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Colony stimulating factors (CSFs): Complex roles in atherosclerosis

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ABSTRACT

Colony stimulating factors (CSFs) play a central role in the development and functional maturation of immune cells besides having pleiotropic effects on cells of the vascular wall. The production of CSFs is induced by multiple atherogenic and inflammatory stimuli and their expression levels are often correlated positively with advanced atherosclerotic plaques and adverse cardiovascular events in humans suggesting that CSFs play a critical role in the pathophysiology of atherosclerosis progression. Interestingly, recombinant CSFs as well as anti-CSFs are being increasingly used for diverse clinical indications. However, the effect of these novel therapeutics on atherosclerotic plaque progression is not well understood. Herein, we summarize the currently available literature on the complex role of CSFs in various stages of atherosclerosis and emphasize the necessity for conducting further mechanistic studies in animal models of atherosclerosis as well as the need for evaluating the cardiovascular safety of CSF-based therapies in humans.

1. Introduction

Colony stimulating factors (CSFs) are glycosylated cytokines that are produced and secreted by a number of different cell types including immune and non-immune cells. Initially, these factors were identified based on their *in vitro* ability to promote differentiation and survival of hematopoietic precursors into distinct immune cell lineages as indicated by their names. For example, granulocyte colony stimulating factor (G-CSF) promotes differentiation, proliferation, and survival of neutrophils; macrophage colony stimulating factor (M-CSF) promotes development of monocytes and macrophages from hematopoietic precursors; granulocyte macrophage colony stimulating factor (GM-CSF) influences differentiation of stem cells into monocytic and granulocytic lineage including neutrophils, eosinophils, and basophils; interleukin-3 (IL-3) or multi-colony stimulating factor (multi-CSF) aids the differentiation of multipotent hematopoietic stem cells into myeloid and lymphoid lineages. However, it is increasingly clear that these CSFs play roles above and beyond their presumed function in immune cell lineage specification and development. Recent data from animal and human studies have indicated the functional immunomodulatory role for CSFs as both pro-inflammatory and anti-inflammatory mediators in several pathological contexts including atherosclerosis. In this review, we have comprehensively surveyed the available data on the roles of different CSFs in the progression of experimental and clinical atherosclerosis as well as discuss the potential mechanistic basis for their action. Given the emerging use of CSFs and anti-CSFs in the clinical setting, we highlight the importance of conducting studies that examine the cardiovascular effects of these therapies in humans.

2. Granulocyte colony stimulating factor (G-CSF)

G-CSF (colony stimulating factor-3, CSF3) is essential for the development and maturation of granulocytic neutrophils from hematopoietic precursors in the bone marrow [1]. Besides, G-CSF also plays a role in mobilization of hematopoietic stem cells from the bone marrow into the peripheral blood [1]. Interestingly, G-CSF is produced by several cell types including endothelial cells, vascular smooth muscle cells, and macrophages under basal conditions and at an increased level under inflammatory conditions [2]. G-CSF receptor (CSF3R) is expressed on multiple responder cell types including hematopoietic and non-hematopoietic cells and signals upon ligand binding through activation of JAK-STAT and ERK1/2 signaling pathways [3].

2.1. Effect of G-CSF on atherogenic processes

Since atherosclerosis was considered a predominantly monocyte-macrophage driven disease, the role of neutrophils and its associated growth factors in the pathogenesis of the disease have remained largely understudied. Recently, several groups have demonstrated the pivotal role of neutrophils in all stages of atherosclerosis, wherein they promote the entry of monocytes into the vascular wall [4], increase macrophage activation and inflammatory mediator production [5], contribute to lesional ROS [6], degrade extracellular matrix via secretion of matrix metalloproteases [7] eventually leading to plaque progression

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and rupture [8]. Interestingly, increased levels of G-CSF and concomitant neutrophilia was observed in humans with coronary artery disease and was an independent predictor of major adverse clinical events (MACE) [9,10]. Similar to patients with CAD, *Apoe*$^{-/-}$ mice rendered hyperlipidemic by high fat diet feeding demonstrate neutrophilia and increased levels of serum G-CSF [11]. Patients with CAD as well as animal models of atherosclerosis demonstrate increased serum levels of TNF-α and IL-1β [11], cytokines that are known to stimulate production of G-CSF [12], raising the possibility that chronic inflammation may drive G-CSF secretion and neutrophilia in atherosclerosis, although this hypothesis has not been experimentally tested. In addition to its classical role in neutrophil production, G-CSF is also known to promote platelet activation and aggregation [13], cellular processes that are known to promote endothelial cell activation and atherosclerosis progression [14].

2.2. Clinical trials and animal studies

Recombinant human G-CSF (filgrastim and lenograstim) has immense clinical applications, particularly to boost peripheral blood neutrophil levels in cancer patients following cancer chemotherapy [15] as well as to enhance mobilization of bone marrow stem cells into peripheral blood to promote myocardial repair following myocardial infarction [16,17]. The risk-benefit ratio of G-CSF therapy has been called into question following observation of major adverse vascular events in 2 out of 16 patients with CAD and 1 in 5 patients with ischemic heart disease following administration of G-CSF [18,19]. These initial reports prompted further animal studies to understand the mechanistic basis of the effect of G-CSF in atherosclerosis.

While clinical studies and most *in vitro* studies suggest mechanisms whereby G-CSF promotes atherosclerosis progression via enhancing leukocyte count as well as direct pro-inflammatory effects, *in vivo* data from animal studies have been equivocal. Haghighat et al. demonstrated that administration of recombinant murine G-CSF at a dose of 10 μg/kg/day for 5 days a week for 8 alternate weeks to *Apoe*$^{-/-}$ mice resulted in increased atherosclerotic lesion area without significant changes in serum cholesterol, lesional macrophage numbers, and pro-inflammatory gene expression [20]. Interestingly, G-CSF administration led to increased plaque neo-vascularization, which is known to be associated with plaque rupture in humans [21,22]. In contrast, Sinha et al. found that administration of G-CSF to *Apoe*$^{-/-}$ mice led to a decrease in atherosclerotic lesion size, which was associated with decreased lesional macrophage content and lower serum cholesterol [23]. The mechanistic basis for the lower cholesterol levels in these mice is not known. It is important to note that this study utilized recombinant G-CSF at a dose of 300 μg/kg/day, which is 30 times higher than the dose conventionally used in humans and hence it is difficult to directly extrapolate these findings to humans with atherosclerosis. Also, Uchiyama et al. demonstrated that the atheroprotective effects of G-CSF administration was mediated through induction of regulatory T cells, but the mechanistic basis is currently unknown [24]. In addition to these reports, the effect of G-CSF has also been studied in rabbit and mini-swine models of atherosclerosis. G-CSF administration to rabbits (100 μg/kg/day for 7 days) led to a decrease in atherosclerotic lesion size in the coronary artery which was associated with decreased lipid accumulation within the plaque but without systemic changes in total cholesterol or triglycerides [25]. Additionally, Matsumoto et al. tested the dosage effect of G-CSF on atherosclerosis progression and observed that all doses tested led to increased collagen deposition and plaque stabilization [26]. In contrast, another study in a rabbit model of atherosclerosis demonstrated that G-CSF (15 μg/kg/day, 5 days a week for 3 weeks) led to increased atherosclerosis extent in thoracic aorta associated with early accelerated increase in total cholesterol, LDL-cholesterol, and triglycerides. However, the peak levels and final levels of cholesterol at the end of the study was unaffected [27]. Mechanistically, G-CSF administration was demonstrated to increase endothelial cell apoptosis, which may precipitate atherosclerosis progression [28]. In contrast to the above studies, G-CSF administration (10 μg/kg/day for 10 days) in high-cholesterol fed mini-swine was not associated with either changes in blood lipid levels or extent of atherosclerosis despite increased levels of peripheral blood leukocytes [29].

3. Granulocyte-macrophage colony stimulating factor (GM-CSF)

GM-CSF (CSF2) is produced by multiple cell types including macrophages, T-cells, B-cells, endothelial cells, and vascular smooth muscle cells under both basal and inflammatory conditions [30–33]. Based on *in vitro* studies, GM-CSF was considered to be important for the development of granulocytes, macrophages, and dendritic cells. However, *in vivo* data from animal studies demonstrated that GM-CSF was dispensable for most immune cell development under homeostatic conditions except for the development of alveolar macrophages and CD103^{+} dendritic cells [34–36]. GM-CSF receptor (CD116) is a heterodimer comprising of the unique GM-CSF binding α-subunit and a common β-subunit which is shared with receptors for IL-3 and IL-5 [37]. Interestingly, the downstream signaling following ligand binding seems to be fine-tuned to the concentration of the ligand, partially explaining the pleiotropic cellular effects of GM-CSF ranging from proliferation to cell survival [37,38].

3.1. Role of GM-CSF in atherosclerosis

GM-CSF is highly expressed in advanced atherosclerotic plaques of humans [39] and its expression is inducible by atherogenic oxidized-LDL [40]. The interest in examining the cardiovascular effects of GM-CSF arose from early animal studies that demonstrated the specific arteriogenesis capability of this cytokine [41,42] raising the possibility of its clinical use to promote collateral artery development in patients with CAD. Indeed, Seiler et al. demonstrated that the collateral flow index was increased in 11 CAD patients administered GM-CSF as compared with placebo [43]. However, another clinical trial was prematurely terminated because of the development of acute coronary syndrome in 2 of the 7 patients who were administered GM-CSF raising issues about the cardiovascular safety of this therapeutic regimen [44]. Interestingly, several studies suggest that GM-CSF plays a major role as a pro-inflammatory cytokine and aggravates multiple chronic autoimmune and inflammatory diseases including rheumatoid arthritis, multiple sclerosis, and atherosclerosis [45]. Not surprisingly, several antagonistic monoclonal antibodies that target GM-CSF or GM-CSF receptor are currently under clinical trial for the treatment of chronic inflammatory diseases [46]. With this background, it is evident that the vascular effects of either supplementing or inhibiting GM-CSF requires a deeper understanding to facilitate its safe development as a therapeutic strategy for several chronic inflammatory diseases. Importantly, several *in vitro* studies have suggested a pro-atherogenic role for GM-CSF. For example, GM-CSF promotes generation of reactive oxygen species [47] and myeloperoxidase in macrophages [39], increases collagen production by smooth muscle cells [48], decreases macrophage secretion of ApoE [49], increases intimal CD11c^{+} cell proliferation [50], increases apoptosis in vascular smooth muscle cells [51] and lesional macrophages [31], and induces inflammatory cytokine production by macrophages [31,52]. In contrast, additional *in vitro* studies suggest that GM-CSF may actually polarize macrophages to function in an atheroprotective manner. For example, GM-CSF promotes the production of anti-inflammatory IL-1 receptor antagonist [53], suppress IFN-γ responses [54], increase secretion of soluble VEGF receptor [55], thereby inhibiting atherosclerosis progression. In addition, serum cholesterol concentrations is lowered in patients administered GM-CSF [56] probably via elevation of expression of VLDL receptors [57] and blocking hepatic cholesterol biosynthesis [58].

The effect of GM-CSF on atherosclerosis has been studied from both a pharmacological perspective as well as to understand its

pathophysiological role. The administration of pharmacological dose of GM-CSF (10 μg/kg/day, 5 days a week for 7.5 months) to atherosusceptible rabbits led to a decrease in atherosclerotic lesion area specifically in the aortic arch without systemic changes in the levels of cholesterol, leukocytes, and platelets [51]. Although the lesion area was decreased, there was increased smooth muscle cell apoptosis, decreased smooth muscle cell numbers and decreased lesional collagen content suggesting the development of an unstable plaque [51].

Injection of GM-CSF (10 μg/kg/day, 5 days a week, alternate weeks for 8 weeks) in high-fat diet fed *Apoe*$^{-/-}$ mice led to an increase in atherosclerotic lesion area which was associated with plaque neovascularization [20]. Interestingly, in humans, neovascularization is associated with intraplaque hemorrhage and plaque rupture [22,59]. To glean the pathophysiological role of GM-CSF in atherosclerosis progression, mice deficient in GM-CSF have been extensively studied.

Two independent studies analyzed the effect of GM-CSF deficiency in the progression of atherosclerosis in LDLR knockout mice. Both studies demonstrated a decrease in lesional dendritic cell (DC) numbers and decreased lesional T-cells [31,60]. Shaposhnik et al. demonstrated that GM-CSF deficiency was associated with a modest decrease in atherosclerotic lesion size, an effect that was observed specifically in female mice [60]. Interestingly, in the study by Subramanian et al., there was no significant difference in the atherosclerotic lesion size in the GM-CSF deficient mice [31]. However, there was a significant decrease in plaque necrosis in the GM-CSF deficient mice, which in humans is correlated with a stable plaque phenotype [61]. Mechanistically, it was shown that GM-CSF stimulates the production of IL-23, a pro-inflammatory cytokine, which decreases the expression of the anti-apoptotic protein Bcl-2, thereby increasing the susceptibility of macrophages to stress-induced death in advanced atherosclerotic plaques [31].

In contrast to the above studies, deficiency of GM-CSF in *Apoe*$^{-/-}$ mice led to an increase in lesion size without alteration in plasma cholesterol levels [62]. There was increased accumulation of macrophages and decreased lesional collagen suggesting the development of a vulnerable plaque. Mechanistically, the changes were attributed to decreased expression of macrophage PPAR-γ and ABCA-1 leading to decreased cholesterol efflux. In addition, there was an increase in the expression of pro-inflammatory cytokines MCP-1, TNF, and VCAM-1, which are known to promote progression of atherosclerosis [62]. The mechanistic basis of why deficiency of GM-CSF has such divergent effects on atherosclerosis progression between *Ldlr*$^{-/-}$ *vs. Apoe*$^{-/-}$ mice is currently unknown. However, it is possible that the difference may arise due to the pleiotropic role of ApoE in functions besides lipoprotein metabolism. For example, ApoE has been demonstrated to play an anti-inflammatory role in macrophages wherein it skews their responses towards an M2 phenotype [63]. Also, ApoE suppresses mitogen-induced lymphocyte proliferation via suppression of biologically active IL-2 [64]. In addition, ApoE is known to decrease hematopoietic stem cell proliferation, thereby controlling monocytosis and neutrophilia associated with hypercholesterolemia [65], factors that are known to impact atherosclerosis progression.

4. Macrophage colony stimulating factor (M-CSF)

M-CSF (CSF1) is produced by both immune and non-immune cells and its expression is increased under several inflammatory conditions [66]. Interestingly, M-CSF is known to be expressed in several isoforms including a soluble, membrane-bound, and matrix associated forms [67]. M-CSF binds to its cognate receptor c-fms, a proto-oncogene and a tyrosine kinase to mediate its downstream activities. M-CSF plays a critical role in monocyte-macrophage development, differentiation, and function [67]. M-CSF deficient mice have decreased monocyte numbers in the peripheral blood as well as severely decreased macrophage numbers in several tissues [68]. Interestingly, the M-CSF deficient mice show a significantly decreased numbers of osteoclasts leading to their characteristic osteopetrosis and associated skeletal deformities.

4.1. Role of M-CSF in atherosclerosis

M-CSF levels are increased in atherosclerotic arteries as compared with normal arteries in humans as well as experimental animals [69,70]. Also, vascular endothelial cells as well as macrophages show increased expression of M-CSF when exposed to atherogenic oxidized-LDL [71,72]. Importantly, increased concentration of plasma M-CSF reflects advanced atherosclerosis progression and future cardiovascular events in patients with CAD [73]. M-CSF influences atherogenesis and atherosclerosis progression by increasing monocyte numbers [74], increasing monocyte migration to inflammatory sites [75], increasing macrophage differentiation, proliferation and survival [74,76], and increased lipid uptake by macrophages mediated by alteration of expression levels of scavenger receptors [69,77]. Besides, injection of recombinant M-CSF leads to a decrease in serum cholesterol levels in rabbits, primates, and humans, suggesting a potential role for M-CSF in the maintenance of cholesterol homeostasis [78–80]. The potential mechanisms of cholesterol lowering effect of M-CSF include up-regulation of APOE expression by macrophages [69] and increased expression of scavenger receptors [77] and LDL receptor related protein (LRP1) expression on macrophages [81]. The M-CSF deficient *op/op* mouse has been a great tool to understand the pathophysiological role of M-CSF in atherosclerosis. Initial study using the M-CSF deficient *op/op* mice in an *Apoe*$^{-/-}$ background revealed the critical role of M-CSF in atherogenesis [82]. Consistent with previous studies wherein M-CSF injection led to a decrease in cholesterol levels [78–80], the M-CSF deficient mice showed a 3-fold increase in systemic cholesterol accounted for by an increase in both VLDL and LDL cholesterol fractions [82]. Interestingly, despite the increased cholesterol levels, the M-CSF deficient mice had about 7-fold decrease in atherosclerotic lesion size [82]. Consistent with a role for M-CSF in monocyte development, the *op/op* mice demonstrated a 50% decrease in peripheral blood monocyte numbers. Although the phenotype of the *op/op* mice was dramatic and suggested a critical role for M-CSF in atherogenesis, this study was confounded by the mixed genetic background of these mice and the loss of body weight due to decreased food consumption secondary to absence of teeth [82]. To overcome these confounding factors, subsequent studies bred the *op/op* mice to a pure *C57BL/6* background as well as provided the mice with a specialized liquid diet to maintain their body weight [83,84]. Interestingly, these studies also demonstrated that M-CSF deficiency was associated with decreased atherosclerotic lesion development despite significant increase in systemic cholesterol levels. Additional insights were obtained by examination of phenotype of the heterozygous *op/+* mice. The *op/+* mice, which had a 50% decrease in M-CSF levels as compared with control mice, demonstrated a robust 100-fold decrease in atherosclerotic lesion size without any systemic changes in cholesterol or peripheral blood monocyte numbers [84,85]. This observation suggested that M-CSF influences atherogenesis and atherosclerosis progression independent of its effect on cholesterol and monocyte development and raised the possibility that vascular M-CSF levels rather than systemic M-CSF levels to be the critical determinant of atherosclerosis progression [84]. Interestingly, M-CSF within the vascular wall could be derived either from lesional macrophages or vascular endothelial cells and smooth muscle cells. To understand the relative contribution of hematopoietic vs non-hematopoeitic sources of M-CSF to the pathogenesis of atherosclerosis, Shaposhnik et al. adoptively transferred bone marrow cells from *Csf1*$^{-/-}$ or *Csf1*$^{+/+}$ mice into lethally irradiated *Ldlr*$^{-/-}$ mice [85]. Interestingly, the *Csf1*$^{-/-}$ bone marrow transplanted *Ldlr*$^{-/-}$ mice showed similar M-CSF levels and atherosclerotic lesion area as compared with control mice. In contrast, transplantation of *Csf1*$^{+/+}$ bone marrow into *Csf1*$^{+/-}$*Ldlr*$^{-/-}$ mice led to a 50 decrease in aortic root atherosclerosis and a 35% decrease in systemic M-CSF levels [85]. These data together suggest that M-CSF derived from non-hematopoietic arterial wall cells to be the

Table 1
The table provides a comprehensive analysis of the effects of various CSFs in animal models of atherosclerosis. PMN, Polymorphonuclear neutrophils; Mϕ, macrophage; Treg, regulatory T cells; SMC, smooth muscle cells; ND, not determined; ↑, increase; ↓, decrease; ↔, no change.

Gene	Model	Species (strain)	Diet	Duration of diet	Blood lipids		Atherosclerotic lesion parameters		Leukocytes		Inflammatory parameters		Reference
					Total cholesterol	Triglycerides	Lesion size (μm2)	Fibrous cap/ collagen/SMC	Peripheral blood	Lesion	Systemic	Atherosclerotic lesion	
G-CSF (CSF3)	rG-CSF	Mice (*Apoe*$^{-/-}$)	High fat diet	4 weeks	↔	ND	↓	ND	ND	↑ Tregs	ND	↓ TNFα, IFNγ; ↑ IL10	[24]
	rG-CSF	Rabbits (WHHL)	High fat diet	10 weeks	↑	↔	↑	ND	↑	ND	ND	ND	[27]
	rG-CSF	Swine	High fat diet	12 weeks	↑	ND	↔	↑ collagen, SMC	↑ PMN				[29]
	rG-CSF (variable dose)	Rabbits (WHHL)	High fat diet	14 weeks	↑	↑	Variable	↑collagen, ↑SMCs	↑ WBCs, PMN	↓ foam cells	ND	↔	[26]
	rG-CSF	Rabbit (WHHL-MI)	High fat diet		↔	↔	↓	↔ collagen, SMCs	↑ WBCs	↔ Mϕ, PMN	ND	ND	[25]
	rG-CSF	Mice (*Apoe*$^{-/-}$)	High fat diet		↔	↔	↑	↔ fibrous cap	ND	ND	↔	ND	[20]
GM-CSF (CSF2)	*Csf2*$^{-/-}$	Mice (*Apoe*$^{-/-}$)	High fat diet	12 weeks	↔	ND	↑	↓ collagen	ND	↑ Mϕ	ND	↑ VCAM1, TNFα, MCP1; ↓ IL1ra	[62]
	Csf2$^{-/-}$	Mice (*Ldlr*$^{-/-}$)	High fat diet	12–13 weeks	↔	↔	↓	↔ collagen, SMCs	↔ in DCs	↓ DCs, T cells	ND	↔	[60]
	rGM-CSF	Rabbit (WHHL)	Chow diet	30 weeks	↔	↔	↓	↓ SMCs, collagen	↔	ND	ND	ND	[51]
	rGM-CSF	Mice (*Ldlr*$^{-/-}$)	High fat diet	2 weeks	ND	ND	ND	ND	↔	↑ Monocytes	ND	ND	[50]
	rGM-CSF	Mice (*Apoe*$^{-/-}$)	High fat diet	8 weeks	↔	↔	↑	↔ fibrous cap	ND	ND	↑ TNFα	ND	[20]
	CSf2$^{-/-}$	Mice (*Ldlr*$^{-/-}$)	High fat diet	12 weeks	↔	↔	↔	↔ collagen cap	↔ WBCs	↓ DCs, T cells	↓IL23, IL2, IFNγ	↓ IFNγ, IL2, IL23, IL17, IL6	[31]
MCSF (CSF1)	*Csf1*$^{-/-}$	Mice (*Apoe*$^{-/-}$)	Chow diet	16 weeks	↑	ND	↓	ND	↓ Monocytes	ND	ND	ND	[82]
	Csf1$^{-/-}$	Mice (*Apoe*$^{-/-}$)	High fat diet	9 weeks	↑	ND	↓	ND	↓ Monocytes	↓ Mϕ	ND	ND	[77]
	Csf1$^{-/-}$	Mice (*Apoe*$^{-/-}$)	High fat diet	12 weeks	↑	ND	↓	ND	ND	ND	ND	ND	[83]
	Csf1$^{-/-}$	Mice (*Ldlr*$^{-/-}$)	High fat diet	16 weeks	↑	ND	↓	ND	↓ Monocytes	ND	ND	ND	[84]
	Csf1$^{-/-}$	Mice (*Ldlr*$^{-/-}$)	High fat diet	16 weeks	↔	ND	↓	ND	↓ Monocytes	ND	ND	ND	
	Csf1$^{+/-}$	Mice (*Ldlr*$^{-/-}$)	High fat diet	13 weeks	↔	↔	↓	↔	↔	↓ Mϕ	↓ IL6, IL1β	ND	[85]
	Anti-CSF1R mAb	Mice (*Apoe*$^{-/-}$)	High fat diet	6 weeks	↔	ND	↓	ND	↔	ND	ND	ND	[86]
	Anti-CSF1R mAb	Mice (*Apoe*$^{-/-}$)	High fat diet	12 weeks	↔	ND	↔	ND	↔	ND	ND	ND	

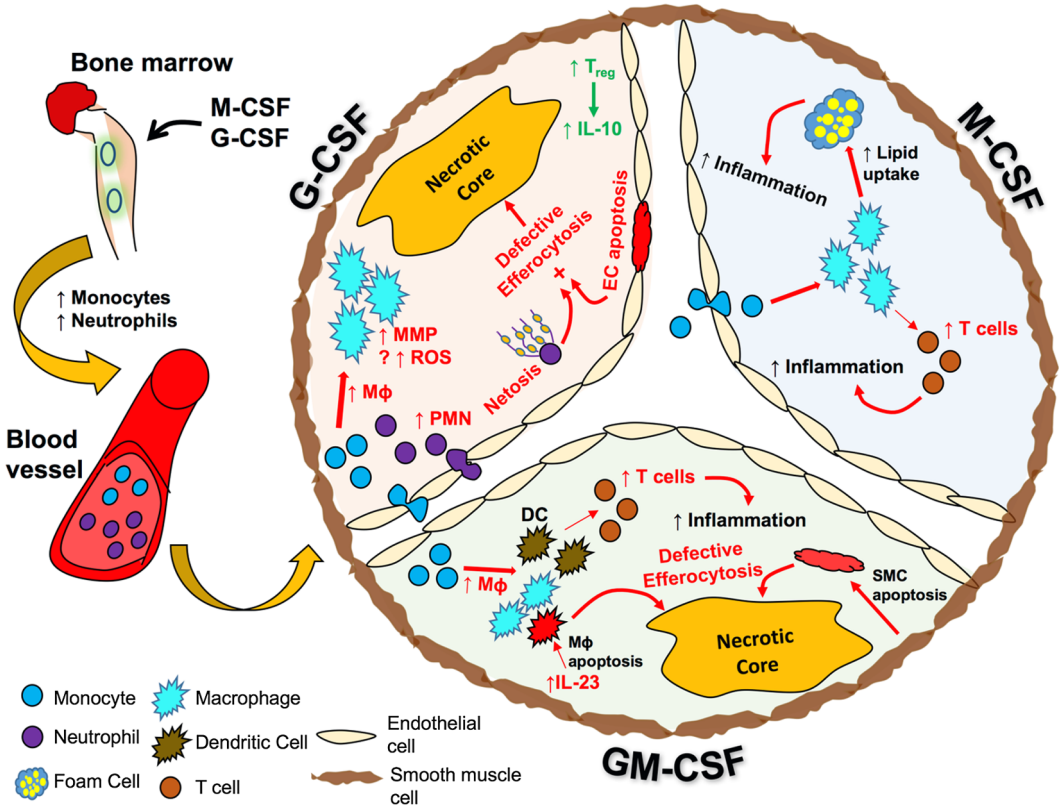

Fig. 1. Schematic illustration of the pleiotropic effects of various CSFs in bone-marrow, peripheral blood, and atherosclerotic lesions based on evidence from *in vitro* studies, *in vivo* studies, and human atherosclerosis. Pro-atherosclerotic events are labeled in red color while anti-atherosclerotic events are labeled in green color. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
The table summarizes the atheroprotective and pro-atherogenic effects of CSFs based on *in vitro* and *in vivo* studies. EC, endothelial cells; DC, dendritic cells; Mϕ, macrophage; SMC, smooth muscle cells; ROS, reactive oxygen species; ↑, increase; ↓, decrease.

	Atheroprotective effects	Atherogenic effects
G-CSF	↓ IL-1β, TNF-α, IFN-γ ↑ Tregs ↑ IL-10 ↑ Collagen	↑ Monocytes and Neutrophils ↑ Foam cell formation ↑ Apoptosis of ECs
GM-CSF	↓ Cholesterol ↓ Hepatic cholesterol Biosynthesis ↓ SMCs	↑ DCs and T Cells ↑ ROS ↑ Inflammatory Cytokines ↑ Angiogenesis ↑ Lesional cell apoptosis and Plaque necrosis
M-CSF	↓ Cholesterol	↑ Monocytes ↑ Mϕ differentiation ↑ Foam cells ↑ Th_1T cells

major contributor to the pathogenesis of atherosclerosis. Given the critical role of M-CSF in atherosclerosis, two groups tested the possibility of using either an c-fms tyrosine kinase activity inhibitor or an antagonistic antibody against c-fms (M-CSF receptor) as a potential therapeutic strategy for atherosclerosis. *Ldlr*$^{-/-}$ mice that were administered GW2580, an orally bioavailable selective inhibitor of c-fms, the lesion size and tissue macrophage numbers were significantly decreased without affecting blood lipid levels [85]. Similarly, *Apoe*$^{-/-}$ mice that were fed a high-fat diet and injected with antagonistic antibody to c-fms for 6 weeks demonstrated a 70% reduction in accumulation of macrophage foam cells in the aortic root [86]. However, in mice that had pre-established lesions, administration of the c-fms antagonistic antibody was associated with only a minimal protective effect suggesting that M-CSF might be critical particularly during the early stages of atherosclerosis development [86].

5. Multi-colony stimulating factor (interluekin-3)

IL-3 is produced by several cell types with a major contribution from T-lymphocytes and mast cells [87,88]. Despite being considered a growth factor that regulates the differentiation and survival of hematopoietic progenitors to mast cells and basophils, IL-3 deficient mice demonstrate normal numbers of mast cells and basophils under homeostatic conditions [89]. Interestingly, IL-3 is a pro-inflammatory cytokine and is associated with several chronic inflammatory and allergic disorders. Also, plasma IL-3 level was found to be elevated in patients with CAD, particularly in symptomatic patients who underwent percutaneous coronary intervention [90]. Additionally, it was observed that atherosclerotic lesional T cells from patients expressed IL-3 and IL-3 *in vitro* stimulated VEGF production and smooth muscle cell proliferation and migration [91]. However, there has been no studies examining the specific role of IL-3 in experimental atherosclerosis. Wang et al. utilized the common beta subunit (*Cbs*$^{-/-}$) knockout mice, which are deficient in the common beta subunit of the GM-CSF/IL-3/IL-5 receptor and observed that adoptive transfer of *Cbs*$^{-/-}$*Apoe*$^{-/-}$ bone marrow cells into *Ldlr*$^{-/-}$ mice was associated with decreased bone marrow hematopoietic stem cell proliferation, decrease blood monocyte and neutrophil counts, decreased atherosclerotic lesion area and macrophage content [92]. However, given that the *Cbs*$^{-/-}$ mice are deficient in GM-CSF, IL-3, and IL-5 signaling, it is currently not known which of these cytokines/growth factors lead to the above phenotype.

6. Conclusions

It is evident that the colony stimulating factors play pleiotropic role

in the development of various immune cells and actively modulate their functional properties. In addition, several of these CSFs also act upon vascular wall endothelial cells and smooth muscle cells to mediate functional changes that could influence the progression of atherosclerosis (Table 1 and Fig. 1). Despite a plethora of studies that have individually examined the activities of different CSFs in atherosclerosis (Table 2), we do not yet comprehend how these factors interact with each other to mediate their effects *in vivo*. For example, M-CSF polarizes macrophages to a M2 phenotype [93] which is atheroprotective, while GM-CSF polarizes macrophages to an M1 phenotype [93] which is pro-inflammatory and leads to atherosclerosis progression. Interestingly, exposure of macrophages to GM-CSF or M-CSF leads to distinct transcriptional and phenotypic changes [94] such that macrophages exposed to GM-CSF demonstrate increased expression of ABCA1/ABCG1 and increased lipid efflux while macrophages exposed to M-CSF demonstrate increased uptake of lipids via scavenger receptors [95]. It is important to note that although macrophages exposed to GM-CSF or M-CSF demonstrate such extreme contrasting phenotypes, there is considerable plasticity and reversal of phenotypes when another CSF is added to the milieu. For example, when M-CSF differentiated macrophages (M2-type) are exposed to GM-CSF, they quickly transform their phenotype to a M1-like macrophage [66] and demonstrate down-regulation of M-CSF receptor and M-CSF-mediated signaling suggesting the existence of extensive cross-talk between the pathways. Based on these *in vitro* studies, it is likely that the relative levels of these CSFs *in vivo* will determine the macrophage phenotype as well as other cellular phenotypes and functional outcomes. Nevertheless, how these factors balance each other and what determines the net outcome of their interaction *in vivo* is an area that requires intensive investigation. This aspect gains particular significance given that the levels of both M-CSF and GM-CSF predict the development of adverse cardiovascular events.

In addition, given the pro-inflammatory role of GM-CSF in atherosclerosis and the available evidence from both animal and human studies that GM-CSF may lead to plaque progression and adverse clinical outcomes, it is imperative to examine the cardiovascular safety profile in future clinical trials that administer GM-CSF to patients, many of whom may have sub-clinical CAD. Also, novel antagonists to GM-CSF, which are currently in clinical trial for the treatment of rheumatoid arthritis and psoriasis [46], may have clinical benefits in patients with atherosclerosis via its ability to suppress lesional cell apoptosis and necrotic core formation. Future clinical trials in this direction are highly warranted. In summary, CSFs play a pivotal role in all stages of atherosclerosis and may thus serve as novel therapeutic targets to promote atherosclerotic plaque stabilization.

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