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REVIEW

Molecular and cellular mechanisms of macrophage survival in atherosclerosis

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Abstract Macrophages play a key role in the initiation and progression of atherosclerotic plaques. Although a significant number of macrophages undergoes cell death during plaque development as a result of atherogenic stressors, advanced plaques are characterized by a large macrophage content. Macrophage accumulation is mediated by continuous recruitment of monocytes, reduced emigration of macrophages and poor phagocytosis of dead cells which may trigger secondary necrosis and amplification of plaque inflammation. Moreover, an increasing body of evidence indicates that macrophages have developed several strategies to survive and to proliferate in the adverse environment of an advanced atherosclerotic plaque. Macrophages contain organic molecules or enzymes that provide enhanced antioxidant protection. In addition, synthesis of anti-apoptotic proteins is upregulated and several cellular protection mechanisms such as the unfolded protein response and autophagy are activated in macrophages to promote cellular survival. In this review, we discuss these macrophage survival mechanisms that allow growth and destabilization of advanced atherosclerotic plaques.

Keywords Macrophage · Atherosclerosis · Survival · Cell death

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Introduction

Atherosclerosis is a non-resolving inflammatory disease marked by atheromatous plaques in the intima of medium- and large-sized arteries [108]. Critical to plaque formation is the infiltration of circulating monocytes in the subendothelial space, where they differentiate into macrophages [50, 65]. Subsequently, macrophages internalize modified lipoproteins and turn into foam cells [50, 65]. Although a significant number of macrophage-derived foam cells undergoes apoptosis as a result of prolonged endoplasmic reticulum stress and other pro-apoptotic stimuli, an advanced plaque is characterized by a strong accumulation of macrophages. It should be noted that plaques with a large macrophage content are considered dangerous as they may easily rupture. Indeed, a large body of evidence indicates that macrophages contribute to plaque destabilization through cytokine secretion, the induction of smooth muscle cell (SMC) death and the release of matrix degrading enzymes [19, 24, 65]. A good understanding of the mechanisms leading to macrophage accumulation is therefore important to prevent plaque rupture and life-threatening clinical complications such as myocardial infarction and stroke. Several mechanisms contributing to macrophage accumulation have recently been identified. First, there is a persistent recruitment of monocytes into established atherosclerotic lesions [41, 75], particularly in the setting of hypercholesterolemia [94]. These monocytes can differentiate in two major types of macrophages, those that further promote inflammation, referred to as classically activated (or M1) macrophages, and those that promote resolution, referred to as alternatively activated (or M2) macrophages. In vitro experiments as well as limited in vivo data suggest that M1 and M2 macrophages coexist in human atheromas [7, 10], but an imbalance in this ratio

may cause impaired resolution [25, 26, 55]. A third macrophage subset, namely Mox, has recently been identified in plaques of LDL receptor (LDLR) knockout mice [39], though little is known about their functionality in vivo [10]. Secondly, emigration of macrophages from atheromata is suppressed during hypercholesterolemia [54] and may lead to prolonged production of proteases, cytokines and procoagulant/thrombotic factors. Thirdly, apoptotic macrophages are not effectively cleared by viable phagocytes in advanced lesions [85], a condition known as defective efferocytosis, leading to secondary necrosis and amplification of inflammatory responses [95]. Finally, macrophages have developed several strategies to survive and to proliferate in the adverse environment of an advanced atherosclerotic plaque. In this review, we provide a comprehensive overview of these macrophage survival mechanisms that allow growth and destabilization of advanced atherosclerotic plaques.

Enhanced macrophage survival mediated by modified LDL (Table 1)

An initiating event in atherosclerosis is the accumulation of low density lipoprotein (LDL) in the subendothelial matrix where it undergoes minor modifications such as proteolysis, aggregation, hydrolysis of phosphatidylcholine and cholesteryl esters as well as oxidative changes [72]. Aggregated LDL (agLDL) stimulates foam cell formation in macrophages, but at the same time suppresses apoptosis by downregulating caspase-1 and -3 as well as by upregulating the anti-apoptotic cytokine interleukin-1β [27, 47]. Moreover, a human homologue of bovine ubiquitin-conjugating enzyme E2-25K, termed LIG (LDL-inducible gene), is upregulated in human macrophages after treatment with agLDL [27, 43]. Increased LIG mRNA expression is followed by polyubiquitination and increased ubiquitin-dependent degradation of intracellular pro-apoptotic proteins such as p53 [27, 43]. Because apoptosis caused by oxidized LDL (oxLDL) is p53 dependent [44], increased degradation of ubiquitinated p53 may represent an important anti-apoptotic event in agLDL-laden macrophages.

OxLDL exhibits a dramatic cytotoxic effect to vascular cells leading to apoptosis, as mentioned above, but may also stimulate necrosis and autophagic death [56, 60]. OxLDL is therefore involved in cell loss, formation of a lipid core, plaque rupture and subsequent thrombotic events. However, a large body of evidence indicates that low doses of oxLDL (<100 μg/ml) or short oxLDL treatment (4–6 h) leads to macrophage survival, DNA synthesis, and an enhanced proliferative response to macrophage-colony stimulating factor (M-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF) [32, 33]. Moreover, aggregation of lightly oxidized LDL potentiates dramatically its ability to stimulate macrophage DNA synthesis [31]. Importantly, exposure of macrophages to 100 μg/ml of minimally oxidized LDL induces proliferation and macrophage activation, whereas extensively oxidized LDL induces cell death at the same concentration [33]. Two hundred μg/ml of oxLDL causes cell death regardless of the oxidation degree [33]. These results suggest that oxLDL-induced cell death of macrophages is not self-evident, but indicate that the macrophage response to oxLDL (proliferation or cell death) largely depends on the oxidation degree, aggregation status, exposure time and concentration of oxLDL. Most interestingly, it is the lipid component of oxLDL that promotes macrophage survival and DNA synthesis [31]. As a consequence, also lipids isolated from human plaques promote macrophage survival [31].

Uptake of oxLDL occurs via a group of macrophage scavenger receptors (MSRs) such as class A MSR (MSR-A). Circulating monocytes elaborate MSR-A at undetectable levels, but when the cells differentiate into macrophages, they express high levels of MSR-A. It has been proposed that increased MSR-A expression promotes not only the uptake of lipid components, but also long-term protection of macrophages from apoptosis [52]. Because cholesterol-induced macrophage apoptosis requires engagement of MSR-A [20] and overexpression of MSR-A induces non-macrophage cell apoptosis [110], this hypothesis is unlikely. Indeed, recent evidence suggests that neither MSR-A nor other major pattern recognition receptors including CD36, Toll-like receptor 2 or 4, CD14 and RAGE are responsible for activating the oxLDL prosurvival pathway, and that the anti-apoptotic effect is not dependent on the uptake of oxLDL [79]. Therefore, several alternative mechanisms mediating oxLDL-induced macrophage survival have been proposed, even though not all of them are widely accepted. For example, oxLDL stimulates macrophages to release GM-CSF, which promotes autocrine macrophage growth. GM-CSF expression has been immunohistochemically confirmed in rabbit and human atherosclerotic plaques, and according to some groups, could suppress apoptotic death of macrophages [101]. However, others reported that the prosurvival effect of oxLDL is not inhibited by neutralizing GM-CSF antibodies and that macrophage survival induced by oxLDL does not depend on secretion of growth factors or cytokines [32, 37]. Eukaryotic elongation factor-2 (eEF2) kinase is activated in response to oxLDL, resulting in the inhibition of protein synthesis, and these effects have been considered to be part of a signaling pathway in which oxLDL can block macrophage apoptosis [14]. However, because macrophages in atherosclerotic plaques are stimulated, and not inhibited to

Table 1 Overview of intracellular molecules that contribute to macrophage survival in atherosclerotic plaques

Molecule	Effect	Reference
Protection against modified LDL		
LDL-inducible gene (LIG)	Induces polyubiquitination and ubiquitin-dependent degradation of pro-apoptotic proteins	[27, 43]
PI3K/Akt	Inactivates pro-apoptotic proteins via phosphorylation	
	Causes changes in gene expression through effects on NF-κB and p53	
Toso	Binds the death adaptor FADD and disrupts DISC formation	[92, 68]
	Activates MAPK and NF-κB	
Protection against oxidative stress		
Heme oxygenase	Confers resistance against oxidant stress	[3]
DNA repair enzymes (e.g. Ref-1, PARP-1, DNA-PK, p53)	Prevent accumulation of oxidative DNA damage	[57, 58]
Ascorbate	Oxidant scavenger	[30]
α-tocopherol	Oxidant scavenger	[30]
Glutathione	Oxidant scavenger	[30]
7,8-dihydroneopterin	Scavenger of hydroxyl and peroxyl radicals	[29, 30]
	Decreases CD36-mediated uptake of oxLDL	[29]
3-hydroxyanthranilic acid	Inhibitor of LDL oxidation	[30]
Mer	Anti-apoptotic signaling via Akt en Erk1/2 in response to H_2O_2	[1]
Arachidonic acid	Causes mitochondrial translocation of protein kinase Ca and cytosolic accumulation of Bad and Bax in response to peroxynitrite	[11, 12]
Manganese superoxide dismutase (MnSOD)	Catalyzes dismutation of superoxide anions into water and hydrogen peroxide	[30, 45]
Uncoupling protein 2	Uncouples ATP production from mitochondrial respiration and superoxide anion formation	[5, 102, 103]
Protection against apoptosis		
Bcl-2 or its anti-apoptotic family members	Multiple anti-apoptotic effects	[83, 99]
Members of the inhibitor of apoptosis (IAP) protein family	Inhibit caspase activity	[6]
FLICE-inhibitory protein (FLIP)	Protects against Fas-induced apoptosis	[73]
Short isoform of caspase-2 (caspase-2S)	Multiple anti-apoptotic effects	[59]
p38 MAPK	Activates Akt	[87]
Protection against hypoxia		
Hypoxia-inducible factors (Hifs)	Transcription factors	[74, 91]

undergo apoptosis after treatment with protein synthesis inhibitors [15, 16], also this mechanism seems unlikely. At present, the most plausible explanation for oxLDL-mediated macrophage survival, reported by several groups, is activation of the PI3K/Akt signaling pathway [8, 37]. Akt promotes cell survival directly by its ability to phosphorylate and inactivate several pro-apoptotic targets, including Bad and the forkhead transcription factors. Akt also exerts its anti-apoptotic effects indirectly by changing the expression level of genes that encode components of cell death, such as the Bcl-2 family members and through its effects on NF-κB and p53 [38]. According to recent evidence, activation of Akt by oxLDL requires engagement of the platelet-activating factor (PAF) receptor and a $G\alpha_i$-coupled protein [81]. PAFR is involved in the uptake of oxLDL [80], but may require additional receptors (e.g. CD36) to induce oxLDL-mediated downstream events such as IL-8 and MCP-1 expression. Moreover, immune complexes of IgG with oxLDL engage Fcγ receptors and thereby activate Akt-dependent prosurvival mechanisms, also in monocytes [69]. Finally, it is noteworthy that Akt signaling in macrophages depends to a significant extent on constitutive Ca^{2+} influx, presumably through a mechanism that involves calmodulin and calmodulin-dependent kinase II [97].

Besides aggregation and oxidation, partial enzymatic degradation of LDL by hydrolases secreted by vascular cells may take place in the subendothelium. Macrophages treated with enzymatically modified LDL (E-LDL) upregulate the anti-apoptotic gene Toso and show less apoptosis

[90]. Toso directly binds the death adaptor FADD and this may disrupt the formation of the death-inducing signaling complex (DISC) [92]. Recent evidence showed that the anti-apoptotic function of Toso depends on RIP1 ubiquitination and involves the recruitment of FADD to a Toso/RIP1 protein complex [68]. In response to CD95L and TNFα, Toso also promotes the activation of MAPK and NF-κB signaling pathways [68].

Finally, it is noteworthy that hemoglobin (Hb)-modified LDL (HbLDL) can be found in atherosclerotic plaques. HbLDL is highly susceptible to oxidation and induces heme oxygenase (HO-1) in macrophages due to its lipid peroxide (LOOH) and heme content [3]. HO-1 induction in HbLDL-treated macrophages confers resistance against oxidant stress [3].

Enhanced antioxidant protection (Table 1)

Numerous studies suggest that oxidative stress and the production of intracellular reactive oxygen species (ROS) are key elements in the progression of atherosclerosis. Excessive synthesis of ROS and their byproducts are capable of causing oxidative damage to biomolecules including proteins, lipids and DNA. In accordance with this concept, elevated levels of base modifications and DNA strand breaks have been identified in both human and experimental atherosclerosis [57, 58]. Such damage is of potential pathobiologic significance, because many ROS-induced DNA modifications are promutagenic. DNA strand breaks, on the other hand, may account for a transient or permanent cell cycle arrest, chromosome rearrangements and, in case of extensive damage, induction of cell death or transformation events. Several enzymatic defense systems including base excision repair (Ref-1, PARP-1) or non-specific repair pathways (DNA-PK, p53) are upregulated particularly in plaque macrophages to prevent formation of oxidative DNA damage (Fig. 1) [57, 58]. Moreover, ascorbate, α-tocopherol and glutathione provide a considerable oxidant scavenging capacity to macrophages [30]. Yet, additional antioxidant mechanisms appear to be involved. For example, γ-interferon stimulation of macrophages by Th-1 cells causes the enzymatic breakdown of intracellular GTP to 7,8-dihydroneopterin which can either inhibit protein hydroperoxide formation by scavenging hydroxyl and peroxyl radicals, thereby generating 7,8-dihydroxanthopterin, or by rapidly reacting with the potent oxidant HOCl to form neopterin [30]. 7,8-dihydroneopterin also protects macrophages by scavenging oxidants generated in response to oxLDL and by decreasing CD36-mediated uptake of oxLDL [29]. Apart from 7,8-dihydroneopterin, γ-interferon catalyzes degradation of the amino acid tryptophan to a range of products including 3-hydroxyanthranilic acid (3HAA). This compound is a potent inhibitor of macrophage-mediated LDL oxidation, and thus may also reduce oxLDL concentrations within plaques [30].

Mer tyrosine kinase plays several important roles in normal macrophage physiology such as regulation of cytokine secretion and clearance of apoptotic cells, but mediates also a prosurvival function in macrophages under conditions of oxidative stress. Indeed, Gas6-dependent phosphorylation of Mer has been reported in response to H_2O_2 treatment and leads to increased downstream anti-apoptotic signaling via Akt and Erk1/2 [1]. Protection against peroxynitrite in macrophages is mediated, at least in part, by cytosolic phospholipase A(2)-released arachidonic acid. This lipid messenger is metabolized by 5-lipoxygenase to 5-hydroxyeicosatetraenoic acid and causes the mitochondrial translocation of protein kinase Cα, an event associated with cytosolic accumulation of Bad and Bax [11, 12]. Under these conditions, the anti-mitochondrial permeability transition (MPT) activity of Bcl-2 is fully active and promotes macrophage survival. Finally, it is noteworthy that manganese superoxide dismutase (MnSOD) is upregulated in macrophages of atherosclerotic plaques [45]. This enzyme catalyzes the dismutation of superoxide anions into water and hydrogen peroxide. The latter compound is then broken down by catalase or glutathione peroxidase [36]. Potential triggers of MnSOD are TNF-α, IL-1 and oxLDL [30].

Probably the most important biological source of superoxide anions is the mitochondrial electron transport chain. Mitochondria possess a mechanism called 'mild' uncoupling, which reduces the production of ROS by the respiratory chain. This mechanism is in part regulated by the mitochondrial uncoupling protein 2 (UCP2) that uncouples ATP production from mitochondrial respiration and thereby converts the loss of potential energy in heat production [103, 106]. Differentiation of monocytes into macrophages increases UCP2 expression [102]. In line with this finding, it has been observed that UCP2 is abundantly expressed in subendothelial macrophages of atherosclerotic plaques and provides protection against oxidative damage [102]. Conversely, lack of UCP2 in macrophages accelerates atherosclerotic plaque development [5].

Enhanced production of anti-apoptotic proteins (Table 1)

Apoptosis is a major event in advanced atherosclerosis targeting all cell types, including foam cells of macrophage origin [65]. Inhibition of macrophage apoptosis can occur by upregulating the multidomain protein Bcl-2 or one of its anti-apoptotic family members (Bcl-xL, Bcl-w, Mcl-1, A1/Bfl-1, Boo/Diva and NR-13). In particular, Bcl-2 is a

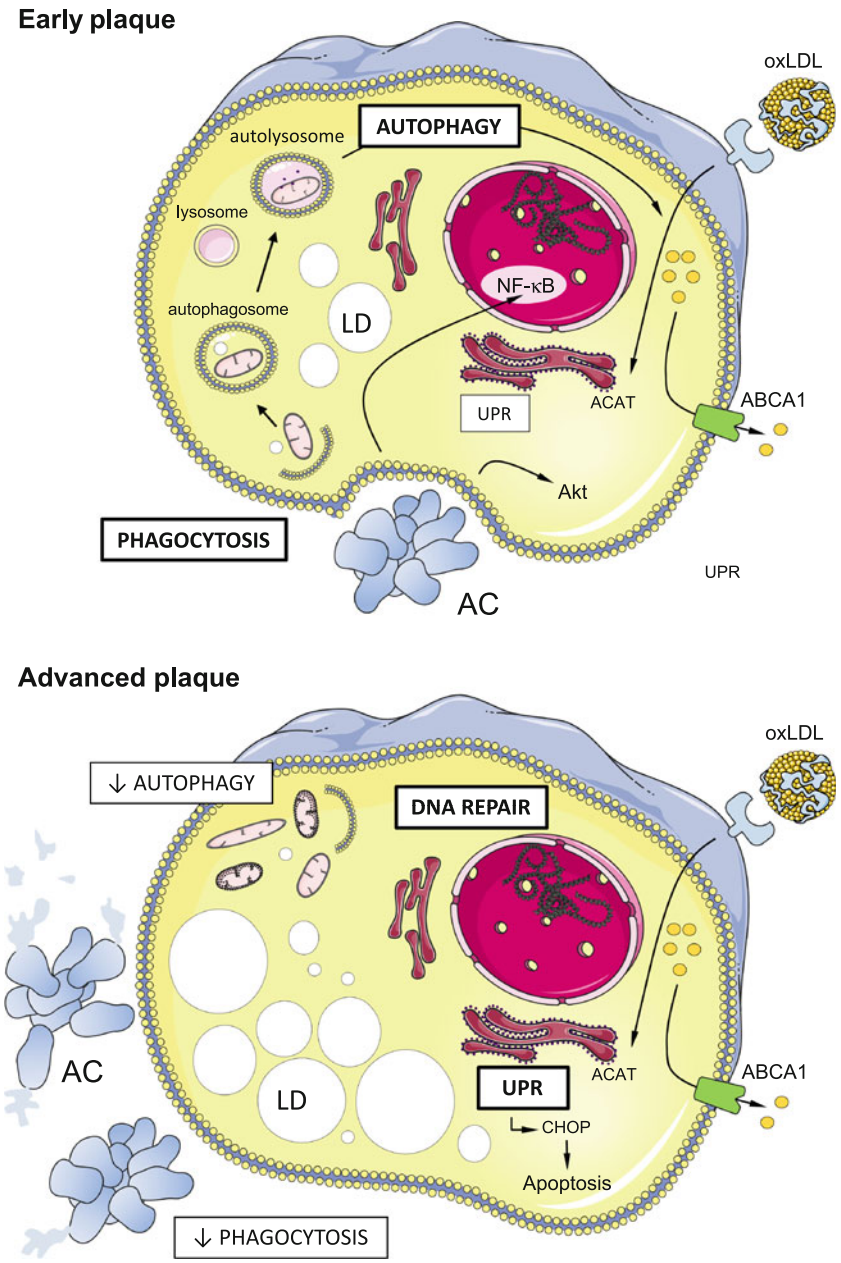

Fig. 1 Schematic overview of the cellular mechanisms that promote macrophage survival in early and advanced atherosclerotic plaques. In early plaques, activation of autophagy protects macrophages against adverse environmental effects (e.g. oxidative stress) by removing damaged proteins and organelles. Moreover, lipid droplets (LD) are a substrate for autophagy. Cholesteryl esters in LD are hydrolyzed in autolysosomes to generate free cholesterol for efflux via cholesterol transporter ABCA1. Apart from autophagy, the unfolded protein response (UPR) is activated in early plaques to protect macrophages against apoptosis by ER stress-inducing agents such as the accumulation of free cholesterol in the ER membrane. Macrophages esterify the cholesterol and try to prevent its toxic effects through efficient trafficking of the internalized cholesterol to acyl-CoA:cholesterol acyltransferase (ACAT) in the ER. If apoptosis would occur, apoptotic cells (AC) are rapidly cleared via phagocytosis. This event protects against cell death via several survival pathways involving PI3K/Akt and NF-κB. In advanced plaques, both autophagy and AC phagocytosis are defective. Defective autophagy results in the accumulation of damaged organelles and may sensitize macrophages to undergo cell death. Free, nonphagocytised AC will undergo secondary necrosis, contribute to the enlargement of the necrotic core and promote plaque progression. Furthermore, overstimulation of the UPR may occur in advanced plaques, which may lead to synthesis of the protein CHOP and induction of apoptosis. Because ROS-induced base modifications and DNA strand breaks frequently occur in advanced lesions, several enzymatic defense systems including base excision repair or non-specific repair pathways are upregulated, particularly in macrophages, to prevent formation of oxidative DNA damage or chromosome rearrangements

key cell survival molecule and its almost all-round protective capacity has been interpreted as the result of an enormous variety of anti-apoptotic effects. Activities of Bcl-2 include a reduction in ROS levels [35], stabilization of lysosomal and mitochondrial membrane integrity [93, 112], enhancement of the proton efflux from mitochondria [89], regulation of intracellular calcium homeostasis [48], and prevention of cytochrome *c* release from mitochondria [111]. According to an immunohistochemical study using human coronary and carotid plaques, expression of Bcl-2 is similar in both control and atherosclerotic specimens, while the expression of Bcl-xL is higher in the plaque [83]. These findings suggest that Bcl-xL, but not Bcl-2, may act as an inducible protective factor in plaque macrophages. Indeed, recent evidence shows that the inactivation of the Bcl-x gene in macrophages stimulates their sensitivity to apoptosis, and results in more advanced atherosclerotic lesions in apoE-deficient mice [88]. However, even at basal levels, also Bcl-2 plays a protective role against macrophage apoptosis, specifically in advanced lesions, as macrophage-targeted deletion of Bcl-2 in apoE-deficient mice results in a 40–45 % increase in apoptotic cells [99].

Besides anti-apoptotic Bcl-2 proteins, macrophage apoptosis can be inhibited by the members of the inhibitor of apoptosis (IAP) protein family such as cIAP1, cIAP2, XIAP, NAIP, livin, survivin and apollon, which inhibit caspase activity. Survivin is clearly detectable by immunohistochemistry in macrophages infiltrating early lesions, but absent in advanced plaques [6]. In contrast, XIAP and particularly cIAP2 are both strongly upregulated in advanced plaques. Macrophages in culture upregulate survivin after stimulation with M-CSF [6], an inflammatory growth factor released by the atherosclerotic vessel wall. Conversely, prolonged treatment with oxLDL abolishes macrophage survivin expression and triggers apoptosis [6]. Raising XIAP or cIAP2 is not sufficient to block oxLDL-induced apoptosis, even though XIAP is important for macrophage survival. Survivin may thus have a biphasic role in atherosclerosis: it may promote macrophage survival and plaque progression in the early stages of atherosclerosis, but loss of survivin expression in advanced plaques, possibly due to prolonged oxLDL exposure, may contribute to apoptosis and plaque instability.

Next to the activity of IAPs, caspases can also be regulated by blocking their activation. For example, FLICE-inhibitory protein (FLIP), a caspase-8 homolog lacking proteolytic activity, is upregulated during macrophage differentiation and confers resistance to Fas-mediated apoptosis [73]. Furthermore, macrophages around the necrotic core of advanced human plaques show enhanced levels of the short isoform of caspase-2 (caspase-2S) that acts as an endogenous inhibitor of apoptotic cell death [59]. Indeed, overexpression of caspase-2S can inhibit nuclear changes associated with apoptosis [23]. Caspase-2S also prevents the maturation of apoptotic bodies, delays phosphatidylserine externalization on the plasma membrane of dying cells and prevents cleavage and activation of procaspase-2L [23]. Interestingly, the treatment of macrophages in culture with the DNA strand-breaking agents etoposide or camptothecin stimulates caspase-2S expression [59]. Given the high number of DNA strand breaks in advanced plaques [57, 58], these findings provide evidence for a survival factor in macrophage-derived foam cells of human atherosclerotic plaques that may be upregulated in response to DNA damage.

Induction of the unfolded protein response

Nascent proteins of the secretory pathway or proteins designed for trafficking to the cell membrane are post-translationally modified and folded in the endoplasmic reticulum (ER). Chaperones such as the glucose-regulated protein 78 (GRP78/BiP) are present in the ER lumen and associate with the newly synthesized peptides to improve their maturation. When folding is unsuccessful, the malfolded proteins are directed towards the cytoplasm where they are recognized by the proteasome and degraded. However, in stressful conditions, when there is an overload of malfolded proteins, a unique signaling cascade referred to as the unfolded protein response (UPR) is activated that confers cytoprotective advantage [34] (Fig. 1). Indeed, in vitro experiments showed that UPR activation occurs after monocyte to macrophage differentiation, particularly after a significant increase in de novo protein synthesis, and reduces macrophage cell death by ER stress-inducing agents [21]. Moreover, UPR markers are dramatically increased in very early intimal macrophages [113]. Because these cells rarely undergo apoptosis, it has been suggested that UPR activation is an important cellular mechanism for macrophage survival within early atherosclerotic lesions [21]. Interestingly, advanced lesions contain a number of molecules (e.g. oxidized lipids, saturated fatty acids) and processes (e.g. oxidative stress) that are known ER stressors [96]. Consistent with this finding, markers of UPR activation are not only markedly upregulated in macrophages from early lesions, but also in macrophage foam cells from fatty streaks and advanced atherosclerotic lesions [113]. However, unlike the UPR in early intimal macrophages, activation of the UPR in macrophages of advanced plaques leads to elevated levels of CHOP which triggers apoptosis execution pathways through a number of mechanisms including NADPH oxidase activation and subsequent ROS generation (Fig. 1) [96]. Using CHOP-deficient mice crossbred with ApoE or LDLR knockout mice, a causal link between CHOP expression, apoptosis and plaque necrosis

has been demonstrated [98]. Thus at an early stage, the UPR may protect macrophages against death, but when ER stress is prolonged or severe, it causes cell death and this makes plaques more vulnerable and even prone to rupture.

Stimulation of autophagy (Fig. 1)

Autophagy is a subcellular mechanism for bulk destruction of cytoplasmic components such as protein aggregates and complete organelles via lysosomes. It occurs at basal levels in most tissues, but is activated by environmental stimuli (such as nutrient deprivation, low oxygen and hormones) or stress-related signals (e.g. accumulation of mutant proteins) [64]. The autophagic process thus maintains the balance between biogenesis and production of cell organelles, and destroys unwanted or damaged intracellular structures. In this way, cell survival is promoted in an unfavorable environment. LDLR knockout mice with a macrophage-specific deletion of the essential autophagy gene Atg5 develop plaques with increased apoptosis and oxidative stress as well as enhanced plaque necrosis, indicating that autophagy is anti-apoptotic and present in atherosclerotic plaques to protect macrophages against various atherogenic stressors including 7-ketocholesterol, ROS and inducers of ER stress (Fig. 1) [53]. Also of note, lipid droplets in macrophage foam cells are delivered to lysosomes via autophagy, where lysosomal acid lipase acts to hydrolyze lipid droplet-derived cholesteryl esters in order to generate free cholesterol mainly for ABCA1-dependent efflux (Fig. 1) [70]. This process is specifically induced upon macrophage cholesterol loading and may contribute to macrophage survival. Recent evidence suggests that basal levels of macrophage autophagy play an essential role in atheroprotection during early atherosclerosis [77]. However, autophagy becomes dysfunctional in the more advanced stages of atherosclerosis (Fig. 1) and its deficiency promotes atherosclerosis in part through activation of the inflammasome [77]. Autophagy can be intensified selectively in macrophages by specific drugs such as mammalian target of rapamycin (mTOR) inhibitors (e.g. everolimus) [62, 107] or Toll-like receptor 7 (TLR7) ligands (e.g. imiquimod) [19]. However, moderate drug-induced stimulation of macrophage autophagy via everolimus or imiquimod may trigger cytokine production, though in an autophagy-independent manner (via activation of p38 MAP kinase or NF-κB, which are off-target effects of everolimus and imiquimod, respectively) [19, 63]. As a consequence, imiquimod stimulates expression of vascular adhesion molecule-1, infiltration of T-lymphocytes, accumulation of macrophages and plaque enlargement [19]. These findings suggest that both inhibition and moderate drug-induced stimulation of macrophage autophagy is detrimental for the structure of the plaque. Nonetheless, extensive stimulation of macrophage autophagy, as demonstrated after stent-based delivery of everolimus, causes degradation of major portions of the cytosol which in turn leads to autophagy-mediated, non-apoptotic cell death and a marked reduction in macrophage content [62, 63, 107]. Given the numerous detrimental effects of macrophages in advanced plaques, this approach seems to be a promising strategy for the treatment of coronary artery disease [86].

Other survival strategies

In addition to the strategies described above, many other cellular processes keep macrophages alive during atherogenesis. For example, phagocytic clearance of apoptotic cells by macrophages is very efficient in early plaques and protects against cell death (Fig. 1) [17, 78]. Phagocytosis of apoptotic cells also profoundly inhibits the proliferation of macrophages stimulated to proliferate by growth factors such as M-CSF [78]. The ability of apoptotic cells to promote macrophage survival and to inhibit proliferation appears to be the result of several survival pathways involving PI3K/Akt and NF-κB as well as inhibition of Erk1/2 [17, 78]. Conversely, phagocytosis of apoptotic cells in advanced plaques is defective and may result in secondary necrosis, expansion of the necrotic core and increased plaque vulnerability [85, 95]. Mechanisms that are responsible for defective clearance of apoptotic cells in advanced plaques include enhanced levels of oxidative stress [84, 85], competition with oxLDL for binding on macrophages [84], impaired autophagy [51, 76] and loss of UCP2 protein in deeper layers of the plaque [71, 102].

In contrast to phagocytosis of apoptotic cells, phagocytosis of modified LDL leads to large intracellular amounts of free cholesterol, which can be cytotoxic if not handled properly. Indeed, free cholesterol accumulates in the ER membrane, triggers ER dysfunction and activates an UPR-associated apoptotic response. Macrophages esterify the cholesterol and try to prevent its toxic effects through efficient trafficking of the internalized cholesterol to acyl-CoA:cholesterol acyltransferase (ACAT) in the ER [13]. Even when ACAT is rendered dysfunctional, as might occur in advanced atherosclerotic plaques, macrophages often remain viable through massive efflux of cholesterol via cholesterol efflux transporters such as ABCA1 and ABCG1.

Monocytes express the chemokine receptor CX_3CR1 that binds to its ligand CX_3CL1, also known as fractalkine. Absence of either CX_3CR1 or CX_3CL1 leads to reduced circulating monocyte numbers in mice, particularly the $Gr1^{low}$ subset that displays significantly higher CX_3CR1 amounts [49]. Enforced expression of the human Bcl-2 transgene rescues the wild-type phenotype [49], suggesting

that binding of CX3CL1 to its receptor is an important survival signal that promotes monocyte homeostasis and atherogenesis. Because there are significant differences in signaling downstream of mouse versus human CX_3CR1, it remains to be determined whether the survival function of CX_3CL1, as shown in several murine disease models, is also applicable in humans [109]. Moreover, the mechanism of monocyte survival via the CX_3CR1–CX_3CL1 axis is unclear and needs to be investigated [109].

Another important issue is that advanced atherosclerotic plaques reveal areas of low pH or low oxygen. A reduction in extracellular pH from 7.4 to 7.0 inhibits oxLDL-induced apoptosis of macrophages in vitro [28]. This inhibition of apoptosis is attributable at least in part to a decrease in the binding of oxLDL to cell surface receptors at low pH, thereby reducing endocytosis of oxLDL [28]. On the other hand, hypoxia prolongs macrophage survival by enhancing glycolysis [82]. Moreover, hypoxia mediates accumulation of hypoxia-inducible factors (Hifs) such as Hif-1α in macrophages [91]. Hifs are key proteins of the cellular adaption to low oxygen tensions. Recent evidence, however, showed that inhibition of Hif-signaling in combination with oxLDL treatment dramatically induces apoptosis independently of the oxygen tension [74]. This finding suggests that the Hif pathway is also involved in blocking the apoptotic effects of oxLDL.

Finally, it is noteworthy that prostaglandin E2, a major product of activated macrophages, regulates macrophage survival through EP4 receptor-dependent activation of PI3K/Akt and NF-κB signaling pathways. EP4 deficiency compromises macrophage survival and suppresses early atherosclerosis in LDLR knockout mice [4]. Furthermore, p38 MAPK is activated during atherogenesis [66]. Although p38 MAPK has been shown to play a crucial role in regulating apoptosis, p38 can have both pro- and anti-apoptotic functions depending on the apoptotic stimulus. Recent evidence showed that genetic ablation of p38 in macrophages suppresses activation of Akt and leads to enhanced macrophage apoptosis in advanced plaques [87]. These data demonstrate an important prosurvival role for p38 in macrophages during atherosclerosis.

Concluding remarks

Macrophages in atherosclerotic plaques possess a remarkable ability to survive different types of cellular stress. In this way, macrophages may accumulate in the vascular wall and contribute to plaque development. Because the mechanisms of macrophage survival are very diverse and not necessarily restricted to macrophages, inhibition of prosurvival pathways is probably not an ideal approach to prevent macrophage accumulation and plaque progression. Nonetheless, macrophages have become an excellent therapeutic target and interventions mediating macrophage loss can yield beneficial results [100]. Promising therapeutic strategies that provoke macrophage elimination in different stages of atherosclerosis include (1) lipid lowering, either in the circulation or from macrophages in the plaque, (2) direct and indirect targeting of adhesion molecules and chemokines involved in monocyte adhesion and transmigration, or (3) selective induction of macrophage death [18]. Even though these approaches are promising in animal studies (but not all, see for example [19, 104]), clinical trials are so far disappointing due to side effects or the lack of additional benefits [18]. Nonetheless, macrophages remain more than ever an important focus for ongoing research, not only for macrophage-specific delivery of drugs which may help to reduce adverse effects [2, 40], but also for modulation of the macrophage phenotype with growth factors or other compounds [9, 46], and for the detection or imaging of vulnerable plaques [22, 105]. Because the promotion of macrophage death (or the inhibition of macrophage survival) in atherosclerosis remains controversial [61], and hitherto, nobody really knows whether this strategy is beneficial for plaque regression, inhibition of monocyte infiltration or monocyte retention in the neointima may represent the best clinical targets, as demonstrated recently [42, 67, 75].

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