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Efferocytosis signaling in the regulation of macrophage inflammatory responses

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Abstract

Since the pioneering work of Elie Metchnikoff and the discovery of cellular immunity, the phagocytic clearance of cellular debris has been considered an integral component of resolving inflammation and restoring function of damaged and infected tissues. We now know that the phagocytic clearance of dying cells (efferocytosis), particularly by macrophages and other immune phagocytes, has profound consequences on innate and adaptive immune responses in inflamed tissues. These immunomodulatory effects result from an array of molecular signaling events between macrophages, dying cells and other tissue-resident cells. In recent years, many of these molecular pathways have been identified and studied in the context of tissue inflammation, helping us better understand the relationship between efferocytosis and inflammation. We review specific types of efferocytosis-related signals that can impact macrophage immune responses and discuss their relevance to inflammation-related diseases.

Keywords

Macrophage; Phagocytosis; Apoptosis; Efferocytosis; Inflammation

Introduction

Tissue injury or infection leads to the prompt activation of a local inflammatory response to mediate pathogen clearance and limit tissue damage. The proper termination of this inflammatory cascade is important to allow execution of tissue repair and resolution responses required to restore normal tissue function. Improper resolution can result in excessive scarring and organ damage as well as chronic inflammation and loss of self-tolerance that contributes to disease states such as cardiovascular disease, cancer and autoimmunity (1). In healthy, immune competent hosts, most inflammatory episodes are self-limiting, with endogenous host mechanisms successfully orchestrating the onset and resolution of inflammation. The identification and molecular characterization of these endogenous resolution pathways has been a promising and intense area of research in recent

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years (1–3). In this review, we will discuss how some of the signals that regulate apoptotic cell clearance (efferocytosis) can also function to modulate macrophage immune responses in the context of tissue inflammation. While we primarily focus on macrophage responses in this review, it is important to emphasize that many different types of hematopoietic and non-hematopoietic cell types can carry out efferocytosis and that apoptotic cells can exert important and sometimes cell type-specific immunoregulatory consequences on these phagocytes (reviewed in (4–6))

Cell death in inflamed tissues

Cell death is a universal feature of infected and damaged tissues. Cell death can occur by unregulated, “accidental” means such as mechanical or osmotic lysis, particularly in the early stages of inflammation. However inflammation-associated cell death can also result from the activation of specific biochemical pathways (e.g. apoptosis, necroptosis) (7). Apoptosis is the most prominent mechanism of programmed cell death seen in inflamed tissues for both hematopoietic and non-hematopoietic cells (1, 8). The stimuli that trigger apoptosis within inflamed tissues vary widely depending on the type of inflammation, and can be due to pathogen-derived, host-derived or iatrogenic stimuli. However, it is important to note that inflammation-associated apoptosis is most often multifactorial and in most inflammatory settings the precise cellular and molecular mechanisms of apoptosis induction are poorly understood. Nonetheless, it is clear that apoptotic cell death plays an integral role in the outcome of tissue inflammation.

Sepsis is one of the best-studied examples where apoptosis has clear and profound consequences on the resolution of inflammation. A life-threatening condition responsible for ~250,000 U.S. deaths annually, sepsis can occur when a localized infection fails to properly resolve, leading to systemic inflammation, organ failure and death (2, 3, 9). In humans and mice, sepsis is associated with widespread apoptosis in lymphoid organs causing dramatic reductions in hematopoietic cell numbers, including thymic T cells, bone marrow B cells and peripheral lymphocytes (7, 10). This depletion can cause persistent lymphopenia that contributes to mortality (11). In mice, blockade of apoptosis using caspase inhibitors or overexpression of anti-apoptotic genes improves survival following polymicrobial sepsis (12, 13). Similarly, most myeloid cell populations are also depressed in sepsis (e.g. monocytes, macrophages, DC), although neutrophil survival is increased (14). Consequently, sepsis-associated leukocyte apoptosis is thought to transiently impair immunity and contribute to the generation of an immunosuppressed state known as compensatory anti-inflammatory response syndrome (15). The extent to which immunomodulatory effects of efferocytosis contribute to sustained immune suppression is an important but poorly understood area of sepsis research (16).

Leukocyte apoptosis is also a characteristic feature of self-limiting inflammation resulting from acute, localized and moderate injury or infection. However, unlike sepsis, apoptotic cells are thought to be beneficial in self-resolving inflammation by helping to reprogram tissue macrophages from a pro-inflammatory to a pro-resolution state (17). Tissue resident macrophages are key immune sentinels and thus are among the first immune cells to respond to tissue damage by producing cytokines that initiate and orchestrate the recruitment of

granulocytes from the blood into the tissue (18). Neutrophils typically accumulate in significant numbers within minutes to a few hours depending on the nature and severity of the injury. Once in the tissue, most neutrophils rapidly undergo apoptosis and begin to accumulate as uncleared apoptotic cells. The time at which viable neutrophil numbers are highest in the tissue is often considered the “peak” of a self-limiting inflammatory episode (17). Importantly, the maximum number of uncleared apoptotic neutrophils in the tissue typically coincides with this “peak”, followed by macrophage-mediated efferocytosis and clearance of the neutrophils during the resolution stage (19). Such observations support the theory that efferocytosis of apoptotic neutrophils by resident macrophages is an important immune trigger for the onset of resolution (20, 21). However confirmation of this theory has been hampered by the difficulty in tracking the fate of engulfed endogenous apoptotic cells. Nonetheless, there is abundant evidence that apoptotic cells can suppress macrophage inflammatory responses in vitro and that failure to clear apoptotic cells exacerbates inflammation in vivo, indicating efferocytosis plays a crucial role in modulating the inflammatory response of macrophages (and other phagocytes) to promote resolution (5, 6, 22).

Homeostatic cell clearance

Approximately 1×10^{10} cells undergo apoptosis daily in the human body; however, few free apoptotic cells are seen at homeostasis, indicating the presence of highly efficient efferocytosis mechanisms (23). This efficiency depends on the execution of the three main efferocytosis signaling programs depicted in Figure 1: 1) find-me signaling – chemoattractant-mediated recruitment of phagocytes to apoptotic cells, 2) eat-me signaling – receptor-mediated recognition and engulfment of apoptotic cells, and 3) post-engulfment signaling – signals related to the phagolysosomal processing of engulfed cellular material. The most obvious anti-inflammatory effect of efferocytosis is the physical sequestration of dying cells to limit the release of intracellular DAMPs that can drive inflammation (7, 24, 25). Indeed, there is now very strong evidence that disrupting homeostatic efferocytosis in mice, either genetically or chemically, can lead to accumulation of uncleared apoptotic cells in situ along with spontaneous inflammation and autoimmune disease (reviewed in (6, 26, 27)). However, efferocytosis can also directly alter the inflammatory signaling pathways in the engulfing macrophage. Here we will discuss how many of the signals that mediate the three stages of efferocytosis also play important roles in driving the immunomodulatory effects of apoptotic cells on macrophages.

Find-me signaling in inflammation

In order for phagocytosis to occur, phagocytes must first “find” a cell undergoing apoptosis. This find-me stage of efferocytosis is mediated by the release of numerous soluble factors that attract macrophages to the site of cell death (6, 28, 29). Key find-me signals identified to date include triphosphate nucleotides (ATP, UTP) (30, 31), the chemokine CX3CL1 (32), and the signaling lipids lysophosphatidylcholine (lysoPC) and sphingosine-1-phosphate (S1P) (33–35). While all of these factors can stimulate macrophage migration to apoptotic cells, the relevance of individual find-me signals in efferocytosis depends on many factors, including phagocyte and apoptotic cell type as well as the apoptotic stimuli and the stage of

apoptosis being studied (reviewed in (36)). As discussed below, some of these find-me signals also function as key modulators of macrophage inflammatory responses.

Apoptotic cells release adenine and uridine nucleotides through caspase-3/7-activated hexameric pannexin-1 channels on their surface (37). Extracellular nucleotides ATP and UTP act as find-me signals to promote cell clearance by stimulating recruitment of motile phagocytes expressing P2Y purinergic receptors and also by causing upregulation of phagocytic receptors (30, 38–40). Extracellular nucleotides can also exert immunomodulatory effects on macrophages via the breakdown of ATP into adenosine – a well known and potent modulator of macrophage inflammation (41, 42). Recent reports have shown that during efferocytosis, G_s-linked A2a and A2b adenosine receptors on macrophages mediate suppression of pro-inflammatory cytokines (e.g. CXCL1, CXCL2) and upregulation of pro-resolution factors (e.g. *Nr4a1*, *Thbs1*) (43, 44) (Figure 1). Interestingly, the precise mechanisms responsible for generating extracellular adenosine during efferocytosis are poorly understood. Although adenosine can be released directly from macrophages (43, 45), in many tissue settings the accumulation of extracellular adenosine is largely due to the hydrolysis of extracellular adenine nucleotides (i.e. ATP, ADP, AMP) by ecto-enzymes such as CD39 (ATP/ADP→AMP) and CD73 (AMP→adenosine) (41, 42). How these ecto-enzymes contribute to adenosine generation and immune modulation of macrophages during efferocytosis remains an open question.

The chemokine CX3CL1 was identified as an apoptotic find-me signal by Gregory and colleagues (32), and since has been implicated in cell clearance and tissue repair in a number of tissue settings. Apoptotic cell-derived CX3CL1 stimulates recruitment of CX3CR1+ phagocytes to clear apoptotic debris in lymphoid tissues and the brain (32, 46). Additionally, CX3CL1 from apoptotic cells enhances efferocytosis by stimulating phagocytes to express the eat-me ligand MFG-E8 (47–49). In the context of tissue repair, CX3CL1-CX3CR1 signaling has been shown to play a beneficial role by reducing expression of inflammatory cytokines and enhancing expression of pro-survival (e.g. BCL-2) and antioxidant genes (HO-1) (49–51). Thus release of CX3CL1 from dying cells not only serves to promote ‘clean-up’ of damaged tissues, but also aids in driving expression of genes that are beneficial to the restoration of tissue homeostasis.

Lipid find-me signals also double as chemoattractants and immune regulators during efferocytosis. S1P is released by apoptotic cells through the caspase-dependent upregulation of S1P kinases (SphK) 1 and 2 (34, 35). Chemotaxis of myeloid cells to S1P is mediated by the S1P family of GPCRs (S1PR1-5) (52). Beyond its role as a macrophage chemoattractant, Brunë and colleagues found that S1P derived from apoptotic cells provokes an anti-apoptotic and anti-inflammatory gene expression program in macrophages, characterized by suppression of TNF and IL-12 along with increased production of IL-10, VEGF and PGE_2 (53–55). Moreover, S1PR signaling in macrophages promotes an alternative “M2-like” anti-inflammatory phenotype, including increased cAMP and COX-2 and suppression of NFkB signaling (52). More recently, Luo et al reported that apoptotic cell-derived S1P can stimulate erythropoietin-EPR autocrine signaling in macrophages that stimulates activation of the M2-promoting nuclear receptor PPARγ (56). Like S1P, lysoPC is also a potent chemoattractant find-me signal for monocytes and macrophages during efferocytosis (33),

and deletion of a putative lysoPC receptor G2A in mice leads to increased tissue inflammation and hallmarks of systemic autoimmunity (57, 58). However, it is presently unclear what role apoptotic cell-derived lysoPC plays in the immunoregulatory functions of G2A in vivo.

Eat-me signaling in inflammation

Macrophages primarily recognize apoptotic cells via high levels of surface PtdSer. Normally confined to the inner leaflet of viable cells, PtdSer is enriched on the exofacial side of the membrane as a result of caspase-mediated changes in the activity of several key phospholipid transport enzymes (reviewed in (59)). At least 12 PtdSer efferocytosis receptors have been identified to date and comprise a structurally heterologous group of surface proteins that bind either directly to PtdSer or indirectly via recognition of soluble PtdSer-binding opsonins (reviewed in (6, 60)). In 2002 Henson and colleagues first showed that PtdSer on apoptotic cells was not only a critical ‘eat-me’ ligand, but also that PtdSer recognition by macrophages has profound immunomodulatory effects on macrophages and other phagocytes (61). Since this time, many efferocytic receptors have been identified and subsequently found to play a role in regulating macrophage immune responses (Figure 1).

The four best-studied *bona fide* PtdSer efferocytosis receptors are: BAI, TIM, Stabilin, and CD300. The GPCR BAI1 directly binds PtdSer via a series of N-terminal thrombospondin repeats (TSR) and subsequently stimulates actin polymerization and phagocytosis via recruitment and activation of the Elmo-Dock bipartite Rac-GEF (62). While *Bai1* expression is restricted to specific phagocyte populations (62), it appears particularly important for efferocytosis by gastric and intestinal phagocytes (63). Using a mouse model of colitis, Lee et al recently reported that Bai1-deficient mice displayed increased colonic inflammation, tissue damage and mortality (64). It was also shown that *Bai1* deletion in macrophages and colonic epithelial cells leads to higher levels of IL-1α, IL-6 and TNF in the presence of apoptotic cells in vitro (64). These findings are in line with previous studies showing that Elmo1-Dock signaling not only regualtes actin dynamics but can also influence the expression of inflammatory genes (65–67). Presently it is not clear how Bai/Elmo/Dock signaling alters inflammatory gene expression (64). Interestingly, separate studies have shown that Elmo can localize to the nucleus (68) and interact with transcriptional machinery (66), but whether these functions are relevant to changes in macrophage gene expression during efferocytosis is not known.

The TIM family of receptors (hTIM-1, 3, 4) are type I cell-surface glycoproteins that bind PtdSer via N-terminal IgV domains to mediate apoptotic cell engulfment (69–71). TIM-1 and TIM-3 possess one or more tyrosine phosphorylation sites in their C-terminal cytoplasmic domains that control ligand-dependent recruitment and suppression of Src family kinases (70). TIM expression in murine immune phagocytes is highly subset- and tissue-restricted, and mice lacking individual TIM molecules, while viable, display a number of organ-specific immune defects (70, 72–75). In a renal ischemia/reperfusion injury model, TIM-1 binding of PtdSer is required for apoptotic cells to suppress NFkB activation and production of TNF, IL-6, CCL5 in proximal tubular cells (76, 77). In these studies, tyrosine phosphorylation of the TIM-1 cytoplasmic tail caused increased recruitment of the p85

subunit of PI3K and inhibition of NFkB activation downstream of TLR4 (77). TIM-3 is expressed on multiple macrophage and DC populations, and can evoke both immune-activating and immune-suppressing outcomes during efferocytosis (78). In TLR-stimulated CD8+ dendritic cells (DC), TIM-3 can promote cross-presentation of corpse antigens (79), However, signaling via TIM-3 can also inhibit NFkB and inflammatory cytokine production via tyrosine phosphorylation of the TIM-3 cytoplasmic tail (78). TIM-4, by contrast, has a short cytoplasmic tail that is dispensable for apoptotic cell engulfment, suggesting that TIM-4 functions as a high affinity PtdSer binding/tethering receptor rather than *bona fide* engulfment signaling receptor (80, 81). Still, Martinez et al recently showed that TIM-4-mediated corpse engulfment can trigger recruitment of specific autophagy components to the phagosome in a distinct form noncanonical autophagy termed LC3-associated phagocytosis (LAP) (82). Subsequently Green and colleagues reported that genetic disruption of LAP in myeloid cells (via deletion of NOX2 or RUBCN) leads to a chronic autoinflammatory disorder and development of SLE-like disease in mice (83). In these studies LAP-deficient macrophages stimulated with apoptotic cells in vitro were found to produce much higher levels of inflammatory cytokines (IL-1β, IL-6, CXCL10) and lower levels of IL-10 compared to LAP-sufficient macrophages (83). Given that TIM-4-deficient mice also develop hallmarks of tissue inflammation and autoimmunity (73, 74), it will be important to delineate the precise roles of TIM-4 in conventional efferocytosis versus LAP in shaping macrophage inflammatory responses to apoptotic cells.

Of the seven human *CD300* genes, three have been shown to bind PtdSer and modulate efferocytosis: CD300A, CD300B, CD300F (84). However, the immunomodulatory effects of CD300 receptors during efferocytosis are not straightforward, as these receptors can trigger either activating or inhibitory outcomes depending on their association with different cytoplasmic signaling modules. For example, although signaling via the ITIM domain of CD300A inhibits phagocytosis of apoptotic cells, this domain is important for suppression of inflammatory cytokines during efferocytosis (85, 86). CD300F can both stimulate and inhibit efferocytosis depending on the type of phagocyte, and CD300F-deficient mice develop symptoms of autoimmunity (87). CD300B signaling via the adaptor protein DAP12 positively regulates efferocytosis, and Voss et al recently showed that LPS treatment of macrophages causes CD300B to interact with TLR4 and that this interaction augments TLR4-dependent production of pro-inflammatory cytokines (88). Along these lines, the CD300F receptor was recently shown to interact directly with the common chain of IL-4/IL-13 receptor to enhance production of IL-4 and thus could potentially promote M2 polarization in macrophages (89). Although CD300 molecules have been shown to regulate immune responses in many different tissue settings, it remains to be seen exactly how CD300-signaling during efferocytosis impacts immune signaling and cytokine production in macrophages during the resolution of tissue inflammation.

Stabilin receptors (*Stab1, Stab2*) are type I surface receptors that trigger efferocytosis through direct interaction with PtdSer (90–92). In mice, Stabilin receptors are expressed in a number of macrophage populations (93), while in humans *Stab1* was found to be highly expressed in endothelial cells and steroid-treated tissue macrophages (94). In vitro, expression of *Stab2* in cultured cells stimulates the binding and Rac-dependent internalization of apoptotic cells and also stimulates TGFβ production (91). Interestingly,

Stab1/Stab2 double knockout mice have shorter lifespans and increased tissue inflammation compared to wild-type mice, although these results likely stem from the loss of hyaluronic acid scavenging mediated by these receptors (95, 96). How Stabilin signaling regulates cytokine expression during efferocytosis is still unclear, although STAB1-mediated engulfment requires the intracellular adaptor protein GULP which has been shown to regulate cytokine production downstream of other receptors including LRP1 (97, 98).

A number of efferocytic receptors indirectly recognize PtdSer on apoptotic cells via interaction with PtdSer-binding 'bridging' proteins such as MFG-E8, CCN1, GAS6 and Protein S (ProS1) (6, 60). Upon binding to PtdSer on apoptotic cells MFG-E8 and CCN1 are recognized by $\alpha_v\beta_{3/5}$ integrin/vitronectin receptors on phagocytes, leading to Rac activation and corpse internalization (99, 100). It is clear that α_v-mediated engulfment of MFG-E8 opsonized apoptotic cells is important for limiting tissue inflammation and maintenance of self-tolerance in mice (99, 101). However, it is less clear whether $\alpha_v\beta_{3/5}$ signaling directly regulates cytokine production in macrophages during efferocytosis. Studies using RGD mimetics to manipulate α_v–mediated apoptotic cell recognition have reported significant effects on macrophage TNF and IL-10 production (102, 103). By contrast, studies using $\alpha_v\beta_{3/5}$ blocking antibodies or macrophages deficient in β_3 or β_5 did not find substantial effects on cytokine production during efferocytosis (104, 105). Still, it is important to note that the role of α_v integrins in efferocytosis is quite complex owing to its interactions with multiple β chains (i.e. β_1, β_6, β_8) and the formation of signaling complexes with other efferocytosis molecules such as CD36, thrombospondin and HMGB1 (106, 107). Thus α_v integrins play key roles in macrophage adhesion, motility and phagocytosis, but more investigation is required to define their contribution to immune signaling during efferocytosis.

The TAM (Tyro3, Axl, Mer) tyrosine kinases are another family of efferocytosis receptors that recognize PtdSer on apoptotic cells indirectly through binding to the PtdSer-binding serum proteins GAS6 and ProS1. All three TAM receptors are capable of mediating efferocytosis, with Axl and Mer being particularly important for efferocytosis by dendritic cells and macrophages (108, 109). Disruption of TAM signaling in vivo leads to increased levels of uncleared apoptotic cells in multiple tissues, and TAM-deficient mice display exacerbated responses to apoptotic cells and inflammatory stimuli in vivo (108, 110, 111). At the molecular level, recognition of Gas6/ProS1-opsonized apoptotic cells causes TAM receptor dimerization shown to function as key negative regulators of inflammation and adaptive immunity by controlling multiple immune signaling pathways and gene expression programs. In the context of efferocytosis, signaling via Axl and Mer can directly suppress TLR- and type I IFN-driven inflammatory signaling pathways via distinct mechanisms. In dendritic cells, activation of Mer by apoptotic cells inhibits IKK activity downstream of TLR4, leading to reduced NFkB binding at the *Tnf* promoter (113). In a separate study, Sharif et al reported that suppression of TLR-driven TNF by apoptotic cells required Axl-mediated upregulation of the *Twist* transcriptional repressors that bind E-box elements and inhibits *Tnf* promoter activity (110). Interestingly, TAM receptors also function as negative regulators of type I IFN-induced inflammatory signaling in DC by suppressing IFNα-mediated STAT1 signaling and by upregulation of the E3 ubiquitin ligases suppressor of cytokine signaling 1 and 3 (SOCS1 and SOC3) (111). Presently, the precise mechanisms

regulating these responses and what roles they play in efferocytosis-mediated immune modulation remain to be fully defined. Nevertheless it is clear that in macrophages and DCs TAMs are key mediators of both apoptotic cell phagocytosis and anti-inflammatory signaling. As such, TAMs are very attractive targets for modulation of efferocytosis responses in controlling innate and adaptive immune responses.

Post-engulfment signaling in inflammation

The engulfment of apoptotic cell by macrophages results in the acquisition of substantial quantities of excess cellular materials such as lipids, carbohydrates, proteins and nucleic acids, and macrophages can adjust to this increased metabolic load by activating degradation and efflux pathways (114). In addition, a number of recent studies have shown that engulfment of apoptotic cells engages multiple metabolic sensing pathways in macrophages that play important roles in controlling phagocytosis and immune signaling (115–118). Among these metabolite-sensing mechanisms, the nuclear receptor (NR) family of transcriptional regulators stand as the best studied link between sensing of ingested apoptotic cells and the macrophage efferocytosis machinery. Studies using genetic and pharmacologic manipulation have identified multiple NR family members as critical regulators of macrophage efferocytosis in vitro and in vivo, including *LXRα, LXRβ, PPARγ, PPARδ, RXRα* (reviewed in (6, 119)). An important and consistent finding from these studies is that NR activation, both homeostatically and during efferocytosis, enhances transcription of engulfment-related genes in macrophages, including PtdSer receptors, PtdSer binding soluble proteins and key intracellular engulfment signaling molecules (Figure 1). Accordingly, mice lacking one or more of these NRs display defective clearance of apoptotic cells, enhanced tissue inflammation and autoimmunity (120–122). However, it is important to note that while internalization of apoptotic cells can modulate the activity of multiple NRs, we presently do not know the cellular source or precise molecular nature of the NR-modulating ligands relevant to efferocytosis.

In addition to promoting phagocytosis, NRs are involved in the regulation of macrophage inflammation during efferocytosis (119). For example, treatment of LXRα/β-deficient macrophages with apoptotic cells does not induce TGFβ and IL-10 production nor suppress IL-1β and IL-12 production like their WT counterparts (121). Similarly, macrophages lacking PPARδ fail to produce IL-10 and produce more TNF and IL-12 than WT macrophages when cultured with apoptotic cells (120). Also, macrophages from mice lacking PPARγ and RXRα are refractory to the immunosuppressive effects of apoptotic cells in vitro (122). More recently, the nuclear receptor Nr4a1 was found to be rapidly upregulated in macrophages cultured with apoptotic cells, and Nr4a1-deficient macrophages cultured with apoptotic cells showed enhanced NFkB activation and IL-12 production compared to WT macrophages (44, 123). Interestingly, Nr4a1 upregulation by apoptotic cells can occur via adenosine activation of the A2a receptor, suggesting regulation of this NR could be stimulated by nucleotide find-me signals as well as via phagosomal signaling. (44, 124).

Conclusions

The discovery of the immunomodulatory activity of apoptotic cells on macrophages nearly 20 years ago transformed our view of efferocytosis from a simple garbage disposal process to one capable of actively shaping immune responses and influencing tissue restorative programs. This paradigm shift has led to promising new therapeutic avenues built on manipulating efferocytosis-related processes, as evidenced by the ongoing trials of anti-PtdSer antibodies in several clinical settings (125, 126). The efferocytosis mechanisms highlighted here illustrate the ability of macrophages to engage specific molecular pathways that control both phagocytosis and immune signaling. This linkage supports the idea that the process of dead cell clearance is not simply an end unto itself, but rather that the efferocytosis process provides key physiologic status information regarding cell death and tissue health to the immune system via macrophages (and other phagocytes). Finally, it is important to note that while efferocytosis signaling is often framed as a chronological three-step process (Figure 1), physiologic efferocytosis by macrophages almost certainly involves the simultaneous integration of many different efferocytosis signaling processes. As such, understanding how the spatiotemporal dynamics and potential synergistic relationships of these different signaling pathways affect cell clearance and immunity stands as an important, albeit very challenging, area for future studies. Considering the ubiquity of cell death during inflammation and the inherent signaling complexities of inflamed tissues, major mechanistic advances in this area will likely require new experimental approaches to tease apart the precise immunomodulatory roles of dead cell-derived signals.

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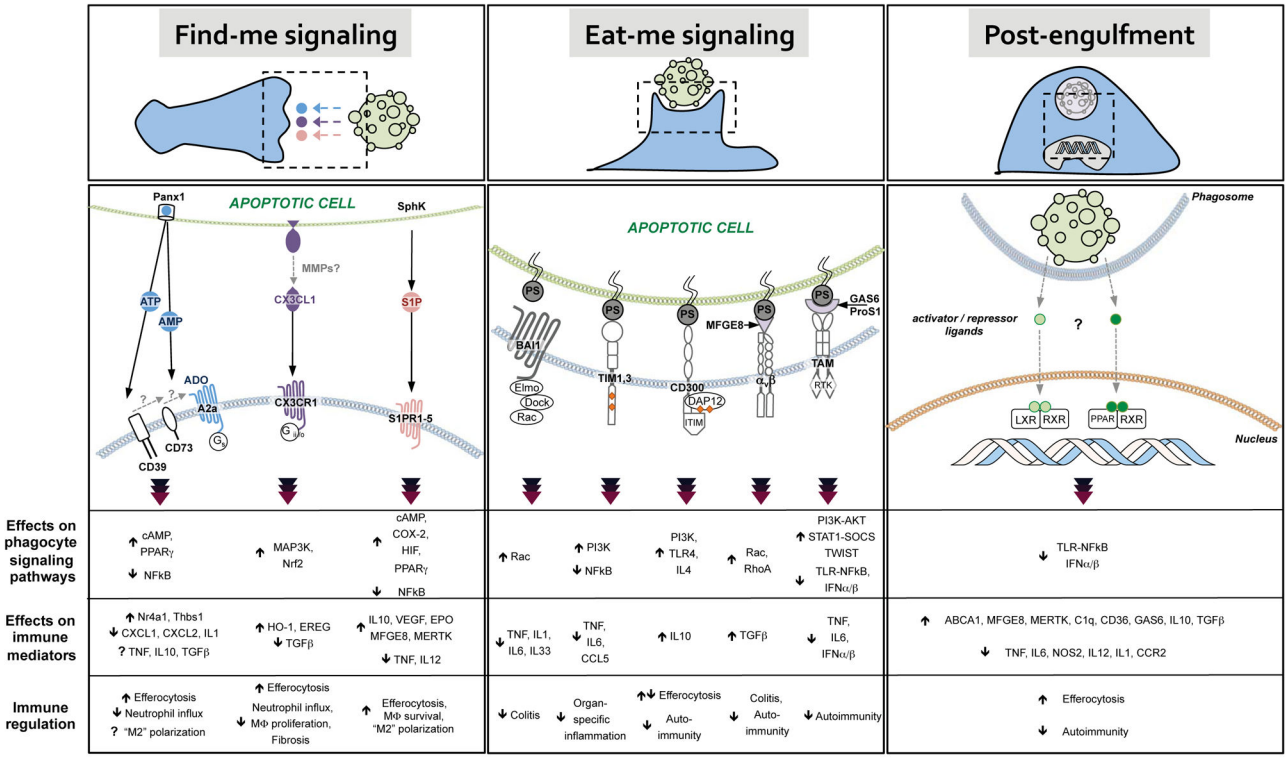

Figure 1. Immunomodulatory roles of efferocytosis signals

Depiction of the three key stages of efferocytosis (dashed boxes) with detailed illustrations showing some of the known signaling molecules/pathways relevant to immune modulation for each of the three stages. The lower table indicates some of the key effects of these efferocytosis signaling mechanisms on: immune signaling in phagocytes, production of immune mediators, and some of the prominent immune outcomes of the indicated molecules/pathways. Arrows in the table indicate whether signaling via these pathways generally increases or decreases the effects listed. Please note this diagram represents only a portion of the efferocytosis signals and their immunoregulatory effects that have been described. More comprehensive lists of these signaling pathways can be found in references 6, 61. MMP (matrix metalloprotease) PS (PtdSer), RTK (receptor tyrosine kinase), ITIM (immune-tyrosine based inhibitory motif).