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Macrophage proliferation and apoptosis in atherosclerosis

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Abstract

Purpose of review: Atherosclerosis is driven by cardiovascular risk factors that cause the recruitment of circulating immune cells beneath the vascular endothelium. Infiltrated monocytes differentiate into different macrophage subtypes with protective or pathogenic activities in vascular lesions. We discuss current knowledge about the molecular mechanisms that regulate lesional macrophage proliferation and apoptosis, two processes that occur during atherosclerosis development and regulate the number and function of macrophages within the atherosclerotic plaque.

Recent findings: Lesional macrophages in early phases of atherosclerosis limit disease progression by phagocytizing modified lipoproteins, cellular debris and dead cells that accumulate in the plaque. However, macrophages in advanced lesions contribute to a maladaptive, nonresolving inflammatory response that can lead to life-threatening acute thrombotic diseases (myocardial infarction or stroke). Macrophage-specific manipulation of genes involved in cell proliferation and apoptosis modulates lesional macrophage accumulation and atherosclerosis burden in mouse models, and studies are beginning to elucidate the underlying mechanisms.

Summary: Despite recent advances in our understanding of macrophage proliferation and apoptosis in atherosclerotic plaques, it remains unclear whether manipulating these processes will be beneficial or harmful. Advances in these areas may translate into more efficient therapies for the prevention and treatment of atherothrombosis.

Key words: atherosclerosis, macrophage, cell proliferation, apoptosis.

1. Introduction

Atherosclerosis and associated cardiovascular disease (myocardial infarction and stroke) are the leading causes of mortality and morbidity in developed countries, and projections of global mortality and disease burden predict that by 2020-2030 these disorders will be the main cause of death world-wide [1-3]. Cardiovascular risk factors (for example, dyslipidemia, hypertension, diabetes and smoking) instigate a chronic inflammatory response that causes endothelial dysfunction, a key process in the initiation and progression of atherosclerosis. Dysfunctional endothelial cells trigger the recruitment of circulating leukocytes to the subendothelial space [4, 5]. Infiltrated monocytes differentiate into macrophages and dendritic cells, which are essential mediators of the local immune response underlying the accumulation of lipids, cells and extracellular matrix components in the injured vessel wall [6]. During the first steps of atherosclerosis, intimal macrophages and dendritic-like cells phagocytose matrix-retained oxidized low-density lipoproteins (oxLDL) though scavenger receptors and become foam cells, the main components of the “fatty streak”. The prolonged accumulation of lipid-derived apoptotic cells, cell debris and cholesterol crystals leads to the formation of the necrotic core, a hallmark of advanced plaques.

Lesional macrophages acquire specialized phenotypes in response to signals from the local microenvironment that polarize them towards a specific activation state [7]. Macrophage polarization in the atherosclerotic plaque has attracted much interest in the light of evidence that distinct macrophage subtypes, with different protective or pathogenic functions, predominate at different stages of atherosclerosis [6, 8, 9]. Accumulation of alternatively-activated macrophages (M2 or AAM) in early lesions might

be a mechanisms to limit disease progression in the initial phases, whereas increased polarization towards pro-inflammatory classically-activated macrophages (M1 or CAM) is thought to may contribute to the expansion and vulnerability of advanced plaques. M2 macrophages support a number of key anti-inflammatory activities that limit atherosclerosis progression, including inhibition of immune-cell recruitment through TGFβ production, IL-10-dependent reduction of IFNγ synthesis, and clearance of apoptotic cells and tissue debris, a process known as efferocytosis [10-12]. In advanced phases of the disease, however, secretion of metalloproteinases, a characteristic of M2-like macrophages, contributes to matrix degradation and the formation of unstable plaques, which can rupture and trigger life-threatening myocardial infarction or stroke.

Key factors controlling neointimal macrophage accumulation—and consequently plaque growth and vulnerability—include monocyte infiltration, intimal macrophage proliferation and apoptosis, and monocyte/macrophage egress from the lesion to the bloodstream. Although recruitment from the blood has traditionally been considered the main route by which immune cells reach inflamed tissues [7], recent studies suggest that tissue resident macrophages can expand rapidly in situ during T helper 2 (Th2)-dependent inflammation [13], a key process in atherosclerosis [5]. This review discusses recent knowledge about the mechanisms that control macrophage proliferation and apoptosis in the atherosclerotic plaque.

2. Macrophage proliferation and atherosclerosis

Studies in human atherosclerotic plaques have detected expression of proliferation markers in intimal macrophages, which may be the predominant proliferative cell type in the atherosclerotic plaque [14, 15]. Expression of positive cell-cycle regulators has also been detected in macrophages in the restenotic lesions of patients with peripheral artery disease, although these accounted for only a small proportion of proliferating cells compared with vascular smooth muscle cells (VSMCs) [16]. Cell proliferation is also evident in macrophages within atherosclerotic plaques from dyslipidemic rabbits and mice [17-21]. This section discusses evidence from mouse models and human studies for the roles of tumor suppressor genes, myeloid growth factors and oxLDLs in macrophage proliferation and atherosclerotic lesion growth **(Figure 1).**

Tumor suppressor genes in macrophage proliferation and atherosclerosis. The importance of lesional macrophage proliferation in atherosclerosis development has been investigated in genetically-modified mice lacking tumor suppressor genes expressed in animal and human atherosclerotic lesions [22] **(Table 1).** Systemic and hematopoietic-restricted inactivation of $p27^{Kip1}$ increase intimal macrophage proliferation and accelerate atherosclerosis in apolipoprotein E-null *(apoE-KO)* mice [19, 20]. Similarly, macrophage-specific deficiency for retinoblastoma protein in *apoE-KO* mice increases lesional macrophage proliferation and enhances atherosclerosis development without affecting apoptosis [23]. Systemic inactivation of the growth suppressor and pro-apoptotic protein p53 in *apoE-KO* mice also increases intimal cell proliferation and enhances atherosclerosis [21, 24]. However, studies using different mouse strains and strategies to manipulate p53 expression in macrophages have yielded conflicting

results. Atherosclerosis was increased in lethally-irradiated *Apoe*3-Leiden* transgenic mice reconstituted with p53-null bone marrow (BM), correlating with increased lesional macrophage content and a tendency towards decreased apoptosis without affecting proliferation [25]. Atherosclerosis was also increased upon transfer of p53-null BM cells into LDL receptor-null *(Ldlr-KO)* mice; however, immunohistopathological analysis revealed increased intimal cell proliferation and vulnerable-appearing lesions marked by augmented tissue necrosis and reduced collagen deposition, without effects on apoptosis [26]. Contrasting with these findings, macrophage-specific p53 inactivation using the Cre-loxP system did not affect atherosclerosis burden in *apoE-KO* mice, despite an increased lesional macrophage content that coincided with reduced macrophage apoptosis, unaltered proliferation and reduced accumulation of cholesterol in the lesion [27]. Transplantation of p53 BM to mice lacking p53 and apoE reduced intimal cell proliferation and apoptosis and plaque formation [21]; but in another gain-of-function approach, *Super-p53/apoE-KO* mice, which have an extra copy *p53*, showed no changes in intimal cell proliferation, apoptosis or atherosclerosis burden [28].

Similar confusion surrounds the role in atherosclerosis of the tumor suppressor $p21^{Cip1}$, a downstream target of p53. Merched et al. reported that whole-organism or BM–restricted deletion of $p21^{Cip1}$ protects against atherosclerosis in *apoE-KO* mice [29]. Plaques in mice lacking both $p21^{Cip1}$ and apoE are more stable than plaques in mice lacking only apoE, with increased apoptosis but unaltered cellular proliferation. Interestingly, $p21^{Cip1}$-null macrophages also show increased phagocytic activity towards latex microspheres and apoptotic cells, suggesting that $p21^{Cip1}$ promotes atherogenesis in part by impeding this phagocytotic activity. In marked contrast, however, Akyurek et al. found accelerated atherogenesis in *apoE-KO* mice systemically deficient for $p21^{Cip1}$, consistent with the growth suppressive role of this protein [30].

Atherosclerosis risk in humans has been associated with several single-nucleotide polymorphisms (SNPs) in a region of chromosome 9p21 near the *Ink4/Arf* locus. This locus includes the growth suppressor genes *CDKN2A* (encoding $p16^{Ink4a}$ and ARF: human $p14^{Arf}$, mouse $p19^{Arf}$) and *CDKN2B* (encoding $p15^{Ink4b}$), and the antisense noncoding RNA *ANRIL*, which silences *Ink4/Arf* expression [42]. Interestingly, some studies have demonstrated correlations between the 9p21 risk-associated SNPs and expression of *ANRIL* and *Ink4/Arf* in circulating leukocytes [42], suggesting a role for *Ink4/Arf* growth suppressors in the identified genetic association. However, Holdt et al. found no clear association between 9p21 genotype and *CDKN2A* and *CDKN2B* expression in human atherosclerotic plaques [43]. Recent mouse studies have investigated the role of the *Ink4/Arf* growth suppressors in atherosclerosis **(Table 1).** Systemic $p19^{Arf}$ deficiency in *apoE-KO* mice attenuates macrophage and VSMC apoptosis and aggravated atherosclerosis without affecting intimal cell proliferation, perhaps reflecting a compensatory up-regulation of $p16^{Ink4a}$ [31]. Kuo et al. found that *Ldlr-KO* mice transplanted with $p16^{Ink4a}$/$p19^{Arf}$ haplodeficient BM also show accelerated atherosclerosis, with increased intimal monocyte/macrophage proliferation but no changes in apoptosis [32]. Moreover, although $p16^{Ink4a}$ inactivation decreases inflammatory signalling in mouse macrophages [44], transplantation of $p16^{Ink4a}$-null BM into *Ldlr-KO* mice does not affect atherosclerosis [33].

Myeloid growth factors and oxLDL in macrophage proliferation and atherosclerosis. Macrophage and granulocyte/macrophage colony-stimulating factors (M-CSF and GM-CSF) are key regulators of myeloid cell proliferation and survival during homeostasis and inflammation [45]. M-CSF expression is elevated in atherosclerotic lesions in rabbits and pigs [46, 47] and in the plasma of patients with

angina pectoris [48]. Moreover, M-CSF release and macrophage proliferation are induced by C-reactive protein [49], a biomarker of inflammation and a predictor of future risk of cardiovascular disease [50]. Notably, M-CSF deficiency inhibits atherogenesis in *apoE-KO* mice [34, 35], and treatment of mice with M-CSF after wire-mediated femoral artery denudation accelerates the formation of neointimal lesions, which mostly consist of BM-derived cells [36]. However, in hypercholesterolemic rabbits M-CSF treatment reduces cholesterol ester accumulation in the aorta and prevents atherosclerosis [51, 52] **(Table 1).**

The role of GM-CSF in atherosclerosis is less clear. Systemic injection of GM-CSF in *Ldlr-KO* mice markedly increases cell proliferation in nascent atherosclerotic lesions and proliferation is inhibited by function-blocking anti-GM-CSF antibody [37]. More than 90% of the proliferating intimal cells in *Ldlr-KO* mice are dendritic cells [37], and GM-CSF deficiency in this model reduce the content of intimal dendritic cells and cause a 20%-50% decrease in atheroma size, depending on the location of the lesions [38]. While these results suggest a proatherogenic role of GM-CSF in *Ldlr-KO* mice, studies in *apoE-KO* mice showed accelerated atherosclerosis by both GM-CSF genetic disruption [39] and treatment with GM-CSF [40]. Finally, studies in hyperlipidemic rabbits showed reduced atherosclerosis upon GM-CSF treatment [53]. The use of different animal models and approaches to manipulate GM-CSF expression and function might explain these seemingly conflicting findings.

Hypercholesterolemia results in the accumulation of oxLDL in the arterial wall, and its internalization by macrophages in the atherosclerotic plaque converts these cells into foam cells [6]. oxLDL can induce macrophage proliferation, a process inhibited by statins in vitro and in vascular lesions in vivo [54-57]. GM-CSF plays an essential role in oxLDL-induced macrophage proliferation [58-60], with both protein kinase C (PKC) [61,

62] and extracellular signal-regulated kinase (ERK) [63] being involved in oxLDL-dependent GM-CSF production. In contrast, GM-SCF production and macrophage proliferation are inhibited by AMP-activated protein kinase (AMPK), through increases in the levels of p53, $p21^{Cip1}$ and $p27^{Kip1}$ and a decrease in RB phosphorylation [64]. Interestingly, a combination of hyperglycemia and hyperlipidemia in *Ldlr-KO* mice stimulates macrophage proliferation in atherosclerotic lesions via a pathway that may involve glucose-dependent LDL oxidation [41].

3. Macrophage apoptosis and atherosclerosis

Macrophage apoptosis has been identified as a prominent feature of atherosclerotic plaques at all stages of the disease. In early atherosclerosis, rapid efferocytosis by intimal phagocytes (mainly M2 macrophages) reduces the accumulation of apoptotic cells and thus limits local inflammation and lesion growth by preventing secondary cellular necrosis [65, 66]. As disease progresses, defective efferocytosis and the ensuing accumulation of apoptotic macrophages and VSMCs impedes the resolution of inflammation and thus promotes plaque necrosis and instability. The pro-apoptotic effect of oxLDL on M2 macrophages may contribute to defective efferocytosis [67].

The impact of macrophage apoptosis on atherosclerosis at early and advanced stages of plaque progression has been investigated using several genetically-engineered mouse models **(Table 2).** Studies on the role of the growth suppressor and pro-apoptotic protein p53 have been discussed above. In *Ldlr-KO* mice, reconstitution with BM from mice lacking the pro-apoptotic protein Bax reduces macrophage apoptosis and increases the size of aortic root lesions [68]. Similarly, increased aortic atherosclerosis in *apoE-KO* mice lacking $p19^{Arf}$ is associated with attenuated

macrophage and VSMC apoptosis, while intimal cell proliferation is unaffected [31]. Conversely, inactivation of the pro-survival protein AIM (Spα/Api6) in *Ldlr-KO* mice increases macrophage apoptosis and inhibits atherosclerosis [69]. A similar coincidence of increased intimal apoptosis with reduced aortic atherosclerosis is seen in *Ldlr-KO* mice transplanted with fetal liver cells lacking EP4, the prostaglandin E(2) receptor involved in macrophage survival [70]. Moreover, intimal macrophage apoptosis induced in hypercholesterolemic rabbits by treatment with the nitro-oxide precursor L-arginine is accompanied by regression of preestablished atherosclerotic lesions [75]. Remarkably, using a number of approaches to manipulate macrophage apoptosis in *apoE-KO* mice, Gautier et al. demonstrated that macrophage apoptosis is antiatherogenic during the early stages of atherosclerosis, but accelerates plaque progression in more advanced lesions [76].

Endoplasmic reticulum stress (ERS) and macrophage apoptosis. Macrophage apoptosis is thought to promote the formation of the necrotic core in advanced atheromas, thus increasing plaque vulnerability and the risk of thrombotic vascular disease [77]. However, the underlying cellular and molecular mechanisms remain poorly characterized. ERS has emerged as a general mediator of vascular inflammation and endothelial dysfunction in atherosclerosis that contributes to plaque vulnerability through the induction of macrophage and VSMC apoptosis [78, 79]. Signals that can lead to ERS and apoptosis in macrophages include the excessive accumulation of modified LDLs, which dysregulates calcium homeostasis and activates the mitochondrial apoptotic pathway, and palmitic acid, which upregulates oxidized LDL receptor-1 (LOX-1) and enhances oxLDL uptake [80-82] **(Figure 2)**. Alleviation of ERS with the chemical

chaperone 4-phenyl butyric acid results in marked protection against lipotoxic death in macrophages and reduces atherosclerosis in *apoE-KO* mice [71].

Prolonged ERS induces inflammation in macrophages and activates several ERS-related proteins, including activating transcription factor-6 (ATF-6), inositol requiring protein-1 (IRE-1) and protein kinase RNA-like kinase (PERK) [83, 84]. Signaling pathways activated by these proteins cause the accumulation of unfolded proteins in the ER, which initiates the unfolded protein response (UPR) to restore normal ER function [85, 86]. However, if the stress is prolonged, or the adaptive response fails, apoptotic cell death ensues. A key effector of UPR-dependent apoptosis is the transcription factor CHOP (GADD153), which causes calcium release from the ER lumen to the mitochondria and the release of pro-apoptotic caspases [87]. CHOP also activates calcium/calmodulin-dependent protein kinase II (CaMKII), which in turn promotes cell death by activating both the extrinsic (death receptor/Fas) and the intrinsic (mitochondria/caspases) apoptosis pathways [88]. Remarkably, fat-fed *apoE-KO* and *Ldlr-KO* mice lacking CHOP exhibit reduced lesional apoptosis and plaque necrosis and smaller atherosclerotic lesions [72].

Apoptosis of ER-stressed macrophages in advanced atheromas appears to be triggered by cooperation between macrophage pattern recognition receptors (PRRs), such as A-type scavenger receptor (SRA) and toll-like receptor 4 (TLR4), through a process involving signal transducer and activator of transcription-1 (STAT1), CaMKII, and cytosolic calcium [73, 86, 89]. Macrophage apoptosis and plaque necrosis are decreased in advanced plaques of *Ldlr-KO* mice transplanted with STAT1-null BM, although atherosclerosis burden was not affected [73]. Targeted deletion of SR-A and CD36 similarly reduce lesional macrophage apoptosis and plaque necrosis in *apoE-KO*

mice, but loss of these PRRs does not substantially diminish macrophage foam cell formation or atherosclerosis burden [74].

4. Conclusions

Although blood monocytes have traditionally been considered the main source of macrophages in atherosclerotic lesions, local proliferation and apoptosis have emerged as important regulators of macrophage number and atherosclerosis development. The role in atherosclerosis of cell-cycle regulators, myeloid-specific growth factors and apoptosis regulators has been extensively investigated using genetically-modified mouse strains **(Table 1, Table 2).** Macrophage-specific manipulation of genes involved in cell proliferation and apoptosis has yielded inconclusive and sometimes conflicting results in different atherosclerosis models, possibly reflecting the complex network of regulatory circuits that orchestrate cellular hyperplasia and apoptosis and the diversity of macrophage functions in different stages of atherosclerosis. There is now compelling evidence that the phagocytic activity of lesional macrophages limits local inflammation and lesion growth by preventing secondary cellular necrosis. However, phagocytosis by lesional macrophages of lipoproteins, unwanted or dead cells and cellular debris also has strong proatherogenic effects that contribute to plaque destabilization and rupture. It is therefore unclear whether more benefit would be achieved by promotion of macrophage accumulation or by their removal from the atherosclerotic plaque. Mouse studies suggest that correct coupling of macrophage apoptosis and efferocytosis in early atherosclerosis limits atherosclerosis burden, whereas intimal macrophage and VSMC apoptosis and defective efferocytosis might promote necrosis in advanced lesions. Clearly, more research is needed to conclusively determine whether manipulation of proliferation and apoptosis in lesional macrophages has therapeutic

potential. Further insight into these important processes will require a more complete understanding of the molecular and cellular mechanisms that regulate macrophage growth and apoptosis in atherosclerotic lesions. Achievement of this will require the generation of new mouse models with inducible macrophage-specific gene alterations to achieve gain- or loss-of-function in specific phases of atherosclerosis. Work has begun on the pharmacological manipulation of these pathways to provide novel treatments for inflammatory disorders [90, 91], and new advances in basic and pre-clinical research may translate into innovative therapies to combat atherothrombosis. In addition, because macrophage apoptosis is an important feature of advanced atherosclerotic plaques with a major impact on plaque stability, an increasing number of studies are attempting to identify macrophage apoptotic proteins as markers of plaque vulnerability [92, 93].

5. Key points

- Accumulation of macrophages in atherosclerotic lesions is regulated by the equilibrium between proliferation and apoptosis.

- In early lesions, macrophages appear to limit disease progression through rapid clearance of apoptotic cells (efferocytosis); however, as disease progresses, defective efferocytosis and the ensuing accumulation of apoptotic cells promote plaque inflammation, necrosis and instability.

- Preclinical studies show that lesional macrophage accumulation and atherosclerosis burden are modulated by macrophage-specific manipulation of

genes involved in cell proliferation and apoptosis and by treatment with myeloid-specific growth factors.

- ERS has emerged as a key effector of macrophage apoptosis, and recent studies have begun to elucidate the mechanisms that induce ERS in the plaque and the ensuing apoptotic response.

- Despite the recent advances in our understanding of how intimal macrophage proliferation and apoptosis are regulated in atherosclerotic lesions, additional work is needed to translate this knowledge into new therapies to combat atherothrombosis.

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7. Conflict of interest

None.

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Table 1. Role of cell-cycle regulators and myeloid-specific growth factors in mouse models of atherosclerosis

Mouse model	Genetic modification or treatment	Effect on cell proliferation	Effect on atheroma size	Ref.
apoE-KO	$p27^{Kip1}$ global inactivation	Increased macrophage proliferation	Increase	[19]
apoE-KO	$p27^{Kip1}$-/- BMT in $p27^{Kip1}$+/+ mice	Increased macrophage proliferation	Increase	[20]
apoE-KO	Macrophage-specific RB protein deficiency	Increased macrophage proliferation	Increase	[23]
apoE-KO	p53 global inactivation	Increased macrophage proliferation (brachiocephalic)	Increase (aorta) None (brachiocephalic)	[21]
apoE-KO	p53 global inactivation	Increased intimal cell proliferation	Increase	[24]
apoE*3-Leiden	p53-/- BMT in p53+/+ mice	None	Increase	[25]
Ldlr-KO	p53-/- BMT in p53+/+ mice	Increased macrophage proliferation	Increase	[26]
apoE-KO	Macrophage-specific p53 deficiency	None	None	[27]
apoE-KO	p53+/+ BMT in p53-/- mice	Reduced macrophage proliferation (brachiocephalic)	Reduction (aorta) None (brachiocephalic)	[21]
apoE-KO	One extra p53 allele (normally regulated)	None	None	[28]
apoE-KO	$p21^{Cip1}$ global inactivation	None	Reduction	[29]
apoE-KO	$p21^{Cip1}$-/- BMT in $p21^{Cip1}$+/+ mice	Not reported	Reduction	[29]
apoE-KO	$p21^{Cip1}$ global inactivation	Not reported	Increase	[30]
apoE-KO	$p19^{Arf}$ global inactivation	None	Increase	[31]
Ldlr-KO	$p16^{Ink4a}$ and $p19^{Arf}$ haplodeficient	Increased macrophage proliferation	Increase	[32]

	BMT in $p16^{Ink4}$/ $p19^{Arf}$ +/+ mice			
Ldlr-KO	$p16^{Ink4a}$ BMT in $p16^{Ink4}$ +/+ mice	None	None	[33]
apoE-KO	M-CSF deficiency	Not reported	Reduction	[34, 35]
Wild-type	M-CSF treatment	Not reported	Increase	[36]
Ldlr-KO	GM-CSF treatment	Increased intimal cell proliferation	Not reported	[37]
Ldlr-KO	Antibody anti-GM-CSF treatment	Reduced intimal cell proliferation	Not reported	[37]
Ldlr-KO	GM-CSF global inactivation	Reduced intimal dendritic cell proliferation	Reduction	[38]
apoE-KO	GM-CSF global inactivation	Increased macrophage plaque content	Increase	[39]
apoE-KO	GM-CSF treatment	Not reported	Increase	[40]
Ldlr-KO	Hiperlipidemia and hyperglicemia treatment	Increased macrophage proliferation	Increase	[41]

BMT: bone marrow transplant

Table 2. Role of apoptosis regulators in mouse models of atherosclerosis

Mouse model	Genetic modification or treatment	Lesion stage	Effect on apoptosis	Effect on atheroma size	Ref.
Ldlr-KO	Bax-/- BMT in Bax+/+ mice	early	Reduced macrophage apoptosis	Increase	[68]
apoE-KO	$p19^{Arf}$ global inactivation	early	Reduced macrophage and VSMC apoptosis	Increase	[31]
Ldlr-KO	AIM-/- BMT in AIM+/+	early	Increased macrophage apoptosis	Reduction	[69]
Ldlr-KO	EP4-/- fetal liver cells in EP4+/+	early	Increased macrophage apoptosis	Reduction	[70]
apoE-KO	Treatment with the chemical chaperone 4-phenyl butyric acid	late	Reduce macrophage apoptosis	Reduction	[71]
apoE-KO	*aP2* global inactivation	late	Reduce macrophage apoptosis	Reduction	[71]
apoE-KO	CHOP global inactivation	late	Reduced intimal cell apoptosis and plaque necrosis	Reduction	[72]
Ldlr-KO	CHOP global inactivation	late	Reduced intimal cell apoptosis and plaque necrosis	Reduction	[72]
Ldlr-KO	STAT1-/- BMT in STAT1+/+	late	Reduced macrophage apoptosis and plaque necrosis	None	[73]
apoE-KO	SR-A and CD36 global inactivation	late	Reduced macrophage apoptosis and plaque necrosis	No affected	[74]

BMT: bone marrow transplant

Figure 1. Macrophage proliferation in atherosclerosis. Endothelial cell dysfunction caused by oxLDL accumulation within the arterial wall triggers the recruitment of circulating leukocytes to the subendothelial space. Infiltrated monocytes differentiate to macrophages that phagocytose oxLDL. Once internalized, oxLDL induce the production and release of GM-CSF. The pro-inflammatory C-reactive protein also induces M-CSF production and release by endothelial cells and neointimal macrophages. The myeloid-specific growth factors M-CSF and GM-CSF induce, in a autocrine and paracrine manner, neointimal macrophage proliferation through the activation of positive cell-cycle regulators and inhibition of CKIs. **CKIs:** Cyclin-dependent kinase Inhibitors**; GM-CSF:** Granulocyte macrophage colony-stimulating factor; **M-CSF**: Macrophage colony-stimulating factor; **oxLDLs:** oxidized LDLs.

Figure 2. Apoptosis of ER-stressed macrophages in advanced atheromas. Excessive accumulation of oxLDL upregulates LOX-1, which in turn enhances oxLDL uptake and provokes ERS. Prolonged ERS activates ER-related proteins such as ATF-6, IRE-1 and PERK, which initiate the UPR. A key effector of UPR is the transcription factor CHOP, which causes the release of calcium and pro-apoptotic caspases from the ER. CHOP also activates CaMKII, which also promotes apoptosis by triggering the extrinsic (death receptor/Fas) and the intrinsic (mitochondria/caspases) apoptosis pathways. Apoptosis of ER-stressed macrophages in advanced atheromas appears to need a “second hit” induced by PRRs, such as SRA and TLR4, through a process involving STAT1 and CaMKII. **ATF-6:** transcription factor-6; **CaMKII:** calcium/calmodulin-dependent protein kinase II; **CHOP:** Endoplasmic reticulum stress-induced transcription factor; **ERS:** Endoplasmic reticulum stress; **IRE-1:** inositol requiring protein-1; **LOX-1:** oxidized LDL receptor-1; **oxLDLs:** oxidized LDLs; **PERK:** protein kinase RNA-like kinase; **PRRs:** pattern recognition receptors; **SRA:** A-type scavenger receptor; **STAT1:** activator of transcription-1; **TLR4:** toll-like receptor 4.

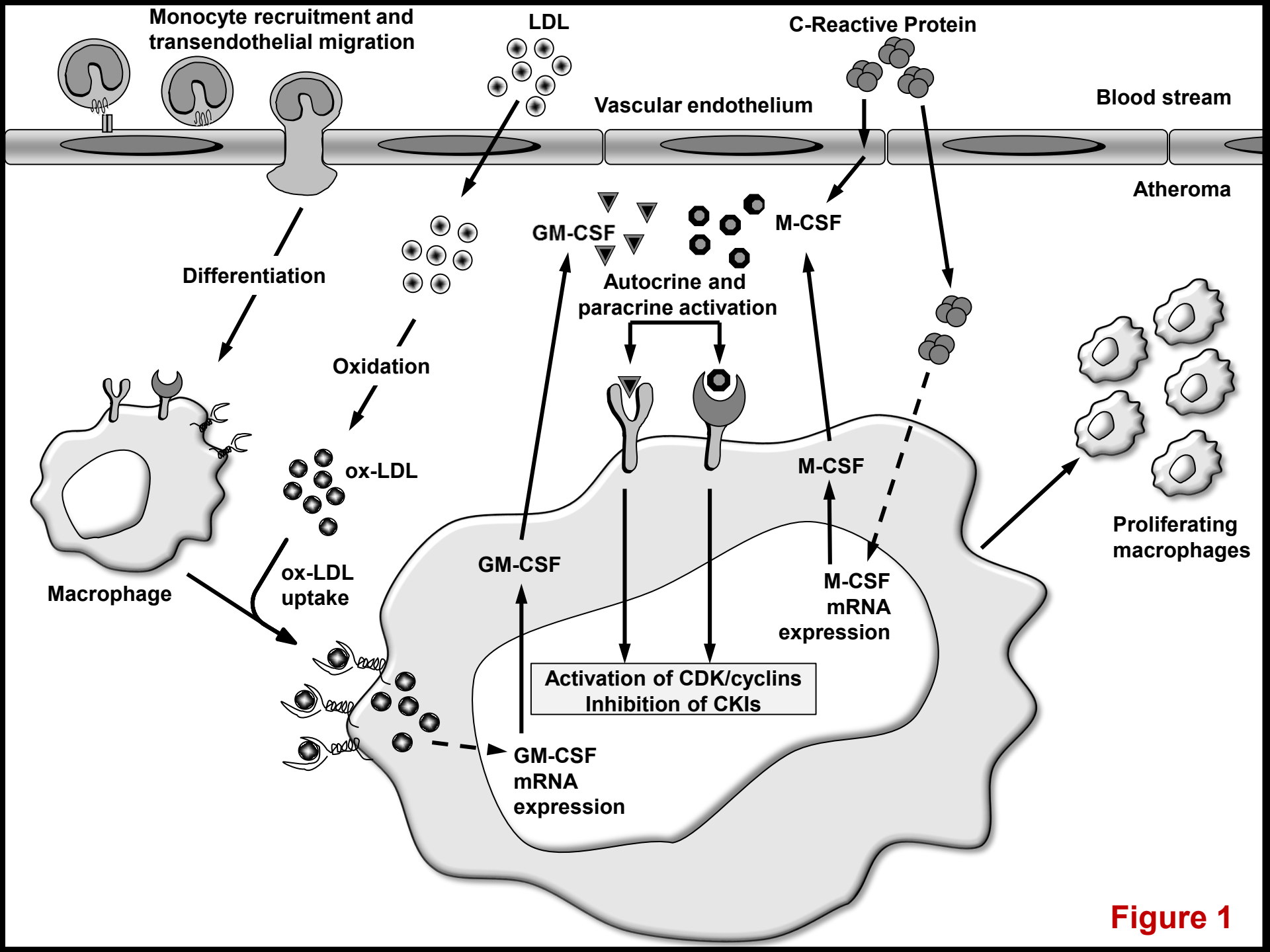
Monocyte recruitment and transendothelial migration
LDL
C-Reactive Protein
Vascular endothelium
Blood stream
Atheroma
Differentiation
Oxidation
GM-CSF
M-CSF
Autocrine and paracrine activation
ox-LDL
Macrophage
ox-LDL uptake
GM-CSF
M-CSF
M-CSF mRNA expression
Activation of CDK/cyclins
Inhibition of CKIs
GM-CSF mRNA expression
Proliferating macrophages

Figure 1

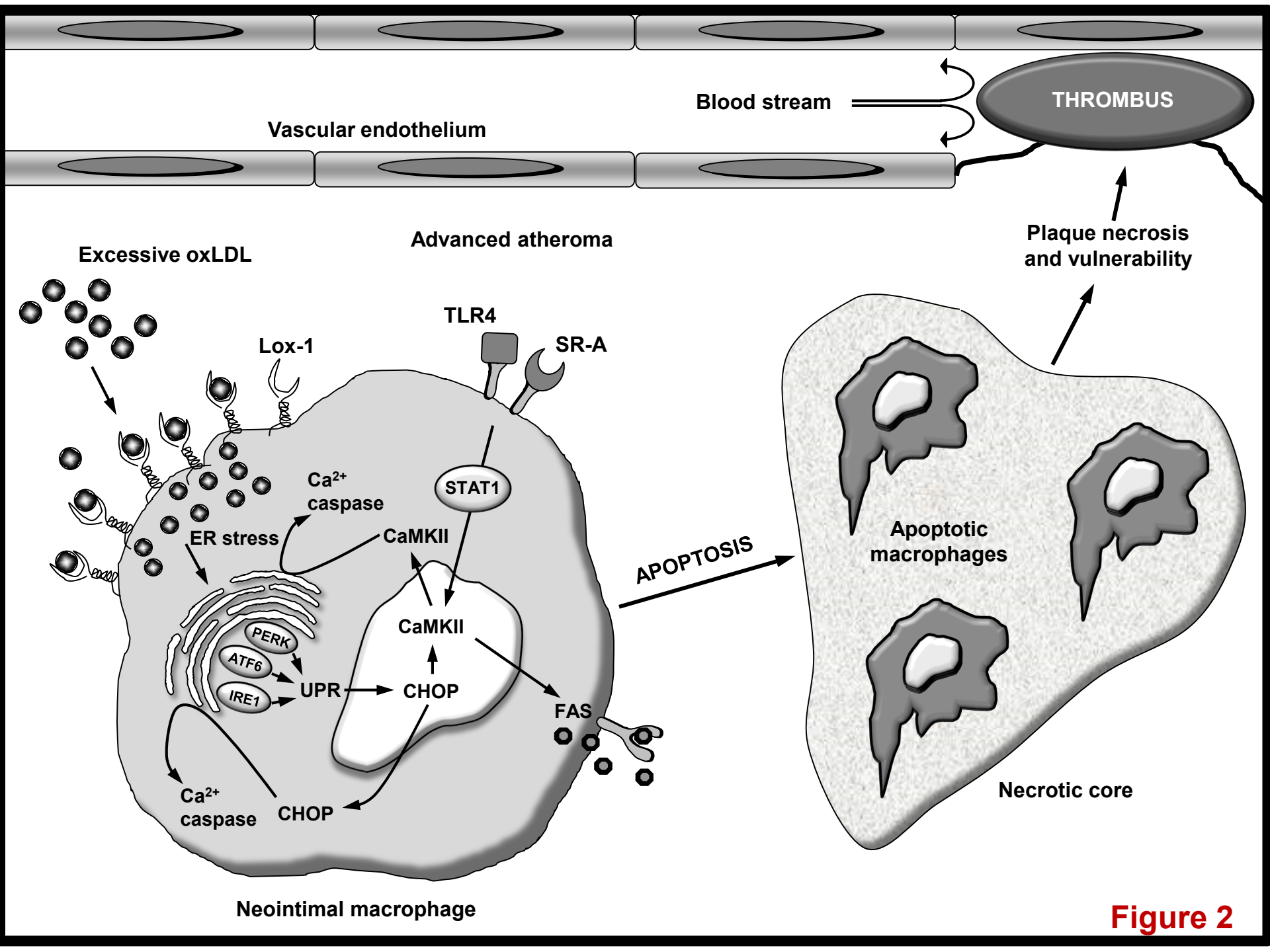
Blood stream
THROMBUS
Vascular endothelium
Advanced atheroma
Plaque necrosis
and vulnerability
Excessive oxLDL
TLR4
Lox-1
SR-A
Ca2+
caspase
STAT1
ER stress
CaMKII
APOPTOSIS
Apoptotic
macrophages
CaMKII
PERK
ATF6
IRE1
UPR
CHOP
FAS
Ca2+
caspase
CHOP
Necrotic core
Neointimal macrophage

Figure 2