Apolipoprotein E (APOE) is a protein that plays a critical role in the metabolism of lipids and lipoproteins. APOE is predominantly synthesized by the liver and transported to the peripheral tissues, where it is involved in the clearance of cholesterol from the blood. Its function is essential for maintaining cholesterol homeostasis and preventing the development of atherosclerosis. Variations in the APOE gene are associated with disparities in cardiovascular disease risk, emphasizing the genetic basis for the development of atherosclerosis.

The APOE gene is located on chromosome 19 and consists of three common alleles: APOE ε2, ε3, and ε4. These alleles differ in their ability to bind and transport lipids, with APOE ε4 being the most efficient at removing cholesterol from the bloodstream. Individuals who inherited at least one APOE ε4 allele have a higher risk of developing cardiovascular disease, including coronary artery disease and stroke. In contrast, individuals with two APOE ε2 alleles have a reduced risk of cardiovascular disease.

The association between APOE genotype and cardiovascular disease risk is thought to be mediated through its impact on lipid metabolism. APOE ε4 increases the risk of atherosclerosis by promoting the formation of cholesterol-rich plaques in the arterial walls, which can lead to the development of cardiovascular disease. Meanwhile, APOE ε2 has been linked to a lower risk of cardiovascular disease by facilitating the clearance of excess cholesterol from the bloodstream.

The genetic variation in the APOE gene underscores the importance of understanding the genetic predispositions to cardiovascular disease. This knowledge can guide personalized strategies for prevention and management, including lifestyle modifications and targeted interventions. Further research is needed to elucidate the molecular mechanisms underlying the association between APOE genotype and cardiovascular disease risk, facilitating the development of more effective prevention and treatment strategies.
Apoptosis and efficient efferocytosis in early atherosclerosis: protective

Apoptosis and defective efferocytosis in advanced atherosclerosis: detrimental

interferon-γ, and cholesterol overload (in case of macrophages). Apoptosis can affect all the cell types in the atherosclerotic plaque: vascular cells, such as endothelial and smooth muscle cells, as well as inflammatory cells, such as T-lymphocytes and macrophages. The consequences of apoptosis within atherosclerotic plaques will depend on the cell type that is involved, the plaque stage, and apoptotic cell localization. Death of endothelial cells, in contact with the blood flow, may initiate plaque erosion, resulting in clinical events because of thrombosis in the absence of plaque rupture. Regarding smooth muscle cells, apoptosis could destabilize the fibrous cap and induce rupture. Macrophages represent the majority (more than 40%) of dead cells in the atherosclerotic lesions. The studies on the modulation of macrophage apoptosis during atherosclerosis development seem controversial at first glance. Some animal studies strongly suggest that macrophage apoptosis is a negative regulator of plaque growth. Transplantation of low-density lipoprotein (LDL) receptor−/− mice with bone marrow of apoptotic resistant bax−/−, or apoptosis signal-regulating kinase 1−/− mice induced an increase of vascular lesion size. Also G2a−/−apolipoprotein E (ApoE)−/− mice that have decreased macrophage apoptosis and therefore an accumulation of lesional macrophages demonstrate increased atherosclerosis. Selective inhibition of the antiapoptotic gene Bcl-2 in macrophages resulted in increased apoptosis and plaque necrosis in advanced lesions. However, other experiments suggest that macrophage apoptosis promotes necrotic core formation and atherosclerosis. C/EBP homologous protein−/− mice have less macrophage apoptosis in response to cholesterol-induced endoplasmic reticulum stress and have reduced necrotic core and lesion development in both ApoE−/− and LDL receptor−/− background, whereas ApoE−/− mice with heterozygosity for a protein involved in cholesterol trafficking demonstrate decreased macrophage apoptosis, lesional necrosis, and atherosclerosis, suggesting that macrophage apoptosis increases plaque progression. These contradictory observations might be explained by different effects of macrophage apoptosis depending on atherosclerotic plaque stage, as was illustrated by Gautier et al and schematically represented in the Figure. In the model of Gautier et al, an increase of macrophage apoptosis susceptibility induced a decrease of plaque size after 5 weeks of Western diet but an increase after 15 weeks of Western diet. Also, Babaev et al demonstrated that increased macrophage apoptosis reduces early atherosclerosis. We could speculate that macrophage apoptosis in early lesions would limit plaque development through a negative regulation of inflammation. In more advanced plaques, macrophages and foam cells are specialized in and necessary for AC clearance and scavenging in a microenvironment where efferocytosis mechanisms are compromised (see below). Subsequently, their death will further alter cellular debris removal and promote plaque growth.

On top of this, other studies demonstrate a major role for apoptosis in the thrombogenicity of atherosclerotic plaques. First, apoptotic intimal cells in plaques are a source of tissue factor, a potent inducer of the coagulation cascade. In addition, ACs externalize phosphatidylserine to their outer cell membrane, thereby enhancing tissue factor activity. In atherosclerotic lesions ACs colocalize with tissue factor, whereas shed apoptotic bodies from plaques show procoagulant activity, which can stimulate thrombosis locally as well as systemically. Finally, unstable lesions contain more tissue factor than stable lesions, mostly in macrophage-rich regions close to the necrotic core, and are associated with increased plaque thrombogenicity. Furthermore, atherosclerotic plaques from diabetic patients, which are known to be more unstable than those of nondiabetic patients, are rich in apoptotic debris.

Clearance of ACs: Mechanisms of Efferocytosis

Apoptosis is rarely observed in normal histological sections because prompt clearance mechanisms narrow the observa-
Efferocytosis in Atherosclerosis

Van Vré et al

The effect of cell death on the immune system is discussed by Zheng et al. In many occasions, ACs were shown to produce anti-inflammatory factors, as well as directly induce tolerogenic responses in antigen presenting cells, through the release of anti-inflammatory cytokines such as interleukin-10 and transforming growth factor-β. Meanwhile, primary and secondary necrotic cells are associated with inflammation. Nevertheless, there still remains controversy regarding these assumptions. Generally, the nature of the immune response to apoptosis and its subsequent impact on atherosclerosis will depend on the intrinsic antigenicity of the cells, the history of activation or stress before cell death, the nature of the cell death inducer, the cell death pathway that is engaged, and the availability of phagocytes and immune cells that can respond. Indeed, though many tolerogenic responses are attributed to ACs, some ACs can exert proinflammatory effects, as is described for ACs with oxidative epitopes. Another illustration of diverse immune responses to ACs is the observation that free cholesterol-
induced apoptosis of macrophages results in a proinflammatory response, whereas oxidized LDL (oxLDL)–induced or UV-induced apoptosis results in a more tolerogenic immune response.43 Finally, as mentioned above, the impact of apoptosis is beneficial in early stages of atherosclerosis, whereas in advanced lesions, it contributes to plaque instability and thrombogenicity.18 It is assumed that less efficient apoptosis is beneficial in early stages of atherosclerosis, in gld mice, which demonstrate higher AC levels because of over, infusion of lysophosphatidylcholine reduced AC uptake and foam cell formation might substantially alter efferocytosis,51 and macrophages containing abundant apoptotic material are detected in plaques, supporting that efferocytosis occurs in atherosclerosis. Yet the occurrence of apoptotic debris extracellularly and the very high numbers of free ACs encountered in atherosclerotic lesions44 suggest either increased AC death or insufficient mechanisms for the removal of ACs in atherosclerosis. It should be acknowledged that studies on efferocytosis in atherosclerosis are based on microscopic analysis of AC and phagocytes colocalization in histological sections. This might be hampered by difficult discrimination between AC adherence compared with uptake by efferocytes. Moreover, conclusions are merely based on observations and associations at a certain time point because functional data on in vivo kinetics of AC clearance in plaques are difficult to obtain.

Increased cell death may result from the omnipresence of apoptotic triggers such as oxLDL and interferon–γ, especially in advanced atherosclerotic lesions, but sufficient numbers of phagocytes seem to be present and colocalize with ACs in plaques. Alternatively, several in vitro studies suggest a major role for impaired efferocytosis in atherosclerosis. For instance, competition between ACs and oxidized lipids for clearance by phagocytes was repeatedly observed.44,45 Moreover, infusion of lysophosphatidylcholine reduced AC uptake in gld mice, which demonstrate higher AC levels because of genetically impaired Fas-ligand.46 Because ACs and oxidized lipids share antigenic properties, autoantibodies directed against oxLDL also fix on ACs and are able to inhibit their engulfment.52 However, a more recent in vivo study demonstrated that natural IgM against oxLDL might not inhibit but facilitate the uptake of ACs by macrophages.47 Moreover, Ogden et al showed that IgM is required for optimal complement dependent efferocytosis of ACs.48 Besides competitive inhibition with oxLDL, oxidative stress44 and transformation into foam cells49 also impair the capacity to engulf apoptotic debris. Interestingly, the modulation of cholesterol transport in macrophages affects the expression of molecules implicated in efferocytosis such as MFGE8.49 Moreover, using immunohistochemistry, we have demonstrated a decrease of MFGE8 protein expression in macrophages around the necrotic core.50 These data suggest that changes in macrophage phenotype during atherogenesis and foam cell formation might substantially alter efferocytosis. Furthermore, it was shown that increased fatty acids in macrophage membrane lipid composition can cause impaired efferocytosis. This was illustrated in obese ob/ob LDL receptor−/− mice in which a fish oil–rich diet reversed the effects.51 Finally, inflammation is also likely to have pro-

Impaired Efferocytosis in Atherosclerosis

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found effects on efferocyte function. For instance, mertyrosine kinase (MERTK) is cleaved under inflammatory conditions, and soluble MERTK can compete for the ligands of membrane bound MERTK and therefore impede efferocytosis.52

Apart from macrophages, increased numbers of immature DCs are present in atherosclerotic lesions53 and might also be implicated in efferocytosis in atherosclerosis. As recently discussed,54 different efferocytes might have different effects on plaque evolution. DCs are less well equipped than macrophages to digest internalized proteins but are more specialized in the overall interpretation of the microenvironmental signals, the induction of adequate immune responses after AC uptake, and the migration to lymphoid organs. Interestingly, ACs contained in DC phagosomes can be found in afferent lymphatics.55 Immature DCs are specialized in uptake of substances but lose their phagocytic capacity after maturation as soon as their antigen presenting functions are induced.56 As for macrophages, they are considered less migratory but could transform into DCs to transport cell death material to lymphoid organs.57 Although in vitro studies have shown that AC uptake can induce tolerogenic responses in the phagocyte,51 at the moment, studies on efferocytosis by different phagocytes in atherosclerotic plaques are lacking, and it is unknown whether they undergo a phenotypic transformation, alter their function, and emigrate from the plaque after AC uptake.

It is widely assumed that delayed clearance of ACs leads to secondary necrosis, membrane leakage of inflammatory cell contents, and triggering of autoimmunity. Although mice lacking the phagocytic receptor CD14 challenge this hypothesis by showing an accumulation of apoptotic bodies without autoimmune pathogenesis or autoantibodies.58 Other mouse models of decreased apoptosis engulfment, such as mice lacking the receptor MER59 and the bridging molecules MFGE860,61 and C1q,62 do demonstrate an association between increased nonphagocytosed apoptotic bodies and autoimmune pathology with increasing age. Therefore, it was suggested that besides their role in efferocytosis, signaling through the MER receptor or integrins via MFGE8 is required for direct immune suppressive effects following AC recognition.63 Interestingly, MER attenuated the inflammatory response to free cholesterol loading of macrophages in vitro43 and ligated tyro3/axl/mer receptors negatively regulate inflammatory pathways through the induction of suppressors of cytokine signaling proteins.64 Also, MFGE8 directly influences regulatory immune responses.50,65 Finally, other evidence that clearance of ACs is important for the maintenance of immune tolerance is seen for the receptor peroxisome proliferator–activated receptor-δ, which is induced when macrophages engulf ACs. Deletion of peroxisome proliferator–activated receptor-δ results in decreased expression of oposinins, such as the complement factor C1q, MFGE8, and MERTK, impaired efferocytosis and autoimmune responses in mice.66 Similarly, liver X receptor (LXR) is induced by AC uptake and enhances MERTK expression. LXR deficiency results in impaired efferocytosis and increased inflammation.67 Moreover, it is probable that in atherosclerotic lesions, which are already a proinflammatory milieu in which engul-

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ment mechanisms are very much solicited, impairment of efferocytosis pathways will have more pronounced effects. Indeed, we believe that atherosclerotic lesions represent a very particular, pathological microenvironment in which very high apoptosis rates demand a fully functional and available efferocytosis system. Therefore, blockage of just 1 efferocytosis pathway in atherosclerosis is likely to have much more extensive effects. Indeed, this was illustrated by recent studies analyzing deficiency of either MFG8, MERTK, or C1q efferocytosis pathways in models of atherosclerosis.50,68–70 In these studies, invalidation of only 1 pathway had strong effects on atherosclerosis with enhanced accumulation of ACs, even in early lesions, an increase of necrotic core, and in some cases an increase in plaque size (as illustrated in the Figure). We showed that the absence of the bridging molecule MFG8 in a mouse model of atherosclerosis leads to accelerated atherosclerosis with enlarged necrotic cores, because of an accumulation of AC debris. In addition, MFG8 deficiency coincided with a switch of the immune response toward a Th1 proinflammatory phenotype.50 Furthermore, studies by Tabas’s group68 and ours69 showed that defect in the phagocytic receptor MER leads to increased accumulation of ACs, resulting in enlarged plaque necrosis, a proinflammatory phenotype, and aggravated atherosclerosis. Finally, the complement factor C1q was also shown to be an essential bridging molecule for efficient efferocytosis and limited atherosclerosis.70 Other experimental studies indicating the importance of defective efferocytosis in atherosclerosis include mouse models that might undergo increased apoptosis susceptibility in addition to deficient AC engulfment. Even so, G2a-deficient mice with decreased macrophage apoptosis as well as efferocytosis demonstrate increased atherosclerosis.14 Furthermore, inactivated Fas-ligand in ApoE−/− mice (gld ApoE−/− mice) resulted in more ACs, less efferocytosis, and enhanced atherosclerosis.46 Besides, deficiency in ApoE itself modulates clearance in vitro and in vivo independently of its role in lipoprotein metabolism,34 potentially adding to the increased susceptibility of ApoE−/− mice to develop atherosclerosis. Finally, transglutaminase-2–deficient macrophages demonstrate deficient AC ingestion in vitro, whereas transglutaminase-2–deficient bone marrow resulted in increased atherosclerosis in mice.72

Of note, the above-described observations on impaired efferocytosis in atherosclerosis can be of interest for other major autoimmune diseases, such as lupus erythematosus, which is also characterized by increased cell death and defective efferocytosis. As in atherosclerosis, inadequate engulfment and digestion of dead cells may result in secondary necrosis, improper presentation of antigens, and subsequent chronic activation of the immune system. Interestingly, patients with lupus erythematosus are at increased risk to develop cardiovascular events.73

Concluding Remarks

Along with cell proliferation and apoptosis equilibriums, clearance of dying/dead cells is vastly important for the development and homeostasis of organisms. Endogenous components secreted from or expressed on dead cells can induce innate and adaptive immune responses. It seems that the complex, debris-rich environment of the advanced atherosclerotic lesion challenges the many engulfment mechanisms. Indeed, deletion of efferocytosis pathways leads to aggravated inflammation and increased atherosclerosis.

The current knowledge on the many molecular pathways of efferocytosis, the different effector subsets (macrophages and DCs), and their interaction with the immune system require further exploration to better understand the determinants of plaque progression, as well as plaque instability. In the end, this will allow us to identify new therapeutic targets to treat atherosclerosis. As discussed above, ACs can induce peroxisome proliferator–activated receptorγ and LXR receptors, which result in upregulation of opsonins and efferocytosis-related pathways, such as C1q or MERTK receptor. Consequently, administration of ligands to peroxisome proliferator–activated receptorγ or LXR might become a therapeutic strategy to promote efferocytosis. Interestingly, administration of a synthetic agonist for LXR did ameliorate lupus autoimmunity in a mouse model,67 suggesting that at least part of the beneficial effect of LXR agonists in cardiovascular disease might be related to their efferocytosis-dependent immunomodulatory effect.

Sources of Funding

This work was supported by the Fondation de Recherche Médicale (France), Institut National de la Santé et de la Recherche Médicale (France), and the British Heart Foundation (United Kingdom).

Disclosures

None.

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Arterioscler Thromb Vasc Biol. 2012;32:887-893; originally published online February 9, 2012;
doi: 10.1161/ATVBAHA.111.224873
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272
Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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