Efferocytosis in health and disease

Amanda C. Doran [iD] *[1,2,3], Arif Yurdagul Jr[4] and Ira Tabas* [iD] *[4,5,6] ***

Abstract | The clearance of apoptotic cells by professional and non-professional phagocytes — a process termed 'efferocytosis' — is essential for the maintenance of tissue homeostasis. Accordingly, defective efferocytosis underlies a growing list of chronic inflammatory diseases. Although much has been learnt about the mechanisms of apoptotic cell recognition and uptake, several key areas remain incompletely understood. This Review focuses on new discoveries related to how phagocytes process the metabolic cargo they receive during apoptotic cell uptake; the links between efferocytosis and the resolution of inflammation in health and disease; and the roles of efferocytosis in host defence. Understanding these aspects of efferocytosis sheds light on key physiological and pathophysiological processes and suggests novel therapeutic strategies for diseases driven by defective efferocytosis and impaired inflammation resolution.

[1]*Department of Medicine, Division of Cardiovascular Medicine, Vanderbilt University Medical Center, Nashville, TN, USA.*

[2]*Vanderbilt Institute for Infection, Immunology and Inflammation, Vanderbilt University, Nashville, TN, USA.*

[3]*Department of Molecular Physiology and Biophysics, Vanderbilt University, Nashville, TN, USA.*

[4]*Department of Medicine, Columbia University Irving Medical Center, New York, NY, USA.*

[5]*Department of Pathology and Cell Biology, Columbia University Irving Medical Center, New York, NY, USA.*

[6]*Department of Physiology and Cellular Biophysics, Columbia University Irving Medical Center, New York, NY, USA.*

*e-mail: iat1@ cumc.columbia.edu

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Every day, approximately 0.4% of the estimated 37.2 trillion cells in an adult human die[1]. However, even in tissues where cell turnover is high, apoptotic cells (ACs) are scarce, indicating very high efficiency of and capacity for AC clearance. The clearance of ACs, termed 'efferocytosis', is an essential process for the maintenance of tissue homeostasis in normal physiology and for the restoration of homeostasis following disease[2,3]. In a number of non-resolving, chronic inflammatory diseases, efferocytosis becomes defective, and accumulation of dead cells ensues[4]. The dead cells can become secondarily necrotic, which can lead to autoimmunity, tissue necrosis and pathological inflammation[5,6]. Accordingly, a major area of biomedical research is emerging to understand the mechanisms by which efferocytosis is successfully carried out in normal physiology and how it becomes defective in disease.

Genetic, biochemical and imaging approaches have revealed that efferocytosis is morphologically and mechanistically distinct from classic forms of phagocytosis[7]. Clearance of ACs requires phagocyte expression of receptors that recognize AC-associated ligands, reorganization of the phagocyte cytoskeleton to engulf cell-bound ACs and induction of phagosome–lysosome fusion to degrade AC cargo[8] (BOX 1). In response to AC ingestion, macrophages restrain the production of proinflammatory cytokines and enhance the production of molecules that dampen inflammation and mediate resolution and repair[9,10] (BOX 2), processes which fail in settings of defective efferocytosis.

Over the last three decades, much has been learnt about the mechanisms of AC recognition and uptake by professional and non-professional phagocytes, how phagocyte biology is altered by AC uptake to avoid inflammatory responses and the role of impaired efferocytosis in disease. We refer the reader to elegant reviews

on these topics[2,7,11–15]. However, several key areas related to efferocytosis have remained incompletely understood, including how phagocytes respond to the large amounts of metabolic cargo following the degradation of ingested ACs, links between efferocytosis and the resolution of inflammation in health and disease and roles of efferocytosis in successful as well as impaired host defence. The last few years have seen exciting progress in these three areas, which is the focus of this Review.

Continual efferocytosis and metabolism

An interesting and important feature of efferocytosis is the ability of phagocytes to ingest multiple ACs over a relatively short period, referred to as continual efferocytosis. This capability of efferocytes is essential when the AC-to-phagocyte ratio is high (for example, after an acute inflammatory response). There are several challenges faced by phagocytes undergoing continual AC uptake. First, each AC uptake event internalizes a substantial amount of plasma membrane as the phagosome enters the cell, and so subsequent rounds of efferocytosis require rapid restoration of cell surface area[16]. Second, phagolysosomal degradation of ACs results in large amounts of metabolic cargo, including amino acids, lipids and nucleic acids, that must be processed in a safe and productive manner[17].

Membrane recycling. The phagocytosis of large particles, including ACs, requires adequate cell surface area to engulf and seal the particle[16,18,19]. For the initial event, inositol phospholipids and phosphoinositide 3-kinases (PI3Ks) promote the fusion of intracellular vesicles, such as recycling endosomes and possibly portions of endoplasmic reticulum (ER)[20,21], with the plasma membrane near the site of the developing phagosome. For a subsequent phagocytic event, cell surface area must
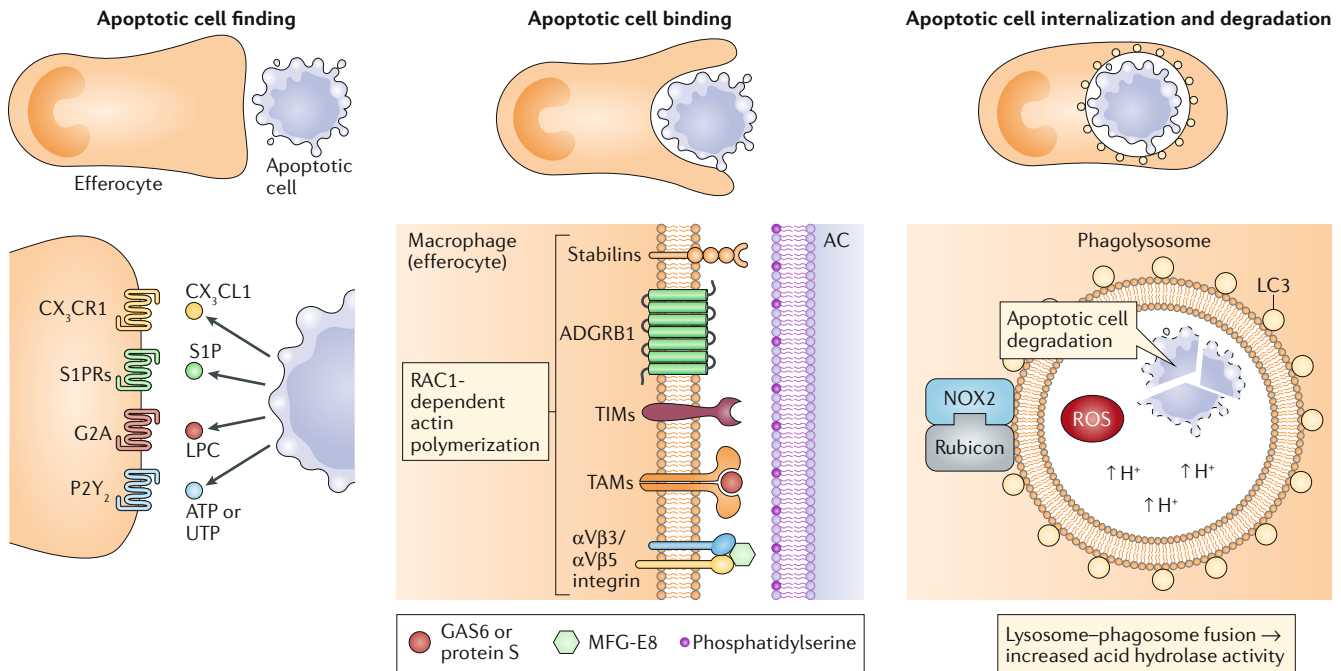
Box 1 | **How phagocytes carry out efferocytosis**

The process of efferocytosis is mechanistically distinct from classic forms of phagocytosis and involves several phases: apoptotic cell (AC) finding, AC binding, AC internalization and AC degradation[4] (see the figure). In the first phase, ACs induce rapid mobilization of efferocytic immune cells by releasing chemokines (such as CX₃C-chemokine ligand 1 (CX₃CL1))[137], lipids (such as sphingosine 1-phosphate (S1P)[138] and lysophosphatidylcholine (LPC)[139]) and nucleotides (ATP and UTP)[140]. Second, efferocytes engage ACs through cell-surface receptors that either directly bind molecules on the AC surface or bind bridging molecules that interact with the AC surface[7]. Efferocyte receptors that directly bind ACs include stabilin 1, stabilin 2, adhesion G protein-coupled receptor B1 (ADGRB1; also known as BAI1), T cell immunoglobulin mucin receptor 1 (TIM1), TIM3, TIM4 and low-density lipoprotein receptor-related protein 1 (LRP1). The receptors that function through bridging molecules include the protein tyrosine kinases TYRO3, AXL and MER proto-oncogene tyrosine kinase (MERTK) (also known as TAMs), the αVβ3 and αVβ5 integrins and the scavenger receptor CD36. The most important bridging molecules are growth arrest-specific protein 6 (GAS6; also known as AXL ligand), protein S and milk fat globule-EGF factor 8 (MFG-E8)[7]. The most important AC-associated signal is externalized phosphatidylserine, but others include calreticulin, intercellular adhesion molecule 3 (ICAM3) and glycosylation moieties[141,142].

Whether direct or indirect, engagement of cell-surface receptors on efferocytes activates the RHO family of small GTPases to mediate AC internalization. For example, the small GTPase RAC1 is activated when the ADGRB1–phosphatidylserine interaction promotes the assembly of a complex of engulfment and cell motility protein 1 (ELMO1) and dedicator of cytokinesis protein 1 (DOCK180)[143] or when the stabilin 2–phosphatidylserine interaction drives an association between the PTB domain-containing engulfment adaptor protein 1 (GULP) and thymosin β4 (REFS[144,145]). On activation, RAC1 localizes to the site at the efferocyte plasma membrane that has engaged the AC and stimulates verprolin homology domain-containing protein 1 (WAVE1)-dependent activation of actin-related protein 2/3 (ARP2/3) complex, which then polymerizes actin to form the phagocytic cup and internalize the AC[146–148].

In the next phase, efferocytes undergo mitochondrial fission to increase the level of cytoplasmic calcium, which is necessary for phagosome sealing, and assemble the class III phosphoinositide 3-kinase (PI3K) complex to synthesize phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P₃) and stabilize the NADPH oxidase 2 (NOX2) complex to produce reactive oxygen species (ROS)[22,149]. Both PtdIns(3,4,5)P₃ and ROS are necessary for conjugating autophagy-related protein LC3-II to lipids at the phagosomal membrane, a process termed 'LC3-associated phagocytosis'. LC3-associated phagocytosis promotes phagolysosomal assembly and acidification and thereby facilitates AC degradation[52]. NOX2 deficiency in chronic granulomatous disease has been associated with impaired efferocytosis[150].

Apoptotic cell finding

Efferocyte

Apoptotic cell

CX₃CR1 — CX₃CL1
S1PRs — S1P
G2A — LPC
P2Y₂ — ATP or UTP

Apoptotic cell binding

Macrophage (efferocyte)

RAC1-dependent actin polymerization

Stabilins
ADGRB1
TIMs
TAMs
αVβ3/ αVβ5 integrin

AC

⬤ GAS6 or protein S   ⬡ MFG-E8   • Phosphatidylserine

Apoptotic cell internalization and degradation

Phagolysosome

LC3

Apoptotic cell degradation

NOX2
Rubicon
ROS
↑H⁺   ↑H⁺   ↑H⁺

Lysosome–phagosome fusion → increased acid hydrolase activity

G2A, G protein-coupled receptor G2A; S1PR, sphingosine 1-phosphate receptor.

be restored by membrane recycling from the engulfed phagosome[16,18,19]. One mechanism involves mitochondrial fission, which occurs after AC engulfment owing to an increase in the level of the mitochondrial fission protein dynamin-related protein 1 (DRP1)[22]. Mitochondrial fission causes the mitochondria to dissociate from the ER, which facilitates ER calcium release into the cytoplasm rather than into the mitochondria. An increased level of cytoplasmic calcium, probably acting through calcium-dependent vesicular transport proteins such as synaptotagmin VII (REF.[23]), promotes phagolysosome-to-plasma membrane vesicular transport, which allows phagosome formation around the subsequent AC. In vivo relevance for this process was demonstrated in low-density lipoprotein receptor-deficient mice (LDLR-deficient mice) fed a Western diet and lacking myeloid DRP1 (REF.[22]). In these mice, atherosclerotic lesional efferocytosis is compromised and plaque necrosis is increased compared with control mice[22]. An interesting topic for future study is whether atherosclerosis and other diseases driven by defective efferocytosis, such as autoimmune and neurodegenerative disease, acquire defects in mitochondrial fission that contribute to impaired efferocytosis.

Vesicular recycling in cells is also regulated by the RAB family of GTPases. A recent report provided evidence

Box 2 | How efferocytosis prevents inflammation

During development, organ structure, limb formation and negative selection of T cells demand that cells undergo physiological apoptosis[7]. However, because apoptotic cells (ACs) can undergo secondary necrosis and trigger immune responses, they must be swiftly removed in a non-inflammatory manner[10]. Efferocytosis quells inflammation by stimulating the production of anti-inflammatory cytokines while simultaneously repressing proinflammatory cytokines, and it promotes inflammation resolution[9]. These processes occur through integrated but mechanistically distinct pathways. For example, AC binding to the efferocyte receptor T cell immunoglobulin mucin receptor 1 (TIM1) suppresses the production of tumour necrosis factor (TNF), IL-6 and CC-chemokine ligand 5 (CCL5) by blocking activation of nuclear factor-κB (NF-κB), whereas AC binding to the efferocyte receptor stabilin 2 stimulates the production of transforming growth factor-β (TGFβ)[151–153]. Binding of ACs to the protein tyrosine kinases MER proto-oncogene tyrosine kinase (MERTK) and AXL can suppress Toll-like receptor (TLR) and type 1 interferon-mediated proinflammatory signalling pathways[12]. Activation of MERTK by ACs blocks the activity of IκB kinase (IKK), which prevents TLR4-induced, NF-κB-dependent TNF expression[154]. AXL activation upregulates the transcriptional repressors Twist-related proteins to prevent TLR4-induced TNF promoter activity[155]. These efferocyte receptors also upregulate the E3 ubiquitin ligases suppressor of cytokine signalling 1 (SOCS1) and SOCS3 and thereby block IFNα-mediated signal transducer and activator of transcription 1 (STAT1) signalling and proinflammatory gene expression[156].

Sterols delivered to efferocytes following phagolysosomal AC degradation activate nuclear sterol receptors, such as peroxisome proliferator-activated receptor-γ (PPARγ), PPARδ and liver X receptor-α (LXRα), which stimulate production of the anti-inflammatory cytokines IL-10 and TGFβ and induce differentiation of regulatory T cells and T helper 2 cells to resolve immune responses[41,43,57,157–159]. Additionally, PPARγ and LXRs bind to nuclear receptor corepressor, which prevents its removal from the promoter sequences of genes encoding certain proinflammatory cytokines[160,161], such as TNF and IL-1β. Moreover, interactions between efferocytes and ACs enhance the synthesis of specialized proresolving mediators while suppressing the production of proinflammatory leukotrienes[10]. For example, engagement of the AC receptor MERTK leads to the translocation of lipoxygenase 5 from the nucleus to the cytoplasm, promoting the synthesis of lipoxin A4 (REF.[49]). Together, these actions prevent inflammation and promote resolution.

Low-density lipoprotein receptor-deficient mice
(LDLR-deficient mice). A mouse model of atherosclerosis in which hypercholesterolaemia is induced by a targeted deletion of the gene encoding LDLR, which functions to clear LDL from the circulation. *Ldlr*−/− mice fed a high-fat, high-cholesterol diet have a very high level of plasma LDL and develop aortic lesions that are morphologically similar to human atherosclerotic plaques.

Western diet
A commonly used rodent diet that contains a higher fat, sucrose and cholesterol content than standard chow diet, akin to the fast food diet encountered in the Western hemisphere. Ingestion of this diet by *Ldlr*−/− mice results in weight gain, high glucose levels and elevated levels of circulating cholesterol and triglycerides that drive the development of atherosclerotic plaques.
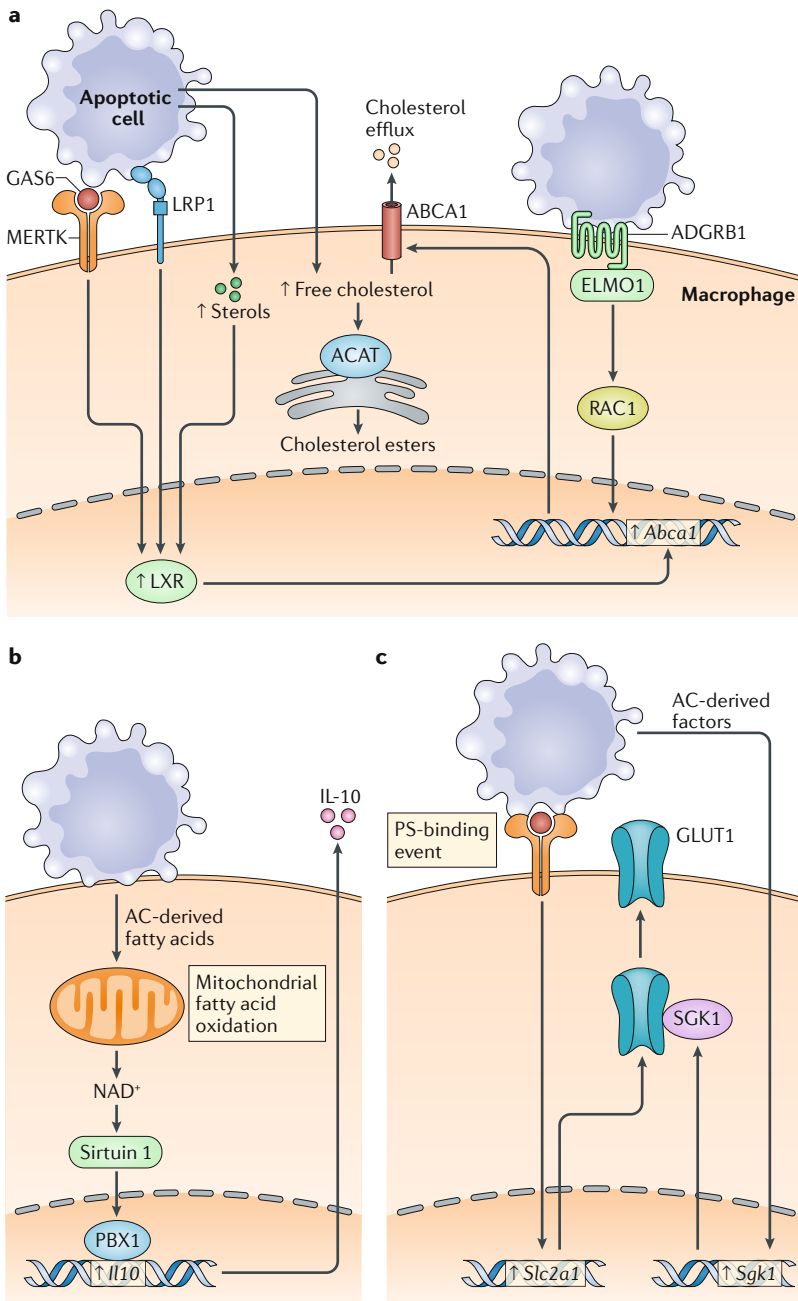
in vitro that RAB17 is recruited to AC-engulfing phagosomes to mediate vesicular transport from AC-containing phagolysosomes to recycling endosomes, which then fuse with the plasma membrane[24]. This process avoids the intermixing of vesicles containing degraded ACs with the MHC class II-loading compartment, thereby preventing antigen presentation to T cells[25]. Further studies will be needed to establish the role of RAB17 in continual efferocytosis and avoidance of T cell activation in vivo.

Cholesterol metabolism. Following the phagolysosomal degradation of ACs, efferocytes are confronted with a large quantity and diversity of potentially cytotoxic macromolecules that require efflux or metabolism (FIG. 1a). A prime example is related to how efferocytes handle free (unesterified) cholesterol[26]. Each time an efferocyte degrades an AC, intracellular cholesterol level increases markedly, which is exacerbated if the AC is loaded with cholesterol, as occurs in macrophage foam cells in atherosclerosis[27]. When macrophages are incubated with apoptotic foam cells in vitro, efficient trafficking of phagolysosomal cholesterol to acyl-CoA cholesterol acyltransferase (ACAT) in the ER catalyses the esterification of free cholesterol to cholesterol fatty acid esters, thereby preventing the membrane-damaging effects of excess free cholesterol[28]. In addition, macrophages are able to carry out massive cholesterol efflux after ingesting ACs, in part due to induction of the cholesterol efflux

transport protein ATP-binding cassette transporter A1 (ABCA1)[29]. AC-derived sterols, likely through metabolism to oxysterols, can activate the nuclear receptors liver X receptor-α (LXRα) and LXRβ, which induce *Abca1* expression. Oxysterols resulting from efferocytosis may have other functions, including blocking inflammasome activation by suppressing mitochondrial oxidative stress and promoting AC internalization by stabilizing the actin GTPase RAC1 (REF.[30]).

Another LXR–ABCA1 pathway is mediated by LDLR-related protein 1 (LRP1)-mediated efferocytosis, which activates peroxisome proliferator-activated receptor-γ (PPARγ), an inducer of LXR- and ABCA1-mediated cholesterol efflux[31]. Finally, engagement of adhesion G protein-coupled receptor B1 (ADGRB1; also known as BAI1) by ACs was also found to induce ABCA1 expression and stimulate cholesterol efflux in cultured cells[32]. The mechanism was dependent on ADGRB1-mediated AC internalization but, surprisingly, was independent of LXRs, as efflux was not blocked by inhibition or genetic targeting of LXRs[32].

Energy metabolism. Mitochondria are key responders to ingested metabolic loads. One study found that soon after phagocytes degrade ACs, the mitochondrial membrane potential (MMP) increases[33]. This is related to the delivery of AC cargo, as the uptake of beads does not affect the MMP. Importantly, the increase in MMP is only transient, suggesting that a mechanism eventually becomes activated to guard against excessive MMP. Indeed, this MMP-modulating mechanism was found to involve upregulation of expression of the mitochondrial uncoupling protein UCP2 after the initial increase in MMP[33]. When *UCP2* is silenced using small interfering RNA, MMP remains high, and efferocytosis is impaired. Mice deficient in UCP2 have increased numbers of ACs in two experimental models of apoptosis, which is consistent with defective efferocytosis. Although the mechanism of UCP2 upregulation and the signalling mechanisms linking UCP2-mediated modulation of MMP to specific processes in continual efferocytosis remain to be determined, these findings are important not only for revealing the role of UCP2 but also for showing the importance of continual efferocytosis in vivo.

The mitochondrial response to fatty acids was recently discovered as a novel proresolving mechanism. In efferocytic macrophages isolated from mouse myocardium following infarction as well as in cultured macrophages exposed to ACs in vitro, NAD+ produced by mitochondrial fatty acid oxidation and complex III subunit 5-dependent mitochondrial electron transport leads to sirtuin 1-mediated activation of PBX1, which is an inducer of the gene encoding the resolution mediator IL-10 (REF.[34]) (FIG. 1b). In mice lacking complex III subunit 5 in myeloid cells, myocardial damage after myocardial infarction is worse and is associated with markedly decreased levels of IL-10. It remains to be determined whether fatty acids originate from degraded ACs, as predicted, and whether the NAD+–sirtuin 1–PBX1 pathway, which was elegantly dissected in vitro, is functional in vivo.

Unlike oxidative phosphorylation, glycolysis is generally considered to be proinflammatory[35]. A study examining

Fig. 1 | **Mechanisms for handling AC-derived metabolic cargo in macrophage efferocytes.** Apoptotic cell (AC)-derived cargo can be cytotoxic, and therefore it needs to be released from the efferocyte or metabolized. Metabolic changes resulting from these processes can be used by the efferocyte to optimize further AC uptake and to trigger resolution pathways. Three examples are illustrated here. **a** | AC-derived free cholesterol can be esterified by acyl-CoA cholesterol acyltransferase (ACAT) into cholesterol esters and sequestered in neutral lipid droplets or effluxed from the cell to prevent its accumulation. Efflux is facilitated by various efferocytosis receptor signalling pathways and sterol pathways that lead to the induction of cholesterol efflux transporters, such as ATP-binding cassette transporter A1 (ABCA1). **b** | AC-derived fatty acids undergo oxidation in a mitochondria-mediated pathway that promotes upregulation of expression of proresolving IL-10. **c** | Binding of ACs to efferocyte receptors that recognize phosphatidylserine (PS) leads to the upregulation of glucose transporter 1 (GLUT1; also known as SLC2A1) and serum/glucocorticoid regulated kinase 1 (SGK1), which is required for GLUT1 transport to the plasma membrane. ADGRB1, adhesion G protein-coupled receptor B1; ELMO1, engulfment cell motility 1; GAS6, growth arrest-specific protein 6; LRP1, LDL-related protein 1; LXR, liver X receptor; MERTK, MER proto-oncogene tyrosine kinase; PBX1, pre-B cell leukaemia homeobox 1.

gene changes in efferocytes exposed to ACs versus IgG-coated cells found an increase in glycolysis and a decrease in oxidative phosphorylation in AC-exposed efferocytes, and whereas blocking aerobic glycolysis suppresses efferocytosis, blocking oxidative phosphorylation does not[36]. Binding of the AC recognition motif, phosphatidylserine, to efferocytes leads to the induction of *Slc2a1* mRNA (which encodes the glucose transporter GLUT1), whereas other factors released from ACs induce *Sgk1*, which encodes a kinase necessary for the localization of GLUT1 at the cell surface (FIG. 1c). Experimental inhibition of *Slc2a1* or *Sgk1* induction results in the suppression of both initial and continual efferocytosis in vitro and in vivo, including in the setting of atherosclerosis in Western diet-fed *Ldlr*^{-/-} mice. Inhibition of glycolysis blocked AC-induced actin polymerization, suggesting that blocking GLUT1 inhibits glycolysis and impairs efferocytosis. Furthermore, the later stages of efferocytosis are associated with an increase in the level of lactate, a by-product of glycolysis, and this is associated with an increased level of mRNA encoding the lactate transporter SLC16A1 (also known as MCT1). Silencing of *Slc16a1* leads to an increase in cellular lactate level and to impairment of efferocytosis. Furthermore, medium collected from wild-type efferocytes but not from *Slc16a1*-silenced efferocytes induces *Il10* expression in bone marrow-derived macrophages, suggesting a proresolving role of lactate and perhaps other factors released by SLC16A1. Although the identity of the AC-released factors that induce *Sgk1*, the mechanism linking phosphatidylserine binding to *Slc2a1* induction and the molecular pathway downstream of lactate–SLC16A1 remain to be elucidated, this key study is the first to link glycolysis and lactate to efferocytosis and resolution. Although it is somewhat surprising to ascribe a proresolving role to glycolysis, these results are not necessarily at odds with prior findings. Resolution is a natural response to inflammation, and so it may be that inflammation-mediated glycolysis acts as an early trigger to initiate the resolution cascade. Alternatively, glycolysis may be necessary to allow an efferocyte to process the metabolic load resulting from the ingestion of an AC. Additional studies will be needed to more precisely determine the relationship between metabolism and efferocytosis.

Efferocytosis in resolution and repair

In addition to preventing secondary necrosis, efferocytosis has three overall outcomes: termination of inflammatory responses, promotion of self-tolerance and activation of proresolving pathways. When efferocytosis is impaired, these functions are compromised, leading to heightened inflammation, impaired resolution and development of disease. As the effects of efferocytosis on anti-inflammatory and tolerogenic pathways have been reviewed in depth[12,37–40], we focus on the proresolving roles of efferocytosis and emerging mechanisms of the failure of this process in disease.

Cell polarization. Efferocytosis promotes a proresolving phenotype by downregulating proinflammatory cytokine expression, increasing the levels of proresolving mediators, inhibiting inducible nitric oxide synthase

Foam cells
Macrophages that localize at sites of early atherosclerotic lesion development and that subsequently ingest apolipoprotein B-containing lipoproteins in the subendothelium. They are called foam cells because lipoprotein uptake and metabolism by these macrophages leads to the accumulation of cholesterol ester droplets in the cytoplasm, which gives the cells a 'foamy' appearance.

Peroxisome proliferator-activated receptor-γ
(PPARγ). A member of a group of nuclear receptor proteins involved in altering lipid and glucose metabolism and inflammation. Their ligands include free fatty acids and eicosanoids.

Fatty acid oxidation
An important metabolic process used to derive energy through the mobilization and oxidation of fatty acids, mainly in the mitochondrial matrix. Fatty acid oxidation is positively and negatively regulated by 5' AMP-activated protein kinase and mechanistic target of rapamycin, respectively.

Myocardial infarction
An episode of acute cardiac ischaemia that leads to the death of heart muscle cells. It is usually caused by the rupture or erosion of an atherosclerotic plaque leading to occlusive clot formation.

Oxidative phosphorylation
The metabolic pathway that occurs at the inner mitochondrial membrane and uses an electrochemical gradient created by the oxidation of electron carriers to generate ATP.

Glycolysis
A metabolic pathway that generates the cellular high-energy store ATP by oxidizing glucose to pyruvate. In eukaryotic cells, pyruvate is further oxidized to CO_2 and H_2O in a process known as aerobic respiration, which results in a net yield of 36–38 molecules of ATP per metabolized molecule of glucose.

(iNOS) and enhancing the production of angiogenic growth factors. These effects occur in part through activation of LXRs, PPARγ and PPARδ[41–43]. As described in the preceding section, ACs also promote proresolving macrophages through a fatty acid oxidation–IL-10 pathway[34]. Efferocytosis may also enhance tissue repair by specifying the cell fate of bipotent progenitor cells during chronic liver injury[44]. In vitro, engulfment of sonicated hepatocyte debris by liver macrophages induces WNT3A expression in these cells[44]. In vivo, WNT3A promotes canonical WNT signalling in neighbouring progenitor cells and leads to the generation of hepatocytes that repopulate the diseased parenchyma[44]. Further studies will be needed to link the in vitro and in vivo data, for example, to show that efferocytosis of dead hepatocytes by liver macrophages in vivo is responsible for WNT signalling. In a converse scenario, macrophage polarization to more efficient efferocytes may be triggered by ACs themselves. Apoptotic neutrophils are a rich source of ACs for efferocytosis in inflammation[45]. In the setting of experimental myocardial infarction, acute depletion of neutrophils resulted in increased fibrosis, greater accumulation of ACs within infarcts and worse cardiac function. Macrophages from these mice express lower levels of the protein tyrosine kinase MERTK, and MERTK expression could be restored in vitro by incubation of macrophages with neutrophil gelatinase-associated lipocalin[45].

Production of proresolving mediators. Efferocytosis also leads to altered production of lipid mediators that play a central role in inflammation resolution. Macrophages incubated with apoptotic neutrophils or neutrophil microparticles increase expression of long-chain fatty acid-derived lipids termed 'specialized proresolving mediators' (SPMs), including lipoxin A4, and resolvins D1, D2 and E2, while concomitantly decreasing expression of proinflammatory prostaglandins and leukotriene B4 (REF.[46]). Lipoxin A4 and resolvin D1 further enhance efferocytosis, setting up a cycle by which they augment their own production[46]. The increase in SPM production occurs by several mechanisms. First, ACs and their microparticles contain precursors of SPMs, and when ACs are ingested by macrophages, the SPM precursors are converted to mature lipid mediators[46]. In addition, expression of 12/15-lipoxygenase, a key biosynthetic enzyme for SPMs, is upregulated by efferocytosis[47]. MERTK–extracellular signal-regulated kinase (ERK) signalling promotes SPM synthesis by increasing the cytoplasmic-to-nuclear ratio of 5-lipoxygenase, another key enzyme in SPM biosynthesis[48,49]. In vitro and in vivo studies show that deletion of MERTK or cleavage to render MERTK non-functional results in impaired resolution, whereas prevention of MERTK cleavage enhances resolution[48]. Moreover, activation of LXRα after AC engulfment induces MERTK expression, which implicates MERTK and the SPMs in the proresolving activity of LXR[41]. MERTK-binding bridging molecules have also been implicated in efferocytosis-induced resolution. For example, protein S is upregulated by macrophages during resolution, and when protein S is deleted from macrophages, they express higher levels of tumour necrosis factor (TNF) and lower levels of IL-10, arginase 1 and resolvin D1 (REF.[50]).

Two other examples warrant mention. First, after macrophages internalize ACs, MYC is cleaved into MYC-nick, which promotes the recruitment of acetyltransferases to α-tubulin, facilitating LC3-associated phagocytosis[51]. LC3-associated phagocytosis promotes phagolysosomal assembly and acidification[52], and in this study MYC-nick improved efferocytosis and upregulated *Il10*, *Arg1*, *Retnla* and *Mrc1* (REF.[51]). Second, resolving macrophages in sterile peritonitis increase expression of the secretory integrin-binding protein DEL1 (also known as EDIL3), which promotes efferocytosis by bridging phosphatidylserine on ACs with αVβ3 integrin on macrophages. This process leads to LXR-dependent upregulation of anti-inflammatory and proresolving cytokines and production of lipoxin A4, resolvin D1 and resolvin E1. DEL1 also promotes the proefferocytic function of resolvin D1, suggesting a feedforward mechanism[53].

Crosstalk with regulatory T cells. CD4+CD25+FOXP3+ regulatory T cells (T_{reg} cells) are potent modulators of the immune response. They interact with cells of both the innate immune system and the adaptive immune system to limit T cell proliferation, promote tolerance by deleting autoreactive cells and producing anti-inflammatory cytokines, limit excessive inflammation, foster alternative activation of macrophages towards a proresolving phenotype[54,55] and promote resolution[56]. More recently, T_{reg} cells have been shown to enhance macrophage efferocytosis in vitro and in models of acute lung injury, peritonitis and atherosclerosis[57]. The mechanism involves T_{reg} cell secretion of IL-13, which upregulates macrophage production of IL-10. IL-10 then promotes macrophage efferocytosis by inducing the GTP-exchange factor VAV1, which enhances RAC1-dependent actin assembly at the phagosome and subsequent AC internalization[57]. Macrophage efferocytosis also increases the number of T_{reg} cells in vivo, as infusion of ACs leads to expansion of T_{reg} cells in a manner dependent on macrophage-derived transforming growth factor-β (TGFβ)[58]. A separate in vitro study showed that ingestion of ACs increases phagocyte production of TGFβ and IL-10, which increase the differentiation of T_{reg} cells[40]. More recently, experimental induction of intestinal epithelial cell apoptosis resulted in uptake of ACs by CD103+ dendritic cells (DCs), leading to the upregulation of genes involved in T_{reg} cell generation and a concomitant increase in the number of T_{reg} cells in the mesenteric lymph nodes[59]. When considered together, these studies suggest that T_{reg} cells and macrophages participate in a positive feedback system linked by clearance of ACs.

Prevention of immune activation. Although efferocytosis of infected ACs by DCs can lead to the presentation of pathogen antigens and activation of effector T cells as part of host defence (see the following section), this process does not usually occur when resolving macrophages ingest non-infected ACs. This concept can be illustrated by a recent study showing that CX_3CR1+MERTK+ macrophages in the T cell zone of lymph nodes are inefficient at priming CD4+ T cells, a function instead delegated to phagocytic DCs in the region[60]. Several mechanisms

to explain this observation have been investigated. First, as discussed earlier, internalization of ACs via efferocytosis leads to shunting of cargo towards recycling endosomes and away from the MHC class II-loading compartment, thus avoiding antigen presentation of AC-derived peptides[25]. Second, phagosomes in pro-resolving macrophages undergo acidification more efficiently than those in proinflammatory macrophages, leading to proteolysis of AC-derived proteins to a degree that prevents the generation of suitable peptides for MHC class II presentation[61].

On the basis of these findings, it is not surprising that autoimmunity can develop when efferocytosis fails (FIG. 2a). There are a few examples of lymphocyte activation and autoimmunity in the setting of defective efferocytosis in mouse models. First, genetic targeting of the AC–efferocyte bridging molecule milk fat globule-EGF factor 8 (MFG-E8) leads to the development of autoantibody-mediated glomerulonephritis that mimics lupus nephritis[62]. Second, targeting of MERTK results in impaired efferocytosis and increased titres of nuclear autoantibodies[63]. Third, targeting of complement C1q subcomponent subunit A leads to an accumulation of ACs, high titres of autoantibodies and glomerulonephritis[64]. Fourth, targeting of PPARδ in macrophages, which impairs efferocytosis owing to defective PPARδ-mediated upregulation of bridging molecules, notably C1q, promotes the production of autoantibodies and lupus-like autoimmune disease[43]. These data suggest that defective efferocytosis leads to impaired self-tolerance, but it is also possible that uncleared ACs become secondarily necrotic and act as a proinflammatory trigger. Further studies will be necessary to determine the relative contribution of these mechanisms.

Defective efferocytosis in cardiometabolic disease
As illustrated in the previous sections, genetically engineered defects in efferocytosis can impair resolution and predispose an organism to disease. However, there are also a variety of mechanisms by which disease processes themselves cause defective efferocytosis[6,65–67]. We present here a few examples, focusing on aspects of cardiometabolic disease.

Atherosclerosis. Atherosclerotic plaques form when modified lipoproteins accumulate within the subendothelial layer of arteries, generating an inflammatory stimulus that drives leukocyte influx into the vessel wall. Many of these leukocytes become apoptotic, and although early in lesion formation they are cleared efficiently, efferocytosis begins to fail in advanced plaques, leading to an accumulation of secondarily necrotic cells in an area of the plaque called the necrotic core[68–70]. In humans, large necrotic cores are associated with the type of high-risk plaques that are prone to cause myocardial infarction and stroke[71]. Therefore, elucidating the mechanisms of defective efferocytosis is an important objective of atherosclerosis research[4].
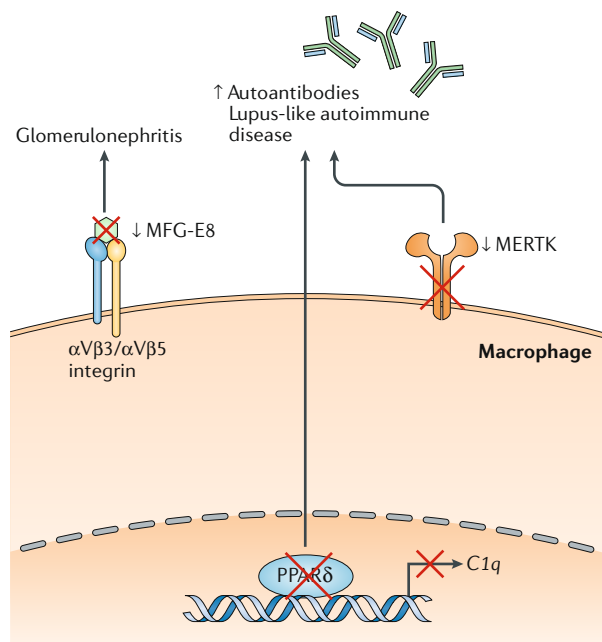
One mechanism of defective efferocytosis in atherosclerosis is proteolysis of efferocytosis receptors, such as MERTK and LRP1 (FIG. 2b). MERTK is important in lesional efferocytosis, as Western diet-fed *Mertk*$^{-/-}$*Ldlr*$^{-/-}$ mice have increased lesion size, larger necrotic cores and reduced production of SPMs[72,73]. Lesional macrophages in advanced lesions in humans and mice express high levels of the enzyme calcium/calmodulin-dependent protein kinase IIγ (CaMKIIγ), which suppresses an ATF6–LXRα–MERTK pathway[74]. Accordingly, the atherosclerotic lesions of Western diet-fed *Ldlr*$^{-/-}$ mice lacking myeloid CaMKIIγ had higher levels of macrophage MERTK expression, improved efferocytosis and smaller necrotic cores[74].

Another potential mechanism of impaired MERTK function in advanced lesions is cell-surface shedding of the receptor on inflammatory macrophages by the protease disintegrin and metalloproteinase domain-containing protein 17 (ADAM17), which leads to defective efferocytosis and the generation of the cleaved extracellular fragment called soluble MER[75,76]. This soluble fragment can bind to the bridging molecule growth arrest-specific protein 6 (GAS6; also known as AXL ligand), which may prevent its interaction with intact MERTK receptors[75], but a role for soluble MER has not yet been demonstrated in an actual disease setting. In progressing atherosclerotic lesions in mice and humans, surface expression of MERTK on macrophages declines, and levels of ADAM17 and soluble MER increase[72,77–79], suggesting that MERTK cleavage may be a mechanism for impaired efferocytosis in advanced atherosclerosis. The first evidence suggesting a causative role of MERTK in atheroprogression came from a study using Western diet-fed *Ldlr*$^{-/-}$ mice in which endogenous MERTK had been replaced by a cleavage-resistant mutant[77]. The plaques of these mice show enhanced lesional efferocytosis, smaller necrotic cores and higher levels of SPMs compared with control Western diet-fed *Ldlr*$^{-/-}$ mice[77]. Genetic targeting of the efferocytosis receptor LRP1 in macrophages or haematopoietic cells in atheroprone mice also leads to increased lesion area and necrotic core size[80–82]. LRP1-mediated efferocytosis may become impaired during atherosclerosis progression through exposure of lesional macrophages to oxidized LDL, as treatment of cultured macrophages with oxidized LDL promotes epsin-mediated, ubiquitin-dependent internalization and downregulation of LRP1 (REF.[83]). Consistent with this finding, genetic targeting of myeloid epsin 1 and epsin 2 in apolipoprotein E-deficient mice (*Apoe*$^{-/-}$ mice) leads to smaller necrotic cores and plaque size[83].

Defects in signals derived from ACs have also been identified in atherosclerosis. Inflammatory signalling leads to inappropriate expression of the 'don't-eat-me' signal CD47 in lesional ACs, rendering them resistant to internalization by lesional efferocytes[84] (FIG. 2b). Administering an anti-CD47-blocking antibody to atheroprone mice leads to smaller necrotic cores and improves lesional efferocytosis[84]. Consistent with the links between efferocytosis and inflammation resolution, a more recent study showed that anti-CD47 antibody treatment of *Ldlr*$^{-/-}$ mice increases the levels of SPMs, particularly resolvin D1, in atherosclerotic lesions[85]. The study authors also showed that lesional necroptotic cells express very high levels of CD47, which
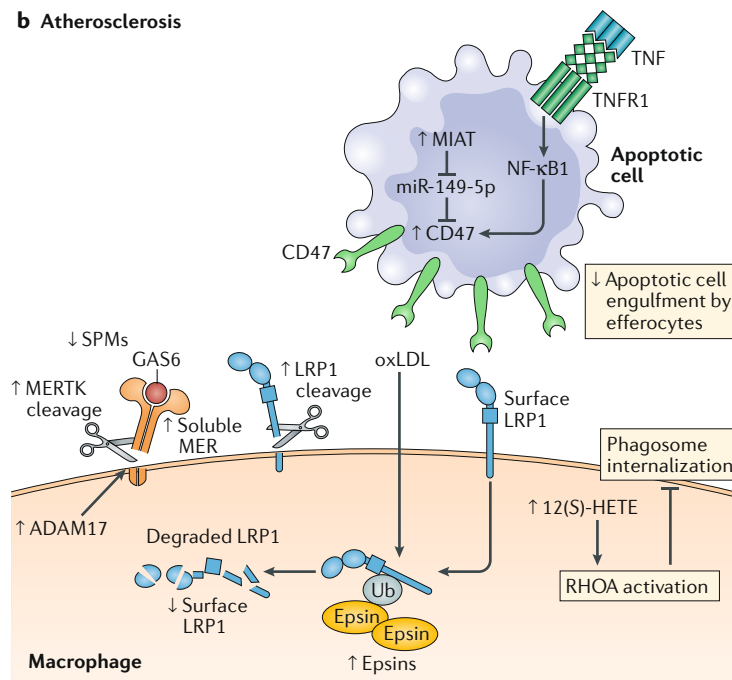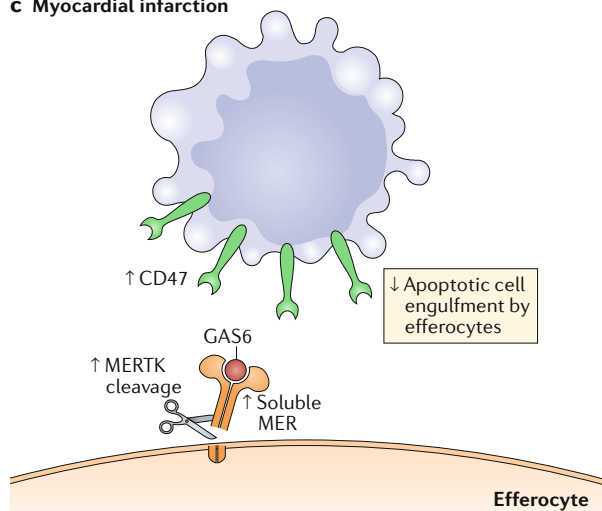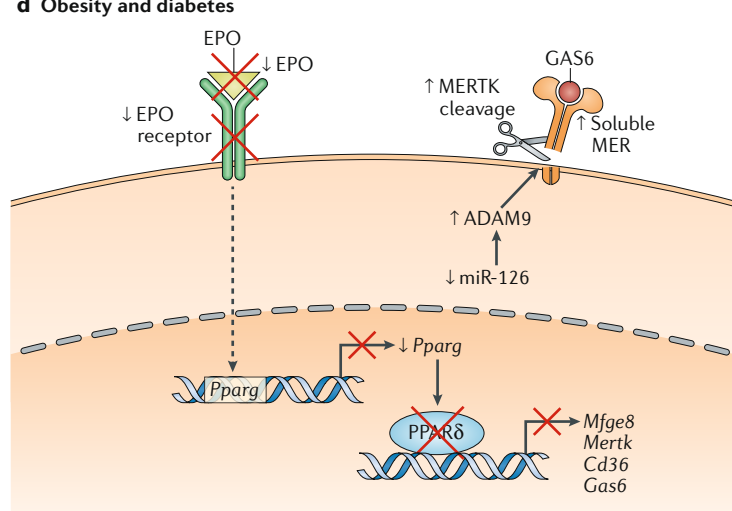
Fig. 2 | **Mechanisms of impaired efferocytosis in disease.** A variety of impairments contribute to defective macrophage efferocytosis and the development and/or progression of disease. **a** | In the autoimmune disease glomerulonephritis, efferocytosis is impaired due to loss of the efferocytosis bridging molecule milk fat globule-EGF factor 8 (MFG-E8). The development of autoantibodies and lupus-like autoimmune disease in mice is associated with impaired efferocytosis due to reduced levels of the efferocytosis receptor MER proto-oncogene tyrosine kinase (MERTK) or to impaired transcription of the efferocytosis bridging molecule C1q by peroxisome proliferator-activated receptor-δ (PPARδ). **b** | In atherosclerosis, proteolytic cleavage of the efferocytosis receptors MERTK and low-density lipoprotein receptor-related protein 1 (LRP1) compromises the ability of plaque macrophages to clear dead cells in atherosclerotic lesions, which contributes to plaque necrosis and impaired resolution. The MERTK cleavage product, soluble MER, may be able to compete for the bridging molecule growth arrest-specific protein 6 (GAS6), sequestering it from intact MERTK receptors. Cell-surface LRP1 levels are also decreased by epsin-mediated internalization of the receptor. A particular lipid that accumulates in atherosclerotic lesions called arachidonic acid-derived 12(S)-hydroxyeicosatetraenoic acid (12(S)-HETE) suppresses apoptotic cell internalization by activating RHOA. Finally, apoptotic cells within atherosclerotic plaques upregulate the 'don't-eat-me' signal CD47, impairing

their ability to be recognized by phagocytes. Several mechanisms have been proposed, including induction of CD47 expression by a tumour necrosis factor (TNF)–TNF receptor 1 (TNFR1)–nuclear factor-κB1 (NF-κB1) pathway and loss of a CD47-suppressing microRNA, miR-143-5p. **c** | Similarly to atherosclerosis, both MERTK cleavage in cardiac macrophages and inappropriately high levels of CD47 on apoptotic cardiomyocytes contribute to an impaired resolution response after experimental myocardial infarction. **d** | In obesity and diabetes, MERTK cleavage also plays an important role in the impaired ability of diabetic macrophages to clear apoptotic cells, although in contrast to the situation in atherosclerosis, cleavage is driven by disintegrin and metalloproteinase domain-containing protein 9 (ADAM9) owing to downregulation of miR-126. Diabetic macrophages also have a downregulation of a proefferocytic pathway involving erythropoietin (EPO)–EPO receptor signalling. Under normal conditions, EPO receptor signalling increases PPARγ expression, leading to enhanced PPARγ-mediated transcription of a number of efferocytosis receptors and bridging molecules. In diabetic mice, however, there is a reduction in levels of both EPO and the EPO receptor that interrupts this pathway and impairs efferocytosis efficiency. MIAT, myocardial infarction-associated transcript; oxLDL, oxidized low-density lipoprotein; SPM, specialized proresolving mediator; Ub, ubiquitin.

leads to macrophages 'nibbling' the cells rather than fully engulfing them. Full engulfment of necroptotic cells was restored by treatment with either anti-CD47 antibody or the resolving mediator resolvin D1, which facilitates the engagement of the 'eat-me' signal calreticulin to overcome the CD47 don't-eat-me signal[85].

The mechanism of increased CD47 expression on lesional apoptotic and necroptotic cells is not known, but in vitro studies have suggested contributions of an inflammatory signalling pathway involving the TNF receptor TNFR1 and its downstream mediator nuclear factor-κB1 (REF.[84]) and of a long non-coding RNA called 'myocardial infarction-associated transcript' (MIAT)[86]. MIAT, the levels of which are increased in patients with symptomatic atherosclerosis, interferes with the post-translational processing of the microRNA miR-149-5p. In vitro, miR-149-5p lowers levels of CD47, and genetic targeting of MIAT in $Apoe^{-/-}$ mice decreases CD47 expression, improves efferocytosis and reduces necrosis in plaques[86]. Another study showed that CD47 can be therapeutically downregulated by a Toll-like receptor 9 (TLR9) agonist, CpG oligonucleotides. TLR9 signalling produces a metabolic shift in macrophages that is associated with enhanced efferocytosis of tumour cells in a mouse model of pancreatic cancer. In this model, CpG oligonucleotide-activated macrophages redirect exogenous fatty acids and glucose towards acetyl-CoA generation and de novo lipid biosynthesis. These macrophages engulf apoptotic tumour cells despite expression of CD47 on the ACs, suggesting that macrophage metabolism can be altered to circumvent the inhibitory function of CD47 (REF.[87]). Although this has not specifically been investigated in atherosclerosis, TLR9 signalling is known to be athero-protective[88], which raises the possibility of a novel therapeutic avenue through which efferocytosis could be enhanced to suppress atherosclerosis progression. In this context, it is interesting to recall the aforementioned study demonstrating a role for fatty acid oxidation in the generation of IL-10 (REF.[34]), which is consistent with a link between fatty acid metabolism and resolution programmes in macrophages.

More generally, advanced human atherosclerotic lesions have a lower content of SPMs than earlier-stage lesions[89], and this defect likely contributes to impaired efferocytosis, as SPMs boost efferocytosis[48,90–95]. Indeed, treatment of mice with advanced atherosclerosis with a resolving mediator improves lesional efferocytosis[96]. Furthermore, as mentioned above, resolvin D1 promotes the engulfment of necroptotic cells[85]. The increase in proinflammatory lipid mediators in advanced atherosclerosis may also compromise efferocytosis. For example, arachidonic acid-derived 12(S)-hydroxyeicosatetraenoic acid, the level of which is increased in progressing atherosclerotic lesions and in serum from individuals with coronary artery disease versus controls, impairs efferocytosis in human monocyte-derived macrophages by activating RHOA, which blocks phagosome internalization[97] (FIG. 2b). This defect could be corrected by co-incubation with statins, which inhibit RHOA by blocking a key RHOA-activating modification, isoprenylation.

Myocardial infarction and heart failure. Efferocytosis plays a key role in the repair of the myocardium after myocardial infarction (FIG. 2c). Mice lacking macrophage MERTK have an exaggerated response to ischaemia–reperfusion injury of the left anterior descending artery, characterized by accumulation of ACs, larger infarct size and more depressed cardiac function. Conversely, mice expressing cleavage-resistant MERTK have a decreased level of ACs in the heart and were more resistant to myocardial injury[98,99]. A recent study demonstrated that extracellular vesicles secreted by a cardiac progenitor cell population, known as cardiosphere-derived cells, promote the expression of MERTK by macrophages, thereby enhancing efferocytosis and healing after myocardial infarction in rat and mouse models[100]. As in atherosclerosis, ACs generated during myocardial injury also express inappropriately high levels of CD47. Treatment with anti-CD47 antibody in the period immediately after myocardial infarction leads to improved resolution of cardiac inflammation, reduced infarct size and preserved cardiac function[101]. In response to ligation of the left anterior descending artery, mice lacking the efferocytosis-bridging molecule MFG-E8 have more cardiac necrosis and inflammation and worse cardiac function compared with control mice, and these effects are attenuated by intracardiac injection of MFG-E8 (REF.[102]). Finally, a population of cardiac myofibroblasts that appears in the heart after myocardial infarction secretes MFG-E8, which promotes their ability to recognize ACs[102]. As a consequence, these myofibroblasts downregulate $Il1b$, $Cxcl2$ and $Il6$ expression and upregulate $Tgfb$ expression[102]. Although TGFβ is generally proresolving when produced by macrophages, production by myofibroblasts may promote excessive fibrosis, which could be deleterious in this setting. Further study will be necessary to determine the myofibroblast-specific role of MFG-E8.

Obesity and diabetes. Macrophages derived from leptin-deficient ob/ob mice or from mice with diet-induced obesity demonstrate impaired efferocytosis[103,104]. If this finding were to translate to humans, it might help to explain the predisposition of patients with obesity and diabetes to complications associated with defective resolution, such as impaired wound healing and atherosclerosis. In the case of atherosclerosis, $Ldlr^{-/-}$ ob/ob mice have impaired lesional efferocytosis and larger necrotic cores compared with $Ldlr^{-/-}$ mice. When $Ldlr^{-/-}$ ob/ob mice are fed a diet rich in ω-3 fatty acids, their macrophages show improved efferocytosis[104]. Although the mechanism is not fully elucidated, it is interesting to hypothesize that SPMs derived from the ω-3 fatty acids are at least partially responsible for the observed improvement. With regard to wound healing, macrophages isolated from wounds of a variety of diabetic mouse models (such as db/db, non-obese diabetic and Akita mice) have impaired efferocytosis, which is associated with higher numbers of ACs and greater expression of proinflammatory cytokines within wounds[105].

Another mechanism of defective efferocytosis in obesity may involve loss of MERTK expression. Although ADAM17 is the main protease that cleaves MERTK,

a recent study showed that increased expression of ADAM9 in macrophages from *db*/*db* mice (which have a genetic dysfunction of the leptin receptor) or macrophages exposed to high levels of exogenous glucose leads to MERTK cleavage and impaired efferocytosis[106] (FIG. 2d). The increase in ADAM9 expression is ascribed to decreased expression of miR-126. The translational relevance of these findings is suggested by data comparing hearts from individuals without diabetes and individuals with diabetes and showing that individuals with diabetes had lower expression of both miR-126 and the active form of MERTK (phosphorylated MERTK) and higher expression of ADAM9 (REF.[106]).

Alternatively, defective efferocytosis in obesity may be related to impaired erythropoietin (EPO) signalling (FIG. 2d). Under normal conditions, the interaction of sphingosine 1-phosphate on ACs with its cognate receptor on macrophages promotes an EPO–EPO receptor–PPARγ pathway that enhances efferocytosis. The mechanism involves a JAK2–ERK–phosphorylated CEBP signalling cascade that induces PPARγ, which in turn increases the expression of a variety of efferocytic molecules, including MFG-E8, GAS6, MERTK and CD36 (REF.[107]). In a model of zymosan-induced peritonitis, macrophages from mice with diet-induced obesity have impaired efferocytosis and reduced levels of both EPO and EPO receptor, which is associated with delayed resolution. Intraperitoneal administration of exogenous EPO to these mice before and during the experimental peritonitis improved efferocytosis and resolution to levels seen in lean mice[103]. These data raise the possibility that EPO may be a novel therapeutic target for enhancing efferocytosis in obesity.

Efferocytosis in host defence

A common consequence of pathogen invasion is death of host cells, and pathogens often remain alive in these dying cells. If infected dead cells are not rapidly engulfed by phagocytes, live pathogens can be released as a result of pathogen-induced pore formation or overall plasma membrane leakage, such as occurs in secondary cell necrosis or necroptosis, resulting in spread of the infection. If rapid efferocytosis occurs, the fate of the pathogen and the subsequent immune response differ depending on the type of pathogen and the dying cell in which it lives; the type of efferocyte, such as macrophage versus DC; and other host factors, including genetic polymorphisms[108,109]. In this section, we provide a few examples.

Fate of bacteria after efferocytosis. When *Mycobacterium tuberculosis* invades macrophages, bacteria remain alive in phagosomes owing to *M. tuberculosis*-induced suppression of lysosome–phagosome fusion. Relatively low-virulence strains of *M. tuberculosis* often induce apoptosis, and when macrophages engulf these ACs, lysosome fusion with AC-containing phagosomes promotes bacterial killing[110] (FIG. 3a). Perhaps related to this mechanism, *M. tuberculosis* infection worsens in mice given a neutralizing antibody against the efferocytosis receptor T cell immunoglobulin mucin receptor 4 (TIM4). Another example of efferocytosis-mediated host

defence occurs when certain bacteria, such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium and some strains of *Klebsiella pneumoniae*, trigger pyroptosis in neutrophils[111,112]. Efferocytosis of the infected neutrophils by macrophages in vitro, which is facilitated by the interaction of pore-induced intracellular traps on pyroptotic cells with scavenger and complement receptors on macrophages[112], leads to bacterial neutralization (FIG. 3b). Inhibition of pyroptosis worsens *K. pneumoniae* infection in mice, but a direct link to impaired efferocytosis has not been shown. This point is important, as molecules released by ACs can affect phagocytes independently of efferocytosis, and this may be particularly important with pore-bearing pyroptotic cells.

The current literature also contains important examples of how efferocytosis fails to neutralize bacteria. Virulent strains of *M. tuberculosis* induce mostly cell necrosis, which decreases efferocytosis of infected cells and promotes bacterial spread[113]. Moreover, an in vitro study suggested that when engulfment of *M. tuberculosis*-infected necrotic neutrophils by macrophages does occur, it can actually promote *M. tuberculosis* growth[114]. In the case of the parasite *Leishmania major*, macrophage engulfment of infected ACs promotes transformation of promastigotes to amastigotes, which are able to avoid phagolysosomal degradation[109]. Moreover, promastigotes themselves may impair phagosome maturation. Another example occurs when macrophages engulf apoptotic T cells infected with the parasite that causes Chagas disease — *Trypanosoma cruzi*. Similarly to uptake of *M. tuberculosis*-infected necrotic cells, uptake of *T. cruzi*-infected apoptotic T cells actually promotes parasite replication in macrophages[115]. *T. cruzi*-infected ACs engage the vitronectin receptor on macrophages, which stimulates the secretion of prostaglandin E$_2$ (PGE$_2$) and TGFβ, which then increase the infectivity of *T. cruzi*. Part of the mechanism involves the ability of these molecules to activate ornithine decarboxylase (ODC) in macrophages: ODC converts ornithine into polyamines, and polyamines promote *T. cruzi* replication. Treatment of *T. cruzi*-infected mice with inhibitors of PGE$_2$ production markedly suppressed parasitaemia, although a direct link to ODC was not shown[115]. Finally, the efferocytosis machinery can be usurped by some pathogens to promote direct infection of macrophages. This type of 'apoptotic mimicry' is used by vaccinia virus, in which phosphatidylserine expression on the virus membrane facilitates efferocytosis receptor-mediated uptake by macrophages, leading to intracellular viral replication[116].

In other cases, pathogens can escape efferocytosis-mediated killing by preventing macrophage engulfment of infected ACs. For example, internalization of methicillin-resistant *Staphylococcus aureus* by neutrophils induces expression of the don't-eat-me signal CD47 (REF.[117]) (FIG. 3c). As a result, dying, infected neutrophils are not engulfed by macrophages, and subsequent necrosis of the neutrophils releases viable bacteria. Although some *K. pneumoniae* infections are controlled by successful efferocytosis-mediated bacterial killing through the mechanism discussed above, more virulent strains can escape this fate. One such strain of *K. pneumoniae*
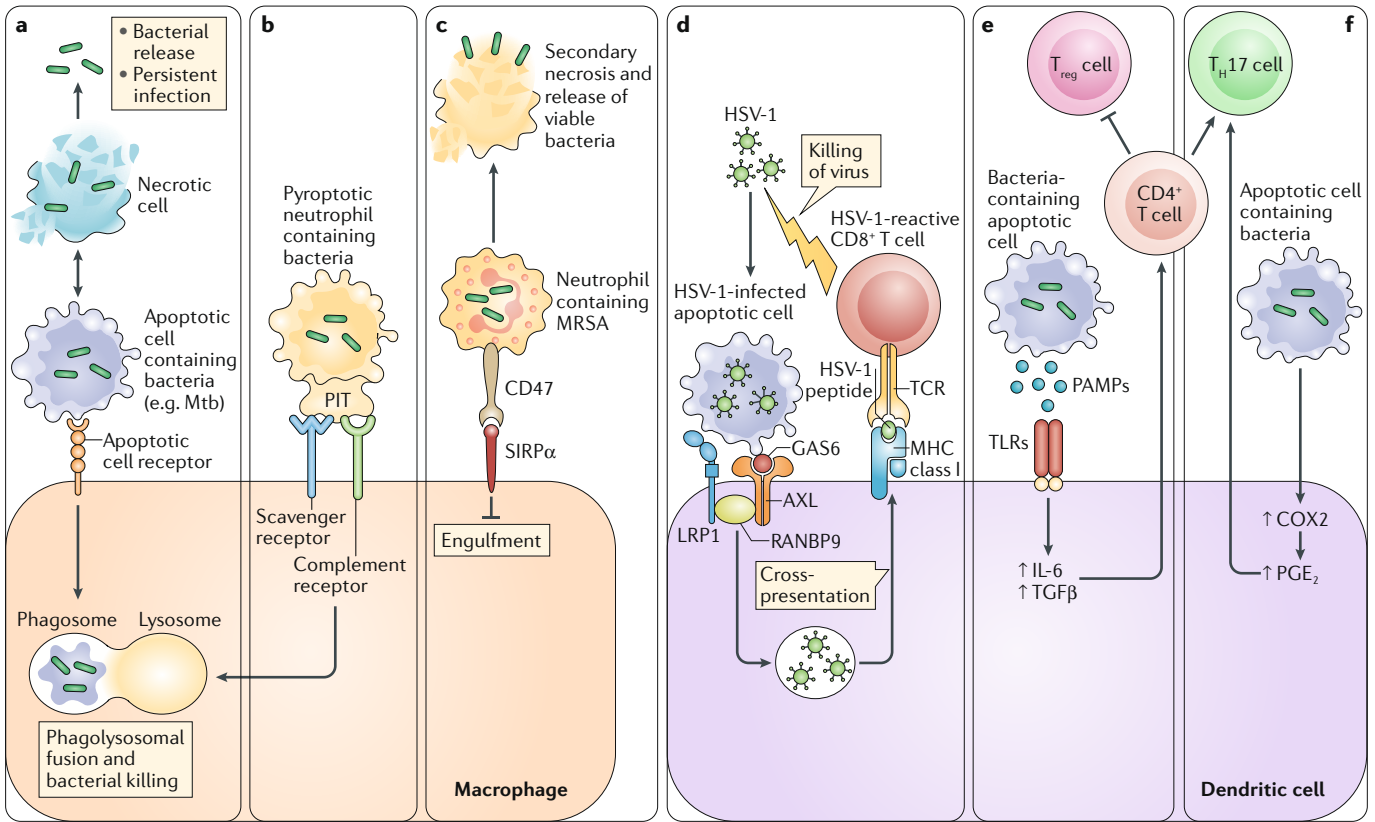
Fig. 3 | **The role of efferocytosis in infection and host defence.** Efferocytosis can mediate host defence against pathogens in some settings but, in other settings, efferocytosis can be usurped by pathogens to promote their spread. Several examples are illustrated. **a** | Engulfment of infected apoptotic cells by macrophages leads to bacterial killing and prevents secondary necrosis of the infected cells, thereby preventing bacterial release. **b** | A similar pathway occurs when certain bacteria induce pyroptosis in infected cells, in which pore-induced intracellular traps (PITs) in these cells are recognized by efferocytosis receptors. **c** | Other types of bacteria, such as methicillin-resistant *Staphylococcus aureus* (MRSA), can evade efferocytic uptake and killing by inducing expression of the don't-eat-me signal CD47 on the cells they infect. Efferocytosis of infected cells by dendritic cells can also participate in host defence (not shown). **d** | Recognition and internalization of herpes simplex virus 1 (HSV-1)-infected apoptotic cells by a protein complex comprising AXL, low-density lipoprotein receptor-related protein 1 (LRP1) and RAN-binding protein 9 (RANBP9) leads to cross-presentation of viral antigen on MHC class I molecules and subsequent generation of antiviral CD8+ T cells. Other types of effector T cells are activated by dendritic cells engulfing bacteria-infected apoptotic cells. **e** | Pathogen-associated molecular patterns (PAMPs) expressed on infected apoptotic cells activate the production of IL-6 and transforming growth factor-β (TGFβ) via Toll-like receptors (TLRs), which promotes the differentiation of CD4+ T cells to IL-17-producing T helper cells (T_H17 cells) and suppresses the generation of regulatory T cells (T_{reg} cells). **f** | T_H17 cells are also activated by prostaglandin E_2 (PGE_2) resulting from the engulfment of apoptotic cells. COX2, cyclooxygenase 2; GAS6, growth arrest-specific protein 6; Mtb, *Mycobacterium tuberculosis*; SIRPα, signal-regulatory protein-α; TCR, T cell receptor.

blocks pyroptosis in neutrophils and thereby prevents infected neutrophil efferocytosis and subsequent bacterial killing by macrophages[118]. Another strain of *K. pneumoniae* activates phospholipid transporter flippases in infected neutrophils, which, when the neutrophils die, prevents externalization of phosphatidylserine and recognition by efferocytic macrophages[118]. Similarly to methicillin-resistant *S. aureus*, the bacteria eventually induce necroptosis of the neutrophils, resulting in bacterial spread.

Antigen presentation from infected ACs. Efferocytosis of infected ACs can help to mount an adaptive immune response against bacteria through the process of antigen cross-presentation. This principle was initially established in vitro by showing that efferocytosis of influenza

virus-infected apoptotic monocytes by DCs leads to cross-presentation of influenza virus antigens on MHC class I and activation of CD8+ T cells[119]. Additional in vitro studies reported similar results using ACs infected with *Salmonella* Typhimurium, human cytomegalovirus, vaccinia virus, *M. tuberculosis*, herpes simplex virus (HSV) and HIV-1 (REFS[120–125]). Engulfment of HIV-1-infected or HIV-1-antigen-transduced ACs by DCs in vitro led not only to cross-presentation of antigen on MHC class I, with activation of CD8+ T cells, but also to presentation of antigen on MHC class II, with activation of CD4+ T cells[122].

In vivo, proof of this concept has proven to be more challenging. In one study, mice were injected with influenza virus-infected apoptotic 3T3 cells, and this led to T cell activation directed against influenza

Cross-presentation
The ability of certain antigen-presenting cells to load peptides that are derived from exogenous antigens onto MHC class I molecules. This property is atypical, because most cells exclusively present peptides from their endogenous proteins on MHC class I molecules. Cross-presentation is essential for the initiation of immune responses to viruses that do not infect antigen-presenting cells.

virus antigen[126]. However, a direct a link between DC efferocytosis-mediated cross-presentation and protective antipathogen adaptive immunity in an actual infectious setting was not shown. This critical link was finally established following the discovery of a cell-surface protein complex on CD8α+ DCs, comprising AXL, LRP1 and RAN-binding protein 9 (RANBP9), that binds and internalizes HSV-1-infected ACs in a manner that leads to cross-presentation of AC-derived viral antigens to CD8+ T cells[127] (FIG. 3d). AXL was necessary for AC binding, LRP1 promoted AC internalization via its interaction with the RAC GTP adaptor GULP1 and RANBP9 facilitated the functional interaction between AXL and LRP1. Genetic targeting of any of these molecules on DCs in mice infected with HSV-1 leads to accumulation of infected ACs, impaired activation of splenic CD8+ T cells directed against HSV-1 antigen, increased viral load and decreased mouse survival[127]. As these DC-targeted gene knockouts do not affect HSV-1 infectivity itself, the data provide direct molecular-genetic causation evidence linking DC efferocytosis of infected ACs with cross-presentation of antigen and adaptive immunity in pathogen-infected mice.

A subsequent study investigated the role of annexin 1 in efferocytosis-mediated *M. tuberculosis* cross-presentation in vitro and in vivo[128]. In cultured DCs exposed to *M. tuberculosis*-infected ACs, annexin 1 facilitates both AC uptake and antigen cross-presentation. Accordingly, holoannexin 1-deficient mice infected with *M. tuberculosis* show impaired activation of CD8+ T cells targeting *M. tuberculosis* antigen, higher bacterial load and lower survival compared with wild-type mice. Further studies are needed to test whether other DC efferocytosis effectors implicated in pathogen antigen cross-presentation in vitro have a role in host defence in vivo.

Efferocytosis of infected ACs can also activate inflammatory IL-17-producing T helper cells (T$_H$17 cells)[129] (FIG. 3e). In vitro studies showed that efferocytosis of *Escherichia coli*-infected ACs by DCs induces the production of several proinflammatory cytokines via TLR activation by AC-associated pathogen-associated molecular patterns (PAMPs). The cytokines, notably IL-6 and TGFβ, promote differentiation of CD4+ T cells into T$_H$17 cells and inhibit production of inflammation-suppressing T$_{reg}$ cells. A subset of these IL-17+CD4+ T cells also produce IL-10, which likely acts as a counter-regulatory mechanism to limit the inflammatory response[129]. In mice infected with the diarrhoea-causing bacterium *Citrobacter rodentium*, apoptotic intestinal epithelial cells stimulate the generation of *C. rodentium*-specific T$_H$17 cells. A recent study comparing DC efferocytosis of sterile ACs versus *E. coli*-infected ACs corroborated most of these findings, most notably the activation of T$_H$17 cells[130]. This study also showed that infected ACs increase the expression of CD86 and CCR7 in DCs, which promote antigen presentation and DC migration to lymph nodes, respectively.

The results of these studies seem to imply that the T$_H$17 cell response is a host defence mechanism to limit pathogen damage. However, host defence may be compromised by another consequence of infected AC uptake in this setting; namely, an increase in expression of cyclooxygenase 2 (COX2) and its product PGE$_2$ by efferocytic DCs[130] (FIG. 3f). Despite being able to activate T$_H$17 cells in other settings, PGE$_2$ here may actually limit the extent of the antipathogen T$_H$17 cell response[131]. The mechanism involves a PGE$_2$–EP4 receptor pathway in T$_H$17 cells that downregulates IL-1 receptor and suppresses T$_H$17 cell differentiation. This idea is supported, although not proven, by the finding that treatment of *C. rodentium*-infected mice with an EP4 receptor antagonist increases colonic T$_H$17 cell numbers and *Il17a* mRNA levels, suppresses *C. rodentium* infection and protects mice from colitis. Recall that PGE$_2$ also promotes the replication of *T. cruzi* in macrophages that have engulfed *T. cruzi*-infected ACs[115], and another study showed that PGE$_2$ resulting from efferocytosis by lung macrophages compromises the ability of these cells to kill phagocytosed *S. pneumoniae* in vitro[132]. Thus, whereas postefferocytosis PGE$_2$ production may play a protective role in certain settings[133], in other settings it may be maladaptive.

Further complexity is illustrated by the recent finding in *C. rodentium*-infected mice that efferocytosis of infected ACs by DCs leads to the presentation of AC-derived self-antigens on MHC class II and activation of a subpopulation of self-reactive T$_H$17 cells in the colon[134]. This subpopulation, which coexists with the *C. rodentium*-specific T$_H$17 cells described previously[129], leads to the production of IgA and IgG1 autoantibodies and autoimmune colitis. As with the *C. rodentium*-specific T$_H$17 cell response, the autoimmune response is triggered by bacterial ligand-induced TLR activation, which optimizes the ability of DCs to present phagocytosed antigens, and does not occur in mice infected with a strain of *C. rodentium* that does not cause epithelial cell apoptosis. What determines the proportion of T$_H$17 cells in each subpopulation and how these findings relate to the aforementioned PGE$_2$ pathway[131] remain to be determined. Nonetheless, these findings highlight a crucial issue in our understanding of DC efferocytosis of infected ACs; namely, the importance of the balance among immune tolerance, protective inflammation and maladaptive autoinflammation. Further studies will be necessary to fully understand the mechanisms and possible genetic variations that determine whether DC efferocytosis of infected ACs in infected hosts elicits a proper antipathogen inflammatory response versus one that is either too weak or too strong.

Conclusions

Efferocytosis is crucial for maintaining tissue homeostasis and promoting resolution in response to inflammation and injury. Failed efferocytosis is emerging as a key mechanism driving the development and progression of chronic inflammatory diseases, including atherosclerosis, obesity, diabetes, heart failure, chronic lung disease, neurodegenerative disease and cancer. Accordingly, therapeutic strategies aimed at improving efferocytosis would be predicted to dampen inflammation and improve resolution. As one example, data from genetically engineered mice suggest that inhibiting the cleavage of MERTK and LRP1 would improve efferocytosis, and

in the case of MERTK, this strategy would also enhance the production of proresolving mediators. In addition, it may be possible to compensate for MERTK cleavage by increasing its expression. As an example, the endogenous fatty acid amide palmitoylethanolamide increases MERTK expression by activating G protein-coupled receptor 55 (REF.[135]). When administered to *Apoe*$^{-/-}$ mice, palmitoylethanolamide promotes features of plaque stability in advanced atherosclerotic lesions, including smaller necrotic cores and increased level of lesional collagen[135]. As another strategy, antibody-mediated neutralization of CD47 can ameliorate experimental atherosclerosis, and this type of therapy has been used in clinical trials for cancer.

As with all new therapeutic strategies, challenges will emerge. For example, as illustrated in this Review, efferocytosis can promote pathogen virulence in certain settings. Moreover, the fibrotic response following efferocytosis may prove to be damaging in diseases such as pulmonary fibrosis and non-alcoholic steatohepatitis.

With anti-CD47 antibody, the major challenge is the development of anaemia due to excessive clearance of erythrocytes. Furthermore, a recent study reported that CD47-deficient mice, which were predicted to be protected from atherosclerosis, actually develop increased lesion formation owing in part to increased lymphocyte activation[136]. Meeting these challenges will require increased understanding of the molecular and cellular mechanisms and consequences of efferocytosis. One important area that has attracted recent attention and will require further study is how efferocytes process and react to AC-derived metabolic cargo. This area is particularly relevant to chronic inflammatory diseases, in which efferocytes are confronted with large numbers of ACs. Further understanding of efferocyte metabolism may suggest new therapeutic opportunities for diseases driven by excessive apoptosis, defective efferocytosis and impaired resolution and repair.

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Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

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