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Macrophage proliferation and apoptosis in atherosclerosis

Vicente Andrés, Oscar M. Pello*, Carlos Silvestre-Roig*

Department of Epidemiology, Atherothrombosis and Imaging, Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

* Authors with equal contribution

Send correspondence to:

Vicente Andrés
CNIC
Melchor Fernández Almagro 3, 28029 Madrid, Spain
Telephone: +34-914531200
Fax: +34-914531265
e-mail: vandres@cnic.es
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 p27Kip1 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\textsuperscript{Cip1}, a downstream target of p53. Merched et al. reported that whole-organism or BM–restricted deletion of p21\textsuperscript{Cip1} protects against atherosclerosis in apoE-KO mice [29]. Plaques in mice lacking both p21\textsuperscript{Cip1} and apoE are more stable than plaques in mice lacking only apoE, with increased apoptosis but unaltered cellular proliferation. Interestingly, p21\textsuperscript{Cip1}-null macrophages also show increased phagocytic activity towards latex microspheres and apoptotic cells, suggesting that p21\textsuperscript{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\textsuperscript{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<sup>Cip1</sup> and p27<sup>Kip1</sup> 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<sup>Arf</sup> 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.
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.

6. Acknowledgements

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7. Conflict of interest

None.
8. References


   An excellent review that discusses the role of macrophages during the different stages of atherosclerosis development.

   An excellent review that discusses the four stages of orderly inflammation mediated by macrophages, the protective and pathogenic functions of macrophages, and macrophage heterogeneity in humans.


This study demonstrates for the first time that rapid in situ proliferation of tissue macrophages in Th2-related pathologies increases density of these immune cells. The authors propose that proliferation in situ is an alternative mechanism of inflammation that allows macrophages to accumulate in sufficient numbers to perform critical functions such as parasite sequestration or wound repair in the absence of potentially damaging cell recruitment.


This study demonstrates that the tumor suppressor p19Arf is a critical regulator of lesional macrophage and VSMC apoptosis and lesion burden in the apoE-KO mouse model of atherosclerosis.


This study demonstrates that transplantation of p16^Ink4a/p19^Arf haplodeficient BM into Ldlr-KO mice increases monocyte/macrophage proliferation and Ly6C proinflammatory monocyte accumulation in atheromas, resulting in accelerated atherosclerosis.


This study demonstrates that transplantation of p16^Ink4a-deficient BM into Ldlr-KO mice does not affect plasma lipids, obesity, glucose tolerance or atherosclerosis, despite previous studies showing that this tumor suppressor regulates macrophage polarization and inflammatory signalling.

[34] Smith JD, Trogan E, Ginsberg M et al. Decreased atherosclerosis in mice deficient in both macrophage colony-stimulating factor (op) and apolipoprotein E. Proc Natl Acad Sci U S A 1995; 92:8264-8268.


This study demonstrates that expression of genes located near the chromosome 9p21 locus of atherosclerosis susceptibility are abundantly expressed in human atheromas. Their expression levels show no clear association with 9p21 genotype, but high p16\textsuperscript{INK4a} and low MTAP expression are associated with a less stable plaque phenotype.


[53] Shindo J, Ishibashi T, Yokoyama K et al. Granulocyte-macrophage colony-stimulating factor prevents the progression of atherosclerosis via changes in the cellular and extracellular


This study demonstrates that alleviation of ERS with the chemical chaperone 4-phenyl butyric acid protects against lipotoxic death in macrophages and inhibits atherosclerosis in hyperlipidemic apoE-KO mice. This beneficial effect involves changes in the expression of macrophage fatty acid-binding protein-4 (aP2) and upregulation of liver X receptor.

Using two different mouse models of atherosclerosis, this study provides direct evidence for a causal link between the ERS effector CHOP and plaque apoptosis and necrosis.


Using macrophage-like THP-1 cells, this study shows that activation of ERS is involved in palmitic-acid-induced upregulation of LDL receptor-1 (LOX-1), a scavenger responsible for oxLDL uptake in macrophages. Moreover, oleic acid and linoleic acid are shown to inhibit palmitic-acid-dependent LOX-1 induction through the suppression of ERS.


This paper identifies a novel pro-apoptotic function for the calcium/calmodulin pathway, which links ER stress to the expression of Fas death receptor and mitochondrial-dependent apoptosis via calcium/calmodulin-dependent protein kinase II gamma.


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

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Genetic modification or treatment</th>
<th>Effect on cell proliferation</th>
<th>Effect on atheroma size</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE-KO</td>
<td>p27^{Kip1} global inactivation</td>
<td>Increased macrophage proliferation</td>
<td>Increase</td>
<td>[19]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>p27^{Kip1/-} BMT in p27^{Kip1/+} mice</td>
<td>Increased macrophage proliferation</td>
<td>Increase</td>
<td>[20]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>Macrophage-specific RB protein deficiency</td>
<td>Increased macrophage proliferation</td>
<td>Increase</td>
<td>[23]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>p53 global inactivation</td>
<td>Increased macrophage proliferation (brachiocephalic)</td>
<td>Increase (aorta) None (brachiocephalic)</td>
<td>[21]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>p53 global inactivation</td>
<td>Increased intimal cell proliferation</td>
<td>Increase</td>
<td>[24]</td>
</tr>
<tr>
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<td>Increase</td>
<td>[25]</td>
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<tr>
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<td>p53/- BMT in p53+/+ mice</td>
<td>Increased macrophage proliferation</td>
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<td>[26]</td>
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<td>None</td>
<td>[27]</td>
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<td>p53+/+ BMT in p53/- mice</td>
<td>Reduced macrophage proliferation (brachiocephalic)</td>
<td>Reduction (aorta) None (brachiocephalic)</td>
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</tr>
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<td>None</td>
<td>[28]</td>
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<td>[29]</td>
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<td>apoE-KO</td>
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<td>Reduction</td>
<td>[29]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>p21^{Cip1} global inactivation</td>
<td>Not reported</td>
<td>Increase</td>
<td>[30]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>p19^{Arf} global inactivation</td>
<td>None</td>
<td>Increase</td>
<td>[31]</td>
</tr>
<tr>
<td>Ldlr-KO</td>
<td>p16^{ Ink4a} and p19^{Arf} haplodeficient</td>
<td>Increased macrophage proliferation</td>
<td>Increase</td>
<td>[32]</td>
</tr>
<tr>
<td>Treatment</td>
<td>Effect</td>
<td>Ref.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>--------------------------</td>
<td>-------------------------------</td>
<td>---------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMT in p16(^{ink4}/p19^{Arf}+/+) mice</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Ldlr-KO p16(^{ink4a}) BMT in p16(^{ink4}+/+) mice</td>
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<td>[33]</td>
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<td>[34, 35]</td>
<td></td>
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</tr>
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<td>[36]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ldlr-KO GM-CSF treatment</td>
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<td>[37]</td>
<td></td>
<td></td>
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<tr>
<td>Ldlr-KO Antibody anti-GM-CSF treatment</td>
<td>Reduced intimal cell proliferation</td>
<td>Not reported</td>
<td>[37]</td>
<td></td>
</tr>
<tr>
<td>Ldlr-KO GM-CSF global inactivation</td>
<td>Reduced intimal dendritic cell proliferation</td>
<td>Reduction</td>
<td>[38]</td>
<td></td>
</tr>
<tr>
<td>apoE-KO GM-CSF global inactivation</td>
<td>Increased macrophage plaque content</td>
<td>Increase</td>
<td>[39]</td>
<td></td>
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<tr>
<td>apoE-KO GM-CSF treatment</td>
<td>Not reported</td>
<td>Increase</td>
<td>[40]</td>
<td></td>
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<tr>
<td>Ldlr-KO Hiperlipidemia and hyperglicemia treatment</td>
<td>Increased macrophage proliferation</td>
<td>Increase</td>
<td>[41]</td>
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BMT: bone marrow transplant
Table 2. Role of apoptosis regulators in mouse models of atherosclerosis

<table>
<thead>
<tr>
<th>Mouse model</th>
<th>Genetic modification or treatment</th>
<th>Lesion stage</th>
<th>Effect on apoptosis</th>
<th>Effect on atheroma size</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ldlr-KO</td>
<td>Bax-/- BMT in Bax+/- mice</td>
<td>early</td>
<td>Reduced macrophage apoptosis</td>
<td>Increase</td>
<td>[68]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>p19Arf global inactivation</td>
<td>early</td>
<td>Reduced macrophage and VSMC apoptosis</td>
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<td>[31]</td>
</tr>
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<td>Ldlr-KO</td>
<td>AIM-/- BMT in AIM+/-</td>
<td>early</td>
<td>Increased macrophage apoptosis</td>
<td>Reduction</td>
<td>[69]</td>
</tr>
<tr>
<td>Ldlr-KO</td>
<td>EP4-/- fetal liver cells in EP4+/-</td>
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<td>Increased macrophage apoptosis</td>
<td>Reduction</td>
<td>[70]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>Treatment with the chemical chaperone 4-phenyl butyric acid</td>
<td>late</td>
<td>Reduce macrophage apoptosis</td>
<td>Reduction</td>
<td>[71]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>aP2 global inactivation</td>
<td>late</td>
<td>Reduce macrophage apoptosis</td>
<td>Reduction</td>
<td>[71]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>CHOP global inactivation</td>
<td>late</td>
<td>Reduced intimal cell apoptosis and plaque necrosis</td>
<td>Reduction</td>
<td>[72]</td>
</tr>
<tr>
<td>Ldlr-KO</td>
<td>CHOP global inactivation</td>
<td>late</td>
<td>Reduced intimal cell apoptosis and plaque necrosis</td>
<td>Reduction</td>
<td>[72]</td>
</tr>
<tr>
<td>Ldlr-KO</td>
<td>STAT1-/- BMT in STAT1+/-</td>
<td>late</td>
<td>Reduced macrophage apoptosis and plaque necrosis</td>
<td>None</td>
<td>[73]</td>
</tr>
<tr>
<td>apoE-KO</td>
<td>SR-A and CD36 global inactivation</td>
<td>late</td>
<td>Reduced macrophage apoptosis and plaque necrosis</td>
<td>No affected</td>
<td>[74]</td>
</tr>
</tbody>
</table>

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

Blood stream

Vascular endothelium

Differentiation

Oxidation

ox-LDL

Macrophage

ox-LDL uptake

GM-CSF

M-CSF

Autocrine and paracrine activation

M-CSF mRNA expression

Activation of CDK/cyclins

Inhibition of CKIs

C-Reactive Protein

Atheroma

Figure 1
Blood stream

Vascular endothelium

Advanced atheroma

Excessive oxLDL

Lox-1

ER stress

CaMKII

Ca2+

caspase

FAS

STAT1

TLR4

SR-A

CHOP

UPR

Excessive oxLDL

THROMBUS

Plaque necrosis and vulnerability

Neointimal macrophage

APOPTOSIS

Apoptotic macrophages

Necrotic core

Figure 2