactic signals (such as MCP1) secreted from susceptible endothelium and subsequent infiltration of these cells into the tissue. In the tissue, monocytes differentiate into macrophages with distinct phenotypic and functional properties dependent upon local cytokine stimuli. These macrophages release cytokines and mediators such as TNFα, MCP1, IL8, IL1, MMPs, and TGFβ which collectively contribute to the local inflammatory and fibrotic responses. Monocytes and macrophages are known to be major drivers of the inflammatory and fibrotic processes in cardiac disease and HF [57,58]. Increased activation of monocytes and abundant monocyte/macrophage infiltrates are seen in pressure-overloaded hearts in early and late stage HF and associate with exaggerated inflammation, tissue injury, fibrosis, but also tissue repair and revascularization [59-61] signifying a complex dual role of monocytes/macrophages in HF [57].

Mechanisms of platelet and monocyte activation and interactions with the endothelium

Platelet and endothelial activation

As described above, the main function of platelets is hemostasis by formation of blood clots from activation of coagulation cascades as well as preservation of the endothelial balance and contribution to inflammation. Under physiological conditions NO derived from the L-

arginine pathway and cyclooxygenase (COX)-2-derived prostacyclin (PGI_2) are secreted from intact endothelium to oppose platelet activation and adhesion [62]. In malfunctioning, inflamed or disrupted endothelium, the release of inflammatory and stimulatory factors (adenosine diphosphate (ADP), Von Willebrand factor (VWF), tissue factor (TF), MCP1), the upregulation of adhesion molecules (E- and P-selectin, integrins (intercellular and vascular cell adhesion molecules (ICAM1 and VCAM1)), and the exposure and release of extracellular matrix proteins (collagen, fibrinogen, fibronectin) promote platelet shape change. This results in increased expression of pro-inflammatory and adhesion molecules (P-selectin, sCD40L, platelet integrins: glycoprotein (GP) 1b, GP1b/V/IX. GPIIb/IIIa, GPVI), stimulating platelet activation, adherence and aggregation (reviewed in Jennings 2009, Davi 2007, and van Gils 2009 [63-65]). Other signals promoting platelet activation include bacterial and viral infection, leukocyte activation, hematologic diseases affecting erythrocytes (e.g. anemia), immune and autoimmune disorders. In HF, circumstances that have the potential to promote platelet activation include hemodynamic changes and vascular factors, vascular endothelial dysfunction and reduced NO formation, renin-angiotensin system activation, increased catecholamine and cytokine release. These biochemical hallmarks are associated with co-morbidities including hypertension, iron deficiency, diabetes, ischemia, peripheral vascular disease, and valvular disorders [5,39,40].

Activated platelets roll along the endothelium and attach to the site of injury via the platelet integrin receptors GP1b/V/IX, GPVI, and GP1b which recognize exposed VWF, collagen, and P-selectin on endothelial cells (reviewed in Varga-Szabo 2008 [66]). Firm adhesion to the endothelium is mediated via the subsequent formation of additional contacts between platelets, endothelial cells and secreted extracellular matrix proteins such as GPIIbIIIa – fibrinogen and GPVI / GP1a – collagen [66]. Following initial adhesion, platelets respond to mediators that sustain and amplify the initial activation. The main amplifiers of platelet

activation are the soluble agonists ADP, thromboxane A2 (TXA2), and thrombin. The actions of ADP and TXA2 are the targets of the most commonly prescribed antiplatelet drugs. Both ADP and TXA2 are released from adherent platelets to promote the activation, recruitment and accumulation of additional platelets. In physiological hemostasis and, mainly, in acute pathology this may lead to the formation of a growing thrombus. ADP interacts with the platelet receptor $P2Y_{12}$ promoting platelet activation, an interaction antagonized by the $P2Y_{12}$ blockers clopidogrel, prasugrel, ticlopidine, ticagrelor, and cangrelor used for prevention of major vascular events in at-risk ACS and MI patients [67,68]. TXA2 is a transient metabolite of arachidonic acid produced by successive actions of prostaglandin- endoperoxide synthase-1 (PTGS-1 or COX1) and thromboxane synthase [69]. TXA2 exerts its effects by binding to its receptor expressed in various cells (platelets, endothelial cells, monocytes, macrophages, smooth muscle cells) and tissues (heart, kidney, spleen) [70]. TXA2 is a key early stage platelet activating signal and its suppression provides the standard explanation for the cardio-protective effects of low dose aspirin post-MI. Drugs that target the TXA2 pathways and their mechanisms of action have been reviewed by Fontana *et al.* [71]. These include not just the COX1 inhibitors (aspirin, triflusal) but also TXA2 synthase inhibitors (ozagrel, picotamide, ridogrel, EV-077) and TXA2 receptor antagonists (seratrodast, ramatroban, terutroban, picotamide, ridogrel, EV-077). Those have shown varying degrees of clinical efficacy in the treatment of peripheral artery disease, atherosclerosis, ACS, and asthma [72-76]. Thrombin, known for cleaving fibrinogen to fibrin, is also a potent platelet activator that is rapidly produced at sites of vascular injury [77]. It binds G-protein-coupled protease-activated receptors (PARs), mainly PAR1, on human platelets. Binding of thrombin to PAR1 leads to receptor cleavage and exposure of an active ligand. Anti-thrombin agents (bivalirudin, fondaparinaux, rivaroxaban, apixaban) and PAR1 inhibitors (vorapaxar, atopaxar) have been evaluated and have shown mixed effectiveness in clinical studies in patients with ACS, stable

atherosclerotic disease, MI, stroke, and ischemia [68,78-85]. As described above, in HF, anti-platelet therapy is still indicated almost exclusively based on the presence of concomitant vascular disease. The use of anti-thrombotic therapy is also indicated in the setting of atrial fibrillation and data beyond this in HF are limited. Further discussion of clinical studies to date is provided below in the final section of this review ("Future perspectives on evaluation of drug therapy directed at monocyte-platelet interactions in HF").

Monocyte activation

Monocytes and macrophages are essential components of the innate immune system with key functions in host defense to pathogens , inflammation, immune regulation, tissue remodelling, homeostasis, and metabolism (phagocytosis/removal of cell debris, iron recycling for reuse by the host, wound healing). Upon sensing of an activating signal, monocytes migrate through the endothelium into respective tissues and differentiate into macrophage effector cells and replenish long-lived resident tissue macrophages in the liver (Kupffer cells), spleen, peritoneum, central nervous system (microglial cells), bone (osteoclasts), and connective tissue (histiocytes) [86]. There are different types of activating signals including infection components (bacterial LPS, lipopeptide, flagelin, and deoxyribonucleic acid (DNA); viral envelope glycoproteins; fungal zymosan and β-glycan), endogenous danger signals (cytokines: TNFα, IL1β, MCP1; soluble mediators: CD40 ligand, TXA2, platelet activating factors (PAF), leukotrienes, low-density lipoprotein, glucose), necrotic cells, and pathophysiological events (hypoxia, ischemia/reperfusion). These signals act on specific signal receptors on monocytes/macrophages which may or may not trigger an associated immune response. Homeostatic clearance of cells generated during tissue remodelling, clearance of apoptotic cells, and iron recycling from senescent erythrocytes is mediated by scavenger receptors, integrins, complement receptors, phosphatidyl serine

receptors, and thrombospondin receptor (reviewed in Kono 2008 [87]). These regulatory processes do not generate an immune response or mediator release. Meanwhile, clearance of necrotic material containing endogenous activators like histones, DNA, heat-shock and nuclear proteins generated as a result of trauma and stress, as well as stress signals from chronic and acute inflammation and remodelling (ischemia/hypoxia, hypertension, cardiac stiffness/fibrosis, high cardiac filling pressures) are regulated by Toll-like receptors (TLR), the IL1 receptor, intracellular pattern recognition receptors, and chemokine receptors. In addition, bacteria and virus sensing activates pattern recognition receptors like TLR, cytoplasmic Nod-like receptors and retinoic acid inducible gene I-like helicase receptors, scavenger receptors (like CD163), the LPS receptor CD14, and C-lectins. This promotes pathogen elimination or neutralization by phagocytosis, antigen presentation, and cytokine release [87]. These interactions induce an immune response with increased monocyte activation and monocytosis, which are characteristic for (auto)-immune and inflammatory diseases as well as for chronic inflammation associated with cardiovascular diseases [88,89]. Ultimately, monocyte activation is reflected by a sequence of events including: 1) increased expression of monocyte surface proteins (MCP1 receptor CCR2, fractalkine receptor CX3CR1, P-selectin glycoprotein 1 (PSGL1), L-selectin, integrins, and CD40); 2) activation of intracellular inflammatory signalling cascades (nuclear factor kappa B (NFκB), intracellular response factors, signal transducer and activator of transcription); 3) release of pro-inflammatory cytokines (MCP1, TNFα, IL1β, IL8, IL6, TF) and ROS; 4) expression of remodelling and angiogenesis-associated mediators (MMP, TIMP, vascular endothelial growth factor); and 5) a change in monocyte shape and actin cytoskeleton rearrangements (Rho, Rac and Cdc42 GTPases). Overall this results in monocyte mobilization and migration into tissue via increased MMP expression, upregulation of endothelial adhesion molecules (selectins, ICAM1, VCAM1), and NO synthesis.

Mutual Platelet-Monocyte-Endothelial Cell activation

A striking feature of monocyte/macrophage activation during cardiovascular stress or injury is the complex, dynamic communication network between circulating monocytes and activated platelets; circulating monocytes and activated endothelial cells; and platelets and endothelial cells, outlined in a recent review by van Gils *et al*. [65]. The precise sequence of the events remains unclear and may indeed be heterogeneous, but the importance of this mutual platelet-monocyte-endothelial cell activation is established in cardiovascular pathophysiology [90-93].

Figure 1 presents the main events taking place within the platelet – monocyte – endothelial cell network. Under pathophysiological conditions, activated platelets adhere to the endothelium, secrete chemokines (MCP1, IL1β, chemokine C-C motif ligand 5 (CCL5), TXA2, TF, PAF, macrophage inflammatory protein), and increase expression of adhesion molecules (P-selectin, GP receptors, CD40L) to promote the recruitment of circulating monocytes. The latter roll, adhere (mainly via P-selectin/PSGL1, GP/integrin, and CD40L/CD40 interactions), and eventually migrate through the endothelium into adjacent tissues, facilitated by MMPs. In other circumstances, platelets may be activated while in the circulation, for example by cytokines released in systemic inflammation or thromboembolism (acute MI), by soluble agents released from platelets present at unstable thrombi [94], or as a result of turbulent flow. These activated platelets bind preferentially to circulating monocytes in a P-selectin/PSGL1-mediated fashion and form monocyte-platelet complexes (MPCs) which show increased adhesive and migratory properties and aid the recruitment and activation of other, non-complexed monocytes [95]. Some of the mechanisms involved include NFκB pathway activation, L-selectin shedding, increased integrin expression and activity,

increased secretion of pro-inflammatory mediators and TF expression [96,97]. MPCs are therefore regarded as functionally important inflammatory mediators.

Briefly, in addition to the interactions between monocytes and platelets, platelet adhesion to the endothelium causes both platelet activation and endothelial activation. The interaction mediates the release of inflammatory chemokines (CCL5, platelet factor 4 (PF4), IL1β, macrophage migration inhibitory factor (MIF)) and mediators (TF, thrombin, PAF, ADP, TXA2), and upregulation of adhesion molecules (CD40L, P-selectin) from adherent platelets. In endothelial cells it activates NFκB and ROS production, upregulates endothelial adhesion molecules (VCAM1, ICAM1, E- and P-selectin), and regulates the secretion of different cytokines and mediators (MCP1, VWF, IL6, IL8, MMPs, granulocyte-macrophage colony stimulating factor) aimed at further monocyte and platelet activation, monocyte transmigration and macrophage differentiation [64,98].

Another important aspect of monocyte/macrophage activation is the polarization of circulating monocytes to tissue macrophages of either the classical/M1 or the alternative/M2 subset. This process is dependent on the type of monocyte activating signal and determines the phenotypic and functional traits of these cells and therefore the outcome of an immune-inflammatory response. Classical/M1 macrophages are induced by pro-inflammatory mediators like interferon gamma (IFNγ), TNFα, and pathogen-associated TLR ligands (LPS). They express high levels of pro-inflammatory cytokines (IL1, TNFα, IFNγ, IL6, IL8, IL12, IL23), produce high levels of reactive nitrogen and oxygen intermediates, stimulate T-helper type 1 responses, have strong anti-microbial and anti-tumor activity, are involved in intracellular parasite killing, and mediate tissue destruction [99,100]. M1 macrophage polarization regulates and is regulated by acute inflammation and infection, such as viral and bacterial infection, arthritis, atherosclerosis, diabetes (insulin resistance), and glomerulonephritis. Alternative/M2 macrophage activation is more complex due to the

existence of several M2 subtypes. M2 macrophages can be induced by IL4, IL13; immune complexes, glucocorticoids, TLR and IL1 receptor ligands; or IL10, TGFβ, IL1β, and IL6 and are involved in parasite containment, T-helper type 2 responses, and tumor promotion. They are highly phagocytic, and express high levels of scavenger, mannose and galactose receptors [99,100]. M2 polarization is mostly associated with chronic infection and inflammation, such as granuloma, helminths, cancers, renal and liver fibrosis, asthma, dermatitis, and wound healing (reviewed in Sica 2012 [100]). M2 are also involved in matrix deposition, tissue remodelling, angiogenesis, immune regulation, and immune suppression which is of importance in chronic fibro-inflammation observed in chronic HF.

The importance of monocyte-platelet interactions and complexes in Heart Failure

As described above, platelets and monocytes have been separately implicated in HF pathogenesis and pathophysiology [37,39,40,58].

It is possible that a crucial, but insufficiently explored pathophysiological aspect of HF is the interaction between the endothelium, platelets, and monocytes in the setting of chronic, low grade inflammation arising from myocardial damage. A dysregulated, augmented cross talk between monocytes and platelets may be a critical factor influencing both the development and the progression of HF.

The ability of activated platelets to interact with leukocytes, particularly monocytes, and form complexes in the peripheral circulation has been described long ago [101]. MPC formation is increased in patients with autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematosus, and antiphospholipid syndrome [102]. MPCs are also increased and can be detected in the peripheral blood of patients with acute thrombotic disorders including acute

MI [91,103,104], stroke [105-107], ACS [90,108], stable CAD [109], atherosclerosis [110,111], as well as in patients with atherothrombotic risk factors such as hypertension [112] and type I diabetes [113]. The significance of MPCs in cardiovascular disease is further supported by the increased levels of MPCs found in the blood of patients following cardiovascular intervention (cardiopulmonary bypass) and by the positive correlation of MPCs with cardiovascular disease severity and prognosis [92,114,115].

Since these conditions are both risk and etiological factors for HF, the importance of MPCs in HF development has also been recognized [46,116]. Research in this area, however, is scarce and is challenged by the diverging etiologies and pathophysiology of the two types of HF, HFREF and HFPEF. While most research, including monocyte and platelet research, has traditionally been orientated to resolving interactions and disease mechanisms in the setting of ischemic heart disease and HFREF, accumulating new knowledge in HFPEF has highlighted the contribution of circulating factors, including leukocytes and platelets, to disease development and progression [5]. Indeed evidence of monocyte and platelet activation separately has been shown in pre-HF and minimally-symptomatic phases of HFPEF [117]. However, to date, mutual monocyte-platelet interaction/activation has not been investigated in HFPEF.

While myocardial damage in HFREF was shown to be driven by oxidative stress to the myocardium originating from within the cardiomyocyte, in HFPEF the myriad of existing comorbidities and systemic and vascular inflammation (i.e. leukocyte and endothelial inflammation, platelet activation) are known to orchestrate cardiac remodeling and dysfunction [5,6]. In this regard, the contribution of monocyte-platelet interactions and MPCs may also be important in the pathophysiology of HFPEF.

Despite this, the few existing studies that have looked at MPCs in HF have examined only patients with HFREF. Increased MPC formation has recently been reported in ischemic HF in

patients with acute as well as chronic stable HFREF (LVEF < 40%) [92]. This was associated with increased MPC formation preferentially with pro-inflammatory monocyte subsets ($CD14^{++}CD16^{-}$ and $CD14^{++}CD16^{+}$) in these HFREF patients compared with patients with stable CAD but no HF [92]. Generally, the extent of MPC formation reflects the level of platelet activation and hyperactivity and is an index of blood thrombogenicity [103]. More recently, platelet-monocyte interactions have emerged as an important pathophysiological link between thrombosis and inflammation, mainly due to platelet-induced inflammatory cytokine and prostanoid production from monocytes as well as increased monocyte endothelial adhesiveness [64,95-97,118,119]. Those features highlight a likely regulatory role of monocyte-platelet interactions and MPCs not only in acute cardiac ischemia, vascular disease and thrombosis but also in chronic non-ischemic HF [93]. Indeed, abnormal platelet activity in chronic stable non-ischemic HFREF has been reported in one study, but these abnormalities were not predictive of outcome, notwithstanding the relatively small sample size [47]. Furthermore, the authors concluded that platelet activation may simply be related to the comorbidities. Whether related to comorbidities or HF, it remains plausible that MPC formation contributes to the progression of fibro-inflammation and worsening of outcomes in HF, not only in HFREF, but also in HFPEF, which requires further evaluation.

Areas for future research include the circulation time, clearance, and exact role of these complexes *in vivo*, in the setting of HF. Mechanistically, even though much is known about the triggers of MPC formation in the blood, the lack of data on MPCs *in vivo* hinders progress in defining the significance of MPCs in HF pathophysiology. The magnitude of MPC formation is primarily dependent on platelet activation and to some extent also on monocyte activation [95,120]. The main protein interaction controlling platelet-monocyte binding at the vascular wall and in the circulation (MPC formation) is the one between P-selectin on activated platelets and PSGL1 on monocytes [95,121] (Figure 1). The crucial role of this

interaction for MPC formation was verified by the use of P-selectin blocking antibodies which abrogated platelet adhesion to monocytes, whereas blocking other ligands had only minor effects [95,122]. As described above, binding of monocytes to activated platelets to form MPCs induces expression of activating, pro-inflammatory cytokines and mediators from monocytes including IL1β, IL8, MCP1, and intracellular NFκB inflammatory signalling (Figure 1); and anti-P-selectin antibodies reduced cytokine production [118]. In addition, an increase in high-sensitivity CRP, enhancement of pro-inflammatory monocytes subsets ($CD14^{++}CD16^{+}$), and increased monocyte adhesion to endothelial cells was reported as a result of increased platelet activation and MPC formation [119]. Those effects were reduced by the COX2 selective inhibitor NS-398, aspirin, and the -selective antagonist of prostaglandin E receptors 1 and 2, AH6809 [119]. Monocytes within MPCs show increased stable adhesiveness to activated endothelium due to increased expression and activity of β1 and β2 integrins and decreased expression of L-selectin which is involved in early monocyte rolling along the endothelium [96]. These result in increased monocyte adhesion to ICAM1, VCAM1, and fibronectin and facilitate monocyte transendothelial migration.

The circulation time and clearance of MPCs are not well defined and differ between humans and animals. In apolipoprotein-E-deficient mice, MPC formation was caused by injection of activated platelets. This was accompanied by increased CCL5, PF4, and increased VCAM1-mediated monocyte binding to atherosclerotic endothelium. MPCs were found to be relatively short-lived (3-4 h) and cleared upon monocyte transmigration [121]. Similarly, in primates MPC lifespan upon injection of thrombin-activated platelets was approximately 30 min while in patients with percutaneous coronary intervention, MPCs were detectable for up to 24 h [103]. Similarly, acute MI patients registered higher levels of MPC formation with no increase in circulating P-selectin–expressing platelets. Of note, the lifespan of MPCs did not relate to P-selectin shedding from platelet surface, which occurs several hours after MPC formation but

may be related to increased adhesive capacity of these complexes [103]. A paper by van Gils *et al.* [123] has shed some light on the regulation of MPCs during transendothelial migration. The authors demonstrated *in vivo* that platelets localize to PSGL1 regions at the uropod of monocytes upon migration and detach from migrating monocytes and remain at the endothelial surface. MPC dissociation was associated with monocyte PSGL1 redistribution and mechanical stress, but not with reduced PSGL1 expression, reduced platelet-binding capacity of monocytes, or the type of endothelial matrix protein.

Finally, the circulation time and clearance of MPCs might also depend on the extent of platelet phagocytosis mediated by activated monocytes, but this issue requires further study in the setting of HF.

Future perspectives on evaluation of drug therapy directed at monocyte-platelet interactions in HF

Taken together, the available evidence shows heart failure is a hypercoagulable state independently of the presence of sinus rhythm and might support the hypothesis that monocyte-platelet-endothelial interactions and MPCs have an important role in HFREF as well as HFPEF pathogenesis and progression. Accepting this hypothesis would further point to putative clinical benefit of therapies directed at low-grade, chronic inflammation as well as platelet activation in the setting of HF. However, there are few conclusive clinical studies to support this hypothesis and, indeed, data from several large clinical trials have shown conflicting and even adverse outcomes with anti-thrombotic and anti-inflammatory therapy in HF (Table 1).

In reconciling these observations several factors must be considered. Firstly, many of the studies to date have not been appropriately powered, prospective, randomized studies

designed to address the hypothesis. Of the prospective, randomized studies, the HELAS [124] and WASH [50] studies of anti-platelet/anti-coagulant strategies in HF were underpowered, as were anti-inflammatory studies of thalidomide, IV-IG therapy and IL-1 receptor antagonist anakinra in HF populations [125-127]. In the larger WATCH study [51], which was terminated prematurely arising from recruitment difficulties, achieving 1587 of a planned 4500 participants, there were no differences in the primary endpoint of death, non-fatal MI and non-fatal stroke between aspirin, warfarin and clopidogrel. However, this study raised a concern about excess hospitalizations for HF associated with aspirin versus warfarin and was in direct contrast to the subsequent WARCEF study [36], which was adequately powered, also showed no difference in primary endpoint between aspirin and warfarin, yet showed a trend to increased hospitalizations for HF in the warfarin versus aspirin treated patients.

Secondly, almost all of the reported HF studies were carried out in HFREF patients, frequently with advanced disease, whereas there is some evidence from post-hoc analyses of the ACCLAIM study that anti-inflammatory therapies are likely to be most effective and beneficial in early stage HF [23]. Furthermore, the possible benefit of anti-thrombotic therapy in HFPEF has yet to be formally tested in prospective, controlled studies. A small number of retrospective or observational studies have suggested that platelet activation is a feature of HFPEF and may be modifiable (Table 1) [34,127,128]. There is only one small study of anti-inflammatory therapy with IL-1 receptor antagonist anakinra in HFPEF [127] and one small prospective study of the same anti-inflammatory therapy in HFPEF currently recruiting (clinicaltrials.gov identifier NCT02173548).

Thirdly, inappropriate dosing which can cause off-target or adverse effects and risks for the patient that eventually outweigh any clinical benefits may be an important reason for failure of anti-thrombotic/anti-inflammatory therapy in HF to date. For example, aspirin has proven anti-platelet effects at low doses (< 80 mg) commonly used in Europe and dose-related

adverse effects at higher doses. Of particular concern in HF, modulation of vasodilating prostaglandins can occur at higher aspirin doses and it has been shown that there are dose-dependent adverse renal effects of aspirin at doses > 80 mg daily [129]. Despite this, in all of the prospective, randomized studies of aspirin in HF to date, higher daily doses were used. Similarly, it was shown in the ATTACH study that there is a significant increase in death and HF hospitalization with higher dose and longer treatment of the TNFα antagonist infliximab [22]. Given the chronic, low grade nature of inflammation in HF, doses and duration of anti-inflammatory therapy should be considered in the study design.

A forth consideration is that many of the trials include patients who may fall into the category of "indication for anti-thrombotic or anti-inflammatory therapy" independently of HF (including patients with ischemic heart disease, peripheral vascular disease, MI, atherosclerosis, stroke, atrial fibrillation), which makes evaluation of the benefits of anti-thrombotic/anti-inflammatory drugs for HF very difficult. A related concern is the highly prevalent use of medications such as statins and aspirin among at-risk populations that persists long after the development of HF. However, as HF is a syndrome arising from other cardiovascular abnormalities, and involves multi-system pathology, the distinction between comorbidity and etiological factors is blurred and it may be unrealistic or even unwise to exclude patients with other conditions responsive to anti-thrombotic/anti-inflammatory therapy.

Finally, more work is needed to expand our understanding of platelet–targeting agents beyond simple anti-coagulation/thrombotic agents, but also as means for regulation / modulation of other platelet functions, as well as leukocyte (monocyte) and endothelial function. The emerging importance of the platelet and endothelium in modulating tumor cell intravasation and extravasation [130] may have parallels with monocyte/macrophage intravasation in the myocardium as a key step in the pathogenesis of myocardial dysfunction

in HF. Furthermore, it may be rational to use agents that interfere not with a single type of cell or event but with intercellular communication and actions. Therefore, there may be a role for modulating myocardial fibrosis using pharmacological agents that target monocyte as well as platelet function, the interaction of these cells in the circulation, MPC formation and the intravasation of monocyte-derived macrophages via inflamed vascular endothelium into the failing heart.

This concept has been applied to atherosclerosis, where binding of platelet P-selectin to monocyte PSGL1 has been shown to promote activation of the interacting cells, release of pro-inflammatory mediators, endothelial adhesiveness and activation, monocyte transmigration into adjacent tissues, and thrombogenicity while its blockage had beneficial cardiovascular effects in the setting of atherosclerosis [110,120-123]. While it is long recognized that the severity of interstitial fibrosis closely correlates with the extent of LV hypertrophy and impairment of ejection fraction [131,132], there is now a recognition of the potential importance of perivascular fibrosis in non-ischemic HF [133]. In addition, suppressed NO production and responsiveness, increased P-selectin and circulating MPCs in hypertension, the main etiological factor associated with HFPEF [112], and increased serum soluble P-selectin in patients with diastolic dysfunction (independent of diabetes or CAD) [134] indicate a possible important contribution of the P-selectin – PSGL1 pathway in driving chronic HF, particularly HFPEF. Soluble, platelet-bound, and total P-selectin are also significantly increased in congestive HFREF (LVEF < 50%) [47]. While the prognostic significance of this has yet to be determined it is interesting to note that a recent 10-year long-term follow-up study showed that soluble P-selectin has prognostic value in predicting cardiac events including cardiac death, non-fatal MI, and ACS with hospitalization in patients with preserved LVEF > 50% [135]. However, no study to date has evaluated the relationship between P-selectin levels and outcome in HF, nor explored the potential benefits of direct P-selectin – PSGL1 inhibition in

therapy of chronic HF in patients. Interesting evidence from a transgenic mouse model of chronic HF with cardiac-specific overexpression of TNFα clearly showed that targeted disruption of P-selectin gene alongside ICAM-1 expressed by immune-inflammatory and endothelial cells improves cardiac function and survival [136]. This may point to the benefit of modifying both platelet and monocyte activation in patients as outlined by Moertl *et al.* who showed that treatment with high dose (4 g/day) omega-3 polyunsaturated fatty acids reduced P-selectin, TF, and inflammatory cytokine release (IL6, TNFα) in patients with advanced non-ischemic, chronic HFREF [137]. From a non-pharmacological perspective, exercise training (20 weeks) also significantly decreased soluble P-selectin and CD40 levels reflecting monocyte and platelet activation in patients with mild to moderate chronic HF [138].

Other pharmacological agents aimed at inhibiting platelet or monocyte function, or both, with a potential to regulate monocyte-platelet interaction and MPC formation include anti-thrombin agents, nitrates, PAR1 inhibitors, ADP antagonists, and TXA2 antagonists. The clinical benefits of these drugs in the context of wider, largely acute cardiovascular disease including peripheral artery disease, atherosclerosis, ACS, MI, and ischemia have been extensively. However, in the setting of HF, the evidence is scant. For example, a combination of aspirin (325 mg/day) and ADP $P2Y_{12}$ blocker clopidogrel (75 mg/d) in advanced congestive HFREF (PLUTO-CHF trial) (LVEF < 40%, NYHA ≥ 2) resulted in significant inhibition of platelet activation (collagen-induced aggregation in plasma and whole blood) and expression of adhesion molecules (PECAM1, GPIb, GP IIb/IIIa antigen, GP IIb/IIIa, CD151) including P-selectin when compared with patients taking only aspirin [139]. The combined therapy also reduced formation of platelet-leukocyte microparticles, an index of increased MPC formation [139]. These effects were sustained in the broad spectrum of patients with HF independent of its etiology, severity (NYHA), or myocardial contractility [140]. Similar effects on platelet function and platelet-leukocyte microparticles were also achieved by a

combination of aspirin (325 mg/d) and selective serotonin reuptake inhibitors in congestive HFREF (LVEF < 40%, NYHA ≥ 2) [141]. Similarly, treatment of stable, severe HFREF patients (NYHA III/IV) with the oral direct Factor Xa inhibitor rivaroxaban in a small study successfully reduced platelet activation and hypercoagulability, thus minimizing risk and improving clinical prognosis [142]. Finally, in a study of 25 chronic HFREF patients awaiting transplantation, 11 received oral anti-thrombotic agents (target INR 2-3) associated with reduced fibrinolysis, inflammation, and endothelial dysfunction [143]. These data once again suggest that important links exist between platelet, monocyte and endothelial cell function in HF and that it may be possible to modulate not only platelet function, but platelet-monocyte interactions using available pharmacological therapy. Furthermore, from the perspective of HF management, not only is evidence scant, but also almost exclusively in HFREF, rather than HFPEF patients. Although retrospective, observational data provide evidence of an association between antiplatelet therapy using COX1 inhibition with low dose aspirin (75 mg/d) and improved HF outcomes in an unselected, mixed HFREF and HFPEF population (average LVEF: 40 ± 15%) [34] more prospective, randomized data are needed to explore the mechanisms and optimal pharmacological and non-pharmacological management of the adverse consequences of platelet-monocyte interactions in HF, particularly with preserved ejection fraction.

Conclusion

There are several emerging paradigms in the understanding of the pathophysiology of HF which implicate cardio-metabolic risk factors, inflammation, endothelial dysfunction, myocardial fibrosis, and myocyte dysfunction. The monocyte – platelet interaction has emerged in limited studies to date as a potentially important pathophysiological link between inflammation, thrombosis, endothelial activation, and myocardial dysfunction. This

interaction may play a crucial role in promoting cardiac dysfunction by modulating thrombogenicity, inflammation, endothelial dysfunction, and oxidative stress and facilitating monocyte to macrophage infiltration in the myocardium promoting fibrosis and dysfunction. This may also be of particular importance in HFPEF, which has been under-investigated to date and is now acknowledged as a syndrome with a strong inflammatory component in pre- and minimally symptomatic phases, promoting a reactive cardiac fibrosis and dysfunction [5-7,9]. It is entirely plausible to draw the conclusion that inflammation is a correlate and not causative in HF from the clinical work to date in HFREF [21] and that therapies targeting the platelet are of little value in HF without established underlying indications. However, there may be lessons to learn in the design of future studies from the evidence base to date. Furthermore, the expanding knowledge in our understanding of immune modulation as well as molecular profiling to identify target patient subsets in a more personalized strategy offer hope. Finally, more studies in chronic HF, particularly HFPEF, are needed to properly assess the value of including therapeutic agents which target not only platelets and platelet activation, as current therapies do, but also monocytes [117], and more specifically platelet-monocyte interactions.

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Tables

Table 1 Clinical trials in Heart Failure with Preserved and Reduced Ejection Fraction utilizing anti-platelet and anti-inflammatory therapies

Ref.	Study	Type of HF	Patients	Therapy	Study results
Anti-platelet therapy					
[124]	HELAS; multicenter, randomized, double-blind, placebo-controlled trial; mean follow-up – 19.5 +/- 1.6 months (group-dependent)	HFREF	197 HFREF patients with IHD or DCM	IHD patients – aspirin (325 mg/d) or warfarin (2.5-10 mg/d, INR 2-3). DCM patients – warfarin (2.5-10 mg/d, INR 2-3) or placebo	No significant difference among the groups in the incidence of embolic events
[139]	PLUTO-CHF; prospective, randomized trial; mean	HFREF	88 outpatients with congesti	Aspirin (325 mg/d) and clopidogrel (75 mg/d) versus aspirin	1) Combination therapy with aspirin and clopidogrel inhibits platelet activation and expression of adhesion molecules including

	follow-up – 1 month		ve HFREF	(325 mg/d) alone	P-selectin when compared with aspirin alone therapy
[50]	WASH; open-label, randomized, controlled trial; mean follow-up – 27+/- 1 months	HFREF	279 patients with HFREF in sinus rhythm	Aspirin (300 mg/d) vs. warfarin (INR 2.5) vs. no therapy	1) No difference in primary clinical outcome (death, nonfatal MI, or nonfatal stroke) between aspirin, warfarin or non-treated group 2) Increased risk of all cause (re)hospitalization (secondary endpoint) in aspirin group
[51]	WATCH; multinational, prospective, randomized trial; mean follow-up – 1.9 yrs.	HFREF	1587 patients with HFREF in sinus rhythm	Double-blind aspirin (162 mg/d) or clopidogrel (75 mg/d) vs. open-label warfarin (INR 2.5-3.0)	1) All 3 drugs have equal beneficial effects with respect to primary end point (reduced all-cause mortality, nonfatal MI, and nonfatal stroke) 2) Warfarin is superior to aspirin and clopidogrel in reducing secondary endpoints (non-fatal stroke and (re)hospitalizations due to worsening HF), but associates with increased risk of minor bleeding
[36]	WARCEF; double-blind,	HFREF	2305 patients with	Aspirin (325 mg/d) vs. warfarin	1) Similar beneficial effect with either drug on primary outcome

	multicenter trial; mean follow-up – 3.5±1.8 yrs.		HFREF in sinus rhythm	(INR 2.0-3.5)	(ischemic stroke, intracerebral hemorrhage, or all-cause death) 2) No difference in primary outcome between treatment with warfarin or aspirin 3) reduced risk of ischemic stroke with warfarin, offset by an increased risk of major hemorrhage 4) a trend toward an increased rate of hospitalization for heart failure in the warfarin group, in direct contrast to the results of the WASH and WATCH trials
[34]	Observational retrospective community-based study; median follow-up – 2.6 (0.8-4.5) yrs.	HFREF and HFPEF	1476 patients with HF comorbidities attending a HF disease management program	low-dose aspirin (75 mg/d) vs. non-aspirin and high-dose aspirin (>75 mg/d)	1) low-dose aspirin associates with reduced mortality risk (primary endpoint) compared with non-aspirin use 2) low-dose aspirin associates with reduced risk of HF hospitalization (secondary endpoint) compared with non-aspirin use in the total population 3) no difference in mortality or HF hospitalization between high-

					dose aspirin users (>75 mg/d) and non-aspirin users
Anti- inflammatory therapy					
128	Health ABC; observational community-based study; median follow-up – 9.4 yrs.	HFPEF and HFREF	2610 (older) patients with HF comorbidities attending a HF disease management program	Standard anti-hypertensive, antithrombotic, and anti-inflammatory HF therapy	1) Strong association of inflammatory markers (IL6, TNFα, CRP) with HF – particularly HFPEF – risk 2) Monitoring of and intervention with inflammatory markers may improve risk stratification and reduce mortality in HFPEF
144	RENEWAL (including RENAISSANCE and RECOVER); double-blind, randomized, placebo-controlled	HFREF	1123 patients (RENAISSANCE) and 925 patients (RECOVER) with moderate	Etanercept (25 mg/2x week) vs. etanercept (25 mg/3x week) vs. placebo (RENAISSANCE)	1) The TNFα inhibitor etanercept had no effect on clinical status at 24 weeks (primary endpoint) in RENAISSANCE or RECOVER 2) Etarnecept had no effect on the death or chronic HF hospitalization end point in RENEWAL

	multicenter trial; mean follow-up – 24 weeks		to severe HFREF	Etanercept (25 mg/ week) vs. etanercept (25 mg/2x week) vs. placebo (RECOVER)	
[22]	ATTACH; randomized, double-blind, placebo-controlled trial; mean follow-up – 28 weeks	HFREF	150 patients with moderate to severe HFREF	Infliximab (5 mg/kg) vs. infliximab (10 mg/kg) vs. placebo at 0, 2, and 6 weeks after randomizatio n	1) Neither dose of the TNFα inhibitor infliximab improved clinical status at 14 weeks (primary endpoint) despite suppression of inflammatory markers and a modest increase in ejection fraction 2) Significant increase in death and HF hospitalization at 28 weeks in the patients who received 10 mg/kg infliximab
[23]	ACCLAIM; double-blind, placebo-controlled randomized	HFREF	2426 patients with HFREF and HF hospitali	non-specific immunomod ulation therapy (IMT)	1) IMT was associated with a significant reduction in the risk of primary endpoint events (composite of time to death from any cause or first hospitalization for cardiovascular reason)

	trial; mean follow-up – 10.2 months		zation or iv drug therapy in an outpatient setting within the past 12 months	vs. placebo by intragluteal injection on days 1, 2, 14, and every 28 days thereafter	2) Such benefits were seen also in patients without a history of MI (irrespective of NYHA) and patients within NYHAII.
[125]	Double-blind, placebo-controlled randomized trial; mean follow-up – 12 weeks	HFREF	56 patients with HFREF secondary to IDCM or CAD	Thalidomide (25 mg QD increasing to 200 mg QD) vs. placebo for 12 weeks	1) The TNFα antagonist thalidomide significantly improved cardiac function (LVEF, in LV end-diastolic volume, heart rate) and improved matrix-stabilization by decreasing matrix metalloproteinase-2 (with no change in its inhibitor). These effects on LVEF were more marked in IDCM than in CAD 2) Thalidomide had both pro- and anti-inflammatory effects (lower total neutrophil count, higher TNFα

[126]	Double-blind, placebo-controlled randomized trial; mean follow-up – 26 weeks	HFREF	40 patients with HFREF stratified according to cause (ICM and IDCM)	Intravenous immunoglobulin (IVIG) vs. placebo for 26 weeks	1) IVIG increased anti-inflammatory mediators (IL10, IL1 receptor antagonist, soluble TNF receptors) and decreased N-terminal pro-atrial natriuretic peptide favoring a net anti-inflammatory effect in HFREF 2) IVIG significantly improved LVEF, independent of the cause of HF
[127]	D-HART; double-blind, randomized, placebo-controlled, crossover trial; mean follow-up – 28 days	HFPEF	12 patients with HFPEF (LVEF ≥ 50%) and evidence of systemic inflammation	Anakinra (100 mg) or placebo) for 14 days and an additional 14 days of the alternate treatment (placebo or anakinra)	1) IL1 receptor blockade with anakinra significantly improved peak oxygen consumption (aerobic exercise capacity) and reduced plasma CRP (systemic inflammation) from baseline to the post-treatment follow-up point (primary endpoint). 2) CRP reduction correlated with the improvement in peak oxygen consumption (secondary endpoint).

INR, international normalized ratio; iv, intravenous; IDCM, idiopathic dilated cardiomyopathy; DCM, dilated cardiomyopathy; CAD, coronary artery disease; ICM, ischemic cardiomyopathy; IHD, ischemic heart disease

Figure legends

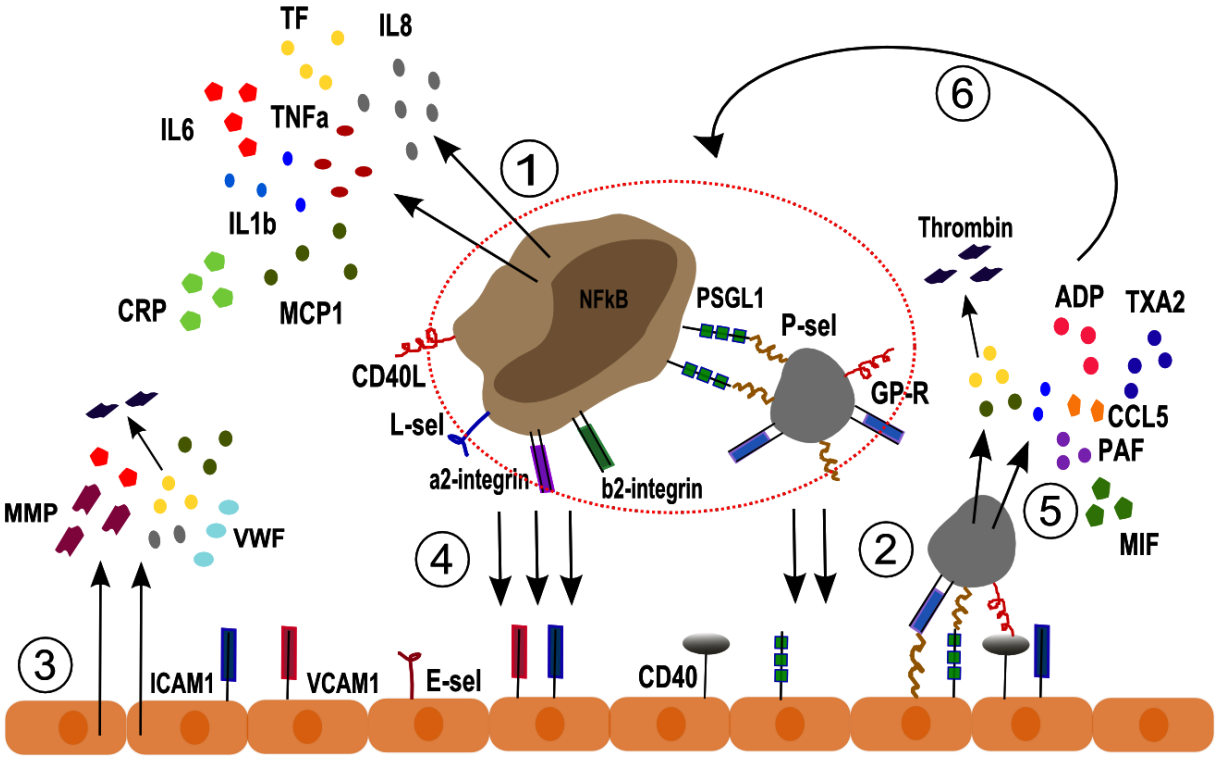

Figure 1 The monocyte – platelet – endothelial cell communication network. (1) Pathophysiological signals promote monocyte and platelet activation in the blood. Cell activation results in release of inflammatory mediators (IL1β, TNFα, IL6, IL8, MCP1, CRP, TF) and upregulation of adhesion molecules (P-sel, L-sel, CD40L, α/β-integrins, GP-R) in both monocytes and platelets, however with major contribution of monocytes. These aid monocyte – platelet interactions and formation of complexes (mainly in a P-selectin – PSGL1). Subsequent events include (2) platelet adhesion to the endothelium, (3) expression of adhesion (PSGL1, CD40, E-sel, ICAM1, VCAM1) and inflammatory (MMP, MCP1, VWF, IL6, IL8, TF, thrombin) mediators from activated endothelium, and (4) recruitment, adhesion and transmigration of monocytes across the endothelium. The precise order of the latter three events is unclear as they may happen simultaneously and each one may precede

another of happen as a result of it. In either case, (5) endothelium-adherent platelets secrete an array of inflammatory chemokines and mediators (ADP, TXA2, PAF, TF, thrombin, MCP1, IL1β, MIF, CCL5) aimed at recruitment and adhesion of more platelets (in physiological and pathological thrombus formation) and recruitment and activation of monocytes, which (6) back-loops to further boost monocyte – platelet interactions.