Phagocytosis in atherosclerosis: Molecular mechanisms and implications for plaque progression and stability

Dorien M. Schrijvers⁎, Guido R.Y. De Meyer, Arnold G. Herman, Wim Martinet

Division of Pharmacology, University of Antwerp, Universiteitsplein 1, B-2610 Wilrijk, Belgium

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Abstract

Macrophages play a key role in atherosclerotic plaque destabilization and rupture. In this light, selective removal of macrophages may be beneficial for plaque stability. However, macrophages are phagocytic cells and thus have an important additional role in scavenging of modified lipoproteins, unwanted or dead cells and cellular debris via phagocytosis. The concept of phagocytosis as well as the underlying mechanisms is well defined but the effect of phagocytosis in terms of plaque stability remains poorly understood. Recent findings point towards a complex role of macrophage phagocytosis in atherogenesis. Macrophages are necessary for removal of apoptotic cells from plaques, but exert strong proatherogenic properties upon phagocytosis of lipoproteins, erythrocytes and platelets. Apart from heterophagy, autophagocytosis better known as autophagy may occur in advanced atherosclerotic plaques. Several lines of evidence indicate that autophagy is initiated in plaque smooth muscle cells as a result of cellular distress. Since autophagy is well recognized as a survival mechanism, autophagic smooth muscle cells in the fibrous cap may reflect an important feature underlying plaque stability. All together, phagocytosis is a crucial process involved in atherogenesis that may significantly affect the stability of the atherosclerotic plaque.

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1. Introduction

One of the earliest events in atherosclerosis is the entry of monocytes into the arterial intima [1]. These monocytes differentiate into macrophages and start to accumulate large amounts of lipid through the uptake of modified lipoproteins which results in foam cell formation. Macrophage-derived foam cells in turn stimulate smooth muscle cell (SMC) migration from the media into the intima, and in this way contribute to the progression of atherosclerosis. At this point, the atherosclerotic lesion is considered stable. It consists of a small necrotic core, if present at all, separated from the blood flow by a firm fibrous cap of SMCs and extracellular matrix. However, the release of matrix metalloproteinases by macrophages in the plaque leads to progressive collagen break-down and thinning of the fibrous cap. They also produce pro-inflammatory cytokines with pro-apoptotic potential which may contribute to cell death of surrounding cells. Accordingly, stable atherosclerotic lesions slowly progress over a period of decades to advanced and unstable lesions. These lesions are characterized by a thin fibrous cap containing few SMCs overlying a large necrotic core composed of dead cells, lipid deposits and cellular debris. In contrast to stable lesions, advanced human atherosclerotic plaques also show intraplaque neovascularization. These newly formed vessels are fragile and often reveal signs of leakage, thereby introducing platelets and red blood cells into the plaque [2].

The presence of macrophages, however, may not be entirely detrimental for the structure of the plaque. Macrophages are also professional phagocytes and thus designed to remove pathogens, small particles as well as dead or damaged cells from the atherosclerotic tissue via phagocytosis. Although the concept of this process is known for decades...
and the mechanisms underlying phagocytosis are well defined [3], the link with the pathogenesis of atherosclerosis remains poorly understood. In this review, we have outlined some of the most interesting recent findings concerning phagocytosis of lipoproteins, apoptotic cells, red blood cells and platelets by macrophages in atherosclerotic plaques and how this may affect plaque progression and stability. Furthermore, we present evidence for the initiation of autophagocytosis in atherosclerosis.

2. Phagocytosis of lipoproteins

Low density lipoproteins (LDL) are the main source of excess cholesterol deposition in foam cells. In early lesions, foam cells are derived from macrophages, but in more advanced lesions, also SMCs can undergo foam cell transformation. The latter phenomenon can be explained, at least in part, by the finding that hypoxia, due to increased wall thickness and reduced oxygen diffusion [4], significantly increases LDL uptake and lipid accumulation in SMCs [5]. Because uptake of native LDL via the LDL receptor (LDLR) is subject to negative feedback regulation, accumulation of excessive amounts of LDL-derived cholesterol by macrophages is thought to require modification of LDL that permits rapid unregulated internalization [6]. Several types of LDL modification have been shown to permit cholesterol uptake in vitro, which includes acetylation, oxidation and aggregation, producing acetylated LDL (AcLDL), oxidized LDL (oxLDL) and aggregated LDL (agLDL), respectively [7–9]. Acetylation of LDL in vitro can induce cholesterol accumulation in macrophages. However, there is no evidence that acetylation of LDL occurs to any extent in vivo. In contrast, oxidative modification of LDL, catalyzed by all contrast, oxidative modification of LDL, catalyzed by all

acLDL and oxLDL are both ligands for the scavenger receptor class A type I/II (SRA). However, there is considerable evidence for the existence of morphologically and functionally distinct uptake pathways that deliver these two ligands to different endocytic compartments. This is most likely due to the involvement of different receptors. Approximately 80% of acLDL phagocytosis is mediated by SRA, whereas 70% of the oxLDL uptake is mediated by receptors different from SRA (e.g. CD36 [14], macrosialin/CD68 [15] and lectin-like oxidized low density lipoprotein receptor-1 (LOX-1) [16]) (Fig. 1). Mild oxidation of LDL produces minimally modified LDL (mmLDL), which is still recognized by the LDL receptor but not by the scavenger receptors. It binds specifically to CD14 (LPS receptor) and induces enhanced CD36 expression, which leads to enhanced uptake of oxLDL [17]. In SMCs, the scavenger receptors are barely expressed, but LDL receptor-related protein 1 (LRP1) is

![Fig. 1. Phagocyte recognition of lipoproteins (LDL), apoptotic cells (AC), red blood cells (RBC) and platelets (PLT). Several receptors on the phagocyte membrane are involved in the phagocytic process. They interact either directly with their ligands or via bridging molecules. CD14, lipopolysaccharide receptor; LOX-1, lectin-like oxidized low density lipoprotein receptor-1; CD36, thrombospinrin receptor; CD68, macrosialin; SRA, scavenger receptor class A; FcR, Fc fragment of immunoglobulin G receptor; LRP, LDL receptor-related protein; SREC, scavenger receptor of endothelial cells; MARCO, macrophage receptor with collagenous structure; CD91, calreticulin; GAS-6, growth arrest-specific gene 6; β2-GPI, beta 2-glycoprotein 2; ABC-1, ATP-binding cassette-1; TSP, thrombospinrin; PS, phosphatidylserine; PSR, PS receptor; αβ, vitronectin receptor.](https://academic.oup.com/cardiovascres/article-abstract/73/3/470/367562)
and accumulates along with oxLDL cholesterol within the oxLDL protein is partially resistant to lysosomal degradation compartments, a process called patocytosis [19]. The process that delivers agLDL into a labyrinth of surface-connected this type of cells. A different mode of agLDL entry has been terolemia and mediates uptake of aggregated lipoproteins in highly expressed [18]. LRP1 is upregulated by hypercholes-
terolemia [20]. This accumulation is, at least partly, responsible for the transformation of macrophages into foam cells. Foam cell formation in atherosclerotic plaques is initiated when the cholesterol removal becomes limited, either because the amount of plasma cholesterol that enters the arterial wall is too large for macrophages to process, or because the ability of macrophages to excrete cholesterol becomes limited (e.g. low bio-availability of high density lipoproteins [HDL]). Therefore, by lowering the level of plasma lipoproteins or by increasing the plasma levels of HDL, accumulation and phagocytosis of excess amounts of lipoproteins in the arterial wall are prevented.

It is generally well accepted that accumulation of lipoproteins in the vessel wall, in particular oxLDL, contributes to apoptotic and/or necrotic cell death [21]. It remains, however, unclear whether oxLDL stimulates cell death upon binding to the cell surface or after phagocytosis. Recent evidence suggests that oxLDL-induced macrophage cell death does not require uptake of oxLDL and probably occurs upon binding to the cell surface. Firstly, it is known that aggregation enhances uptake of oxLDL by macrophages via SRA and that it decreases oxLDL-induced cell injury [22]. Secondly, inhibitors of phagocytosis such as cytochalasin B and D and latrunculin A, prevent uptake of oxLDL and subsequently restore oxLDL-induced cytotoxicity [22]. In this regard, enhanced phagocytosis of oxidized and/or aggregated LDL not only contributes to foam cell formation, but also to plaque progression and formation of an unstable plaque phenotype due to macrophage survival. In line with these findings it is important to note that oxLDL stimulates endothelial cells to produce a number of pro-inflammatory molecules such as adhesion molecules and growth factors, resulting in the recruitment of monocytes to the vessel wall [23]. In addition, oxLDL modulates gene expression in macrophage-derived foam cells [24]. Pro-inflammatory genes such as interleukin-8 (IL-8) are upregulated whereas tissue inhibitors of metalloproteinases (TIMPs) are down-regulated. Furthermore, macrophage-derived foam cells isolated from hypercholesterolemic rabbits produce reactive oxygen species (ROS) that activate the latent zymogen of matrix metalloproteinase-2 [25], thereby inducing a shift towards weakening of the fibrous cap. At present, it is unknown whether these effects occur upon binding of oxLDL to macrophages or after phagocytosis.

Despite the detrimental effects of foam cell formation in plaque macrophages on atherogenesis, approaches that target pharmacological management of atherosclerosis through the use of acyl-coenzyme A:cholesterol acyltransferase 1 (ACAT1) inhibitors and inhibition of foamy cell formation paradoxically report increased atherosclerosis [26,27]. ACAT1 esterifies free cholesterol after intracellular degra-
dation of lipoproteins so that it can be stored as lipid droplets in macrophages. Inhibition of ACAT1 may reduce the cholesterol burden in plaque macrophages through increased efflux of free cholesterol into the HDL pathway, but inefficient efflux or compartmentalization of free cholesterol to sites disconnected from the efflux machinery may induce macrophage apoptosis owing to the toxic effects of free cholesterol [28]. Increased generation of apoptotic cells may stimulate atherogenesis as outlined below.

3. Phagocytosis of apoptotic cells

Advanced atherosclerotic lesions are characterized by the presence of apoptotic cells (AC) derived from a variety of cell types, including macrophages, T lymphocytes and SMCs [29]. Mechanisms involved in the initiation of apoptosis include accumulation of high concentrations of intracellular free cholesterol and extracellular oxLDL, production of pro-apoptotic cytokines such as tumor necrosis factor-α (TNF-α) and the release of excessive amounts of ROS by macrophages, growth factor withdrawal, hypoxia and direct cell–cell interactions (e.g. binding of Fas-ligand to Fas) [30]. When cells undergo apoptosis, they are rapidly recognized and phagocytized by professional phagocytes, such as macrophages and dendritic cells. Phagocytosis of AC inhibits the production of pro-inflammatory cytokines by macrophages such as IL-1β, IL-8 and TNF-α through a suppressive mechanism involving the autoinframe/paracrine secretion of transforming growth factor-β (TGF-β) [31,32]. TGF-β secretion in turn inhibits recruitment of circulating monocytes [33]. In addition, AC phagocytosis strongly inhibits expression of the pro-inflammatory IL-12 family of cytokines (IL-12, IL-23, IL-27) whereas the production of the anti-inflammatory cytokine IL-10 is stimulated [34]. In this way, tissues are protected from harmful exposure to inflammatory responses as well as from the immunogenic content of the dying cells after undergoing postapoptotic necrosis.

AC express many cell surface changes which may serve as “eat me” signals for recognition by macrophages (Fig. 1). A large number of surface molecules, ligands and receptors have also been identified on the macrophage [3,35–38]. The best characterized recognition mechanism is the exposure of phosphatidylserine (PS) on AC [39–42], directly followed by binding of PS to the PS receptor on the macrophage membrane [43,44]. Interestingly, a number of PS-binding proteins exist that can act as a bridge between AC and macrophages (Fig. 1). Among these bridge molecules, it is worth mentioning (i) plasma–protein β2-glycoprotein 1 (β2-
GPI), (ii) growth arrest-specific gene 6 (Gas6) protein that binds to the Mer kinase and (iii) milk-fat globule epidermal growth factor 8 (MFG-E8, also known as lactadherine) that connects vitronectin receptor integrin (αvβ3) to PS (Fig. 1). This multiplicity of ways in which phagocytes recognize and engulf AC suggests that a hierarchy of engulfment mechanisms and back-up systems may exist. Indeed, upon blocking PS with a specific antibody or annexin V, phagocytosis of AC decreases but this inhibition is never complete, suggesting cooperation between different recognition mechanisms [45]. It should be noted that exposure of PS has been documented on many different cell types undergoing apoptosis. Some membrane changes, however, have only been described in certain cell types (e.g. expression of altered carbohydrates on apoptotic thymocytes and hepatocytes, ICAM-3 expression on apoptotic B-cells and loss of CD16 on apoptotic neutrophils).

Recent evidence suggests that phagocytosis of AC by macrophages is impaired in atherosclerosis. One of the first discoveries in the quest for possible mechanisms that contribute to defective clearance of AC in atherosclerosis was made by Steinberg, Witztum and coworkers, who showed that oxLDL competes with AC for macrophage binding (Fig. 2). AC and oxLDL share oxidatively modified moieties on their surfaces that serve as ligands for macrophage recognition [46,47]. Moreover, oxLDL is immunogenic and anti-oxLDL auto-antibodies are commonly found in atherosclerotic lesions of both animals and patients [48]. These auto-antibodies also specifically bind to the surface of apoptotic cells, but not to normal cells, thereby inhibiting their phagocytosis by macrophages [49]. Another factor that contributes to diminished phagocytosis of AC in atherosclerotic plaques is the presence of oxidative stress mediators such as peroxynitrite. Recent evidence indicates that the peroxynitrite donor SIN1A concentration-dependently decreases phagocytosis of AC in vitro [50]. PS or other membrane associated factors present on macrophages or AC that are required for phagocytosis may be sensitive to oxidative treatment. Furthermore, accumulation of indigestible particles in the cytoplasm of macrophages results in a nearly complete inhibition of AC phagocytosis [50]. Moller et al. reported that uptake of indigestible, rigid particles by macrophages induces cellular ‘stiffening’ so that changes in cellular shape, which are needed to form pseudopodia for phagocytosis, are inhibited [51]. Electron microscopy images from macrophage-derived foam cells in atherosclerotic plaques show that these cells are often crammed with large lipid droplets. Because accumulation of indigestible debris in the cytoplasm of macrophages inhibits phagocytosis, it is tempting to speculate that these lipid-laden

Fig. 2. Schematic overview of the different mechanisms that are responsible for diminished phagocytosis of apoptotic cells (AC) by macrophages in atherosclerotic plaques. (1) The cell death stimulus that results in the formation of AC determines the extent to which dying cells are recognized and removed by phagocytes. (2) Oxidative stress exerted by peroxynitrite (and probably also other ROS) inhibits phagocytosis of AC by macrophages. (3) Monoclonal auto-antibodies to various epitopes of oxLDL bind specifically to the surface of AC and not to normal cells. Once bound, these antibodies profoundly inhibit uptake of AC by macrophages. (4) Both oxRBC and oxLDL compete with AC for the same epitopes on the surface of macrophages, thereby profoundly inhibiting AC phagocytosis. (5) Free cholesterol loading in macrophages downregulates the secretion of lactadherine, a macrophage-derived molecule that facilitates phagocytosis of AC. (6) Accumulation of indigestible material (e.g. lipid droplets in foam cells) causes macrophage ‘stiffening’, thereby decreasing macrophage phagocytosis capacity.
macrophages are no longer able to engulf AC ("full is full"). However, it should be noted that in vitro generated foam cells (e.g. macrophages after phagocytosis of agLDL or platelets) can efficiently engulf AC because the degree of foam cell formation as observed in plaques is very hard to accomplish in vitro. In addition, cholesterylester-loaded macrophages, the most prominent type of phagocytes in early lesions, can effectively recognize and ingest AC [52]. Also of note, secretion of MFG-E8, a macrophage-derived molecule that facilitates phagocytosis of AC [53], is downregulated upon free cholesterol-loading in macrophages [54]. Finally, it is worth mentioning that success or failure of adequate AC phagocytosis is determined not only by phagocyte availability, but also by the nature of the cell death trigger [55]. Indeed, AC are not equally marked for safe clearance, which implies that clearance of AC depends on the cell death initiator that is used.

Reduced uptake of AC promotes a number of processes that may contribute to plaque instability (Fig. 3). Firstly, impaired phagocytosis of AC results in enhanced secretion of pro-inflammatory cytokines, including TNF-α and IL-12, and a reduced release of anti-inflammatory proteins such as TGF-β and IL-10. Deregulated expression of these cytokines may result in inflammatory autoimmune responses, as seen in systemic lupus erythematosus and rheumatoid arthritis, two nonatherosclerotic inflammatory disorders with a similar defect in AC phagocytosis by macrophages. Secondly, PS often becomes oxidized during apoptosis. These oxidized phospholipids are known to induce secretion of pro-inflammatory proteins such as MCP-1 and IL-8, thereby contributing to a persistent state of chronic inflammation [56]. Thirdly, tissue factor (TF) expression colocalizes with AC, especially around the lipid core of human atherosclerotic plaques [57,58]. TF is a key element in the initiation of the coagulation cascade and mediates thrombus formation after rupture of an unstable plaque, when free AC are exposed to the blood stream. It binds coagulation factor VII and its activated form factor VIIa. This complex proteolytically

Fig. 3. Schematic overview of the differential effects of phagocytosis by macrophages on plaque progression and stability. One of the earliest events in atherosclerosis is the retention of LDL in the intima, where LDL transforms into aggregated or oxidized LDL (agLDL and oxLDL, respectively). Uptake of modified LDL protects macrophages (MΦ) against oxLDL cytotoxicity but results in foam cell formation and plaque progression. Binding of oxLDL to MΦ stimulates cell death. Apoptotic cell death is a prominent feature of advanced plaques. In general, MΦ clear apoptotic cells (AC) via phagocytosis prevent inflammatory reactions caused by secondary necrosis of AC. However, phagocytosis of AC in plaques is severely impaired in various ways (e.g. oxLDL and oxidized red blood cells compete with AC for binding on the PS receptor; see also Fig. 2). Accordingly, AC remain unremoved and contribute to plaque progression. Moreover, phosphatidylserine (PS) exposed on the surface of AC can initiate thrombus formation upon contact with platelets (PLT). These PLTs enter the plaque through leaky neovessels or after plaque rupture and are phagocytized by MΦ. Platelet phagocytosis activates macrophages (as shown by upregulation of inducible nitric oxide synthase (iNOS) and secretion of TNFα) and contributes to an unstable plaque phenotype. Leaky neovessels also release red blood cells (RBC) in the plaque. RBC are rapidly oxidized and phagocytized by MΦ. Upon phagocytosis of oxRBC, ceroid depositions accumulate in the MΦ cytoplasm. Moreover, oxRBC uptake leads to macrophage activation and exocytosis of iron. Extracellular iron has the ability to oxidize LDL resulting in further plaque destabilization.
activates factors IX and X, which in turn leads to thrombin generation. TF activity is highly dependent on the presence of PS because PS increases the catalytic activity of the TF/ factor VIIa complex [59]. Finally, expansion of the necrotic core is stimulated due to secondary necrosis of free AC. The interaction of macrophages with necrotic cells often results in an additional inflammatory response [60].

The exact number of non-phagocytized AC required for a detectable inflammatory response is presently unknown. It is therefore unclear whether free AC in advanced human atherosclerotic plaques are frequent enough to induce significant inflammation. There are, however, several lines of evidence that accumulation of free AC in plaques contributes to lesion progression. The impact of impaired phagocytosis of AC on atherosclerosis was recently demonstrated in different mouse models. For example, aortic valve lesions in LDLR−/− recipients of transglutaminase 2 (TG2)−/− bone marrow are larger than in recipients of TG2+/+ bone marrow [61]. TG2 deficiency fundamentally impairs the capacity of macrophages to ingest apoptotic cells [62] which supports the general hypothesis that accumulation of undigested AC may function as a pro-inflammatory factor that stimulates expansion of atherosclerotic plaques. Besides TG2, apolipoprotein E (apoE) modulates clearance of AC both in vitro and in vivo, resulting in a systemic pro-inflammatory state in apoE−/− mice, independent of its role in lipoprotein metabolism [33]. In addition, gldapoE−/− mice, lacking the genes for Fas-ligand and apolipoprotein E, have high levels of free AC in tissues and in the circulation which was due, at least in part, to an impaired ability to scavenge apoptotic debris [63]. These mice display enhanced atherosclerosis compared with apoE−/− mice.

4. Erythrophagocytosis

Intraplaque hemorrhage is common in advanced human atherosclerotic plaques and arises from the disruption of thin-walled microvessels penetrating the atherosclerotic lesion from the adventitial microvascular network through the media into the thickened intima [2]. Acute hemorrhagic events may also occur after rupture of the fibrous cap. These events promote infiltration of erythrocytes and deposition of large amounts of iron. It is presently unclear why newly formed microvessels become fragile and prone to rupture. Possibly, ongoing inflammation, release of matrix-degrading metalloproteinases by macrophages or proteases secreted by mast cells are responsible for proteolytic damage of the microvessels [64,65]. Once inside the plaque, erythrocytes rapidly undergo lipid peroxidation followed by phagocytosis via macrophages. Binding and phagocytosis of oxidized red blood cells (oxRBC) by macrophages are predominantly mediated by PS recognition but the macrophage receptors that are involved remain to be elucidated (Fig. 1). Importantly, oxRBC competes with both oxLDL and AC for macrophage binding since they all share oxidatively modified moieties on their surface that serve as ligands for macrophage recognition (Fig. 2) [46,47,66]. Upon phagocytosis of oxRBC in vitro, macrophages (i) transform to foam cells, (ii) become activated as indicated by elevated iNOS activity and (iii) produce autofluorescent pigment with the characteristics of ceroid (insoluble mixture of oxidized lipids and proteins) that can be found frequently in foam cells around microvessels in human plaques [67]. Loegering et al. reported that phagocytosis of IgG coated erythrocytes inhibits certain macrophage functions, including the respiratory burst, killing of bacteria as well as phagocytosis itself [68]. A challenge of macrophages with IgG coated erythrocytes is followed by an increase in lipid peroxidation as assessed by thiobarbituric acid-reactive substances (TBARS). Since phagocytosis of IgG coated erythrocyte ghosts lacking iron-containing hemoglobin does not affect macrophage function, it is tempting to speculate that iron may interact with ROS of the macrophage respiratory burst to cause oxidative damage to the macrophage. However, several lines of evidence question this theory. First, phagocytosis of IgG coated erythrocytes fails to deplete glutathione, suggesting that the level of oxidative stress is minimal [69]. Secondly, iron is exocytosed by the macrophage and gives rise to Fenton reactions outside the cell, thereby triggering peroxidation of LDL and formation of extracellular ceroid deposits [70,71]. Thirdly, phagocytic challenge with complement coated erythrocytes causes neither an increase in TBARS nor an inhibition of macrophage function [69]. It should be noted that an increase in TBARS following complement receptor mediated phagocytosis of erythrocytes may occur when the respiratory burst is stimulated with phorbol myristate acetate (PMA) [68]. This finding suggests that hemoglobin iron and phagocyte generated oxidants may collaborate to induce inhibition of macrophage function following erythrocyte phagocytosis.

5. Phagocytosis of platelets

Besides erythrocytes, platelets can infiltrate in the atherosclerotic plaque via leakage and/or rupture of newly formed microvessels. Subsequent clearance of platelets by macrophages is mediated through class A scavenger receptors (Fig. 1) [72]. Cell surface expression of PS by platelets suggests that phagocytosis also involves the PS receptor. Moreover, CD36 might be required in platelet recognition by macrophages as CD36 associates with CD9 and integrins on human platelets [72]. Phagocytosis of platelets by macrophages is known as an alternative mechanism of foam cell formation [73,74]. A recent study, however, established a link between platelet phagocytosis and macrophage activation via proteolytic processing of platelet-derived amyloid precursor protein (APP) and generation of β-amyloid (Aβ)-like peptides [74]. Hitherto, APP and Aβ have been investigated almost exclusively in brain tissue from patients with Alzheimer’s disease. Nonetheless, APP is abundantly present in α-granules of platelets and consequently also accumulates along with APP derived fragments in human
atherosclerotic plaques, more specifically in macrophages associated with platelet phagocytosis in the vicinity of neovascularization [74]. These macrophages often express iNOS, suggesting that APP and its processing to Aβ((like) peptides can play a major role in macrophage activation after platelet phagocytosis. The finding that platelet phagocytosis evokes macrophage activation in advanced human atherosclerotic plaques and the possible role of APP and Aβ((like) peptides in this process has been confirmed in cell culture experiments [74]. Phagocytosis of platelets by IFNγ-primed mouse J774 macrophages or human THP-1 monocytes in vitro, as demonstrated with confocal microscopy, leads to macrophage activation, as indicated by the upregulation of iNOS mRNA and protein in the macrophages and the production of high amounts of nitrite and TNF-α (Fig. 3). Because uptake of platelets from APP knockout mice by macrophages does not result in macrophage activation [75], APP or derived fragments can be considered the effector molecules that induce macrophage activation after platelet phagocytosis. Interestingly, macrophage activation after platelet phagocytosis is increased in the presence of thrombin, which is a substance that evokes platelet degranulation. In this light, it can be assumed that platelets not necessarily need to be phagocytized by macrophages to mediate their activation. Interaction with platelet collagen that activates platelets might be sufficient to induce APP and Aβ’s release. The observation that a rim of platelets often surrounds iNOS-positive macrophages without clear signs of internalization supports this view [74].

6. Autophagocytosis

Autophagocytosis, also known as macroautophagy or simply autophagy, is a regulated mechanism for bulk degradation of long-lived proteins and the only way known for the degradation of organelles [76]. The process of autophagy involves the formation of autophagic vacuoles, named autophagosomes, containing portions of the cytoplasm and organelles typically surrounded by two membrane layers. Autophagosomes fuse with pre-existing lysosomes and mature to become degradative autolysosomes. The content and inner membranes are then degraded by lysosomal hydrolases. In most cells, autophagy is suppressed to a basal level, but under specific conditions of stress (e.g. starvation, cellular injury, treatment with certain hormones or toxic chemicals) formation of autophagosomes is induced. This suggests that autophagy is in the first place an adaptive survival mechanism that allows a mechanism for recycling of cellular components in addition to its catabolic role providing energy in times of famine. However, when autophagy is triggered continuously, progressive degradation of vital cytoplasmic components will occur and the cell will literally digest itself to death as a suicide strategy. Although autophagy in mammalian cells is known for at least forty years, we are only beginning to understand its physiological role and participation in different human pathologies. This is at least partly related to the fact that only a limited number of specific marker proteins and diagnostic methods are available to monitor the autophagic process [77]. Nevertheless, some observations indicate that autophagy may be involved in human atherosclerosis. Transmission electron microscopy of disintegrating SMCs in the fibrous cap reveals certain features of ‘programmed’ cell death unrelated to apoptosis but typical of autophagy, such as formation of myelin figures [78] and severe vacuolization [79] (Fig. 4A,B). The latter phenomenon was already described in dying SMCs by Stehbens as granulovesicular degeneration [80]. More recently, Kockx and Herman classified these cells as type C SMCs [81] and considered them similar to dying SMCs found in unstable plaques of saphenous vein grafts [82]. Interestingly, vacuolated SMCs in the fibrous cap often contain granular ubiquitin inclusions in their cytoplasm representing insoluble protein aggregates of diverse origin (Fig. 4C–E) [83]. Accumulation of ubiquitinated inclusions in the cytosol is currently an established marker for autophagic degeneration of cardiomyocytes during heart failure [84,85]. Because ubiquitinated SMCs in the fibrous cap are surrounded by a thick layer of basal lamina [79], it is tempting to speculate that these “caged” cells undergo autophagy as a result of starvation effects. Further evidence for autophagy in atherosclerotic plaques comes from in vitro studies. 7-ketocholesterol (7KC) is one of the major oxysterols present in atherosclerotic plaques. Treatment of SMCs in culture with 7KC results in extensive vacuolization, intense protein ubiquitination and processing of cytosolic microtubule-associated protein 1 light chain 3 (LC3-I) into the autophagosomal specific isoform LC3-II [83]. The latter event has never been observed in apoptosis or necrosis but is characteristic of autophagy. All together, these findings strongly suggest that autophagy occurs in human atherosclerotic plaques, particularly in SMCs. However, the specific role and regulation of autophagy in atherosclerosis remain to be determined. Since autophagy is well recognized as a survival mechanism and not as a death pathway [86], one can speculate that autophagy of SMCs in the fibrous cap is an important mechanism underlying plaque stability.

7. Phagocytosis in atherosclerosis: beneficial or detrimental?

Given the complex role of macrophage phagocytosis in atherosclerosis, it would be interesting to know whether a general change in the rate of phagocytosis has a beneficial or detrimental effect on plaque progression and whether the ultimate effect of phagocytosis depends on the type and/or complexity of the lesion. However, direct in vivo evidence from studies using phagocytosis inhibitors such as cytochalasin B or latrunculin A is lacking so that we can only formulate preliminary conclusions based on indirect observations as outlined above. Early lesions do not contain large amounts of infiltrated platelets or erythrocytes and reveal undetectable levels of apoptosis. Therefore, phagocytosis in
early lesions is mainly focused on lipoproteins and might be proatherogenic. In advanced lesions, where different particles or cellular debris can be phagocytized including platelets, erythrocytes, AC and lipoproteins, only phagocytosis of AC seems beneficial for plaque stability. Because phagocytosis of AC by macrophages in advanced plaques is significantly impaired (Fig. 2), the benefit resulting from AC uptake would not overrule the negative aspects associated with phagocytosis of lipoproteins, platelets or erythrocytes, especially in complicated lesions with significant neovascularization or in plaques that have ruptured. As a consequence, both in early and advanced lesions, phagocytosis may stimulate rather than inhibit plaque formation. If true, nonspecific enhancement of phagocytosis in atherosclerosis is not desirable. Nonetheless, apoptotic clearance is a beneficial process and selectively enhancing AC uptake might help to prevent the progression of advanced atherosclerotic lesions, on condition that the proclearance intervention would not alter the fundamental property of phagocytes to selectively recognize and ingest only apoptotic cells. Glucocorticoids are known to stimulate phagocytic clearance of AC, but the serious adverse effects of glucocorticoid therapy render this approach impractical [35]. Examples of other pharmaceuticals that promote phagocytic clearance of AC are the cholesterol-lowering agent lovastatin [87], the macrolide antibiotic azithromycin [88] and members of the lipoxin family [89]. Future studies are needed to determine whether these drugs may provide the basis for a novel therapeutic strategy to prevent the progression of advanced atherosclerotic plaques via interference with the process of phagocytosis. Indeed, it has recently been proposed that too much phagocytosis of AC might be associated with the production of reactive oxygen species and tissue injury which has prompted the search for attenuation mechanisms of phagocytosis [90].

8. Conclusion

It is generally assumed that macrophages play a crucial role in plaque destabilization and rupture. From this perspective, selective removal of macrophages might be beneficial for plaque stability. However, macrophages also have an important role in scavenging of modified lipoproteins, senescent cells and cells that have died by apoptosis. Loss of macrophages would therefore reduce scavenging activity resulting in the accumulation of extracellular oxLDL and AC. Unphagocytized oxLDL is detrimental for the plaque due to cytotoxic effects, but also the consequences of impaired phagocytosis of AC are likely to be substantial. Indeed, free AC promote (secondary) necrosis, inflammation and thrombosis and may contribute to acute clinical complications after plaque rupture. On the other hand, uptake of erythrocytes and platelets leads to macrophage activation and plaque instability. These findings suggest that macrophage phagocytosis in atherogenesis might act as a double edged sword. Macrophages are necessary for removal of AC from plaques, but exert strong proatherogenic properties upon phagocytosis of lipoproteins, erythrocytes and platelets.

Besides heterophagocytosis, evidence is emerging for the initiation of autophagy in atherosclerosis. The paucity of markers is the greatest limitation in studying autophagy in
mammalian tissue including atherosclerotic plaques. At present, autophagy is linked to various human pathologies such as Alzheimer’s disease, different forms of cancer, muscular disorders and cardiomyopathies [91], resulting in increased attention to this process. Continued progress on the detection methods and the proteins involved in the autophagic process will undoubtedly provide more insight into the role of autophagy in atherosclerosis.

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