Monocytes play an important role in immune defence, inflammation, and tissue remodelling. Nevertheless, the role of monocytes in cardiovascular disease is obscure. Indeed, monocytes infiltrate dysfunctional tissue and augment tissue damage and are actively involved in tissue regeneration and healing. In support of the latter, recent studies have provided data on the functional and structural plasticity of monocytes. Monocytes are also actively involved in processes associated with tissue regeneration such as angiogenesis and vasculogenesis, either by producing pro-angiogenic factors or even by evolving to structural components of the vascular wall. This review article provides an overview on whether monocytes represent deteriorating immune overreaction in heart failure (HF), or a desperate attempt for tissue repair or physiological compensation in the failing heart. Perhaps, it is time to reconsider our attitude towards monocytes and consider more ‘monocyte activation’ rather than ‘monocyte suppression’ as a potential therapeutic target in HF.

Keywords
Monocytes • Heart failure • Angiogenesis • Inflammation • Endothelial progenitor cells

1. Introduction
Heart failure (HF) is no longer regarded as an isolated cardiac entity, but a systemic disorder involving several, initially adaptive and later detrimental, neurohumoral and inflammatory compensatory mechanisms. Our knowledge of the pathophysiology and treatment of HF has rapidly increased in the last decades. Despite effective medical interventions that have targeted neurohumoral activation, mortality and morbidity rates remain considerably high. Attempts at neurohumoral blockade have reached the limits of effectiveness and new therapeutic targets have to be found in relation to the complex pathophysiological pathways of HF.

However, HF is a functional consequence of a wide variety of diseases. Several diverse and potentially modifiable mechanisms contribute to the establishment and progression of HF including structural and functional abnormalities of the heart, vascular injury, endothelial dysfunction, neurohormonal factors, and oxidative stress. Inflammation as an important pathway of endothelial dysfunction or as part of ischaemia-induced tissue damage has been also implicated in the progression of HF. Inflammatory activation may be different in HF occurring in the early stages after myocardial infarction (MI) compared with chronic ischaemic HF, or even HF occurring in the course of viral myocarditis.

Monocytes play an important role in immune defence, inflammation, and tissue remodelling and they do so by phagocytosis, antigen processing and presentation, and by cytokine production. Monocytes consist of a heterogeneous multifunctional cellular population. The plasticity of monocytes is a well-documented phenomenon, perhaps first observed by Metchnikoff, who described an evolution of the infiltrating cells in inflammatory exudates. It has also long been recognized that monocytes isolated from different anatomical sites display a diversity of phenotypes and capabilities. Given that monocyte function is dependent in part on signals received from the immediate microenvironment, it is suggested that MC heterogeneity may arise from unique conditions within specific tissues.

In the present review, we shall discuss the potential role of monocytes and their progenitors among bone marrow-derived mononuclear cells (MNCs), in the pathways involved in HF. Emphasis will be directed at pathways related to myocardial and vascular injury as a consequence of ischaemia. In this respect we attempt to answer the question: Are monocytes involved in
regeneration of damaged cardiac and vascular tissue or monocyte infiltration represents just an immune overreaction with merely deteriorating effects? To provide a substantiated answer to this question, two major monocyte-mediated pathways related to the pathophysiology of HF will initially be reviewed.

2. Monocytes: mediators of inflammation and angiogenesis

2.1 Inflammation and HF

It is widely accepted that inflammation is strongly implicated in almost every aspect of cardiovascular disorders including HF.8–11 Levels of circulating cytokines have been correlated with short- and long-term outcome and in several instances, with the response to treatment.8–11 Even in the milder and more initial stages of HF, altered cytokine activity—especially increased levels of interleukin (IL)-6 and tumour necrosis factor-α (TNF-α)—are present and have been evaluated as potential marker for risk stratification, early diagnosis and prognosis. TNF-α has been implicated in HF progression as a mediator of myocardial dysfunction and adverse remodelling.12 Indeed, animal studies have demonstrated increased cardiac expression of TNF-α in failing myocardium and the overexpression of myocardial TNF-α results in left ventricular (LV) dysfunction and dilatation.13,14

Case-control studies have also shown activation of inflammatory cytokines, particularly TNF-α, in the myocardium and peripheral monocytes in patients with HF and have suggested that increased circulating levels are associated with increased mortality.15,16 In fact, pro-inflammatory molecules are activated earlier in HF than are the classic neurohormones which tend to be activated in the latter stages of HF. For example, Seta et al.17 reported significantly higher TNF-α levels in patients in the early stages of HF (NYHA II) compared with normal subjects. Moreover, a linear association between TNF-α levels and functional HF classification has been shown.8,17 It has been hypothesized that a sustained increase in cytokines, including TNF-α and its receptor, results in a monocyte phenotype transition, as well as cardiomyocyte apoptosis, and activation of matrix metalloproteinases—eventually leading to cardiac hypertrophy and adverse LV remodelling.18,19 However, attempts to utilize TNF-α as a therapeutic target in patients with severe systolic dysfunction have resulted in an increase in all-cause mortality.20,21 Despite its many deleterious effects, TNF-α has also been demonstrated to exert some protective effects, namely against acute ischaemic injury.22,23 To date, the role that TNF-α plays in HF has not been fully elucidated and warrants further investigation.

The chronic release of reactive oxygen species (ROS) is linked to the development and progression of HF. Experimental and clinical HF trials have shown an increased production of ROS, such as superoxide, hydrogen peroxide and hydroxyl radicals. Other different pathways are also implicated in the increased ROS production in the failing heart, including NAD(P)H and xanthine oxidase, dysfunctional nitric oxide synthase and the mitochondrial electron transport chain.24 Abnormal activation of the phagocytic NAD(P)H oxidase in response to neurohormonal activation can contribute to cardiomyocyte apoptosis and matrix metalloproteinase activation, leading to remodelling of the failing LV.

Nonetheless, the major consequences of high ROS levels in HF may relate to endothelial dysfunction.25 Among HF patients with severe endothelial dysfunction and reduced nitric oxide availability, there is a close association between the degree of endothelial dysfunction and the risk of adverse clinical outcomes.26,27 Finally, ROS are a potential target of drugs with known pleiotropic anti-inflammatory and anti-oxidative effects; for example, several studies have demonstrated the important role of the antioxidant effects of statins on cardiac hypertrophy and on endothelial dysfunction in patients with HF.28,29

Monocytes represent both the main cellular source and one of the main cellular targets of pre-inflammatory cytokines (Table 1).12–17,18–23 However, their role in HF is not restricted to cytokine signalling. There is good evidence implicating monocytes in various cardiovascular disorders associated with HF.

Under certain stimuli, circulating monocytes differentiate into macrophages. Macrophages are mobile, phagocytic cells specialized in removing, by various forms of endocytosis, unwanted cellular and extracellular debris, invading microorganisms, and other foreign matter.28

Monocytes have been involved in atherosclerosis, the pathophysiological process underlying coronary artery disease and subsequently of ischaemic cardiomyopathy.2 Their initial protective inflammatory

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Major action in relation to vascular inflammation</th>
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<tr>
<td>TNF-α</td>
<td>Induces inflammation</td>
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<tr>
<td></td>
<td>Induces apoptotic cell death</td>
</tr>
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<td></td>
<td>Potent chemoattractant for neutrophils</td>
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<tr>
<td>IL-1α/β</td>
<td>Induces chemokine/cytokine expression</td>
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<tr>
<td></td>
<td>Promotes the expression of adhesion molecules on ECs</td>
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<tr>
<td>IL-6</td>
<td>Induces chemokine/cytokine production</td>
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<td></td>
<td>Major mediator of the acute phase response</td>
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<tr>
<td>IL-8</td>
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<td></td>
<td>SMCs proliferation and migration</td>
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<tr>
<td>IL-10</td>
<td>Anti-inflammatory cytokine</td>
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<tr>
<td></td>
<td>Down-regulates the expression of Th1 cytokines</td>
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<td></td>
<td>Inhibits NF-κB activity</td>
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<td>IL-12</td>
<td>Induces cell-mediated immunity</td>
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<tr>
<td></td>
<td>T cell stimulating factor</td>
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<tr>
<td>IL-18</td>
<td>Induces the production of TNF-α and INF-γ</td>
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<tr>
<td>MIP 1α/β</td>
<td>Activate and chemotaxis of human granulocytes</td>
</tr>
<tr>
<td>MCP</td>
<td>Induces the synthesis and release of pro-inflammatory cytokines</td>
</tr>
</tbody>
</table>

TNF, tumour necrosis factor-α; IL, interleukin; EC, endothelial cells; INF, interferon; MIP, monocyte inflammatory protein; MCP, monocyte chemoattractant protein; MC, monocytes; SMC, smooth muscle cells.
response starts to damage the arterial wall, and dysfunctional endothelial cells (ECs) and macrophages release cytokines, chemokines, and growth factors, which promote the migration of smooth muscle cells into the intima of the arterial wall forming the intermediate or fibro-fatty lesion.2,11 At this stage, the lesion can contain multiple layers of smooth muscle cells, connective tissue, macrophages, and T cells.2,11 Remodelling of the vessel wall occurs, resulting in advanced lesion formation.

Ischaemia-induced myocardial damage and subsequently myocardial remodelling and HF have also been associated with intense inflammatory activity and monocyte infiltration to the damaged tissue.28,29 In the early 1980s the mainstream theory indicated a rather catastrophic role of monocyte accumulation in the ischaemic myocardium, whereby hypoxia induces necrosis in the cardiac myocytes, which subsequently stimulates the complement cascade and initiates an inflammatory response.28,29 Once activated, monocytes/macrophages produce many cytokines, chemokines, and growth factors, including IL-1α and -β, IL-6, TNF-α, and macrophage inflammatory proteins 1α/β.28,29 The theory was further supported by studies in animal models showing that the infarct size was reduced if blood was depleted of white cells or if lipoxygenase metabolism of arachidonic acid was inhibited.30 More recent studies have suggested a deteriorating effect of monocyte infiltration in myocardial tissue. For example, Maekawa et al.31 reported a strong association between peripheral monocytosis, LV dysfunction, and LV aneurysm formation after MI. Consequently, inhibition of MC activation is a tempting therapeutic target in the prevention of ischaemia-related HF (Figure 1).

2.2 Angiogenesis and HF

Angiogenesis is a physiological process involving the growth of new blood vessels from the pre-existing ones.32 Recently, the term arteriogenesis has also been introduced to describe outgrowth of pre-existing arterioles into large conductance collateral arteries.32 Both angiogenesis and arteriogenesis have been involved in the pathophysiology of almost every aspect of cardiovascular disease including coronary artery disease and HF.32

Perhaps, the best characterized of the pro-angiogenic agents is vascular endothelial growth factor (VEGF), which is relatively unique among growth factors in terms of its specificity for the vascular endothelium.33 VEGF causes a massive signalling cascade in ECs. Indeed, VEGF is a major contributor to angiogenesis, increasing the number of capillaries in a given network. In vitro studies have demonstrated that bovine capillary ECs will proliferate and show signs of tube structures upon stimulation by VEGF.33 Moreover, altered VEGF levels have been demonstrated in patients with HF compared with healthy controls.34 and implicated in the prognosis of patients with congestive HF.35

More recently, a second family of growth factors specific for the vascular endothelium has been identified.36 The angiopoietins are protein growth factors acting through interaction with their receptors: Tie-1 and Tie-2.37 Similar to VEGF, the specificity of the Angs for the vascular endothelium results from the restricted distribution of their receptors to these cells. Tie1 and Tie2 are receptor tyrosine kinases just as are the receptors for VEGF.37 The actions of the angiopoietins seem however to be quite different from those of VEGF. In fact, the angiopoietins act in a complementary

Figure 1 The role of monocytes either as mediators of vascular tissue injury or as mediators of vascular tissue repair.
and coordinated fashion with VEGF, playing a later role in vascular development. Angiopoietin (Ang)-1 is also a chemotaxin and—in conjunction with VEGF—recruits ECs to initiate and accelerate angiogenesis. Ang-2, in the presence of VEGF, promotes a rapid increase in capillary diameter, remodelling of the basal lamina and new vessel growth. In contrast, if VEGF is inhibited, Ang-2 leads to EC death and vessel regression.44,45 Chong et al.20 demonstrated increased levels of VEGF, Ang-2, and Tie-2, but normal levels of Ang-1, in both stable and decompensated HF patients thus indicating a potential role for these angiogenic factors in the pathophysiology of HF.

Fibroblast growth factor (FGF) family, which include at least 22 known members, are also important mediators of angiogenesis.36,37 FGFs stimulate a variety of cellular functions by binding to cell surface FGF-receptors in the presence of heparin proteoglycans.38 Receptor activation gives rise to a signal transduction cascade that leads to gene activation and diverse biological responses, including cell differentiation, proliferation, and matrix dissolution, thus initiating a process of mitogenic activity critical for the growth of ECs, fibroblasts, and smooth muscle cells. There are currently no data available demonstrating increased plasma FGF levels in HF; however the potential importance of the FGF family in HF has been underscored in a number of interventional studies.40,41 At this point FGF proteins are the most promising therapeutic agent with respect to acute as well as sustained benefits in HF patients.40,41

Increased levels of angiogenic factors in HF are not direct proof of enhanced angiogenesis per se occurring in HF. There is evidence coming mostly from animal models suggesting that impaired angiogenesis leads to HF.42 For example, Hilfiker-Kleiner et al.42 reported that STAT3-deficiency in mice is associated with reduced myocardial capillary density leading to increased interstitial fibrosis, dilated cardiomyopathy and premature death. Moreover, delivery of angiogenic factors improves symptoms and slows progression of HF.43,44 Pearlman et al.43 demonstrated that VEGF improves collateral blood supply, promoting recovery of cardiac global and regional function after coronary artery occlusion in swine. Similarly Lazarous et al.44 have shown on the dog modal coronary artery occlusion that short-term treatment with basic FGF-enhanced collateral development without increasing neointimal accumulation at sites of vascular injury.

2.3 Monocytes and angiogenesis

Available data indicate a potential role of monocytes in vascular tissue repair. van Amerongen et al.45 have found that macrophage depletion markedly impaired wound healing and enhanced remodelling and mortality after myocardial injury, thus identifying the macrophages as key players in myocardial healing. More recently, Zandbergen et al.46 revealed that the lack of macrophages was associated with earlier development of myocardial dysfunction in hypertensive rats. In contrast, MG activation may be important in repair processes and debris clearance; hence, the depletion of monocytes/macrophages may not necessarily be an effective strategy in the prevention of HF.46 Indeed, these studies found a beneficial role of these cells in the prevention of ischaemia-related myocardial remodelling probably because of their involvement in angiogenesis.

Angiogenesis stimulated by tissue hypoxia is mediated by activation of hypoxia-inducible factor 1α gene expression and usually leads to the development of capillaries.47,48 Angiogenesis is tightly regulated and occurs within the context of a fine balance between conditions that promote (pro-angiogenic) and inhibit (anti-angiogenic) vessel formation.48 Angiogenesis correlates positively with the number of macrophages in various injury models, including MI and stroke.49,50 In fact, Manookitiwongsa et al.49 proposed a simplified hypothesis to explain the relationship between macrophage infiltration and angiogenesis in the brain following stroke. The authors speculated that new blood vessels are formed to promote macrophage infiltration in order for necrotic tissue to be removed. This theory is supported by the fact that tissue healing is associated with up-regulation of DNA synthesis in ECs at sites of macrophage accumulation. Moreover, macrophages isolated from sites of damaged tissue induce angiogenesis in vitro, and media from activated macrophage culture acts as strong stimulus of angiogenesis.50,51 In conclusion, activated macrophages have the capability to influence each phase of angiogenesis, mostly by producing pro-angiogenic factors (e.g. VEGF, FGF-2) or by responding to angiogenic factors for instance, by expressing Tie-2,52,53 However, the prospect of MCs to act as endothelial progenitor cells (EPCs) themselves has been also investigated.

2.4 Monocytes as EPCs

EPCs have been characterized as circulating myeloid-derived cells sharing stem cell surface markers (e.g. CD34) and endothelial marker (usually VEGF receptor 2 [KDR]).54 The surface marker CD133 has been detected in an immature subset of EPCs and was suggested as an additional marker of human angioblast-like EPCs distinguishing these from mature endothelial or monocytic cells. As a result, the combined phenotype of CD34+/CD133+/KDR+ is now often used alongside to CD34+/KDR+ definition of EPCs (Figure 2).54

Admittedly, the origin as well as the phenotypic and functional characterization of EPCs remains obscure. Several cell types obtained either from bone marrow, peripheral blood, or tissue-resident stem cells may differentiate into ECs and CD14+ myeloid monocyte cells are also a common source of EPCs, at least in vitro. Furthermore, EPCs may change their phenotype undergoing the process of maturation, initiated in bone marrow and continues into circulation (e.g. loss of stem cell marker, CD133, and increased expression of endothelial markers). More recently, Ingram et al. redefined EPCs, using their in vitro angiogenic potency as EC colony-forming units, with limited potential for proliferation, and endothelial colony-forming cells, with almost unlimited ability to proliferate in culture, and concluded that endothelial colony-forming cells are the ‘real EPCs’ being able to form perfused vessels in vivo.55 It becomes progressively apparent that EPCs represent a heterogeneous population in terms of origin and their angiogenic properties. This cell heterogeneity should be cautiously considered in future cell therapy studies and studies that aim at identification of pharmacological target molecules.

Despite their obscure origin, EPCs theoretically poses some functional characteristics that have attracted much attention over the last decade. These cells reflect a functional subpopulation
within the blood MNCs with a proven potential to differentiate into an endothelial phenotype in vitro. By extrapolating these findings in vivo, EPCs could incorporate into newly forming blood vessels, a process often referred to as vasculogenesis (as opposed to angiogenesis), which is the sprouting of new vessels from the existing ones. Also, EPCs can reach the areas of blood vessel injury, differentiate into mature ECs, and repair the damaged endothelium.54

Much attention has also been focused on the capacity of monocytes to act as potential EPCs.56,57 Circulating monocytes have the capacity to differentiate into a variety of phagocytes, including macrophages, dendritic cells, osteoclasts, microglia, Kupffer cells.7 Until recently, the differentiation potential of monocytes was believed to be restricted to cells possessing phagocytic or antigen-presenting properties. However, several lines of evidence showing that circulating CD14+ monocytes have the potential to differentiate into various non-phagocytes, including mesodermal and neuroectodermal lineages.57 Surprisingly, this phenomenon was probably first described almost 150 years ago. In 1867, Cohnheim and co-workers demonstrated that peripheral blood monocytes participated in tissue renewal in various organs.

The ability of circulating cells of haematopoietic origin to differentiate into vascular ECs in areas of vascular remodelling has been demonstrated by Crosby et al.,58 who observed that blood-derived cells represent approximately 10% of ECs in mice neovasculature formed in response to surgical sponge implantation or hind limb ischaemia. Almost concurrently Fernandez-Pujol et al.59 demonstrated that under appropriate in vitro conditions, CD14+ cells (which are typically CD34−) differentiate into EC-like cells exhibiting characteristics of both ECs and monocytes. In accordance, Moldovan et al.60 reported that when macrophages were induced to infiltrate the heart by overexpression of monocyte chemotactant protein (MCP-1), the invading macrophages formed erythrocyte-containing vascular-like channels reminiscent of the tumour cell-derived vascular channels.

However, the authors noted that cells of the monocytic channels lacked some EC antigens and may themselves be subsequently colonized by EC or angioblasts. Indeed, Harraz et al.61 reported that CD14+ monocytes (either CD34+ or CD34−) have the potential to be incorporated into the endothelium of blood vessels in mouse ischaemic limbs and to transdifferentiate into ECs; also, monocytes differentiated into macrophages, dendritic cells, or ECs depending on environmental cues. Schmeisser et al.62 reported that under angiogenic stimulation, including VEGF and basic FGF, monocytes develop an endothelial phenotype with expression of specific surface markers and even form cord- and tubular-like structures in vitro suggesting possible implication in vasculogenesis.

Finally, Kuwana et al.57 described a primitive cell population termed monocyte-derived multipotential cells (MOMC) and introduced the concept of the multipotential nature of circulating monocytes. This cell population contains progenitors that can differentiate into several distinct mesenchymal cell types, including bone, cartilage, fat, and skeletal and cardiac muscle cells, as well as neurons. MOMCs are generated in vitro by culturing circulating CD14+ monocytes on fibronectin in the presence of soluble factors derived from circulating CD14+ cells. MOMCs express several endothelial markers, including vascular endothelial cadherin
and VEGF type 1 receptor, and have the ability to take up acetyl-
ated low-density lipoproteins. More recently, the same authors
examined the endothelial differentiation potential of human
MOMCs, and their capacity to induce in vitro and in vivo vasculariza-
tion.63 They concluded that co-transplantation of the MOMCs
promoted the formation of blood vessels, and more than 40% of
the new formed vessel sections incorporated human ECs derived
from MOMCs.63 These findings indicate that human MOMCs can
proliferate and differentiate along the endothelial lineage in a
specific permissive environment and thus could represent an au-
tologous transplantable cell source for therapeutic vasculogenesis.

These data clearly demonstrate the intimate relationship between
monocytes and ECs suggesting that specific monocyte population
may be recruited for vasculogenesis and may represent an endo-
thelial precursor population. However, it remains to be determined
how EPCs are implicated in the pathophysiology of heart disease and
how can EPC activity be manipulated in beneficial ways.

Altered numbers of circulating EPCs are evident in patients with
HF and have been associated with HF functional class (Table 2).
Additionally, patients with HF also exhibit impaired function of
EPCs. This was exceptionally demonstrated both in vivo and in vitro by Heeschen et al.64 who showed that bone marrow-
derived MNCs isolated from patients with chronic ischaemic
cardiomyopathy had a significantly reduced migratory and colony-
forming activity in vitro and a reduced neovascularization capacity
in vivo despite similar content of haematopoietic stem cells.

3. Monocytes and monocyte
progenitors: markers or
therapeutic targets in HF

3.1 Bone marrow-derived cells and HF

The preservation of tissue and organ integrity is an ongoing
process throughout an individual’s life. This repair process is
mediated, in part, by reserved multipotential cells found within
most organs and/or in bone marrow (stem cells or progenitor
cells [PCs]).65 As tissue damage occurs, pro-inflammatory cyto-
kines are produced and serve as a stimulus to mobilization of
PCs.65 If sufficient and appropriate cell recruitment occurs, inflam-
mation extinguishes and tissue’s structural and functional integrity
is restored. However, with extended tissue damage, the demand
for PCs exceeds the capacity of the body to respond resulting in
a failure of intrinsic tissue repair. This failure of endogenous
repair is accompanied by a secondary repair process, resulting in
irreversible structural and functional tissue impairment often
described as scarring.56 Exogenous supply of appropriate PCs can
overcome this pre-existing failure of repair, reduce inflammation,
and restore tissue’s structural and functional integrity.65

Currently, several sources have been utilized for harvesting PCs
for the purpose of tissue repair including skeletal muscle, heart,
and fat but mostly bone marrow. Bone marrow-derived MNCs
include a large amount of monocytic cells. The proven functional
and structural plasticity of cells of monocytic origin make us specu-
late that they are probably the cellular population most actively
involved in tissue regeneration.

The observation that bone marrow elements contribute to
cardiac repair in the setting of ischaemia-induced myocardial
necrosis served as the rationale for adult bone marrow cell
therapy after MI. In vitro-derived data and studies in animal
models of HF (Table 3) have encouraged the conduct of clinical
trials investigating the therapeutic potential of bone marrow-
derived PCs in the setting of the human failing heart.57–63,66
Early studies applied intracoronary delivery of bone marrow
MNCs in post-MI patients based on data derived from animal
studies that demonstrate benefit in subjects with ischaemia-related
tissue damage. For example, Strauer et al.67 have shown that in-
tracoronary transplantation of autologous, bone marrow MNCs in
addition to standard therapy after MI significantly decreased
infarct area and improved local contractility compared with the
standard therapy group. The authors concluded that selective

<table>
<thead>
<tr>
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<th>Study design and definition of HF</th>
<th>Definition of EPCs</th>
<th>Outcome</th>
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<tbody>
<tr>
<td>Valgimigli et al.66</td>
<td>Case–control, observational Objective ventricular impairment NYHA I–IV</td>
<td>CD34+ CD133+</td>
<td>† EPCs in early stages of HF</td>
</tr>
<tr>
<td>Nonaka-Sarukawa et al.82</td>
<td>Case–control observational Objective ventricular impairment NYHA I–IV</td>
<td>VEGF receptor2+</td>
<td>† EPC in advanced HF</td>
</tr>
<tr>
<td>Michowitz et al.83</td>
<td>Prospective observational Primary endpoint: survival NYHA I–IV</td>
<td>‘Early’ EPC colonies (flk-1+, Tie-2+ CD31+)</td>
<td>The number of colonies was an independent predictor of mortality</td>
</tr>
<tr>
<td>Geft et al.84</td>
<td>Case–control observational NYHA I–IV</td>
<td>CD34+</td>
<td>Number of apoptotic EPCs positively correlated with NYHA class</td>
</tr>
<tr>
<td>Fritzenwanger et al.85</td>
<td>Case–control, observational NYHA I–IV</td>
<td>CD34+ CD133</td>
<td>Number of EPCs inversely correlated with NYHA class</td>
</tr>
</tbody>
</table>

NYHA, New York Heart Association; EPC, endothelial progenitor cells; VEGF, vascular endothelial growth factor; HF, heart failure; EC, endothelial cell.
intracoronary transplantation of autologous, bone marrow MNCs was safe, effective, and mostly related to myocardial regeneration and neovascularization.

Based on the encouraging results from non-randomized small scale trials, The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute MI (TOPCARE-AMI) investigators randomized 59 patients after acute MI to receive intracoronary infusion of bone marrow MNCs or ex vivo expanded circulating PCs. They delivered the cells into the infarct-related artery 4 days after MI and demonstrated significant improvement in LV ejection fraction, as well as significantly enhanced myocardial viability and regional wall contractility in the infarct area. No difference was observed between the two active cell treatment groups.

In the bone marrow transfer to enhance ST-elevation infarct regeneration (BOOST) trial, Wollert et al. randomized 60 patients after successful percutaneous coronary intervention for acute MI to receive either intracoronary bone marrow MNCs or standard therapy. The authors demonstrated a difference in LV systolic function improvement between the two groups, which was significant after 6 months but not after 18 months of follow-up. The data indicated that the application therapy although might not be able to provide long-term benefit on LV systolic function after acute MI, may accelerate the recovery of the myocardium. From the above studies and others that followed more recently (summarized in Table 4), it became clear that implantation of bone marrow MNCs is a safe procedure that may improve cardiac function by a substantial and clinically meaningful degree following MI.

Table 4 provides long-term benefit on LV systolic function after acute MI to receive either intracoronary bone marrow MNCs or standard therapy. The Transplantation of Progenitor Cells and Regeneration Enhancement in Acute MI (TOPCARE-AMI) investigators randomized 60 patients after successful percutaneous coronary intervention for acute MI to receive either intracoronary bone marrow MNCs or standard therapy. The authors demonstrated a significant improvement in overall LV systolic function. These data are certainly encouraging and a number of phase II studies in the field are currently being conducted.

Several technical and safety issues still need to be considered. For instance, it is likely that the observed effects after autologous transfusion of cells in patients with cardiovascular risk factors and cardiovascular disease are limited because of a significant impairment of cells. The latter theory is supported by the findings of Heeschen et al., who reported reduced number and impaired function of bone marrow MNCs from patients with chronic ischemic cardiomyopathy and concluded that this functional impairment may limit their therapeutic potential. On the other hand, allogeneic transfusion of PCs from healthy donors bears the problem of immunological incompatibilities. No data are available assessing efficiency and safety of allogeneic transfusion of PCs in the management of heart disease. Instead of isolating PCs from peripheral blood or bone marrow and re-transfuse them, we perhaps need studies evaluating methods for intrinsic stem cell mobilization. In our opinion, the use of selective PC stimulators might be a realistic future strategy.

More recently, monocyte chemoattractant protein (MCP)-1 has been implicated in the pathways of angiogenesis by promotion of accumulation of cells with angiogenic potential. Indeed, the delivery of a small number of inflammatory monocytes in sites of ischemia results in marked increase in the local production of MCP-1, which in turn, is associated with a secondary, more robust wave of monocyte recruitment. Studies of mice genetically deficient in MCP-1 or CCR2 (MCP-1 receptor) indicate that although not required for the early mobilization of monocytes, the secondary wave of monocyte recruitment and subsequent
stimulation of angiogenesis are dependent upon CCR2 signalling. Collectively, these data suggest a novel role for MCP-1 in the inflammatory and angiogenic response to ischaemia. 73

Granulocyte-macrophage colony-stimulating factor (GM-CSF), is a cytokine with pleiotropic functions. 74,75 The beneficial effect of GM-CSF in the treatment of ischaemia-induced tissue damage has been well documented. In fact, GM-CSF has been successfully employed in the treatment of chronic skin ulcers. 74 However, the biological effects underlying GM-CSF action in impaired wound healing have been only partly clarified. An appealing theory suggests that GM-CSF augments collateral flow, as shown in a short-term administration of the cytokine in occlusive peripheral artery disease, although responses in the GM-CSF group were quite variable. 75

### 3.2 Monocyte-related molecules as biomarkers in HF

The diagnosis and risk-stratification of patients with HF is dependent upon the availability of specific, accurate, and effective disease- or risk-markers. Thus, there is an increasing interest in the development of new cardiovascular biomarkers. Potential markers of HF include neurohormonal mediators, markers of myocyte injury, and indicators of systemic inflammation.

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**Table 4** Studies assessing the impact of bone marrow-derived mononuclear cell infusion on patients with HF

<table>
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<tr>
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<th>Patients/controls</th>
<th>Type of patients</th>
<th>Way of delivery</th>
<th>Positive outcome</th>
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<tbody>
<tr>
<td>Strauer et al. 67</td>
<td>10/0</td>
<td>Post-MI</td>
<td>IC</td>
<td>S, E</td>
</tr>
<tr>
<td>TOPCARE-AMI 68</td>
<td>59/0</td>
<td>Post MI</td>
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<td>S, E</td>
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<tr>
<td>Stamm et al. 94</td>
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<td>Post-MI</td>
<td>S</td>
<td>S, E</td>
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<tr>
<td>Tse et al. 95</td>
<td>8/0</td>
<td>Post-MI</td>
<td>EM</td>
<td>S, E</td>
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<tr>
<td>Perin et al. 70</td>
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<td>BOOST 69</td>
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<td>IACT study 97</td>
<td>18/18</td>
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<td>Janssens et al. 98</td>
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<td>Post-MI</td>
<td>IC</td>
<td>S, E</td>
</tr>
<tr>
<td>HEBE trial 100</td>
<td>ongoing</td>
<td>Post-MI</td>
<td>IC</td>
<td>ongoing</td>
</tr>
<tr>
<td>Nasseri et al. 101</td>
<td>10/0</td>
<td>Implantation of LVAD</td>
<td>EM</td>
<td>-</td>
</tr>
<tr>
<td>van Ramshorst et al. 102</td>
<td>14/10</td>
<td>Post-MI</td>
<td>EM</td>
<td>S, E</td>
</tr>
<tr>
<td>Beeres et al. 103</td>
<td>15/0</td>
<td>Post-MI</td>
<td>EM</td>
<td>S, E</td>
</tr>
<tr>
<td>TABMMI study 104</td>
<td>10/0</td>
<td>Post-MI</td>
<td>EM</td>
<td>S, E</td>
</tr>
<tr>
<td>Assmus et al. 105</td>
<td>121/0</td>
<td>Post-MI</td>
<td>IC</td>
<td>S, E</td>
</tr>
</tbody>
</table>

BMMNC, bone marrow mononuclear cell; Post-MI, post-myocardial infarction; IHD, ischaemic heart disease; IC, intracoronary delivery; S, surgical delivery; EM, endomyocardial delivery; S safety; E, efficiency; LVAD, left ventricular assist device.

**Table 5** Studies assessing the therapeutic potential of stimulator-induced mobilization of bone marrow cells

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental setting</th>
<th>Type of stimulator</th>
<th>Main effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takahashi et al. 106</td>
<td>Hind limb ischaemic rabbits</td>
<td>GM-CSF</td>
<td>Enhanced EPC mobilization in response to ischaemia; augmented neovascularization of ischaemic tissues</td>
</tr>
<tr>
<td>Natori et al. 107</td>
<td>Colon cancer cells inoculated into mice subcutaneous space</td>
<td>GM-CSF</td>
<td>Enhanced tumour neovascularization</td>
</tr>
<tr>
<td>Westenbrink et al. 108</td>
<td>Infarcted Fischer F344 rats</td>
<td>EPO</td>
<td>Neovascularization in post-MI heart failure through EPC recruitment and increased myocardial expression of VEGF</td>
</tr>
<tr>
<td>Westenbrink et al. 109</td>
<td>Infarcted Fischer F344 rats</td>
<td>EPO</td>
<td>Stimulation of normal EPC-mediated endothelial turnover; improvement of cardiac microvascularization and function only in the presence of ischaemia</td>
</tr>
<tr>
<td>Shmilovich et al. 110</td>
<td>HF patients NYHA II–IV</td>
<td>BNP</td>
<td>Enhanced vessel growth by increasing the number of endothelial progenitors and improvement of their functional properties</td>
</tr>
</tbody>
</table>

GM-CSF, granulocyte-macrophage colony-stimulating factor; EPC, endothelial progenitor cells; EPO erythropoietin; VEGF, vascular endothelial growth factor; HF, heart failure; NYHA, New York Heart Association; BNP, brain natriuretic peptide; MI, myocardial infarction.
Early in the 1990s, Levine et al. described elevated circulating TNF-α levels in patients with HF. Since then, numerous interleukins (including IL 1, 6, and 18) have been shown to be produced by nucleated cells of the failing heart. More recently, new molecules have been introduced as potential markers in HF. For example, Fas (also termed APO-1) is a member of the TNF-α receptor family that is expressed on a variety of cells, including myocytes. When Fas is activated by the Fas-ligand, it mediates apoptosis and plays an important role in the development and progression of HF. Another molecule, ST2 is a member of the IL-1 receptor family, and is a protein secreted by cultured myocytes subjected to mechanical strain. Also, the galectins are a family of soluble beta-galactoside-binding lectins that play regulatory roles in inflammation and immunity. Recently, a role for galectin-3 in the pathophysiology of HF has been suggested with its upregulation in hypertrophied hearts, stimulatory effects on macrophage migration, fibroblast proliferation, and the development of fibrosis. Thus, serum levels of TNF-α, IL-1, -6, and -18, soluble Fas, ST2, and galectin-3 have been reported elevated in HF patients and these might become useful markers for the risk-stratification of patients with HF and in screening to identify asymptomatic subjects at risk for HF.

4. Conclusion

There is convincing evidence that monocytes possess preserved plasticity, and are able to transdifferentiate into other non-phagocytic cell types under diverse microenvironments. Recent literature—mostly by in vitro derived data—supports the fact that monocytes include a population of potent PCs actively involved in postnatal vasculogenesis, although, it is not certain that monocytes can become fully functional ECs. There is also convincing evidence to support a significant role of monocytes in the pathways of angiogenesis—a significant step in the process of tissue repair—following ischemic myocardial injury. Post-ischemic injury increased monocyte activation is more likely an attempt for tissue repair rather than a deteriorating immune overreaction. Thus, induction rather than inhibition of monocyte activation is a potential therapeutic target (Figure 3).

However, the available data do not sufficiently establish a causative relation between altered monocyte-derived pathways and progression of HF. It is not clear whether this is a pathway leading to HF or a consequence of the systemic nature of HF. In the first case, mobilization of monocytes could be a potential therapeutic target while in the second case, monocytes number and function could be an excellent disease marker. Moreover, it must be elucidated whether MCs can differentiate to a fully functional EC phenotype or their contribution in angiogenesis and/or vasculogenesis is restricted to the production of vascular growth factors. We also need to identify the stimulus that will lead to monocytes’ differentiation to PCs, and such stimuli have to be cell-specific and organ-specific in order to limit their action in the desired area. Brain natriuretic peptides, GM-CSF and erythropoietin that have been used up to now as PC activators are neither cell- nor organ-specific.

The greater gap in research is the lack of in vivo evidence supporting that monocytes/macrophages infiltrating the dysfunctional vascular wall or the injured myocardium are potent PCs. Perhaps, certain stimuli are required for monocytes/macrophages to trans-differentiate into functional PCs. The possibility arises...
that these stimuli are not present in the actual dysfunctional area and need to be externally applied. Alternatively, only a subset of these cells may possess the capacity to transdifferentiate into PCs. In our opinion it will be some time before monocyte-derived PC-based therapies will be applied in clinical practice. Nonetheless, much progress has been made the last years in understanding the role of monocyte in cardiovascular disease. We believe that their presence in the sites of vascular or myocardial injury is not a catastrophic immune overreaction but a desperate attempt for tissue repair.

Finally, anti-inflammatory treatment strategies for the management of HF are promising but the initial trials that tested therapies targeting inflammation in the overall HF population have had disappointing results. As highlighted in a recent statement issued by the Translational Research Committee of the Heart Failure Association of the European Society of Cardiology, the most successful trials have been those where small (and very carefully selected) groups of patients have been treated. Indeed, the idea of a common inflammatory pathway that underlies all different forms of HF appears unlikely. It is perhaps more realistic to design specific anti-inflammatory approaches for different types and/or stages of HF. Thus determination of the specific inflammatory pathways in different forms of HF is essential. Greater knowledge of basic mechanisms, detailed preclinical studies, and justified patient selection for clinical trials are clearly required for the future development of successful anti-inflammatory approaches in the management of HF.

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