# Macrophage death and defective inflammation resolution in atherosclerosis

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Abstract | A key event in atherosclerosis is a maladaptive inflammatory response to subendothelial lipoproteins. A crucial aspect of this response is a failure to resolve inflammation, which normally involves the suppression of inflammatory cell influx, effective clearance of apoptotic cells and promotion of inflammatory cell egress. Defects in these processes promote the progression of atherosclerotic lesions into dangerous plaques, which can trigger atherothrombotic vascular disease, the leading cause of death in industrialized societies. In this Review I provide an overview of these concepts, with a focus on macrophage death and defective apoptotic cell clearance, and discuss new therapeutic strategies designed to boost inflammation resolution in atherosclerosis.

Efferocytosis
The phagocytic clearance of apoptotic cells (from the Latin 'effero', meaning to take to the grave or bury) before they undergo secondary necrosis. The process usually triggers an anti-inflammatory response.

Alternatively activated macrophage
(M2 macrophage).
A macrophage stimulated by IL-4 or IL-13 that expresses arginase 1, the mannose receptor CD206 and IL-4 receptor-α. There may be pathogen-associated molecular patterns expressed by helminths that can also drive the alternative activation of macrophages.

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The successful resolution of inflammatory disease processes, often known as 'catabasis', requires a distinct series of processes, including inhibition of inflammatory cell recruitment, promotion of inflammatory cell egress and clearance of apoptotic cells by phagocytes (a process known as efferocytosis)[1,2]. These processes are mediated by a wide array of molecules, including anti-inflammatory cytokines, lipoxygenase-derived bioactive lipids and transcription factors. Inflammation resolution also involves subsets of immune cells, such as alternatively activated macrophages (M2 macrophages), that have specific functional characteristics related to suppressing inflammation and engulfing cellular debris[3].

Understanding the principles of inflammation resolution is important to determine the complex process of atherosclerosis progression. Atherothrombotic vascular disease (ATVD) is the most frequent cause of death in the industrialized world, and this problem is growing owing to the worldwide increase in obesity and insulin resistance[4]. Insulin resistance is thought to be a major risk factor for ATVD owing to both its effects on systemic risk factors, such as plasma lipoprotein levels and blood pressure, and direct effects on the arterial wall[5]. Atherogenesis is triggered by the retention of apolipoprotein B-containing lipoproteins in the subendothelium of the arterial wall[6–8] (BOX 1). These retained lipoproteins, perhaps after oxidative modification, trigger a chronic inflammatory response that initially involves monocyte-derived macrophages and then involves other inflammatory cells, including T cells and mast cells[6–8]. In the early stages of disease development, the atherosclerotic lesions are small and asymptomatic and are not at risk of causing atherosclerotic plaque disruption and luminal thrombosis. Moreover, at least one key event in inflammation resolution — efferocytosis — seems to function normally in these early lesions (see below). However, the few lesions that do progress to the type of dangerous atherosclerotic plaque that can cause ATVD have all the hallmarks of defective resolution of inflammation, including defective efferocytosis, a persistent inflammatory state and defective egress of inflammatory cells[9–12] (FIG. 1). Defects in any of these processes promote the formation of highly inflamed and necrotic plaques that are referred to as 'vulnerable plaques' because they are vulnerable to structural disruption and atherothrombosis, which are the immediate precursors of acute cardiovascular clinical events[13]. For example, the failure of macrophage egress leads to prolonged production of collagen-degrading matrix proteases and coagulation-promoting tissue factor by these cells[7,8]. The failure of efferocytosis leads to post-apoptotic secondary necrosis, which amplifies the inflammatory response and eventually leads to the generation of the plaque-disrupting necrotic core of vulnerable plaques[9,10]. In this Review, I discuss the processes of immune cell entry and egress, the anti-inflammatory signalling induced through mediators of inflammation resolution and the role of macrophage death and defective efferocytosis in plaque progression.

Box 1 | **How atherosclerotic plaques develop**

Certain areas of medium-sized arteries are prone to the permeation and then subendothelial accumulation of apolipoprotein B-containing lipoproteins, such as low-density lipoprotein and remnant lipoproteins[6]. For reasons that are not completely understood, but are probably related to an initial innate immune response to the accumulated and often subendothelially modified lipoproteins, the overlying endothelium is activated to secrete chemokines and express adhesion molecules that attract and bind monocytes[7,8]. These processes are followed by entry of the monocytes into the subendothelial space. Once in the subendothelium, the monocytes differentiate into macrophages and ingest the retained lipoproteins[6–8]. Lipoprotein uptake promotes the intracellular accumulation of various lipids, including cholesterol, oxysterols and fatty acids, which promotes the accumulation of lipid droplets in the cytoplasm of macrophages. This accumulation causes the macrophages to become foam cells and induces an inflammatory response[6–8].

Over weeks, months and even years, the process continues and even amplifies. For example, foam cells can secrete additional extracellular matrix molecules that further promote lipoprotein retention, and the inflammatory response leads to the recruitment of more monocytes, as well as T cells, mast cells and possibly neutrophils[6–8]. The lesion enlarges, but at this stage the arterial lumen remains large enough to feed the distal organ owing to outward remodelling of the arterial wall. Moreover, in most cases, the lesion is contained by a subendothelial scar-like structure referred to as the fibrous cap, which is produced by collagen-secreting myofibroblasts that populate the intima and are derived from precursor cells in the media, adventitia and/or blood[7,8].

However, some of these lesions undergo a process of arterial wall breakdown. The types of lesions that are susceptible to this process of arterial wall breakdown are often referred to as 'vulnerable plaques' and are characterized by large areas of necrosis, thin fibrous caps and a heightened state of inflammation[13]. Disruption of the intima can expose pro-coagulant and pro-thrombotic contents within the intima, such as tissue factor, to coagulation factors and platelets in the blood[7,8]. In the worst-case scenario, an occlusive thrombus forms, leading to acute oxygen and nutrient deprivation of distal tissues fed by the artery. When these events occur in the coronary arteries, the region of heart muscle tissue fed by the involved artery becomes injured, and the result is unstable angina, myocardial infarction or sudden cardiac death.

Atherosclerosis
A process whereby lipids, inflammatory cells and extracellular matrix accumulate in the subendothelial space (intima) of focal areas of medium-sized arteries, which finally leads to plaque formation.

Atherothrombotic vascular disease
(ATVD). Disease caused by acute occlusive arterial thrombosis overlying areas of chronic atherosclerosis. The occlusive thrombosis starves the tissue that the involved artery feeds of oxygen and nutrients. For example, if the involved artery feeds the heart muscle, myocardial infarction (death of heart muscle cells) can ensue.

Insulin resistance
A state in which signalling through insulin receptors is impaired. The cause can be exposure to high levels of insulin, which downregulates insulin receptors by a negative homeostatic mechanism, or disruption of signalling molecules downstream of the insulin receptor, such as insulin receptor substrate 1 (IRS1) and IRS2. Insulin resistance, caused by high levels of insulin in the bloodstream, is responsible for a substantial portion of the pathology associated with type 2 diabetes, including ATVD.

Atherosclerotic plaque
The name given to an atherosclerotic lesion without precise designation of lesion stage, but usually referring to a lesion that has developed beyond the early foam cell stage, particularly a lesion that is raised and fibrotic.

Atherothrombosis
Following the rupture of unstable atherosclerotic plaques, thrombogenic material becomes exposed or released to mediate thrombus formation and eventually occlusion of an artery.

## Monocyte entry and macrophage egress

Two key processes in the resolution phase of inflammation are the decreased entry of new inflammatory cells and the egress of cells from the site of inflammation[1,12]. However, the entry of monocytes into pre-existing inflammatory atherosclerotic lesions in mice is enhanced (instead of suppressed as would normally occur during inflammation resolution)[14]. Moreover, numbers of LY6C$^{hi}$ monocytes, which are precursors of inflammatory macrophages, increase in the blood during hypercholesterolaemia in mice, in part owing to their impaired conversion into less inflammatory LY6C$^{low}$ monocytes[15]. These studies suggest that there is persistent recruitment of inflammatory monocytes to established atherosclerotic lesions, particularly during hypercholesterolaemia, which is consistent with defective inflammation resolution.

Monocytes can differentiate into two main types of macrophages: those that promote inflammation, which are referred to as classically activated (M1) macrophages, and M2 macrophages, which promote resolution of inflammation[3]. Thus, an imbalance in the ratio of M1 and M2 macrophages in advanced atherosclerosis may cause or at least reflect impaired resolution. Among the factors that can shift the balance in favour of M2 macrophage differentiation are an increase in T helper 2 ($T_H2$) cell-secreted molecules, such as interleukin-4 (IL-4)[3], the transcription factors peroxisome proliferator-activated receptor-γ (PPARγ) and PPARδ[16,17], and the bioactive lipid sphingosine 1-phosphate (S1P)[18]. Studies in mice have shown that processes that promote $T_H2$ cell polarization[19], activate PPARγ[20] or activate SIP signalling[18] have beneficial effects on atherosclerosis, but the degree to which these results reflect M2 macrophage polarization, other aspects of inflammation resolution or other processes in atherogenesis remains to be fully explored.

Egress of inflammatory cells from atheroma is also impaired[21]. An important step in the egress of inflammatory myeloid cells is their conversion into migratory cells, such as dendritic cells (DCs)[22]. Many growth factors and cytokines (such as granulocyte–macrophage colony-stimulating factor (GM-CSF), tumour necrosis factor (TNF), IL-4 and IL-6) have been shown to promote DC differentiation and/or maturation, perhaps forming a link between the initial inflammatory cytokine stage and the subsequent resolution stage of inflammation[23]. In the atherosclerotic lesions of apolipoprotein E (*Apoe*)$^{-/-}$ mice, a model of atherosclerosis, inflammatory cell egress is not observed in the setting of hypercholesterolaemia. However, when plaques are exposed to low levels of cholesterol through transplantation of plaque-bearing aortae from the *Apoe*$^{-/-}$ mice into wild-type mice, DC-like cells migrate through adventitial lymph vessels to local lymph nodes in a process that is dependent on the DC migratory molecule CC-chemokine receptor 7 (CCR7) (REF. 24). Although the quantitative importance of this DC egress pathway is not yet known, these data suggest that one factor that impairs key processes of inflammation resolution is the hypercholesterolaemic state, perhaps through the induction of inflammatory molecules that block the resolution phase.

## Inflammation-resolving mediators

Anti-inflammatory cytokines, signalling molecules and transcription factors have important roles in the resolution of inflammation[1,2]. The most important of these are IL-10; transforming growth factor-β (TGFβ); lipid mediators such as lipoxins, resolvins, protectins, maresins and prostaglandins; and, in the case of macrophages in atherosclerosis, liver X receptors (LXRs) and the PPAR transcription factors[25–30]. The main target cells of IL-10 are macrophages and DCs, in which IL-10 receptor signalling

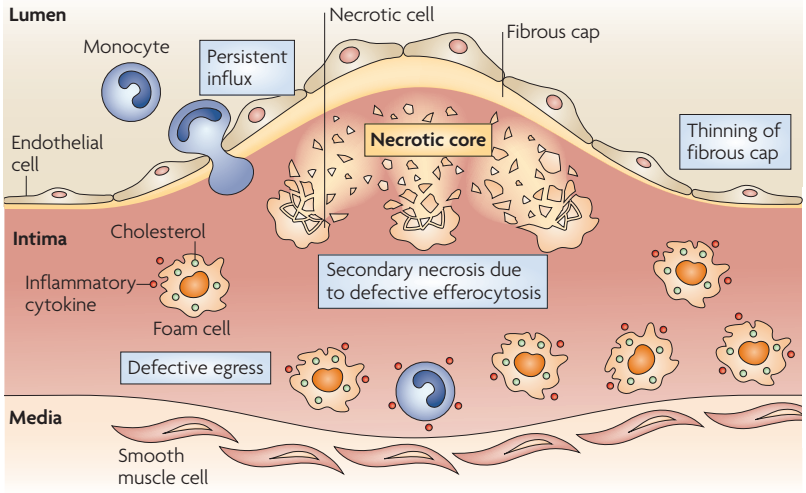


Figure 1 | **A vulnerable atherosclerotic plaque showing the hallmarks of defective resolution of inflammation.** Inflammatory cells, including lipid-laden macrophage foam cells, accumulate in the intima owing to the persistent influx of new cells, particularly monocytes, and defective egress of the resident cells. Moreover, apoptotic macrophages are not efficiently cleared by efferocytosis and so they undergo secondary necrosis. This process contributes to the formation of the necrotic core, which promotes plaque disruption, particularly thinning of the fibrous cap. If the process continues, the fibrous cap breaches, leading to lumenal thrombosis and arterial occlusion.

induces the expression of the anti-inflammatory molecule suppressor of cytokine signalling 3 (SOCS3) and inhibits the nuclear factor-κB (NF-κB) pathway[25]. The net result is the suppression of macrophage-mediated activation of inflammatory T cells and decreased production of pro-inflammatory cytokines (IL-1, IL-6, IL-12 and TNF) and matrix metalloproteinases by macrophages. IL-10 also activates signal transducer and activator of transcription 3 (STAT3), which inhibits endoplasmic reticulum (ER) stress-induced apoptosis in macrophages by inducing the expression of cell-survival molecules[31]. IL-10 can also enhance efferocytosis both *in vitro* and *in vivo*[32]. When fat-fed low-density lipoprotein receptor $(Ldlr)^{-/-}$ mice, which develop hypercholesterolaemia and atherosclerotic plaques, were transplanted with IL-10-transgenic bone marrow cells, there was a 47% decrease in the atherosclerotic lesion size and an 80% decrease in the necrotic core area compared with mice that received wild-type bone marrow cells[33]. Moreover, IL-10 levels in the serum are lower in humans with acute coronary syndromes and inversely correlate with future atherothrombotic events in survivors of myocardial infarction[34]. Thus, deficiency in the amounts and/or functions of IL-10 may contribute to the defect in inflammation resolution in atherosclerosis.

TGFβ has two key roles in inflammation resolution: suppression of inflammation, particularly as mediated by $CD4^+CD25^+$ regulatory T cells and by phagocytes during the process of apoptotic-cell clearance[35,36], and stimulation of a protective 'scar' response in resolving lesions by inducing collagen production by fibroblasts[37]. A defect in the protective scar response in intimal myofibroblast-like smooth muscle cells may contribute to a key feature of vulnerable atherosclerotic plaques, namely thinning of the fibrous cap[13]. When TGFβ signalling was interrupted in $Apoe^{-/-}$ mice by administration of a decoy soluble TGFβ receptor or TGFβ-specific neutralizing antibodies, plaque progression towards a vulnerable phenotype was accelerated and was associated with a heightened state of inflammation, large necrotic cores and thin fibrous caps[38,39]. By contrast, transgenic overexpression of TGFβ in $Apoe^{-/-}$ mice stabilized atheroma by decreasing these three detrimental end points[37].

A recent study has suggested that macrophage 12,15-lipoxygenase, an enzyme that is involved in arachidonate metabolism, has a role in lesional inflammation resolution in $Apoe^{-/-}$ mice through the synthesis of the pro-resolving lipid mediators lipoxin A4, resolvin D1 and protectin D1 (REF. 11). In particular, 12,15-lipoxygenase deficiency promoted lesion formation, and an inverse correlation between 12,15-lipoxygenase expression and blood level of certain inflammatory cytokines was observed. *In vitro* experiments showed that lipoxin A4, resolvin D1 and protectin D1 suppressed the production of atherosclerosis-associated inflammatory cytokines by lipopolysaccharide (LPS)-activated macrophages and enhanced the ability of macrophages to ingest apoptotic cells[11]. Moreover, TNF-mediated activation of endothelial cells, which has a crucial role in monocyte chemotaxis and adhesion during atherogenesis, was also suppressed by lipoxin A4 and resolvin D1 (REF. 11). Other models have substantiated these findings. For example, in a rabbit model of inflammatory periodontal disease, which is a significant risk factor for ATVD in humans[40], overexpression of 15-lipoxygenase (the rabbit enzyme involved in lipoxin biosynthesis) and the subsequent production of lipoxin A4 were associated with a decrease in high-fat-diet-induced atherosclerosis[41]. In addition to the 12,15-lipoxygenase pathway, there is evidence that a 14-lipoxygenase pathway can generate maresin (macrophage mediator in resolving inflammation), which has potent anti-inflammatory effects[28]. The cyclooxygenase pathway can also participate in inflammation resolution[27,42]. For example, in LPS-stimulated human macrophages *in vitro*, prostaglandin $E_2$ ($PGE_2$) interaction with the EP4 receptor suppressed the expression of inflammatory cytokines and chemokines[43]. This effect was shown to be independent of the canonical EP4–cyclic AMP–protein kinase A pathway[43], but other anti-inflammatory effects of $PGE_2$ involve cAMP–protein kinase A-mediated induction of IL-10 and suppression of NF-κB[44]. Macrophage EP4 deficiency in high-fat-diet-fed $Ldlr^{-/-}$ mice is associated with a decrease in phosphorylated AKT family members and an increase in early lesional macrophage apoptosis[45], suggesting that the predominant effect of the macrophage $PGE_2$–EP4 pathway in this context is induction of an AKT-mediated cell survival pathway.

Another mediator of inflammation resolution, and one that has particular relevance to atherosclerosis, is the LXR family of transcriptional activators. LXRs have anti-inflammatory effects in macrophages[29,46], which include inhibition of NF-κB-mediated gene induction (such as of pro-atherogenic IL-6), suppression of antigen-induced T cell proliferation and suppression of matrix metalloproteinase 9 production (which is thought to mediate plaque disruption)[47,48]. LXR activation is also involved

**Secondary necrosis**
A process that occurs in apoptotic cells that are not cleared by phagocytes. The integrity of the plasma membrane is lost and the constituents of the cell are released.

**Atheroma**
An advanced atherosclerotic plaque, particularly one that is rich in cholesterol-filled macrophage foam cells and has areas of plaque necrosis.

**Apolipoprotein E *(Apoe)*$^{-/-}$ mice**
A widely used mouse model that is prone to develop atherosclerosis because the mice have high levels of types of atherogenic lipoprotein called remnant lipoproteins. This lipoprotein abnormality is cause by the genetic absence of apolipoprotein E (APOE), which normally clears remnant lipoproteins from the bloodstream by interacting with hepatocytes.

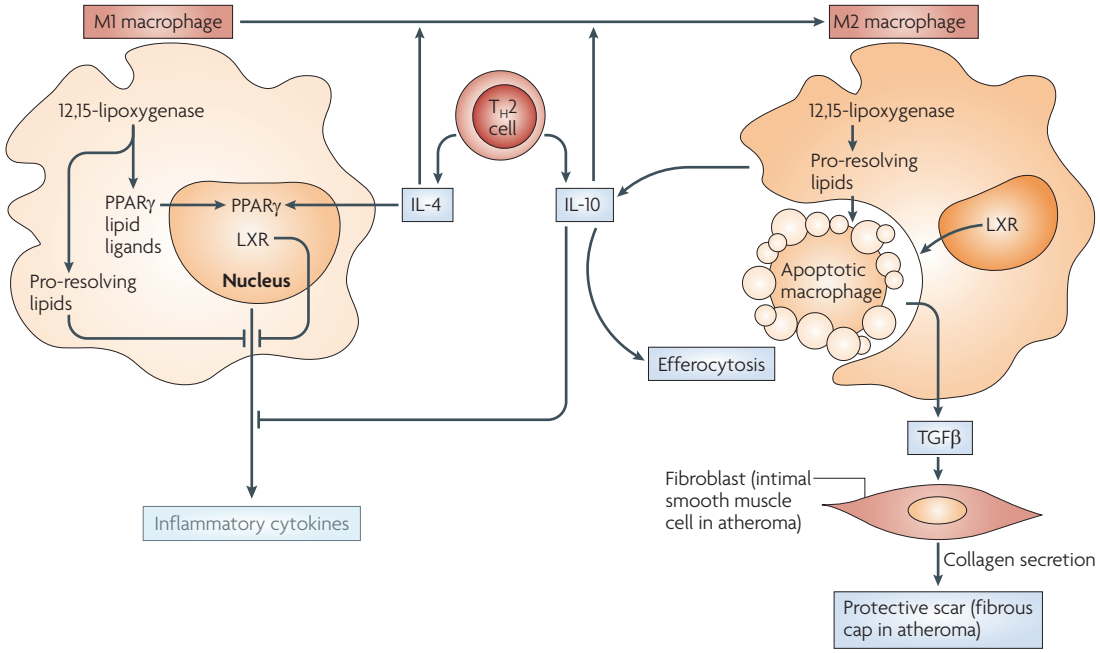


Figure 2 | **Examples of integration of inflammation resolution by resolution mediators.** Interleukin-10 (IL-10), which is secreted by T helper 2 ($T_H2$) cells and by efferocytes during apoptotic cell clearance, blocks inflammatory responses in classically activated M1 macrophages, stimulates the conversion of M1 macrophages to alternatively activated M2 macrophages and enhances efferocytosis itself. $T_H2$ cells also secrete IL-4, which (similarly to IL-10) promotes M2 macrophage formation. IL-4 also induces the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ), which suppresses inflammation in macrophages. Moreover, lipid ligand activators of PPARγ are synthesized through the action of 12,15-lipoxygenase, which also leads to the synthesis of pro-resolving lipids, including lipoxins, resolvins and protectins. These lipid mediators suppress inflammatory cytokine production and stimulate efferocytosis. Activation of another family of transcription factors, the liver X receptors (LXRs), links two key features of inflammation resolution: suppression of inflammatory cytokine production and enhancement of efferocytosis. Successful efferocytosis leads to the production of transforming growth factor-β (TGFβ), which stimulates formation of scar tissue in wound healing during inflammation resolution and the protective fibrous cap in atheroma.

Endoplasmic reticulum (ER) stress
Perturbation of ER function, such as that which occurs during a high level of protein translation or when newly synthesized proteins become misfolded, resulting in the activation of a corrective signal transduction pathway called the unfolded protein response.

Low-density lipoprotein receptor *(Ldlr)*$^{-/-}$ mice
Another widely used mouse model of atherosclerosis. These mice accumulate high levels of low-density lipoprotein (LDL) when on a high-fat diet because their hepatocytes lack LDL receptors and cannot efficiently rid the bloodstream of atherogenic LDL particles.

Atherosclerotic lesion
A collection of lipids, cells and extracellular matrix in a focal area of the arterial subendothelium. These lesions are triggered by the accumulation of apolipoprotein B-containing lipoproteins and a maladaptive, macrophage-dominant inflammatory response to these lipoproteins. With further intracellular lipid accumulation and formation of foam cells, atherosclerotic plaques develop. The later-stage lesions contain a core of extracellular lipid surrounding the cholesterol-laden cells, many of which undergo apoptosis or necrosis.

Periodontal disease
A bacteria-mediated inflammatory disease of the gums that has an epidemiological association with atherothrombotic vascular disease, perhaps through promoting systemic inflammation.

in a key step in atherosclerosis regression, namely cholesterol efflux from cholesterol-loaded macrophages[49,50]. In mouse models of atherosclerosis, LXR agonists markedly suppress lesion progression, even after lesions have become established, and deletion of *Lxr* promotes lesion progression[51–53].

Importantly, the various cellular processes of inflammation resolution can be linked by these inflammation-resolving factors (FIG. 2). For example, IL-10 not only directly blocks inflammatory responses in M1 macrophages but also stimulates the conversion of M1 macrophages to the M2 subtype and enhances efferocytosis[3]. Efferocytosis, in turn, promotes further IL-10 production[36]. LXR activation in macrophages links two key features of inflammation resolution: suppression of inflammatory cytokine production[29] and enhancement of efferocytosis through the induction of at least two efferocytosis receptors, transglutaminase 2 (encoded by *TGM2*) and proto-oncogene tyrosine-protein kinase MER (MERTK)[54,55]. As a third example of integration, successful efferocytosis leads to the production of TGFβ[36], which stimulates the formation of the protective fibrous cap in atheroma[37]. 12,15-lipoxygenase not only leads to the synthesis of lipid mediators of inflammation resolution, including those that enhance efferocytosis[26], but also mediates the induction of lipids, such as 13-hydroxyoctadecadienoic acid (13-HODE) and 15-hydroxyeicosatetraenoic acid (15-HETE), that are activators of anti-inflammatory PPARγ[30]. In this regard, PPARγ itself is induced by IL-4, which promotes the formation of M2 macrophages[3].

## Macrophage death and efferocytosis

***Early lesional macrophage death.*** Although the mechanisms of macrophage death in early lesions are not well known, apoptosis occurs in macrophage-rich regions of early lesions[56]. Several studies have used genetic engineering in mouse models of atherosclerosis to either increase or decrease macrophage apoptosis in early lesions, and these studies have shown that apoptosis in this setting is associated with smaller lesion size and less plaque progression[45,57–60]. A likely explanation for this phenomenon is that apoptotic cells are efficiently cleared by neighbouring macrophages in early lesions and therefore the lesions would have fewer inflammatory cells and post-apoptotic necrotic cells, as well as more efferocyte-derived anti-inflammatory mediators. Support for this concept comes from a study in which the efferocytosis mediator complement component 1q (C1q) was genetically targeted in *Ldlr*$^{-/-}$ mice[61]. In these mice, the appearance of apoptotic macrophages in early lesions increased dramatically, suggesting that the normal paucity of detectable apoptotic cells in early lesions is due to efficient efferocytosis.

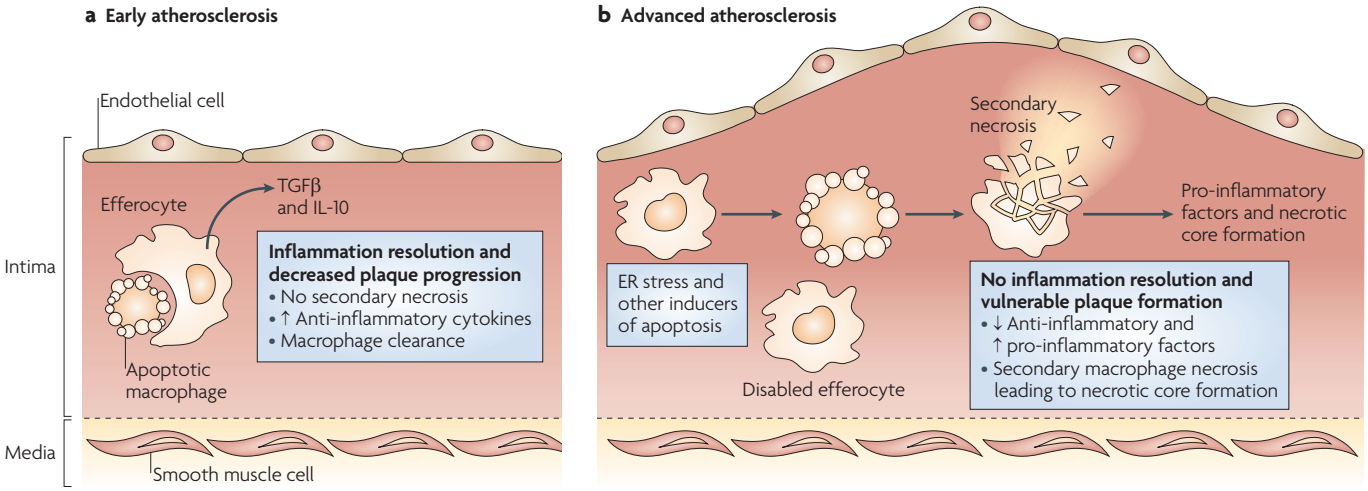


Figure 3 | **Efferocytosis and inflammation resolution in early and advanced atherosclerosis. a** | In early atherosclerotic lesions, efferocytosis is efficient, leading to rapid clearing of apoptotic macrophages. This process prevents secondary necrosis, elicits the production of anti-inflammatory cytokines and clears macrophages from the lesions. The result of this inflammation resolution process is decreased plaque progression. **b** | In advanced lesions, efferocytes do not function properly and thus apoptotic macrophages, which arise in part from endoplasmic reticulum (ER) stress-induced apoptosis, become secondarily necrotic. The necrotic material is a stimulus for inflammation, and the normal anti-inflammatory signalling associated with efferocytosis does not occur. Moreover, an important mechanism for ridding the lesion of inflammatory macrophages is lost. Thus, inflammation resolution fails to occur normally, and necrotic macrophages coalesce into necrotic cores. These features define plaques that are vulnerable to rupture, which in turn can trigger acute lumenal thrombosis and arterial occlusion. IL-10, interleukin-10; TGFβ, transforming growth factor-β.

The corollary of this concept is that the elements of defective inflammation resolution known to exist in advanced lesions, notably defective efferocytosis, have not yet taken hold in early atherosclerosis[9,10] (FIG. 3a).

***Advanced lesional macrophage death.*** Several hypotheses have been proposed to explain macrophage apoptosis in advanced lesions, and undoubtedly more than one mechanism is involved. Examples include growth factor deprivation and the presence of toxic cytokines and oxidized lipids or lipoproteins[62], but there is currently little evidence for these hypotheses *in vivo*. However, there is increasing *in vivo* evidence supporting the concept that ER stress may contribute greatly to advanced lesional macrophage death (FIG. 3b). ER stress activates a branch of the unfolded protein response (UPR) pathway that increases the expression of the pro-apoptotic protein CEBP-homologous protein (CHOP; also known as DDIT3)[63]. The expression of CHOP can lead to apoptosis by several mechanisms, but recent work points to a specific apoptotic mechanism that involves release of $Ca^{2+}$ from the ER lumen[64,65]. There are many ER stressors present in advanced atherosclerotic lesions, and expression of ER stress proteins — including CHOP — is closely correlated with apoptosis and plaque vulnerability in human coronary arteries[66]. *In vitro* studies have suggested that some of these atherosclerosis-relevant ER stressors, such as 7-ketocholesterol, are sufficient by themselves to trigger this apoptotic pathway if they are present in sufficient amounts[64]. However, in cases of more subtle ER stress a second signal is needed to trigger apoptosis[67,68]. An example includes excess accumulation of lipoprotein-derived cholesterol by macrophages, in which the ER stress signal arises from excess cholesterol accumulation in the ER membrane, and the second signal is engagement of pattern recognition receptors (PRRs), which enhance pro-apoptotic processes and suppress compensatory cell-survival signalling in the setting of ER stress[67,68].

There are *in vivo* molecular–genetic causation data supporting these concepts. Although mouse models of atherosclerosis, such as high-fat-diet-fed *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, are not useful for studying plaque disruption or acute atherothrombosis, they are valuable for studying atherosclerosis up to and including the stage of plaque necrosis[69]. Using these models, it has been possible to test the pathophysiological relevance of specific mechanisms of macrophage apoptosis, as well as the suggestion that advanced lesional macrophage death promotes plaque necrosis (TABLE 1). For example, advanced lesional macrophage apoptosis and plaque necrosis are reduced in a mouse model with a heterozygous mutation in *Npc1* (which facilitates ER stress in the setting of excess cholesterol), and in mice with null mutations in *Stat1* (which is a key pro-apoptotic signalling molecule downstream of the ER stress and PRR pathway), *Chop*, or *Sra1* and *Cd36* (which encode PRRs that can serve as second signals for ER stress-induced apoptosis)[70–73]. Moreover, in the context that insulin resistance is a major risk factor for ATVD in industrialized societies[4,5], activation of the macrophage ER stress–apoptosis pathway, advanced lesional macrophage apoptosis and plaque necrosis are enhanced in the setting of macrophage insulin resistance[74,75]. Finally, apoptotic processes in general, and ER stress-induced apoptosis in particular, usually results from an imbalance between apoptotic and compensatory cell-survival pathways. For example, ER stress

Unfolded protein response (UPR). A response that increases the ability of the endoplasmic reticulum (ER) to fold and translocate proteins, decreases the synthesis of proteins degrades misfolded proteins and corrects disturbances in calcium and redox imbalance in the ER. If prolonged, the UPR can trigger apoptosis.

Table 1 | **Mouse models of ER stress-induced macrophage apoptosis and defective efferocytosis in atherosclerosis**

| Mouse model | Description of mutation | Weeks on high-fat diet | Effect of mutation on macrophage apoptosis and plaque necrosis in advanced aortic root lesions | Refs |
|---|---|---|---|---|
| ***Models affecting ER stress-induced macrophage apoptosis*** | | | | |
| *Npc1*$^{+/-}$ → *Apoe*$^{-/-}$ mice* | NPC1 is involved in intracellular cholesterol trafficking; the heterozygous mutation results in a partial defect of cholesterol trafficking to the ER and thus protects the ER from cholesterol-induced ER stress | 18 or 25 | ↓ | 70 |
| *Stat1*$^{-/-}$ → *Ldlr*$^{-/-}$ mice* | STAT1 is activated during ER stress and promotes macrophage apoptosis | 10 or 12 | ↓ | 71 |
| *Chop*$^{-/-}$*Apoe*$^{-/-}$ mice or *Chop*$^{-/-}$*Ldlr*$^{-/-}$ mice | CHOP is induced during ER stress and triggers apoptosis in ER-stressed macrophages | 10 or 12 | ↓ | 72 |
| *Insr*$^{-/-}$ → *Ldlr*$^{-/-}$ mice* | Macrophages lacking insulin receptors, as a model of macrophage insulin resistance, undergo ER stress and are more susceptible to ER stress-induced apoptosis | 8 or 12 | ↑ | 75 |
| *Sra*$^{-/-}$*Cd36*$^{-/-}$*Apoe*$^{-/-}$ mice | Activation of SRA and CD36 synergizes with ER stress to cause apoptosis in macrophages | 12 | ↓ | 73 |
| *p38a*$^{fl/fl}$*Lysm*$^{Cre+/-}$*Apoe*$^{-/-}$ mice‡ | ER stress activates p38 in macrophages, which can trigger a compensatory cell survival pathway through activation of AKT | 9 | ↑ | 76 |
| ***Models affecting efferocytosis*** | | | | |
| *Tg2*$^{-/-}$ → *Ldlr*$^{-/-}$ mice* | TG2 mediates recognition of apoptotic cells by efferocytic macrophages | 16 | ↑ | 88 |
| *Mfge8*$^{-/-}$ → *Ldlr*$^{-/-}$ mice* | MFGE8 is a molecule that can bridge apoptotic cells and efferocytes, thus facilitating efferocytosis | 8, 15 or 20 | ↑ | 89 |
| *Mertk*$^{KD}$*Apoe*$^{-/-}$ mice§ | MERTK is a receptor on efferocytes for apoptotic cells; the kinase domain mutation renders the receptor non-functional | 8, 10, 15 or 16 | ↑ | 90,91 |
| Gld-*Apoe*$^{-/-}$ mice | The mutation in Gld mice inactivates the ligand for the Fas receptor. Mice with this mutation acquire an autoimmune syndrome characterized by defective efferocytosis | 12 | ↑ | 92 |

*Apoe*, apolipoprotein E; *Chop*, CEBP-homologous protein (also known as *Ddit3*); Cre, cre recombinase; ER, endoplasmic reticulum; *Insr*, insulin receptor; *Ldlr*, low-density lipoprotein receptor; *Lysm*, lysozyme M; *Mertk*, proto-oncogene tyrosine-protein kinase MER; *Mfge8*, milk fat globulin E8; *Npc1*, Niemann-Pick C1 protein; *Sra*, type A scavenger receptor; *Stat1*, signal transducer and activator of transcription 1; *Tg2*, transglutaminase 2. *This designation indicates transplantation of bone marrow from mutant mice into lethally irradiated *Apoe*$^{-/-}$ or *Ldlr*$^{-/-}$ mice to create chimeric mice in which only bone marrow-derived cells carry the mutation. Bone marrow cells from wild-type mice are used to generate control mice. Using this method, the main type of lesional cell carrying the mutation is the macrophage. ‡fl/fl refers to both alleles of the indicated gene being flanked by loxP sites. §*Mertk*$^{KD}$ is a kinase domain mutation of the MERTK protein.

activates the mitogen-activated protein kinase p38 family, which promote cell survival in certain settings of ER stress by activating Akt family members, known mediators of cell survival[76]. Macrophage-targeted knockout of p38α in *Apoe*$^{-/-}$ mice enhanced both advanced lesional macrophage apoptosis and plaque necrosis[76].

Advanced plaques in humans also show markers of ER stress and evidence of macrophage apoptosis[77–79]. In one study, autopsy specimens and fresh atherectomy specimens of human coronary arteries from patients with heart disease were divided into five stages of atherosclerosis, from very early lesions to vulnerable plaques, and analysed for UPR markers and apoptosis. The results showed a close correlation between UPR marker expression (including CHOP), apoptosis and advanced plaque morphology[78]. These correlative findings set the stage for genetic studies, such as genome-wide association studies, to identify a more causative relationship between genes in the ER stress–apoptosis pathway and human coronary artery disease.

***Efferocytosis.*** A key step in the resolution of inflammation is efferocytosis of apoptotic inflammatory cells[36,80]. In acute inflammation, this process mostly involves the clearance of short-lived apoptotic neutrophils. However, in the chronic inflammatory process of atherosclerosis, most of the apoptotic cells that need to be cleared are apoptotic macrophages[9,61]. Efficient clearance of apoptotic cells prevents secondary necrosis and triggers an anti-inflammatory response through the induction of TGFβ, IL-10 and other anti-inflammatory cytokines[80]. Inflammation can also be suppressed in phagocytes by molecules released by the apoptotic cells themselves, such as protein S100A9 released by apoptotic neutrophils[81]. Of relevance to ATVD, uncleared apoptotic cells also shed plasma membrane microparticles, which can stimulate thrombosis[82]. In early atherosclerotic lesions, as mentioned previously, efferocytosis seems to be efficient. In advanced lesions, however, increased apoptosis is associated with plaque necrosis, which suggests inefficient efferocytosis[9,10] (FIG. 3b). Indeed, a clear

defect in efferocytosis in advanced human atherosclerotic lesions compared with a control tissue (human tonsils) was revealed by a study in which *in situ* efferocytosis was assessed through quantification of apoptotic cells that were either associated with or free from neighbouring phagocytes in these tissues[83].

What are the molecular mechanisms of efferocytosis in advanced atheroma? And what goes wrong in this setting? Efferocytosis involves a complex interplay between several sets of proteins that enable the recognition and engulfment of apoptotic cells by phagocytes[80,84]. In the early stages of apoptosis, cells secrete factors, referred to as 'find me' signals, that attract phagocytes (for example, lysophosphatidylcholine[85]), and they suppress the secretion of other molecules, referred to as 'don't eat me' signals, that normally prevent the phagocytosis of non-apoptotic cells (for example, CD47 (REF. 86)). Apoptotic cells also express a unique set of surface molecules that recognize and bind to cognate receptors on the apoptotic cell (for example, phosphatidylserine[87]). In several cases, the interaction between the surface molecule and receptors involves an intermediary bridging molecule. For example, αVβ3 integrin and MERTK on phagocytes interact with apoptotic cells through the bridging molecules milk fat globulin E8 (MFGE8; also known as lactadherin) and growth arrest-specific protein 6 (GAS6), respectively[80,84]. Contact between apoptotic cells and phagocytes enables engulfment of the apoptotic cells, leading eventually to digestion in phagolysosomes. In addition, as mentioned above, receptor engagement on the phagocyte often triggers an anti-inflammatory response[36,80]. Mouse studies have revealed specific roles for several efferocytosis receptors or bridging molecules in advanced atherosclerosis, including transglutaminase 2 (REF. 88), MFGE8 (REF. 89), MERTK[90,91] and FAS ligand (also known as CD95L)[92] (TABLE 1). For example, *Apoe*$^{-/-}$ mice lacking MERTK have a defect in efferocytosis in advanced atheroma that correlates with an increase in plaque inflammation and plaque necrosis[90,91].

What seems to be defective efferocytosis in advanced atheroma could, in theory, instead be extensive apoptosis that overwhelms the phagocytic capacity of macrophages. However, the normal high capacity of macrophages for efferocytosis, including that seen in early atherosclerotic lesions, suggests that this is, at most, only part of the explanation[59,80]. A second possibility is that advanced lesional macrophage death *per se* limits efferocytosis by limiting the pool of competent efferocytes. However, advanced lesions have a large population of macrophages that could presumably carry out this role[93]. Furthermore, even when macrophages accumulate large amounts of cholesteryl fatty acid esters and become foam cells, which is a common feature of lesional macrophages, their ability to recognize and engulf apoptotic cells is not compromised[94]. In addition, efferocytes possess a remarkable ability to remain viable even after they engulf apoptotic cells that are extremely rich in potentially toxic cholesterol, because the process of efferocytosis activates several cholesterol efflux and cell survival pathways that protect the cells[95]. A third possibility is that advanced lesional apoptotic macrophages are poor substrates for efferocytic recognition and engulfment. For example, the nature of the apoptotic stimulus or some other feature in these lesions might lead to poor display of efferocytosis ligands or find me signals and/or inappropriately increased expression of don't eat me signals on the apoptotic cells. A fourth possibility is that expression or function of bridging molecules or efferocytosis receptors is deficient in atheroma. In this context, MERTK is susceptible to sheddase-mediated cleavage, leading to release of the extracellular portion of the receptor, which is referred to as soluble MER[96]. MERTK cleavage inhibits efferocytosis by two mechanisms — destruction of MERTK and competitive inhibition of efferocytosis by soluble MER[96]. As another example, the expression of the bridging molecules GAS6 and MFGE8 may be downregulated in advanced lesions by the processes of lesional smooth-muscle cell death and inflammation, respectively[97]. There may also be inappropriate activation of negative regulators of efferocytosis, such as a recently discovered lipid phosphatase called myotubularin[98]. Some of these changes in efferocyte function may be related to alterations in phagocyte subpopulations in advanced lesions: that is, an increase in DCs, which are less efficient at clearing apoptotic cells than macrophages[99], or an increase in the ratio of M1/M2 macrophages[3]. In this context, a deficiency in PPARδ and/or its activators in advanced lesions could increase the M1/M2 ratio and decrease efferocytosis[100,101].

Defective efferocytosis may also be associated with the ageing process, which is a major risk factor for ATVD[102]. For example, clearance of apoptotic cells is defective in the tissues of aged mice and in isolated DCs from elderly humans[103,104]. The mechanism of this defect is not known, but the defect in the mouse study could be reproduced *in vitro* by exposure of macrophages from young mice to serum from old mice, suggesting the presence of an efferocytosis-inhibitory factor or the absence of a soluble efferocytosis effector in the aged mouse serum[103]. In the human DC study there was a general defect in phagocytic signalling[104]. Finally, to the extent that some degree of efferocytosis does occur in advanced lesions, a crucial component of anti-inflammatory signalling may be defective, for example owing to a defect in secretion of, or response to, anti-inflammatory cytokines. Whatever the mechanisms, increasing evidence suggests that defective efferocytosis in advanced atheroma is a major cause of necrotic core formation and thus contributes substantially to the formation of vulnerable plaques.

Foam cell
A macrophage in the arterial wall that ingests cholesterol-rich apolipoprotein B-containing lipoproteins and thereby accumulates cholesteryl fatty acid esters. These cells secrete various substances involved in plaque growth.

## Future directions and therapeutic implications

The ability to translate the complex process of plaque progression into an integrated molecular and cellular concept of defective inflammation resolution provides a useful way to understand how atherosclerosis leads to clinical disease and how plaque progression may be prevented by new therapeutic approaches. In this context, it is important to outline areas within this paradigm that

need further investigation. Most importantly, we need a more complete understanding of the molecular and cellular mechanisms for defective inflammation resolution in advanced atherosclerosis. Although this Review has focused on upstream mediators of inflammation resolution, complex negative feedback mechanisms at the transcriptional and translational levels are crucial sequelae of the inflammatory response[105,106]. At a fundamental level, it will be important to elucidate what portion of the defect in inflammation resolution in advanced atherosclerosis is due to defective production of inflammation-resolving effectors and what portion is due to the defective function of these effectors at the cellular level; for example, decreased activity of receptor signal transduction pathways.

Now that we possess a reasonably complete list of these effectors, and with a good knowledge of how they act on cells, we can probe plaques during the most important period of progression to the necrotic (thin fibrous cap) stage for transcription factors and enzymes involved in effector formation and for the receptors and signalling molecules that are activated by these effectors. A good knowledge of the cell types involved is also crucial, and more work is needed in this area. T cells are involved in the production of certain inflammation-resolving effectors, such as IL-10, and in the production of factors that regulate the function of these effectors. For example, $T_H2$- and $T_H1$-type cytokines influence the balance between M2 and M1 macrophages, respectively[3]. The main inflammation resolution effector cells in lesions are macrophages and DCs, and understanding how each of the various subsets of these cell types influences the overall resolution response — and the defects of these cells in advanced lesions — is an important goal for the future. Moreover, it will be interesting to assess the roles of mast cells and neutrophils in normal and defective inflammation resolution, because studies are increasingly implicating these two cell types in atherogenesis and plaque progression[107,108].

The answers to these questions will increasingly inform the field as to the best therapeutic approaches to prevent defective inflammation resolution in progressing atherosclerotic plaques and to reverse the defect in those plaques that are already advanced. On the basis of our current knowledge, we can begin to propose strategies. With regard to lipid mediators of inflammation resolution, omega-3 long-chain fatty acids, which occur naturally in fish oils, are precursors of protectins, resolvins and maresins[26,28], and the fish oil eicosapentaenoic acid has been shown to have beneficial effects on ATVD in humans[109]. More in-depth understanding of the mechanisms of these effects may suggest more specific and potent drugs to enhance inflammation resolution in advanced atherosclerotic lesions. In this context, exposure of macrophages to saturated fatty acids *in vitro* and exposure of obese mice to a saturated fatty acid-enriched diet led to defects in macrophage efferocytosis, including in advanced atherosclerotic lesions, and one mechanism may be the displacement of the omega-3 fatty acids in macrophages by the saturated fatty acids[110]. Activators of LXRs and PPARs are already available as oral-based formulations, although off-target systemic effects, notably hepatosteatosis and heart failure, respectively, limit their current usefulness[47]. As another example, the orally available drug FTY720, a mimetic of the pro-resolution lipid S1P, has been shown to increase the proportion of M2 macrophages in atherosclerotic lesions and slow the lesion progression in mice[18]. In a mouse model of peritonitis, an analogue of the pro-resolution lipid lipoxin A4 enhanced efferocytic clearance of apoptotic neutrophils[111]. Endogenous and exogenous cannabinoids, which are fatty acid derivatives, can trigger inflammation resolution, perhaps through stimulation of the biosynthesis of lipid mediators[112]. Indeed, oral administration of the cannabinoid receptor 1 agonist rimonabant suppressed atherosclerosis and plaque inflammation in high-fat-diet-fed *Ldlr*$^{-/-}$ mice[113]. However, there are significant side effects to rimonabant[114] and so, as with LXR and PPAR agonists, the safest application of these potent agents would be in a setting that optimizes the specific accumulation of the drug in atherosclerotic plaques. Thus, future developments in plaque-targeted drug technology will be important with these agents.

Therapeutic administration of bioactive proteins is an even greater technical challenge but one with great promise. For example, causation data in mice and genetic association data in humans suggest that the inflammation-resolving effectors IL-10 and TGFβ can prevent plaque progression[33,34,37–39]. The protein annexin I, which is an anti-inflammatory downstream effector molecule of glucocorticoids, has been shown to increase the efferocytosis of apoptotic neutrophils in the inflamed joints of a mouse model of systemic lupus erythematosus[115]. The challenge in these cases is how to administer a bioactive protein and how to do it in a plaque-targeted manner. New approaches in plaque-targeted protein delivery systems, such as nanoparticle-based therapy, may offer great promise in this area[116]. A different type of innovative strategy to enhance efferocytosis was suggested by Silverman and colleagues[117], who showed that immunization of mice with apoptotic cells boosted the production of natural IgM antibodies to apoptotic cell surface proteins, leading to C1q recruitment and increased efferocytosis. Others have suggested that if efferocytosis could be enhanced, purposefully inducing inflammatory cell apoptosis could speed the process of inflammation resolution[118].

Successful inflammation resolution-based therapies for atherosclerosis may necessitate non-oral therapeutic regimens; thus, the primary niche may be short-term therapy after an acute coronary event or in subjects with severe ATVD risk factors who have not been on long-term risk reduction therapy. The foundation of chronic ATVD preventative therapy is risk reduction through cholesterol lowering, mainly by using statins, blood pressure control, treatment of insulin resistance (including lifestyle changes) and elimination of environmental risk factors, notably smoking. However, it takes up to two years for such therapy to show maximum beneficial effects, and even with these therapies, many patients remain at high risk for recurrent disease[119]. In this

**Statins**
A family of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA reductase), an enzyme that catalyses the conversion of HMG-CoA to L-mevalonate. These molecules are mainly used as cholesterol-lowering drugs, but they also have immunoregulatory and anti-inflammatory properties, the clinical significance of which is not fully known. L-mevalonate and its metabolites are implicated in cholesterol synthesis and other intracellular pathways.

context, one can envision that safe and effective therapy directed specifically at preventing further plaque progression and promoting plaque regression could substantially lower events during this period. Moreover, therapy directed at improving inflammation resolution would be expected to alter the overall inflammatory milieu of vulnerable plaques in a manner that should improve the effectiveness of conventional, chronic plaque-stabilizing therapies, such as statins. Given the high risk of morbidity and mortality during this period, the benefits of periodic systemic therapy should outweigh the inconvenience.

## Summary and conclusions

In industrialized societies, almost all individuals have subclinical atherosclerotic lesions by the time they enter their early twenties[120]. Although only a minority of the lesions in these individuals will progress to cause ATVD, the number of lesions is so high that this progression accounts for the leading cause of morbidity and mortality in this population. Therefore, defining the molecular and cellular processes that cause the transition of subclinical atherosclerotic lesions to dangerous plaques is essential. In this Review, I have discussed recent studies providing evidence that defects in specific molecular and cellular events that normally function to limit or resolve inflammatory processes can account for a substantial portion of this transition. In contemplating how this knowledge can lead to useful therapy, it is important to understand that atherosclerosis progression in general, and defective inflammation resolution in particular, are complex processes, and it is unlikely that only one approach will be effective. Instead, therapeutic approaches that target complementary processes in defective inflammation resolution are likely to have the most success. Fortunately, several of these processes are mediated by common effector molecules (FIG. 2), which could be feasible targets for both conventional types of drugs and drugs that will require new means of formulation, delivery and tissue targeting. Given the increasingly prevalent pressures of ageing and obesity on ATVD in our society and the rest of the world, even modest progress in this area over the next decade could have a huge beneficial effect.


1. Serhan, C. N. *et al.* Resolution of inflammation: state of the art, definitions and terms. *FASEB J.* **21**, 325–332 (2007).
**This review presents an outstanding summary of the principles of inflammation resolution, many of which apply to the concept of resolution failure in atherosclerosis.**
2. Lawrence, T. & Gilroy, D. W. Chronic inflammation: a failure of resolution? *Int. J. Exp. Pathol.* **88**, 85–94 (2007).
3. Martinez, F. O., Helming, L. & Gordon, S. Alternative activation of macrophages: an immunologic functional perspective. *Annu. Rev. Immunol.* **27**, 451–483 (2009).
4. [No authors listed]. 2007 NHLBI Morbidity and Mortality Chart Book. *NHLBI Morbidity and Mortality Chartbook* [online], http://www.nhlbi.nih.gov/resources/docs/cht-book.htm (2007).
5. Grundy, S. M. Obesity, metabolic syndrome, and cardiovascular disease. *J. Clin. Endocrinol. Metab.* **89**, 2595–2600 (2004).
6. Tabas, I., Williams, K. J. & Boren, J. Subendothelial lipoprotein retention as the initiating process in atherosclerosis: update and therapeutic implications. *Circulation* **116**, 1832–1844 (2007).
7. Lusis, A. J. Atherosclerosis. *Nature* **407**, 233–241 (2000).
8. Glass, C. K. & Witztum, J. L. Atherosclerosis: the road ahead. *Cell* **104**, 503–516 (2001).
9. Tabas, I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. *Arterioscler. Thromb. Vasc. Biol.* **25**, 2255–2264 (2005).
10. Schrijvers, D. M., De Meyer, G. R., Herman, A. G. & Martinet, W. Phagocytosis in atherosclerosis: Molecular mechanisms and implications for plaque progression and stability. *Cardiovasc. Res.* **73**, 470–480 (2007).
**References 9 and 10 present the concept that efferocytosis, a key process in inflammation resolution, is defective in advanced atherosclerotic lesions and is an important factor in plaque necrosis.**
11. Merched, A. J., Ko, K., Gotlinger, K. H., Serhan, C. N. & Chan, L. Atherosclerosis: evidence for impairment of resolution of vascular inflammation governed by specific lipid mediators. *FASEB J.* **22**, 3595–3606 (2008).
**This paper provides evidence that lipid mediators of inflammation resolution derived from 12,15-lipoxygenase, including lipoxin A4, resolvin D1 and protectin D1, suppress atherosclerotic plaque progression in the *Apoe*$^{-/-}$ mouse model of atherosclerosis.**
12. Randolph, G. J. Emigration of monocyte-derived cells to lymph nodes during resolution of inflammation and its failure in atherosclerosis. *Curr. Opin. Lipidol.* **19**, 462–468 (2008).
13. Virmani, R., Burke, A. P., Kolodgie, F. D. & Farb, A. Vulnerable plaque: the pathology of unstable coronary lesions. *J. Interv. Cardiol.* **15**, 439–446 (2002).
14. Swirski, F. K. *et al.* Monocyte accumulation in mouse atherogenesis is progressive and proportional to extent of disease. *Proc. Natl Acad. Sci. USA* **103**, 10340–10345 (2006).
15. Swirski, F. K. *et al.* Ly-6C$^{hi}$ monocytes dominate hypercholesterolemia-associated monocytosis and give rise to macrophages in atheromata. *J. Clin. Invest.* **117**, 195–205 (2007).
**References 14 and 15 show that inflammatory monocytes continually enter atherosclerotic lesions even after inflammation has been long-standing. This finding supports the concept that a key process in inflammation resolution (eventual suppression of inflammatory cell influx) is defective in atherosclerosis.**
16. Chawla, A. *et al.* PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nature Med.* **7**, 48–52 (2001).
17. Odegaard, J. I. *et al.* Macrophage-specific PPARγ controls alternative activation and improves insulin resistance. *Nature* **447**, 1116–1120 (2007).
**Although not dealing directly with atherogenesis, this paper provides a link between the PPAR family of transcriptional activators and a key process in inflammation resolution, namely the alternative activation of macrophages.**
18. Nofer, J. R. *et al.* FTY720, a synthetic sphingosine 1 phosphate analogue, inhibits development of atherosclerosis in low-density lipoprotein receptor-deficient mice. *Circulation* **115**, 501–508 (2007).
19. Laurat, E. *et al. In vivo* downregulation of T helper cell 1 immune responses reduces atherogenesis in apolipoprotein E-knockout mice. *Circulation* **104**, 197–202 (2001).
20. Li, A. C. *et al.* Differential inhibition of macrophage foam-cell formation and atherosclerosis in mice by PPARα, β/δ, and γ. *J. Clin. Invest.* **114**, 1564–1576 (2004).
**This study showed that PPARγ, a mediator of inflammation resolution and cholesterol efflux in atherosclerotic macrophages, has a beneficial effect on lesion progression.**
21. Angeli, V. *et al.* Dyslipidemia associated with atherosclerotic disease systemically alters dendritic cell mobilization. *Immunity* **21**, 561–574 (2004).
22. Llodra, J. *et al.* Emigration of monocyte-derived cells from atherosclerotic lesions characterizes regressive, but not progressive, plaques. *Proc. Natl Acad. Sci. USA* **101**, 11779–11784 (2004).
**This paper provides *in vivo* evidence that macrophage egress, a key process in inflammation resolution, is defective in mouse atherosclerotic lesions but that it can occur in a setting in which regression of atherosclerosis is promoted.**
23. Quah, B. J. & O'Neill, H. C. Maturation of function in dendritic cells for tolerance and immunity. *J. Cell. Mol. Med.* **9**, 643–654 (2005).
24. Trogan, E. *et al.* Gene expression changes in foam cells and the role of chemokine receptor CCR7 during atherosclerosis regression in ApoE-deficient mice. *Proc. Natl Acad. Sci. USA* **103**, 3781–3786 (2006).
25. Mosser, D. M. & Zhang, X. Interleukin-10: new perspectives on an old cytokine. *Immunol. Rev.* **226**, 205–218 (2008).
26. Serhan, C. N., Chiang, N. & Van Dyke, T. E. Resolving inflammation: dual anti-inflammatory and pro-resolution lipid mediators. *Nature Rev. Immunol.* **8**, 349–361 (2008).
27. Willoughby, D. A., Moore, A. R., Colville-Nash, P. R. & Gilroy, D. Resolution of inflammation. *Int. J. Immunopharmacol.* **22**, 1131–1135 (2000).
28. Serhan, C. N. *et al.* Maresins: novel macrophage mediators with potent antiinflammatory and proresolving actions. *J. Exp. Med.* **206**, 15–23 (2009).
29. Joseph, S. B., Castrillo, A., Laffitte, B. A., Mangelsdorf, D. J. & Tontonoz, P. Reciprocal regulation of inflammation and lipid metabolism by liver X receptors. *Nature Med.* **9**, 213–219 (2003).
30. Huang, J. T. *et al.* Interleukin-4-dependent production of PPAR-γ ligands in macrophages by 12/15-lipoxygenase. *Nature* **400**, 378–382 (1999).
31. Li, Y. *et al.* Extracellular Nampt promotes macrophage survival via a nonenzymatic interleukin-6/STAT3 signalling mechanism. *J. Biol. Chem.* **283**, 34833–34843 (2008).
32. Lingnau, M., Hoflich, C., Volk, H. D., Sabat, R. & Docke, W. D. Interleukin-10 enhances the CD14-dependent phagocytosis of bacteria and apoptotic cells by human monocytes. *Hum. Immunol.* **68**, 730–738 (2007).
33. Pinderski, L. J. *et al.* Overexpression of interleukin-10 by activated T lymphocytes inhibits atherosclerosis in LDL receptor-deficient mice by altering lymphocyte and macrophage phenotypes. *Circ. Res.* **90**, 1064–1071 (2002).
**This paper directly links the inflammation-resolving cytokine IL-10 with the key end point of plaque necrosis in mouse atherosclerosis.**

34. Seljeflot, I., Hurlen, M., Solheim, S. & Arnesen, H. Serum levels of interleukin-10 are inversely related to future events in patients with acute myocardial infarction. *J. Thromb. Haemost.* **2**, 350–352 (2004).
35. Wahl, S. M., Swisher, J., Cartney-Francis, N. & Chen, W. TGF-β: the perpetrator of immune suppression by regulatory T cells and suicidal T cells. *J. Leukoc. Biol.* **76**, 15–24 (2004).
36. Huynh, M. L., Fadok, V. A. & Henson, P. M. Phosphatidylserine-dependent ingestion of apoptotic cells promotes TGF-β1 secretion and the resolution of inflammation. *J. Clin. Invest.* **109**, 41–50 (2002).
37. Frutkin, A. D. *et al.* TGF-β1 limits plaque growth, stabilizes plaque structure, and prevents aortic dilation in apolipoprotein e-null mice. *Arterioscler. Thromb. Vasc. Biol.* **29**, 1251–1257 (2009).
38. Mallat, Z. *et al.* Inhibition of transforming growth factor-β signalling accelerates atherosclerosis and induces an unstable plaque phenotype in mice. *Circ. Res.* **89**, 930–934 (2001).
39. Lutgens, E. *et al.* Transforming growth factor-β mediates balance between inflammation and fibrosis during plaque progression. *Arterioscler. Thromb. Vasc. Biol.* **22**, 975–982 (2002).
40. Bahekar, A. A., Singh, S., Saha, S., Molnar, J. & Arora, R. The prevalence and incidence of coronary heart disease is significantly increased in periodontitis: a meta-analysis. *Am. Heart J.* **154**, 830–837 (2007).
41. Van Dyke, T. E. Resolution of inflammation-unraveling mechanistic links between periodontitis and cardiovascular disease. *J. Dent.* **37**, S582–S583 (2009).
42. Peters-Golden, M. Putting on the brakes: cyclic AMP as a multipronged controller of macrophage function. *Sci. Signal.* **2**, pe37 (2009).
43. Takayama, K. *et al.* Prostaglandin E2 suppresses chemokine production in human macrophages through the EP4 receptor. *J. Biol. Chem.* **277**, 44147–44154 (2002).
44. Wall, E. A. *et al.* Suppression of LPS-induced TNF-α production in macrophages by cAMP is mediated by PKA-AKAP95-p105. *Sci. Signal.* **2**, ra28 (2009).
45. Babaev, V. R. *et al.* Macrophage EP4 deficiency increases apoptosis and suppresses early atherosclerosis. *Cell Metab.* **8**, 492–501 (2008).
46. Marathe, C. *et al.* The arginase II gene is an anti-inflammatory target of liver X receptor in macrophages. *J. Biol. Chem.* **281**, 32197–32206 (2006).
47. Hong, C. & Tontonoz, P. Coordination of inflammation and metabolism by PPAR and LXR nuclear receptors. *Curr. Opin. Genet. Dev.* **18**, 461–467 (2008).
48. Castrillo, A., Joseph, S. B., Marathe, C., Mangelsdorf, D. J. & Tontonoz, P. Liver X receptor-dependent repression of matrix metalloproteinase-9 expression in macrophages. *J. Biol. Chem.* **278**, 10443–10449 (2003).
49. Venkateswaran, A. *et al.* Control of cellular cholesterol efflux by the nuclear oxysterol receptor LXRα. *Proc. Natl Acad. Sci. USA* **97**, 12097–12102 (2000).
50. Tall, A. R. Cholesterol efflux pathways and other potential mechanisms involved in the athero-protective effect of high density lipoproteins. *J. Intern. Med.* **263**, 256–273 (2008).
51. Joseph, S. B. *et al.* Synthetic LXR ligand inhibits the development of atherosclerosis in mice. *Proc. Natl Acad. Sci. USA* **99**, 7604–7609 (2002).
**This paper provides *in vivo* evidence linking LXR family members, mediators of inflammation resolution and cholesterol efflux in atherosclerotic macrophages, with a beneficial effect on lesion progression.**
52. Levin, N. *et al.* Macrophage liver X receptor is required for antiatherogenic activity of LXR agonists. *Arterioscler. Thromb. Vasc. Biol.* **25**, 135–142 (2005).
53. Bradley, M. N. *et al.* Ligand activation of LXRβ reverses atherosclerosis and cellular cholesterol overload in mice lacking LXRα and apoE. *J. Clin. Invest.* **117**, 2337–2346 (2007).
54. Rebe, C. *et al.* Induction of transglutaminase 2 by a liver X receptor/retinoic acid receptor-α pathway increases the clearance of apoptotic cells by human macrophages. *Circ. Res.* **105**, 393–401 (2009).
55. Gonzalez, N. *et al.* Apoptotic cells promote their own clearance and immune tolerance through activation of the nuclear receptor LXR. *Immunity* **31**, 245–258 (2009).
56. Kockx, M. M. *et al.* Apoptosis and related proteins in different stages of human atherosclerotic plaques. *Circulation* **97**, 2307–2315 (1998).
57. Liu, J. *et al.* Reduced macrophage apoptosis is associated with accelerated atherosclerosis in low-density lipoprotein receptor-null mice. *Arterioscler. Thromb. Vasc. Biol.* **25**, 174–179 (2005).
58. Boesten, L. S. *et al.* Macrophage p53 controls macrophage death in atherosclerotic lesions of apolipoprotein E deficient mice. *Atherosclerosis* **88**, 780–786 (2009).
59. Arai, S. *et al.* A role for the apoptosis inhibitory factor AIM/Spα/Api6 in atherosclerosis development. *Cell Metab.* **1**, 201–213 (2005).
60. Wang, B. Y. *et al.* Regression of atherosclerosis: role of nitric oxide and apoptosis. *Circulation* **99**, 1236–1241 (1999).
61. Bhatia, V. K. *et al.* Complement C1q reduces early atherosclerosis in low-density lipoprotein receptor-deficient mice. *Am. J. Pathol.* **170**, 416–426 (2007).
62. Tabas, I. Apoptosis and plaque destabilization: the role of macrophage apoptosis induced by cholesterol. *Cell Death. Differ.* **11**, S12–S16 (2004).
63. Zinszner, H. *et al.* CHOP is implicated in programmed cell death in response to impaired function of the endoplasmic reticulum. *Genes Dev.* **12**, 982–995 (1998).
64. Li, G. *et al.* Role of ERO1α-mediated stimulation of inositol 1, 4, 5-triphosphate receptor activity in endoplasmic reticulum stress-induced apoptosis. *J. Cell Biol.* **186**, 783–792 (2009).
65. Timmins, J. M. *et al.* Calcium/calmodulin-dependent protein kinase II links endoplasmic reticulum stress with Fas and mitochondrial apoptosis pathways. *J. Clin. Invest.* **119**, 2925–2941 (2009).
66. Seimon, T. & Tabas, I. Mechanisms and consequences of macrophage apoptosis in atherosclerosis. *J. Lipid Res.* **50**, S382–S387 (2009).
67. DeVries-Seimon, T. *et al.* Cholesterol-induced macrophage apoptosis requires ER stress pathways and engagement of the type A scavenger receptor. *J. Cell Biol.* **171**, 61–73 (2005).
68. Seimon, T. A., Obstfeld, A., Moore, K. J., Golenbock, D. T. & Tabas, I. Combinatorial pattern recognition receptor signalling alters the balance of life and death in macrophages. *Proc. Natl Acad. Sci. USA* **103**, 19794–19799 (2006).
69. Rosenfeld, M. E. *et al.* Animal models of spontaneous plaque rupture: the holy grail of experimental atherosclerosis research. *Curr. Atheroscler. Rep.* **4**, 238–242 (2002).
70. Feng, B. *et al.* Niemann-Pick C heterozygosity confers resistance to lesional necrosis and macrophage apoptosis in murine atherosclerosis. *Proc. Natl Acad. Sci. USA* **100**, 10423–10428 (2003).
71. Lim, W. S. *et al.* STAT1 is critical for apoptosis in macrophages subjected to endoplasmic reticulum stress *in vitro* and in advanced atherosclerotic lesions *in vivo*. *Circulation* **117**, 940–951 (2008).
72. Thorp, E. *et al.* Reduced apoptosis and plaque necrosis in advanced atherosclerotic lesions of *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice lacking CHOP. *Cell Metab.* **9**, 474–481 (2009).
**This paper provides *in vivo* evidence that the UPR effector CHOP has an important role in macrophage apoptosis and plaque necrosis in advanced atherosclerotic lesions in mice.**
73. Manning-Tobin, J. J. *et al.* Loss of SR-A and CD36 activity reduces atherosclerotic lesion complexity without abrogating foam cell formation in hyperlipidemic mice. *Arterioscler. Thromb. Vasc. Biol.* **29**, 19–26 (2009).
74. Liang, C. P. *et al.* Increased CD36 protein as a response to defective insulin signalling in macrophages. *J. Clin. Invest.* **113**, 764–773 (2004).
75. Han, S. *et al.* Macrophage insulin receptor deficiency increases ER stress-induced apoptosis and necrotic core formation in advanced atherosclerotic lesions. *Cell Metab.* **3**, 257–266 (2006).
76. Seimon, T. A. *et al.* Macrophage deficiency of p38α MAPK promotes apoptosis and plaque necrosis in advanced atherosclerotic lesions in mice. *J. Clin. Invest.* **119**, 886–898 (2009).
77. Gargalovic, P. S. *et al.* The unfolded protein response is an important regulator of inflammatory genes in endothelial cells. *Arterioscler. Thromb. Vasc. Biol.* **26**, 2490–2496 (2006).
78. Myoishi, M. *et al.* Increased endoplasmic reticulum stress in atherosclerotic plaques associated with acute coronary syndrome. *Circulation* **116**, 1226–1233 (2007).
79. Sanson, M. *et al.* Oxidized low-density lipoproteins trigger endoplasmic reticulum stress in vascular cells: prevention by oxygen-regulated protein 150 expression. *Circ. Res.* **104**, 328–336 (2009).
80. Henson, P. M., Bratton, D. L. & Fadok, V. A. Apoptotic cell removal. *Curr. Biol.* **11**, R795–R805 (2001).
81. De Lorenzo, B. H. *et al.* Macrophage suppression following phagocytosis of apoptotic neutrophils is mediated by the S100A9 calcium-binding protein. *Immunobiology* 3 Aug 2009 (doi:10.1016/j.imbio.2009.05.013).
82. Mallat, Z. *et al.* Shed membrane microparticles with procoagulant potential in human atherosclerotic plaques: a role for apoptosis in plaque thrombogenicity. *Circulation* **99**, 348–353 (1999).
83. Schrijvers, D. M., De Meyer, G. R., Kockx, M. M., Herman, A. G. & Martinet, W. Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **25**, 1256–1261 (2005).
**This study used an *in situ* assay to provide evidence that efferocytosis is defective in advanced human atherosclerotic lesions.**
84. Ravichandran, K. S. & Lorenz, U. Engulfment of apoptotic cells: signals for a good meal. *Nature Rev. Immunol.* **7**, 964–974 (2007).
85. Peter, C. *et al.* Migration to apoptotic "find-me" signals is mediated via the phagocyte receptor G2A. *J. Biol. Chem.* **283**, 5296–5305 (2008).
86. Gardai, S. J. *et al.* Cell-surface calreticulin initiates clearance of viable or apoptotic cells through transactivation of LRP on the phagocyte. *Cell* **123**, 321–334 (2005).
87. Zhou, Z. New phosphatidylserine receptors: clearance of apoptotic cells and more. *Dev. Cell* **13**, 759–760 (2007).
88. Boisvert, W. A. *et al.* Leukocyte transglutaminase 2 expression limits atherosclerotic lesion size. *Arterioscler. Thromb. Vasc. Biol.* **26**, 563–569 (2006).
89. Ait-Oufella, H. *et al.* Lactadherin deficiency leads to apoptotic cell accumulation and accelerated atherosclerosis in mice. *Circulation* **115**, 2168–2177 (2007).
90. Thorp, E., Cui, D., Schrijvers, D. M., Kuriakose, G. & Tabas, I. Mertk receptor mutation reduces efferocytosis efficiency and promotes apoptotic cell accumulation and plaque necrosis in atherosclerotic lesions of *Apoe*$^{-/-}$ mice. *Arterioscler. Thromb. Vasc. Biol.* **28**, 1421–1428 (2008).
91. Ait-Oufella, H. *et al.* Defective mer receptor tyrosine kinase signalling in bone marrow cells promotes apoptotic cell accumulation and accelerates atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **28**, 1429–1431 (2008).
**References 90 and 91 provide *in vivo* evidence that the phagocyte receptor MERTK has an important role in mediating efferocytosis and thus preventing plaque necrosis in advanced mouse atheroma.**
92. Aprahamian, T. *et al.* Impaired clearance of apoptotic cells promotes synergy between atherogenesis and autoimmune disease. *J. Exp. Med.* **199**, 1121–1131 (2004).
93. Libby, P. *et al.* Macrophages and atherosclerotic plaque stability. *Curr. Opin. Lipidol.* **7**, 330–335 (1996).
94. Li, Y. *et al.* Cholesterol-induced apoptotic macrophages elicit an inflammatory response in phagocytes, which is partially attenuated by the Mer receptor. *J. Biol. Chem.* **281**, 6707–6717 (2006).
95. Cui, D. *et al.* Pivotal advance: macrophages become resistant to cholesterol-induced death after phagocytosis of apoptotic cells. *J. Leukoc. Biol.* **82**, 1040–1050 (2007).
96. Sather, S. *et al.* A soluble form of the Mer receptor tyrosine kinase inhibits macrophage clearance of apoptotic cells and platelet aggregation. *Blood* **109**, 1026–1033 (2007).
97. Komura, H., Miksa, M., Wu, R., Goyert, S. M. & Wang, P. Milk fat globule epidermal growth factor-factor VIII is downregulated in sepsis via the lipopolysaccharide–CD14 pathway. *J. Immunol.* **182**, 581–587 (2009).
98. Zou, W. *et al. Caenorhabditis elegans* myotubularin MTM-1 negatively regulates the engulfment of apoptotic cells. *PLoS. Genet.* **5**, e1000679 (2009).
99. Xu, W. *et al.* IL-10-producing macrophages preferentially clear early apoptotic cells. *Blood* **107**, 4930–4937 (2006).

100. Odegaard, J. I. *et al.* Alternative M2 activation of Kupffer cells by PPARδ ameliorates obesity-induced insulin resistance. *Cell Metab.* **7**, 496–507 (2008).
101. Mukundan, L. *et al.* PPARδ senses and orchestrates clearance of apoptotic cells to promote tolerance. *Nature Med.* **15**, 1266–1272 (2009).
102. *Wilson*, P. W., Castelli, W. P. & Kannel, W. B. Coronary risk prediction in adults (the Framingham Heart Study). *Am. J. Cardiol.* **59**, 91G–94G (1987).
103. Aprahamian, T., Takemura, Y., Goukassian, D. & Walsh, K. Ageing is associated with diminished apoptotic cell clearance *in vivo*. *Clin. Exp. Immunol.* **152**, 448–455 (2008).
104. Agrawal, A. *et al.* Altered innate immune functioning of dendritic cells in elderly humans: a role of phosphoinositide 3-kinase-signalling pathway. *J. Immunol.* **178**, 6912–6922 (2007).
105. Liew, F. Y., Xu, D., Brint, E. K. & O'Neill, L. A. Negative regulation of Toll-like receptor-mediated immune responses. *Nature Rev. Immunol.* **5**, 446–458 (2005).
106. Foster, S. L., Hargreaves, D. C. & Medzhitov, R. Gene-specific control of inflammation by TLR-induced chromatin modifications. *Nature* **447**, 972–978 (2007).
107. Liu, J. *et al.* Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. *Nature Med.* **15**, 940–945 (2009).
108. Zernecke, A. *et al.* Protective role of CXC receptor 4/CXC ligand 12 unveils the importance of neutrophils in atherosclerosis. *Circ. Res.* **102**, 209–217 (2008).
109. Saito, Y. *et al.* Effects of EPA on coronary artery disease in hypercholesterolemic patients with multiple risk factors: sub-analysis of primary prevention cases from the Japan EPA Lipid Intervention Study (JELIS). *Atherosclerosis* **200**, 135–140 (2008).
110. Li, S. *et al.* Defective phagocytosis of apoptotic cells by macrophages in atherosclerotic lesions of ob/ob mice and its reversal by a fish oil diet. *Circ. Res.* 15 Oct 2009 (doi:10.1161/CIRCRESAHA.109.199570).
111. O'Sullivan, T. P. *et al.* Aromatic lipoxin A4 and lipoxin B4 analogues display potent biological activities. *J. Med. Chem.* **50**, 5894–5902 (2007).
112. Burstein, S. H. & Zurier, R. B. Cannabinoids, endocannabinoids, and related analogues in inflammation. *AAPS J.* **11**, 109–119 (2009)
113. Dol-Gleizes, F. *et al.* Rimonabant, a selective cannabinoid CB1 receptor antagonist, inhibits atherosclerosis in LDL receptor-deficient mice. *Arterioscler. Thromb. Vasc. Biol.* **29**, 12–18 (2009).
114. Moreira, F. A. & Crippa, J. A. The psychiatric side-effects of rimonabant. *Rev. Bras. Psiquiatr.* **31**, 145–153 (2009).
115. Maderna, P., Yona, S., Perretti, M. & Godson, C. Modulation of phagocytosis of apoptotic neutrophils by supernatant from dexamethasone-treated macrophages and annexin-derived peptide $Ac_{2-26}$. *J. Immunol.* **174**, 3727–3733 (2005).
116. Wickline, S. A., Neubauer, A. M., Winter, P. M., Caruthers, S. D. & Lanza, G. M. Molecular imaging and therapy of atherosclerosis with targeted nanoparticles. *J. Magn. Reson. Imaging* **25**, 667–680 (2007).
117. Chen, Y. *et al.* Regulation of dendritic cells and macrophages by an anti-apoptotic cell natural antibody that suppresses TLR responses and inhibits inflammatory arthritis. *J. Immunol.* **183**, 1346–1359 (2009).
118. Hallett, J. M. *et al.* Novel pharmacological strategies for driving inflammatory cell apoptosis and enhancing the resolution of inflammation. *Trends Pharmacol. Sci.* **29**, 250–257 (2008).
119. Rouleau, J. Improved outcome after acute coronary syndromes with an intensive versus standard lipid-lowering regimen: results from the Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis in Myocardial Infarction 22 (PROVE IT-TIMI 22) trial. *Am. J. Med.* **118**, S28–S35 (2005).
120. Strong, J. P., Malcom, G. T., Oalmann, M. C. & Wissler, R. W. The PDAY study: natural history, risk factors, and pathobiology. Pathobiological Determinants of Atherosclerosis in Youth. *Ann. NY Acad. Sci.* **811**, 226–237 (1997).



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**Competing interests statement**
The author declares no competing financial interests.

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