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Functional alterations of myeloid cell subsets in hyperlipidaemia: relevance for atherosclerosis

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Abstract

Atherosclerosis is a chronic inflammatory disease wherein the infiltration of myeloid cells of the vessel wall is a hallmark event. Lymphocytes, platelets and endothelial cells stand out as prominent suspects being involved in atherosclerosis. However, recent advances suggest a crucial role for myeloid leucocytes, specifically monocyte subsets, neutrophils, dendritic cells and endothelial progenitor cells. These cell types are not just rapidly recruited or already reside in the vascular wall, but also initiate and perpetuate core mechanisms in plaque formation and destabilization. Hyperlipidaemia is an independent risk factor for atherosclerosis. Herein, hyperlipidaemia skews myeloid cell haemostasis, phenotype and transcriptional regulation of pro-inflammatory factors ultimately promoting myeloid cell extravasation and atherosclerosis. We here review the role of myeloid cells in atherosclerosis as well as the effects of hyperlipidaemia on these cells.

Keywords: atherosclerosis • hyperlipidaemia • monocyte • neutrophil • dendritic cell • endothelial progenitor cell

Introduction

Atherosclerosis is an inflammatory disease characterized by intense immunological activity, which increasingly threatens human health worldwide [1]. With primary risk factors such as smoking, hypertension, hyperlipidaemia and hyperglycaemia, the incidence of atherosclerosis-related diseases is expected to further increase. The pathophysiology of atherosclerosis involves plaque formation in arteries characterized by inflammation, lipid accumulation, cell death and fibrosis [2, 3]. Although clinical complications of atherosclerosis can arise from plaques causing flow-limiting stenosis, the most severe clinical events follow the rupture of a plaque, which exposes the pro-thrombotic material in the plaque to the blood and causes sudden thrombotic occlusion of the artery at the site of disruption. In the heart, atherosclerosis can lead to myocardial infarction and heart failure, whereas in the arteries that perfuse the brain, it can cause ischemic stroke and transient ischemic attacks. If atherosclerosis affects other arterial branches, it can result in renal impairment, hypertension, abdominal aortic aneurysms and critical limb ischaemia.

Development of atherosclerotic plaques

Atherosclerosis is initiated by activation and dysfunction of endothelial cells subsequently leading to adhesion of leucocytes and activated platelets as well as increased permeability for plasma lipids [2]. Monocytes emigrating to the inflamed vessel wall transform into foam cells to form early plaques, the fatty streaks. Following the accumulation of additional inflammatory cells fatty streaks progress into mature atherosclerotic plaques characterized by a core region that is surrounded by a cap of

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smooth muscle cells and a collagen-rich matrix. The secretion of cytokines and growth factors by immune cells within the plaque and the continued deposition of extracellular matrix components, contribute to plaque progression causing narrowing of the arterial lumen. The central core of the mature plaques can become necrotic, and neovascularization within the plaques can allow leakage of blood components and haemorrhage [2, 4]. Over time, the secretion of matrix-degrading proteases and cytokines by plaque cells causes thinning of the fibrous cap. Ongoing weakening of the cap provokes plaque erosion and rupture which by exposure of tissue factor triggers the coagulation cascade and the formation of a thrombus.

The concept of atherosclerosis being a chronic inflammatory disease is widely accepted in the field of cardiovascular research. This understanding is in principle based on the observation of leucocyte infiltration throughout the different stages of atherosclerosis. As such, activated $CD4^+$ memory T cells of the T helper 1 (T_H1)-cell type traffic to atherosclerotic lesions and contribute to plaque progression. Unanticipated roles for infiltrating neutrophils in atherogenesis were recently discovered [5–7]. In addition, endothelial progenitor cells (EPCs) that respond to local endothelial-cell damage and early regenerative homing signals can affect the composition, vascularization and stability of plaques. The most prominent leucocyte subset in atherosclerosis is, however, represented by monocytes and monocyte-derived macrophages [2, 3]. In this review article we highlight mechanisms by which hyperlipidaemia skews the phenotype and function of myeloid cell subsets to promote atherosclerosis.

Dyslipidaemia is a major risk factor for atherosclerosis

Hyperlipidaemia, especially hypercholesterolemia, is regarded as an independent risk factor in the development of ischemic heart disease including myocardial infarction. Epidemiological studies showed that there is a strong relationship between the elevation of serum total cholesterol concentration and the morbidity and mortality of myocardial infarction [8, 9]. Apart from these significant studies several additional findings point at the importance of hypercholesterolemia in atherosclerosis. Animals, with some rare exceptions, do not spontaneously develop atherosclerosis. The low-density lipoprotein (LDL) level in most animals is 50 mg/dl or even less as compared to an average range of 130–160 mg/dl in human beings [10]. In addition, in Japan in the 1960s LDL levels were only about 100 mg/dl. This was associated with a mortality from cardiovascular diseases that was about 10% of that in the United States and the rest of the developed countries. This was true despite the fact that the Japanese held the world record for the average nicotine consumption per day, a higher incidence of hypertension and a prevalence of diabetes similar to that in other developed countries. More solid support stems from randomized clinical trials of lipid-lowering therapy. These have produced up to 30% reduction of major coronary events, thereby firmly vindicating the significance of high lipid levels as major contributing risk factor in atherosclerosis [11].

Myeloid cells in atherosclerosis and effects of hyperlipidaemia

Monocytes/macrophages

Monocytes and monocyte-derived macrophages as well as foam cells are the most abundant leucocyte subset in the atherosclerotic plaque. Depletion of monocytes from the circulation significantly reduced plaque formation providing direct evidence for the importance of monocytes and their descendents in atherosclerosis [12]. In a more recent study, depletion of monocytes and macrophages at early time-points of atherosclerosis formation reduced the activity and number of macrophages within the plaque, inhibited early lesion development and altered plaque composition, reducing collagen content and necrotic core formation [13]. Depletion of monocytes and macrophages at later time-points, however, did not have any of these effects, suggesting an important role of monocytic cells in early stages of lesion formation [13]. This is in line with the early appearance of monocytes in fatty streaks, the earliest visible lesion in human and experimental atherosclerosis [14].

In human beings, monocytes can be differentiated by expression levels of CD14 and CD16 (Table 1) [15–17]. Classical monocytes are defined as $CD14^+CD16^-$, while non-classical monocytes are $CD14^{lo}CD16^+$. The presence of murine monocyte subsets has more recently been described [18]. Murine monocytes can be divided into either $Gr1^+$ monocytes expressing high levels of CCR2, L-selectin (CD62L) and low levels of CX_3CR1, or the $Gr1^-$ monocytes which are characterized by high expression of CX_3CR1, LFA-1 and the lack of expression of CCR2 or CD62L (Table 1). The first are thought to correspond to human classical monocytes, while the latter are believed to correspond to the set of human non-classical monocytes. The differential expression levels of chemokine receptors between the murine subsets, which are similar in the human monocyte populations [19], are crucial for their extravasation behaviour [3]. Importantly, inflammatory monocytes employ CX_3CR1, CCR2 as well as CCR5 to emigrate into the atherosclerotic lesion [20]. As CCR2, CCR5 and CX_3CR1 are all associated with atheroprogression [21–23], these chemokine receptors and the inflammatory monocytes may stand out as important partners in atherosclerosis. While the involvement of selectins and CAMs has traditionally been investigated for monocytes as such [24, 25], recent data indicate distinct engagement patterns of these receptors for the monocyte subsets. An *et al.* demonstrated that inflammatory monocytes express higher levels of PSGL-1, which was found to be crucially involved in the interaction of $Gr1^+$ monocytes with the atherosclerotic

Table 1 Monocyte subsets in human beings and mice

	Human		Murine	
	Classical	**Non-classical**	**Classical**	**Non-classical**
Phenotypical markers	$CD14^{++}$ $CD16^{-}$	$CD14^{+}$/$CD14^{int}$ $CD16^{+}$	$CD115^{+}$ $Gr1^{+}$ $7/4^{hi}$	$CD115^{+}$ $Gr1^{-}$ $7/4^{lo}$
Adhesion molecules and chemokine receptors	$CCR2^{+}$ $CCR1^{+}$ $CXCR1^{+}$ $CXCR2^{+}$ $CX3CR1^{int}$ PSGL-1^{+} $CD62L^{+}$ $CD11a^{+}$ $CD11c^{+}$ $CD43^{+}$	$CCR2^{-}$ $CCR1^{-}$ CX_3CR1^{hi} $CD62L^{-}$ $CD43^{++}$ $CD11a^{++}$ $CD11c^{++}$	$CCR2^{+}$ $CCR1^{+}$ CX_3CR1^{int} PSGL-1^{+} $CD62L^{+}$ $CD43^{+}$ $CD11a^{+}$ $CD11c^{-}$	$CCR1^{-}$ CX_3CR1^{hi} $CCR5^{+}$ $CD62L^{-}$ $CD43^{++}$ $CD11a^{++}$ $CD11c^{+}$
Alternative names		Pro-inflammatory monocytes	Inflammatory monocytes	Resident monocytes

endothelium [26]. In contrast to inflammatory monocytes, the role and recruitment pattern of resident monocytes are not as clear. In human beings, resident monocytes are more potent in presenting antigens, which may be in line with the acquisition of the dendritic cell marker CD11c by murine resident monocytes [18]. Recently it has been shown that murine resident monocytes patrol healthy tissues through CX_3CR1-dependent crawling on non-activated endothelium, which allows their rapid tissue infiltration at sites of infection or injury [27].

A prominent function of macrophages in atherosclerosis is the handling of altered self-components. To accomplish this, macrophages are equipped with a wide array of pattern recognition receptors such as scavenger receptors. These allow for the uptake of modified LDL and contribute thereby to the foam cell formation. Among various scavenger receptors, SR-AI and CD36 have been regarded as the most important receptors in the uptake of oxLDL [28]. While data for CD36 are less consistent, it seems that mice deficient in SR-AI show reduced plaque sizes [29]. A more recent publication indicates that deficiency of CD36 or SR-AI does not lead to decreased plaque sizes, but rather reduced plaque complexity as demonstrated by lower numbers of lesional apoptotic macrophages [30]. Thus, this study indicates that CD36 and SR-AI may be important in pro-inflammatory processes in the vessel wall and their deletion promotes plaque stability. Notably, human inflammatory monocytes show higher SR-AI expression levels but similar CD36 expression as compared to resident monocytes [31].

Hyperlipidaemia exhibits apparent effects on monocyte haemostasis, expression of pro-inflammatory factors, chemokines and other adhesion molecules thus promoting a more severe progression of atherosclerosis. Clinical data have shown a positive correlation between white blood cell count and acute myocardial infarction [32–34]. More recently, however, a study showed that monocytosis is an independent risk factor for coronary artery disease [35]. Interestingly, pravastatin treatment was shown to induce plaque regression along with decreased total leucocyte counts. Out of the leucocyte subsets, however, only reduction in monocyte count was found to correlate with the reduced plaque size [36]. Early work demonstrated the expansion of circulating monocytes in animals fed a high fat diet some of which was due to increased monocyte production in the bone marrow [37, 38]. A recent study on atherosclerotic mice provides more detailed evidence for the induction of monocytosis in mice receiving high fat diet. Interestingly monocytosis in these mice resulted from an increase in the $CD115^{+}Gr1^{+}$ subset, while $CD115^{+}Gr1^{-}$ monocytes were not affected (Fig. 1) [39]. Hypercholesterolemia-associated monocytosis resulted from continued bone marrow production of $Gr1^{+}$ monocytes, increased survival of these cells in the periphery, and impaired conversion to the $Gr1^{-}$ subset and could be directly correlated to the serum cholesterol levels. Of note, statin treatment partially reversed the increase in peripheral inflammatory monocytes. Future research will determine whether human beings with elevated serum cholesterol exhibit $CD14^{hi}CD16^{-}$ monocytosis, and whether this variable relates to atheroma burden and its complications. Furthermore, Wu *et al.* recently found an expansion of $CD11c^{+}$ monocytes under high fat diet in atherosclerotic mice [40]. Monocytic CD11c was further shown to be important in firm adhesion thus contributing to monocyte emigration *via* VCAM-1 and E-selectin. Of note, CD11c-deficient mice in an atherosclerotic background exhibited less plaque and decreased macrophage content. Although it is not really clear if that effect is solely due to this described monocyte subset since CD11c is also expressed by dendritic cells and the receptor itself can be looked upon as a key receptor in progression of atherogenesis. As the expression pattern of CD11c is different in mouse and man it remains to be investigated how these data relate to the human situation.

A crucial factor in monocyte recruitment to atherosclerotic lesions is the level of adhesion molecules expressed on endothelial cells as well as on leucocytes. In a recent study hyperlipidaemia has been directly correlated to a significant increase not only in CAM levels released from endothelial cells but also in some CAMs located at the monocyte surface in human beings [41]. Monocyte CD18 and CD54 showed significantly higher expression in hypertriglyceridemic patients than in controls. Hypercholesterolemia, and specifically high LDL levels, was further found to result in

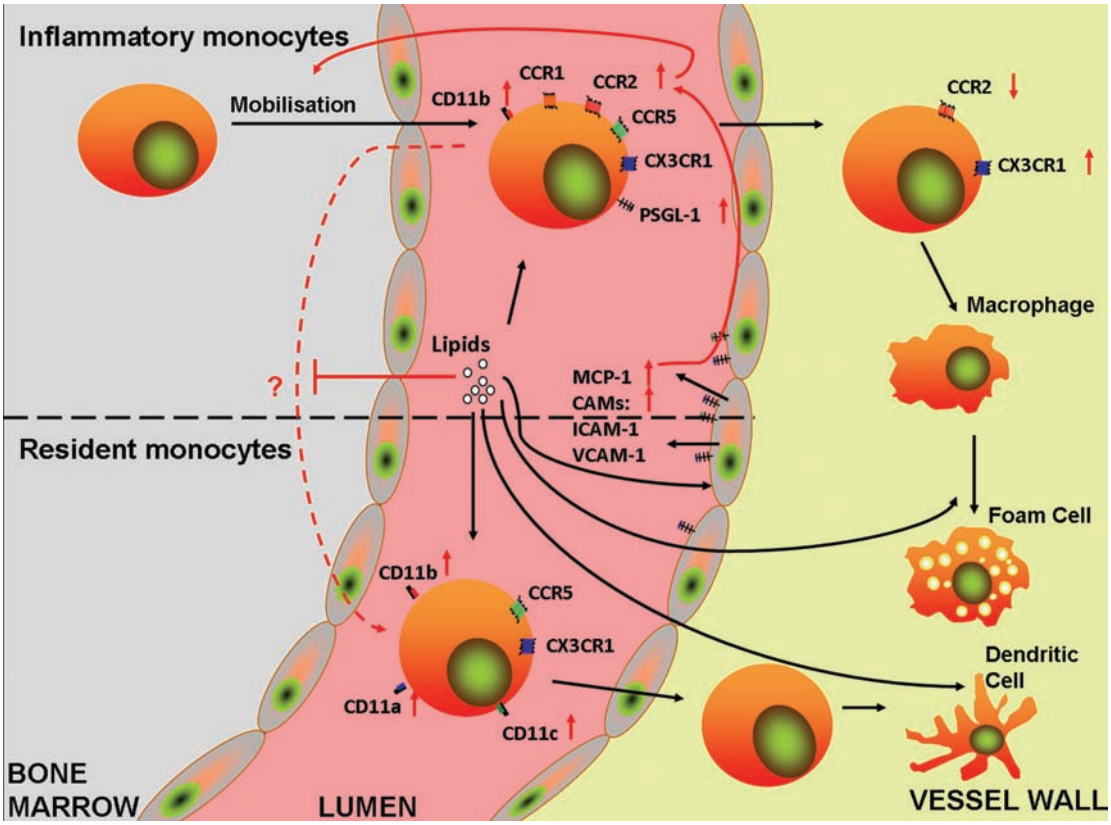

Fig. 1. Role of monocytes in atherosclerosis and their affection by in hyperlipidaemia. Inflammatory monocytes are characterized by higher expression levels in PSGL-1, CCR1 and CCR2. Therefore these molecules were proposed to be essential for recruitment of this monocyte subset. In contrast, CCR5 and CX_3CR1 seem to be of major importance for extravasation of resident monocytes. Inflammatory monocytes will differentiate to macrophages, which take up oxLDL forming foam cells. These cells are critically involved in atheroprogression by releasing cytokines (*e.g.* TNF, IL-6), chemokines (*e.g.* MCP-1), proteases (*e.g.* MMPs), and lipid mediators (*e.g.* leukotrienes). Resident monocytes on the other hand may acquire DC-markers such as CD11c. Hyperlipidaemia skews monocyte counts and function in several ways (in red). Monocytosis of inflammatory monocytes may be due to reduced conversion to resident monocytes or enhanced mobilisation from the bone marrow, some of which may be due to higher levels of circulating MCP-1. Furthermore, CCR2, PSGL-1, and CD11b on inflammatory monocytes is enhanced by hyperlipidaemia. The emigrated monocyte will retain in the vessel wall due to decrease of CCR2 and increase in CX_3CR1.

enhanced monocytic CCR2 expression [42, 43]. Similarly, it was shown that circulating $Gr1^+$ monocytes in atherosclerotic mice have higher levels of CCR2 (Fig. 1) [20]. Application of statins was further shown to reduce monocytic CCR2 expression in man, mice and rats ultimately leading to reduced arterial infiltration [44]. Additionally statins seem to have an influence on chemokine and chemokine receptor expression in human endothelial cells and macrophages. In both cell types, treatment with simvastatin significantly decreased CCR4 and MCP-1 expression in a dose dependent manner as well as MCP-1 secretion [45]. Likewise CCR1, CCR2 and CCR5 mRNA expression is down-regulated by simvastatin.

oxLDL was shown to have several pro-atherogenic effects on monocytes and macrophages. Not just are monocytes chemoattracted to the site of inflammation [46], oxLDL also enhances proliferation [47] and differentiation [48, 49] of monocytes and macrophages. With regard to the latter it has just recently been described that peritoneal oxLDL inoculation induced monocyte shape changes towards macrophages as well as expression of the scavenger receptor CD36 [49]. Inhibition of M-CSF-R abolished these phenotypical changes suggesting an autocrine loop, wherein oxLDL promotes monocyte differentiation *via* induction of M-CSF. The oxLDL-induced expression of CD36 was further found to be partially dependent on PPAR-gamma and protein kinase C [50, 51]. While the axis of oxLDL-PPAR-gamma is critically involved in enhancing monocytic scavenger receptor expression, it may on the other hand reduce CCR2 expression [52], which may help retain monocytes at sites of inflammation. This switch-CCR2 off goes along with switch-CX_3CR1 on (Fig. 1), causing cessation of CCR2-dependent migration and activation of CX3CR1-dependent retention mechanisms [53].

Taken together, hyperlipidaemia induces monocytosis and skews the phenotype of circulating monocytes to a more adhesive phenotype. Once adherent, emigrated monocytes are attracted by oxLDL, which will not only induce differentiation and proliferation, but also retain monocytes within the vessel wall.

Neutrophils

Despite the detection of polymorphonuclear leucocytes (PMN) in atherosclerotic lesions during the early 1980s [54], proof for the pro-atherogenic effect of PMN is scarce. Recently, however, Zernecke *et al.* provided evidence for the pro-atherogenic role of PMN [6]. In this study the size of murine atherosclerotic plaques has been shown to be closely correlated with the number of PMN in the peripheral blood. Disruption of the CXCR4/CXCL12 axis by injection of a small-molecule antagonist increased the number of peripheral PMN being associated with aggravated lesion formation. In contrast, depletion of PMN significantly reduced the plaque size indicating a functional role of PMN in atherosclerotic lesion formation. Interestingly, a single nucleotide polymorphism close to the CXCL12 locus has recently been identified to be associated with premature myocardial infarction [55]. In line with this, a positive correlation between peripheral PMN count and acute coronary events as well as the degree of atherosclerosis has previously been shown [56, 57]. Nevertheless, mechanistic insights underlying PMN-driven atherosclerosis is limited and to a large degree derived from microcirculatory and basic inflammatory models. Besides their physiological functions such as uptake and digestion of bacteria, PMN-driven pro-inflammatory mechanisms largely depend on interaction with neighbouring cells [58]. PMN do so by release of preformed granule proteins, acting as mediators in the crosstalk between PMN and endothelial cells [59], monocytes [60], dendritic cells [61] and T cells [62]. Prominent members of PMN-derived granule proteins are LL-37, α-defensins (human neutrophil peptides, HNPs), HBP (azurocidin, CAP37) as well as the serprocidins (elastase, cathepsin G, proteinase-3), all of which have also been identified in the atherosclerotic lesion [63–65]. Luminally detected granule proteins may have been deposited by PMN. In this location these proteins may activate monocytes in flow and induce adhesion and regulate endothelial permeability and cell adhesion molecule expression [66–69]. In contrast, granule proteins released in the vessel wall, may attract inflammatory monocytes and activate macrophages to produce and release pro-inflammatory cytokines such as tumour necrosis factor (TNF)-α and IL-6 [70–72]. In addition to the activation of adjacent cells, PMN granule proteins with enzymatic activity may exert effects with direct effects on plaque vulnerability. PMN are rich in matrixmetalloproteinases (MMP)-9, cathepsin G, elastase and proteinase-3. Through their proteolytic activity they may contribute to weakening of the fibrous cap and ultimately promote plaque rupture. Mice devoid of MMP-9 indeed show reduced plaque size [73]. Murine models of aneurysm formation further support a role of PMN and PMN-derived cathepsin G, elastase and proteinase-3 in arterial pathophysiology [74, 75]. Besides granule proteins, PMN release vast amounts of oxygen produced *via* myeloperoxidase, lipoxygenases and NADPH oxidase. Much of the oxygen radicals are secreted extracellularily, where they contribute to oxidation of LDL to oxLDL. However, knock out models for myeloperoxidase and gp91 do not lend strong support for the importance of oxygen radicals produced *via* these two molecules [76, 77].

The presence of primed PMN in hyperlipidaemia has been reported by Araujo *et al.* There they show a positive correlation between plasma triglycerides and LDL and PMN ROS formation [78]. In a more recent study, Mazor *et al.* found an increased rate of superoxide release and CD11b surface expression positively correlating with the severity of hyperlipidaemia [79]. In addition, circulating PMN contained less MPO, while plasma MPO levels were elevated, indicative of granule discharge from PMN in patients with hyperlipidaemia. Finally, the authors showed that hyperlipidaemia resulted in a slight (non-significant) increase in circulating PMN counts associated with enhanced levels of PMN apoptosis suggesting a higher turnover of PMN. Taken together these studies indicate a priming of PMN in hyperlipidaemia.

OxLDL in the vessel wall may further activate PMN, thus modulating PMN-mediated pro-inflammatory effects. As for monocytes, oxLDL was shown to stimulate PMN adhesion [80, 81] and transendothelial migration [82]. Increased adhesion may be due to enhanced β_2-integrin mobilization from intracellular stores [81], thus allowing for firm arrest. As indicated above, major pro-inflammatory tools of PMN are granule proteins and reactive oxygen species. Therefore it is of interest that oxLDL was shown to induce release of secondary and primary granules as indicated by discharge of lactoferrin and MPO, respectively [82]. Similarly, oxLDL, but not native LDL, evoked a clear-cut increase in production and release of superoxide by PMN. This increase was preceded by a rapid rise in intracellular Ca^{2+}. Interestingly, both the transient Ca^{2+} increase and the superoxide production were abolished by presence of fucoidan, an unspecific scavenger receptor antagonist [83]. Thus these data suggest that PMN, contrary to common believe, may possess biological active scavenger receptors.

Interestingly, it has consistently been reported that statins positively affect PMN-mediated pro-inflammatory mechanisms. However, most of these effects were found to be independent of the lipid-lowering effect of statins. Maher *et al.* demonstrated that pravastatin, simvasatatin and atorvastatin reduced PMN transendothelial migration by reducing the levels of active GTP-bound Rho, which is required for cytoskeletal rearrangements during migration [84]. Furthermore, Eccles *et al.* showed that simvastatin and fluvastatin selectively reduce P- and E-selectin mediated interaction between human endothelial cells and human PMN *in vitro*, by blocking cell surface expression of both selectins [85]. Also, several findings indicate that statins may modulate the endothelial production of various chemokines that attracts and activates neutrophils, such as epithelial neutrophil-activating peptide (CXCL5 or ENA-78) and interleukin-8 (CXCL8) [85, 86]. These findings are further corroborated in ApoE-deficient mice subjected to ligation of the common carotid artery, followed by collar positioning. There, fluvastatin treatment was found to attenuate PMN infiltration in neointimal carotid lesions, independent of plasma cholesterol lowering [87].

Little is known about the PMN in atherosclerosis and therefore only limited attention has been drawn to the effect of hyperlipidaemia on PMN. However, it has consistently been reported that hyperlipidaemia primes circulating PMN as indicated by granule mobilization and ROS formation. Similar effects have been observed for oxLDL.

Mast cells

In human beings, mast cells are derived from $CD34^{+}CD117^{+}CD3^{+}FceRI^{-}CD14^{-}$ progenitor cells [88–90]. These committed progenitors circulate in the blood until they reach peripheral tissues where differentiation and maturation takes place. Early work suggested a role for mast cells in human atherosclerosis [91], and this has been confirmed by more recent studies that identified mast cells in coronary and carotid-artery plaques at sites of plaque erosion, rupture or haemorrhage [92]. More direct evidence for the contribution to plaque progression comes from two recent studies. Local application of mast cell activators was shown to induce intraplaque haemorrhage, apoptosis of macrophages, vascular permeability and monocyte recruitment to mouse plaques [93]. Much of this activity was dependent on mast cell degranulation and further research identified chymase and CXCR2 ligands as active compounds. In another approach development of atherosclerosis was studied in mice lacking mast cells. These mice exhibited decreased lesion size, lipid deposition, T cell and macrophage numbers, cell proliferation and apoptosis, but increased collagen content and fibrous cap development [94]. Adoptive transfer of wild-type mast cells, but not transfer of mast cells lacking IL-6 or IFN-γ, restored plaque formation, thus indicating that IL-6 and IFN-γ are primary effectors of mast cells. Secondarily, these mediators induce expression and release of matrix-degrading proteases thus resulting in plaque formation and destabilization. The same group demonstrated just recently that mast cells actively participate in the formation of aortic aneurysms by release of IL-6 and IFN-γ, which induced apoptosis of smooth muscle cells and protease expression [95].

As for PMN, the cytoplasm of mast cells is filled with preformed granules, which are readily released after cell activation. The instant availability of these mediators may attribute an important role in the onset of atherosclerosis [92]. Recently it has been shown that oxLDL induces activation and degranulation of mast cells [96]. In addition, mast cells are involved in accumulation of LDL in the vascular wall by increasing transendothelial permeability [97]. By means of oxidative modification granule proteins of mast cells contribute to the retention of LDL in the sub-endothelial space, thus enhancing foam cell formation [98].

Dendritic cells

Dendritic cells (DC) are a heterogeneous set of antigen presenting cells consisting of conventional DC, plasmocytoid DC as well as inflammatory DC. While DCs form a network in the arterial intima of young healthy individuals [99], the numbers of DCs were found to be increased in advanced human plaques [100, 101]. There they form clusters with T cells residing in the plaque shoulder and regions prone to rupture. Yilmaz *et al.* found that up to 70% of DCs in the shoulders of vulnerable carotid plaques express markers of DC activation such as CD83 and DC-LAMP [102]. Accumulation of DC in atherosclerotic lesions was further shown to be associated with plaque growth and inflammation. In contrast to macrophages, PMN, and mast cells, however, no depletion (or even subset-specific depletion) studies are available supporting the importance of DC in atherosclerosis. Despite the lack of solid evidence for the involvement of DC in atheroprogression, several mechanisms have been suggested by which these cells contribute to the immune response in the plaque. As in peripheral tissue, DC may be involved in antigen capture and processing as well as T-cell stimulation. LDL and modified lipoproteins, such as oxLDL, represent an antigen that could trigger such a mechanism resulting in IFN-γ production from T cells. In agreement with a potential axis of oxLDL–DC–T-cell stimulation is the finding that oxLDL enhances expression of costimulatory molecules by DC with subsequent T-cell proliferation [103, 104]. However, this notion is controversial inasmuch as it has been suggested that hyperlipidaemia inhibits activation of DC. Therein, it was shown that hyperlipidaemia impaired DC migration to lymph nodes thus suppressing immunological priming [105]. Reduced migration resulted from inhibitory signals generated by platelet-activating factor or oxLDL that acts as a platelet-activating factor (PAF) mimetic. Therefore, hyperlipidaemia may sequester activated DC in the periphery where they can aggravate local inflammation. Additionally, it was shown that hyperlipidaemia inhibits Toll-like receptor-induced production of pro-inflammatory cytokines and up-regulation of costimulatory molecules by a subset of DCs [106]. Subsequent Th1 responses were found to be profoundly diminished. Further analysis has proven oxLDL to be the key active component in this, as it could directly uncouple TLR-mediated signalling on DCs and inhibit NF-κB nuclear translocation [106]. Contrasting these observations, it has recently been shown that DC from mice fed high fat diet maintain antigen processing and antigen presentation capabilities as well as T-cell priming efficacy [107]. These conflicting data may result from differences in models applied and the focus on different DC subsets. Hence, this calls for further investigations on the effect of hyperlipidaemia on DC subsets in man and mouse.

Plaques of atherosclerotic patients on statins contain lower numbers of DCs as compared to control patients [108], which is likely due to inhibited adhesion and transmigration of DC [109]. In addition, statins were shown to reduce DC maturation and subsequent T-cell stimulation [110] providing an alternative explanation for the beneficial effects of statins in atherosclerosis.

Endothelial progenitor cells

EPCs circulate in peripheral blood of human beings and mice (Table 2). These cells can be considered as the currently most

Table 2 EPCs in human beings and mice

	Human	Murine
Phenotypical markers	(CD133), CD34, VEGFR2 CD14, CD31, Tie2, VE-cadherin, vWF	c-kit, sca-1, Flk-1, CD31, Tie2, vWF
Adhesion molecules and chemokine receptors	CD11a, CD11b, CD49e, PSGL-1 CCR2, CCR5 CXCR2, CXCR4	
Alternative names	Angiogenic outgrowth cells early EPCs myeloid EPCs	

intensively studied adult progenitor cell population with relevance to endothelial regeneration and neovascularisation of ischemic tissue [111–113]. Besides some heterogeneity in origin and characterization, EPCs in human peripheral blood have most commonly been defined as low granular cells positive for CD34 and for the vascular endothelial growth factor receptor-2 (VEGFR2), but negative for the common leucocyte antigen CD45 [113–115]. Although some groups have previously highlighted CD133 as an EPC specific marker [116, 117], recent data revealed that $CD133^+CD34^+VEGFR2^+$ cells from peripheral blood rather reflect primordial haematopoietic precursors [118]. Functionally, EPCs engraft into neo-endothelium and differentiate into mature endothelial cells. Recent reports have further provided growing evidence about some circulating myeloid $CD14^+$ subpopulations ($CD14^+CD34^{lo}$, $CD14^+VEGFR2^+CXCR2^{+/-}$, $CD14^+CD16^+Tie2^+$) as potent paracrine players in angiogenesis [119–122]. Particularly, the Tie2-expressing monocytes have been intensively studied as key regulators in tumour angiogenesis [123]. Another type of cells can be isolated from peripheral blood mononuclear cells by using adhesion on fibronectin and culture in medium with endothelial growth factors [124]. Such a procedure results in obtaining of spindle-shaped cells or colony-forming units within 4–7 days after isolation. Although published articles have frequently characterized these cells as EPCs, their 'progenitor' properties are rather dubious as most of the adherent cells are negative or dimly positive for progenitor markers but intensively co-express endothelial and myeloid antigens. Therefore, cultured 'EPCs' even refer to myeloid angiogenic outgrowth cells [113, 125]. Hence, a re-evaluation of the definition for *in vitro* expanded 'EPCs' is urgently required. Nevertheless and beyond terminological inconsistencies, cells with an EPC-like phenotype have been largely recognized to reflect endogenous angiogenic capacity and to maintain tissue regeneration after therapeutic infusion.

Several clinical studies have shown that number and functional capacity of circulating or cultured EPCs were differentially affected in diseases associated with accelerated arterial remodelling, such as coronary artery disease, diabetes or cancer [112, 115, 126]. Notably, reduced numbers of EPCs has been shown to correlate with the Framingham risk factor score and to predict future cardiovascular events [127, 128]. Functional defect of EPCs has also been described in diabetic patients [129]. Similarly, dysfunction of EPCs was reported in obesity and hypercholesterolemia [130, 131]. Oxidized LDL has been shown to decrease EPC survival and tube-formation capacity [132], while HDL induced nitric oxide production and improved the engraftment of EPCs [133, 134]. So far, the current clinical concept claims a protective role of EPCs during the progression of atherosclerotic disease and further suggests that these cells might predict endogenous vascular repair capacity. This, however, is contrasted by a study where infusion of EPC into atherosclerosis-prone mice resulted in increased plaque size and decreased plaque stability [135]. Circulating angiogenic cells express CXCR2 and the expression of its ligands CXCL1, CXCL7 and MIF is elevated during inflammation. Increased expression of these ligands correlates with plaque instability, whereas lack of CXCR2 enhances plaque stability. These data suggest that mobilization of EPCs and/or $CD14^+$ subsets might associate with plaque instability during advanced atherosclerosis, much of which could be attributed to the proteolytic, pro-inflammatory and pro-angiogenic properties of $CXCR2^+$ monocyte subsets. Together, a potential protective role of EPCs is primarily related to endothelial dysfunction in early stages of atherosclerosis or secondary to regional endothelial repair, *e.g.* after arterial injury. In contrast, a widespread EPC mobilization and influx may associate with destabilization of advanced atherosclerotic plaques.

Conclusions

Hyperlipidaemia exhibits apparent effects on circulating myeloid cells affecting haemostasis, expression of pro-inflammatory factors, chemokine receptors and other adhesion molecules thus inducing cellular activation or dysfunction. However, due to discovery of unexpected roles of myeloid subsets in atherosclerosis (*e.g.* PMN) or due to the recently discovered diversity within myeloid subsets (*e.g.* monocytes) much work remains to be done to elucidate effects and mechanisms of hyperlipidaemia on individual myeloid subsets.

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